TABLE 4. Time to Onset, Action Taken, and Outcome of ADRs in Patients Receiving Erlotinib

Event	Median Time to Onset, Days (Range)	Actions Taken With Erlotinib, %				Patients With Recovery/ Improvement, % (*n*)	Median Time to Recovery/ Improvement, Days (Range)
		Continued	Discontinued	Suspended	Dose Decreased		
Skin disorder							
Rash	8 (1–494)	71.9	8.5	5.8	12.0	81.8 (1798/2199)	34 (1–605)
Dry skin	15 (1–185)	85.9	3.0	2.6	7.0	72.6 (196/270)	47 (2–535)
Pruritus	11 (1–220)	79.5	6.1	9.1	5.3	84.8 (112/132)	33 (2–243)
Paronychia	32 (2–558)	70.0	8.1	10.0	10.0	84.3 (177/210)	55 (4–564)
Diarrhea	8 (1–365)	77.0	7.7	6.5	7.7	94.7 (776/819)	12 (1–538)
Hepatic function disorder	13 (1–448)	54.0	19.5	11.8	9.9	77.0 (288/374)	20 (3–450)
ILD	23 (1–339)	1.9	88.0	5.1	0.6	54.4 (86/158)	22 (4–208)
Eye disorder	15 (1–362)	80.6	7.2	6.5	4.3	87.8 (122/139)	20 (2–545)
Hemorrhage	13 (2–259)	53.7	31.5	7.4	3.7	77.8 (42/54)	10 (1–86)

Patients with missing data for the time from the start of erlotinib treatment to the onset of ADR were excluded. The analysis includes only the results of action taken with erlotinib ADRs. Percentages do not indicate the final treatment status of erlotinib.
ADR, adverse drug reaction; ILD, interstitial lung disease.

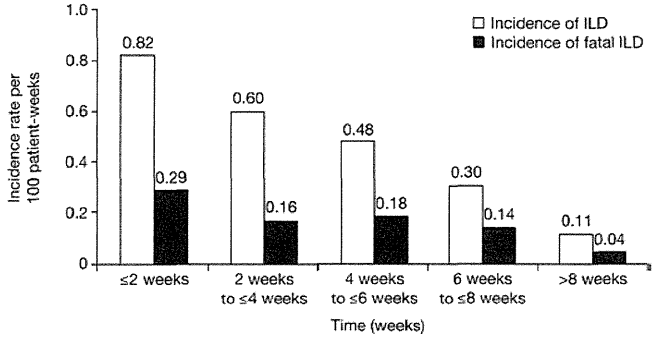

FIGURE 2. Time to ILD onset from first erlotinib dose and outcome of ILD by duration of observation. The eight patients without data for either the duration of observation or the time from the start of erlotinib treatment to the onset of ILD were excluded from the analysis. Value determined by dividing the number of patients developing ILD during the specified duration of observation by the patient-days during the observation period (total duration [number of days] of observation of all patients receiving erlotinib during the specified duration of observation). Expressed per 100 patient-weeks. ILD deaths are also expressed per 100 patient-weeks. ILD, interstitial lung disease.

Japan remain unclear, but this may reflect genetic or biological differences between the Japanese population and the rest of the world. Similar trends have been observed with gefitnib[6] and other drugs.[6,8–10]

ILD was typically characterized by an early onset after initiation of erlotinib, with the highest incidence occurring during the first 2 weeks, followed by a marked decrease 8 weeks after treatment initiation. Physicians should be actively aware of the symptoms of ILD, which usually, but not exclusively occur within 8 weeks of treatment initiation. Notably, a number of patients in this study developed ILD after 8 weeks of treatment. These findings are consistent with those reported in Japanese NSCLC studies with gefitinib.[5,6] In our study, multivariate Cox regression analysis showed that concomitant or previous ILD, smoking history, concomitant or previous lung infection, and an ECOG PS of 2 to 4 were significant risk factors for ILD. This is consistent with poor PS and a history of smoking being cited as risk factors for the development of ILD among gefitinib-treated patients,[5,6] whereas a previous history of ILD seems to be a risk factor in both gefitinib-treated patients and patients prescribed other drugs.[11–13] In contrast to our study, studies on gefitinib did not identify a previous history of lung infection as a risk factor for ILD.[5,6] This was possibly a result of patients with a previous history of lung infection not being among the patient-selection variables in the gefitinib studies. Although some reports suggest that sex (male) and tumor histology (squamous) could be risk factors for ILD, because of the high rate of smoking history in these groups, these findings were not corroborated in our study.[14,15] There was no two-factor interaction for any of these risk factors, which implies that patients with two risk factors are not exponentially more at risk than those with only one risk factor.

Erlotinib was generally well tolerated with rash and diarrhea, the most frequently observed ADRs, a fact that is consistent with findings of phase III trials of erlotinib for NSCLC. These events were predominantly grade 1 to 2, manageable, and their frequency was comparable to that reported in Japanese phase II clinical trials of erlotinib[2–4] and in gefitinib clinical studies.[16,17]

Median OS and PFS were 260 days (approximately 8.5 months) and 64 days (approximately 2.1 months), respectively, in our study. These results are broadly comparable to those reported in the global TRUST trial in pretreated NSCLC (7.9 and 3.25 months, respectively)[7] and the global phase III BR.21 trial (6.7 and 2.2 months, respectively),[1] although median OS in our study was approximately 2 months longer than that seen in BR.21. Meanwhile, two Japanese phase II studies of erlotinib in NSCLC showed more favorable results with 13.5 and 14.7 months for median OS, and 75 and 77 days for median PFS,[2,3] similar to results achieved in a subgroup analysis of East/South-East Asian patients from the TRUST

TABLE 5. Risk Factors for ILD Detected in Cox Regression Analysis (Univariate Analysis and Multivariate Analysis)[a]

			Univariate Analysis		Multivariate Analysis	
Demographics	**Total Number of Patients**	**Incidence of ILD, %**	**Hazard Ratio (95% CI)**	***p***	**Hazard Ratio (95% CI)**	***p***
Total[b]	3488	4.5				
Sex						
Male	1792	6.3				
Female	1696	2.7	0.389 (0.275–0.551)	<0.0001		
Age (yrs)						
<55	561	3.6				
≥55	2926	4.7	1.208 (0.755–1.933)	0.4308		
BMI (kg/m^2)						
<25	2631	5.2				
≥25	450	3.3	0.564 (0.325–0.978)	0.0415		
Histology						
Adenocarcinoma	2891	4.3				
Other	587	5.6	1.440 (0.975–2.125)	0.0667		
Time from the date of first diagnosis of NSCLC to the start of treatment (days)						
<360	849	4.2				
≥360	2517	4.6	0.953 (0.653–1.391)	0.8015		
Concomitant disease or medical history						
Emphysema or COPD: No	3184	4.1				
Emphysema or COPD: Yes	266	10.9	3.254 (2.159–4.905)	<0.0001		
ILD: No	3320	3.9				
ILD: Yes	168	17.3	4.724 (3.118–7.158)	<0.0001	4.074 (2.622–6.331)	<0.0001
Lung infection: No	3221	4.2				
Lung infection: Yes	227	9.3	2.457 (1.550–3.894)	0.0001	1.972 (1.180–3.296)	0.0095
Concomitant disease						
Hepatic dysfunction: No	3191	4.4				
Hepatic dysfunction: Yes	267	6.4	1.654 (0.999–2.738)	0.0505		
Renal dysfunction: No	3191	4.4				
Renal dysfunction: Yes	267	6.4	1.579 (0.953–2.614)	0.0760		
Cardiovascular disease: No	2536	4.3				
Cardiovascular disease: Yes	935	5.0	1.104 (0.780–1.562)	0.5770		
History of allergies						
No	2980	4.3				
Yes	462	5.4	1.234 (0.803–1.895)	0.3376		
Smoking history						
No	1662	2.5				
Yes	1777	6.4	2.910 (2.032–4.168)	<0.0001	2.991 (1.988–4.498)	<0.0001
ECOG PS						
0,1	2576	4.3				
2–4	910	5.2	1.677 (1.183–2.378)	0.0037	1.628 (1.105–2.398)	0.0137
History of chest radiotherapy						
No	2488	3.8				
Yes	989	6.4	1.823 (1.321–2.517)	0.0003		
Pretreatment LDH (IU/l)[c]						
<250	2167	4.0				
≥250	1187	5.6	1.861 (1.346–2.571)	0.0002		
Number of chemotherapy regimens for primary disease[c]						
1	666	4.1				
≥2	2780	4.7	1.208 (0.792–1.842)	0.3805		
History of gemcitabine						
No	1761	4.5				
Yes	1684	4.6	1.085 (0.791–1.488)	0.6149		

(Continued)

TABLE 5. *(Continued)*

Demographics	Total Number of Patients	Incidence of ILD, %	Univariate Analysis Hazard Ratio (95% CI)	Univariate Analysis *p*	Multivariate Analysis Hazard Ratio (95% CI)	Multivariate Analysis *p*
History of gefitinib						
No	1550	5.9				
Yes	1905	3.5	0.606 (0.440–0.834)	0.0021		

BMI, body mass index; ECOG PS, Eastern Cooperative Oncology Group performance status; ILD, interstitial lung disease; LDH, lactate dehydrogenase; COPD, chronic obstructive pulmonary disease.

[a]Exploratory variables included: selected baseline demographics, risk factors reported in clinical studies of gefitinib,[5,6] history of the chemotherapies with high risk of ILD in Japan, and the statistically significant factors in univariate analysis from which some factors were excluded if they are correlated to other factors.

[b]Eight patients whose observation periods were unknown were excluded from the univariate and multivariate analyses.

[c]LDH and number of chemotherapy regimens were analyzed as a continuous variable in the multivariate analysis.

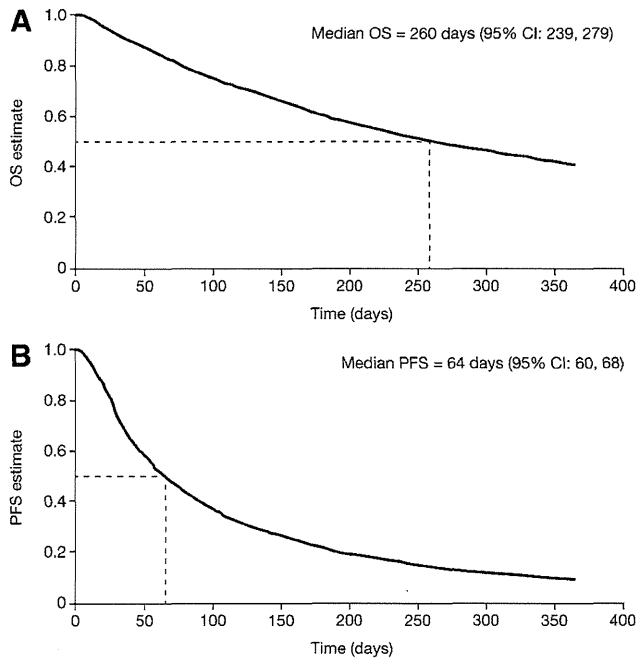

FIGURE 3. Kaplan-Meier curves for (*A*) OS and (*B*) PFS in the efficacy analysis population. *A*, OS (Information on final outcome was unavailable for one patient who was excluded from the OS calculation). *B*, PFS (Information on disease progression was unavailable for seven patients who were excluded from the PFS calculation).

study (14.7 and 5.75 months for median OS and median PFS, respectively).[18] A possible reason for this difference could be the inclusion in our study of a relatively high proportion of patients with a poor ECOG PS (2–4), a history of previous gefitinib therapy, and a high number of patients who had received multiple lines of chemotherapy, making this a relatively poor prognosis group. In the Japanese phase II studies, the majority of patients had an ECOG PS of 0 to 1 (99.1%; 107 of 108 patients) and patients with a history of gefitinib were excluded.[2,3] Poor PS is an independent prognostic factor for shorter survival in patients with NSCLC.[19] Interestingly, in the current study, patients with an ECOG PS of 0 to 1 had a median OS of 370 days and a median PFS of 80 days, suggesting that these data are not inferior to those from the Japanese phase II clinical trials. Furthermore, consistent with the BR.21, TRUST, and Japanese phase II studies,[1–3,7] OS and PFS were longer in women, nonsmokers, patients with nonsquamous NSCLC, patients with a PS 0 to 1, and those with a rash classified as grade 2 or more. Prolonged survival in patients with rash versus in those without rash has also been described in gefitinib-treated patients with NSCLC.[20,21] Mohamed et al. reported significantly longer median survival in patients experiencing any grade of skin rash compared with those without rash (10.8 versus 4.0 months, respectively; hazard ratio 0.41; $p < 0.0001$), whereas Janne et al. reported better survival for patients with gefitinib-induced skin rash, but the correlation did not reach statistical significance.

Several factors should be taken into account when considering our results. First, this was a single-arm surveillance study with no control group; second, unlike a clinical trial, our study did not include a strict period of observation for a defined investigation; and third, no patient-selection criteria were used: all the 3488 patients treated with erlotinib in the postapproval period up to the cutoff date were included in the interim analysis. Consequently, our study population was probably more representative of the general population in Japan than a clinical study population, which is typically limited to patients with a good PS or without selected comorbidities, and/or to patients without a history of EGFR TKI treatment. Notably, the efficacy results from the current study are comparable to those from clinical trials of erlotinib, despite our study population having a high proportion of patients who are usually excluded from study enrollment, i.e., patients who have received multiple lines of chemotherapy, patients with a history of gefitinib therapy, and patients with a relatively poor PS.

In conclusion, this large postmarketing surveillance study conducted in more than 3000 Japanese patients provides valuable information about the efficacy and safety of erlotinib, which would be difficult to obtain from phase III trials. The study also provides an insight into the treatment profile of erlotinib in a previously underevaluated population. Our data confirm that erlotinib has an acceptable safety profile in Japanese patients during routine clinical practice, which is comparable to that reported in other study populations. No new safety signals were detected and the risk/benefit balance of erlotinib was considered favorable, suggesting

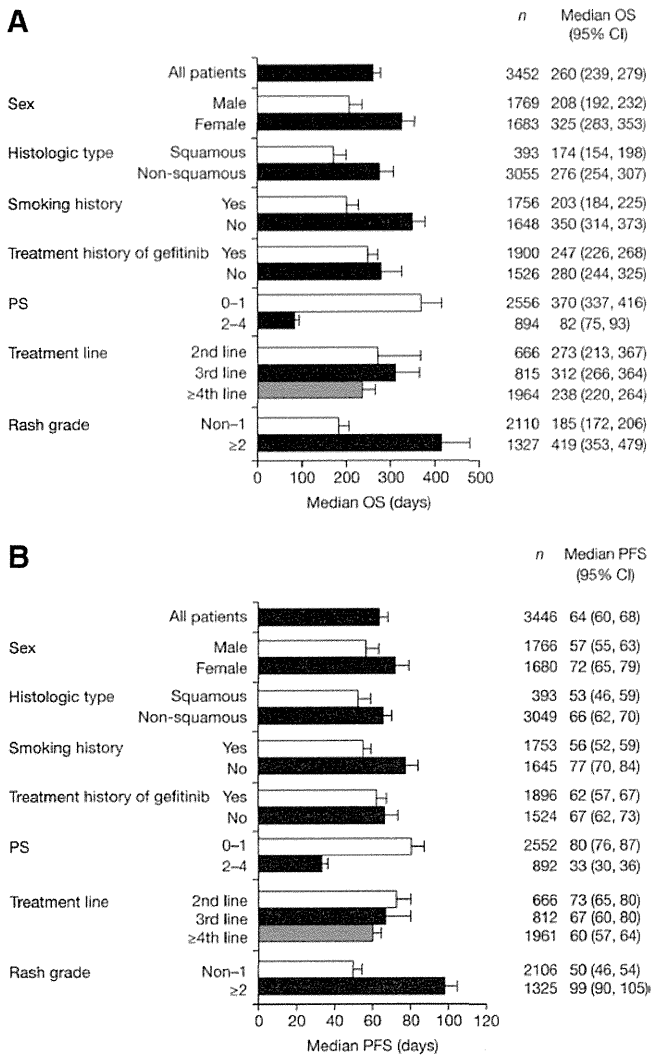

FIGURE 4. Subgroup analyses for (*A*) OS and (*B*) PFS. OS, overall survival; PFS, progression-free survival.

that erlotinib is a generally well-tolerated treatment option for Japanese patients with NSCLC. After study completion, we aim to present the results of the final analyses for more than 10,000 registered patients.

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ORIGINAL ARTICLE

Pharmacokinetics of sepantronium bromide (YM155), a small-molecule suppressor of survivin, in Japanese patients with advanced solid tumors: dose proportionality and influence of renal impairment

Yumiko Aoyama · Tetsuya Nishimura · Taiji Sawamoto · Taroh Satoh · Masataka Katashima · Kazuhiko Nakagawa

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Abstract
Purpose The purpose of this analysis was to investigate the pharmacokinetics (PK) of sepantronium (YM155), a small-molecule suppressor of the expression of the antiapoptosis protein survivin, in Japanese patients with advanced solid tumors and to evaluate the effect of renal impairment on the PK profile of sepantronium.
Methods Sepantronium was administered as a continuous intravenous infusion of 1.8–10.6 mg/m^2/day for 168 h (7 days) to 33 patients. PK parameters were estimated via non-compartmental method. Renal function was categorized for the analysis based on the chronic kidney disease guidance using eGFR values at pre-dose.
Results The PK of sepantronium was dose proportional in the dose range of 1.8–10.6 mg/m^2/day. Age and sex did not significantly affect the PK of sepantronium. Results suggested that total clearance and renal clearance in patients with moderate renal impairment were 0.7-fold lower than those in patients with normal renal function, resulting in 1.3-fold higher steady-state concentration and area under the curve values. The PK parameters of sepantronium in patients with mild renal impairment were comparable to those in the patients with normal renal function.
Conclusions While age and sex did not significantly affect the PK of sepantronium, moderate renal impairment increased exposure of sepantronium by about 30 %. The results suggest that no dose adjustment is required for patients with mild renal impairment.

Keywords Sepantronium bromide · YM155 · Pharmacokinetics · Renal impairment · Advanced solid tumor

Y. Aoyama (✉) · T. Nishimura · T. Sawamoto · M. Katashima
Clinical Pharmacology, Development, Astellas Pharma Inc.,
3-17-1, Hasune, Itabashi-ku, Tokyo 174-8612, Japan
e-mail: yumiko.aoyama@astellas.com

T. Satoh
Department of Frontier Science for Cancer and Chemotherapy,
Graduate School of Medicine, Osaka University, Osaka, Japan

K. Nakagawa
Department of Medical Oncology, Kinki University,
Faculty of Medicine, Osaka, Japan

Introduction

Sepantronium bromide (sepantronium, YM155, 1-(2-methoxyethyl)-2-methyl-4,9-dioxo-3-(pyrazin-2-ylmethyl)-4,9-dihydro-1*H*-naphtho[2,3-*d*]imidazolium bromide), a small-molecule survivin suppressant, was identified by cell-based, high-throughput screening and lead optimization. Sepantronium bromide selectively suppresses survivin expression, resulting in activation of caspases and apoptosis induction in hormone refractory prostate cancer cells. Sepantronium bromide showed broad spectrum antitumor activity and induced tumor regressions in various xenograft models rather than uncertainness of mode of action. Continuous infusion of sepantronium bromide has also been found to induce tumor regression and intratumoral survivin suppression in established human hormone refractory prostate cancer (HRPC), non-Hodgkin lymphoma (NHL), and non-small cell lung cancer (NSCLC) tumor xenografts [1–4].

Phase 2 studies to evaluate the safety, efficacy, and pharmacokinetics (PK) of sepantronium were conducted with a continuous intravenous infusion (CIVI) for 7 days and showed modest single-agent clinical activity in patients with NSCLC, HRPC, or unresectable stage III or IV melanoma, respectively [5–7]. Sepantronium is currently being

investigated in phase 1 and phase 2 studies in combination therapy, enrolling patients with diffuse large B-cell lymphoma and other solid tumors [8–10].

The results of a monotherapy phase 1 study to evaluate tolerability, safety, efficacy, and PK of sepantronium in Japanese patients with advanced solid tumors have been reported previously [11]. Sepantronium was administered by CIVI for 7 days (168 h) every 21 days at 1.8, 3.6, 4.8, 6.0, 8.0, and 10.6 mg/m^2/day. Sepantronium was generally well tolerated, and the maximum tolerated dose was estimated to be 8.0 mg/m^2/day when administered as the CIVI for 7 days in Japanese patients. Dose-limiting toxicities (DLT) observed in the study were increased blood creatinine, grade 3 increased serum aspartate aminotransferase (AST), and grade 4 anemia. Steady-state conditions were achieved 24 h after start of infusion for all doses administered. The concentrations of unchanged drug declined rapidly following a biphasic manner after termination of infusion. It appeared that systemic exposure increased with increasing doses and no accumulation was noted with repeated doses. Mean values for elimination half-life ($t_{1/2}$) and total body clearance (CL) of sepantronium seemed constant across the dose ranges. The urinary excretion ratio of unchanged drug ranged from 25 to 42 % and showed no relationship with the dose administered. Non-clinical studies in rats showed that three metabolites were identified in bile and urine after a single intravenous dose of sepantronium; however, sepantronium was minimally metabolized when incubated with human cryopreserved hepatocytes [12].

Given these characteristics, it is anticipated that a substantial reduction in renal function may affect the renal clearance of sepantronium, and consequently its PK, as well as possibly, its safety, and tolerability.

The present analysis was performed to evaluate the effect of renal impairment on PK of sepantronium using a linear mixed effect model with data obtained from a previously reported phase 1 study in Japan. In addition, dose proportionality of sepantronium was evaluated, and an exploratory analysis to investigate the effect of demographics on the PK of sepantronium was performed.

Methods

A retrospective analysis of data obtained from the previously reported phase 1 study in Japanese subjects [11] was performed.

Study design

The study was an open-label, single center, phase 1, dose-escalation study with administration of CIVI dose of sepantronium as monotherapy over 7 days (168 h) every 21 days. The safety, tolerability, efficacy, and PK of sepantronium were evaluated in male and female Japanese patients with advanced solid tumors. Sepantronium was prepared for administration by dilution of the appropriate volume of concentrated stock solution in 5 % dextrose in a light- and temperature-controlled environment.

The study consisted of 6 dose cohorts of 3–6 patients each treated with 1.8, 3.6, 4.8, 6.0, 8.0, or 10.6 mg/m^2/day. Doses were expressed as those of the cationic moiety of sepantronium bromide. Each 21-day cycle included a 7-day (168-h) administration period and a 14-day observation period (1 cycle). This study was conducted at the Department of Medical Oncology, Kinki University Hospital, Osaka, Japan. The protocol was approved by an independent ethics committee for the study site, and the study was conducted in accordance with the principles of the Declaration of Helsinki. Results regarding the tolerability, safety, and basic PK profile of sepantronium have been reported previously [11].

Population

The study enrolled Japanese male and female patients with advanced solid tumors. Eligibility criteria for patients enrolled in the study included refractory advanced solid tumors for which no standard therapy was available; histologic or cytologic diagnosis of cancer; age at least 20 years; life expectancy of at least 12 weeks; Eastern Cooperative Oncology Group performance status of <3; and adequate hematopoietic, hepatic, and renal functions (absolute neutrophil count of $\geq 1.5 \times 10^9$/L, platelets of $\geq 100 \times 10^9$/L, hemoglobin of ≥ 9 g/dL, bilirubin within 1.5 × upper limit of normal, transaminases of $\leq 2.5 \times$ upper limit of normal, and creatinine of $\leq$ upper limit of normal) [11].

Blood and urine sampling

Venous blood samples were collected in tubes containing heparin sodium from a site other than the infusion site before and at 0.25, 0.5, 1, 2, 3, 4, 6, 12, 24, 48, 72, 96, 120, and 144 h after start of infusion, as well as at the end of infusion (168 h), and at the following time points thereafter: 168.25, 168.5, 169, 170, 171, 172, 174, 180, 192, and 216 h after the start of infusion. Blood samples were centrifuged immediately, and the plasma samples obtained were stored at −20 °C before analysis. To determine the urinary concentration of unchanged sepantronium, urine samples were collected over the 216-h period after start of CIVI and stored at −20 °C before analysis. Blood and urine samples for PK evaluation were collected during cycle 1 and cycle 2 [11].

Table 1 Patient characteristics

Descriptive statistics for patient demographics		Number of PK data sets and frequency of renal function			
	No. of patients		Cycle 1	Cycle 2	Total
Total patients	33	Total	31	15	46
Male/female	23/10	Renal function			
Age (years)		Normal	12	7	19
Median (range)	59 (26–81)	Mild	14	7	21
Body weight (kg)		Moderate	5	1	6
Median (range)	54 (40–88)				
BSA					
Median (range)	1.6 (1.3–2.0)				

Thirty-two of 33 patients who received sepantronium administration had concentration data and were PK evaluable patients. Fourteen of 32 had both cycle 1 and cycle 2 data, 17 patients had cycle 1 data, and remaining 1 patient had cycle 2 data

Bioanalytical procedures

Measurement of sepantronium concentration in plasma and urine samples was performed by Astellas Europe B.V. EDD using liquid chromatography tandem mass spectrometry (LC–MS/MS). The lower limit of quantitation for sepantronium was 0.05 ng/mL in plasma and 1.0 ng/mL in urine. Concentrations were expressed as those of the cationic moiety of sepantronium bromide [11, 13]. The precision and the accuracy of inter- and intra-assay for the LC–MS/MS methods were within ±20 % (unpublished data).

Pharmacokinetic analysis

PK parameters of sepantronium in plasma and urine were calculated using WinNonlin Professional® version 5.0.1 (Pharsight Corporation, Mountain View, CA, USA) and the SAS® system (SAS Institute Inc., Cary, NC, USA). The area under the curve (AUC) was calculated according to the linear trapezoidal rule from zero to time t of the last measurable concentration above the lower limit of quantitation. Steady-state concentration (C_{SS}) was the mean value of daily concentrations taken through 7-day CIVI (the mean value of concentration at 24, 48, 72, 96, 120, 144, and 168 h after start of infusion). Terminal elimination half-life ($t_{1/2}$) of sepantronium was calculated as follows: $t_{1/2}$ = ln 2/terminal elimination rate constant. CL is the total systemic clearance, estimated by: CL = total amount of dose/AUC from time zero to infinity. CL_R is renal clearance, estimated by: CL_R = cumulative amount excreted in urine/AUC. V_d is apparent volume of distribution, estimated by: V_d = CL/terminal elimination rate constant. Ae is amount excreted in urine. Fe is fraction excreted in urine estimated by: Fe = Ae/Dose.

Statistical analysis

Statistical analysis was performed using the SAS® system. Dose proportionality in PK parameters of sepantronium was evaluated via power model regression using a mixed effect model. The effect of cycle, dose, and demographics (age and sex) upon PK parameters as a fixed effect was investigated.

Renal function was categorized into normal renal function with eGFR of ≥90 mL/min/1.73 m^2 (normal group), mild decrease in eGFR (60–89 mL/min/1.73 m^2) (mild renal impairment group), or moderate decrease in eGFR (30–59 mL/min/1.73 m^2) (moderate renal impairment group) based on the chronic kidney disease guidance [14]. The eGFR for a Japanese population was calculated using the following equation [15]:

$$\mathrm{eGFR}\left(\mathrm{mL/min/1.73\,m^2}\right) = 194 \times (\text{serum creatinine})^{-1.094} \times (\text{age})^{-0.287} (\times 0.739 \text{ if female})$$

PK parameters among renal function groups were compared using geometric mean ratios (GMRs) and their 90 % confidence intervals (CIs) with renal function and cycle as fixed effects and subject as a random effect in the mixed effect model. Results of patients with normal eGFR (normal group) were considered as reference data. Analysis was performed using natural-log transformed PK parameters. The point estimates and their 90 % CIs were exponentiated, and the results were presented as a natural scale. Analysis was performed using dose-normalized AUC (AUC/Dose), dose-normalized C_{SS} (C_{SS}/Dose), CL, and CL_R.

Results

Study populations

Patient characteristics are summarized in Table 1. A total of 33 male (n = 23) and female (n = 10) patients with advanced solid tumors received sepantronium as a CIVI for at least 1 cycle. The PK was evaluated based on data from cycle 1 and cycle 2. After exclusion of 1 patient who had

Table 2 Descriptive Statistics for PK Parameters

	Cohort 1 1.8 mg/m^2/day (n = 6)	Cohort 2 3.6 mg/m^2/day (n = 10)	Cohort 3 4.8 mg/m^2/day (n = 9)	Cohort 4 6.0 mg/m^2/day (n = 8)	Cohort 5 8.0 mg/m^2/day (n = 8)	Cohort 6 10.6 mg/m^2/day (n = 5)
AUC (ng h/mL)	538 ± 119	1,186 ± 385	1,738 ± 685	2,239 ± 952	2,233 ± 489	3,235 ± 526
$t_{1/2}$ (h)	7 ± 3	21 ± 9	15 ± 10	16 ± 6	21 ± 9	29 ± 14
CL (L/h)	42 ± 7	39 ± 13	35 ± 11	34 ± 11	41 ± 14	34 ± 7
C_{SS} (ng/mL)	3 ± 1	7 ± 2	10 ± 4	14 ± 6	13 ± 3	19 ± 3
V_d (L)	436 ± 175	1,197 ± 568	759 ± 541	795 ± 347	1,169 ± 484	1,544 ± 955
	(n = 6)	(n = 6)	(n = 9)	(n = 7)	(n = 4)	(n = 4)
Ae (mg)	7 ± 1	16 ± 5	16 ± 5	19 ± 6	25 ± 2	46 ± 8
Fe (%)	31 ± 4	35 ± 8	30 ± 9	28 ± 6	29 ± 4	42 ± 8

Values are mean ± standard deviation. *AUC*, area under the curve from zero to time t of the last measurable concentration above the limit of quantitation: $t_{1/2}$, terminal elimination half-life, *CL* total systemic clearance, C_{SS} steady-state concentration, V_d apparent volume of distribution, *Ae* amount excreted in urine, *Fe* fraction excreted in urine

no concentration data, 32 patients were included in the analysis.

Fourteen of 32 patients had both cycle 1 and cycle 2 data, while the 17 patients had cycle 1 data and remaining 1 had cycle 2 data, yielding a total of 46 data sets for use in the analysis. The baseline of serum creatinine for enrolled patients ranged from 0.4 mg/dL to 1.2 mg/dL, and were under the upper limit of normal level (1.3 mg/dL). The number of data sets in moderate renal impairment group (30–59 mL/min/1.73 m^2) was 6. The number of data sets in normal group (≥90 mL/min/1.73 m^2) (n = 19) and mild renal impairment group (60–89 mL/min/1.73 m^2) (n = 21) was comparable. All dose cohorts included normal group and mild renal impairment group. Moderate renal impairment group were in the dose cohorts of 3.6 (n = 3), 4.8 (n = 1), or 6.0 mg/m^2/day (n = 2). There were no patients who had severe decrease in eGFR or who required dialysis. Renal function in 2 patients changed from cycle 1 to cycle 2 (from mild to normal in one patient and vice versa in the other).

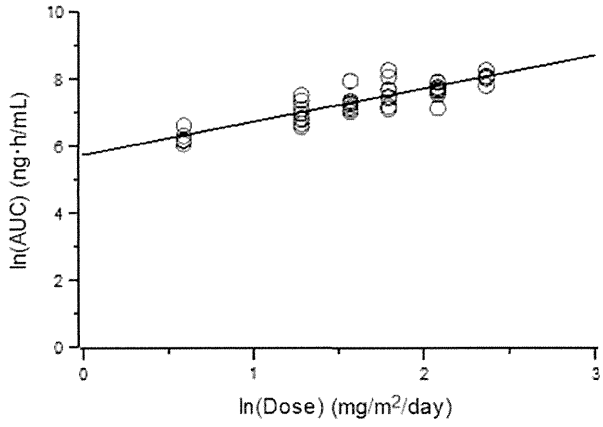

Fig. 1 Relationship between dose and AUC of sepantronium. *Line*: power model regression, *empty circle*: individual value. Slopes and their 90 % confidence intervals obtained from power model regression between the dose and AUC at a dose range of 1.8–10.6 mg/m^2/day was 0.981 (0.868–1.094)

Pharmacokinetics

Descriptive statistics for PK parameters of sepantronium by dose cohort were presented in Table 2. The relationship between dose and AUC was presented in Fig. 1.

There was no difference in PK parameters between cycle 1 and cycle 2 [11], and the analysis was performed using combined data from cycle 1 and cycle 2. Inter-individual variability of sepantronium PK was moderate as shown in Table 2. Slopes [90 % CIs] by power model regression for AUC and C_{SS} versus dose were 0.981 [0.868–1.094] and 0.998 [0.885–1.110], respectively. The results suggested that AUC and C_{SS} increased in a dose proportional manner.

Significance as a fixed effect of age and sex was evaluated by adding to the power model; however, for these models, either fit statistics were not improved by adding age and sex as fixed effects, or the effect was not significant (age, $p > 0.1$; sex, $p > 0.04$). No demographics were therefore added to the model as a fixed effect.

Summary statistics of PK parameters by renal function are presented in Table 3. Mean plasma concentration versus time profile of sepantronium is presented in Fig. 2. The relationship between PK parameter and renal function is presented in Fig. 3.

Mean plasma concentrations in the moderate renal impairment group were slightly higher than the concentrations in other groups after termination of the sepantronium infusion. Mean PK parameters in the mild impairment group were comparable to those in the normal group. The GMR for the mild renal impairment group versus normal group was nearly equal to 1, and the 90 %

Table 3 Summary of pharmacokinetic parameters of sepantronium after continuous intravenous infusion of 1.8, 3.6, 4.8, 6.0, 8.0, or 10.6 mg/m^2/day for 168 h

PK parameters (mean ± SD)	Normal (n = 19)	Mild (n = 21)	Moderate (n = 6)
AUC/Dose (ng h/mL/mg)	29 ± 14	27 ± 7	37 ± 10
C_{SS}/Dose (ng/mL/mg)	0.17 ± 0.08	0.16 ± 0.04	0.22 ± 0.07
CL (L/h)	39 ± 11	39 ± 11	28 ± 9
	(n = 14)	(n = 14)	(n = 4)
CL_R (L/h)	13 ± 5	13 ± 2	9 ± 4

PK Parameter comparison, GMR (90 % CI)	Mild/normal	Moderate/normal
AUC/Dose (ng h/mL/mg)	0.976 (0.819–1.162)	1.340 (1.033–1.738)
C_{SS}/Dose (ng/mL/mg)	0.989 (0.824–1.187)	1.273 (0.969–1.672)
CL (L/h)	1.021 (0.857–1.217)	0.740 (0.570–0.960)
CL_R (L/h)	1.107 (0.887–1.383)	0.695 (0.499–0.968)

SD standard deviation, *AUC/Dose* dose-normalized area under the curve from zero to time t of the last measurable concentration above the limit of quantitation, *C_{SS}/Dose* dose-normalized steady-state concentration, *CL* total systemic clearance, *CL_R* renal clearance, *GMR* geometric mean ratio, *CI* confidence interval

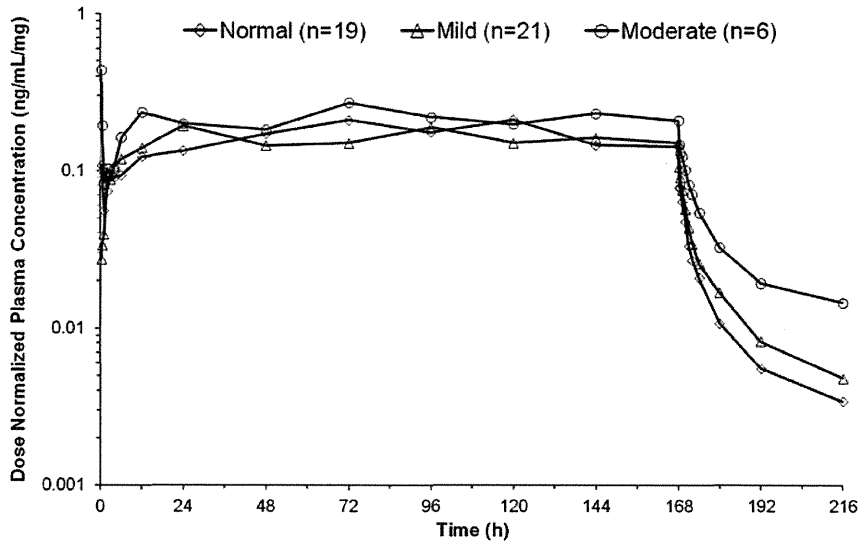

Fig. 2 Mean dose-normalized plasma concentration versus time profile of sepantronium after continuous intravenous infusion of 1.8–10.6 mg/m^2/day for 168 h in Japanese patients with advanced solid tumors

CIs were almost in the range of 0.8–1.25. Renal function in 2 patients changed from cycle 1 to cycle 2 (from mild to normal in one and vice versa in the other), and PK parameters in these patients were similar for both cycles. Results for the moderate renal impairment group showed lower mean CL and CL_R compared to the normal group, and AUC/Dose and C_{SS}/Dose in the moderate renal impairment group were 1.3-fold higher than those in the normal group.

Two of the five patients in the highest dose cohort (10.6 mg/m^2/day) had DLT of increased blood creatinine. Of note is the fact that these two patients with the DLT had mild renal impairment. The AUC values of these two patients were 3975 and 3098 ng h/mL, respectively, and were equal to or greater than the values in the other patients in the same dose cohort (2510–3351 ng h/mL).

Discussion

We evaluated the effect of renal impairment on PK of sepantronium in patients with advanced solid tumors using the data obtained from an open-label, phase 1 study. Sepantronium was administered as CIVI at a dose and rate of 1.8–10.6 mg/m^2/day over 7 days. Overall, PK parameters of sepantronium were similar in patients with mild impairment and patients with normal renal function; however, patients with moderate impairment had a slightly

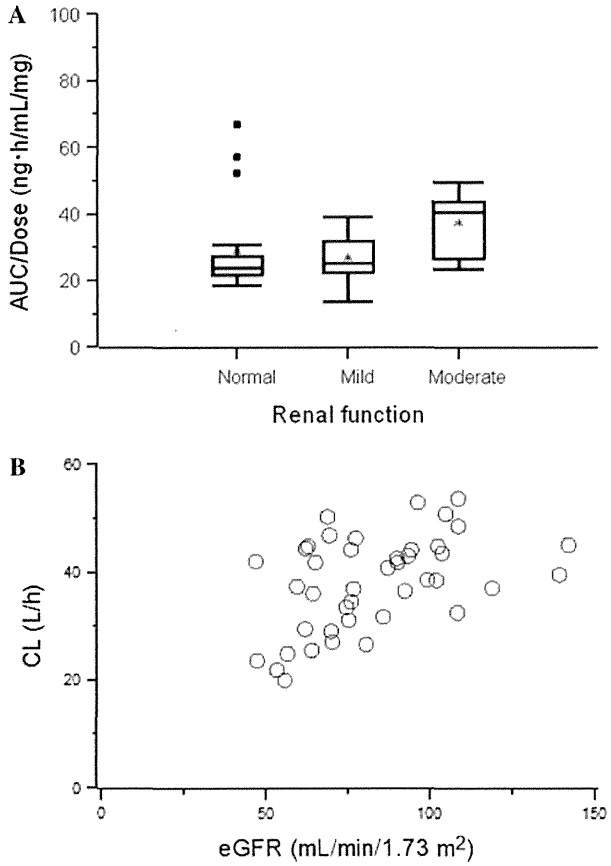

Fig. 3 Relationship between renal function and PK parameters of sepantronium. *Star* represents a mean value: *box* represents a range of 50 % interval: a *bar* in each *box* represents a median value: *bar* under the *box* represents a 25 percentile: *bar* over the *box* represents a 75 percentile: *fixed circle* represents outlier in **a**. Pearson's correlation coefficient between CL and eGFR = 0.46, $p = 0.0021$ in **b**

lower clearance of sepantronium. The GMR for the mild renal impairment group compared to the normal group was nearly equal to 1, and the 90 % CIs were in the range of 0.8–1.25 which is commonly accepted as an equivalence range. The results indicated that there was no clinically significant difference in PK between patients with normal renal function and patients with mild renal impairment.

Two phase 1 studies have consistently reported excretion ratios of sepantronium as unchanged drug into urine of approximately 30 %, results which indicate that urinary excretion is an important elimination routes of sepantronium [11, 13]. The results that moderate renal impairment reduced the CL_R of sepantronium and mild renal impairment had no effect on the PK are in agreement with the above assumption.

Impaired renal function often alters a drug's PK profile, when the drug is eliminated primarily by renal excretion. In vitro and animal studies have suggested that renal impairment may affect or down-regulate various CYP enzymes and transporters that may lead to clinically relevant changes in non-renal clearance [16, 17]. The CL and CL_R of sepantronium decreased in parallel in the moderate renal impairment group in the present analysis. Although the elimination mechanism of sepantronium is yet not fully known, it is assumed that a decrease in CL reflects a reduction in CL_R linearly.

The majority of the PK data sets were comprised of normal group ($n = 19$) and mild renal impairment group ($n = 21$). Only 6 were in the moderate renal impairment group. None of the patients had severe renal impairment. Inter-subject variability of sepantronium PK parameters was moderate as shown in Table 3, and there were three outliers in the normal renal function group which were presented as fixed circle in Fig. 3a. Figure 3b shows a relationship between the eGFR and CL of sepantronium. A weak correlation was observed between eGFR and CL of sepantronium when outliers were excluded (Pearson's correlation coefficient between CL and eGFR = 0.46, $p = 0.0021$). There is a possibility that a strong relationship will be observed between eGFR and CL of sepantronium if further investigation is conducted using comparable number of patients with moderate and severe renal impairment compared to patients with normal renal function.

Most patients enrolled in the study had normal serum alanine aminotransferase and AST values, and an analysis to evaluate the effect of hepatic impairment on PK of sepantronium was not performed. Of interest was a weak correlation observed between the CL_R and baseline value of alkaline phosphatase (ALP), which was above upper normal range in 15 of 46 PK data sets at the baseline (Pearson's correlation coefficient = 0.40, $p = 0.0229$). However, no similar relationship was observed between CL and ALP (Pearson's correlation coefficient = 0.15, $p = 0.3106$). The reason for this finding is unclear.

It was reported that 3 metabolites were identified in bile and urine samples obtained after a single intravenous dose of sepantronium to rats. The proposed metabolic pathways of sepantronium in rats involve *N*-dealkylation, *o*-demethylation, and the oxidation of a methyl group to a carboxylic acid. Sepantronium is minimally metabolized when incubated with human cryopreserved hepatocyte [12]. It was suggested that human organic cation transporter 1 (OCT1) was the predominant transporter for the hepatic uptake of sepantronium, and that excretion into bile was an important elimination pathway of sepantronium in humans. It has also been reported that the transporter-mediated uptake clearance observed in vitro may account for the in vivo intrinsic hepatic clearance [18]. The contribution ratio of hepatic metabolism to metabolic clearance in humans is unclear presently; however, given these results from in vitro and non-clinical studies, the

contribution ratio of hepatic metabolism in humans is likely small. Further investigation for metabolites in human will help to clarify the effect of hepatic impairment on PK of sepantronium.

Frequently, the effects of renal impairment are clarified by conducting a clinical study enrolling patients with renal impairment and comparing them with those in matched healthy subjects, or by performing a model analysis using a non-linear mixed effect model [17]. The present analysis is another approach to investigate the effect of renal or hepatic impairment on the PK of compounds early in the clinical trials.

Dose proportionality of the PK of sepantronium was evaluated via the power model regression. Slopes and their 90 % CIs for AUC and C_{SS} versus dose were within the range of 0.8–1.25, indicating that exposure of sepantronium increased in a linear dose proportional manner. Other PK parameters were similar among dose cohorts. These results suggested that the PK of sepantronium was linear at a dose range from 1.8 to 10.6 mg/m^2/day. In agreement with the present findings, an earlier phase 1 study for sepantronium conducted in the United States found that the values of C_{SS} and AUC increased in a dose proportional manner, and CL was independent of dose over the range of 1.8 to 4.8 mg/m^2/day [13]. The effect of cycle on sepantronium PK was evaluated by adding cycle numbers to the model as a fixed effect; however, the effect was not significant ($p > 0.1$). These results indicate that there was no difference in PK parameters between cycle 1 and cycle 2 and accumulation with repeated dosing was not observed. Demographics such as age and sex did not significantly affect the PK of sepantronium.

A previous non-clinical toxicology study found that short-term exposure at high plasma concentrations caused nephrotoxicity [11]. Two of five patients in the highest dose cohort (10.6 mg/m^2/day) had DLT of increased blood creatinine [11]. Of note is the fact that these two patients with the DLT had mild renal impairment. The AUC values of these 2 patients were equal or greater than the values in the other patients in the same dose cohort. The PK of sepantronium was linear, and individual AUC and C_{SS} values in lower-dose cohorts were not in excess of those patients receiving 10.6 mg/m^2/day, although inter- and intra-patient variability was moderate.

In conclusion, while age and sex did not significantly affect the PK of sepantronium; moderate renal impairment increased exposure of sepantronium by about 30 %. The CL and CL_R of sepantronium were lower in patients with moderate renal impairment relative to the patients with normal renal function. The PK in patients with mild renal impairment was comparable to those for patients with normal renal function. The results suggest that no dose adjustment is required for patients with mild renal impairment. It will be necessary to monitor the safety in patients with moderate renal impairment.

Conflict of interest Yumiko Aoyama, Tetsuya Nishimura, Taiji Sawamoto, and Masataka Katashima are employees of Astellas Pharma Inc.

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Phase I trial of OTS11101, an anti-angiogenic vaccine targeting vascular endothelial growth factor receptor 1 in solid tumor

Hidetoshi Hayashi,[1] Takayasu Kurata,[1,4] Yasuhito Fujisaka,[1] Hisato Kawakami,[1] Kaoru Tanaka,[1] Takafumi Okabe,[1] Masayuki Takeda,[1] Taroh Satoh,[1] Koji Yoshida,[2] Takuya Tsunoda,[2] Tokuzo Arao,[3] Kazuto Nishio[3] and Kazuhiko Nakagawa[3]

[1]Department of Medical Oncology, Kinki University Faculty of Medicine, Osaka-Sayama, Osaka; [2]Onco Therapy Science, Kanagawa Science Park R&D, Takatsu-ku, Kawasaki City, Kanagawa; [3]Department of Genome Biology, Kinki University Faculty of Medicine, Osaka-Sayama, Osaka, Japan

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OTS11101 is a novel peptide vaccine that acts as an angiogenesis inhibitor by inducing cytotoxic T lymphocyte (CTL) cells that specifically target vascular endothelial cells expressing vascular endothelial growth factor (VEGF) receptor 1. We conducted a phase I study to evaluate the safety, tolerability, maximum tolerated dose, and pharmacodynamic biomarker status of this vaccine. Nine patients with advanced solid tumors received 1.0, 2.0, or 3.0 mg of OTS11101 subcutaneously, once a week in a 28-day cycle. Three patients experienced grade 1 injection site reactions, which were the most frequent adverse events. Grade 2 proteinuria and hypertension each occurred in one patient. As other toxicities were generally mild, the maximum tolerated dose was not reached. Furthermore, we explored the induction of specific activated CTLs, and biomarkers related to angiogenesis. A pharmacodynamics study revealed that induction of specific CTLs was observed for a dose of 2.0 and 3.0 mg. The serum concentrations of soluble VEGF receptor 1 and 2 after vaccination increased significantly compared with baseline. A microarray was performed to give a comprehensive analysis of gene expression, suggesting that OTS11101 vaccination resulted in T cell activation in a clinical setting. In conclusion, OTS11101 was well tolerated in patients up to 3.0 mg once weekly and our biomarker analysis suggested that this anti-angiogenesis vaccine is biologically active. (*Cancer Sci* 2013; 104: 98–104)

Angiogenesis, defined as the formation of new blood vessels from a pre-existing vasculature, is essential for tumor growth and the spread of metastases.[1, 2] Therefore, angiogenesis inhibition is considered to be an effective strategy to treat cancer, and clinical application of this strategy is pursued using multiple modalities, which include specific inhibitors of the signaling pathways of vascular endothelial growth factor (VEGF), including their corresponding receptors (VEGFR). The approval of a new broad family of molecularly targeted anticancer drugs, such as anti-VEGF antibody and VEGF receptor tyrosine kinase receptor inhibitors (VEGFR-TKIs), represents one of the most significant recent advances in clinical oncology. However, these existing anti-angiogenic agents have significant shortcomings, including side-effects and the requirement of frequent or continuous administration. To overcome these deficits, novel alternative therapies with different mechanisms of action would be of great value for anti-cancer therapy.

Vascular endothelial growth factor receptor 2 is expressed on endothelial cells of vessels in various types of primary tumor and metastases. This receptor has therefore been a major target to date.[3] However, it has been emphasized that tumor angiogenesis mediated by the VEGFR1 pathway is also important.[4,5] Previous studies have demonstrated that VEGFR1, but not VEGFR2, is upregulated by hypoxic conditions,[6,7] and similar patterns of distribution of both VEGFR1 and VEGFR2 are not always observed in tumors.[8–10] Thus, VEGFR1 is a promising target for anti-angiogenic cancer treatments, and further clinical development of this strategy is warranted.

Recently, several specific immunotherapies have been attempted and some clinical trials have shown promising efficacy. In particular, epitope peptides that have been used for the induction of CTL responses in patients with cancer in numerous clinical studies.[11] We have previously identified epitope peptides of human VEGFR1 and demonstrated that CTLs induced by these peptides have a potent and specific cytotoxicity, in a human leukocyte antigen (HLA) class I-restricted manner, against not only peptide-pulsed target cells but also endothelial cells endogenously expressing VEGFR1.[12] Furthermore, vaccination using these epitope peptides *in vivo* was shown to inhibit tumor-induced angiogenesis, resulting in significant suppression of tumor growth without fatal adverse effects.[12]

OTS11101 (VEGFR1-1084) is a novel peptide vaccine restricted with HLA-A*24:02, which acts as an angiogenesis inhibitor by specifically targeting VEGFR1. Here, we conducted a phase I dose-escalation study to determine the maximum tolerated dose (MTD), tolerability, and antitumor effect of OTS11101 administered by a weekly subcutaneous injection. To identify biomarkers that reflect the pharmacodynamics and doseresponse relationship of OTS11101, we further evaluated the CTL response induced with OTS11101 and the serum concentration of soluble VEGFR1, soluble VEGFR2, and various types of cytokines related to angiogenesis. Comprehensive gene expression analysis was performed using a microarray.

Methods

Patient eligibility. HLA-A*24:02-positive individuals aged ≥20 years with a histologically confirmed diagnosis of an advanced tumor refractory to standard therapy were included in the study. Within 21 days of study registration, patients had to have an absolute white blood cell count of ≥3000 or ≤12 000, platelets ≥100 000/mm^3, and hemoglobin ≥9.0 g/dL. Baseline creatinine and total bilirubin had to be less than 1.5 times the upper limit of normal (ULN) and both aspartate aminotransferase (AST) and alanine aminotransferase (ALT) had to be 2.5 times the ULN. Other inclusion criteria included

[4]To whom correspondence should be addressed.
E-mail: t-kurata@med.kindai.ac.jp
Trial registration identification number: UMIN000002700.

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an Eastern Cooperative Oncology Group performance status of <2 and adequate organ function. Individuals were excluded if they had a symptomatic brain tumor or brain metastases, active bleeding, or serious illness or concomitant nononcologic disease that was difficult to control by medication. All subjects received information about the nature and purpose of the study, and they provided written informed consent in accordance with institutional guidelines.

Study design. This study was designed as a single-center, open-label, dose-escalation phase I trial. The primary objectives of this dose-escalation trial were to determine if OTS11101 given subcutaneously once weekly at 1.0–3.0 mg could be confirmed as safe and tolerable treatment, and to collect overall safety data. The secondary objectives included the determination of the MTD, pharmacodynamics, and preliminary information about the antitumor activity and the efficacy on angiogenic peripheral blood biomarkers. The study was reviewed and approved by the Institutional Review Board. OTS11101 was emulsified with Incomplete Freund's adjuvant. Dose levels of OTS11101 were 1.0, 2.0, and 3.0 mg once weekly in a 28-day cycle. If a patient experienced a drug-related dose-limiting toxicity (DLT), excluding injection site reaction, the treatment with OTS11101 was discontinued. The dose escalation/reduction scheme was based on the occurrence of drug-related DLTs within the first treatment course. If a DLT was not observed in any of the first three patients, the dose was escalated to the next level. If a DLT was observed in one of the first three patients, three additional patients were recruited to that dose level. If a DLT occurred in only one of six patients, dose escalation was permitted. If two or more of six patients experienced a DLT, an independent data monitoring committee determined the dose escalation or reduction decision or stopped the recruitment of additional patients.

Safety and efficacy assessments. The safety and tolerability of OTS11101 were assessed according to the Common Toxicity Criteria for Adverse Events version 3.0. The following adverse events were defined as DLTs: grade 4 hematologic and grade 3/4 nonhematologic toxicity except for grade 3 anorexia, nausea, vomiting, diarrhea, constipation, and γ-glutamyl transpeptidase (γ-GTP) increases that could be controlled by supportive treatment. Objective tumor response was evaluated according to the Response Evaluation Criteria in Solid Tumors version 1.1.[13]

Measurement of CTL responses. An enzyme-linked immunospot (ELISPOT) assay was performed to measure the specific CTL response against the peptide.[14] Peripheral blood mononuclear cells (PBMC) were obtained from patients before the vaccination treatment and at the end of first course. Frozen PBMC cultured with OTS11101 and IL-2 Peptide was added into the culture at days 0 and 7 and cells were harvested after 2 weeks. After harvesting, CD4-positive cells were depleted using a Dynal CD4 positive isolation kit (Invitrogen, Carlsbad, Canada) and were used as responder cells in the ELISPOT assay. A human interferon (IFN)-γ ELISpot PLUS kit (MabTech, Nacka Strand, Sweden) was used to measure the CTL responses. HIV-A24 peptide (RYLRDQQLL)-pulsed TISI cells were used as negative control stimulator cells.[15] The number of peptide-specific spots was calculated by subtracting the spot number in the control well from the spot number of well with OTS11101-pulsed TISI cells. The CTL response was estimated as positive when the average count of peptide-specific spots was greater than 20 spots/well. The method in this section is described in detail in data S1.

Circulating levels of soluble VEGFR and angiogenesis-related cytokines. The concentrations of serum-soluble VEGFR (sVEGFR) 1 and 2 were measured by ELISA before the vaccination on day 1 and after the vaccination on days 8 and 29 (VERSA max; Molecular devices, Sunnyvale, CA, USA). Furthermore, plasma cytokines related to angiogenesis were measured using an antibody suspension array full system (Bio-Rad Laboratories, Hercules, CA, USA) as previously described[16] and included the following: angiopoietin II (ANG-2), follistatin, granulocyte colony-stimulating factor (G-CSF), interleukin-8 (IL-8), leptin, platelet-derived growth factor-BB (PDGF-BB), platelet endothelial cell adhesion molecule-1 (PECAM-1), VEGF, and hepatocyte growth factor (HGF).

Microarray procedure and sample preparation. The PBMC were obtained from whole blood using an ACCUSPIN System (Sigma–Aldrich, St. Louis, MO, USA). The RNA extraction method and the quality check protocol have been described previously.[17] The microarray procedure was performed according to the Affymetrix protocols (Affymetrix, Santa Clara, CA, USA) and has been previously described.[18]

Statistical analysis for microarray. The microarray analysis was performed using the BRB Array Tools software version 4.2.0 Beta 1 (http://linus.nci.nih.gov/BRB-ArrayTools.html). In brief, a log base 2 transformation was applied to the raw microarray data, and global normalization was used to calculate the median over the entire array. Genes were excluded if the percentage of data missing or filtered out exceeded 50%. Genes that passed the filtering criteria were then considered for further analysis. In gene ontology and BioCarta pathway analyses, the statistical significance of global gene expression changes was assessed by mean negative natural logarithm of the *P*-values of the respective single gene univariate test (LS)/Kolmogorov-Smirnov (KS) permutation tests and the Efron-Tibshirani's GSA MaxMean test. Statistical significance levels for gene ontology analysis and pathway analysis were set at $P = 0.0001$ and $= 0.001$, respectively. The gene set comparison tool analyzes pre-defined gene sets for differential expression among pre-defined classes (Pre versus Post). This indicates which gene sets contain more differentially expressed genes than would be expected by chance. "BioCarta" is a trademark of BioCarta Inc.

Statistical analysis. Student's paired *t*-test was used to compare serum sVEGFR1 and sVEGFR2 levels or plasma cytokine levels at baseline (pre-treatment) and on days 8 and 29, to evaluate the significance of changes induced by OTS11101. A *P*-value of <0.05 was considered statistically significant.

Results

Patient demographics. From December 2009 to November 2010, nine HLA-A*24:02-positive patients were treated with OTS11101. The baseline demographics of these patients are shown in Table 1. All patients had previously received one or more chemotherapy regimens for advanced disease. All patients completed the first cycle of four injections of OTS11101 and six patients were subjected to further cycles of vaccination. The median (range) number of vaccinations in the whole cohort was nine (4–44).

Safety. All nine patients received at least one dose of study treatment and were evaluated for safety (Table 2). One patient with cholangiocellular cancer given a dose of 2.0 mg experienced a grade 3 γGTP increase. His hepatic metastases had progressed and invaded the common bile duct; therefore, the investigators and independent data monitoring committee judged the γGTP increase as an adverse event related to the primary disease and agreed to escalate to the third dose level of 3.0 mg. No other patients showed any toxicities of grade 3 or greater. Three patients (33.3%) (two in the 1.0-mg cohort and one in the 2.0-mg cohort) developed injection site reactions (grade 1). In the 1.0-mg cohort, one patient reported grade 2 proteinuria. Grade 2 hypertension was identified in a patient receiving the 3.0-mg dose. No DLTs were observed in this trial.

Tumor response. All patients were evaluated for tumor response. Although no complete or partial responses were

Table 1. Clinical characteristics of the eligible patients

Characteristics	No. patients
Median age (range)	68 (range: 44–78) years
ECOG performance status	
0	5
1	4
Cancer type	
Hypopharynx	1
Vulvar paget's disease	1
Thyroid (papillary carcinoma)	1
Uterine cervix	1
GIST	1
Orbital (pleomorphic adenocarcima)	1
Cholangiocellular	1
Biliary	1
Pancreas	1
Duration of disease, months (median)	39.9
Prior therapy	
Chemotherapy	
≤2 regimens	6
>3 regimens	3
Surgery	8

ECOG, eastern cooperative oncology group; GIST, gastrointestinal stromal tumor.

Table 2. Summary of adverse events

OTS11101 dose	1 mg (*n* = 3)		2 mg (*n* = 3)		3 mg (*n* = 3)		Total
CTCAE grade	G1/2	G3	G1/2	G3	G1/2	G3	
Hypothyroidism	2						2
Skin rush	1						1
Fatigue	1				1		2
Fever	1						1
Hypertension					1		1
Reaction of injection site	2		1				3
Hematoma of injection site	1						1
Urticaria					1		1
Proteinuria	1						1
LDH increased					1		
Lymphopenia					1		
Fibrinogen increase			1				1
γ-GTP increased				1	1		2
Fibrin D-dimer increased					1		1
TAT increased					1		1

Presented is the highest ever reached CTCAE grade. One patient may have experienced >1 event. CTCAE, common terminology criteria for adverse events; LDH, lactase dehydrogenase increased; γ-GTP, γ-glutamyl transferase; TAT, thrombin-antithrombin complex.

observed, five patients (55.5%) had stable disease (SD) for at least one treatment courses (28 days). The median duration of progression free survival (PFS) for all patients was 50 days (range: 31–201 days).

CTL response. An IFN-γ ELISPOT assay was conducted using PBMC periodically obtained from patients to assess the cellular immune responses to OTS11101. Positive CTL responses specific to the vaccinated peptide were determined as described in the Methods section. Positive CTL responses were seen in one of the three patients in both the 2.0 and 3.0-mg dose cohorts (Table 3, Fig. S1). The positive CTL response in the 2.0-mg cohort was seen in a 68-year-old female patient with follicular thyroid cancer who had multiple metastases in the lungs. She achieved stable disease that persisted for >3 months after initiating the vaccination. A 62-year-old female patient with uterine cervix cancer receiving 3.0 mg who had multiple metastases in the lungs also showed a positive CTL response. As the tumor continued to grow, despite chemotherapy, she was enrolled in this study. After initiating the vaccination, the tumor size evaluated by computed tomography remained stable for 6 months.

Serum levels of sVEGFR1, sVEGFR2, and other plasma cytokines related to angiogenesis during OTS11101 peptide vaccination. We investigated serum sVEGFR1, sVEGFR2, and other plasma cytokines related to angiogenesis as biomarker for OTS11101 vaccination (Fig. 1). The serum concentrations of sVEGFR1 on day 29 increased significantly over the first 4 weeks of treatment ($P < 0.001$, *t*-test). The serum concentrations of sVEGFR2 were also upregulated on days 8 and 29 ($P < 0.016$ and 0.044, respectively). Greater increases in sVEGFR1 levels on day 8 were seen at the higher dose levels of OTS11101.

Additionally, according to the analysis of angiogenesis-related cytokines, the level of IL-8, PECAM-1, and VEGF were significantly elevated on day 29 compared with the baseline (Fig. 1, Fig. S2).

Microarray analysis of gene expression change in PBMC after OTS11101 vaccination. To determine whether the OTS11101 vaccination induced any systemic immunological effects, we examined the gene expression profiles of PBMC of all nine patients on days 1 and 8, using microarray analysis. A gene set analysis was performed by comparing the overall significance of gene groups defined by GO categories. Out of the 4121 GO classes, 281 were found to be significantly affected by OTS11101 vaccination, as shown by at least one of the three tests used to assess the significance of the differences (Table S1). Six out of the 280 cellular component categories, 24 of 655 molecular function (MF) categories and 251 of 3186 biological process (BP) categories were significant. Interestingly, the gene ontology categories of "T cell activation" and "immune response" were selected. These results suggested that the genes related to T cell activation or immune response might be associated with vaccination response. Furthermore, pathway analysis of gene expression microarray data revealed that 19 pathways were significantly selected from 288 BioCarta pathways at the nominal 0.01 level of the LS permutation test or KS permutation test than expected by chance (Table 4). The most represented classes of differently expressed genes included genes involved in T cell function-related pathways, strongly supporting the notion that OTS11101 vaccination actually gives rise to T cell activation in clinical settings.

Discussion

To our knowledge, the clinical trial described here is the first human trial to consist of a vaccine strategy that has used the HLA-A*24:02-restricted epitope-peptide derived from VEGFR1 (VEGFR1-1084: OTS11101). The current phase I study showed that OTS11101 can be safely given to patients with advanced solid tumors, and no DLT was observed in any cohort. Analysis of the ELISPOT assay results indicated that peptide-specific CTL could be induced by the OTS11101 vaccination. Furthermore, biomarker analysis provided evidence that OTS10111 treatment actually provoked an anti-angiogenic effect and T cell activation in a clinical setting.

Targeting tumor angiogenesis with vaccines has potential advantages over targeting tumor cells. First, tumor endothelial cells are more accessible to the immune system than are tumor cells at a distance from the vessels.[19] Cancer endothelial cells are readily accessed by lymphocytes in the bloodstream, and CTLs can directly damage endothelial cells without the

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Table 3. Cytotoxic T lymphocyte (CTL) response in patients

Dose level	Patient No.	Type of cancer	Duration of PFS	Best response	Peptide-specific spots
1.0 mg	1-01	Biliary	31	PD	−10
	1-02	GIST	32	PD	−13
	1-03	Vulvar paget's disease	73	SD	−5
2.0 mg	2-01	Thyroid (papillary carcinoma)	110	SD	103
	2-02	Cholangiocellular	31	PD	3
	2-03	Hypopharynx	194	SD	0
3.0 mg	3-01	Uterine cervix	201	SD	24
	3-02	Pancreas	43	PD	NA
	3-03	Orbital (pleomorphic adenocarcima)	50	SD	1

GIST, gastrointestinal stromal tumor; NA, not analyzed; PD, progression disease; PFS, progression free survival; SD, stable disease.

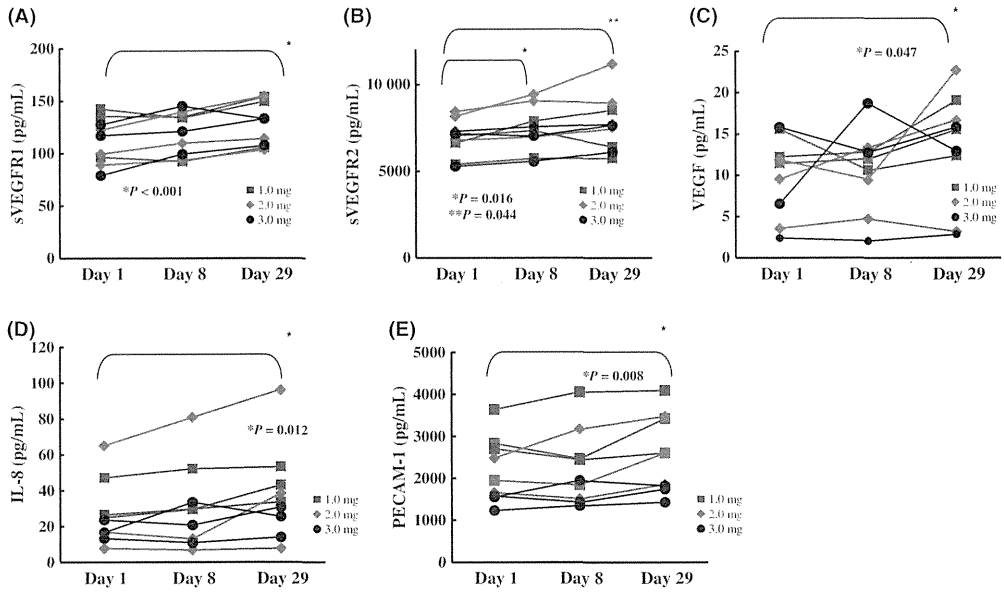

Fig. 1. Changes in angiogenesis-related biomarker concentrations in the blood after OTS11101 vaccinations: Evaluated at pretreatment, and after one and four vaccinations. The serum concentrations of serum-soluble vascular endothelial growth factor receptor 1 (sVEGFR1) increased significantly over the first 4 weeks of treatment (A). The serum concentrations of sVEGFR2 were also upregulated on days 8 and 29 (B). The plasma concentrations of VEGF (C), interleukin (IL)-8 (D), and platelet endothelial cell adhesion molecule-1 (PECAM-1) (E) were significantly elevated on day 29 compared with the baseline. The experiment was performed in triplicate.

penetration of any other tissue type. In addition, the lysis of even a small number of endothelial cells within the tumor vasculature may result in the destruction of vessel integrity, thus leading to the inhibition of numerous tumor cells.[20] Therefore, endothelial cells could be a good target for cancer immunotherapy. Second, the loss or downregulation of HLA molecules on tumor cells is considered to be one of the major reasons for limited clinical efficacy.[21–23] Since such HLA loss has not been reported for endothelial cells of newly formed vessels in tumors, the development of vaccines against vascular endothelial cells in tumor tissues may overcome the immune-escape of tumor cells.

In the present trial, we observed no severe adverse effects related to the treatment; as anticipated, patients experienced local injection site reactions, and a few experienced low-grade constitutional symptoms such as fever and fatigue. With respect to the specific toxicity of anti-angiogenic treatment, such as hypertension, proteinuria, and bleeding, which is often observed with anti-VEGF antibody and VEGF tyrosine kinase receptor inhibitors,[24,25] low-grade toxicity occurred in two patients (grade 2 proteinuria and hypertension in each, respectively). VEGFR1 has multiple functions not only in angiogenesis but also in hematopoiesis. Previous studies have reported that VEGFR1 is expressed on hematopoietic stem cells and that it plays functional roles in the recruitment of hematopoietic stem cells and reconstitution of hematopoiesis.[26] However, our results showed that bone marrow hematopoiesis was not affected by immunization with OTS11101 in a clinical setting. Thus, this vaccination is considered to be very safe and well tolerated compared with existing anti-angiogenic treatments.

In addition, the current trial demonstrated that clinical SD was achieved in five different types of cancer in which the VEGF pathway plays a potentially major role for angiogenesis or tumor progression.[27–32] We were unable to reach any firm conclusion from the small size of this phase I trial; therefore, further studies are required to investigate the efficacy of OTS11101 vaccination.

Table 4. Results of pathway analysis (Biocarta database)

	Pathway description	Number of genes	LS permutation *P*-value	KS permutation *P*-value	Efron-Tibshirani's GSA test *P*-value
1	CTL-mediated immune response against target cells	24	**0.00001**	0.00322	**<0.005**
2	Ras-independent pathway in NK cell-mediated cytotoxicity	38	**0.00001**	0.02009	**<0.005**
3	Selective expression of chemokine receptors during T-cell polarization	31	**0.00001**	**0.00001**	0.085
4	Dendritic cells in regulating TH1 and TH2 development	15	**0.00002**	**0.00001**	**<0.005**
5	HIV-induced T cell apoptosis	14	**0.00014**	0.00332	0.125
6	Granzyme A-mediated apoptosis pathway	23	**0.00023**	0.23511	**<0.005**
7	Bystander B cell activation	19	**0.00025**	**0.00014**	0.01
8	The role of eosinophils in the chemokine network of allergy	10	**0.00045**	0.01152	**<0.005**
9	Regulation of spermatogenesis by CREM	7	**0.00052**	0.00876	**<0.005**
10	Antigen-dependent B cell activation	23	**0.00057**	**0.00008**	0.025
11	D4-GDI signaling pathway	25	**0.00066**	0.08035	**<0.005**
12	Keratinocyte differentiation	85	0.00157	**0.00039**	0.045
13	Pertussis toxin-insensitive CCR5 signaling in macrophage	40	0.0018	0.02627	**<0.005**
14	Th1/Th2 differentiation	32	0.00339	**0.00001**	0.01
15	Eicosanoid metabolism	28	0.00511	**0.00002**	0.28
16	TSP-1-induced apoptosis in microvascular endothelial cell	21	0.00536	**0.00026**	0.305
17	Alpha-synuclein and parkin-mediated proteolysis in Parkinson@	5	0.01157	**0.00008**	0.14
18	IFN gamma signaling pathway	20	0.02591	**0.00072**	0.065
19	Steps in the glycosylation of mammalian N-linked oligosaccarides	23	0.05822	**0.00036**	0.05

Data considered significant ($P < 0.001$) are shown in bold. CCR5, C-C chemokine receptor type 5; CREM, cyclic AMP response element modulator; CTL, cytotoxic T cell lymphocyte; D4-GDI, Rho GDP dissociation inhibitor beta; HIV, human immunodeficiency virus; KS, Kolmogorov-Smirnov; LS, mean negative natural logarithm of the *P*-values of the respective single gene univariate test; NK, natural killer; TSP-1, thrombospondin-1.

Proof of concept (POC) based on immunologic monitoring is crucial for the rational design of cancer vaccination studies. Peptide vaccine trials have focused predominately on stimulating CTLs, through the administration of 8–10 mers binding on HLA class I alleles and such vaccine-induced CTLs can directly kill tumor cells.[33] The specific CTL responses against the OTS11101 vaccination were observed at 2-mg and 3-mg dose levels, clearly demonstrating that CTLs against VEGFR1 could be induced by the vaccination.

Additionally, it is important to address the toxicity to endogenous antigen-expressing cells. In the current study, we did not monitor the CTL response against the endogenous target. However, a previous study demonstrated that the CTLs induced with the VEGFR1 peptide induced strong cytotoxicity against target cells endogenously expressing VEGFR1.[12]

Several parameters measured in the blood circulation of patients with cancer might hold pharmacodynamic biomarker value. Naturally, VEGF, which is the most extensively explored biomarker, has been examined in numerous clinical trials. The present study demonstrated that serum VEGF levels were elevated after OTS11101 vaccination. The increases in these key mediators of angiogenesis are similar to those seen in the study of VEGF TKIs and anti-VEGF antibody in cancer patients or in preclinical models.[34–38] We also observed a time-dependent change in serum sVEGFR1 and sVEGFR2 levels. Several clinical trials have detected changes in the serum levels of sVEGFR1 and sVEGFR2.[39] In contrast to the VEGFR TKIs, all of which significantly decrease sVEGFR2 levels,[34,36,40–45] OTS11101 induced a mild but significant increase in serum sVEGFR1 and sVEGFR2. OTS11101 acts as an angiogenesis inhibitor by inducing CTLs that target vascular endothelial cells expressing VEGFR1, whereas VEGFR TKIs and anti-VEGF antibody directly inhibit the VEGF/VEGFR pathway. We speculate that this difference in pharmacological function between the vaccination and existing VEGF/VEGFR targeted agents creates such discrepancies in the kinetics of sVEGFR1 and sVEGFR2. Although the biological significance of this is not entirely understood, it suggests that OTS11101 potentially possesses a distinct antitumor mechanism of action compared with VEGFR TKIs. Furthermore, OTS11101 modulated the plasma concentration of inflammatory cytokines such as IL-8 and PECAM-1, the latter of which is a member of the immunoglobulin superfamily of cell surface receptors.

A limitation of biomarker analysis in the present study is the lack of examination of the vasculature in tumor samples. Investigation of changes to the vasculature in tumor tissues after vaccination is an attractive approach to POC, and several studies have reported a reduction in microvessel density in tumor tissues of patients receiving bevacizumab.[46–48] In future clinical trials evaluating the efficacy of OTS11101 vaccination, an investigation of the changes to the vasculature in tumor samples is needed.

While *ex vivo* or *in vitro* studies have provided a wealth of information on the specific effect of immuno-peptide therapy, they cannot substitute for an ultimate understanding of what goes on *in vivo*. There is no valid and widely accepted *in vivo* analysis to date to achieve POC during the clinical development of cancer vaccines. Microarray technology has enabled significant genes to be identified almost throughout the genome using a hypothesis-free approach. The recent introductions of this technology in the field of cancer research are numerous, providing insights into a more accurate classification of cancer, better defined prognosis, and novel approaches for therapy. Microarray analyses have been shown to be powerful tools in identifying and characterizing gene regulation in the ontogeny, differentiation, and activation of immune cells. In the present study, we have used the microarray procedure using PBMC obtained from patients to monitor the biological activity of OTS11101. To effectively interpret the enormous amount of microarray data, we examined biologically relevant gene pathways rather than individual genes. Although it is difficult to determine whether the observed pathways elicited by OTS11101 vaccination led to the activation of CTLs or the suppression of regulatory T-cells, we detected several pathways related to CTL response. For example, in the CTL-mediated immune response against target cell pathways, a set of genes identified as significantly upregulated following OTS11101 vaccination included granzyme B, perforin, CD247, FAS ligand, ICAM1, and integrin. It is known that pathways

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containing cytotoxic proteins, such as perforin and granzyme B can mediate cancer cell death via CTLs. FAS-ligand induced apoptosis also plays a crucial role in the CTL response. Thus, the results of our analysis indicate that several pathways related to cytotoxic function of CTL were significantly affected by a single administration of OTS11101, supporting the notion that this peptide induces an immune response. Our current study introduced a novel direction in which microarray analysis could be useful for achieving POC during early clinical trials of cancer vaccine.

In conclusion, OTS11101 shows an acceptable safety profile for patients with advanced solid tumors. The preliminary evaluations of biological activity of OTS11101 using microarray and biomarker analysis demonstrate that this vaccine is biologically active. Further trials to evaluate the efficacy of this promising vaccination are warranted, and phase II clinical trial evaluating the anti-tumor activity of OTS11101 in combination with gemcitabine in patients with locally advanced or metastatic pancreatic cancer are ongoing.

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Disclosure Statement

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Fig. S1. Representative ELISPOT assay.

Fig. S2. Changes in angiogenesis-related cytokine concentrations.

Table S1. Assessment of overall significance of expression changes in gene groups defined by Gene Ontology (GO) categories.

Data S1. Methods of measurement of CTL responses in detail.

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ORIGINAL ARTICLE

Phase I and pharmacokinetic study of gefitinib and S-1 combination therapy for advanced adenocarcinoma of the lung

Hidemi Kiyota · Isamu Okamoto · Masayuki Takeda · Haruko Daga · Tateaki Naito · Masaki Miyazaki · Hideaki Okada · Hidetoshi Hayashi · Kaoru Tanaka · Masaaki Terashima · Koichi Azuma · Haruyasu Murakami · Koji Takeda · Nobuyuki Yamamoto · Kazuhiko Nakagawa

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Abstract
Background A phase I dose-escalation study was performed to investigate the safety and pharmacokinetics of the combination of S-1 and gefitinib in patients with pulmonary adenocarcinoma who had failed previous chemotherapy.
Methods Patients received gefitinib at a fixed daily oral dose of 250 mg, and S-1 was administered on days 1–14 every 21 days at doses starting at 60 mg/m^2 (level 1) and escalating to 80 mg/m^2 (level 2). The primary end point of the study was determination of the recommended dose for S-1 given in combination with a fixed dose of gefitinib.
Results Twenty patients were enrolled in the study. Two of the first six patients at dose level 2 experienced a dose-limiting toxicity (elevation of alkaline phosphatase of grade 3 in one patient; elevations of aspartate and alanine aminotransferases of grade 3 in the other). The recommended dose was thus determined as level 2, and an additional 11 patients were assigned to this level. All observed adverse events were well managed. The response rate was 50 % (10 of 20 patients), and the median progression-free survival (PFS) and overall survival times were 10.5 and 21.2 months, respectively. In *EGFR* mutation–positive patients ($n = 9$), seven patients achieved an objective response and the median PFS was 12.4 months, whereas none with wild-type *EGFR* ($n = 6$) responded. No pharmacokinetic interaction between S-1 and gefitinib was detected.
Conclusions The combination of S-1 and gefitinib is well tolerated and appears to possess activity against *EGFR* mutation–positive NSCLC.

Keywords Gefitinib · S-1 · Non-small-cell lung cancer · Epidermal growth factor receptor · Phase I study

H. Kiyota · I. Okamoto (✉) · M. Takeda · M. Miyazaki · H. Hayashi · K. Tanaka · M. Terashima · K. Azuma · K. Nakagawa
Department of Medical Oncology, Faculty of Medicine, Kinki University, 377-2 Ohno-higashi, Osaka-Sayama, Osaka 589-8511, Japan
e-mail: chi-okamoto@dotd.med.kindai.ac.jp

H. Daga · H. Okada · K. Takeda
Department of Clinical Oncology, Osaka City General Hospital, Osaka, Japan

T. Naito · H. Murakami · N. Yamamoto
Thoracic Oncology Division, Shizuoka Cancer Center, Shizuoka, Japan

Introduction

Gefitinib was the first molecularly targeted agent to become clinically available for the treatment of non-small-cell lung cancer (NSCLC). Somatic activating mutations of *EGFR* have been identified as a major determinant of the clinical response to treatment with gefitinib, with achievement of a clinical benefit with this drug in NSCLC patients with wild-type *EGFR* having been problematic [1, 2]. Furthermore, despite the therapeutic efficacy of gefitinib for patients with *EGFR* mutation–positive NSCLC, most such patients ultimately develop resistance to the drug. The development of combination therapy with gefitinib and other chemotherapeutic agents is being pursued in an attempt to improve treatment efficacy.

S-1 is an oral fluorinated pyrimidine formulation that combines tegafur (FT), 5-chloro-2,4-dihydroxypyridine (CDHP), and oxonic acid (Oxo) in a molar ratio of 1:0.4:1 [3]. FT is a prodrug that generates 5-fluorouracil (5-FU) in blood largely as a result of its metabolism by cytochrome