Table 7. Prognostic factors (multivariate analysis)

	Hazard ratio (95% CI)	p*
ECOG PS		
2–3	3.786 (1.736–8.259)	<0.01
Primary site		
Lung		
GI tract	1.061 (0.531–2.121)	0.87
HBP	1.390 (0.593–3.255)	0.45
Others	1.364 (0.534–3.480)	0.52
NSE		
≥15 ng/ml	1.480 (0.782–2.803)	0.23
Hb		
<12 g/dl	1.144 (0.740–1.768)	0.55
Alb		
<3.7 g/dl	1.381 (0.890–2.144)	0.15
ALP		
<360 IU/l	1.189 (0.682–2.075)	0.54
Liver involvement		
Presence	1.943 (1.146–3.295)	0.014
Regimen		
CDDP + CPT-11		
CDDP + VP-16	1.990 (1.062–3.731)	0.032
CBDCA + VP-16	1.487 (0.873–2.532)	0.14

Hb = Hemoglobin; Alb = albumin; ALP = alkaline phosphatase; CDDP = cisplatin; CPT-11 = irinotecan; VP-16 = etoposide; CBDCA = carboplatin. * Cox proportional hazards model.

SCLC; however, little is known about treatment efficacy because of the rareness of these cases, unlike SCLC (which accounts for about 20% of all lung cancers). Consequently, we decided to conduct the present investigation. In an attempt to unify the therapeutic procedures among unified patients, the candidates for enrollment were rigidly restricted to patients with pathologically confirmed EP-NECs and SCLC, extended or recurrent disease, and who had been treated with the PE, IP, or CE regimen. The PE or IP regimens are presently considered to be the standard treatment regimens for patients with extended SCLC [33–35]. The CE regimen has been reported to have a similar efficacy to the PE regimen among patients with SCLC and can be considered as an alternative treatment regimen [36].

The first aim of this study was to clarify the efficacy of standard SCLC regimens when used to treat advanced EP-NECs and to compare the treatment outcome with that for SCLC. Our study showed that the response rate of the patients with EP-NECs was lower than that of the patients with SCLC. The objective response rate to platinum-based chemotherapy was 77.8% for the patients with SCLC, and the MST in the present study was 13.6 months, which was similar to previously reported survival times [33, 34, 36]. On the other hand, the efficacy of such regimens against EP-NECs seemed to be worse in the present study, with a response rate of 31.7% compared with 33–66.7% in previous reports [5, 7, 10, 20, 21]. Although the reason for the difference in the treatment efficacies was unknown, possible reasons include the differences in the criteria used to evaluate response and the relatively small number of patients. Additionally, the response rates of EP-NECs with different primary sites were significantly different, with the HBP group showing the lowest response rate. This finding also disagreed with Brenner et al.'s [5] report, which showed no differences in the response to chemotherapy among the different anatomical locations. The patients with EP-NECs tended to have unfavorable outcomes compared with the patients with SCLC in our study. Some reports have shown that the OS of patients with EP-NECs was better than that of patients with SCLC [7, 16]. In the present study, the most common primary sites of the EP-NECs were the stomach, pancreas, and esophagus. On the other hand, relatively few patients had tumors arising in the reproductive tract, breast, or head and neck region, which have been reported to be associated with a better prognosis [4, 9–12]. At our institution, genitourinary EP-NECs tend to be resected, and head and neck EP-NECs are often treated using chemoradiotherapy. In other words, the outcomes of the patients in a particular study might depend on the ratio of the primary sites. Moreover, an interesting finding in the present study was that although liver involvement was identified as a prognostic factor in a multivariate analysis, the primary site was not a prognostic factor. In fact, liver metastasis is a well-documented poor prognostic factor in patients with NETs [22, 37–40]. Actually, 73% of the EP-NECs exhibited liver involvement in the present study, whereas only 22% of the SCLCs exhibited liver involvement.

Another interesting point of the present study is that the IP regimen was associated with a significantly more favorable outcome than the PE regimen in the multivariate analysis. When the outcome of the SCLC patients was examined according to the chemotherapeutic regimen that was used, the MST associated with the IP regimen was 16.6 months, which was significantly longer than that associated with the PE regimen (12.4 months) or the CE regimen (9.3 months) ($p = 0.023$ and $p = 0.023$, respectively; table 5). These results agree with those of a Japanese report by Noda et al. [35]. A similar trend was observed among patients with EP-NECs in our study.

However, this result should be interpreted with caution because of the bias introduced by the different treatment policies with regard to the selection of chemotherapeutic regimens among the divisions of our institute. For example, all the patients in the HBP group were treated using the PE regimen, whereas a large proportion of the patients in the GI and 'others' groups were treated using the IP regimen (table 3).

The objectives of this study included not only patients with 'extrapulmonary small-cell carcinoma' but also those with 'EP-NEC diagnosed based on a high Ki67 index or high mitotic count without small-cell morphological features; NEC other than small-cell carcinoma'. It is possible that 'NEC other than small-cell carcinoma' may be biologically and clinically different from small-cell carcinoma, and these differences could influence the treatment outcomes of NEC in this study. However, although the terms 'small-cell carcinoma' and 'large-cell carcinoma' were specified in the WHO 2010 classification, no definite indicators distinguishing 'NEC other than small-cell carcinoma' from 'small-cell carcinoma' exist. Therefore, we refrained from comparing them in this study. Additionally, some prognostic factors, such as the Ki67 index, mitotic count or degree of necrosis, may also influence the treatment outcomes. Because information regarding these factors could not be obtained for all the patients in this study and because our main purpose in this study was to compare the clinical outcomes according to the primary tumor site, we did not evaluate the chemosensitivity or the patient outcome according to these factors. Nevertheless, these points are very important clinical questions and should be examined in future studies.

Another limitation of this study was its retrospective nature, and a prospective trial with a large number of patients treated using the same chemotherapeutic regimen is needed to confirm our findings.

In conclusion, the response rate and prognosis of patients with extended or recurrent EP-NECs, especially those originating in the liver, biliary tract, or pancreas, were worse than those of the patients with SCLC in this study. The ECOG PS, liver involvement, and treatment regimen had a larger impact on the prognosis than the primary tumor site, as demonstrated by multivariate analysis.

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Disclosure Statement

The authors have no conflicts of interest.

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Original Article

Everolimus for Advanced Pancreatic Neuroendocrine Tumours: A Subgroup Analysis Evaluating Japanese Patients in the RADIANT-3 Trial

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Objective: Everolimus, an inhibitor of the mammalian target of rapamycin, has recently demonstrated efficacy and safety in a Phase III, double-blind, randomized trial (RADIANT-3) in 410 patients with low- or intermediate-grade advanced pancreatic neuroendocrine tumours. Everolimus 10 mg/day provided a 2.4-fold improvement compared with placebo in progression-free survival, representing a 65% risk reduction for progression. The purpose of this analysis was to investigate the efficacy and safety of everolimus in the Japanese subgroup enrolled in the RADIANT-3 study.

Methods: Subgroup analysis of the Japanese patients was performed comparing efficacy and safety between everolimus 10 mg/day orally ($n = 23$) and matching placebo ($n = 17$). The primary endpoint was progression-free survival. Safety was evaluated on the basis of the incidence of adverse drug reactions.

Results: Progression-free survival was significantly prolonged with everolimus compared with placebo. The median progression-free survival was 19.45 months (95% confidence interval, 8.31–not available) with everolimus vs 2.83 months (95% confidence interval, 2.46–8.34) with placebo, resulting in an 81% risk reduction in progression (hazard ratio, 0.19; 95% confidence interval, 0.08–0.48; $P < 0.001$). Adverse drug reactions occurred in all 23 (100%) Japanese patients receiving everolimus and in 13 (77%) patients receiving placebo; most were grade 1/2 in severity. The most common adverse drug reactions in the everolimus group were rash ($n = 20$; 87%), stomatitis ($n = 17$; 74%), infections ($n = 15$; 65%), nail disorders ($n = 12$; 52%), epistaxis ($n = 10$; 44%) and pneumonitis ($n = 10$; 44%).

Conclusions: These results support the use of everolimus as a valuable treatment option for Japanese patients with advanced pancreatic neuroendocrine tumours.

Key words: pancreatic neuroendocrine tumours – mTOR inhibitors – everolimus – Japanese population

INTRODUCTION

Pancreatic neuroendocrine tumours (pNET) are neuroendocrine neoplasms originating from islets of Langerhans cells in the pancreas (1). According to US population-based estimates [Surveillance, Epidemiology and End Results (SEER) program], pNET account for ~1.3% of pancreatic neoplasms in incidence; however, the prevalence represents

10% of pancreatic neoplasms (1). The incidence of pNET has increased significantly in the past three decades, reaching 0.32 per 100 000 patients in the SEER registry during 2000–04 (2). Because these tumours are often not recognized until the advanced stages of the disease progress, the prognosis for patients is poor (2). Most patients with pNET (~64%) present with advanced (metastatic) disease (2). The estimated median survival time for patients with distant metastatic pNET is 24 months (2).

The epidemiology of pNET in Japan is of increasing interest, but data are limited. According to a national survey conducted in 2005, the overall incidence of pNET in Japan was 1.01 per 100 000 people (approximately three times higher than US estimates), and the overall prevalence was 2.23 per 100 000 people (3). The geographical difference in epidemiological data suggests that ethnic differences underlie the disease, but ethnic differences in efficacy and safety of drug therapies have not yet been investigated.

While surgery is the mainstay of treatment for patients with limited disease burden confined to the primary site and regional lymph nodes (4,5) and for patients with resectable liver metastases based on National Comprehensive Cancer Network guidelines (6), it is not an option for patients with advanced or unresectable metastatic disease (6). Systemic chemotherapy with streptozocin (Zanosar®; Keocyt in the EU and Teva Parenteral Medicines, Inc. in the USA) is the approved treatment option for advanced pNET in the USA (6), France, Canada and Israel, but it is not available in Japan. The role of chemotherapy in the treatment of pNET remains inconclusive. Response rates differ among studies (5,7,8), and their rigorousness for response evaluation (e.g. clinical evaluation vs exclusive use of radiologic evaluation) has been questioned (5,7). Modest response rates, coupled with concerning toxicity profiles often associated with the use of chemotherapeutic agents (5,9,10), underscore the continuing unmet need for effective, approved treatment options for advanced pNET.

Liver metastases of NET have high hepatic artery blood flow, and liver-directed therapies are used in the treatment of NET patients despite the lack of randomized controlled trials (11). Transarterial embolization and transarterial chemoembolization are, thus, useful treatments for hepatic metastases of NET, including downstaging for those patients with high tumour burden and controlling the symptoms of hormonal hypersecretion (11–13). It has also been reported that radiofrequency ablation may be useful in conjunction with surgical resection or as an alternative to resection in patients with unresectable tumours and limited numbers of hepatic metastases (14–17). However, this procedure is limited to tumours that measure $\leq$5 cm in diameter and are not near vital structures (11). Reports on hepatic intra-arterial chemotherapy as monotherapy for hepatic metastases are few. Therefore, National Comprehensive Cancer Network (NCCN) guidelines (6) and European Neuroendocrine Tumor Society (ENETS) guidelines (11) do not recommend it. Among the major limitations of liver-directed therapies are that no randomized controlled trial has been conducted and that the complexity and high risk of morbidity make it necessary that the patient use an experienced centre (11).

The molecular pathogenesis of pNET continues to be explored. Current data suggest that constitutional activation of the mammalian target of rapamycin (mTOR) signalling pathway may be a key underlying factor involved in pNET proliferation. mTOR, a cytoplasmic serine/threonine kinase, is a central modulator of the cell cycle (18). Aberrant upstream activation of mTOR by phosphatidylinositol-3-kinase (P13K)/Akt3 (due to various mechanisms causing signalling defects, such as mutations) results in dysregulated downstream signalling, leading to uninhibited cell growth and proliferation, cellular metabolism and angiogenesis (19). This mechanism has been implicated in the development and progression of many cancers (20), including pNET (21). Inhibition of this pathway is, thus, an important therapeutic target in oncology.

Everolimus (Afinitor®; RAD001; Novartis Pharmaceuticals), an orally bioavailable inhibitor of mTOR, was recently approved in the USA and Europe for the treatment of patients with progressive pNET that are unresectable, locally advanced or metastatic. Everolimus has shown promising anti-tumour activity in Phase II studies of patients with pNET (22,23) and in a recently published Phase III trial (24). The RADIANT-3 trial evaluated the efficacy and safety of oral everolimus, 10 mg/day vs placebo, in 410 patients with low- or intermediate-grade advanced pNET (24). In this trial, everolimus demonstrated a statistically and clinically significant 2.4-fold improvement in progression-free survival (PFS). The median PFS was 11.0 months in the everolimus group vs 4.6 months with placebo, representing a 65% risk reduction for progression compared with placebo [hazard ratio (HR), 0.35; $P < 0.001$] (24). Most common adverse drug reactions (ADRs) occurring in at least 30% of patients receiving everolimus were stomatitis (64%), rash (49%), diarrhoea (34%) and fatigue (31%) (24). The subgroup analysis of Japanese patients who participated in the RADIANT-3 trial is presented herein to investigate whether the efficacy and safety of everolimus were comparable in the Japanese population and the overall study population.

PATIENTS AND METHODS

Patients

The RADIANT-3 inclusion and exclusion criteria have been described in detail (24). Briefly, eligibility criteria included age $\geq$18 years, low- or intermediate-grade advanced (unresectable or metastatic) pNET and radiologic documentation of disease progression within 12 months before randomization.

The additional inclusion criteria were presence of measurable disease per Response Evaluation Criteria in Solid Tumors (RECIST), version 1.0; World Health Organization (WHO) performance status (PS) $\leq$2; adequate bone marrow, renal and hepatic function and adequately controlled lipid and glucose levels. The exclusion criteria included hepatic

artery embolization within 6 months before enrolment (within 1 month in the presence of other sites of measurable disease), cryoablation or radiofrequency ablation of hepatic metastasis 2 months before enrolment, presence of severe or uncontrolled medical condition, previous therapy with an mTOR inhibitor or long-term treatment with corticosteroids or other immunosuppressants.

Study Oversight

The study was approved by the institutional review board or ethics committee at each participating centre and was conducted in accordance with Good Clinical Practice principles and applicable local regulations. Written informed consent was obtained from all patients.

Study Design and Treatment

RADIANT-3 was a double-blind, placebo-controlled, randomized, multicentre, Phase III study (NCT00510068) conducted in 82 centres within 18 countries worldwide (24). Patients were randomly assigned to receive either oral everolimus 10 mg/day ($n = 207$) or placebo ($n = 203$), both in conjunction with best supportive care (Fig. 1). Forty Japanese patients from three different centres participated; 23 received everolimus and 17 received placebo. Patients were stratified on the basis of whether they had received chemotherapy and on baseline WHO PS (0 vs 1–2). Treatment was continued until the occurrence of disease progression, development of an unacceptable ADR (treatment-related adverse event), interruption of treatment for ≥ 3 weeks or withdrawal of patient consent. Investigators were unaware of study group assignments; however, disclosure was allowed if an investigator determined disease progression (per RECIST criteria), in which case a patient initially assigned to placebo could switch to open-label everolimus. Dose reductions or delays were permitted in the event of clinically significant ADRs (24).

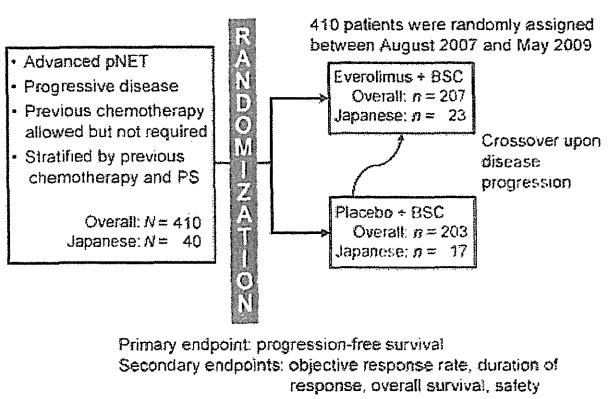


Figure 1. Study design. BSC, best supportive care; pNET, pancreatic neuroendocrine tumours; PS, performance status.

Efficacy and Safety Assessments

The primary endpoint was PFS evaluated by local investigator assessment according to the RECIST criteria, version 1.0. The secondary endpoints included confirmed objective response rate (complete response or partial response), duration of response, overall survival (OS) and safety. All randomly assigned patients were assessed for efficacy (i.e. intention-to-treat analysis). Tumour measurements were performed at baseline and repeated every 12 weeks. PFS by central review was a secondary endpoint in the trial; when needed, adjudication was performed by an independent central adjudication committee composed of a board-certified radiologist and an oncologist, both of whom were unaware of study group assignments (24). To detect radiologic lung changes suggestive of pneumonitis, a central radiology review of chest computed tomography (CT) scans and chest X-rays was performed.

All the patients who received at least one dose of the study drug and had at least one follow-up assessment were included in the safety analysis. Safety assessments included ADRs, laboratory evaluations and physical examinations (24).

Statistical Analysis

Kaplan–Meier methods were used to assess PFS and OS, as reported earlier (24). Statistical comparisons between study groups were made using a one-sided log-rank test. A Cox proportional hazards model was used to assess HR.

RESULTS

Patients and Treatment

As seen in Table 1 (24), baseline demographic characteristics of and previous therapies for the Japanese patients were well balanced between the treatment groups. The median age of the Japanese subgroup (45 years in the everolimus group, 53 years in the placebo group) was similar to that of the overall study population (24), as was the number of disease sites. Disease severity was generally lower in the Japanese subgroup than in the overall population; a higher percentage had a WHO PS of 0, and a slightly higher percentage had a well-differentiated histologic grade.

The percentage of patients receiving previous chemotherapy was similar between the treatment groups for the overall population (50% in the everolimus and 50% in the placebo groups) and slightly higher in the everolimus arms (61%) compared with the placebo arm (53%) in the Japanese subgroup. In addition, a higher percentage of patients in the overall population received previous somatostatin analogue treatment (49% in the everolimus group and 50% in the placebo group) compared with the Japanese subgroup (22 and 35%, respectively). None of the Japanese patients in the

Table 1. Baseline characteristics and previous therapies

	Japanese subgroup		Overall population[a]	
	Everolimus ($n = 23$)	Placebo ($n = 17$)	Everolimus ($n = 207$)	Placebo ($n = 203$)
Median age, years (range)	45 (33–85)	53 (38–77)	58 (23–87)	57 (20–82)
Male/female, %	57:44	47:53	53:47	58:42
WHO PS 0/1/2, %	87/13/0	88/12/0	67/30/3	66/32/3
No. disease sites 1/2/≥3, %	30/35/35	29/29/41	25/41/34	31/32/38
Histologic grade, %				
Well-differentiated	100	94	82	84
Moderately differentiated	0	6	17	15
Unknown	0	0	1	1
Previous therapies, %				
Chemotherapy	61	53	50	50
Radiotherapy	13	12	23	20
Targeted therapy	0	0	5	7
Immunotherapy	0	0	3	4
Hormone therapy	0	0	1	1
Other	4	0	10	13
Somatostatin analogues	22	35	49	50

WHO PS, World Health Organization performance status.
[a]Data previously presented by Yao JC et al. *N Engl J Med.* 2011;364: see also 514–23 (24).

Table 2. Patient disposition

	Japanese subgroup, *n* (%)		Overall population,[a] *n* (%)	
	Everolimus ($n = 23$)	Placebo ($n = 17$)	Everolimus ($n = 207$)	Placebo ($n = 203$)
Treatment ongoing	12 (52)	3 (18)	66 (32)	26 (13)
Patient discontinuation	11 (48)	14 (82)	141 (68)	177 (87)
Disease progression	6 (26)	14 (82)	92 (44)	163 (80)
Adverse events	4 (17)	0	36 (17)	7 (3)
Death	1 (4)	0	4 (2)	3 (1)
Withdrawal of consent	0	0	4 (2)	4 (2)
Other reasons	0	0	5 (2)	0
Duration of exposure, weeks, median (range)	60 (4–103)	12 (4–70)	38 (1–118)	16 (0.4–132)

[a]Data previously presented by Yao JC et al. *N Engl J Med.* 2011;364:514–23 (24).

RADIANT-3 trial received previous targeted therapy, immunotherapy or hormone therapy.

Patient disposition is shown in Table 2 (24). At a median follow-up of 16.1 months for the Japanese subgroup and 17.0 months for the overall population (24), median durations of exposure were 60 weeks (range, 4–103) and 38 weeks (range, 1–118), respectively, in the everolimus groups and 12 weeks (range, 4–70) and 16 weeks (range, 0.4–132), respectively, in the placebo groups. Higher percentages of patients in the placebo groups discontinued treatment (82% in Japanese subgroup and 87% in the overall population) compared with the everolimus groups (48% in the Japanese subgroup and 68% in the overall population).

For both the Japanese subgroup and the overall population, disease progression was the primary reason for treatment discontinuation and occurred in substantially more patients in the placebo groups (82% in the Japanese subgroup and 80% in the overall population) than in the everolimus groups (26% in the Japanese subgroup and 44% in the overall population). In the Japanese subgroup population, one patient in the everolimus group died of acute respiratory distress syndrome caused by sepsis that was considered to be treatment related; no patient in the placebo group died during the study. In the overall population, 12 patients (6%) in the everolimus group and 4 patients (2%) in the placebo group died while receiving the study drug; five deaths in the everolimus group and three deaths in the placebo group were attributed to the underlying cancer or disease progression, and seven deaths in the everolimus group and one death in the placebo group were attributed to adverse events. One death in the everolimus group that was related to the study drug was the Japanese patient described earlier (24).

Efficacy

In the Japanese subgroup, PFS was significantly prolonged in patients treated with everolimus compared with placebo [HR, 0.19; 95% confidence interval (CI), 0.08–0.48; log rank, $P < 0.001$] (Fig. 2a) (24). PFS was achieved for a median of 19.45 months (95% CI, 8.31-not available) in the everolimus group compared with 2.83 months (95% CI, 2.46–8.34) in the placebo group.

These PFS results represent a 16.62 month prolongation in median PFS and an 81% risk reduction in disease progression in the everolimus group. They are consistent with those demonstrated for the overall population (24), wherein median PFS was 11.0 months (95% CI, 8.4–13.9) in the everolimus group compared with 4.6 months (95% CI, 3.1–5.4) in the placebo group, correlating with a 65% risk reduction in progression in the everolimus group (HR, 0.35; 95% CI, 0.27–0.45; $P < 0.001$) (Fig. 2b) (24).

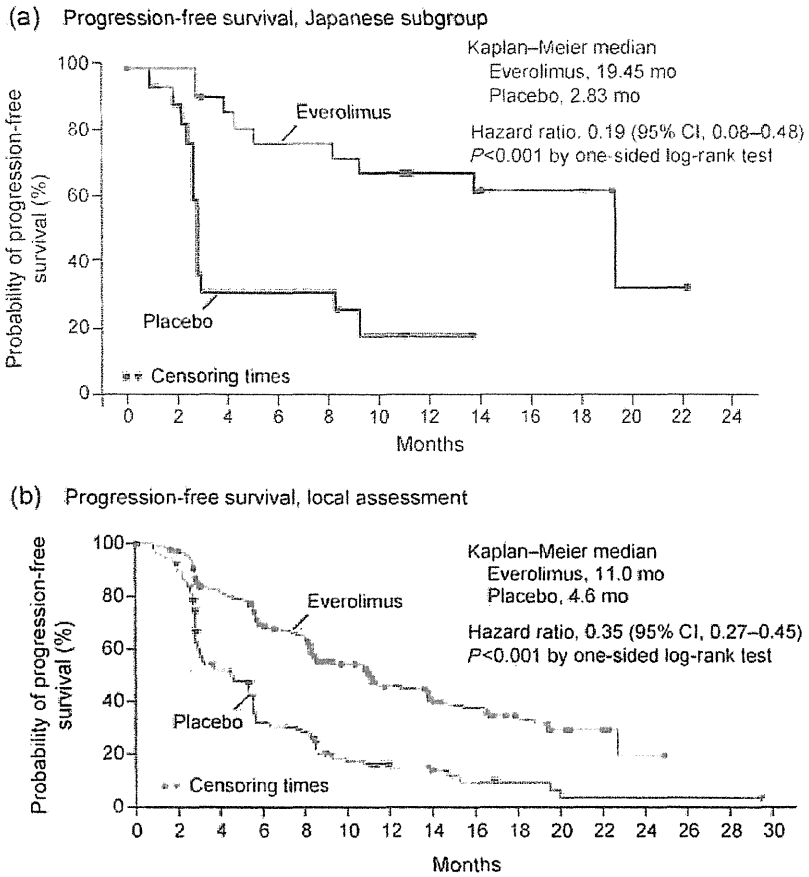


Figure 2. Kaplan–Meier plot of progression-free survival (PFS) for (a) Japanese subgroup and (b) local assessment of the overall population (24). Hazard ratios were obtained from a Cox model. CI, confidence interval; HR, hazard ratio. Reprinted from Yao JC, Shah MH, Ito T, et al. Everolimus for advanced pancreatic neuroendocrine tumors. *N Engl J Med* 2011;364:514–23 (24). Copyright© 2011 Massachusetts Medical Society.

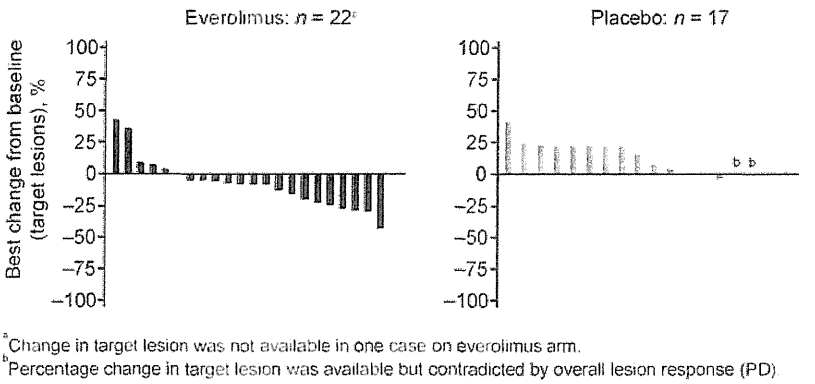
Table 3. Best overall response

Response	Japanese subgroup, *n* (%)		Overall population,[a] *n* (%)	
	Everolimus ($n = 23$)	Placebo ($n = 17$)	Everolimus ($n = 207$)	Placebo ($n = 203$)
Complete response	0	0	0	0
Partial response	1 (4)	1 (6)	10 (5)	4 (2)
Stable disease	19 (83)	5 (29)	151 (73)	103 (51)
Progressive disease	2 (9)	11 (65)	29 (14)	85 (42)
Unknown	1 (4)	0	17 (8)	11 (5)
Objective response rate (CR or PR) [95% CI]	1 (4) [0.1–21.9]	1 (6) [0.1–28.7]	10 (5) [2.3–8.7]	4 (2) [0.5–5.0]

CI, confidence interval; CR, complete response; PR, partial response.
[a]Data previously presented by Yao JC et al. *N Engl J Med.* 2011;364:514–23 (24).

In the Japanese subgroup, 13 patients crossed over from the placebo arm to receive everolimus; 148 patients crossed over in the overall population. Median OS was not reached at the time of this analysis, and no significant difference between treatment groups was observed in the Japanese subgroup (HR, 0.90; 95% CI, 0.20–4.05; $P = 0.45$) or in the overall population (HR, 0.89; 95% CI, 0.64–1.23) (24).

No patient in either treatment group achieved complete response; however, significantly more patients in the everolimus group than in the placebo group achieved stable disease



[a]Change in target lesion was not available in one case on everolimus arm.
[b]Percentage change in target lesion was available but contradicted by overall lesion response (PD).

Figure 3. Best percentage change from baseline in Japanese subgroup. PD, progressive disease.

[83 vs 29%, respectively; Table 3(24)], and fewer patients had progressive disease (9 vs 65%, respectively). Graphical analysis of the best percentage change from baseline in the Japanese subgroup (Fig. 3) illustrates that more patients experienced reduction in target lesion size in the everolimus group than in the placebo group. All these findings were consistent with those determined for the overall population (Table 3) (24).

Safety

As shown in Table 4 (24), in the Japanese subgroup, ADRs occurred in all the 23 (100%) patients treated with everolimus and in the 13 (77%) patients who received placebo; most ADRs were grade 1 or 2 in severity. The most common ADRs in the everolimus group were rash ($n = 20$; 87%), stomatitis ($n = 17$; 74%), infections ($n = 15$; 65%), nail disorders ($n = 12$; 52%), epistaxis ($n = 10$; 44%) and pneumonitis ($n = 10$; 44%), all of which occurred at higher rates than in the overall population (24). Grade 3 ADRs occurred in 15 (65%) patients in the everolimus group and in 5 (29%) patients in the placebo group. One grade 4 infection occurred in the everolimus group (4%); none was reported in the placebo group. The most common grade 3/4 ADRs occurring in Japanese patients in the everolimus group were neutropenia ($n = 4$; 17%), anaemia ($n = 2$; 9%), pneumonitis ($n = 2$; 9%), leucopoenia ($n = 2$; 9%), infections ($n = 2$; 9%) and abnormal hepatic function ($n = 2$; 9%). In the overall population, the most common ADRs affecting $\geq$30% of patients treated with everolimus were stomatitis ($n = 131$; 64%), rash ($n = 99$; 49%), diarrhoea ($n = 69$; 34%) and fatigue ($n = 64$; 31%) (24). Most ADRs were grade 1 or 2. Common grade 3 or 4 ADRs in the everolimus group were stomatitis ($n = 14$; 7%), anaemia ($n = 12$; 6%) hyperglycaemia ($n = 11$; 5%), thrombocytopenia ($n = 8$; 4%) and diarrhoea ($n = 7$; 3%) (24).

In the overall population, 68 (33.3%) patients treated with everolimus had post-baseline findings suggestive of pneumonitis based on the central review of chest X-ray or chest CT scan. In the Japanese subgroup, seven (30.4%) patients treated with everolimus had these post-baseline findings based on the central review, indicating that in this treatment arm Japanese patients did not differ from the overall population in the rate of lung changes. However, only 27 (13.2%) patients treated with everolimus in the overall population were reported by the investigator to have pneumonitis as an adverse event compared with seven (30.4%) patients in the Japanese subgroup (Table 5), indicating that Japanese investigators reported the lung changes more frequently.

DISCUSSION

The RADIANT-3 trial represents the largest placebo-controlled Phase III clinical trial in patients with advanced pNET (24). As was seen with the overall population, the Japanese subgroup demonstrated large and significant increases in PFS [81% ($P < 0.001$) vs 65% ($P < 0.001$) for the overall population] (24). In addition, median PFS in the Japanese subgroup increased nearly 7-fold in the everolimus group compared with the placebo group as against an ~2-fold increase in the overall population (24). Because patients whose disease progressed on placebo were allowed to cross over to open-label everolimus, this study was not designed to analyse differences in OS. RECORD-1, the Phase III trial of everolimus in patients with metastatic renal cell carcinoma, also showed longer PFS in the Japanese subgroup than in the overall population (25,26). However, in the RECORD-1 trial, the ratio of PFS in the everolimus and placebo arms was only 1.6-fold (5.75 months vs 3.61 months, respectively; HR, 0.19; 95% CI, 0.05–0.83) (25,26).

Reasons for the disparity in the PFS ratio in the patients with NET remain inconclusive. The number of disease sites was similar between the everolimus groups for the two populations; however, the Japanese subgroup had a lower percentage of patients with a WHO PS of 1 or 2 than the overall population (13 vs 33%, respectively), a higher percentage of patients with well-differentiated tumours (100 vs 82%, respectively) and a lower percentage of patients who previously received somatostatin analogue therapy (22 vs 49%, respectively) (24). The pharmacokinetics of everolimus in

Table 4. Adverse drug reactions

	Japanese subgroup, *n* (%)				Overall population[a], *n* (%)			
	Everolimus (*n* = 23)		Placebo (*n* = 17)		Everolimus (*n* = 204)		Placebo (*n* = 203)	
	All grades	Grade 3 or 4	All grades	Grade 3 or 4	All grades	Grade 3 or 4	All grades	Grade 3 or 4
Any ADR	23 (100)	16 (70)	13 (77)	5 (29)	195 (96)	92 (45)	151 (74)	28 (14)
Rash	20 (87)	0	2 (12)	0	99 (49)	1 (<1)	21 (10)	0
Stomatitis[b]	17 (74)	0	4 (24)	0	131 (64)	14 (7)	34 (17)	0
Infections[c]	15 (65)	2 (9)	3 (18)	0	46 (23)	5 (2)	12 (6)	1 (<1)
Nail disorders	12 (52)	0	0	0	24 (12)	1 (<1)	2 (1)	0
Epistaxis	10 (44)	0	0	0	35 (17)	0	0	0
Pneumonitis[d]	10 (44)	2 (9)	0	0	35 (17)	5 (2)	0	0
Dysgeusia	8 (35)	0	2 (12)	0	35 (17)	0	8 (4)	0
Fatigue	8 (35)	0	2 (12)	0	64 (31)	5 (2)	29 (14)	1 (<1)
Anaemia	7 (30)	2 (9)	0	0	35 (17)	12 (6)	6 (3)	0
Glossitis	7 (30)	0	0	0	7 (3)	0	0	0
Headache	7 (30)	0	3 (18)	0	39 (19)	0	13 (6)	0
Hyperlipidemia	7 (30)	0	1 (6)	0	9 (4)	0	2 (1)	0
Neutropenia	7 (30)	4 (17)	3 (18)	3 (18)	13 (6)	6 (3)	4 (2)	4 (2)
Vomiting	7 (30)	0	0	0	31 (15)	0	13 (6)	0
Decreased appetite	6 (26)	0	1 (6)	0	40 (20)	0	14 (7)	2 (1)
Diarrhoea	6 (26)	0	2 (12)	0	69 (34)	7 (3)	20 (10)	0
Hyperglycaemia	6 (26)	1 (4)	1 (6)	0	27 (13)	11 (5)	9 (4)	4 (2)
Hypertension	6 (26)	0	1 (6)	1 (6)	10 (5)	1 (<1)	4 (2)	1 (<1)
Leucopenia	6 (26)	2 (9)	2 (12)	0	12 (6)	2 (1)	4 (2)	1 (<1)
Pyrexia	6 (26)	0	0	0	22 (11)	0	0	0
Cheilitis	5 (22)	0	1 (6)	0	8 (4)	0	2 (1)	0
Diabetes mellitus	5 (22)	1 (4)	0	0	17 (8)	5 (2)	0	0
Gingivitis	5 (22)	0	0	0	7 (3)	0	1 (<1)	0
Nausea	5 (22)	0	1 (6)	0	41 (20)	5 (2)	37 (18)	0

Other grade 3/4 ADRs in the everolimus group: abnormal hepatic function, liver abscess, abdominal pain, acute respiratory distress syndrome, aplasia pure red cell, decreased blood phosphorus, cellulitis, decreased haemoglobin, hypophosphatemia, thrombocytopenia, lymphopenia, ileus and staphylococcal sepsis.
ADR, adverse drug reaction.
[a]Data previously presented by Yao JC et al. *N Engl J Med.* 2011;364:514–23 (24).
[b]Including aphthous stomatitis, mouth ulceration and tongue ulceration.
[c]Including all types of infection.
[d]Including interstitial lung disease, lung infiltration, pulmonary fibrosis and restrictive lung disease.

Japanese patients was previously shown to be similar to those in Caucasians (27), who accounted for 79% (*n* = 322) of the patients in the overall population (24). Though the Japanese patients were smaller than the overall population enrolled in RADIANT-3, the similar pharmacokinetics of everolimus in Japanese and Caucasian patients suggest that exposure of everolimus should not have accounted for its increased efficacy in this subgroup. As previously noted, the incidence of pNET is nearly three times higher in Japanese than in Caucasians (3). Ethnic differences that might have been responsible for the different incidence might also have contributed to the different response to everolimus in these groups and continue to be investigated.

Patients with pNET who have mutations in tuberous sclerosis 2 (*TSC2*) and phosphatase and tensin homolog (*PTEN*)—tumour suppressor genes of the P13K/Akt pathway—tend to have more aggressive tumours and subsequent quicker times to disease progression (21). Decreased protein expression of these negative regulators (TSC2 and PTEN) leads to overstimulation of the mTOR pathway by constitutive activation of PI3K/Akt, thus resulting in uninhibited tumour growth (19). Notably, tumours exhibiting

Table 5. Central radiology assessment by combined X rays and CT scans on radiologic lung changes suggestive of pneumonitis

	Japanese subgroup, *n* (%)		Overall population, *n* (%)	
	Everolimus (*n* = 23)	Placebo (*n* = 17)	Everolimus (*n* = 204)	Placebo (*n* = 203)
Patients with radiologic lung changes suggestive of pneumonitis				
Baseline	1 (4.3)	0	15 (7.4)	10 (4.9)
Post-baseline[a]	7 (30.4)	0	68 (33.3)	27 (13.3)
Newly occurring or worsened[b]	7 (30.4)	0	62 (30.4)	23 (11.3)
Without review	0	0	1 (0.5)	2 (1.0)
Pulmonary adverse events in patients with newly occurring or worsened lung changes suggestive of pneumonitis				
Total	7 (30.4)	0	27 (13.2)	0
Pneumonitis	5 (21.7)	0	19 (9.3)	0
Interstitial lung disease	2 (8.7)	0	4 (2.0)	0
Lung infiltration	0	0	5 (2.5)	0
Pulmonary fibrosis	0	0	1 (0.5)	0

CT, computed tomography.
[a]Number of patients with any evidence of pneumonitis at any post-baseline assessment regardless of the baseline status.
[b]Number of patients with newly occurring or worsened pneumonitis compared with baseline.

PTEN mutations have been shown to be particularly sensitive to treatment with mTOR inhibitors (19).

Safety evaluations found everolimus to be generally well tolerated in this Japanese subgroup; no new safety concerns were observed. Select ADRs including epistaxis (44 vs 17%), pneumonitis (44 vs 17%), dysgeusia (35 vs 17%), anaemia (30 vs 17%) and headache (30 vs 19%) tended to occur with greater frequency in the everolimus group of the Japanese subgroup than in the overall population, respectively (24). The higher incidence of ADRs in Japanese patients is attributable to careful monitoring and conservative assessments by Japanese investigators. Incidence of grade 3/4 ADRs in the Japanese subgroup was comparable to that in the overall population, respectively, for select events, including anaemia (9 vs 6%), hyperglycaemia (4 vs 5%) and diabetes mellitus (4 vs 2%) (24). Stomatitis, which affected 7% of patients, was the most commonly occurring grade 3/4 ADR in the everolimus group in the overall population; none of the cases of stomatitis in the Japanese subgroup was rated as grade 3/4. Neutropenia, which affected 17% of patients in the everolimus group compared with 3% of patients in the overall population, was the most commonly occurring grade 3/4 ADR in the Japanese subgroup.

The frequency of interstitial lung disease (ILD)-type events reported as 'adverse events' in the investigator's opinion appeared to be higher in Japanese patients than in the overall population. However, a central radiology review found similar proportions of radiologic findings compatible with pneumonitis in Japanese and non-Japanese patients. Moreover, the presence of the characteristic symptoms associated with ILD, such as cough (4.3% in Japanese patients vs 23.8% in non-Japanese patients) and dyspnoea (13.0% in Japanese patients vs 17.1% in non-Japanese patients), was less common in Japanese than in non-Japanese patients. The higher report in Japanese patients may be explained by Japanese investigators' greater awareness of the disease , which could lead to more frequent radiologic assessments and diagnoses.

In conclusion, in this subgroup analysis of Japanese patients, everolimus demonstrated a clinically meaningful improvement in PFS over placebo and was generally well tolerated, as demonstrated in the larger RADIANT-3 trial (24). These results support the use of everolimus as a valuable treatment option for Japanese patients with advanced pNET.

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Conflict of interest statement

Takeshi Tajima, Akio Kasuga and Yoshie Fujita are employed by Novartis Pharma K.K. Junji Furuse received consulting fees from Bayer, Chugai, Eisai, Eli Lilly, Taiho, Pfizer and Novartis and received honoraria from Bayer, Chugai, Eisai, Eli Lilly, Taiho, Pfizer and Novartis; Takuji Okusaka received honoraria from Novartis; Chigusa Morizane received honoraria from Novartis; Masafumi Ikeda received honoraria from Bayer Yakuhin Ltd. and Dainippon Sumitomo Pharma; Tetsuhide Ito, Hisato Igarashi and Kohei Nakachi declare that they have no conflict of interest.

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Phase I study of TAC-101, an oral synthetic retinoid, in Japanese patients with advanced hepatocellular carcinoma

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Preclinical models have shown that TAC-101 (4-[3,5-bis(trimethylsilyl) benzamide] benzoic acid), an oral synthetic retinoid, has antitumor activity in hepatocellular carcinoma (HCC). We conducted a phase I study in Japanese patients with advanced HCC to examine the pharmacokinetics, recommended dose, safety, and efficacy of TAC-101. The administered dose of TAC-101 was 10 mg/day in four patients (level 1), 20 mg/day in six (level 2), and 30 mg/day in three (level 3). There was no dose-limiting toxicity at level 1. Only one patient each had dose-limiting toxicity at level 2 (grade 2 fatigue, recovery requiring eight or more consecutive days of rest) and at level 3 (grade 3 splenic vein thrombosis). Level 3 (30 mg/day) was considered the maximum tolerated dose and 20 mg/day the recommended dose by a panel of medical experts, placing maximum emphasis on safety. The most frequent adverse events were fatigue, headache, and dermal symptoms such as rash. Pharmacokinetic parameters in Japanese patients with HCC were similar to those in patients in the United States, most of whom were Caucasian. Although no patient had a complete or partial response, the disease control rate was 38.5%. In conclusion, the recommended dose of TAC-101 for patients with HCC is 20 mg/day. TAC-101 had an acceptable toxicity profile, warranting further evaluation in clinical trials. (*Cancer Sci* 2012; 103: 1524–1530)

Hepatocellular carcinoma (HCC) is one of the most common cancers in the world. Outcomes remain poor because disease is usually advanced at diagnosis and associated with hepatic impairment and a high rate of recurrence, resulting from either intrahepatic metastases from the primary tumor or multicentric lesions. Surgical resection, liver transplantation, radiofrequency ablation (RFA) or percutaneous ethanol injection (PEI) are the mainstays of treatment in patients with potentially curable disease. Transcatheter arterial chemoembolization (TACE) is the procedure of choice for noncurative HCC. Currently marketed systemic chemotherapeutic agents, with the exception of sorafenib, provide only marginal benefits.[1–3] Despite the survival benefit demonstrated for sorafenib, more effective systemic therapy for HCC is required.

TAC-101 (4-[3,5-bis(trimethylsilyl) benzamido] benzoic acid) is an orally absorbed synthetic retinoid. This analogue of vitamin A (retinol) binds to nuclear retinoic acid receptor-α (RAR-α), activates RAR-α transcriptional activity, and has shown antitumor activity in primary and metastatic preclinical models of liver cancer.[4,5] TAC-101 inhibits tumor growth in the liver with low toxicity and markedly improves survival in both primary HCC and metastatic colon cancer models.[6]

In the United States, an initial dose-escalation study was performed in patients with advanced cancer. TAC-101 was orally administered daily, without a rest period. The most frequent toxicities were skin and mucosal membrane disorders, myalgia/arthralgia, fatigue, and triglyceridemia. Dose-limiting toxicities (DLT) occurring during the first 28 days of treatment (cycle 1) were fatigue, arthralgia/joint pain, myalgia, and venous thromboembolism (VTE). VTE developed in nine of 29 patients as a characteristic adverse reaction of TAC-101; the dose ranged from 12 to 34 mg/m^2.[7] In a phase I/II study, TAC-101 was administered orally in 21-day cycles (14 days on/7 days off) to patients with advanced HCC. In the phase I portion of the study, the initial dose was 40 mg/day. Two patients had DLT, and the dose was reduced to 20 mg/day. Since only 1 of 6 assessable patients had DLT during the first two cycles of therapy, 20 mg/day was designated as the maximum tolerated dose (MTD) for the 21-day treatment cycle. At this dose level, TAC-101 was generally well tolerated, and the most common drug-related adverse events were increased blood triglyceride levels, fatigue, dermatitis, pruritus, nausea, dry skin, myalgias, dry mouth, arthralgias, anorexia, diarrhea, and headache. Among 21 evaluable patients, no patient had a complete response (CR) or partial response (PR), but 12 (57%) had stable disease (SD). Median progression free survival (PFS) and overall survival (OS) were 3.4 months and 19.2 months, respectively.[8]

We report the results of the first phase I study of TAC-101 in Japanese patients with HCC. Our major goals were to evaluate safe dose levels, tolerability, pharmacokinetics, and efficacy.

Materials and Methods

Eligibility. Eligible patients had pathologically or clinically proved advanced HCC that was not amenable to standard treatments. A hypervascular mass on diagnostic imaging was considered a sufficient non-invasive diagnostic criterion for HCC. At least one measurable lesion on CT or MRI (not including necrotic lesions caused by prior treatment) was required. Other eligibility criteria included an age of 20 to 75 years; an Eastern Cooperative Oncology Group (ECOG) performance status (PS) of 0 to 2; an estimated life expectancy of at least 60 days; adequate hematologic function (white blood cell [WBC] $\geq$3000/mm^3, hemoglobin $\geq$8.0 g/dL, platelets $\geq 5.0 \times 10^4$/mm^3); adequate hepatic function (aspartate aminotransferase [AST] and alanine aminotransferase [ALT] $\leq$5 times the upper limit of normal [ULN], total bilirubin $\leq$2.0 mg/dL, serum albumin $\geq$2.8 g/dL, and prothrombin activity $\geq$40%); adequate renal function (serum creatinine

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Clinical trial registration: This trial was not registered in the clinical trial database because it was an early phase trial and not a controlled study.

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Table 1. Summary of patient background characteristics

Background characteristics		10 mg/day	20 mg/day	30 mg/day	Total (%)
No. eligible patients		*n* = 4	*n* = 6	*n* = 3	*n* = 13
Gender	Male	3	5	3	11 (84.6)
	Female	1	1	0	2 (15.4)
Age (years)	<65	2	0	2	4 (30.8)
	≥65	2	6	1	9 (69.2)
	Median	64	72	60	70
	Min., Max.	59, 70	66, 74	45, 70	45, 74
ECOG, PS	0	4	5	3	12 (92.3)
	1	0	1	0	1 (7.7)
Stage*	Stage I	0	0	0	0 (0.0)
	Stage II	1	1	0	2 (15.4)
	Stage III	2	4	2	8 (61.5)
	Stage IVA	0	0	0	0 (0.0)
	Stage IVB	1	1	1	3 (23.1)
Child–Pugh classification	A	3	2	3	8 (61.5)
	B	1	4	0	5 (38.5)
	C	0	0	0	0 (0.0)
Grade of histological differentiation	Well differentiated	2	1	0	3 (23.1)
	Moderately differentiated	0	3	1	4 (30.8)
	Poorly differentiated	0	0	1	1 (7.7)
	Unknown	2	2	1	5 (38.5)
Extrahepatic metastasis	No	3	5	2	10 (76.9)
	Yes	1	1	1	3 (23.1)
History of hepatectomy	No	1	3	2	6 (46.2)
	Yes	3	3	1	7 (53.8)
History of nonsurgical therapy	No	0	0	0	0 (0.0)
	Yes	4	6	3	13 (100.0)

*According to the staging system of the Liver Cancer Study Group of Japan (4th edition). ECOG, PS Eastern Cooperative Oncology Group performance status.

≤1.5 times the ULN); and a Child–Pugh class of A or B. Resection was permitted if the procedure had been performed at least 180 days before registration in the study. Other prior treatments for HCC were permitted if such treatment had been performed at least 30 days before registration. Patients were excluded if they had tumors involving more than 50% of the liver; brain or bone metastases or vascular invasion of the main trunk and first branch(es) of the portal vein, the main trunk of the left/middle/right hepatic veins, the inferior right hepatic vein, the short hepatic veins, or the inferior vena cava; severe complications; other malignancies; or inability to comply with the protocol requirements. Patients were also excluded if they had a history of VTE. Patients who were receiving anticoagulants or hormone replacement therapy were excluded. Written informed consent was obtained from each patient. The study was approved by the local institutional review boards of the National Cancer Center Hospital, Japan.

Study design and treatment plan. TAC-101 was supplied by Taiho Pharmaceutical Co., Ltd. (Tokyo, Japan). This study evaluated the pharmacokinetics of TAC-101 and established the MTD for two courses of treatment. Patients received the assigned dose of TAC-101 once daily (after breakfast) for 14 consecutive days, followed by a 7-day rest (a 21-day treatment course). If grade 3 or higher hematologic toxicity or grade 2 or higher nonhematologic toxicity occurred, the dose of TAC-101 could be reduced (minimum dose, 10 mg/day). If DLT occurred, treatment with TAC-101 could be temporarily suspended. Treatment was continued until evidence of disease progression. Treatment was terminated if recovery from an adverse event required more than 21 days, if the patient requested treatment to be discontinued, or if unacceptable toxicity developed in the opinion of the investigator.

The starting dose of TAC-101 (level 1) was 10 mg/day, level 2 was 20 mg/day, level 3 was 30 mg/day, level 4 was 40 mg/day, level 5 was 50 mg/day, and level 6 was 60 mg/day. Patients were enrolled in cohorts of three for each dose level. The dose was escalated according to cohort and was not increased in the same patient. If none of the first three patients had DLT during the first two cycles of therapy, the dose was increased to the next dose level. If one or two of the first three patients had DLT, three additional patients were assigned to the same dose level; if only one or two of the first six patients had DLT, the dose was increased to the next dose level; if all of the first three patients or three or more of the first six patients had DLT, the dose was defined as the MTD; the recommended dose (RD) was defined as the level one step below the MTD. A total of six patients received the RD to confirm the safety profile. DLT was defined as any of the following: (i) hematologic toxicity ≥ Grade 4; (ii) nonhematologic toxicity ≥ Grade 3; (iii) AST, ALT ≥ 10 times the ULN; or (iv) a rest period of eight or more consecutive days was required.

Pharmacokinetics. Blood samples for pharmacokinetic analysis were collected before and 2, 4, 6, 8, 10 to 12, and 24 h after administration of TAC-101 on day 1 and after repeated treatment (days 8–13) during the first cycle (approximately 4 mL for each time point). The blood samples were centrifuged, and the resulting plasma samples were stored at −20°C until analysis. Spontaneously voided urine was collected before treatment on day 1 (baseline urine), from 0 to 8 h after treatment (0–8 h pooled urine), and from 8 to 24 h after treatment (8–24 h pooled urine). The urine samples were stored at −20°C until analysis.

Plasma concentrations of TAC-101 were measured using a validated method. The analyte was extracted with *tert*-butyl methyl ether and analyzed by liquid chromatography/tandem mass spectrometry (LC/MS/MS; Waters 2690/Finnigan MAT TSQ7000) with negative ion-electrospray ionization mode, using deuterium-labeled TAC-101 as an internal standard.

Pharmacokinetic parameters were calculated from the plasma concentrations of TAC-101 by non-compartmental analysis using WinNonlin software, version 4.1 (Pharsight, Cary, NC, USA).

For both plasma and urine samples, the metabolites of TAC-101 were preliminarily analyzed using an ultraviolet detector-equipped, high-performance liquid chromatograph and LC/MS/MS (Agilent 1100 series/Applied Biosystems API 4000, Carlsbad, CA, USA). The metabolites TAC-101-M-1, TAC-101-M-2, and TAC-101-M-3 were identified by comparison with authentic samples synthesized by Taiho Pharmaceuticals (Tokyo, Japan). The structures of conjugates were estimated by analyzing the mass fragmentation spectra. Concentrations of TAC-101-M-1 and TAC-101-M-2 were determined using an LC/MS/MS method similar to that used for the assay of TAC-101.

Assessment of efficacy and toxicity. All eligible patients who received at least one dose of the study drug were included in the evaluations of response and toxicity. The criteria of the Japan Society of Clinical Oncology, which closely resemble the World Health Organization criteria, were used to evaluate unblended radiographic tumor responses at the study site. Computed tomography or MRI was used to evaluate measurable disease; the same imaging modality was used at baseline and follow-up. The efficacy endpoints were the overall response rate, the duration of antitumor effect, OS, time to progression (TTP), and time to treatment failure (TTF). Vital signs, physical findings, and the results of hematological and biochemical testing, including thrombosis panel and urine analyses, were assessed at 2-week intervals during treatment and after the 7-day recovery period. The severities of all adverse events were evaluated according to the National Cancer Institute Common Toxicity Criteria, version 2.0 (NCI-CTC Ver. 2.0). The durations of all adverse events and their relations to TAC-101 were initially assessed by the attending physicians. Subsequently, an independent review committee reassessed data on adverse events and evaluated the radiologic tumor responses in a blinded manner using Response Evaluation Criteria in Solid Tumors (RECIST).[9]

Statistical considerations. All data were summarized using descriptive statistics for continuous variables and frequencies and percentages for discrete variables. Median times to events were estimated using the Kaplan–Meier method.

Results

Patient characteristics and treatment. Between October 2003 and May 2005, a total of 13 patients were enrolled at a single site in Japan. All patients were eligible for the evaluation of toxicity and efficacy. The first four patients received dose level 1 (10 mg/day), the next six patients received dose level 2 (20 mg/day), and the last three patients received dose level 3 (30 mg/day). The characteristics of the patients are summarized in Table 1. At study entry, three (23.1%) of the 13 patients had metastatic disease. All 13 patients had received some prior treatment, including one previously given systemic chemotherapy with the oral fluoropyrimidine tegafur-uracil (UFT).

Dose-limiting toxicity and recommended dose. One of the first three patients assigned to level 1 (10 mg/day) discontinued the study medication before completing two courses of treatment because of non-drug-related serious adverse events mentioned in the section of adverse events. Therefore, another patient was assigned to this level, and safety was evaluated in a total of four patients. Because no DLT occurred at this level, the dose was increased to level 2 (20 mg/day). One of the first three patients given level 2 had DLT, and three patients were additionally assigned to this dose level; safety was thus

Table 2. Drug-related adverse events with incidence ≥20% or grade 3–4

Drug-related adverse event	10 mg/day (n = 4)		20 mg/day (n = 6)		30 mg/day (n = 3)		Child–Pugh A (n = 8)		Child–Pugh B (n = 5)		Total (n = 13)	
	All grade (%)	Grade ≥ 3 (%)	All grade (%)	Grade ≥ 3 (%)	All grade (%)	Grade ≥ 3 (%)	All grade (%)	Grade ≥ 3 (%)	All grade (%)	Grade ≥ 3 (%)	All grade (%)	Grade ≥ 3 (%)
Splenic vein thrombosis	0 (0)	0 (0)	0 (0)	0 (0)	1 (33)	1 (33)	1 (13)	1 (13)	0 (0)	0 (0)	1 (8)	1 (8)
Headache	4 (100)	0 (0)	2 (33)	0 (0)	1 (33)	0 (0)	5 (63)	0 (0)	2 (40)	0 (0)	7 (54)	0 (0)
Cough	1 (25)	0 (0)	5 (83)	0 (0)	0 (0)	0 (0)	2 (25)	0 (0)	4 (80)	0 (0)	6 (46)	0 (0)
Rhinorrhea	1 (25)	0 (0)	3 (50)	0 (0)	0 (0)	0 (0)	1 (13)	0 (0)	3 (60)	0 (0)	4 (31)	0 (0)
Alopecia	1 (25)	0 (0)	2 (33)	0 (0)	1 (33)	0 (0)	2 (25)	0 (0)	2 (40)	0 (0)	4 (31)	0 (0)
Eczema	1 (25)	0 (0)	3 (50)	0 (0)	0 (0)	0 (0)	2 (25)	0 (0)	2 (40)	0 (0)	4 (31)	0 (0)
Rash	3 (75)	0 (0)	1 (17)	0 (0)	3 (100)	0 (0)	6 (75)	0 (0)	1 (20)	0 (0)	7 (54)	0 (0)
Arthralgia	1 (25)	0 (0)	3 (50)	0 (0)	1 (33)	0 (0)	3 (38)	0 (0)	2 (40)	0 (0)	5 (39)	0 (0)
Myalgia	2 (50)	0 (0)	0 (0)	0 (0)	1 (33)	0 (0)	3 (38)	0 (0)	0 (0)	0 (0)	3 (23)	0 (0)
Fatigue	1 (25)	0 (0)	4 (67)	0 (0)	1 (33)	0 (0)	3 (38)	0 (0)	3 (60)	0 (0)	6 (46)	0 (0)
Blood cholesterol increased	1 (25)	0 (0)	0 (0)	0 (0)	3 (100)	0 (0)	4 (50)	0 (0)	0 (0)	0 (0)	4 (31)	0 (0)
Blood lactate dehydrogenase increased	1 (25)	0 (0)	1 (17)	0 (0)	3 (100)	0 (0)	4 (50)	0 (0)	1 (20)	0 (0)	5 (39)	0 (0)
Blood triglycerides increased	3 (75)	0 (0)	4 (67)	0 (0)	3 (100)	0 (0)	7 (88)	0 (0)	3 (60)	0 (0)	10 (77)	0 (0)
Fibrin D dimer increased	2 (50)	0 (0)	6 (100)	0 (0)	3 (100)	0 (0)	6 (75)	0 (0)	5 (100)	0 (0)	11 (85)	0 (0)
Thrombin-antithrombin III complex increased	1 (25)	0 (0)	5 (83)	0 (0)	3 (100)	0 (0)	6 (75)	0 (0)	3 (60)	0 (0)	9 (69)	0 (0)
Blood alkaline phosphatase increased	2 (50)	0 (0)	1 (17)	0 (0)	1 (33)	0 (0)	3 (38)	0 (0)	1 (20)	0 (0)	4 (31)	0 (0)

The worst grade was used to calculate the incidence according to grade.

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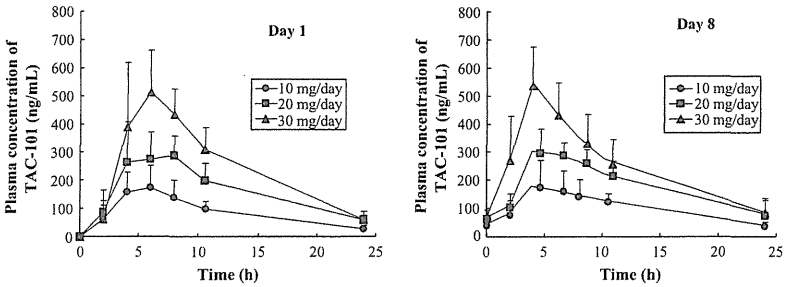

Fig. 1. Plasma concentration-time profile of TAC-101 (4-[3,5-bis(trimethylsilyl) benzamide] benzoic acid) in patients with hepatocellular carcinoma.

Table 3. Pharmacokinetic values of TAC-101 in patients with hepatocellular carcinoma

Blood sampling day	PK parameter	10 mg/day			20 mg/day			30 mg/day		
		No. patients	Mean	SD	No. patients	Mean	SD	No. patients	Mean	SD
Day 1	C_{max} (ng/mL)	4	189.2	85.5	6	333.0	88.6	3	512.1	149.5
	t_{max} (h)	4	5.5	1.0	6	5.7	2.0	3	6.0	0.0
	AUC_{0-24} (ng h/mL)	4	2084	640	6	3900	1140	3	5780	1489
	AUC_{inf} (ng h/mL)	3	2526	497	4	4680	1657	3	6261	1596
	$t_{1/2}$ (h)	3	5.54	0.75	4	6.92	1.67	3	5.57	0.57
	Vd/F (L)	3	32.2	5.6	4	45.5	12.7	3	39.9	9.6
	CL/F (L/h)	3	4.08	0.89	4	4.83	2.15	3	4.98	1.11
Day 8	C_{max} (ng/mL)	4	207.7	71.0	6	326.0	66.9	3	543.9	138.9
	t_{max} (h)	4	6.0	2.9	6	5.7	2.0	3	4.0	0.0
	AUC_{0-24} (ng h/mL)	4	2401	630	6	4191	1063	3	6099	1772
	AUC_{inf} (ng h/mL)	3	2906	755	4	4674	820	3	7257	2410
	$t_{1/2}$ (h)	3	6.47	0.92	4	7.50	0.97	3	8.16	3.59
	Vd/F (L)	3	32.9	5.1	4	46.9	6.4	3	49.3	13.9
	CL/F (L/h)	3	3.62	1.05	4	4.39	0.81	3	4.55	1.87

AUC_{inf}, area under the plasma concentration-time curve up to infinity; AUC_{0-24}, area under the plasma concentration-time curve up to 24 h post-dose; CL/F, oral clearance; C_{max}, maximum plasma concentration; SD, standard deviation; t_{max}, time of maximum concentration; $t_{1/2}$, elimination half-life; Vd/F, apparent volume of distribution.

assessed in a total of six patients. The DLT was grade 2 fatigue requiring eight or more consecutive days of rest for recovery. Because no other patient had DLT, the dose level was increased to level 3 (30 mg/day). Splenic vein thrombosis, a grade 3 drug-related adverse event, occurred in one patient at this level. Although only one patient given 30 mg/day of the study drug had DLT, this dose level was designated as the MTD and 20 mg/day as the RD by a panel of medical experts on an independent monitoring committee, who considered the thromboembolic event to be of great importance on the basis of the results of studies conducted in the United States, in which the event developed in nine of 29 patients and was potentially fatal.

Treatment delivered. Four patients received a total of 14 cycles of treatment at 10 mg/day (median, three cycles per patient; range, 1–7). Six patients received a total of 21 cycles of treatment at 20 mg/day (median, three cycles per patient; range, 2–8). Three patients received a total of seven cycles of treatment at 30 mg/day (median, three cycles per patient; range, 2–3). The dose of TAC-101 was not reduced in any patient. The reasons for terminating treatment were progressive disease in nine patients (69.2%), adverse events in two (15.4%), and other reasons in two (15.4%; one required 21 or more consecutive days of rest, and one withdrew consent).

Adverse events. Drug-related adverse events occurring in the 13 patients are shown in Table 2. Treatment with TAC-101 was generally well tolerated throughout the study. Grade 3 or 4 toxicity (splenic vein thrombosis) occurred in only one patient, who received 30 mg/day of TAC-101. The patient was a 70-year-old, HCV-positive man with Child–Pugh A liver cirrhosis, hypersplenism, and hypertension. He had multiple tumors smaller than 3 cm in diameter in the liver, without vascular invasion or extrahepatic metastasis. Splenic vein thrombosis was noted during a routine restaging CT scan of the target lesion at the end of the third course of therapy. The patient received aspirin, and the thrombosis was considered resolved 85 days after the initiation of treatment with aspirin. The most common toxic effects were fibrin D dimer increased (84.6%), blood triglycerides increased (76.9%), thrombin-antithrombin III complex increased (69.2%), headache and rash (53.8%). Serious adverse events were anorexia, hepatic encephalopathy, renal disorder, aspiration pneumonia, and sepsis in one patient who received 10 mg/day. These events were considered unrelated to the study medication. As for differences in drug-related adverse events between the Child–Pugh A and B groups, the incidences of some events were at least 20 percentage points higher in the Child–Pugh B group than in the Child–Pugh A group, such as cough (25% *vs* 80%), rhinorrhea (13% *vs* 60%), fatigue (38% *vs* 60%), and fibrin D dimer increased (75% *vs* 100%). However, the incidences and severities of most other events were similar in the two groups.

Efficacy. Response could be evaluated in all 13 patients. No patient had a CR or PR. A total of nine patients (69.2%, 9/13) had no change (NC): two of four patients at 10 mg/day, five of six at 20 mg/day, and two of three at 30 mg/day. Four

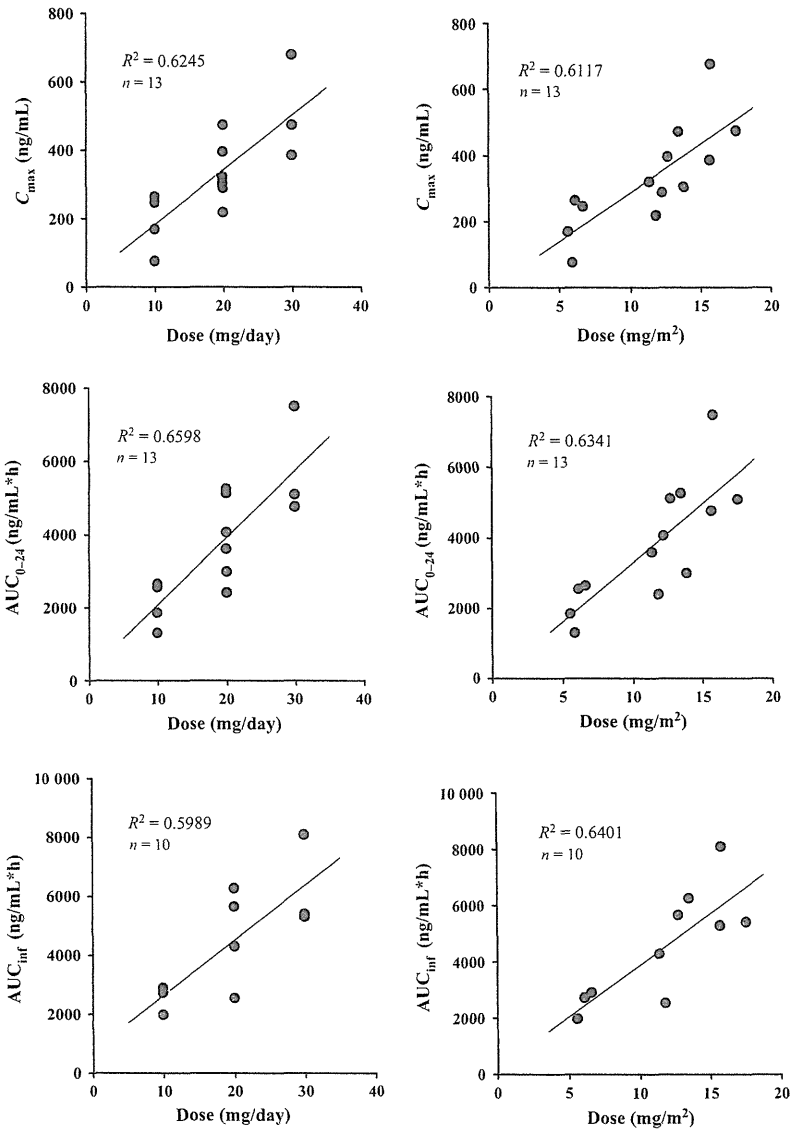

Fig. 2. Relation between TAC-101 (4-[3,5-bis(trimethylsilyl) benzamide] benzoic acid) dose and C_{max} or AUC in patients with hepatocellular carcinoma on day 1 (single dose). The x axes of the left- and right-hand figures represent the dose per day and dose per m^2, respectively.

patients (30.8%, 4/13) had progressive disease (PD): two of four at 10 mg/day, one of six at 20 mg/day, and one of three at 30 mg/day. No change was maintained for 8 weeks (56 days) or longer (long-term NC) in five patients, and the disease control rate (CR + PR + long-term NC) was thus 38.5% (5/13 patients). Median TTF was 60.0 days (95% CI, 46.0-65.0). Median TTP and OS were 86.0 days (95% CI, 58.0 –146.0) and 427.0 days (95% CI, 369.0-unknown), respectively. When response was evaluated according to RECIST, none of the 13 patients had a PR or CR, 7 (53.8%) had SD, and five (38.5%) had PD.

Pharmacokinetic analysis. Mean plasma concentration-time profiles after administration of TAC-101 on day 1 and on day 8 are shown in Figure 1. The calculated pharmacokinetic parameters of TAC-101 are summarized in Table 3. TAC-101 concentrations in plasma reached peak values approximately 6 h after administration and declined with a half-life ($t_{1/2}$) of 5 –8 h. Consistent with the relatively short $t_{1/2}$, increased plasma concentrations after multiple doses of TAC-101 once daily were not apparent. The relations between the dose of TAC-101 and the maximum plasma concentration (C_{max}), area under the plasma concentration-time curve up to 24 h post-dose (AUC_{0-24}), and area under the plasma concentration-time curve up to infinity (AUC_{inf}) after a single dose (day 1) are shown in Figure 2. These variables generally increased proportionally to the dose of TAC-101 (10–30 mg/day). The relation between the dose of TAC-101 and pharmacokinetic parameters was generally unchanged after adjusting the dose according to individual body surface area. The $t_{1/2}$ of 5.54 to 6.92 h, the apparent volume of distribution (Vd/F) of 32.2 to 45.5 L, and the oral clearance (CL/F) of 4.08 to 4.98 L/h after a single dose of TAC-101 did not differ among the dose levels. All pharmacokinetic parameters were generally similar after a single dose and after repeated doses of TAC-101, suggesting that repeated treatment was not

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associated with changes in TAC-101 metabolism or with drug accumulation.

In this clinical study, a total five patients with Child–Pugh class B disease were enrolled, but the oral clearance of TAC-101 was available for only three of the five patients at the dose level of 20 mg/day. The calculated oral clearance of TAC-101 ranged from 3.44 to 5.67 L/h in patients with Child–Pugh class A disease (n = 7, 10–30 mg/day) and from 3.19 to 7.92 L/h in those with Child–Pugh class B disease (n = 3, 20 mg/day). Although there was no apparent difference in oral clearance between the two groups, firm conclusions were precluded by the limited number of patients in this study.

The pooled plasma and urine samples were analyzed to characterize the metabolites of TAC-101. After a single dose of TAC-101, the hydroxylated metabolites TAC-101-M-1 and TAC-101-M-2 were simultaneously detected in plasma samples along with parent TAC-101. The concentrations of TAC-101-M-1 and TAC-101-M-2 in plasma as determined by LC/MS/MS were approximately 16% and 11% of the concentration of unchanged TAC-101 6 h after treatment and approximately 23% and 16% of the concentration of unchanged TAC-101 10.6 h after treatment (mean sampling time; range, 10–12 h), respectively. The respective percentages on day 8 were comparable to those after the initial dose. These results indicate that the majority of absorbed TAC-101 circulates in the body as a parent drug, with minor proportions of metabolites. In urine samples, the hydroxylated metabolite TAC-101-M-3 and the glucuronide conjugates of TAC-101-M-1 and TAC-101-M-2 were detected. The parent drug TAC-101 was not detected in urine, suggesting that hepatic metabolism is the major elimination pathway of TAC-101, which underwent hydroxylation or glucuronide conjugation, followed by partial excretion of metabolites into urine. Based on these exploratory analyses of human plasma and urine, the metabolic pathways of TAC-101 were determined as shown in Figure 3.

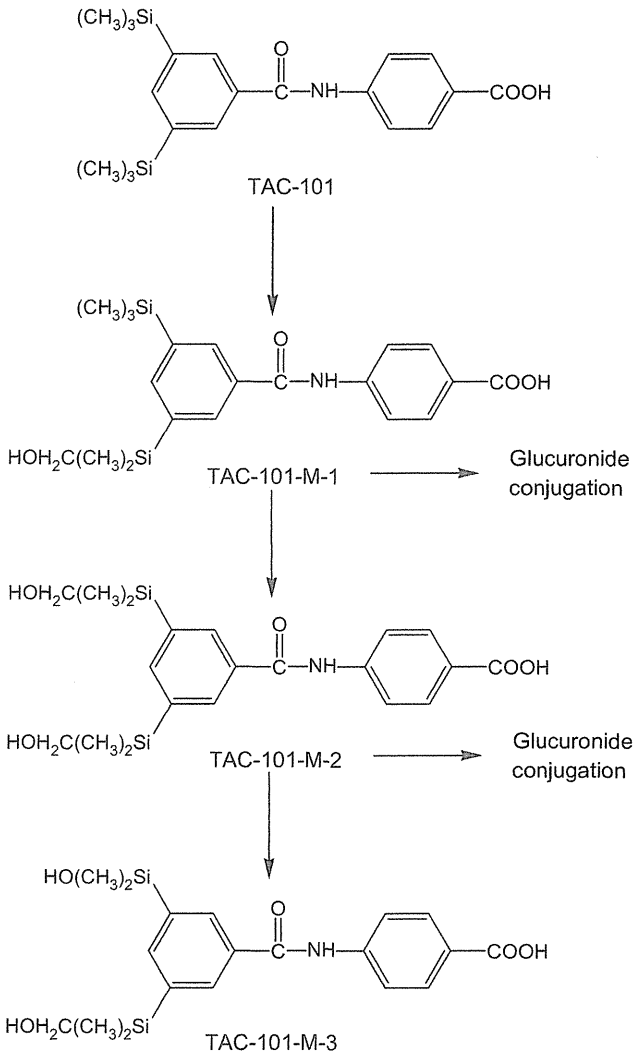

Fig. 3. Metabolic pathways of TAC-101 (4-[3,5-bis(trimethylsilyl) benzamide] benzoic acid) and its metabolites.

Discussion

In one patient given 30 mg/day, CT revealed splenic vein thrombosis, which was considered DLT. Although DLT developed in only one patient receiving 30 mg/day of TAC-101, this dose level was judged to be the MTD by a panel of medical experts who placed maximum emphasis on safety. Mechanisms that potentially trigger thromboembolic events have been studied, but the role of TAC-101 in such events remains unclear.

The most common treatment-related adverse events were fatigue, headache, and dermal symptoms such as rash. However, most adverse events were mild (grade 1 or 2), confirming that TAC-101 is well tolerated at the recommended dose of 20 mg/day. DLT comprised grade 2 fatigue in one patient given 20 mg/day and grade 3 splenic vein thrombosis in one patient given 30 mg/day. The latter was the only grade 3 drug-related adverse event. There were no treatment-related adverse events of grade 3 or higher in patients given 10 or 20 mg/day. In general, the toxic effects of TAC-101 were consistent with those of previous studies.[7,8]

Higginbotham *et al.* reported the results of pharmacokinetic studies of TAC-101 in patients with advanced hepatocellular carcinoma treated in the United States.[8] The mean pharmacokinetic parameters (t_{max}, 4.3 h; C_{max}, 242 ng/mL; AUC_{0-24}, 3067.6 ng h/mL; AUC_{inf}, 4241.1 ng h/mL) obtained for a dose of 20 mg were generally consistent with our data in Japanese patients. The slightly lower C_{max} and AUCs values in the American study might be attributed to general differences in body size between Caucasians and Japanese.

Both maximum (C_{max}) and overall exposures (AUCs) to TAC-101 were generally dose-related within the range of 10 to 30 mg/day. The parent compound TAC-101 was not excreted into urine, suggesting that hepatic metabolism is the major elimination pathway of TAC-101. The primary metabolism of TAC-101 was characterized by hydroxylation of the trimethylsilyl group, producing some primary metabolites, which then underwent glucuronide conjugation. However, the concentrations of the metabolites in plasma were low, suggesting that the biologic activity of TAC-101 is generally attributed to systemic exposure to unchanged TAC-101.

On the basis of the pharmacokinetic and safety profiles of TAC-101 in this study, 20 mg/day, the dose one level below the MTD, was determined to be RD. This dose is the same as the RD in the United States.

As for antitumor effect, no patient had a CR or PR, but nine had NC (69.2%, 9/13 subjects). The median TTF was 60.0 days, the median TTP was 86.0 days, and the MST was 427.0 days. The results for efficacy in this study were also similar to those in the study performed in the United States.[8] Unfortunately, tumor shrinkage was not evident, and the median TTP seemed to be unfavorable on the basis of MST. However, we believe that further evaluations are warranted,

because the mechanisms of action of TAC-101 and other retinoids are considered cytostatic as opposed to cytotoxic, and the TTP in this study may be comparable to those in studies of sorafenib (2.8–5.5 months).[2,3]

In conclusion, our results suggest that TAC-101 is well tolerated at an oral dose of 20 mg/day (dose level 2). This dose, given once daily after breakfast for 14 consecutive days followed by a 7-day rest period, was determined to be the RD for HCC. Additional studies of TAC-101 as a single agent as well as in combination with molecular-targeted agents such as sorafenib are warranted to further delineate potential clinical benefits and risks.

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Disclosure Statement

The authors have no conflict of interest.

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Neural invasion induces cachexia *via* astrocytic activation of neural route in pancreatic cancer

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Pancreatic cancer is characterized by a high frequency of cachexia, pain and neural invasion (N-inv). Neural damage is occurred by N-inv and modulates pain and muscle atrophy *via* the activation of astrocyte in the connected spine. The activated astrocyte by N-inv, thus, may affect cachexia in pancreatic cancer. Clinical studies in patients and autopsy cases with pancreatic cancer have revealed that N-inv is related to cachexia and astrocytic activation. We established a novel murine model of cancer cachexia using N-inv of human pancreatic cancer cells. Mice with N-inv showed a loss of body weight, skeletal muscle and fat mass without appetite loss, which are compatible with an animal model of cancer cachexia. Activation of astrocytes in the spinal cord connected with N-inv was observed in our model. Experimental cachexia was suppressed by disrupting neural routes or inhibiting the activation of astrocytes. These data provide the first evidence that N-inv induces cachexia *via* astrocytic activation of neural route in pancreatic cancer.

Key words: animal model, neural invasion, pancreatic cancer, glial activation, cachexia

Abbreviations: ATCC: American Type Culture Collection; BMI: body mass index; BW: body weight; cAMP: cyclic adenosine-5',3'-monophosphate; CeA: celiac artery; CNS: central nervous system; CRP: C-reactive protein; CT: computed tomography; Fbxo: F-box protein; GFAP: glial fibrillary acidic protein; HE: hematoxylin and eosin; HPLC: high performance liquid chromatography; i.p.: intraperitoneal; Iba1: ionized calcium binding adaptor molecule 1; IHC: immunohistochemistry; L1: the first lumbar spinal cord; LFB: Luxol Fast Blue; N-inv: neural invasion; PBS: phosphate buffer saline; PDE: phosphodiesterase; PDYN: prodynorphin; PPF: propentofylline; PVST: perivascular soft tissue; SAA: serum amyloid A; SC: subcutaneous tumor; SCID: severe combined immunodeficiency; SMA: superior mesenteric artery; Th13: the 13th thoracic spinal cord; UCP: uncoupling protein

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Cachexia is a progressive loss of body weight (BW) mainly caused by the loss of adipose tissue and skeletal muscle mass.[1] BW loss is prevalent in patients with pancreatic cancer (75–85%).[2,3] Metastasis, which is seen in 40–45% of patients with pancreatic cancer,[4] is related to BW loss.[5] The degree of BW loss varies according to the tumor origin, size, type and burden, and the number of metastases.[2,5,6] Neural invasion (N-inv) is a common invasive behavior of pancreatic cancer.[7] The degree of N-inv was associated with cachexia in our previous clinicopathological study[8] and may be a novel cause of cachexia.

Intraneural tumors of pancreatic cancer injure nerve elements[9] and cause pain.[10] Damage to peripheral nerves activates astrocytes and microglia in the connected spinal cord, a phenomenon also known as spinal glial activation.[11,12] The morphological features of activated astrocytes are thickened branches and hypertrophy.[13,14] mRNA for the astrocyte marker glial fibrillary acidic protein (GFAP) is upregulated in activated astrocytes.[15] Glial activation in the central nervous system (CNS) is thought to cause physiological abnormalities, such as allodynia in pancreatitis[16] and muscle atrophy in neurodegenerative disease.[17,18] Neural damage by N-inv may cause systemic abnormalities *via* spinal cord glial activation, thus mediating cachexia in pancreatic cancer (Fig. 1). Here, we tested this hypothesis using autopsy cases, clinical data and an experimental N-inv mouse model of pancreatic cancer.

Clinical N-inv of pancreatic cancer is assessed by the degree of the perivascular soft tissue (PVST) around the superior mesenteric artery (SMA) or the celiac artery (CeA) with dynamic helical computed tomography (CT).[19] Our hypothesis