liver or the spleen. In marked contrast, all IL-12, INF-γ and TNF-α production from liver DCs derived from α-GalCer-treated mice were significantly higher than those from spleen DCs (Fig. 4A–C). To investigate the difference of the antigen-presenting function between liver DCs and spleen DCs, we examined the allostimulatory capacity of liver and spleen DCs using a mixed lymphocyte reaction (MLR). Liver DCs from α-GalCer-treated mice showed higher T cell proliferation ability than those from vehicle-treated or non-treated mice and spleen DCs from all treatment groups. Spleen DCs from all treatment groups and liver DCs from vehicle-treated or non-treated mice showed little T cell proliferation ability (Fig. 4D). These results suggested that α-GalCer treatment increased the function of DCs in the liver more strongly than those in the spleen.

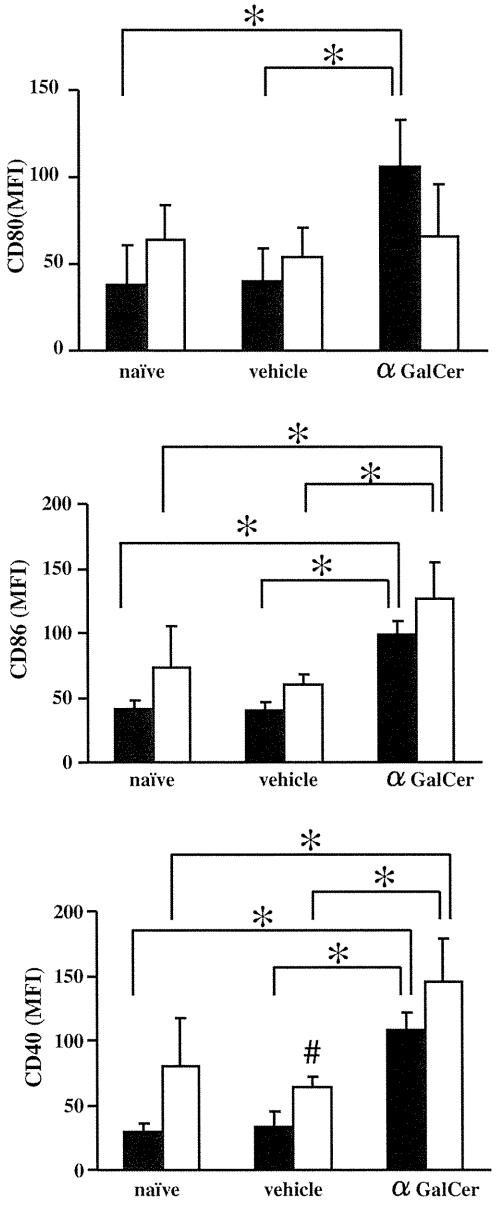

Fig. 3. α-GalCer treatment increased the expression of antigen presenting related molecules on both liver and spleen DCs. DCs were stained with PE- or FITC-conjugated monoclonal antibodies (CD11c, CD40, CD80, CD86 and MHC class II), and the expressions of these molecules were analyzed by flow cytometry. The data are represented as the average of MFI obtained from 5 separate experiments. $^{*}p < 0.05$ for each treatment group, $^{\#}p < 0.05$ between liver DCs (■ black bar) and spleen DCs (□ white bar). Naïve: DCs derived from non-treated mice; vehicle: DCs derived from vehicle-treated mice; α-GalCer: DCs derived from α-GalCer-treated mice.

3.4. Vaccination of $p53_{232-240}$ peptide-pulsed liver DCs isolated from α-GalCer-treated mice resulted in generating $p53_{232-240}$ peptide specific CTLs more efficiently than that of spleen DCs

Based on the above results, liver DCs had more antigen-presenting function than spleen DCs in α-GalCer-treated mice. We next evaluated the potential of tumor associated antigen specific CTL induction by vaccination of peptide-pulsed liver DCs or spleen DCs. We vaccinated normal mice i.p. with peptide-pulsed DC. Five days later, spleen CD8+ T cells were isolated and subjected to IFN-γ ELISPOT assay. As shown in Fig. 5, the numbers of IFN-γ spots observed for T cell responses against $p53_{232-240}$ peptide in mice vaccinated with α-GalCer-activated liver DCs were significantly higher than those in mice with vehicle- or non-treated-liver DCs. There were no detectable spots in mice vaccinated with spleen DCs from all treatment groups, suggesting that spleen DCs displayed no stimulatory activity for CTL induction regardless of the administration of α-GalCer *in vivo*. These results revealed that liver DCs in α-GalCer-treated mice have the highest potential for inducing tumor-associated antigen-specific CTLs, which might be associated with the *in vivo* generation of acquired immunity against liver tumor by α-GalCer treatment shown in Fig. 1.

4. Discussion

We and others previously reported that the early eradication of tumor cells in the liver mainly depended on NKT cells and NK cells [3,4]. In this study, we demonstrated that α-GalCer treatment resulted in generating stronger acquired immunity after eradication of primary CMS4 and MC38 liver tumor, but not after spleen tumor treatment. This suggests that liver, and not spleen, is an unique immunological organ that is favorable for generation of acquired immunity. We examined whether CTLs generated by immunization with peptide- and α-GalCer-pulsed BMDC could show equally antitumor effect in skin, liver and spleen in the normal mice. The generated CTLs in treated mice have equal access to all organs and are capable of killing tumor cells (Sasakawa, unpublished data). Thus, our data encour-

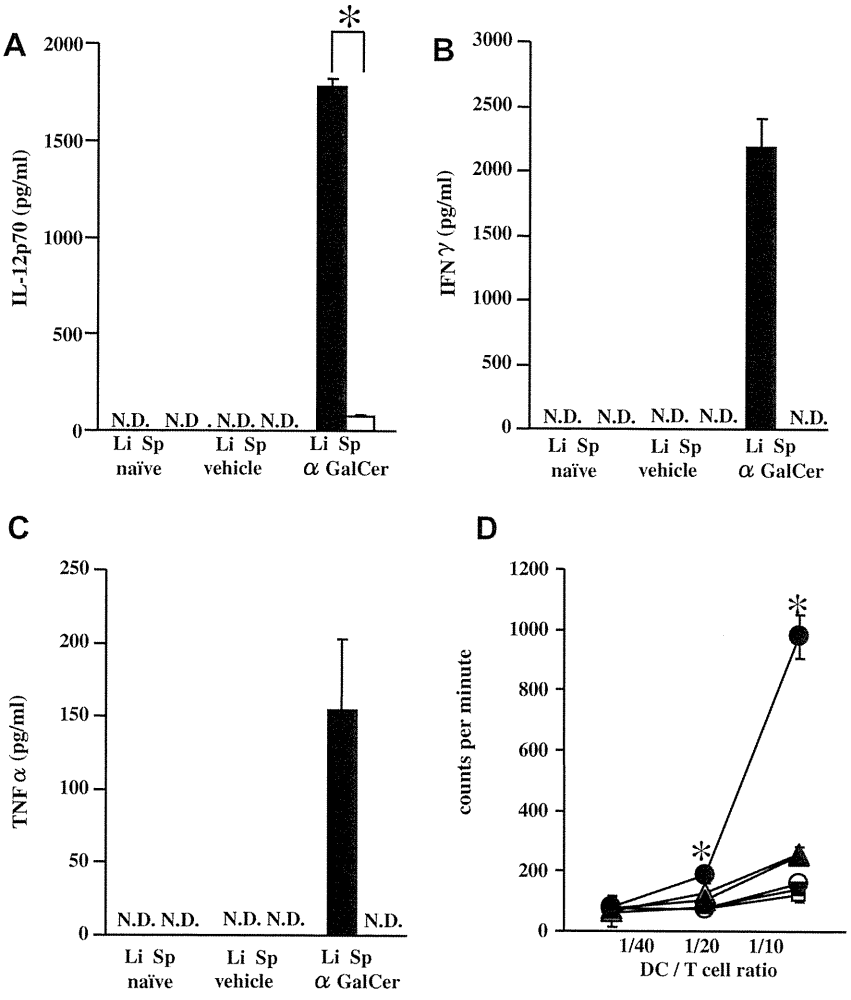

Fig. 4. Th1 type cytokine production of liver DCs from α-GalCer treated mice. Liver and spleen DCs were prepared 24 h after i.p. treatment of α-GalCer or vehicle. 2 × 10^5 DCs were stimulated with LPS (10 μg), and the supernatants of the DC cultures were subjected to specific ELISA. IL-12 (A), IFN-γ (B) and TNF-α (C). N.D., not detected. (D) We examined the allostimulatory capacity of liver and spleen DCs by MLR. Liver DC from non-treated mice (■), vehicle-treated mice (▲), and α-GalCer-treated mice (●). Spleen DC from non-treated mice (□), vehicle-treated mice (△), and α-GalCer-treated mice (○). Each data point represents the mean tumor size ± SD. $^*p < 0.05$ counts per minute (CPM) of liver DCs vs CPM of spleen DCs from α-GalCer, vehicle or non-treated mice, respectively. Similar results were obtained from three separate experiments.

aged us to investigate the ability of liver DC to generate acquired antitumor immunity in comparison with spleen DCs.

In the current study, we investigated the activation of liver and spleen DC function after α-GalCer treatment. The expressions of antigen-presenting related molecules on liver DCs were weaker than those on spleen DCs in normal or vehicle treated mice. Pillarisetty et al. reported that liver DCs are generally weak activators of immunity in contrast to spleen DCs in normal mice and the expressions of MHC and costimulatory molecules on liver DCs were lower than those on spleen DCs in normal mice [22]. This is consistent with our results. In marked contrast, α-GalCer administration resulted in a significant increase of DCs in the liver and the expressions of antigen-presenting related molecules was more strongly upregulated in the liver than in the spleen. It has been reported that the expression of CD8α molecule is an activating marker of conventional DCs from progenitor cells [23]. We demonstrated that α-GalCer administration induced not only an increase of total DCs but also a significant increase of CD8- conventional DCs in the liver, which suggested that α-GalCer treatment resulted in developing progenitor DCs efficiently to matured conventional DCs. More strikingly, the production of Th1 type cytokine from α-GalCer-treated liver DCs were significantly more than from α-GalCer-treated spleen DCs. Previous reports demonstrated that the capacity of Th1 type cytokine to link between innate and adaptive immunity by interacting with DCs and T cells, is important for the induction of adaptive antitumor immune response and long-term therapeutic effect [24]. Furthermore, liver DCs showed higher T cell proliferation ability

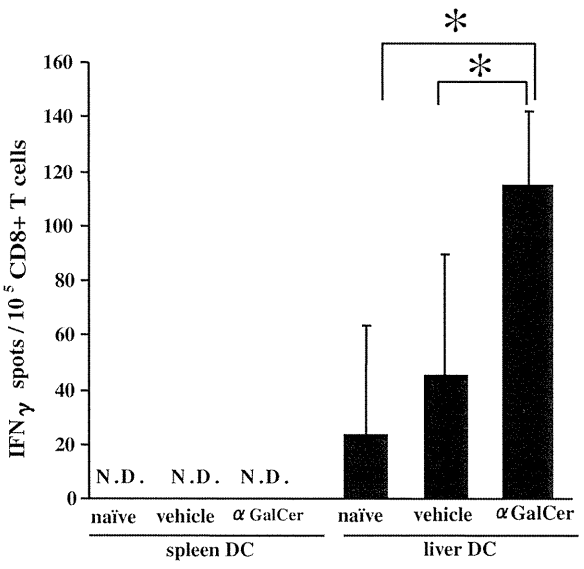

Fig. 5. Evaluation of $p53_{232-240}$ peptide specific CD8+ CTL induction after vaccination of p53 peptide-pulsed DCs from each treated mice. Normal BALB/c mice were immunized i.p. with 1×10^6 $p53_{232-240}$ peptide pulsed liver or spleen DCs isolated from α-GalCer or vehicle treated mice. Five days after vaccination, CD8+ T cells were isolated from the spleen of immunized mice. The frequency of $p53_{232-240}$ peptide specific CD8+ CTL was evaluated by IFN-γ ELSIPOT assay. The results are shown as spots/100,0000 CD8+ T cells; mean ± SD of triplicate samples. CD8+ T cell reactivity against peptide-unpulsed BMDCs served as the negative control in all cases, and this value was subtracted from all experimental determination to determine p53-specific spot numbers. $^*p < 0.05$. N.D., not detected. Similar results were obtained from three separate experiments.

than spleen DCs after α-GalCer treatment. Taken together, these results suggested that α-GalCer treatment resulted in the efficient activation of liver DCs more strongly than spleen DC, which might be associated with the induction of antitumor acquired immunity in the liver.

To examine whether the α-GalCer activated liver and spleen DCs could actually induce acquired immunity, we vaccinated $p53_{232-240}$ peptide-pulsed α-GalCer activated liver and spleen DCs. The frequencies of CD8+ T cells in response to $p53_{232-240}$ peptide were much higher in α-GalCer activated liver DCs vaccinated mice than those in vehicle-treated liver DCs vaccinated mice. Interestingly, the vaccination of $p53_{232-240}$ peptide-pulsed spleen DCs isolated from both α-GalCer and vehicle-treated mice did not generate $p53_{232-240}$ peptide-specific CTL responses. These data suggested that the immunological microenvironment in the spleen may support DCs to be potentially very tolerogenic resulting in inability of generating acquired immunity. In marked contrast, liver DCs potentially have the ability of generating antitumor acquired immunity and that α-GalCer could markedly enhance this ability. A normal mouse liver contains lymphocytes that are usually enriched with 10% NKT cells in contrast to mouse spleen that contains only 2% NKT cells [25]. α-GalCer presented by DCs activates NKT cells upregulating CD40 ligand on NKT cells, which in turn leads to the activation of DCs [17]. Actually we confirmed that i.p. injection of α-GalCer activated equally well in both liver and spleen NKT cells (Sasakawa, unpublished data). Thus, the higher population of NKT cells in the liver may be associated with efficient activation of liver DCs after α-GalCer treatment, which might characterize the unique immunological responses in the liver.

Despite recent progress and early success with various types of immunotherapy, there is still significant room for improvement in these regimens against liver cancer. We demonstrated that liver is an immunologically unique organ that is favorable for generation of acquired antitumor immunity. We propose that α-GalCer treatment may be an attractive strategy for suppressing tumor growth in the liver and promoting regression of metastatic lesions in other organs.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.jhep.2008.12.027.

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EXPERT REVIEWS

Inhibitor of MEK1/2, selumetinib, for biliary tract cancer

Expert Rev. Gastroenterol. Hepatol. 5(5), 579–581 (2011)

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Evaluation of: Bekaii-Saab T, Phelps MA, Li X *et al.* Multi-institutional Phase II study of selumetinib in patients with metastatic biliary cancers. *J. Clin. Oncol.* 29, 2357–2363 (2011).

It is necessary to establish effective chemotherapy to improve the survival of patients with biliary tract cancer. Although the useful of some molecular-targeted agents as first-line therapies has been investigated, none have been found to exert satisfactory efficacy. In this article, we report the results of a Phase II study of selumetinib in patients with metastatic biliary cancer. Selumetinib is an inhibitor of MEK1/2 targeting the RAS/RAF/MEK/extracellular signal-related kinase pathway. Three out of 28 patients showed a confirmed partial response, representing a response rate of 12%. The median progression-free survival was 3.7 months and the median overall survival was 9.8 months. The most common toxicities were rash, xerostomia and nausea. Most toxicities were grade 1 or 2, and the most common grade 3/4 toxicities were diarrhea and nausea. All toxicities were manageable and reversible. The results warrant further evaluation of the use of selumetinib in patients with metastatic biliary cancer.

Keywords: biliary tract cancer • mitogen-activated protein kinase kinase • molecular targeted therapy • Phase II study • selumetinib

Bile duct cancer is subdivided according to the anatomic location of origin into intrahepatic cholangiocarcinoma, gallbladder cancer, extrahepatic cholangiocarcinoma or cancer of the ampulla of Vater. Although surgery currently remains the only potentially curative treatment for each of the aforementioned diseases, many patients are diagnosed at an unresectable advanced stage of the disease. Chemotherapy has been recognized as a recommended therapy for unresectable biliary tract cancer based on the results of comparative studies between chemotherapy and best supportive care.

Despite the numerous Phase II studies conducted on treatments for advanced biliary tract cancer, no accepted standard treatment for this tumor type has been established as yet owing to the low incidence of this cancer, the small number of patients studied and the lack of adequately powered randomized controlled trials. Recently, randomized controlled trials comparing the combination of cisplatin plus gemcitabine with gemcitabine alone have shown the survival benefit of the former regimen [1,2]. Thus, the combination of gemcitabine plus a platinum agent (cisplatin or oxaliplatin) has come to be recognized as standard therapy for unresectable biliary tract cancer.

One of the next issues that needs to be addressed is whether molecular-targeted agents might also be effective against biliary tract cancer. To date, although clinical trials of molecular-targeted therapies as monotherapy or in combination with gemcitabine-based regimens have been conducted, no molecular-targeted agent has been confirmed to be of clinical benefit for biliary tract cancer.

Methods & results

This study was a Phase II study of selumetinib monotherapy in patients with unresectable biliary tract cancer, including intra- or extra-hepatic cholangiocarcinoma and gallbladder cancer [3]. Selumetinib is an inhibitor of MEK1/2 targeting the RAS/RAF/MEK/extracellular signal-related kinase (ERK) pathway, which plays a central role in the regulation of cellular processes, including proliferation, apoptosis and metabolism.

The primary objective of this study was to determine the overall response rate, as defined by the Response Evaluation Criteria In Solid

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Tumors, and the secondary objectives included evaluation of toxicity, overall survival, progression-free survival, assessment of *BRAF* and *KRAS* mutations, and measurement of phosphorylated (p) ERK and pAKT as indicators of activation of the relevant pathways.

With regard to the starting dose and dosing schedule of selumetinib, the drug was administered orally at 100 mg twice daily in 28-day cycles without interruption. Two levels of dose reductions were planned (50 mg twice daily and 50 mg once daily), with patients taken off the study in the case of a need for any additional dose reductions.

A total of 29 patients were enrolled between December 2007 and January 2009. Three patients showed a confirmed partial response, representing a response rate of 12%. In total, 17 patients (68%) showed stable disease. The majority of patients (52%) showed a decrease in the size of the target lesion. The median progression-free survival was 3.7 months (95% CI: 3.5–4.9) and the median overall survival was 9.8 months (95% CI: 5.97–not available).

The most common toxicities were rash (90%), xerostomia (54%) and nausea (51%). Although most toxicities were grade 1 or 2, the most common grade 3/4 toxicities were diarrhea, nausea and fatigue; in particular, grade 4 fatigue was observed in 4% of the patients. All toxicities were manageable and reversible.

Analyses of biologic markers revealed no *BRAF* V600E mutations. Two patients with short-lived stable disease had *KRAS* mutations. Absence of pERK staining was associated with lack of response and positive immunostaining for pERK was associated with improved overall survival.

Expert commentary

Some growth factors, including EGF receptor (EGFR) and VEGF receptor (VEGFR), and various signal transduction pathways that play important roles in the progression, proliferation and metastasis of various cancers, have been identified. Some studies have demonstrated overexpression of EGFR and VEGFR or mutations of their signaling pathways in biliary tract cancer [4]. Furthermore, biliary tract cancer includes various types of cancers, each with different molecular biological characteristics. For example, overexpression of EGFR has been reported to be observed in 10.7, 5.1, 12.4 and 0% of cases of intrahepatic cholangiocarcinoma, extrahepatic cholangiocarcinoma, gallbladder cancer and cancer of the ampulla of Vater, respectively [5]. Relationships between the presence/absence of various genetic mutations and the efficacy of molecular-targeted agents have been identified in various cancers; for example, the efficacy of anti-EGFR antibodies was limited to colorectal cancer patients with wild-type KRAS expression in the tumor. MEK inhibitors, including selumetinib, may be expected to exhibit activity, even against tumors with KRAS mutation. There is as yet, however, no consensus on the molecular–biologic characteristics of biliary tract cancer.

Recently, combined gemcitabine plus cisplatin or oxaliplatin therapy has been established as the standard first-line treatment for biliary tract cancer. Usage of molecular-targeted agents has been focused on as the next step. There are two directions in which molecular-targeted agents can be expected to be applied: one is in combination with standard chemotherapy regimens as first-line therapy, and the other is as monotherapy in second-line chemotherapy. In many patients with progressive disease receiving first-line chemotherapy with the relatively toxic regimen of cisplatin plus gemcitabine or gemcitabine plus oxaliplatin, the general condition is poor and serious cholangitis can easily develop. Less toxic therapy, such as monotherapy with a targeted agent, may be useful in such patients.

In this study, although 11 patients (39%) had a previous history of exposure to prior chemotherapy, there were three objective responses, representing a response rate of 12%, and another 14 patients (68%) showed stable disease [3]. In addition, both the progression-free survival and overall survival compare favorably with published historical controls. These results of selumetinib seem to suggest the promising activity of the drug against biliary tract cancer. Validation is required to confirm the efficacy of MEK inhibitors against biliary tract cancer according to the tumor site or the biological characteristics of the tumor.

Few preclinical studies of molecular-targeted agents for biliary tract cancer have been reported. In an examination conducted using human cholangiocarcinoma cell lines, ZD6474, an inhibitor of VEGFR and EGFR signaling, showed promising anticancer activity [6]. This study revealed that the absence of KRAS mutation and presence of EGFR amplification may be potentially predictive molecular markers of the sensitivity of cholangiocarcinoma to EGFR-targeted therapy [6]. Thus, therapeutically beneficial effects of molecular-targeted agents, including MEK inhibitors, may be expected against tumors with KRAS mutations and further investigations are warranted to confirm the efficacy.

Five-year view

Molecular-targeted therapy should be established based on the biologic features, and it is important to identify the characteristic biologic features of each of the aforementioned types of cancer of the biliary tract. Furthermore, efficient development of targeted therapy should be advanced based on the identification of appropriate biological markers.

Biliary tract cancer is still a difficult disease to treat. Development of new molecular-targeted agents will hopefully allow for improvement of the survival rates in patients with biliary tract cancer, and individualized therapy using targeted agents can be established according to the tumor's biological features.

Financial & competing interests disclosure

The authors have no relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties.

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Key issues

- Selumitinib is an inhibitor of MEK1/2, and a Phase II study of selumetinib showed promising activity against biliary tract cancer.
- The most common toxicities were rash, xerostomia and nausea, and all toxicities were manageable and reversible.
- Analyses of biologic markers suggested the existence of a relationship between the KRAS and BRAF status, and the efficacy of selumitinib.
- The results warrant further evaluation of the use of selumetinib in patients with metastatic biliary cancer.

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• of interest
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• **Preclinical examination of vandetanib in cholangiocarcinoma.**

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Phase II study of erlotinib plus gemcitabine in Japanese patients with unresectable pancreatic cancer

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Erlotinib combined with gemcitabine has not been evaluated in Japanese patients with unresectable pancreatic cancer. This two-step phase II study assessed the safety and pharmacokinetics of erlotinib 100 mg/day (oral) plus gemcitabine 1000 mg/m^2 (i.v. days 1, 8, 15) in a 28-day cycle in the first step, and efficacy and safety in the second step. The primary end-point was safety. One hundred and seven patients were enrolled (first step, n = 6; second step, n = 101). The most common adverse event was RASH (compiled using the preferred terms rash, acne, exfoliative rash, dermatitis acneiform, erythema, eczema, dermatitis and pustular rash) in 93.4% of patients. One treatment-related death occurred. While interstitial lung disease-like events were reported in nine patients (8.5%; grade 1/2/3, 3.8/2.8/1.9%), all patients recovered or improved. The median overall survival, the 1-year survival rate and median progression-free survival were 9.23 months, 33.0% and 3.48 months, respectively. The overall response and disease control rates were 20.3% and 50.0%, respectively. In Japanese patients with unresectable pancreatic cancer, erlotinib plus gemcitabine had acceptable toxicity and efficacy that was not inferior to that seen in Western patients. (*Cancer Sci* 2011; 102: 425–431)

Approximately 232 000 individuals are diagnosed with pancreatic cancer worldwide each year, with an annual death rate estimated at 227 000.[1] In Japan, approximately 22 000 new cases were reported in 2005.[2] Furthermore, data from 2007 show that around 24 000 individuals in Japan died from pancreatic cancer, making this tumor type the fifth leading cause of cancer-related death.[3] The majority of pancreatic cancer cases are diagnosed at an unresectable stage when prognosis is extremely poor.

Current treatment for advanced pancreatic cancer is based on systemic chemotherapy with gemcitabine. Single-agent gemcitabine has been shown to extend median overall survival (OS) to 5.65 months in chemonaïve patients compared with 4.41 months in patients who received fluorouracil.[4] Addition of other cytotoxic agents to gemcitabine has not demonstrated survival benefits over gemcitabine alone.[5–13] The potential of combining gemcitabine with biological agents in patients with advanced pancreatic cancer has also been evaluated in several phase III studies, but these trials failed to show a survival benefit.[14–19]

Epidermal growth factor receptor (EGFR)-mediated signaling is associated with various cellular processes, and the dysregulation of these processes is common in tumorigenesis.[20,21] Furthermore, EGFR is overexpressed in many tumors and its overexpression is often associated with poor prognosis.[22–26] EGFR tyrosine-kinase inhibitors (TKI, such as erlotinib) are used in the treatment of various types of solid tumors.

Erlotinib has demonstrated antitumor activity in pancreatic cell lines[27] and was subsequently assessed as a potential therapeutic agent in pancreatic cancer. In the PA.3 study (n = 569), the risk of death with erlotinib plus gemcitabine was reduced by 18% versus gemcitabine alone (hazard ratio [HR], 0.82; 95% confidence interval [CI], 0.69–0.99; P = 0.038 after adjustment for stratification factors), with a median OS of 6.24 months *vs* 5.91 months, respectively. Erlotinib plus gemcitabine combination therapy provided significant improvements in the 1-year survival rate (23% *vs* 17%; P = 0.023) and progression-free survival (PFS; HR 0.77; 95% CI, 0.64–0.92; P = 0.004).[28] As a result, this combination was approved for use in pancreatic cancer in many countries.

In Japanese patients with non-small-cell lung cancer (NSCLC), a phase II study has specifically shown that erlotinib monotherapy is well tolerated and has promising antitumor activity.[29] However, there are no data on the use of erlotinib combined with gemcitabine in Japanese patients with pancreatic cancer. This phase II study evaluated the safety and efficacy of erlotinib in combination with gemcitabine in Japanese patients with unresectable locally advanced or metastatic pancreatic cancer.

Methods

Patients. Patients aged 20–80 years with histological/cytological evidence of unresectable locally advanced or metastatic adenocarcinoma/adenosquamous carcinoma of the pancreas were eligible for inclusion in the present study. Patients were required to have an Eastern Cooperative Oncology Group (ECOG) performance status (PS) of 0–2, adequate hematological, renal and hepatic function and a life expectancy of at least 2 months. No more than one prior regimen for pancreatic cancer was permitted. Patients who had received prior gemcitabine and/or a TKI were excluded from participation, as were those who had previously been exposed to a human epidermal growth factor receptor 2 (HER2) or EGFR inhibitor. Other key

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Clinical trial registry: JAPIC Clinical Trials Information (see links below). http://rctportal.niph.go.jp/examDetail.php?center=3&center_seq=698 http://www.clinicaltrials.jp/user/cteDetail.jsp?clinicalTrialId=839&language=ja. Trial registration number: JapicCTI-060337.

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exclusion criteria were: symptomatic cerebral metastases; a concurrent lung disorder (such as idiopathic pulmonary fibrosis, interstitial lung disease [ILD] or pneumoconiosis); concurrent or previous drug-induced pneumonia; or a history of radiation to the chest.

The study complied with the Declaration of Helsinki and Good Clinical Practice guidelines. Informed consent was obtained from all patients, and the protocol was approved by ethics committees at all participating institutions.

Study design and treatment. This was a phase II, multicentre, open-label, two-step study. In the first step, six patients were enrolled into the study and treated with oral erlotinib 100 mg/day on days 3–28, plus i.v. gemcitabine 1000 mg/m^2 on days 1, 8 and 15 in a 28-day cycle. The starting doses of erlotinib and gemcitabine were chosen in reference to the PA.3 study. Dose-limiting toxicities (DLT) were assessed in these study participants using the National Cancer Institute Common Terminology Criteria for Adverse Events v3.0 (NCI-CTCAE, National Cancer Institute, Bethesda, MD, USA). Dose-limiting toxicities were defined in conformity to the P1b study as follows:[30] (i) grade 4 decrease (i.e. to <500/mm^3) in neutrophil count >5 days; (ii) grade ≥3 decrease (i.e. to <1000/mm^3) in neutrophil count with associated fever (≥38.5°C); (iii) grade 4 decrease in platelet count (i.e. to <25 000/mm^3); (iv) any grade ILD; (v) grade 4 elevation of alanine transaminase (ALT)/aspartate transaminase (AST) levels, or grade 3 elevation of ALT/AST levels >7 days; (vi) grade ≥3 non-hematological toxicity (excluding rash, hyperglycemia, γ-GTP and events that were judged to be transient/had no effect on study continuation); and (vii) dose-reduction/interruption required due to persistent adverse events (AE), which meant that the second cycle could not be started.

If treatment-related DLT occurred in no more than two of the six patients, transition to the second step of the study was permissible with approval of the Data Safety and Monitoring Committee (DSMC). If DLT occurred in three or more patients, transition to the second step was limited to those cases that were judged to be safe for this study after the DSMC had evaluated the safety data of the patients with a DLT. In the second step, it was planned that 94 patients would be treated with the same dose as the first step. Treatment was continued until disease progression, death, unacceptable toxicity or patient/investigator request.

The primary end-point of the study was safety, with secondary end-points including OS, 1-year survival rate, PFS, overall response rate (ORR), disease control rate (DCR = complete response [CR] + partial response [PR] + stable disease), pharmacokinetics (PK) and correlation of *EGFR* mutation status with outcomes.

Toxicity evaluation. Adverse events were monitored and graded using NCI-CTCAE v3.0. Clinical and laboratory assessments were conducted throughout the study. Adverse events prespecified in the study to be monitored carefully were rash, diarrhea, vomiting, liver dysfunction and ILD-like events. Chest X-ray examination to assess pulmonary toxicity was conducted weekly until week 4 and every 2 weeks thereafter. In addition, chest computed tomography (CT) scan was performed every 4 weeks. The DSMC reviewed the images and clinical data associated with all potential ILD-like events. All ILD-like events were reported to be serious AE (SAE), regardless of the grade.

Efficacy evaluation. The tumor response was assessed using Response Evaluation Criteria in Solid Tumors (RECIST) in patients who had at least one measurable target lesion. Tumors were measured using computed tomography (CT) at baseline and on day 22 of every two cycles thereafter. Median PFS, ORR and DCR were estimated by the extramural review. The relationship between efficacy and the severity of RASH (compiled using the preferred terms rash, acne, exfoliative rash, dermatitis acneiform, erythema, eczema, dermatitis and pustular rash) was also examined.

Pharmacokinetic evaluation. Pharmacokinetic evaluation of erlotinib and its O-desmethylated metabolite (OSI-420) was performed in the six patients enrolled in the first step of the study. Venous blood samples were taken prior to erlotinib dosing on day 3 and day 8 of cycle 1 at 0.5, 1, 2, 4, 6, 8 and 24 h after erlotinib administration. Samples were also taken prior to gemcitabine infusion on days 1 and 8 at 0.5, 0.75, 1, 1.5, 2.5 and 4.5 h after dosing.

The plasma concentrations of erlotinib, OSI-420 and gemcitabine were measured by liquid chromatography, tandem mass spectrometry (LC-MS-MS). The LC-MS-MS analytical methods have been described previously.[31,32] Derived PK parameters included the maximum plasma drug concentration (C_{max}), time to C_{max} (t_{max}), area under the plasma drug concentration-time curve to the last plasma sample (AUC_{last}), terminal half-life ($t_{½}$) and oral clearance (Cl/F).

Biomarker analysis. *EGFR* mutations were assessed in patients with available tumor tissue specimens, which were formalin fixed and paraffin embedded. Samples were analyzed at a central laboratory where DNA was extracted and exons 18–21 sequenced using a nested PCR.

Statistical analysis. Progression-free survival and OS were estimated using the Kaplan–Meier method in all patients who received at least one dose of the study treatment, with 95% CI for the median duration calculated using Greenwood's formula. The Clopper–Pearson method was used to calculate the 95% CI around the ORR, DCR and AE rate. Multivariate analyses were performed for the occurrence of ILD-like events using the logistic regression model. Baseline characteristics investigated for this analysis included gender, age, lung metastasis, emphysema and various baseline laboratory values. The target enrollment was 100 patients, as this was required to evaluate the safety of erlotinib.

Results

Patient characteristics. Between December 2006 and October 2007, a total of 107 patients were enrolled (first step, n = 6; second step, n = 101) from 12 institutions (Fig. 1). One patient who enrolled into the second step did not receive treatment due to deterioration in PS prior to the start of treatment. A total of 106 patients were evaluable for safety (safety population, full analysis set).

The patient demographics and baseline characteristics are shown in Table 1. The median age was 62 years (range, 36–78) and 52.8% of patients were male. Almost all patients were chemonaïve (95.3%). The majority (75.5%) of patients had an ECOG PS of 0 and most (83.0%) had metastatic disease. Over half (63.2%) of the patients had a history of current or past smoking.

Toxicity and dose modifications. The median duration of erlotinib exposure was 102.5 days and its median dose intensity was 100.0 mg/day, with the majority of patients (78.3%) receiving more than 90% of the relative dose intensity. The median duration of gemcitabine treatment was 4.0 cycles and its median dose intensity was 688.0 mg/m^2 per week, with approximately half of the patients (51.4%) receiving more than 90% of the relative dose intensity.

As only one patient had a DLT (grade 3 diarrhea) in the first step, the second step of the study was initiated. One hundred and six patients received at least one dose of erlotinib; these patients were assessable for toxicity. Treatment-related AE and treatment-related changes in laboratory values are summarized in Table 2; most of these were mild to moderate in severity. The most frequently reported AE was RASH, which occurred in

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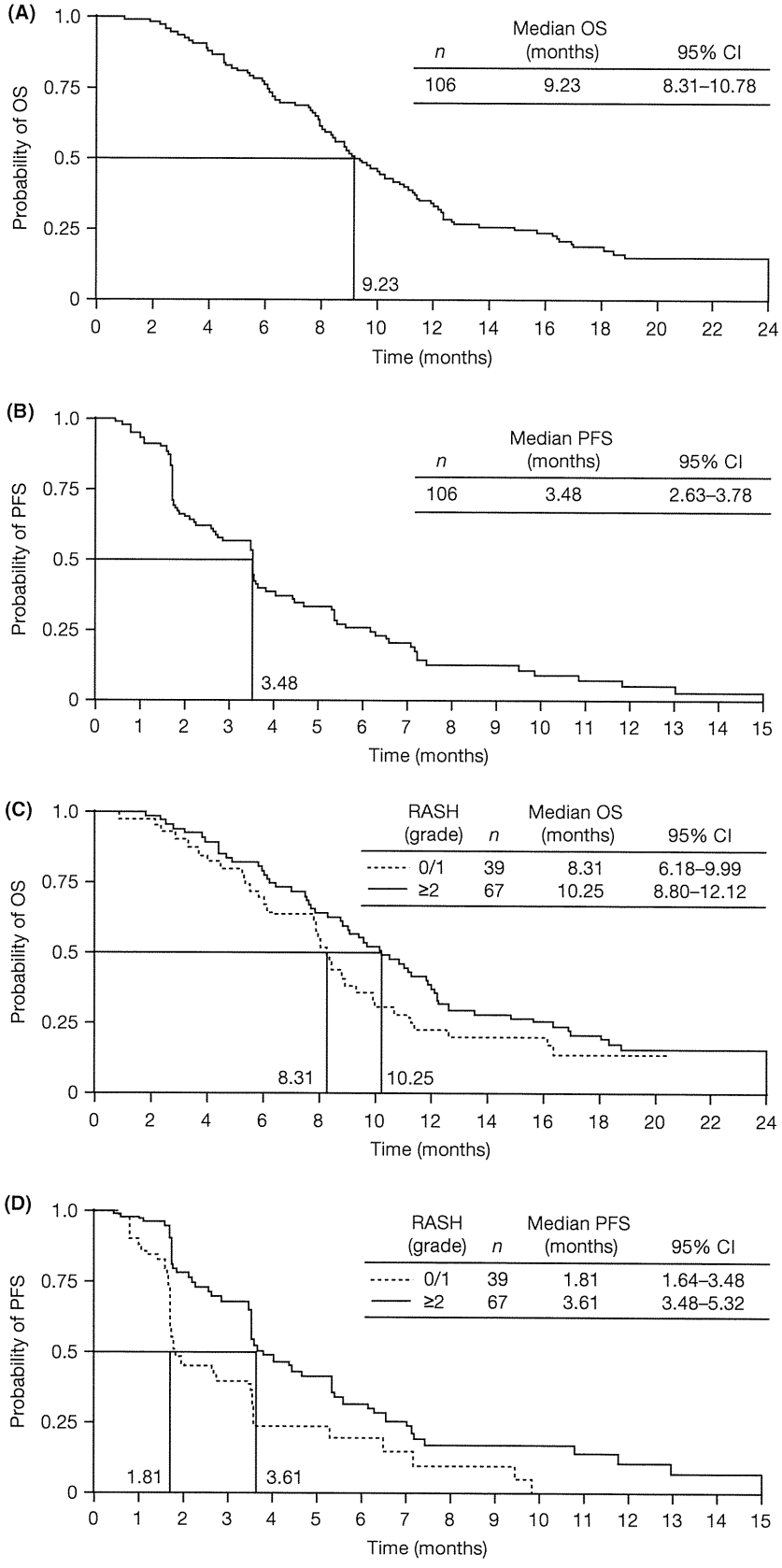

Fig. 1. Kaplan–Meier estimates of (A) overall survival (OS) and (B) progression-free survival (PFS) in the study population (n = 106); (C) OS and (D) PFS according to the severity of RASH (grade ≤1 [n = 39] *vs* grade ≥2 [n = 67]). RASH is a composite of the terms: rash, acne, exfoliative rash, dermatitis acneiform, erythema, eczema, dermatitis and pustular rash. CI, confidence interval.

Table 1. Baseline characteristics and demographics (n = 106)

Characteristic	
Median age (range) (years)	62 (36–78)
Gender, *n* (%)	
Male	56 (52.8)
Female	50 (47.2)
Median bodyweight (range) (kg)	52.3 (33.1–95.0)
Smoking history,† *n* (%)	
Never smoker	39 (36.8)
Past smoker	37 (34.9)
Current smoker	30 (28.3)
ECOG PS, *n* (%)	
0	80 (75.5)
1	26 (24.5)
2	0 (0)
Disease status, *n* (%)	
Metastatic	88 (83.0)
Locally advanced	18 (17.0)
Primary tumor identified, *n* (%)	92 (86.8)
Primary sites, *n* (%)	
Head	46 (43.4)
Body and tail	23 (21.7)
Body	22 (20.8)
Tail	10 (9.4)
Other	5 (4.7)‡
Biliary drainage, *n* (%)	19 (17.9)
Sites of distant metastases, *n* (%)	
Liver	56 (52.8)
Distant lymph nodes	39 (36.8)
Lung	17 (16.0)
Other	26 (24.5)
Prior lines of therapy, *n* (%)	
None	101 (95.3)
One regimen	5 (4.7)§
Median CA19–9 (range) (U/mL)	
Median	776 (0–435 000)
Median CEA (range) (ng/mL)	
Median	4.8 (0.6–1100.1)

†Never smoker, never/hardly smoked; past smoker, passage of at least 1 month since stopping smoking (at the time of registration); current smoker, smoked within 1 month (at the time of registration). ‡Whole of pancreas (n = 1); head and body (n = 3); other (n = 1). §Tegafur, gimeracil, oteracil potassium (S-1) (n = 3); 5-fluorouracil plus leucovorin (n = 2). CA 19–9, carbohydrate antigen 19–9; CEA, carcinoembryonic antigen; ECOG, Eastern Co-Operative Group.

93.4% of the patients; most cases were mild to moderate in severity (87.7%, grade ≤2; 5.7%, grade ≥3). Other common non-hematological AE included anorexia, pruritus, fatigue, nausea and diarrhea. Most patients experienced some degree of hematological toxicity, with grade 3 or 4 neutropenia (neutrophil decreased), leucopenia (white blood cell count decreased) and anemia (hemoglobin decreased) occurring in 34.9%, 29.2% and 14.2% of patients, respectively. Only one treatment-related death occurred (due to gastrointestinal hemorrhage), which was probably due to arterial bleeding caused by the invasion of the primary tumor into the gastrointestinal tract. Although the likelihood of this event being treatment-related was deemed remote, a causal relationship could not be completely excluded because the event occurred during the study treatment administration period.

Treatment-related SAE were reported in 26 (24.5%) patients. These included nine ILD-like events (8.5%), the majority of which (n = 7) were grade 1–2 in severity. Importantly, all of these nine patients recovered or improved, and four of these patients did so without any treatment for ILD-like events. Other

Table 2. Treatment-related adverse events occurring in >30% of patients treated with erlotinib and gemcitabine (n = 106)

	Any grade, *n* (%)	Grade 3, *n* (%)	Grade 4, *n* (%)
Non-hematological			
Rash	78 (73.6)	3 (2.8)	0 (0)
Anorexia	75 (70.8)	15 (14.2)	0 (0)
Pruritus	57 (53.8)	1 (0.9)	0 (0)
Fatigue	56 (52.8)	3 (2.8)	0 (0)
Nausea	56 (52.8)	6 (5.7)	0 (0)
Diarrhea	52 (49.1)	2 (1.9)	0 (0)
Dry skin	49 (46.2)	0 (0)	0 (0)
Stomatitis	38 (35.8)	0 (0)	0 (0)
Pyrexia	32 (30.2)	0 (0)	0 (0)
Hematological			
White blood cell count decreased	85 (80.2)	31 (29.2)	0 (0)
Platelet count decreased	77 (72.6)	9 (8.5)	0 (0)
Hemoglobin decreased	76 (71.7)	13 (12.3)	2 (1.9)
Hematocrit decreased	73 (68.9)	8 (7.5)	0 (0)
Neutrophil decreased	73 (68.9)	32 (30.2)	5 (4.7)
Red blood cell count decreased	72 (67.9)	8 (7.5)	0 (0)
ALT increased	59 (55.7)	10 (9.4)	0 (0)
AST increased	57 (53.8)	4 (3.8)	1 (0.9)
Weight decreased	53 (50.0)	3 (2.8)	0 (0)
Lymphocyte count decreased	46 (43.4)	14 (13.2)	0 (0)
Blood albumin decreased	35 (33.0)	0 (0)	0 (0)
Gamma-glutamyltransferase increased	35 (33.0)	12 (11.3)	1 (0.9)

ALT, alanine amino transferase; AST, aspartate amino transferase.

treatment-related SAE were anorexia (3.8%), vomiting, pyrexia and abnormal hepatic function (1.9% each). The baseline characteristics, treatment and outcomes of patients who developed treatment-related ILD-like events during the study are detailed in Table 3. The onset times of ILD-like events ranged from 7 to 187 days after the start of treatment. In these patients, a relatively long survival was observed (from 119 to 568+ days), and five patients received post-study therapy. All of these nine patients were past or current smokers, and six had emphysema at baseline (not detected prior to treatment, but diagnosed at the extramural review by a radiologist in the DSMC). Multivariate analyses were performed for the occurrence of ILD-like events using the logistic regression model and emphysema at baseline was indicated as a risk factor for onset of ILD-like events (odds ratio [95% CI], 12.13 [1.01–145.7]; P = 0.0491).

Adverse events led to erlotinib discontinuation in 30 patients (28.3%) and gemcitabine discontinuation in 27 patients (25.5%). The main reasons for treatment discontinuation were ILD (n = 6) and anorexia (n = 3); no patient discontinued treatment due to RASH or diarrhea. Due to the onset of AE, a total of 65 patients (61.3%) required one or more interruptions of erlotinib (36 patients [34.0%] for longer than seven consecutive days and 17 patients [16.0%] for longer than 14 consecutive days) and 56 patients (52.8%) had one or more skip of gemcitabine. Modifications in the erlotinib or gemcitabine dosage were required in 17 (16.0%) and 11 (10.4%) patients, respectively, due to AE.

Efficacy. The median OS was 9.23 months (95% CI, 8.31–10.78; Fig. 1A) and the 1-year survival rate was 33% (95% CI, 24–42). Median PFS was 3.48 months (95% CI, 2.63–3.78; Fig. 1B). Among the patients evaluable for tumor response (n = 64), the ORR was 20.3% (13/64; 95% CI, 11.3–32.2) and the DCR was 50.0% (95% CI, 37.2–62.8; CR, n = 0; PR, n = 13; stable disease, n = 19).

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Table 3. Characteristics, treatment and outcomes of patients with treatment-related ILD-like events (n = 9)

Event	Gender	Age (years)	Smoking status†	Days on treatment	ILD maximum grade	Suspicious findings of ILD	Steroids	Oxygen	ILD outcome	Presence of emphysema (assessed by radiologist)	Survival outcome (days)	Post-therapy (chemotherapy)
Lymphoid ILD	M	62	Past	82	1	Pyrexia	None	No	Improved	Yes	362	Yes
ILD	M	42	Current	50	3	Pyrexia	Pulse	Yes	Recovered	Yes	517	Yes
Organising pneumonia	M	60	Past	183	2	Respiratory symptoms	None	No	Improved	Yes	568+	Yes
ILD	F	62	Past	113	2	Cough	Oral	No	Recovered	Yes	376	No
ILD	F	74	Past	111	3	Cough, dyspnea	Pulse	Yes	Improved	None	183	No
ILD	M	60	Current	25	1	Pyrexia	Pulse	No	Recovered	None	119	Yes
ILD	M	77	Past	7	1	X-ray	None	No	Recovered	Yes	255	No
ILD	M	55	Past	187	1	CT	None	No	Recovered	Yes	415	No
ILD	F	60	Current	76	2	Cough	Oral	No	Recovered	None	346	Yes

†Past smoker, passage of at least 1 month since stopping smoking (at the time of registration); current smoker, smoked within 1 month (at the time of registration). CT, computed tomography; F, female; ILD, interstitial lung disease; M, male.

The median OS was longer in patients who experienced RASH of grade ≥2 (n = 67) than in those with RASH of grade ≤1 (n = 39) (10.25 months [95% CI, 8.80–12.12] *vs* 8.31 months [95% CI, 6.18–9.99], respectively; Fig. 1C) and the 1-year survival rate was higher (39% [95% CI, 27–50] *vs* 23% [95% CI, 10–36], respectively). Similarly, the median PFS was longer in patients with RASH of grade ≥2 versus those with RASH grade ≤1 (3.61 months [95% CI, 3.48–5.32] *vs* 1.81 months [95% CI, 1.64–3.48]; Fig. 1D). While there was no notable difference in ORR between patients with RASH grade ≥2 and those with grade ≤1 (21.1% [95% CI, 9.6–37.3] *vs* 19.2% [95% CI, 6.6–39.4]), the DCR was higher in those with more severe RASH (60.5% [95% CI, 43.4–76.0] *vs* 34.6% [95% CI, 17.2–55.7]).

Pharmacokinetics. Plasma sampling for PK analyses was performed in all six patients enrolled in the first step. On day 8, the values of C_{max} were 1760 ± 456.9 ng/mL (mean ± SD) for erlotinib, 169.7 ± 64.5 ng/mL for OSI-420 and 22 700 ± 3272.9 ng/mL for gemcitabine. The AUC_{last} was 29 001 ± 6560 h ng/mL, 2748 ± 788 h ng/mL and 10 717 ± 1458 h ng/mL (mean ± SD), respectively. The mean t_{max} was 8.0 h (range, 2.0–23.9 h), 9.0 h (2.0–23.9 h) and 0.51 h (0.45–0.57 h), respectively. Also on day 8, the mean plasma $t_{1/2}$ was 54.92 h (range, 9.25–144.61 h), 32.79 h (10.36–60.46 h), and 0.63 h (0.31–1.14 h), respectively. The Cl/F of erlotinib and gemcitabine showed interindividual variability; the Cl/F on day 8 was 3972.6 ± 772.1 mL/h (mean ± SD; coefficient of variation 19.4%) and 146 580.4 ± 31 101.3 mL/h (21.2%), respectively.

Biomarker analysis. Of the 106 patients enrolled, *EGFR* mutation status was evaluated in 47 patients (44.3%), all of whom had wild-type *EGFR*. The mutation status of the remaining patients was classified as unknown because samples were not available (30.2%), not examined (9.4%) or the results following sequencing were inconclusive (16.0%).

Discussion

This study was designed to initially assess the safety of erlotinib with gemcitabine for Japanese patients with pancreatic cancer, in whom there had been no prior exposure to either drug. As no significant safety concerns were raised in the first step of the study, enrollment of a further 101 patients was performed. Although the incidence of AE in this study was higher than in the PA.3 study, the incidence of grade 3–4 AE was similar.[28] Despite these results, no new AE specific to Japanese patients were observed. As expected, RASH and gastrointestinal events were among the most common AE in this study, and most of these cases were mild to moderate in severity.

Interstitial lung disease-like events were reported in nine patients (8.5%; grade 1/2/3, 3.8/2.8/1.9%) in the current study, while its incidence was reported to be 2.4% in patients treated in the erlotinib plus gemcitabine arm of the PA.3 study.[28] In addition, in Japanese patients with advanced pancreatic cancer, ILD-like events were reported in two (6.1%) of 33 patients treated with gemcitabine plus S-1, and were reported in three (1.1%) of 264 patients with gemcitabine monotherapy, respectively.[33,34] Likewise, the higher incidence of ILD-like events were documented using S-1 or erlotinib in combination with gemcitabine compared with gemcitabine as monotherapy in patients with pancreatic and biliary tract cancer.[35] On another front, outside of Japan, a high incidence of ILD-like events was reported in gemcitabine and paclitaxel combination therapy in patients with NSCLC.[36] From the above information, considering the higher incidence of ILD when gemcitabine is used in combination, an additive effect from such combinations cannot be ruled out.

In NSCLC, Japanese patients have an increased risk of developing ILD-like events when treated with EGFR TKI.[29,37–39] Fatal cases of ILD-like events have been reported following EGFR TKI administration for the treatment of NSCLC.[37–41] Importantly, however, no patients died due to an ILD-like event in this study. Seven patients experienced ILD-like events of grade 1–2 in severity. This may be due to active management of ILD-like cases during the study period. This management included regular and immediate chest X-rays, in addition to diagnosis with CT scans after any early signs and symptoms were observed (e.g. pyrexia, cough or dyspnea), timely discontinuation of the antitumor drugs (as a precautionary measure in case these drugs were associated with the symptoms) and appropriate treatment for the events (including oral/pulse steroids). By appropriately treating the early symptoms of ILD-like events, patients could restart antitumor therapy (chemotherapy: treatment change). In this study, the onset time for ILD-like events varied markedly between patients (7–187 days). It is therefore necessary to monitor the patients throughout the treatment period.

All of the patients who developed ILD in this study were current or past smokers, and smoking status has been shown to be a risk factor for ILD in the NSCLC population.[38] Results from the multivariate analyses in this study suggest that emphysema is also a risk factor for developing ILD; six of the nine

patients with ILD-like events were diagnosed with emphysema at baseline. Although the number of reports of an ILD-like event may have been artificially elevated due to underlying patient baseline characteristics and the active management of ILD-like events, these results demonstrate the need to consider the risk of ILD-like events in Japanese patients treated with TKI. In particular, it is important that chest CT scans are closely checked for the presence of emphysema or comorbid ILD and that pulmonary status is assessed prior to treatment administration.

This study corroborates the results of the combination of gemcitabine and erlotinib shown in the PA.3 study. The median OS in this study of 9.23 months was longer than those reported in trials with gemcitabine alone. In this study, patients who experienced skin toxicity of grade ≥2 had better outcomes than those with less severe toxicity or the overall study population. Retrospective analyses of data from the PA.3 and AViTA studies have found a significant association between the development of skin toxicity and efficacy in patients with pancreatic cancer treated with erlotinib-based therapy, although the precise mechanisms for the association between skin toxicity and effectiveness are unknown.[28,41,42]

Although the presence of mutations in the tyrosine-kinase region of the *EGFR* gene appears to predict a better response to erlotinib in NSCLC,[43,44] this has not yet been evaluated in pancreatic cancer. *EGFR* mutations are very rare in patients with pancreatic cancer;[45–47] indeed in the present study, no *EGFR* mutations were detected. Further work is required to determine whether *EGFR* mutations can be used as predictive markers for improved survival in Japanese patients receiving erlotinib and gemcitabine as treatment for advanced pancreatic cancer.

In conclusion, the present study shows that erlotinib in combination with gemcitabine is generally well tolerated in Japanese patients with advanced pancreatic cancer. This combination is associated with efficacy and survival outcomes, and the results of this study are consistent with the findings of the global PA.3 study.

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A Phase I/II Study of Combined Chemotherapy with Mitoxantrone and Uracil/Tegafur for Advanced Hepatocellular Carcinoma

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Objective: The aim was to determine the recommended dose of combined chemotherapy with mitoxantrone and uracil/tegafur (Phase I part) and to clarify its efficacy and safety in patients with advanced hepatocellular carcinoma at the recommended dose (Phase II part).
Methods: Patients eligible had histologically confirmed, chemo-naive advanced hepatocellular carcinoma and were amenable to established forms of treatment. The therapy consisted of mitoxantrone administered intravenously at one of three dosages (6, 8 and 10 mg/m^2/day) on day 1 and uracil/tegafur administered orally at 300 mg/m^2 from day 1 through day 21. The treatment was repeated every 4 weeks until evidence of tumor progression or unacceptable toxicity.
Results: A total of 25 patients were enrolled. In the Phase I part, dose-limiting toxicities occurred in all three patients, given mitoxantrone at the dosage of 10 mg/m^2/day, and the recommended mitoxantrone dosage was determined to be 8 mg/m^2/day. Among 19 patients administered the drug at the recommended dosage, 1 patient (5.3%) showed partial response, 8 patients (42.1%) showed stable disease and 10 patients (52.6%) showed progressive disease. The median survival and median progression-free survival were 8.4 and 2.5 months, respectively. The most common toxicities were Grade 3–4 leukopenia (63.2%) and neutropenia (68.4%).
Conclusions: Mitoxantrone at 8 mg/m^2 combined with uracil/tegafur at 300 mg/m^2/day was determined to be the recommended regimen. Although this regimen was generally well tolerated, it appeared to have little activity against advanced hepatocellular carcinoma. These findings do not support the use of this combination regimen in practice.

Key words: hepatocellular carcinoma – chemotherapy Phase I/II – mitoxantrone – uracil/tegafur

INTRODUCTION

Hepatocellular carcinoma (HCC) is one of the most commonly occurring cancers worldwide (1,2). Surgical resection, liver transplantation and local ablation therapy, including radiofrequency ablation and ethanol injection, are considered as curative treatment for HCC (3). Transcatheter arterial chemoembolization (TACE) has been applied to patients with advanced incurable HCC (4,5). However, the majority of HCC patients develop recurrence or metastasis, regardless of the treatment modalities employed. Although patients with HCC at this advanced stage are generally treated by systemic therapy, the prognosis remains poor (6,7). Sorafenib is an orally administered molecular-targeted drug that targets tumor cell proliferation and tumor angiogenesis by inhibiting the serine–threonine kinases Raf-1 and B-Raf and the receptor tyrosine kinase activity of vascular endothelial growth factor receptors 1, 2 and 3 and platelet-derived growth factor

receptor β. This drug was reported to confer an overall survival advantage, with manageable toxicity, in comparison with placebo in a Phase III trial, and it has been accepted worldwide as the first-line chemotherapy for advanced HCC (8). But the advantage is modest. There is urgent need to develop more effective regimens.

5-Fluorouracil (5-FU) has been widely used for the treatment of various gastrointestinal malignancies, including advanced HCC (9,10). A high level of efficacy can be expected when the drug is given as a continuous intravenous infusion (11). However, this would necessitate a permanent intravenous access. Uracil/tegafur (UFT) is an orally administered drug which is a mixture of uracil and tegafur at a molar ratio of 4:1. Tegafur is a prodrug of 5-FU that is hydroxylated and converted to 5-FU by hepatic microsomal enzymes, and uracil prevents the degradation of 5-FU by inhibiting the enzyme dihydropyrimidine dehydrogenase, which results in an increased level of 5-FU in the plasma and tumor tissues (12,13). UFT has been reported to be as effective as intravenous 5-FU for the treatment of malignancies (14,15) and to be effective for the treatment of advanced HCC (16,17).

The therapeutic usefulness of doxorubicin in patients with advanced HCC has also been widely explored since the 1970s. A randomized trial in which doxorubicin was compared with supportive care alone for advanced HCC showed a significant survival benefit in the doxorubicin arm. However, treatment with this drug has not been accepted as a standard chemotherapy because of the high rate of fatal complications reported (18). Mitoxantrone, another anthracycline, has shown similar antitumor activity to that of doxorubicin in both human tumor cell lines and animal models of leukemia and has fewer myelotoxic and cardiotoxic effects than doxorubicin (19). Clinical trials of mitoxantrone have also demonstrated moderate activity against HCC, with a low incidence rate of adverse effects (20,21).

Combination chemotherapeutic regimens composed of a fluoropyrimidine and an anthracycline antibiotic have been reported to show moderate efficacy against HCC with tolerable toxicity (22–24), but combined chemotherapy with UFT and mitoxantrone has not yet been examined. We conducted Phase I/II studies to determine the recommended dosage of the combination of UFT with mitoxantrone (UFM regimen) and to clarify the efficacy and safety when administered at the recommended dose in patients with advanced HCC.

PATIENTS AND METHODS

Eligibility Criteria

The eligibility criteria for study enrolment were: (i) patients with histologically confirmed HCC, who were (ii) unsuitable for surgical resection, local ablation therapy or TACE, (iii) were ≥20 years old, (iv) had an Eastern Cooperative Oncology Group (ECOG) performance status (PS) of 0–2, (v) had adequate bone marrow function (white blood cell ≥3000 cells/mm^3, absolute neutrophil count ≥1500 cells/mm^3, platelet count ≥70 000 cells/mm^3 and hemoglobin ≥8.0 g/dl), renal function [serum creatinine concentration ≤upper limit of normal (ULN)] and hepatic function [serum albumin level ≥3.0 mg/dl, total bilirubin level ≤3.0 mg/dl, serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT) levels ≤5.0 × ULN], (vi) had a life expectancy of at least 12 weeks and (vii) provided written informed consent from each patient.

The exclusion criteria were: clinically evident congestive heart failure, serious cardiac arrhythmia, active or symptomatic coronary artery disease or ischemia, clinically serious infection, seizure disorder requiring medication, prior malignancy (any cancer treated curatively was permitted), clinically evident brain or meningeal metastasis, and pregnant/lactating women. This protocol was approved by the Institutional Review Board for clinical investigation of the National Cancer Center, in conformity with the provisions of the Declaration of Helsinki, Good Clinical Practice guidelines, and local laws and regulations.

Study Treatment

UFT was administered orally at the dose of 300 mg/m^2 per day in two divided doses for 21 consecutive days, followed by a rest period of 7 days (400 mg/body per day in patients with a body surface area of <1.50 m^2 and 500 mg/body/day in patients with a body surface area of ≥1.50 m^2). Mitoxantrone was given as a 60 min intravenous infusion on day 1. This cycle was repeated every 28 days. Patients continued to receive additional courses of this regimen until a cumulative dose of mitoxantrone of 100 mg/m^2, evidence of disease progression or the appearance of unacceptable toxicity.

Phase I Part

The objectives of the Phase I study were to investigate the frequency of dose-limiting toxicity (DLT) and to determine the recommended dose of mitoxantrone and UFT. The criteria of DLT included: Grade 4 leukopenia or neutropenia, Grade 3 neutropenia accompanied by fever (≥38°C) or infection (clinically or biologically confirmed), thrombocytopenia <25 000/mm^3 or necessity of transfusion, Grade 3 or 4 non-hematological toxicity (except nausea/vomiting, anorexia, fatigue and hyperglycemia), AST and ALT >10 times the ULN, suspension of UFT administration for over 3 successive weeks, or an over 6-week delay in the commencement of the next treatment cycle.

Three possible dosage levels of mitoxantrone (Level 1: 6 mg/m^2/day, Level 2: 8 mg/m^2/day and Level 3: 10 mg/m^2/day) were assigned for the Phase I part (Table 1). The first patient to enter the study was started at Level 1. At least three patients were treated at this level and observed for DLT. Dose escalation was continued until at least one-third

Table 1. Dose-escalation schedules of mitoxantrone and uracil/tegafur

Dose level	Mitoxantrone (mg/m^2)	UFT (mg/m^2)	Number of patients enrolled
1	6	300	3
2	8	300	6
3	10	300	3

UFT, uracil/tegafur.

of the patients in a given cohort showed DLT. If none of the first three treated patients developed DLT during the first cycle at a specific dose level, the dose escalation was continued. If one of the first three treated patients developed DLT at any dose level, three additional patients were entered at the same dose level; if only one or two of six patients at a given level experienced a DLT, the dose escalation was continued. The maximum tolerated dose (MTD) was defined as the dose level at which one-third or more of the patients experienced a DLT. The recommended dose for the Phase II study was defined as the dose level preceding the attainment of the MTD.

Phase II Part

The primary endpoint of the Phase II part was the objective response rate. The secondary endpoints were the overall survival, progression-free survival and the frequency and severity of adverse events. The Phase II part was begun after determination of the recommended dosage from the Phase I part.

Assessment of the Response and Toxicity

Physical examination including cardiac symptoms, complete blood cell counts, serum chemistries and urinalysis was performed at the baseline and at least once every 2 weeks after the start of the treatment. Dynamic computed tomography or magnetic resonance imaging was undertaken to evaluate the response at 4- to 6-week intervals after the start of treatment. Tumor response was assessed using the Response Evaluation Criteria in Solid Tumors (25). Toxicity was graded according to the National Cancer Institute common toxicity criteria, version 2.0. Progression-free survival was calculated from the first day of treatment to the appearance of evidence of tumor progression, clinical progression or last date of follow-up. The overall survival was calculated from the first day of treatment until death due to any cause or date of last follow-up. Survival data were analyzed using the Kaplan–Meier method.

Statistical Analysis

In the Phase II part, the primary endpoint was the response rate, and data from at least 19 patients were accrued. The threshold response rate was set at 5% and the expected response rate at 15%. If no responses were observed in the 19 patients and the upper limit of the 90% confidence interval (CI) did not exceed the expected rate of 15%, the UFM regimen was judged to have no activity against HCC. If response was confirmed in one or more of the 19 patients, the decision of whether or not to proceed to a further study using the UFM regimen was taken on the basis of other factors, such as the safety and rate of response, overall survival and time to progression in this study.

RESULTS

Patients

From April 2004 to April 2007, 25 patients were registered for the present study: 12 patients completed the Phase I part (Level 1: 3 patients, Level 2: 6 patients and Level 3: 3 patients). Nineteen patients who received the recommended dose (6 patients received this dose during the Phase I part) were analyzed during the Phase II part. Table 2 shows the baseline characteristics of the patients in the Phase I and Phase II parts of the study of the UFM regimen. There were 19 males and 6 females with a median age of 67 years. All the patients had a good ECOG PS score of 0–1. There were 21 (84%) and 4 (16%) patients with the Child–Pugh Stages A and B, respectively. Thirteen (68%) patients had extrahepatic metastasis, and the major sites of metastasis were lymph node [$n = 7$ (28%)] and lung [$n = 6$ (24%)].

Treatments

In the Phase I part, there was no occurrence of DLT at the Level 1 and Level 2 doses, but all of the three patients who received the Level 3 dose experienced DLT; two of these patients developed Grade 4 neutropenia and one patient developed Grade 3 creatinine elevation. The additional three patients at the Level 2 dose did not experience any DLT. Therefore, Level 3 was considered as the MTD and Level 2 (UFT 300 mg/m^2 and mitoxantrone 8 mg/m^2) as the recommended dose for the Phase II part.

At the recommended dosage level, a total of 69 courses of the UFM regimen were administered with a median of three courses to each patient (range, 1–8 courses). The dose intensity was 98.9% of the planned dosage for mitoxantrone and 97.9% for UFT.

The reasons for treatment discontinuation in the Phase I and Phase II parts were disease progression in 19 patients, liver dysfunction in 1 patient, DLT according to this protocol in 3 patients during the Phase I part and an over 6-week delay in the start of the next course because of the development of leukopenia in 2 patients. After abandoning the UFM regimen, 10 patients received the second-line treatment. Five patients received systemic chemotherapy, one patient received UFT alone and four patients received a combined chemotherapy with UFT and doxorubicin. Two

Table 2. Profile of hepatocellular carcinoma patients population

	Phase I	Phase II
No. of patients	12	19
Gender		
Male	9	14
Female	3	5
Age (years)		
Median	63	67
Range	56–78	56–77
Performance status		
0	11	7
1	1	12
Viral marker		
Hepatitis C antibody+	7	7
Hepatitis B antigen+	2	5
Previous treatment		
Surgical resection	4	10
Percutaneous ablation therapy	3	3
Transcatheter arterial chemoembolization	5	8
Transcatheter arterial infusion	3	5
Radiation therapy	1	2
None	3	3
Child–Pugh classification		
A	8	17
B	4	2
UICC tumor stage[a]		
III	4	6
IVa	3	1
IVb	5	12
Portal vein tumor thrombosis		
(+)	5	4
Extrahepatic metastasis		
Lymph node	5	7
Lung	0	6
Bone	0	3
Adrenal gland	0	1
Peritoneum	0	1
None	7	6

[a]The International Union Against Cancer, 6th edition.

patients received transcatheter arterial infusion with cisplatin, one patient received salvage TACE because of HCC rupture during the follow-up period, one patient received salvage radiofrequency ablation because of rapid growth of HCC that needed control and one patient received immnunotherapy.

Table 3. Toxicity

Toxicity grade	Phase I part									Phase II part		
	Level 1 ($n = 3$)			Level 2 ($n = 6$)			Level 3 ($n = 3$)			Level 2 ($n = 19$)		
	1–2	3	4	1–2	3	4	1–2	3	4	1–2	3	4
Hematological toxicity												
Leukopenia	2	1	0	0	2	0	0	1	1	4	9	3
Neutropenia	0	1	0	0	2	0	0	0	2	4	11	2
Thrombocytopenia	1	1	0	0	0	0	1	0	0	4	1	0
Anemia	0	0	0	1	0	0	0	0	0	1	0	0
Non-hematological toxicity												
Nausea	3	0	0	0	0	0	2	0	0	3	0	0
Anorexia	0	0	0	2	0	0	1	0	0	3	0	0
Elevated bilirubin	2	0	0	0	1	0	1	0	0	6	0	0
Hypoalbuminemia	1	0	0	0	0	0	0	0	0	1	0	0
Fatigue	0	0	0	0	0	0	1	0	0	1	0	0
Hyperpigmentation	0	0	0	0	0	0	0	0	0	1	0	0
Constipation	0	0	0	0	0	0	0	0	0	1	0	0
Elevated creatinine	0	0	0	0	0	0	0	1	0	0	0	0
Elevated AST	0	0	0	1	0	0	0	0	0	2	1	1[a]
Elevated ALT	0	0	0	1	0	0	0	0	0	1	2	1[a]
Liver dysfunction	0	0	0	0	0	0	0	0	0	0	0	1[a]

AST, aspartate aminotransferase; ALT, alanine aminotransferase.
[a]Death related to adverse event.

Toxicity

Table 3 summarizes the toxicities observed in the patients. At the recommended dose (level 2), the major Grade 3–4 hematological toxicities were leukopenia (63.2%) and neutropenia (68.4%). The most common non-hematological toxicities were elevated serum total bilirubin level (31.6%), elevated AST level (26.3%), elevated ALP level (26.3%) and anorexia (21.1%); however, no Grade 3–4 non-hematological toxicities were observed. One patient died of hepatic failure due to hepatitis B virus (HBV) reactivation.

Efficacy

Of the 19 patients who were administered the recommended dosage, 18 died during the follow-up period. All of the 19 patients administered the recommended dosage were evaluable for tumor response; of these, 1 patient achieved partial response (PR), with an overall response rate of 5.3% (95% CI, 0.0–26.0%). Eight patients (42.1%) had stable disease and 10 patients (52.6%) had progressive disease. The 1-year survival rate, median overall survival, median progression-free survival and time to progression were 26.3%, 8.4

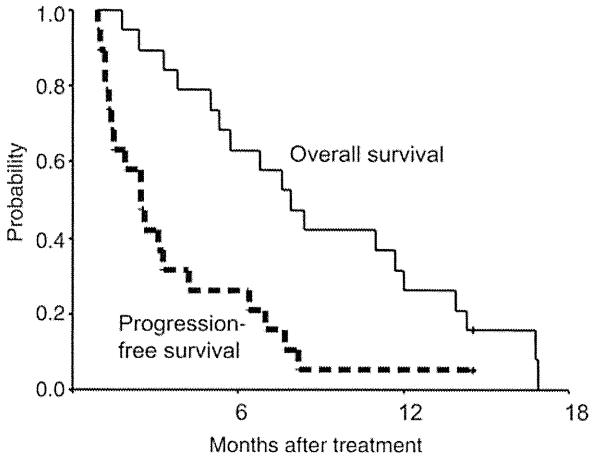

Figure 1. Overall survival and progression-free survival in 19 patients at the recommended dose. Tick marks indicate censored cases.

months (95% CI, 5.4–11.4) and 2.5 months (95% CI, 1.5–3.5), respectively (Fig. 1).

DISCUSSION

Systemic chemotherapy for unresectable HCC is recognized as an important treatment modality, because some patients who have recurrent or very advanced disease are not suitable candidates for effective local treatments such as surgical resection, liver transplantation, local ablation therapy and TACE. Many patients with HCC have underlying chronic liver disease and impaired hepatic function, increasing the toxicity of standard doses of many chemotherapeutic agents and causing difficulty in delivering combination chemotherapies. The results, in terms of the therapeutic efficacy, of investigation of cytotoxic agents for advanced HCC have been disappointing, with few agents have yielded response rates of over 20%, and no cytotoxic agents have produced convincing survival benefits in the Phase III setting (26–28).

In Japan, only five anticancer agents, UFT, adriamycin, cytarabine, mitomycin and 5-FU, had been approved for the systemic chemotherapy of HCC by the Ministry of Health, Labor and Welfare of Japan before sorafenib has been approved. Among these drugs, the results of multiagent regimens containing both a fluoropyrimidine and an anthracycline antibiotic have shown favorable results for advanced HCC (22–24). Thus, it was expected that the combination of mitoxantrone and UFT (UFM regimen) would have effective anticancer activity, and we conducted a Phase I/II study to evaluate this regimen.

In the Phase I part, we determined the recommended dose of mitoxantrone as 8 mg/m^2 on day 1 and of UFT as 300 mg/m^2 from days 1 to 21 of a 28-day cycle. The DLTs observed at Level 3 were Grade 4 neutropenia (two patients) and Grade 3 creatinine elevation (one patient).

Patients with HCC tend to experience more severe myelosuppression and hepatic toxicity than those with other malignant diseases, because most have underlying cirrhosis, which is usually associated with compromised hepatic function, leukopenia and thrombocytopenia (24). In 19 patients treated at the recommended dose level, the most frequently encountered toxicities were leukopenia and neutropenia, which are well-known toxicities of the two drugs. When compared with that in trial of mitoxantrone or UFT for other malignancies, Grade 3 or 4 hematological toxicities occurred more frequently (29–31). However, these toxicities were reversible and generally well tolerated in patients with advanced HCC, except for one case of treatment-related death; this patient developed hepatic failure due to HBV reactivation, because no antiviral drug for HBV infection, such as lamivudine or entecavir, was given. This is a well-recognized complication in patients with HBV infection who received immunosuppressive therapy or chemotherapeutic agents (32,33). Thus, patients with HBV infection should receive prophylactic antiviral treatment before chemotherapy.

In the current study, 1 of the 19 patients showed a PR (response rate, 5.3%). However, the rate of progressive disease was 52.6%. In addition, the result of median time to progression was only 2.5 months. Those results were unfavorable when compared with those reported from other clinical trials (8,21–23). Therefore, this regimen is considered to be ineffective and cannot be recommended for use in clinical practice. There were several reasons for this negative result. One of the reasons was the number of anticancer drugs in the regimen. A regimen containing two drugs may have little activity, and three or more drugs may be needed to obtain activity against HCC, because many of the regimens that have been shown to exert anticancer effect against HCC contain three or more drugs. The other reason was the recommended doses of the drugs in this regimen. We set the criteria of DLT which had included Grade 4 neutropenia or leukopenia. Two patients experienced DLT based on these criteria. However, both recovered soon, with only observation. Therefore, the criteria may be too strict, although the two drugs have been used at these recommended doses for other malignancies. It may be possible to set higher dose levels to obtain higher antitumor effect.

Recently, increasing knowledge of the molecular pathogenesis of HCC as well as the introduction of molecular-targeted therapies has created an encouraging trend in the management of HCC. Combination regimens consisting of molecular-targeted agents such as sorafenib and cytotoxic agents have been reported as promising regimens for patients with advanced HCC and other malignancies (34–37). The UFM regimen itself has little antitumor activity, but the result may be useful in the setting of future clinical trials of cytotoxic agents used in combination with molecular-targeted agents.

In conclusion, the recommended dose was mitoxantrone at 8 mg/m^2 and UFT at 300 mg/m^2/day. A combined chemotherapy with mitoxantrone and UFT appeared to show little activity in patients with advanced HCC, although this regimen was generally well tolerated. These findings do argue against the use of this regimen in clinical practice.

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Conflict of interest statement

None declared.

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