that the efficacy of the combination therapy seemed to be promising, as outlined below.

We recently initiated a phase I and randomized phase II study to evaluate the efficacy and safety of adding the WT1 peptide vaccine to GC in advanced biliary tract cancer (WT-BT trial), since GC has become the new standard and because the WT1 peptide vaccine is an attractive candidate as a partner for chemotherapy to improve survival in patients with advanced biliary tract cancer. WT1 protein is overexpressed in various types of cancer cells, including biliary tract cancer cells [9], and it was ranked as the No. 1 antigen in the cancer antigen prioritization project of the National Cancer Institute [10].

To our knowledge, this is the first randomized clinical trial to evaluate immunotherapy for biliary tract cancer. The study complied with the Declaration of Helsinki. Informed consent was obtained from all the patients, and the protocol was approved by the ethics committees at all participating institutions. This trial was registered at the UMIN Clinical Trials Registry as UMIN 000004886 (http://www.umin.ac.jp/ctr/index.htm). The study was initiated in January 2011.

1. The results of a phase I trial of WT1 peptide vaccine and GEM combination therapy in patients with advanced pancreatic or biliary tract cancer

An open-labeled, dose-escalation phase 1 trial of WT1 vaccine and GEM combination therapy for patients with advanced pancreatic cancer or biliary tract cancer was performed. The primary endpoint was the evaluation of the toxicity, safety, and optimal immunological dose of the vaccine. Human leukocyte antigen (HLA)-A 0201, HLA-A 0206, and/or HLA-A 2402-positive patients with inoperable advanced pancreatic or biliary tract cancer who had not previously been treated with GEM were eligible for this study. Six doses of GEM and 4 doses of WT1 peptide (1 or 3 mg) emulsified in Montanide adjuvant were administered over 2 months. Twenty-five patients (13 male and 12 female) were enrolled. Nine patients had inoperable advanced pancreatic cancer, 8 had gallbladder cancer, 4 had intrahepatic, and 4 had extrahepatic bile duct cancer. The adverse events were comparable to those seen with GEM alone. Delayed-type hypersensitivity test was positive after vaccination in 2 patients, and WT1-specific T cells in peptide-stimulated culture were detected by tetramer assay in 59% (13 of 22) of the patients. The disease control rate at 2 months was 89% for pancreatic cancer and 50% for biliary tract cancer. With a median follow-up time of 259 days, the median survival time for patients with biliary tract cancer was 288 days, and that for patients with pancreatic cancer was 259 days. Although objective clinical efficacy was not apparent, the safety of the WT1 vaccine and GEM combination therapy was confirmed in this study.

2. An ongoing phase I and randomized phase II study to evaluate the efficacy and safety of adding WT1 peptide vaccine to GC in advanced biliary tract cancer (WT-BT trial).

Protocol summary of the WT-BT trial

Study setting

The study is a multi-institutional open-label phase I and randomized phase II trial.

Objectives and endpoints

The aim of this phase I/II study is to determine the recommended dosage of WT1 peptide vaccine when used in combination with GC chemotherapy and to clarify the safety and efficacy of GC plus WT1 peptide vaccine when administered at the recommended dose, in comparison with GC alone.

In the phase I study, we will investigate the frequency of the dose-limiting toxicity (DLT). The criteria for a DLT will include: Grade 4 neutropenia for 8 or more consecutive days, Grade 3 neutropenia accompanied by a fever ($\geq$37.5°C), Grade 4 thrombocytopenia or the need for a transfusion, a Grade 4 aspartate transaminase (AST)/alanine transaminase (ALT) elevation or a Grade 3 AST/ALT elevation for 8 or more consecutive days, Grade 3 or 4 nonhematological toxicity (except for rash, hyperglycemia, gamma-GTP elevation, and any temporary events not affecting the protocol treatment), Grade 3 or 4 local skin inflammation at the vaccine injection sites, or Grade 1 or greater interstitial pneumonia.

In the phase II study, the primary endpoint will be the 1-year overall survival rate for all eligible patients. Overall survival will be defined as the number of days from randomization until death from any cause, and the data will be censored as of the last follow-up day on which the patient was alive. The secondary endpoints will be progression-free survival, response rate, median survival time, 2-year overall survival rate, percentage of adverse events, percentage of serious adverse events, and immunological responses (multimer assay and delayed-type hypersensitivity).

Eligibility criteria

Inclusion criteria

For inclusion in the study, patients are required to fulfill all the following criteria:

1. Clinically diagnosed with biliary tract cancer, including intrahepatic cholangiocarcinoma, extrahepatic cholangiocarcinoma, gallbladder cancer, and ampulla of Vater cancer.
2. Recurrent or unresectable biliary tract cancer.
3. Histologically proven papillary adenocarcinoma, tubular adenocarcinoma, or adenosquamous carcinoma for patients with extrahepatic cholangiocarcinoma, gallbladder cancer, and ampulla of Vater cancer; histologically proven adenocarcinoma for patients with intrahepatic cholangiocarcinoma.
4. Without central nervous system metastasis.
5. Without moderate or greater ascites/pleural effusion.
6. No previous therapy for biliary tract cancer.
7. No previous operation, chemotherapy, or radiotherapy for any other malignancies within the past 5 years.
8. No previous chemotherapy containing gemcitabine or cisplatin for any other malignancies.
9. An Eastern Cooperative Oncology Group (ECOG) performance status of 0 or 1.
10. Sufficient oral intake.
11. Age of 20–80 years.
12. Adequate organ functions.
13. HLA of A2402, A0201, or A0206.
14. Written informed consent.

Exclusion criteria

Patients will be excluded if they meet any of the following criteria:

1. Simultaneous or metachronous (within the past 5 years) double cancers, with the exception of intramucosal tumors curable with local therapy.
2. Pregnant or lactating women or women of childbearing potential and men who wish to father children.
3. Psychosis.
4. Patients requiring systemic steroid medication.
5. Interstitial pneumonia or fibroid lung disease.
6. Active bacterial or fungous infection.
7. Severe complications.
8. Drug allergies to drugs containing iodine compounds and/or gadolinium.
9. Inadequate physical condition, as diagnosed by the primary physician.

Randomization in the phase II study

After the fulfillment of the eligibility criteria has been confirmed, patient registration for both the phase I and II studies will be made by faxing the Data Center. Eligible patients in the phase II study will be stratified according to HLA (A2402/A02XX) and then randomized at the Data Center at a 2:1 ratio, using a minimization method and balancing the study arms according to institution, primary tumor (gallbladder cancer/other than gallbladder cancer), and history of surgical resection for the primary tumor (recurrent/advanced) to receive GC either with or without the WT1 peptide vaccine.

Treatment methods

For the patients in the phase I study, the GC and WT1 vaccine will be administered according to the following schedule: cisplatin (25 mg per m^2 of body-surface area) followed by gemcitabine (1000 mg per m^2) administered intravenously on days 1 and 8 every 3 weeks, with the vaccine (3 mg per body) injected subcutaneously alternating between 2 areas on the unilateral axillary fossa and inguen on days 1 and 8.

For both arms in the phase II study, GC will be administered according to the same dose and schedule as those used in the phase I study, but the vaccine will be administered only for the GC plus WT1 peptide vaccine arm.

The protocol treatments will be continued until disease progression, unacceptable toxicity, or patient refusal, although cisplatin will be continued for only a maximum of 24 weeks.

Follow-up

Enhanced abdominal computed tomography (CT)/magnetic resonance imaging, chest CT/X-rays, and tumor marker levels (carcinoembryonic antigen [CEA] and carbohydrate antigen [CA] 19-9) will be evaluated at least every 6 weeks during the protocol treatment. Patients will be seen on days 1 and 8 of every cycle for a physical examination to monitor their symptoms and the possible toxic effects of treatment. Adverse events will be graded according to the Common Terminology Criteria for Adverse Events (CTCAE) version 4.0.

Study design and statistical analysis

In the phase I study, six patients will be recruited to determine whether a WT1 peptide vaccine dose of 3 mg per body can be recommended for use in combination with GC. A dose of 3 mg per body is the recommended dose for the WT1 peptide vaccine when used in combination with GEM alone, as determined in the previous phase I study. If treatment-related DLTs occur in no more than two of the six patients, transition to the phase II study will be

permissible with the approval of the independent data monitoring committee. If DLTs occur in three or more patients, transition to the phase II study will be terminated.

In the phase II study, 100 patients will be allocated to either of the two arms to evaluate the safety and efficacy of GC plus WT1 peptide vaccine, in comparison with GC alone. The sample size was determined based on the feasibility of the study after considering the research period, the number of participating institutions, and the available financial resources. A total of 66 patients in the GC plus WT1 peptide vaccine arm would enable the 1-year overall survival rate to be estimated with an accuracy of ±10%.

Interim analysis and monitoring

We do not plan to perform an interim analysis in this study. In-house monitoring will be performed every 6 months by the Data Center to evaluate the study progress and to improve the quality of the study.

Discussion

So far, no consensus exists regarding the "best criteria" for evaluating the effectiveness of cancer immunotherapy. Evidence of therapeutic activity may be difficult to obtain in early-phase trials using standard endpoints such as the antitumor response according to the Response Evaluation Criteria in Solid Tumors (RECIST), because most cancer immunotherapies are not expected to result in notable tumor shrinkage. Recently published FDA guidance suggests that the development of a cancer vaccine may present different considerations for clinical trial design than the development of a traditional cytotoxic drug or biological product for the treatment of cancer (http://www.fda.gov/BiologicsBloodVaccines/GuidanceComplianceRegulatoryInformation/Guidances/default.htm).

We retrieved clinical trials using immunotherapy for biliary tract cancer through PubMed (http://www.ncbi.nlm.nih.gov/pubmed) and ClinicalTrial.gov (http://clinicaltrials.gov/), although no reports or ongoing studies were found in this category, except for two trials: our previous phase I study examining GEM plus the WT1 peptide vaccine [8], and another study (phase II) examining chemoradioimmunotherapy, with interleukin 2 and 13-cis-retinoic acid being used for the immunotherapy [11]. Both studies conducted for pancreatic or biliary tract cancer showed some promise for a survival advantage, although the reported evidence was immature. We initiated the current phase I and randomized phase II studies to evaluate the efficacy and safety of adding the WT1 peptide vaccine to GC for the treatment of advanced biliary tract cancer. These studies are only the initial step in the development of immunotherapy for this disease, although we hope that the trial may provide useful data for assessing the true activities of this treatment.

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Conflict of interest None.

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ORIGINAL ARTICLE

Effect of biliary drainage on chemotherapy in patients with biliary tract cancer: an exploratory analysis of the BT22 study

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Abstract

Background/purpose: Complications from biliary drainage in biliary tract cancer (BTC) may influence the relative dose intensity of chemotherapy or increase adverse events during chemotherapy. BT22 was a randomized phase II trial, the results of which were consistent with those of a phase III trial in non-Japanese that demonstrated the effectiveness of gemcitabine plus cisplatin combination therapy (GC) in BTC. The purpose of this exploratory analysis of the BT22 study was to identify the possible effects of biliary drainage on the efficacy and safety of GC or gemcitabine monotherapy (G).

Patients and Methods: The 83 BTC patients who received GC or G in BT22 were retrospectively analysed in two subgroups dependent upon whether biliary drainage was performed before study entry. Efficacy and safety of treatment (GC vs. G) were compared in these two groups.

Results: The GC arm had a higher 1-year survival rate and longer median survival time (MST) than the G arm independent of prior biliary drainage. Patients in the drainage subgroup developed cholangitis more frequently, however, the frequency of grade 3/4 adverse events did not differ between the treatment regimens with/without drainage.

Conclusions: Biliary drainage before chemotherapy did not affect the therapeutic efficacy or tolerability of chemotherapy using G or GC.

Keywords

biliary drainage, chemotherapy, gemcitabine, cholangitis, cisplatin, biliary tract cancer

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Introduction

Biliary tract cancer (BTC), while relatively rare in Western countries, is more common in Japan where it is the sixth leading cause of cancer death with approximately 17 000 deaths every year.[1] The mortality caused by BTC in Japan is higher than any other country, and far exceeds all Western countries.[2]

Developing an effective BTC treatment has become a high priority for Japan. At present the only curative treatment is surgical resection, and although an increasing number of patients undergo surgery each year, outcomes have met with only a varying degree of success. Patients with unresectable disease can only be managed with chemotherapy and supportive care for palliation of disease including biliary decompression. However, prognosis remains extremely poor in these patients.

No standard chemotherapy for BTC has been established. Many clinical trials of systemic chemotherapy have been conducted in BTC patients, but most of these were phase II trials that had small sample sizes and lacked a control group because of the rarity and heterogeneity of BTC. In 2009, the results of a phase III study of gemcitabine plus cisplatin (GC) vs. gemcitabine monotherapy (G) conducted in the United Kingdom (ABC-02 Study) were reported.[3] This previous study included 410 patients and is the largest clinical trial to be conducted in this field. The GC arm had

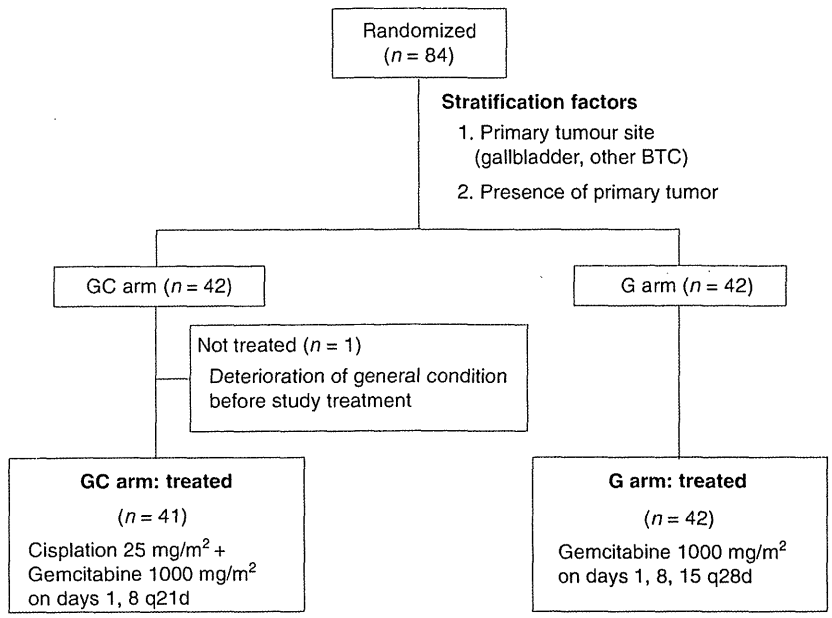

Figure 1 Patient disposition of the BT22 Study. BTC, biliary tract cancer; G arm, gemcitabine monotherapy; GC arm, combination therapy with gemcitabine and cisplatin

a significantly better median survival time (MST) [11.7 months (95% confidence interval (CI) 9.5 to 14.3) vs. 8.1 months (95% CI 7.1 to 8.7); $P < 0.001$] and progression-free survival (PFS) [8.0 months (95% CI 6.6 to 8.6) vs. 5.0 months (95% CI 4.0 to 5.9); $P < 0.001$] than the G arm. A comparison of grade 3 and 4 toxicities showed that the GC combination added little toxicity. From the results of the ABC-02 study, GC was recognized as the standard of care for the treatment of advanced BTC. A randomized phase II study comparing GC and G was also conducted in Japan (BT22 study; Clinical Trial.gov Identifier NCT00380588). Median survival time [11.2 vs. 7.7 months; hazard ratio (HR) 0.69 (95% CI 0.42 to 1.13)] and PFS [5.8 vs. 3.7 months; HR 0.66 (95% CI 0.41 to 1.05)] were similar to the results seen in the ABC-02 study confirming the status of GC as the worldwide standard.[4]

For patients with unresectable disease, biliary decompression is often required if chemotherapy is contemplated.[5] Usually, biliary obstruction will be managed by percutaneous or endoscopic drainage rather than a surgical approach because of the presence of incurable disease and high operative risk. However, complications resulting from insufficient biliary drainage, morbidities such as obstructive jaundice, cholangitis, and sepsis, often require that chemotherapy be interrupted or discontinued.[6] Obstructive jaundice may impact on prognosis by necessitating dose modification of chemotherapy or by complications as a consequence of biliary obstruction.

In the present study, we analysed the data from the BT22 study conducted in Japan to determine the impact of biliary drainage on the efficacy and adverse events associated with gemcitabine-based chemotherapy.

Patients and methods

Patients

This analysis included all patients who received at least one dose of study treatment in the BT22 study, a multicentre study conducted at nine medical institutions in Japan. From September 2006 to October 2008, 84 BTC patients were enrolled. The patients were randomized to either the GC arm [a weekly intravenous (i.v.) infusion of cisplatin 25 mg/m^2 followed by gemcitabine 1000 mg/m^2 for 2 weeks, followed by dose suspension at the third week, repeated as one course] or the G arm (weekly i.v. infusion of gemcitabine 1000 mg/m^2 for 3 weeks followed by dose suspension at the fourth week, repeated as one course). Randomization was stratified by primary tumour site (gallbladder cancer or other BTC) and the presence or absence of a primary tumour. One patient in the GC arm was discontinued before the start of the study treatment for deterioration of a general condition caused by another complication, so the analysis was conducted with 41 GC arm patients and 42 G arm patients (Fig. 1).

The specific criteria for study eligibility have been reported previously[4] and are only summarized here:

- patients with unresectable locally advanced or metastatic intrahepatic bile duct cancer, extrahepatic bile duct cancer, gallbladder cancer, or ampullary carcinoma that is histologically or cytologically confirmed adenocarcinoma (including adenosquamous carcinoma);
- patients with at least one measurable lesion;
- patients with no prior chemotherapy;
- patients with a performance status of 0 or 1;

Table 1 Patient characteristics at baseline

Characteristics	BD (n = 34)		Non-BD (n = 49)	
	n (%)		n (%)	
	GC arm (n = 16)	G arm (n = 18)	GC arm (n = 25)	G arm (n = 24)
Gender				
Male	7 (43.8)	9 (50.0)	11 (44.0)	12 (50.0)
Female	9 (56.3)	9 (50.0)	14 (56.0)	12 (50.0)
Age				
Median (years)	64.5	65.5	65.0	68.5
PS				
0	13 (81.3)	12 (66.7)	21 (84.0)	16 (66.7)
1	3 (18.8)	6 (33.3)	4 (16.0)	8 (33.3)
Primary tumour site				
Gallbladder	6 (37.5)	9 (50.0)	9 (36.0)	8 (33.3)
Non-gallbladder	10 (62.5)	9 (50.0)	16 (64.0)	16 (66.7)
Presence of a primary tumour				
Present	14 (87.5)	18 (100.0)	16 (64.0)	13 (54.2)
Absent	2 (12.5)	0 (0.0)	9 (36.0)	11 (45.8)
Extent of disease				
Locally advanced	5 (31.3)	7 (38.9)	0 (0.0)	1 (4.2)
Metastatic	11 (68.8)	11 (61.1)	25 (100.0)	23 (95.8)

BD, biliary drainage; G arm, gemcitabine monotherapy; GC arm, combination therapy with gemcitabine and cisplatin; PS, performance status.

- patients with an estimated life expectancy of more than 3 months; and
- patients with adequate organ function (e.g. bone marrow, liver and kidney).

Methods

The BT22 study was a randomized study that compared patients from two arms: GC vs. G.

Patients with obstructive jaundice had to achieve a certain degree of jaundice reduction with biliary drainage before study entry (i.e. total bilirubin was three times the upper limit of normal or less). The protocol contained no specific provisions about biliary drainage. The approach (endoscopic or percutaneous transhepatic), drainage type (internal biliary drainage or external biliary drainage) and stent material (plastic stent or metallic stent) could be decided by the investigator. The primary endpoint was 1-year survival rate. Sample size was calculated by the method proposed by Simon *et al.*[7] The 83 treated patients were retrospectively analysed and classified into subgroups of patients who had undergone biliary drainage before the start of the study (BD subgroup) and patients who had not (non-BD subgroup) to compare the efficacy and safety of the treatment regimens (GC vs. G arms). Progression-free survival and overall survival (OS) curves were constructed using the Kaplan–Meier method, and estimates of median OS and the respective 95% CIs were calculated from the Kaplan–Meier estimates. Cox's proportional hazard model was used to estimate the HR. Adverse events were graded according to the Common Terminology Criteria for Adverse Events, version 3.0 (CTCAE v. 3.0). A multivariate Cox proportional hazard model was used to identify prognostic factors.

Results

Of the 83 patients treated in the BT22 study, 34 were in the BD sugroup (16 in the GC arm and 18 in the G arm) and 49 were in the non-BD subgroup (25 in the GC arm and 24 in the G arm). Table 1 shows patient baseline characteristics. More of the patients in the BD subgroup (n = 34) had a primary tumour [GC: (14/16) 87.5%, G: (18/18) 100%], whereas the percentages of patients in the non-BD subgroup (n = 49) without a primary tumour were relatively higher [GC: (9/25) 36.0%, G: (11/24) 45.8%]. However, no substantial imbalances were noted between the two subgroups in gender, age or primary tumour site.

Efficacy

Efficacy data for the subgroups are shown in Table 2 and Fig. 2. In the BD subgroup, a comparison of OS in the GC and G arms showed 1-year survival of 40.9% vs. 27.8% and MST of 11.3 vs. 8.1 months [HR of 0.59 (95% CI 0.27 to 1.30)], respectively. In the non-BD subgroup, a comparison between the GC and C arms showed 1-year survival rate of 37.8% vs. 33.3% and MST of 9.6 vs. 7.5 months [HR of 0.76 (95% CI 0.40 to 1.45)], respectively.

Table 2 Overall survival and progression-free survival with or without biliary drainage by treatment arm

	BD (n = 34)		Non-BD (n = 49)	
	GC arm (n = 16)	G arm (n = 18)	GC arm (n = 25)	G arm (n = 24)
Overall survival				
1-year survival rate	40.9%	27.8%	37.8%	33.3%
Median survival time (months)	11.3	8.1	9.6	7.5
Hazard ratio (95% confidence interval)	0.588 (0.266–1.301)		0.758 (0.397–1.447)	
Progression-free survival				
6-month progression-free survival	53.3%	27.8%	43.7%	27.5%
Median progression-free survival (months)	7.1	3.9	4.5	3.3
Hazard ratio (95% confidence interval)	0.479 (0.222–1.032)		0.748 (0.407–1.374)	

BD, biliary drainage; G arm, gemcitabine monotherapy; GC arm, combination therapy with gemcitabine and cisplatin.

Subgroup analysis results based on HRs for OS by biliary drainage, performance status (PS), primary tumour site, presence of primary tumour and extent of disease are shown in Fig. 3. Hazard ratios (GC vs. G) for OS were less than one in every subgroup.

Safety

Adverse events observed in the GC and G arms with a frequency of at least 30% in the BT22 study have been reported.[4] In this analysis, the frequency of grade 3 and 4 events for the most common adverse events (frequency ≥30%) in the BD subgroup was compared with that in the non-BD subgroup (Table 3). Events in the BD subgroup that were more common in the GC arm were haemoglobin decrease (43.8% vs. 5.6%), thrombocytopenia (37.5% vs. 5.6%) and red blood cell decrease (37.5% vs. 5.6%). Events in the non-BD subgroup that were more common in the GC arm were leukopaenia (32.0% vs. 12.5%), neutoropenia (64.0% vs. 33.3%), and thrombocytopaenia (40.0% vs. 8.3%). There were no significant differences in the incidence of non-haematological events between the GC and G arms in either the BD or non-BD subgroup.

Although the incidence of cholangitis was higher in the BD subgroup than in the non-BD subgroup, the ratio of cholangitis in the GC arm to that in the G arm was not appreciably different in each of the BD and non-BD subgroups. For the BD subgroup, the incidence of Grade 3 and 4 cholangitis in the G arm was relatively higher than that in the GC arm (Table 4).

Prognostic factors

A multivariate Cox's proportional hazard model was used with the following six factors: biliary drainage, PS (0 vs. 1), primary tumour site (gallbladder vs. non-gallbladder), the presence of a primary tumour (present vs. absent), extent of disease (locally advanced vs. metastatic) and chemotherapy regimen (GC arm vs. G arm) (Table 5). The HR of the GC arm to the G arm was 0.72 [95% CI 0.44 to 1.20] after multivariate adjustment for several variables. The primary tumour site of non-gallbladder [HR of gallbladder vs. non-gallbladder 1.72 (95% CI 1.01 to 2.93)] and the absence of a primary tumour [HR of presence vs. absence 2.79 (95% CI 1.40 to 5.56)] were significantly related to a longer OS. Biliary drainage was suggested to have favourable clinical relevance [HR 0.72 (95% CI 0.39 to 1.32)], as well as PS 0 and locally advanced disease.

Discussion

Many studies on unresectable BTC retrospectively investigated whether biliary drainage should be performed endoscopically or with a percutaneous transhepatic approach,[8,9] and whether a plastic or metallic stent should be used.[10–15] Several of the few prospective studies conducted have stent patency or complication-free survival as a primary endpoint. However, only a few of those have considered the impact of drainage on chemotherapy efficacy or adverse events. This analysis represents an important exploratory investigation of the impact of biliary drainage on chemotherapy efficacy in the BT22 study which was a prospectively controlled study.

Insufficient biliary drainage leads to problems during chemotherapy, such as recurrent obstructive jaundice with or without cholangitis, which in turn often results in suspension or discontinuation of chemotherapy. In the BT22 study, the incidence of cholangitis during initial chemotherapy in the GC and G arms in the BD subgroup was higher than that in the respective arms of the non-BD subgroup. Although the profiles of grade 3 and 4 non-haematological adverse events in the GC and G arms did not differ, haematological toxicities were slightly more severe in the GC arm. GC therapy is expected to lead to an increased incidence of cholangitis or progression to severe cholangitis in patients undergoing biliary drainage who are at high risk of cholangitis mainly because the regimen has a more severe haematological toxicity profile than G. These events could undermine the efficacy of GC therapy.

In the BD subgroup, however, MST in the GC and G arms were 11.3 vs. 8.1 months, respectively, with an HR of 0.59 (95% CI 0.27 to 1.30). Median survival time in the GC arm was longer than MST in the G arm. As with adverse events overall, grade 3 and 4 adverse events in the BD subgroup were slightly more severe in the

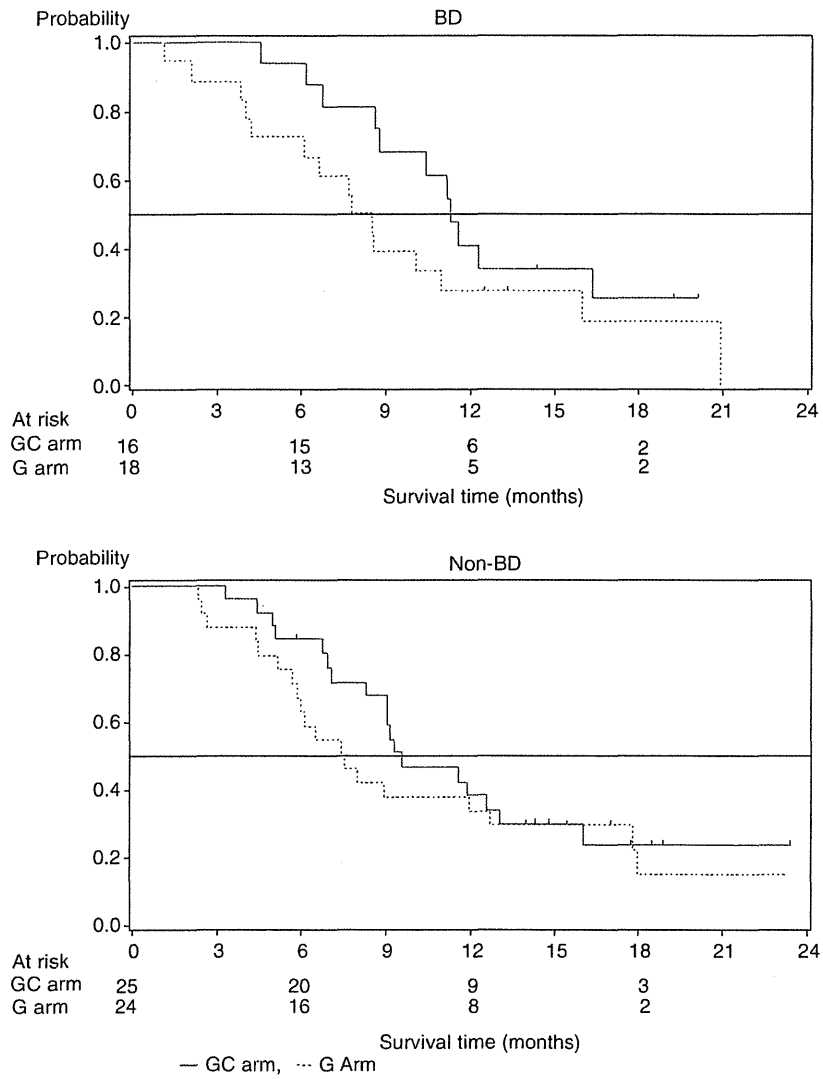

Figure 2 Survival curves in the BD subgroup (a) and non-BD subgroup (b) by the treatment arm. Solid line (—) indicates the GC arm and the broken line (---) the G arm. BD, biliary drainage; G arm, gemcitabine monotherapy; GC arm, combination therapy with gemcitabine and cisplatin

Subgroup		HR*	*P*-value	hazard ratio with 95% confidence interval
Biliary drainage	BD	0.588	0.1902	
	Non-BD	0.758	0.4006	
PS	0	0.759	0.3520	
	1	0.592	0.3344	
Primary tumour site	Gallbladder	0.852	0.6746	
	Non-gallbladder	0.578	0.1097	
Presence of primary tumour	Present	0.722	0.2525	
	Absent	0.602	0.3891	
Extent of disease	Locally advanced	0.591	0.4757	
	Metastatic	0.715	0.2181	

Figure 3 Hazard ratios for overall survival by patient baseline characteristics. HR*, hazard ratio of the combination therapy with gemcitabine and cisplatin (GC) arm to the gemcitabine monotherapy (G) arm

Table 3 Incidence of grade 3 or 4 events among most common adverse events[a]

Most common adverse events	BD (n = 34)		Non-BD (n = 49)	
	n (%)		n (%)	
	GC arm (n = 16)	G arm (n = 18)	GC arm (n = 25)	G arm (n = 24)
Hematologic				
WBC count decreased	4 (25.0)	5 (27.8)	8 (32.0)	3 (12.5)
Neutrophil count decreased	7 (43.8)	8 (44.4)	16 (64.0)	8 (33.3)
RBC decreased	6 (37.5)	1 (5.6)	8 (32.0)	5 (20.8)
Haemoglobin decreased	7 (43.8)	1 (5.6)	8 (32.0)	6 (25.0)
Haematocrit decreased	1 (6.3)	0 (0.0)	1 (4.0)	0 (0.0)
Platelet count decreased	6 (37.5)	1 (5.6)	10 (40.0)	2 (8.3)
Non-haematological				
Anorexia	0 (0.0)	0 (0.0)	0 (0.0)	2 (8.3)
Nausea	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Vomiting	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Constipation	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Diarrhoea	1 (6.3)	0 (0.0)	0 (0.0)	0 (0.0)
Fatigue	0 (0.0)	0 (0.0)	0 (0.0)	1 (4.2)
Pyrexia	0 (0.0)	2 (11.1)	0 (0.0)	0 (0.0)
Weight decreased	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
AST increased	3 (18.8)	5 (27.8)	4 (16.0)	2 (8.3)
ALT increased	4 (25.0)	5 (27.8)	6 (24.0)	2 (8.3)
GGT increased	6 (37.5)	7 (38.9)	6 (24.0)	8 (33.3)
LDH increased	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
ALP increased	1 (6.3)	3 (16.7)	2 (8.0)	4 (16.7)
Blood sodium decreased	4 (25.0)	2 (11.1)	3 (12.0)	2 (8.3)
C-reactive protein increased	0 (0.0)	2 (11.1)	0 (0.0)	1 (4.2)

[a]Most common, incidence ≥30% of all grades; events were graded according to CTCAE v3.0.
AST, aspartate aminotransferase; ALP, alkaline phosphatase; ALT, alanine aminotransferase; GGT, gamma-glutamyltransferase; BD, biliary drainage; G arm, gemcitabine monotherapy; GC arm, combination therapy with gemcitabine and cisplatin.

Table 4 Incidence of cholangitis with or without biliary drainage by treatment arm

	BD (n = 34)		Non-BD (n = 49)	
	n (%)		n (%)	
	GC arm (n = 16)	G arm (n = 18)	GC arm (n = 25)	G arm (n = 24)
Cholangitis (all grades)	5 (31.3)	7 (38.9)	2 (8.0)	3 (12.5)
Cholangitis (≥ grade 3)	2 (12.5)	5 (27.8)	0 (0.0)	1 (4.2)

BD, biliary drainage; G arm, gemcitabine monotherapy; GC arm, combination therapy with gemcitabine and cisplatin.

GC arm than in the G arm, but no particularly frequent events were encountered. In addition, patients with biliary drainage in the G arm had a relatively increased incidence of grade 3 and 4 cholangitis, possibly as a result of a lack of efficacy and inferior biliary drainage. Although careful monitoring of cholangitis is still needed, the above findings indicate GC to be an appropriate standard chemotherapy for unresectable BTC in both patients with and without biliary drainage.

Because this analysis was a retrospective investigation of the BT22 study, several biases could arise after this retrospective approach. Although the biases cannot be completely circumvented using a multivariate Cox's proportional hazard model, the results adjusted with other prognostic factors suggested that biliary drainage would not have a negative impact on the anticancer effect of chemotherapy. The results suggested that baseline biliary drainage did not adversely impact patient prognosis. The presence of a primary tumour (present vs. absent) and primary tumour site (gallbladder vs. non-gallbladder) had the greatest impact on the prognosis, which suggests that the stratification factors of the present study were appropriately selected.

Table 5 Multivariate analysis of prognostic factors using Cox's proportional hazard model

Covariate	*P*-value*	Hazard ratio (95% CI)
Biliary drainage (BD vs. non-BD)	0.2875	0.717 (0.389–1.323)
PS (1 vs. 0)	0.1620	1.532 (0.843–2.785)
Primary tumour site (gallbladder vs. non-gallbladder)	0.0454	1.722 (1.011–2.934)
Presence of primary tumour (present vs. absent)	0.0036	2.789 (1.398–5.564)
Extent of disease (metastatic vs. locally advanced)	0.4333	1.391 (0.609–3.176)
Treatment arm (GC arm vs. G arm)	0.2093	0.724 (0.437–1.199)

*Chi-square test.
BD, biliary drainage; CI, confidence interval; G arm, emcitabine monotherapy; GC arm, combination therapy with gemcitabine and cisplatin; PS, performance status.

As the BT22 study was conducted to compare chemotherapy regimens, the data available for investigating biliary drainage, e.g. the site of bile duct obstruction, the approach (endoscopic or percutaneous transhepatic) and the stent material (plastic stent or metallic stent), were limited. Thus, patient baseline characteristics varied substantially. A detailed subgroup analysis on biliary drainage was unfortunately infeasible with the limited sample size of the study. Moreover, this analysis was conducted to investigate the impact of whether or not biliary drainage was performed before starting chemotherapy, and patients requiring biliary drainage during chemotherapy were consequently included in the non-BD subgroup. Data on adverse events occurring during the primary treatment period are available, but adverse events occurring with more advanced cancer in secondary and subsequent treatments were not investigated. No definite conclusions about the relationship of biliary drainage to chemotherapy may therefore be drawn based on the findings of this analysis alone.

Conclusion

In this analysis, GC combination therapy was safely administered and the therapeutic efficacy of the GC arm was maintained in patients with or without biliary drainage. The presence or absence of biliary drainage was not found to impact the efficacy or adverse events in each treatment arm for unresectable BTC. Based on these results, it appears that adequate efficacy with gemcitabine-based chemotherapy can be expected in patients with BTC even with biliary drainage.

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Conflicts of interest

Masanori Taketsuna and Minori Koshiji are employees of Eli Lilly Japan K.K. Takuji Okusaka has received funding to research and attend/present at conference from Eli Lilly Japan K.K. Junji Furuse has received funding to attend/present at conference from Eli Lilly Japan K.K. The authors report no other conflicts of interest.

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Phase 1 Trial of Wilms Tumor 1 (WT1) Peptide Vaccine and Gemcitabine Combination Therapy in Patients With Advanced Pancreatic or Biliary Tract Cancer

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Summary: An open-labeled, dose-escalation phase 1 trial of Wilms tumor 1 (WT1) vaccine and gemcitabine (GEM) combination therapy for patients with advanced pancreatic cancer or biliary tract cancer was performed. The primary end point was evaluation of toxicity, safety, and optimal immunologic dose of vaccine. Human leukocyte antigen (HLA)-A 0201, HLA-A 0206, and/or HLA-A 2402-positive patients with inoperable advanced pancreatic or biliary tract cancer who had not previously been treated with GEM were eligible for this study. Six doses of GEM and 4 doses of WT1 peptide (1 or 3 mg) emulsified in Montanide adjuvant were administered over 2 months. Twenty-five patients (13 male and 12 female) were enrolled. Nine patients had inoperable advanced pancreatic cancer, 8 had gallbladder cancer, 4 had intrahepatic, and 4 had extrahepatic bile duct cancer. The adverse events were comparable to those with GEM alone. Delayed-type hypersensitivity test was positive after vaccination in 2 patients, and WT1-specific T cells in peptide-stimulated culture were detected by tetramer assay in 59% (13 of 22) of patients. The disease control rate at 2 months was 89% for pancreatic cancer and 50% for biliary tract cancer. With a median follow-up time of 259 days, the median survival time for biliary tract cancer was 288 days, and that for pancreatic cancer was 259 days. Although objective clinical efficacy was not apparent, the safety of WT1 vaccine and GEM combination therapy was confirmed in this study.

Key Words: WT1 peptide vaccine, gemcitabine, pancreatic cancer, biliary tract cancer

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As Wilms tumor 1 (WT1) protein is overexpressed in various types of cancer cell,[1–6] it is an attractive candidate for cancer immunotherapy.[7–11] WT1 has recently been ranked as the number 1 antigen in the cancer antigen prioritization project sponsored by the National Cancer Institute.[12] WT1 peptide-based immunotherapy has been reported for various cancers, including leukemia, myelodysplastic syndromes, lung cancer, renal cell cancer, breast cancer, glioblastoma, and gynecologic cancer.[13–17] In this study, we administered a WT1 peptide vaccine combined with chemotherapy in pancreatic cancer and biliary cancer, as overexpression of WT1 is seen in 65% to 75% of these disorders.[5,6] Moreover, the observation that WT1 protein is present in the cytoplasm of pancreatic ductal adenocarcinoma cells in the majority of cases[5] has encouraged clinical trials of WT1-based immunotherapy.

At present, surgery is the only radical therapeutic option for pancreatic and biliary tract cancers. In addition, gemcitabine (GEM) has been a key drug in chemotherapy for advanced pancreatic cancer resulting in improved survival and clinical benefits with GEM as a first-line therapy.[18] Combination of GEM with other agents is one promising avenue for improving the efficacy of treatment for advanced pancreatic cancer. In fact, a recent randomized phase 3 study of the combination of GEM/erlotinib showed a statistically significant survival benefit in comparison with GEM alone in patients with advanced pancreatic cancer,[19] although there is no worldwide consensus. Furthermore, advanced biliary tract cancer is often treated with GEM[20] and combination therapy with cisplatin has been shown to have survival benefits when compared with GEM monotherapy.[21] Nevertheless, the ultimate effects of chemotherapy alone in pancreatic cancer and biliary tract cancer remain limited, with long-term survival being very rare.[20,22]

The combination of GEM with immunotherapy is therefore attractive, as GEM does not suppress immunologic cells and increases the number of dendritic cells, which serve as antigen-presenting cells. To date, only 1 clinical trial of immunotherapy on pancreatic cancer using a personalized peptide has been reported,[23] and this study is the first reported clinical trial of the combination of WT1 vaccine and GEM chemotherapy.

MATERIALS AND METHODS

Patients

The protocol was approved by the Institutional Ethics Review Board at the National Cancer Center of Japan. Human leukocyte antigen (HLA)-A 0201, HLA-A 0206, and/or HLA-A 2402-positive patients with inoperable advanced pancreatic or biliary tract cancer were eligible for this study.

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Other inclusion criteria were: (1) pathologically confirmed diagnosis of adenocarcinoma or adenosquamous carcinoma; (2) no previous history of treatment by GEM; (3) Eastern Cooperative Oncology Group Performance Status of 0 to 2; (4) expected survival of at least 2 months; (5) aged 20 years or more; (6) adequate main organ function; and (7) provision of written informed consent.

Exclusion criteria were as follows: (1) active infection; (2) severe complications such as heart failure, renal failure, hepatic failure, active gastric ulcer, gastric paralysis, or uncontrollable diabetes; (3) ascites or pleural effusion; (4) severe mental disorder; (5) metastasis to the central nervous system; (6) pregnancy or breast feeding; (7) interstitial pneumonia or pulmonary fibrosis; (8) myeloproliferative disease; (9) history of autoimmune disease; and (10) administration of immunosuppressive drug or corticosteroids.

Study Design

This study was an open-labeled, dose-escalation phase 1 study. The primary end point was evaluation of toxicity, safety, and optimal immunologic dose of combined GEM and WT1 vaccination, and determination of the recommended dose for the phase 2 study. The secondary end point was evaluation of response rate and progression-free survival. Toxicity was evaluated according to the Common Terminology Criteria for Adverse Events (CTCAE v3.0), and treatment efficacy was determined according to the Response Evaluation Criteria in Solid Tumors.

GEM and WT1 vaccine were administered every 28 days as follows: intravenous infusion of GEM (1000 mg/m^2) on days 1, 8, and 15 with 1-week rest. Vaccine (0.1 mL) was injected intradermally into 6 areas (bilateral arms, 2 sites on the lower abdomen and femoral areas) biweekly on day 8 and day 22. Although the scheduled study period was 2 courses, treatment could be continued at the patient's request if there was no disease progression or serious adverse events.

The first vaccination dose (1 mg) was administered to 3 patients, and the dose was increased to the second dose level of 3 mg if no dose-limiting toxicity was observed. When no toxicity was observed in 6 patients who received the second dose level of 3 mg, the study was completed.

WT1 Vaccine Preparation

HLA-A02-restricted WT1 126-134 peptide (RMFP NAPYL) and HLA-A24-restricted WT1 235-243 peptide (CYTWNQMNL) were synthesized at good manufacturing practice grade by NeoMPS (San Diego, CA). WT1 peptides were dissolved in dimethyl sulfoxide (DMSO; Sigma, St Louis, MO) and 5% glucose. Solutions were emulsified with an equal weight of Montanide ISA-51VG adjuvant (Seppic, Paris, France).

Immunologic Analysis

Peripheral blood samples were obtained before vaccination and on day 15 of the first course, on days 1 and 15 of the second course, and on day 1 of the third and fourth courses. Surface marker analysis, multimer assay, and intracellular cytokine staining were performed on the day of sampling. Mixed lymphocyte and peptide culture (MLPC) was performed with the remaining blood preserved as peripheral blood mononuclear cells.

Delayed-type Hypersensitivity (DTH) Test

Delayed-type hypersensitivity (DTH) test was performed before the first vaccination in 20 patients, and after the fourth and tenth vaccinations, if possible. DTH was examined by intradermal injection of 30 μg WT1 peptide dissolved in 50 μL DMSO and saline as a negative control. DTH was measured in terms of maximum diameter of induration or erythema at the injection site at 48 to 72 hours after injection.

Surface Marker Analysis

Whole blood samples were incubated with monoclonal antibodies for 30 minutes at room temperature in the dark. Red blood cells were lysed using PharmLyse [Becton Dickinson (BD), San Diego, CA], and after being washed with Cell Wash (BD), cells were fixed (CellFix, BD) and acquired on a flow cytometer (FACSCalibur, BD). Analyses were performed using CellQuest software.

Multimer Assay

Allophycocyanin-conjugated pentamers and dextramers for WT1/HLA-A*02 (RMFPNAPYL) and WT1/HLA-A*24 (CYTWNQMNL), human immunodeficiency virus/HLA-A*02 (ILKEPVHGV), and human immunodeficiency virus/HLA-A*24 (RYLRDQQLL) as negative controls, and cytomegalovirus (CMV)/HLA-A*02 (NLVPMVATV) and CMV/HLA-A*24 (QYDPVAALF) as positive controls were purchased from Proimmune (Oxford, UK) or provided by Dako Instruments (Glostrup, Denmark).

Whole blood was stained with multimer for 15 minutes, followed by staining with CD8 peridinin chlorophyll protein complex, CD3 fluorescein isothiocyanate (FITC), and CCR7 phycoerythrin for 10 minutes at room temperature in the dark. Subsequent steps were the same as for surface marker analysis.

Intracellular Cytokine Staining

Whole blood (1 mL) was stimulated with 1.0 μM WT1 peptide, DMSO (negative control), or CMV lysates (positive control) for 6 hours at 37°C, in the presence of 10 μg/mL CD28 and CD49d as costimulatory monoclonal antibodies. Breferdin A (Sigma) was added during the last 4 hours of stimulation. After 6 hours of incubation, samples were kept at 4°C overnight and were then lysed, permeabilized, and washed. After staining with CD69 FITC, interferon-γ (or interleukin-4) phycoerythrin, and CD3 allophycocyanin for 30 minutes in the dark, samples were washed, fixed, and acquired on a flow cytometer (FACSCalibur, BD).

MLPC

Peripheral blood mononuclear cells samples were thawed and washed with culture medium (10% fetal bovine serum in Roswell Park Memorial Institute medium). Cells were stimulated with WT1 peptide at a final concentration of 10 μg/mL or with DMSO as a negative control, and were cultured in a 96-well round-bottomed plate at 2×10^5 cells/well. Culture medium containing 100 U/mL interleukin-2 was added on days 2 and 9 or 10. Cultured cells were collected on days 10 to 14, washed and were stained with WT1-tetramer or negative tetramer, CD8 FITC and 7-aminoactinomycin D. Cells were analyzed on a flow cytometer. Results were defined as positive when 7-aminoactinomycin D-negative CD8-positive WT1-tetramer-positive cells were detected in WT1 culture wells, and no CD8-positive tetramer-positive cells were detected in negative controls.

Statistical Analysis

Overall survival and progression-free survival were calculated from the date of assignment into the study to the

date of death or final follow-up and the date of disease progression. Overall survival estimates were calculated using the Kaplan-Meier method, and the survival curves were compared between primary disease arms using the log-rank test. Wilcoxon-Mann-Whitney *U* test was used for the statistical analysis of the immunologic assays.

RESULTS

Patient Characteristics

Between November 2007 and September 2009, 25 patients (13 male and 12 female) were enrolled in this study. Patient characteristics are presented in Table 1. The median age was 65 years (range: 30–79 y). Nine patients (36%) had inoperable advanced pancreatic cancer, 8 (32%) had gallbladder cancer, 4 (16%) had intrahepatic bile duct cancer, and 4 (16%) had extrahepatic bile duct cancer. One patient (4%) had previously received chemotherapy with an oral fluoropyridine (S-1), 6 (24%) had undergone surgery, whereas 11 (44%) had received biliary drainage. Eighteen patients (72%) were at clinical stage IV, and 7 (28%) were at stage III. Fourteen patients positive for HLA-A*2402 were treated with HLA-A24-restricted WT1 235-243 peptide, and 9 HLA-A*0201-positive and 2 HLA-A*0206-positive patients, including 4 patients positive for both HLA-A*0201 and HLA-A*2402, were treated with HLA-A02-restricted WT1 126 to 134 peptide. Seven patients were treated at the first dose level (1 mg/dose) of WT1 vaccine and 18 were treated at the second dose level (3 mg/dose).

Eighteen patients (72%) completed the protocol, and 7 patients (28%) left the study because of rapid disease progression (6 patients) or patient choice (1 patient). Fifteen patients continued compassionate combined GEM and WT1 vaccination therapy after completing the protocol.

Toxicity

As no dose-limiting toxicities were observed at the first dose level, the dose was increased to the second level after 3 patients each completed the HLA-02 and HLA-24 peptide administration at the first dose level. No dose-limiting toxicities were seen throughout the study.

Toxicities documented within the 2 months are shown in Table 2. All patients experienced grade 1 or 2 skin reactions at the site of vaccination; redness and pruritus at the injection site were observed in 25 patients (100%), and induration was seen in 23 patients (92%). Although no patients dropped out of the study due to skin reactions, 2 patients (UPN10 and 19) elected to discontinue treatment because of skin reactions after study completion. In particular, 1 patient (UPN19) discontinued vaccination at 5 months as she developed skin ulcers after the tenth vaccination. Although she continued treatment with GEM alone after the appearance of ulcers, she developed new ulcerations at the injection sites 2 weeks later. Another patient (UPN10) developed severe induration, pruritus, and swelling at the injection site, and had swollen lymph nodes near the vaccination site after 8 months of treatment. Vaccination therapy was terminated at 9 months and treatment with GEM alone was continued because the disease was stable. Despite withdrawal of vaccination treatment, local reactions did not improve and itching, redness, and nodules remained for another 3 months.

Cytopenia, thought to be caused by GEM, was observed in all 25 patients, including 11 with grade 3 to 4 neutropenia and 3 patients with grade 3 anemia. Grade 1 to 2 gastrointestinal symptoms probably because of GEM, such as anorexia (52%), nausea (48%), and vomiting (12%), were also observed.

Clinical Response

Disease status was assessed at the end of the study based on tumor size and metastasis examined by computed tomography. Blood tests for tumor markers such as carcinoembryonic antigen and cancer antigen 19-9 were evaluated as reference data (not considered to be response criteria). The results showed that 15 of the 18 patients who completed the study had stable disease and 3 had progressive disease (PD).

The median survival time of all patients was 278 days: biliary tract cancer, 288 days (gallbladder cancer, 153 days; intrahepatic bile duct cancer, 384 days; and extrahepatic bile duct cancer, 301 days) and pancreatic cancer, 259 days (Fig. 1). Disease control rate at 2 months was 89% for pancreatic cancer, 25% for gallbladder cancer, 100% for intrahepatic bile duct cancer, and 50% for extrahepatic bile duct cancer.

Survival did not significantly differ between patients who received HLA-A02-restricted and HLA-A24-restricted vaccine ($P = 0.39$) (Fig. 2).

Immunologic Responses

No patients exhibited DTH reactivity at pretreatment. Two of the 20 patients showed positive DTH reactions after the fourth vaccination (UPN18 and 19), and 1 patient was positive after the tenth or twelfth vaccination (UPN18).

Surface marker analysis showed that $CD14^+$ monocytes and 2 types of dendritic cells, $CD123^+$ and $CD11c^+$, were significantly elevated whereas the absolute number of most immune cells decreased. The number of natural killer cells and B cells significantly decreased after the fourth course (2 mo). The changes in $CD3^+/CD8^+$ T cells, $CD3^+/CD4^+$ T cells, $CD3^+/CD4^+/CD25^+$, and $CD4^+/CD25^+/GITR^+$ T regulatory cells were not significant (Table 3). WT1-specific T cells were not detectable in uncultured fresh whole blood on either dextramer or pentamer assay. Intracellular interferon-γ production of peripheral lymphocytes stimulated by WT1 peptide was also not significant when compared with negative controls.

MLPC analysis was available from all patients before vaccination, from 20 patients after the second vaccination, from 16 patients after fourth vaccination, and from 9 patients after sixth vaccination or more (Table 4). Positive results were observed at least once after vaccination in 65% (13 of the 20) of the patients. Representative results of MLPC analysis are shown in Figure 3. Only 1 of 25 samples taken before vaccination showed WT1-specific T lymphocytes. The positivity rates for MLPC after the second, fourth, sixth, twelfth, and 30th vaccinations were 25% (5 of 20), 50% (8 of 16), 56% (5 of 9), 33% (2 of 6), and 100% (1 of 1), respectively. Two patients showed positive results for the first time after the sixth and twelfth vaccinations (UPN12 and 19), whereas in another 2 patients, WT1-specific lymphocytes were detected after the fourth vaccination, and these subsequently disappeared during repeated vaccination therapy (UPN1 and 22).

DISCUSSION

In this clinical phase 1 study, we evaluated the safety and efficacy of GEM and WT1 vaccine combination therapy in patients with advanced pancreatic or biliary

TABLE 1. Patient Characteristics

UPN	Stage	Previous Therapy	Age (y)	Sex	HLA	Peptide Dose (mg)	WT1 Dose	GEM Dose	MLPC Response	Response at 2 mo	Day of PD	Survival
Pancreatic cancer												
1	III	BD	59	M	2402	1	25	36	2/5	SD	358	772
2	IV	Chemo	64	M	2402	3	3	5	1/2	PD	43	247*
3	III		71	M	2402	3	2	3	0/1	SD	146	340*
4	III	BD	66	M	2402	3	11	18	2/5	SD	196	275*
5	IV		58	M	2402	3	5	7	1/3	SD	77	259*
6	IV	BD	61	F	0201	3	11	16	0/2	SD	147	217
7	IV	BD	71	M	0206	3	10	15	0/3	SD		225
8	IV	Ope	65	M	0201	3	5	8	0/3	SD	84	141*
9	III		79	F	0206	3	5	9	0/1	SD	77	118*
Gallbladder cancer												
10	IV		75	F	2402	1	17	26	0/5	SD	574	784
11	IV		48	F	0201	1	4	6	1/3	PD	56	278*
12	IV	Ope	76	F	0201	3	21	33	1/4	SD		322
13	IV	BD	61	M	2402	3	3	4	1/2	PD	40	153
14	IV	BD	61	M	0201	1	4	6	2/3	PD	64	146*
15	IV	BD	74	F	2402	3	3	6	0/2	PD	44	107*
16	IV	Ope	51	M	2402	1	1	3	0/1	PD	22	81*
17	IV	BD	68	F	0201	3	1	3	0/1	PD	18	68*
Intrahepatic bile duct cancer												
18	IV		32	F	2402	3	45	70	3/6	SD		720
19	IV	Ope, BD	74	F	0201	3	10	19	1/5	SD	281	384*
20	IV	BD	59	M	0201	1	8	13	3/4	SD	130	363*
21	III	BD	63	F	2402	1	10	18	2/4	SD	174	288*
Extrahepatic bile duct cancer												
22	III	BD	59	F	2402	3	40	43	2/5	SD		686
23	III	BD	69	M	2402	3	13	14	0/3	SD	185	301*
24	IV	Ope	69	M	2402	3	4	6	0/3	PD	56	148*
25	IV	Ope	68	F	0201	3	2	4	0/1	PD	35	63*

UPN 6, 17, 20, and 25 were also positive for HLA 2402.
UPN 10 and 19 discontinued WT1 vaccine because of local skin reactions.
UPN 18 and 19 showed positive delayed-type hypersensitivity reaction.
UPN 3 discontinued WT1 vaccine by choice.
UPN 7, 12, 18, and 22 continue to show SD and are still receiving WT1 vaccine.
*Patient died.
BD indicates biliary drainage; Chemo, chemotherapy with oral fluoropyridine (S-1); F, female; GEM, gemcitabine; HLA, human leukocyte antigen; M, male; MLPC, mixed lymphocyte peptide culture; Ope, operation; PD, progressive disease; SD, stable disease; WT1, Wilms tumor 1.

TABLE 2. Toxicities Within 2 mo (n = 25)

	Grade 1	Grade 2	Grade 3	Grade 4
Fatigue	7	1		
Anorexia	11	2		
Nausea	12			
Vomiting	3			
Fever	2			
Depilation	1			
Generalized rash		4		
Injection site reaction				
Redness	25			
Pruritus	25			
Induration	23			
Stomatitis	2			
Gastromegaly		1		
Leukopenia	5	9	6	
Neutropenia	2	5	7	4
Lymphopenia	6	5		
Anemia	7	11	3	
Thrombocytopenia	10	3		
Hypoalbuminemia	4			
ALT elevation	1			
γ-GTP elevation	1			
Creatinine elevation	1			

γ-GTP indicates glutamyl transpeptidase; ALT, alanine aminotransferase.

tract cancer. This combination therapy was found to be safe with mild toxicity. No dose-limiting toxicities were observed during the study period. Hematopoietic toxicity occurred in all patients; however, the frequency and severity was comparable to that of GEM treatment alone. Grade 1 to 2 gastrointestinal toxicities, which were seen in approximately half of patients, were also considered to be a consequence of GEM toxicity. All other adverse events were of grade 1 and considered to be because of the primary disease. There was no apparent difference in adverse events between the HLA-A02 and HLA-A24-restricted peptide vaccines.

Although some patients showed relatively good clinical outcomes during this study, the clinical efficacy of WT1 vaccine was not apparent from this study. One patient with intrahepatic bile duct cancer and another patient with

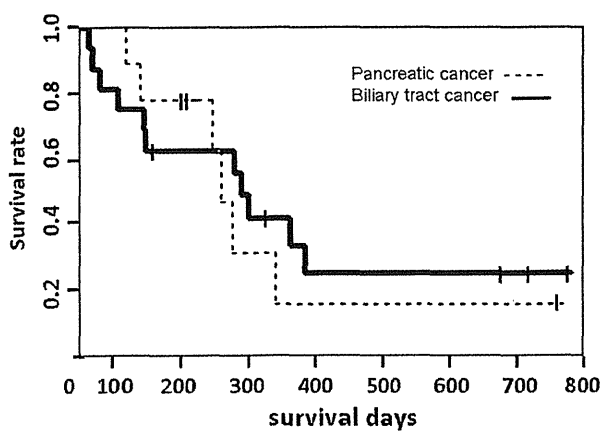

FIGURE 1. Kaplan-Meier estimates of overall survival for biliary tract cancer and pancreatic cancer. Median survival time for biliary tract cancer (n = 16) was 288 days and for pancreatic cancer (n = 9) was 259 days. There were no significant differences between the 2 curves ($P = 0.78$).

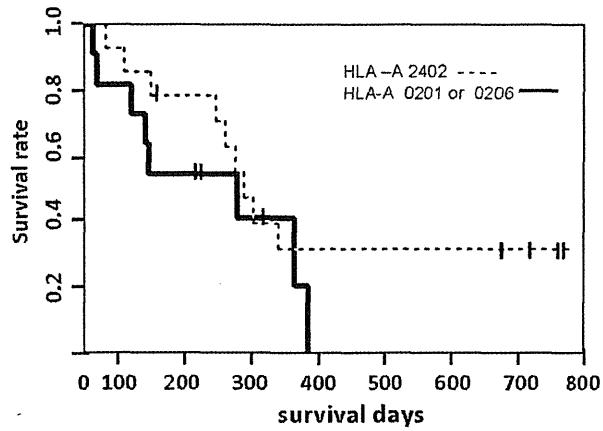

FIGURE 2. Kaplan-Meier estimates of overall survival for patients who received HLA-A 02 and HLA-A24-restricted vaccine. Median survival time in patients who received HLA-A 02 vaccine (n = 11) was 278 days and for those who received HLA-A24 vaccine (n = 14) was 288 days. Survival was not significantly different between the 2 groups ($P = 0.39$). HLA indicates human leukocyte antigen.

extrahepatic bile duct cancer have continued receiving this combination therapy for 22 and 21 months, respectively, and the disease has remained stable. One patient with pancreatic cancer showed a reduction in tumor size at 3 months. However, overexpression of WT1 was not determined in this study, and it is likely that GEM exerted a major effect on this particular patient. GEM monotherapy showed far better survival than historical controls in the Japan Clinical Oncology Group 0506 phase 2 study for locally advanced pancreatic cancer,[24] and survival among patients treated in the 2000s, after the introduction of GEM in Japan, was significantly better than that of patients treated in the 1980s and 1990s.[25]

Six patients could not complete this study because of rapid disease progression. The reason for high PD rate in gall bladder cancer was that most of the patients with gall bladder cancer enrolled in this study had highly advanced disease, whereas 2 patients with relatively well-controlled disease have survived for years. Vaccination therapy seems to have a smaller effect on those with rapid PD, possibly because it takes at least 2 months to induce antitumor effects by vaccination. Administration of vaccine at earlier disease states when adequate immunity is preserved thus seems to be necessary. Vaccine therapy in combination with other treatment modalities that do not suppress host immunity, such as radiation therapy, may also improve efficacy.

Two cases who continued the therapy after the study period showed severe local skin reactions. These severe skin reactions have not been reported earlier with WT1 vaccine therapy, and are considered to be because of the additive effects of GEM on WT1 peptide. Surface marker analysis of peripheral blood showed similar results to our earlier study on the immunologic effects of GEM,[26] confirming an increase in monocytes and dendritic cells during GEM administration. The increase in dendritic cells may have had an effect on local inflammation at the injection sites in the present cases. It was difficult to predict the patients who were likely to develop severe local reactions, as the results of immunomonitoring were not distinguishable from those

TABLE 3. Surface Markers

	Pre (n = 25)	2 mo (n = 17)		7 mo (n = 7)	
	Median (Range)/μL	Median (Range)/μL	*P*	Median (Range)/μL	*P*
$CD14^+$ monocytes	234 (90–641)	376 (182–1090)	0.019*	294 (103–858)	0.16
$CD123^+$ DC $Lin1^-/CD123^+/HLA\text{-}DR^+$	6.4 (1.3–15.0)	10.5 (6.4–29.1)	< 0.001*	11.6 (9.7–35.6)	< 0.001*
$CD11c^+$ DC $Lin1^-/CD11c^+/HLA\text{-}DR^+$	13.7 (2.4–25.2)	16.1 (7.6–35.8)	0.024*	19.3 (13.2–37.7)	0.017*
NK-cell $CD3^-/CD16^+/CD56^+$	207 (91–1235)	95.6 (35.9–443)	0.004*	111 (28.4–868)	0.007*
B-cell $CD14^-/CD20^+$	178 (60.7–414)	149 (58.5–297)	0.032*	119 (49.2–201)	0.018*
$CD4^+$ T-cell $CD3^+/CD4^+/CD8^-$	619 (96.8–1652)	499 (151–959)	0.38	517 (123–869)	0.23
$CD8^+$ T-cell $CD3^+/CD8^+/CD4^-$	461 (159–811)	425 (161–856)	0.37	418 (261–549)	0.59
$CD3^+/CD4^+/CD25^+$	254 (90.7–825)	199 (53.4–510)	0.46	219 (62.0–462)	0.22
$CD4^+/CD25^+/GITR^+$	7.7 (0.29–17.6)	8.6 (1.8–27.6)	0.30	9.9 (1.0–34.0)	0.79

*Statistically significant ($P < 0.05$).
P: Statistical significance of values at 2 and 7 mo in comparison with values before (Pre) vaccination.
DC indicates dendritic cells; HLA, human leukocyte antigen; NK, natural killer.

of other patients.[27] WT1-specific lymphocytes were detectable by MLPC for the first time after 12 vaccinations in the first patient (UPN19), and no WT1-specific lymphocytes were detected throughout the course in the other patient (UPN10). Nevertheless, it is probable that the features of local immunologic status may differ from those of circulating lymphocytes in the peripheral blood.

No WT1-specific lymphocytes were detected on multimer staining in noncultured fresh whole blood. As WT1-specific lymphocytes were detected by MLPC methods, it is likely that the frequency of circulating WT1-specific lymphocytes was very low and below the detection level without expansion. WT1 vaccination is thought to have an expansion effect on precursor WT1-specific lymphocytes, as

TABLE 4. Mixed Lymphocyte Peptide Culture Analysis

No. Vaccination	Pre	2	4	6	12	30
Positive Rate	4% (1/25)	25% (5/20)	50% (8/16)	56% (5/9)	33% (2/6)	100% (1/1)
Pancreatic cancer						
1	0	0	5.26	1.86	0	
2	0	0.81				
3	0					
4	0	0	1.31	0	1.90	
5	0	0	2.75			
6	0	0				
7	0	0	0			
8	0	0	0			
9	0					
Gallbladder cancer						
10	0	0	0	0	0	
11	0	0	2.76			
12	0	0	0	1.40		
13	0	4.58				
14	0	0.56	3.02			
15	0	0				
16	0					
17	0					
Intrahepatic bile duct cancer						
18	0	29.40	9.40	0	0	3.87
19	0	0	0	0	0.35	
20	0	1.47	0.24	0.51		
21	0.16	0	0	5.29		
Extrahepatic bile duct cancer						
22	0	0	1.45	14.94	0	
23	0	0	0			
24	0	0	0			
25	0					

Percentages of Wilms tumor 1 $tetramer^+/CD8^+$ lymphocytes per $CD8^+$ lymphocytes are indicated.

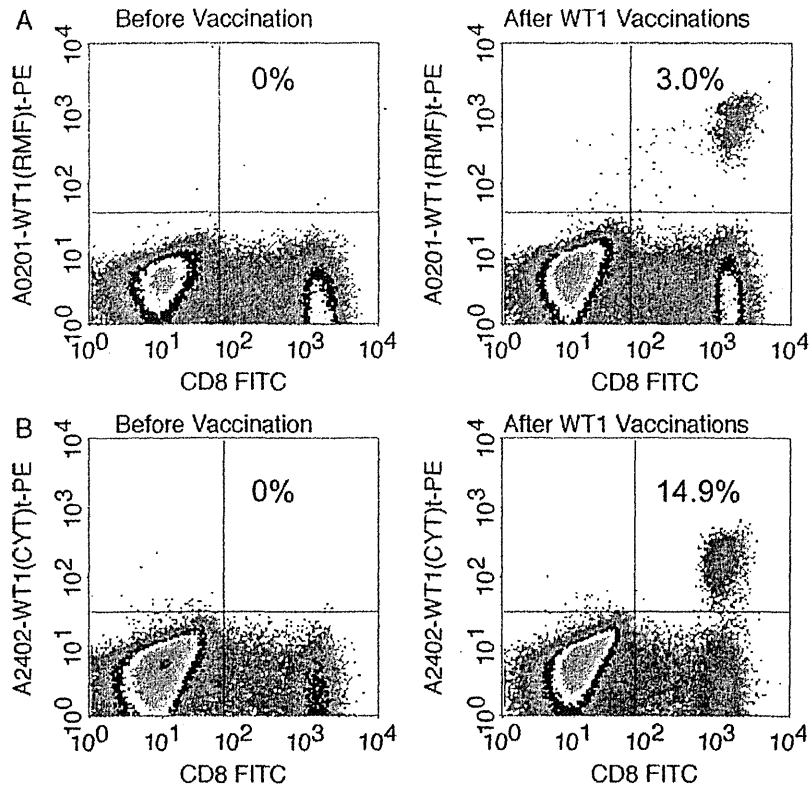

FIGURE 3. Representative results of mixed lymphocyte and peptide culture analysis. A, HLA-A 0201-positive patient with gallbladder cancer (UPN14). B, HLA-A 2402-positive patient with extrahepatic bile duct cancer (UPN22). No $CD8^+$ $WT1\text{-tetramer}^+$ cells were detected before vaccination therapy (left), whereas expansion of WT1-specific T lymphocytes was detected after vaccinations (right). FITC indicates fluorescein isothiocyanate; HLA, human leukocyte antigen; WT1, Wilms tumor 1.

only 1 patient showed positive results before vaccination, whereas 65% of patients showed positive results at least once after vaccination therapy. However, we were unable to show an apparent relationship between the therapeutic effects and the emergence of WT1-specific lymphocytes in this study. Furthermore, induction of WT1-specific lymphocytes required a long period of time in some patients, whereas WT1-specific lymphocytes disappeared during repetition of this combination therapy in some patients. Disappearance of WT1-specific T lymphocytes may be because of T-cell anergy. The optimal immunologic dose of WT1 vaccine may therefore differ among individual patients.

The WT1 peptide dose used in this study was larger than those used in other studies. The second dose level of 3 mg is the maximum dose that can be emulsified in a final volume of 600 μL, which we consider to be the maximum practical and realistic volume that can be injected intradermally at 6 sites (100 μL/site). The vaccine was injected intradermally to enhance immune reactivity, as the Langerhans cells that serve as antigen-presenting cells are distributed in the spinous layer of the epidermis. We were unable to determine the optimal dose for the WT1 vaccine, as the maximum tolerable dose may not be equivalent to the optimal dose, and a dose escalation study, as used in chemotherapy, is not applicable to cancer immunotherapy; thus, development of a realistic immunomonitoring system to determine the optimal vaccine dose is necessary.

Two types of 9-mer peptide, HLA-A02 and HLA-A24-restricted WT1 peptides, were used in this study. These peptides may be applied to the worldwide population, as HLA-A0201 and A2402 accounts for 57% of the Asian population, 56% of White population, and 17% of the African population.[28] The peptide earlier reported as HLA-A0201 restricted was applied to both HLA-A0201 and HLA-A0206 patients, as antigen-specific T cells against this peptide have been detected in relation to graft-versus-tumor effects in HLA-A0206-positive patients who had undergone hematopoietic stem cell transplantation in our earlier studies, thus suggesting the potency of this antigen in HLA-A0206 patients.[29] The HLA-binding motif prediction also showed that this peptide had a common anchor site with HLA-0206, which suggests that it could be applied to HLA-A0206 patients.[30]

In conclusion, although the aim of this study was to assess the safety of the combination of WT1 peptide vaccine and GEM in a small population, our observations indicated that this therapy is safe for patients with advanced pancreatic or biliary tract cancer and may provide long-term survival benefits in some patients.

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Review

Targeted Therapy for Biliary Tract Cancer

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Abstract: It is necessary to establish effective chemotherapy to improve the survival of patients with biliary tract cancer, because most of these patients are unsuitable candidates for surgery, and even patients undergoing curative surgery often have recurrence. Recently, the combination of cisplatin plus gemcitabine was reported to show survival benefits over gemcitabine alone in randomized clinical trials conducted in the United Kingdom and Japan. Thus, the combination of cisplatin plus gemcitabine is now recognized as the standard therapy for unresectable biliary tract cancer. One of the next issues that need to be addressed is whether molecular targeted agents might also be effective against biliary tract cancer. Although some targeted agents have been investigated as monotherapy for first-line chemotherapy, none were found to exert satisfactory efficacy. On the other hand, monoclonal antibodies such as bevacizumab and cetuximab have also been investigated in combination with a gemcitabine-based regimen and have been demonstrated to show promising activity. Furthermore, clinical trials using new targeted agents for biliary tract cancer are also proposed. This cancer is a relatively rare and heterogeneous tumor consisting of cholangiocarcinoma and gallbladder carcinoma. Therefore, a large randomized clinical trial is necessary to confirm the efficacy of chemotherapy, and international collaboration is important.

Keywords: biliary tract cancer; chemotherapy; molecular targeted agent

1. Introduction

Biliary tract cancer is rather common in Latin America and Asia, including Japan, while being relatively rare in European countries and the United States; approximately 16,000 patients in Japan and 5,000 patients in the United States are newly diagnosed as having this cancer each year [1-3]. The varied geographic distribution of the risk factors for biliary tract cancer, including primary sclerosing cholangitis, hepatolithiasis, congenital biliary cystic diseases, chemical agents, and hepatitis virus infections appears to contribute to the differences in the incidence rates among ethnic groups [1-4].

Bile duct cancer is subdivided according to the anatomic location of origin into intrahepatic cholangiocarcinoma, gallbladder cancer, extrahepatic cholangiocarcinoma and ampulla of Vater cancer. Although surgery currently remains the only potentially curative treatment for each of the aforementioned diseases, most patients are diagnosed at an unresectable advanced stage of the disease. While chemotherapy is applicable for all of these diseases, different carcinogenetic pathways and sensitivities to therapies have been demonstrated for each of them. The survival in patients with unresectable biliary tract cancer has been shown to differ by the tumor type, that is, gallbladder carcinoma, intrahepatic cholangiocarcinoma, or extrahepatic cholangiocarcinoma. It would, therefore, be ideal to conduct separate clinical trials in each cancer. However, it is not practical, because each of these biliary tract cancers is relatively rare. For the development of new chemotherapeutic regimens for biliary tract cancer, randomized clinical trials with an appropriate stratification strategy are required, including by the tumor types.

Despite the numerous phase II studies conducted of treatments for advanced biliary tract cancer, no accepted standard treatment for this tumor type has been established yet, because of the low incidence and small number of patients and the lack of adequately powered randomized controlled trials. A number of studies have investigated the usefulness of gemcitabine alone or tegafur/gimeracil/oteracil potassium (S-1) alone, and also gemcitabine-based combination regimens (Table 1) [5-22]. Based on the results of phase II studies, the Japanese guideline for biliary tract and ampullary carcinomas recommends gemcitabine alone or S-1 alone as the first line chemotherapy [23]. Recently, randomized controlled trials comparing the combination of cisplatin plus gemcitabine with gemcitabine alone have shown the survival benefit of the former regimen (Table 1) [24,25]. Furthermore, a randomized controlled trial among best supportive care, fluorouracil plus folinic acid and gemcitabine plus oxaliplatin (Gemox) revealed improved survival with Gemox in patients with unresectable gallbladder cancer as compared with best supportive care and fluorouracil plus folinic acid (Table 2) [26]. 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. Until date, no large clinical trials using targeted agents have been conducted for biliary tract cancer, however, some of these agents appear to offer promise. In this paper, the results of preclinical experiments and clinical trials of molecular targeted therapy for the treatment of biliary tract cancer are reviewed, and the possibilities and future directions of the use of targeted agents are discussed.