20111036A

厚生労働科学研究費補助金

がん臨床研究事業

切除不能胆道がんに対する治療法の確立に関する研究

平成23年度　総括研究報告書

研究者代表　奥坂　拓志

平成24（2012）年３月

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切除不能胆道がんに対する治療法の確立に関する研究

研究者代表者　奥坂　拓志　国立がん研究センター中央病院　副科長

研究要旨：胆道がんは我が国のがん死亡数の第6位を占めており、また切除不能胆道がんの予後はきわめて不良であるため、より有効な非手術療法の開発が求められている。本研究班では「進行胆道がんを対象としたゲムシタビン＋S-1併用療法とS-1単剤療法のランダム化第II相試験（JCOG0805）」を完了し、今後次相試験しての「進行胆道がんを対象としたゲムシタビン+シスプラチン併用療法とゲムシタビン+S-1併用療法（GS療法）の第III相比較試験（JCOG1113）」を開始予定である。さらに現在、「切除不能・再発胆道癌を対象としたゲムシタビン+CDDP+WT1ペプチドワクチン併用化学免疫療法とゲムシタビン+CDDP治療の第I/II相試験」を進行中である。

A．研究目的

切除不能胆道がん患者の予後はきわめて不良であり、その生存期間を向上するためには新しい有効な治療法の確立が必要である。S-1は本邦で開発された新しい抗がん剤であり、切除不能胆道がんに対してもその有用性が期待されている。S-1の一次治療薬としての有効性を検討するために、「進行胆道がんを対象としたゲムシタビン＋S-1併用療法とS-1単剤療法のランダム化第II相試験」を開始した。この試験により有用性が期待できるレジメンを慎重に選択したのちに第Ⅲ相試験を実施して、切除不能胆道がんに対する標準治療法を確立する。また、本研究班では国内外で開発が期待されているWT1ペプチドワクチンを用いた臨床試験も開始し、本疾患に対する有効性と安全性を評価する。

B．研究方法

(1)「進行胆道がんを対象としたゲムシタビン＋S-1併用療法とS-1単剤療法のランダム化第II相試験（JCOG08805）」について：

［研究形式］多施設共同のランダム化第Ⅱ相試験、プライマリーエンドポイントは1年生存割合。

［対象症例］切除不能胆道がんの未治療例、PS 0または1、骨髄・肝・腎などの主要臓器機能が保持され、十分な説明後に本人より文書で同意の得られた症例。

［症例の登録］JCOGデータセンターによる中央登録方式とする。

［治療内容］S-1単独療法群ではS-1をday 1-28に連日経口投与する。これを６週毎に原疾患の悪化または毒性のため中止するまで継続する。S-1とゲムシタビンの併用療法群ではゲムシタビンをday 1，8に静注投与し、S-1はday 1-14に連日経口投与する。これを３週毎に原疾患の悪化または毒性のため中止するまで継続する。

［予定症例数］症例数100例、症例集積期間2年を予定。
［研究の第三者的監視］JCOGに所属する研究班は共同で、Peer reviewと外部委員審査を併用した第三者的監視機構としての各種委員会を組織し、科学性と倫理性の確保に努めている。本研究も、JCOGのプロトコール審査委員会、効果・安全性評価委員会、監査委員会、などによる第三者的監視を受けることを通じて、科学性と倫理性の確保に努める。
(2)「切除不能・再発胆道癌を対象としたゲムシタビン+CDDP+WT1ペプチドワクチン併用化学免疫療法とゲムシタビン+CDDP治療の第I/II相試験」について：
［研究形式］多施設共同の第Ⅰ相/ランダム化第Ⅱ相試験、プライマリーエンドポイントは1年生存割合。
［対象症例］切除不能胆道がんの未治療例、PS 0または1、骨髄・肝・腎などの主要臓器機能が保持され、十分な説明後に本人より文書で同意の得られた症例。
［症例の登録］NPO日本臨床研究支援ユニットによる中央登録方式とする。
［治療内容］3週1コースとしてゲムシタビン、CDDPをday1，day8に投与し，day15は休薬する。WT1ペプチドワクチン群はWT1ペプチドワクチンをゲムシタビン、CDDPと同日に投与する。なお，CDDPは治療開始から最大24週まで、ゲムシタビンとWT1ペプチドワクチンはプロトコール治療中止基準に該当するまで治療を継続する。
［予定症例数］106例（第I相6例、第II相100例）、症例集積期間2年を予定。
倫理面への配慮
参加患者の安全性確保については、適格条件やプロトコール治療の中止変更規準を厳しく設けており、試験参加による不利益は最小化される。また、「臨床研究に関する倫理指針」およびヘルシンキ宣言などの国際的倫理原則を遵守する。

C．研究結果
（1）「進行胆道がんを対象としたゲムシタビン＋S-1併用療法とS-1単剤療法のランダム化第II相試験（JCOG0805）」
1）登録状況：2009年2月より登録を開始し、予定よりおよそ10ヵ月早い2010年4月に登録を完了した（101例）。
2）患者背景：ゲムシタビン＋S-1併用療法（A群、51例）とS-1単剤療法（B群、50例）の主な背景は、年齢中央値（範囲）：66（39-78）歳、62.5（49-79）歳、男/女：27/24、28/22、PS 0/1：39/12、37/13、胆嚢がん/肝内胆管がん/肝外胆管がん/乳頭部がん：19/20/9/3、19/15/11/5、Stage II/III/IV/術後再発：2/6/29/14、0/7/32/11、であり、両群間に大きな偏りは見られなかった。
3）有害事象：A群とB群の主な(10%以上)Grade 3-4の有害事象（%）は、白血球：30、2、ヘモグロビン：12、4、血小板：12、4、好中球：61、4、総ビリルビン：10、14、ALP：8、14、AST：12、14、ALT：14、12、胆管炎：8、12、治療関連死はA群4%（2/51、心筋梗塞、肺炎）、B群0%（0/50）であった。
4）有効性：A群とB群の成績は、1年生存割合：52.9%、40.0%、生存期間中央値：12.5ヵ月、9.0ヵ月、無増悪生存期間中央値：7.1ヵ月、4.2ヵ月であり、A群のB群に対する生存期間のHRは0.859（95%CI：0.543-1.360）であった。
5）総括：ゲムシタビン＋S-1併用療法は主要評価項目である1年生存率においてS-1単剤療法を上回り、有害反応も許容範囲であり、次相試験における試験治療レジメンとすべきと考えられた。

（2）「切除不能・再発胆道癌を対象としたゲムシタビン+CDDP+WT1ペプチドワクチン併用化学免疫療法とゲムシタビン+CDDP治療の第I/II相試験」
第Ⅰ相部分の症例登録を開始し、DLTの評価が6例で実施され、DLTに該当する有害事象は認められなかった。2012年1月現在、4例がプロトコール治療中止、2例が継続中であり、中止の4例のうち3例が腫瘍増大、1例が患者希望（有害事象との関連は否定）であった。

D．考察

我が国における胆道がん死亡数は増加傾向にあり、悪性腫瘍死亡数の第６位となっている。切除不能胆道がんに対しては、ゲムシタビンを中心とする化学療法が行われているが、その治療成績は生存期間中央値が8か月前後ときわめて不良であり、より有効な治療法の開発が切望されている。最近、本邦で開発された経口抗がん剤であるS-1が切除不能胆道がんに対し優れた抗腫瘍効果を示すことが明らかにされ、胆道がんへの適応拡大が承認された。

本研究班では最初に、「進行胆道がんを対象としたゲムシタビン＋S-1併用療法とS-1単剤療法のランダム化第II相試験」を実施し、より有用性が期待できるレジメンを慎重に選択し、続いて第Ⅲ相試験を行って、切除不能胆道がんに対する標準治療法を確立することをめざしている。今回報告したランダム化第Ⅱ相試験（JCOG0805）は一次治療薬としてS-1を用いる場合にゲムシタビンと併用して用いるのがよいのか、あるいはS-1単独で用いて、二次治療としてゲムシタビンを用いるのがよいのかを慎重に判断することを目的としている。このランダム化第II相試験で選択されたレジメンを用いて第III相試験を実施する計画である。最近、ゲムシタビンとシスプラチン併用療法の延命効果が報告されており、来るべき第III相試験ではこのゲムシタビンとシスプラチン併用療法がコントロールレジメンとなるものと考えられている。

胆道がんは依然予後不良な疾患であり、新しい視点からの治療開発戦略も必要と考えられる。我々は別の研究班でWT1ペプチドワクチンの臨床試験を行ってきており、その知見をいかして本研究班において多施設共同試験として本免疫療法の有効性と安全性を検討することとした。胆道がんは我が国には患者が多いにも関わらず新薬開発が遅れており、このような研究を実施することにより本疾患への関心が高まり、新薬治験の導入が促進することも期待したい。

E．結論

「進行胆道がんを対象としたゲムシタビン＋S-1併用療法とS-1単剤療法のランダム化第II相試験（JCOG 0805）」の結果、ゲムシタビン＋S-1併用療法が次相試験の試験レジメンとして選択され、現在、「進行胆道がんを対象としたゲムシタビン+シスプラチン併用療法とゲムシタビン+S-1併用療法（GS療法）の第III相比較試験（JCOG1113）」の開始準備が進められている。「切除不能・再発胆道癌を対象としたゲムシタビン+CDDP+WT1ペプチドワクチン併用化学免疫療法とゲムシタビン+CDDP治療の第I/II相試験」が開始されている。

F．健康危険情報

なし。

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H. 知的財産権の出願・登録状況

１. 特許取得
なし

２. 実用新案登録
なし

３. その他
なし

研究成果の刊行に関する一覧表

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厚生労働科学研究費補助金

がん臨床研究事業

切除不能胆道がんに対する治療法の確立に関する研究

平成２３年度　研究成果の刊行物・別刷

研究代表者　奥坂　拓志
研究分担者　宮川　宏之
菱沼　正一
長瀬　通隆
山口　研成
山口　武人
池田　公史
石井　浩
古瀬　純司
大川　伸一
田中　克明
福冨　晃
山雄　健次
中森　正二
井岡　達也
井口　東郎
杉本　理恵
伊藤　鉄英

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

Miho Kaida,† Yuriko Morita-Hoshi,* Atsuko Soeda,* Takako Wakeda,* Yuni Yamaki,* Yasushi Kojima,‡ Hideki Ueno,‡ Shunsuke Kondo,‡ Chigusa Morizane,‡ Masafumi Ikeda,‡ Takuji Okusaka,‡ Yoichi Takaue,* and Yuji Heike**

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

(*J Immunother* 2011;34:92–99)

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.

Received for publication June 9, 2010; accepted September 3, 2010.
From the *Department of Medical Oncology; ‡Hepatobiliary and Pancreatic Oncology Division, National Cancer Research Center Hospital, Tokyo; and †Department of Pediatrics, Kitasato University Graduate School of Medicine, Kanagawa, Japan.
Supported by Grants-in-Aid for Cancer Research from the Ministry of Health, Labor and Welfare of Japan, and by grants from the Advanced Clinical Research Organization (ACRO).
All authors have declared that there are no financial conflicts of interest in regards to this study.
Reprints: Yuji Heike, Department of Medical Oncology, National Cancer Research Center Hospital, 5-1-1 Tsukiji Chuo-ku, Tokyo 104-0045, Japan (e-mail: yheike@ncc.go.jp).

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. Brefeldin 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

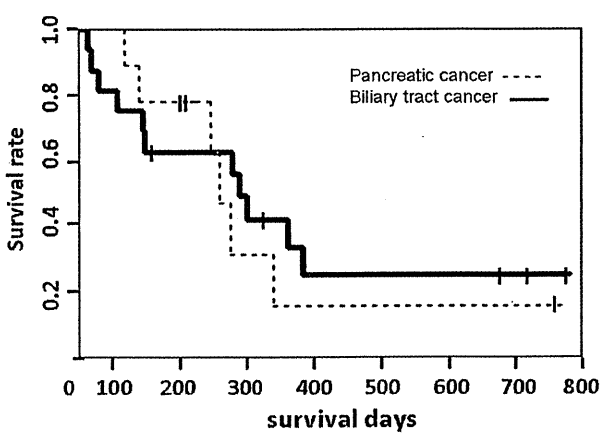

FIGURE 1. Kaplan-Meier estimates of overall survival for biliary tract cancer and pancreatic cancer. Median survival time for biliary tract cancer (n = 16) was 288 days and for pancreatic cancer (n = 9) was 259 days. There were no significant differences between the 2 curves ($P = 0.78$).

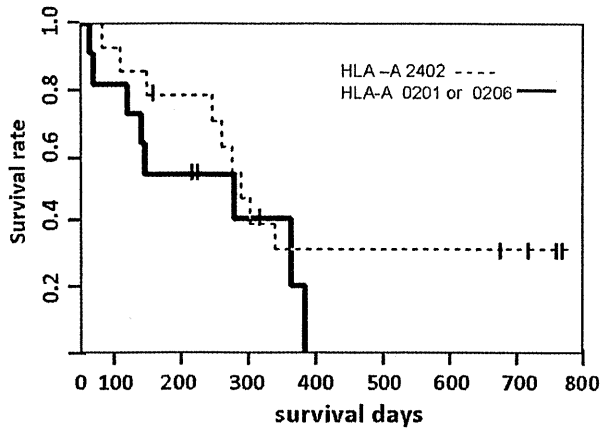

FIGURE 2. Kaplan-Meier estimates of overall survival for patients who received HLA-A 02 and HLA-A24-restricted vaccine. Median survival time in patients who received HLA-A 02 vaccine (n = 11) was 278 days and for those who received HLA-A24 vaccine (n = 14) was 288 days. Survival was not significantly 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