A Phase I Clinical Trial of Vaccination With KIF20A-derived Peptide in Combination With Gemcitabine For Patients With Advanced Pancreatic Cancer

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Summary: KIF20A (RAB6KIFL) belongs to the kinesin superfamily of motor proteins, which play critical roles in the trafficking of molecules and organelles during the growth of pancreatic cancer. Immunotherapy using a previously identified epitope peptide for KIF20A is expected to improve clinical outcomes. A phase I clinical trial combining KIF20A-derived peptide with gemcitabine (GEM) was therefore conducted among patients with advanced pancreatic cancer who had received prior therapy such as chemotherapy and/or radiotherapy. GEM was administered at a dose of 1000 mg/m² on days 1, 8, and 15 in a 28-day cycle. The KIF20A-derived peptide was injected subcutaneously on a weekly basis in a dose-escalation manner (doses of 0.5, 1, and 3 mg/body; 3 patients/cohort). Safety and immunologic parameters were assessed. No severe adverse effects of grade 3 or higher related to KIF20A-derived peptide were observed. Of the 9 patients who completed at least one course of treatment, interferon-γ (IFN-γ)-producing cells were induced in 4 of 9 patients (P2, P3, P6, and P7), and IFN-γ-producing cells were increased in 4 of the 9 patients (P1, P5, P8, and P9). Four of the 9 patients achieved stable disease. The disease control rate was 44%. The median survival time after first vaccination was 173 days and 1-year survival rate was 11.1%. IFN-γ-producing cells were induced by the KIF20A-derived peptide vaccine at a high rate, even in combination with GEM. These results suggest that this combination therapy will be feasible and promising for the treatment of advanced pancreatic cancer.

Key Words: pancreatic cancer, peptide, KIF20A, phase I, immunotherapy

Pancreatic cancer is the fourth leading cause of cancer mortality in the world. The prognosis for patients with pancreatic cancer is extremely poor, with an overall 5-year survival of only 5%. The primary reason for this high mortality rate is the aggressive nature of the malignancy in the absence of early detection. There are few (if any) symptoms that offer an early indication of pancreatic cancer growth; therefore, most such cancers are diagnosed in the advanced stage. As a result, the majority of pancreatic cancers are unreseetable. Other therapies, including radiation and chemotherapy, have limited effects in terms of increased survival. Consequently, median survival time (MST) after the diagnosis of pancreatic cancer is measured in months rather than years. Gemcitabine (GEM) is currently one of the standard therapies for advanced pancreatic cancer, although many chemotherapeutic agents have been used in clinical trials over the past 2 decades. Among these chemotherapeutic agents, GEM is clinically more effective, but the MST is still 6–9 months. The development of new treatment modalities, including specific immunotherapies, is thus required. Recent advances in molecular biology and cellular immunology in the field of tumor immunology have resulted in the identification of a large number of antigens and epitopes recognized by human leukocyte antigen (HLA) class I restricted cytotoxic T lymphocytes (CTL) from melanomas and epithelial cancers. Using cDNA microarray technology coupled with laser microdissection, we recently identified novel HLA-A24-restricted epitope peptides as targets for cancer vaccination for patients with pancreatic cancer. KIF20A (RAB6KIFL) belongs to the kinesin superfamily of motor proteins, which have critical functions in the trafficking of molecules and organelles. Immunotherapy using a new epitope peptide for KIF20A is expected to improve clinical outcomes. A phase I clinical trial combining KIF20A-derived peptide with GEM was therefore conducted for patients with advanced pancreatic cancer who had received prior therapy such as chemotherapy and/or radiotherapy.

MATERIALS AND METHODS

Peptides

The KIF20A-10-66 peptide (KVYLRVRPLL) was synthesized by BCN Peptides (Barcelona, Spain) according to a standard solid-phase synthesis method, thereafter purified by reversed-phase high-performance liquid chromatography (HPLC). The purity (> 90%) and identity of peptides were determined by analytical HPLC and mass spectrometry analysis, respectively. Endotoxin levels and the bioburden of these peptides were tested and determined to be within acceptable levels as Good Manufacturing Practice grade for vaccines.

Patient Eligibility

The institutional review board at Yamaguchi University approved this clinical protocol. Complete written informed consent was obtained from all patients at the time of enrollment. According to the protocol, patients were
required to show positive results for HLA-A*2402. Nine patients diagnosed with metastatic and/or unresectable pancreatic cancer who had received prior therapy such as chemotherapy and/or radiotherapy were enrolled in this trial between January and December 2009 at Yamaguchi University Hospital. Eligibility criteria were as follows: age ≥ 20 years; life expectancy ≥ 3 months; and adequate hepatic, renal, and bone marrow function (serum creatinine level, ≤ 2.0 mg/dL; bilirubin level, ≤ 3.0 g/dL; platelet count, ≥ 75,000/mL; total white blood cell count ≥ 3000/mL and ≤ 15,000/mL). All patients were untreated for ≥ 4 weeks before enrolling into the study and had to have an Eastern Cooperative Oncology Group performance status of 0-2 at the time of enrollment.

Study Design and End-points

This study was a nonrandomized, open-label, phase I clinical trial with dose escalation of the KIF20A-derived peptide combined with GEM for patients with advanced unresectable pancreatic cancer. The primary end-point in this trial was the safety of peptide vaccination combined with GEM. Secondary end-points were clinical outcome, immunologic responses, and determination of the optimal dose of peptide for further clinical trials. The MST is calculated as time after first vaccination. Immunologic responses were assessed by measuring levels of interferon (IFN)-γ production from antigen-specific T cells responding to the KIF20A-derived peptide.

Adverse Events and Clinical Responses

Adverse events were monitored according to the National Cancer Institute Common Terminology Criteria for Adverse Events version 3.0 (CTCAE). Dose-limiting toxicity was defined as a hematological toxicity of ≥ grade 4 and nonhematological toxicity of ≥ grade 3. Clinical response was evaluated based on clinical observations and radiologic findings. All known sites of disease were evaluated on a monthly basis by computed tomography (CT) or magnetic resonance imaging before vaccination and after each course. Tumor size was estimated by direct measurement of the region of abnormal enhancement observed on CT or magnetic resonance imaging or +++) according to the algorithm flow chart described in our previous report (+++: IFN-γ-producing cell is contained 0.02%–0.2%, + + : IFN-γ-producing cell is contained 0.01%–0.02%, − : IFN-γ producing cell is contained < 0.01% in the sample applied for ELISPOT). Sensitivity of our ELISPOT assay was estimated as approximately average level by the ELISPOT panel of the Cancer Immunotherapy Consortium [CIC (http://www.cancerresearch.org/consortium/assay-panels)]. Statistical Analysis

Statistical analysis was performed using the unpaired Student t test for the ELISPOT assay. A value of P < 0.05 was considered statistically significant. OS curves were estimated using Kaplan-Meier methodology. Any correlations with clinical outcomes were estimated using the Wilcoxon rank sum test.

RESULTS

Feasibility and Adverse Reactions

No severe adverse effects of grade 4 or higher were observed. Nine patients satisfying the eligibility criteria were enrolled in this study. Patient characteristics are shown in Table 1. All patients developed grade 1 or 2 local skin reactions with redness and induration at the injection sites. In particular, all 9 patients completed at least 1 course of treatment and all 9 developed immunologic reactions at
the injection sites. G2/G3 leukopenia and neutropenia and G1/G2 thrombocytopenia appeared to be caused by GEM itself. G1–G3 anemia appeared attributable to the progression of pancreatic cancer, although GEM is known to cause anemia as well. No febrile neutropenia was recorded during the course of this study. High-grade fever, fatigue, diarrhea, headache, rash, and itching were not observed in any patients. No hematologic, cardiovascular, hepatic, or renal toxicity was observed during or after vaccination (Table 2). The vaccination protocol was well tolerated in all patients enrolled.

Immunologic Monitoring

The KIF20A-specific T-cell (IFN-γ-producing cells) response was determined using the IFN-γ ELISPOT assay. Representative antigen-specific T-cell responses are shown in Figure 1. In which, PBMC from patients 2, 3, 6, and 7 produced higher level of IFN-γ after vaccine than the level of pre-vaccination (Fig. 1). Positive antigen-specific T-cell (IFN-γ producing cells) responses specific to the vaccinated peptide were determined as described in the Materials and methods section. IFN-γ-producing cells were induced in 4 of 9 patients (P2, P3, P6, and P7), and IFN-γ-producing cells were increased in 4 of the 9 patients (P1, P5, P8, and P9) (Table 3). Antigen-specific T-cell responses were seen in all 3 patients receiving 0.5 mg vaccination; in 2 of the 3 patients receiving 1 mg; and in all 3 patients receiving 3 mg.

TABLE 1. Patients' Characteristics

| Characteristics | Peptide (n = 3) |
|-----------------|----------------|
|                 | 0.5 mg | 1.0 mg | 3.0 mg |
| Age (y)         | 62 (48–74) |
| Sex             | Male/female | 1/2 | 2/1 | 1/2 |
| Performance status (ECOG) | 0/1 | 1/2 | 1/2 |
| Disease stage   | III/IV | 1/2 | 2/1 | 1/2 |
| Prior therapy   | Radical operation | 1 | 0 | 0 |
|                 | Chemotherapy   | 3 | 3 | 3 |
|                 | Radiotherapy   | 1 | 0 | 1 |

UICC-TNM classification of malignant tumors was used for determination of clinical stage.
ECOG indicates Eastern Cooperative Oncology Group.
Antigen-specific T-cell response (IFN-γ-producing cells) could therefore be induced by the KIF20A peptide vaccine at a high rate, even in combination with GEM.

Clinical Responses and OS

Four of the 9 patients achieved stable disease (SD), with the other 5 patients showing progression disease (PD). The disease control rate was 44%. Achievement of SD was seen in 2 of the 3 patients receiving 0.5 mg vaccination, 1 of 3 patients receiving 1 mg, and 1 of 3 patients receiving 3 mg (Table 2). Images from CT of a patient with SD are shown in Figure 2. All 4 patients who achieved SD showed induction of the antigen-specific T-cell responses at a level of 2+ or more (++; or ++++) for the KIF20A peptide (Table 3). In contrast, 3 of the 5 patients who showed PD displayed induction of antigen-specific T-cell responses from negative (−) to reaction (+). No relationship between peptide doses and the antigen-specific T-cell responses or clinical outcome was identified. The MST calculated as time after first vaccination was 173 days and 1-year survival rate was 78%.

**DISCUSSION**

The only cure for pancreatic cancer is surgical resection, although this malignancy is difficult to detect early. At the time of diagnosis, approximately 60% of patients are already beyond the possibility of surgical resection.20–23 GEM is currently used as the standard therapy for unresectable pancreatic cancer. Noninferiority of S-1 compared with GEM was shown in GEST study conducted in Japan, but the superiority of the combination of GEM and S-1 over GEM monotherapy has not yet been conclusively proven.24 The establishment of combination therapy with GEM has been performed many times to date. One large randomized controlled phase III trial with erlotinib showed significantly prolonged survival time (P = 0.038),25 but the difference was only about 10 days. In another study, MST was 11.1 months for the FOLFIRINOX group, compared with 6.8 months in the GEM group, showing a significant difference (P < 0.001). However, markedly more adverse events were noted in the FOLFIRINOX group.26 Taking into account toxicity and economic aspects, the development of new drugs for advanced pancreatic cancer is urgently required.

The present study investigated a novel cancer vaccine therapy for pancreatic cancer using a KIF20A-derived peptide in combination with GEM. To the best of our knowledge, this is the first report to use the KIF20A-derived peptide in a clinical trial. We observed no severe adverse events related to the treatment in this trial (Table 2). Specific adverse events caused by this vaccine treatment were local redness and induration at the injection site; however, no events > grade 3 were observed. In several papers we have examined—their authors show that the intradermic administration of vaccine has proven superior to subcutaneous administrations.27

We tried to administer the KIF20A-derived peptide emulsified with incomplete Freund’s adjuvant as close as possible to the dermis—so as to activate the dendritic cells.

### Table 2. Patients’ Toxicity Assessment and Clinical Outcome

| Patients | Peptide (mg) | Hematologic Toxicity | Local Adverse Effect | RECIST Lesion | Prior Therapy | Frequency of Vaccination | Evaluation | Prognosis (d) |
|----------|--------------|----------------------|---------------------|---------------|--------------|-------------------------|------------|--------------|
| 1        | 61F 0.5      | G2 anemia G3 leukopenia | G2 induration redness | Pancreas uncus tumor | Palliative operation, Chemo | 16 times | SD | PFS:175 d |
| 2        | 53F 0.5      | G2 leukopenia G2 thrombocytopenia G3 neutropenia | G1 induration redness | Liver metastasis | Distal pancreatectomy, Chemo | 8 times | PD | 366 |
| 3        | 49M 0.5      | G1 anemia G3 leukopenia G1 thrombocytopenia | G2 induration redness | Pancreas body tumor | Rad, Chemo | 22 times | SD | PFS:170 d |
| 4        | 70M 1        | G2 anemia G1 thrombocytopenia G2 leukopenia | G0 induration | Pancreas body tumor | Chemo | 7 times | PD | 71 |
| 5        | 72M 1        | G2 leukopenia G2 thrombocytopenia | G1 induration redness | Pancreas uncus tumor | Chemo | 8 times | PD | 208 |
| 6        | 53F 1        | G2 anemia G3 leukopenia G2 thrombocytopenia | G1 induration redness | Pancreas head tumor | Chemo | 8 times | PD | 173 |
| 7        | 74F 3        | G3 anemia G2 leukopenia G2 neutropenia | G2 induration redness | Pancreas head tumor | Chemo | 8 times | PD | 120 |
| 8        | 64F 3        | G1 anemia G2 leukopenia | G2 induration redness | Pancreas head tumor | Chemo | 8 times | PD | 94 |
| 9        | 60M 3        | G2 anemia G3 leukopenia G2 thrombocytopenia | G2 induration redness | Pancreas body tumor | Rad, Chemo | 11 times | SD | PFS: 85 d |

Chemo indicates chemotherapy; PD, progression disease; PFS, progression-free survival; Rad, radiotherapy; SD, stable disease.
could be enhanced by this message. Immunologic responses in this trial were measured by local redness and induration at the injection site and antigen-specific T-cell responses against the vaccinated peptide. No dose-limiting toxicity was observed in any dose cohort. We injected peptide vaccine biweekly after 8 times weekly injection (2 courses) to avoid the risk of exhaustion of the immune response. We chose right inguinal lesion or left inguinal lesion alternately as injection site. Local redness and induration as CTCAE grade 2 at the injection site were observed in all 3 patients with the 3 mg vaccination (Table 2). However, achievement of SD was seen in 2 of the 3 patients receiving 0.5 mg vaccination, 1 of 3 patients receiving 1 mg, and 1 of 3 patients receiving 3 mg (Table 2). In this study, we consider that the optimum peptide dosage for future clinical trials could be set at a level of at least 0.5 mg or more.

As a point of immunologic monitoring, IFN-\(\gamma\)-producing cells were induced in 4 of 9 patients (P2, P3, P6, and P7), and IFN-\(\gamma\)-producing cells were increased in 4 of the 9 patients (P1, P5, P8, and P9). Patient 4 in whom IFN-\(\gamma\)-producing cells response was absent was suffering from acute cholangitis during vaccination. Prior to vaccination, the proportion of lymphocyte in this patient was only 13%. Yamamoto et al\(^{28}\) previously reported that peptide-reactive cellular and humoral responses to vaccinated peptides in postvaccination PBMCs and sera were lower for advanced pancreatic cancer patients than for patients with other solid cancers. They commented that these results suggest that immunity in advanced pancreatic cancer is more depressed than in other epithelial cancers. Alternatively, a more suitable peptide repertoire might be provided for pancreatic cancer patients. Miyazawa et al\(^{29}\) reported that VEGFR2-169 peptide-specific positive CTL responses were observed in 11 of 18 patients who received at least one course of vaccination. Ishikawa et al\(^{10}\) reported URLC10-177 peptide-specific positive CTL responses in 4 of 7 patients. KIF20A peptide vaccine therefore induced or further increased peptide-specific T-cell responses at a higher rate compared with these reports. Four of the 9 patients achieved SD, whereas the other 5 patients showed PD (Table 2). Achievement of SD was seen in 2 of the 3 patients receiving 0.5 mg vaccination, 1 of 3 patients receiving 1 mg, and 1 of 3 patients receiving 3 mg (Table 2). There is no evidence that the SD was mediated by the vaccine. This could simply be the natural history of this disease, but it is interesting to note that all 4 patients who achieved SD showed antigen-specific T-cell response of ++ or +++ reactions for KIF20A peptide. In contrast, 3 of the 5 patients who experienced PD showed antigen-specific T-cell response from negative to 1 + reaction. A tendency toward a correlation between antigen-specific T-cell response and clinical outcome was suggested, but no significant relationship was proved (\(P = 0.074\)). However, the population was too small to be evaluated in this clinical trial. Many prior peptide vaccine studies have demonstrated significant immunogenicity against the peptides utilized in the vaccine without translating into significant clinical benefits. This will be our next focus but

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**TABLE 3. Immunologic Response**

| Dose of Peptides (mg) | Case Number | Course | CTL Reaction | Clinical Response | HLA Typing |
|-----------------------|-------------|--------|--------------|------------------|------------|
|                       |             |        | KIF20A      | CMV              |            |
| 0.5                   | 1           | Pre    | ++          | +++              | SD         | A*2402/A*3303 |
|                       |             | Post 1 | +           | +                |            |             |
|                       |             | Post 2 | +           | +++              |            |             |
|                       |             | Post 3 | +           | +                |            |             |
| 2                     |             | Pre    | -           | +                | PD         | A*2402/A*0201 |
|                       |             | Post 1 | +           | +                |            |             |
|                       |             | Post 2 | +           | +                |            |             |
| 3                     |             | Pre    | -           | +++              | SD         | A*2402      |
|                       |             | Post 1 | -           | +++              |            |             |
|                       |             | Post 2 | +           | +++              |            |             |
|                       |             | Post 3 | +           | +                |            |             |
|                       |             | Post 4 | +           | +                |            |             |
|                       |             | Post 5 | +           | +                |            |             |
| 1                     | 4           | Pre    | -           | +                | PD         | A*2402/A*1101 |
|                       |             | Post 1 | -           | +                |            |             |
|                       |             | Post 2 | +           | +                |            |             |
| 5                     |             | Pre    | +           | +                | SD         | A*2402/A*1101 |
|                       |             | Post 1 | +           | +                |            |             |
|                       |             | Post 2 | +           | +                |            |             |
| 6                     |             | Pre    | -           | +                | PD         | A*2402/A*3303 |
|                       |             | Post 1 | -           | +                |            |             |
|                       |             | Post 2 | +           | +                |            |             |
| 3                     | 7           | Pre    | +           | +                | PD         | A*2402/A*0206 |
|                       |             | Post 1 | +           | +                |            |             |
|                       |             | Post 2 | +           | +                |            |             |
| 8                     |             | Pre    | +           | +++              | PD         | A*2402/A*0206 |
|                       |             | Post 1 | +           | +++              |            |             |
|                       |             | Post 2 | NT          | +++              |            |             |
| 9                     |             | Pre    | +           | +++              | SD         | A*2402/A*2601 |
|                       |             | Post 1 | -           | +++              |            |             |
|                       |             | Post 2 | +           | +++              |            |             |

CMV indicates cytomegalovirus; CTL, cytotoxic T lymphocytes; HLA, human leukocyte antigen; PD, progression disease; SD, stable disease.
prior to that the important thing is to identify a new peptide that possesses high immunogenicity. This protocol was well tolerated, and peptide-specific IFN-γ-producing cells were found to be induced or increased by the KIF20A-derived peptide vaccine at a high rate, even in combination with the anticancer agent, GEM. Although safety and immunogenicity are promising, no conclusions can be made about efficacy at this level of study. We are proceeding on to conduct a phase II clinical trial among patients with advanced pancreatic cancer by combining KIF20A-derived peptide with GEM as the first line. Therefore, additional efficacy data would be required before committing to a large randomized controlled trial.

FIGURE 2. Axial contrast-enhanced computed tomography (CT) scans of patient 3 who showed SD. A, Axial contrast-enhanced CT showing locally advanced tumor of the pancreatic body before vaccination (arrow). B, Axial contrast-enhanced CT after 4 months shows SD of the pancreatic body mass (arrow). SD indicates stable disease.

FIGURE 3. Overall survival measured using the Kaplan-Meier method. The median survival time after first vaccination was 173 days. One-year survival rate was 11.1%.

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CONFLICTS OF INTEREST/FINANCIAL DISCLOSURES

All authors have declared there are no financial conflicts of interest with regard to this work.

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