Long-term Outcome of Immunotherapy for Patients with Refractory Pancreatic Cancer

MASAFUMI NAKAMURA[1], JUNJI WADA[1], HIROYUKI SUZUKI[1], MASAO TANAKA[2], MITSUO KATANO[1] and TAKASHI MORISAKI[1]

Departments of [1]Cancer Therapy and Research and [2]Surgery and Oncology, Graduate School of Medical Sciences, Kyushu University, Fukuoka, Japan

Abstract. *Background: Pancreatic cancer is one of the most fatal human cancers, with a 5-year survival rate of <5%. Although new chemotherapies have been used for pancreatic cancer, the outcome is still poor. Here, we retrospectively analyzed the outcome of immunotherapy in pancreatic cancer patients and revealed the potential of immunotherapy in advanced pancreatic cancer treatment. Patients and Methods: Seventeen pancreatic cancer patients underwent immunotherapy in the Kyushu University and the Yakuin CA Clinic. Six patients had postoperative recurrence, 11 were diagnosed as inoperable because of metastasis, 16 had prior chemotherapy and developed chemotherapy-resistant cancers, while 1 patient had no prior chemotherapy for recurrent cancer after surgical resection because of leukopenia. Immunotherapy was combined with chemotherapy in 11 patients and without chemotherapy in 6 patients. Immunotherapy was classified into two groups; combined dendritic cell (DC) vaccination and intravenous or peritoneal injection of activated lymphocytes (DC vaccine therapy), or injection of lymphokine-activated killer lymphocytes (LAK) alone (LAK therapy). Results: Immunotherapy of refractory pancreatic cancer resulted in a median survival of 9 months. Peritoneal metastasis tended to shorten the survival period. Combination immunotherapy and chemotherapy showed no obvious difference as compared to immunotherapy alone. DC vaccine therapy conferred a significantly better survival period than LAK alone. Conclusion: Our results suggest that immunotherapy utilizing DC vaccination may prolong the survival of refractory pancreatic cancer patients.*

Correspondence to: Masafumi Nakamura, Department of Cancer Therapy and Research, Graduate School of Medical Sciences, Kyushu University, 3-1-1 Maidashi, Higashi-Ku, Fukuoka 812-8582, Japan. Tel: +81 926426941, Fax: +81 926426221, e-mail: mnaka@surg1.med.kyushu-u.ac.jp

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Pancreatic cancer is one of the most fatal human cancers, with an overall 5-year survival rate of <5%, partially because of the difficulty of diagnosis at an early stage (1). However, despite the complete surgical removal of the tumor, most patients developed the disease again as metastases or local recurrence (2-6). Recently, gemcitabine has been reported to improve the survival of inoperable pancreatic cancer patients. Gemcitabine produced a clinical benefit in 24% of patients, with a median survival of 5.6 months and a 1-year survival of 18% (7). There is an increasing body of evidence showing that patients with resectable pancreatic cancer might benefit from adjuvant therapy with gemcitabine (8-15). However, options for patients with relapsed pancreatic cancer are still of limited benefit. Evaluations of single-agent gemcitabine or rubitecan salvage therapies for metastatic pancreatic cancer have reported good patient tolerability but median survivals of only 3.85 and 4.7 months, respectively (16, 17).

Dendritic cells (DCs) are antigen-presenting cells specialized for the induction of a primary T-cell response and can induce antitumor immunity *in vivo* (18-22). We previously reported that combination therapy with tumor cell-pulsed DCs and activated lymphocytes for patients with disseminated carcinomas prolonged the survival of responders (23). This immunotherapy was safe and no evidence of autoimmune disease was noted. No particular adverse reactions, except for low-grade fever, were found. Feasibility is one of the most important factors in investigating a second-line chemotherapeutic agent for refractory pancreatic cancer because there are usually not enough patients available for intense therapy.

Here, we present the outcome of immunotherapy including simple injection of activated lymphocytes and a combination of pulsed DC vaccination and injection of activated lymphocytes in patients with refractory pancreatic cancer (24-26).

Patients and Methods

Patient characteristics. Seventeen pancreatic cancer patients underwent immunotherapy in the Kyushu University and the Yakuin CA Clinic. Six patients had postoperative recurrence and 11 patients were diagnosed as inoperable because of metastasis. The metastatic

Table I. *Patient characteristics*.

Age years	Gender	Metastasis	Prior treatment	Immunotherapy	Combined chemotherapy	Survival time (months)
64	F	Peritoneum, liver	5-FU	DC	None	9
49	M	Peritoneum, lung	GEM	DC	None	20
63	M	Peritoneum	GEM, TS-1	DC	TS-1	7
73	M	Liver	GEM, UFT	DC	GEM	11
61	F	Peritoneum	GEM	DC	GEM	9
59	F	Peritoneum	GEM	LAK	None	7
61	F	Peritoneum	GEM	LAK	GEM	5
70	F	Peritoneum, liver	GEM	DC	GEM	7
65	F	Peritoneum, liver	GEM	DC	GEM	9
58	F	Liver	GEM, TS-1	DC	GEM	19
65	F	Peritoneum	GEM	LAK	None	5
68	F	Liver	GEM, TS-1, CPT-11	DC	None	11
69	F	Peritoneum	none	LAK	None	7
44	M	Peritoneum, liver	GEM, TS-1	DC	GEM+TS-1	8 (alive)
61	M	Peritoneum	GEM	DC	GEM	7 (alive)
74	M	Peritoneum	GEM	LAK	GEM+TS-1	6 (alive)
67	M	Peritoneum	GEM, Radiation	LAK	GEM	6 (alive)

5-FU, 5-Fluorouracil; GEM, gemcitabine; TS-1, tegafur-gimeracil-oteracil potassium; CPT-11, irinotecan; DC, dendritic cell; LAK, lymphokine-activated killer lymphocytes.

sites were the peritoneum in 7 patients, the peritoneum and liver in 2 and the liver in 2. Every patient had prior chemotherapy and had developed chemotherapy-resistant cancer.

Preparation of dendritic cells and activated T-lymphocytes. Autologous tumor-pulsed DCs (DC vaccine) were prepared as described elswhere (23, 27). Briefly, peripheral blood mononuclear cells (PBMC) were collected by leukapheresis with the COBE spectrum apheresis system (GAMBRO BCT, Inc, CL, USA). PBMCs were suspended at a cell density of 4×10^6 cells/ml in GMP-grade RPMI-1640 (Hy-Media; Nipro, Tokyo, Japan) supplemented with 1% human albumin, and 500 μl of cell suspension was added to each well of 24-well culture plates. The adherent cells in the 24-well culture plates were further cultured in Hy-Media containing 1% human albumin, and the immature DCs were prepared in 100 ng/ml of recombinant human granulocyte/monocyte colony-stimulating factor (GM-CSF, 200 ng/ml; Novartis Pharma, Basel, Switzerland) and 50 μl of recombinant human IL-4 (500 U/ml; Ono, Tokyo, Japan) for 7 days. After 7 days, cells were harvested as immature DCs. A total of $2–10\times10^6$ immature DCs were obtained per preparation.

Tumor specimens obtained from the tumor mass or malignant effusions were lysed by five freeze-thaw cycles (necrotic tumor cells). Immature DCs were incubated overnight with necrotic tumor cells for use in 6 patients, with peptides of carcinoembryonic antigen (CEA) and mucin 1 (MUC1) for use in four patients and with peptide of CEA for use in one patient, then cultured for 2 days in medium containing tumor necrosis factor α (TNF-α, 1,000 U/ml; R&D Systems, Minneapolis, MN, USA) and prostaglandin E_2 (PGE_2,1 μg/ml; Sigma, St. Louis, MO, USA).

For the preparation of lymphokine-activated killer cells (LAK), non-adherent mononuclear cells were cultured for 2 weeks with Hy-medium containing 175 JRU/ml human recombinant interleukin (IL)-2 (Nipro) and immobilized monoclonal antibody to CD3 (10 μg/ml) (OKT-3; Jansen-Kyowa, Tokyo, Japan). The final cell products were assessed for viability by the dye-exclusion test and checked twice for possible contamination by bacteria, fungi and endotoxins.

Treatment plan. Methods of immunotherapy were classified into two groups: combined DC vaccination and intravenous or peritoneal injection of activated lymphocytes (DC vaccine), or injection of LAK alone. Patients in the DC vaccine group received an injection of $2\text{-}30\times10^6$ mature DCs loaded with necrotic tumor cells or peptides every 2 or 3 weeks. Intravenous injection of $1\text{-}5\times10^8$ OKT-3/IL-2-activated lymphocytes was combined with the above DC vaccine every 4 weeks. This combination therapy has been named tumor-pulsed DC vaccine therapy. In principle, this tumor-pulsed DC vaccine therapy was continued for as long as possible in the outpatient clinic. Eleven patients were treated with combined immunotherapy and chemotherapy with gemcitabine (GEM) or tegafur-gimeracil-oteracil potassium (TS-1), and six patients with immunotherapy only. LAK cells were injected every 3 weeks. Briefly, GEM (1,000 mg/body in standard) was given every week intravenously for three weeks with one week break and the course was repeated. TS-1 was given (80-120 mg/day in standard) for four weeks by oral administration with two weeks' break.

Study end-points and statistical analysis. The study end-point was overall survival of historically analyzed patients. Statistical analysis was performed by Wilcoxon–Mann-Whitney test with Statview software, SAS Institute Inc., Cary, North Carolina, USA.

Results

Patient characteristics. Seventeen pancreatic cancer patients with postoperative recurrence (n=6) or inoperable cancer (n=11) underwent immunotherapy. Six patients had

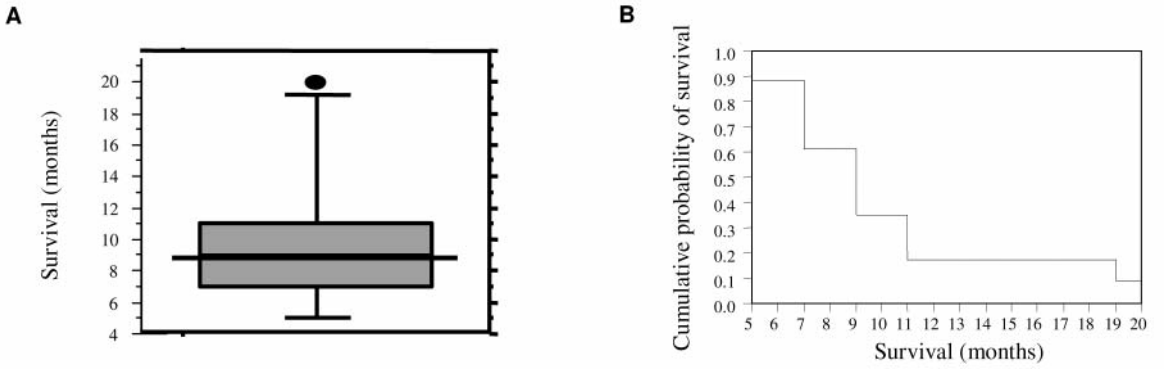

Figure 1. *Overall survival. A, Overall survival of 13 patients, excluding 4 surviving patients. Overall survival time was 9.7 months mean and 9 months median. B, Cumulative probability of survival of all 17 cases including surviving patients. MST was 9 months and the same as in (A) which excluded surviving patients.*

postoperative recurrences: 3 in the peritoneum, 2 in the peritoneum and liver, and 1 in the liver. Eleven patients were diagnosed as inoperable with metastasis in the peritoneum (n=7), peritoneum and liver (n=2) and liver (n=2). The characteristics of patients are shown in Table I.

Overall survival. Overall survival of 13 patients, excluding 4 surviving patients, was a mean of 9.7 months and median of 9 months (Figure 1A).

Analysis of all 17 cases including surviving patients demonstrated a median survival time (MST) of 9 months, which was the same as with exclusion of surviving patients (Figure 1B). The data indicate that immunotherapy is a potential candidate for treating recurrent pancreatic cancer after standard chemotherapy.

Peritoneal metastasis affects the survival time. We further analyzed patients excluding the surviving 4 patients. Ten out of 13 patients developed peritoneal metastasis (PM) at the beginning of our treatment and the other 3 patients were free of PM. The MSTs of patients with PM and without PM were 7 months and 11 months, respectively. PM was a statistically significant factor in MST in our series of pancreatic cancer immunotherapy (p=0.038, Figure 2).

DC therapy improves MST more than LAK therapy. Nine out of 13 patients underwent DC therapy and 4 patients had LAK therapy. The MSTs of patients with DC and LAK therapy were 9 and 6 months respectively and were statistically different (p=0.0116) (Figure 3).

Gemcitabine showed no additional effect on MST in the treatment of refractory pancreatic cancer patients with immunotherapy. Six out of 13 patients underwent combination therapy with immunotherapy and gemcitabine. However, gemcitabine unexpectedly did not confer any additional survival advantage on refractory pancreatic patients (Figure 4A). One of the 7 patients without gemcitabine was administered a combination of immunotherapy and 5-fluorouracil (5-FU). We further compared the MST of 6 patients injected with gemcitabine with 6 patients who underwent immunotherapy without any combination of chemotherapy. The MST of the gemcitabine group and no chemotherapy group were 9 and 8 months, respectively (p=0.87, Figure 4B).

Discussion

The overall survival of historically analyzed refractory pancreatic cancer patients treated by immunotherapy in this study was longer than that previously reported for second-line therapy of pancreatic cancer (8, 28-31). Ottele *et al*. (8) examined the potential effectiveness of second- or third-line therapy with paclitaxel (Taxol) after confirmed progression of pancreatic cancer with a gemcitabine-containing schedule. Paclitaxel was administered at weekly intervals and the MST was 17.5 weeks (range 7-88 weeks). Milella *et al*. (28) treated pancreatic cancer patients with progressive disease after gemcitabine-based chemotherapy with celecoxib and infusional 5-FU. The MST was 15 weeks. Reni *et al*. (29) reported the effect of a mitomycin, docetaxel and irinotecan regimen on gemcitabine-resistant pancreatic cancer patients. The MST was 6.1 months. Cantore *et al*. (30) used irinotecan and oxaliplatin in patients with advanced pancreatic cancer that had progressed despite more than 1 course of a gemcitabine-containing regimen and the MST was 5.9 months. Only Kozuch *et al*. (31) showed a longer MST of 10.3 months with injection of four active single agents into refractory pancreatic cancer patients.

We previously reported immunotherapy as an effective method to treat patients with malignant effusion (32).

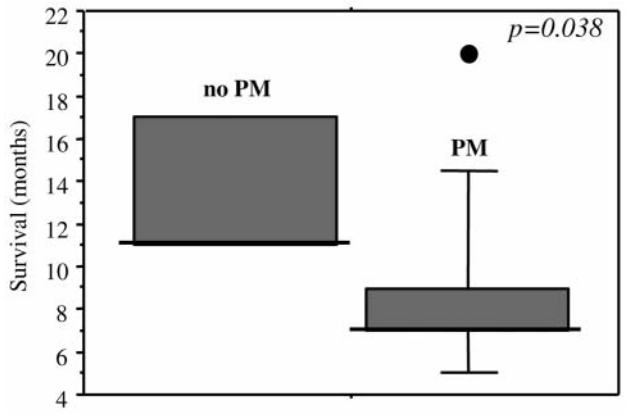

Figure 2. *Median survival time (MST) of the patients with peritoneal metastasis. Thirteen patients, excluding 4 surviving patients, were divided into two groups depending on the existence of peritoneal metastasis (PM) at the beginning of treatment resulting in 10 patients with PM and 3 without PM (no PM). The MSTs of patients with PM and without PM (no PM) were 7 months and 11 months, respectively. PM was a statistically significant factor in MST in our series of pancreatic cancer treated with immunotherapy (p=0.038).*

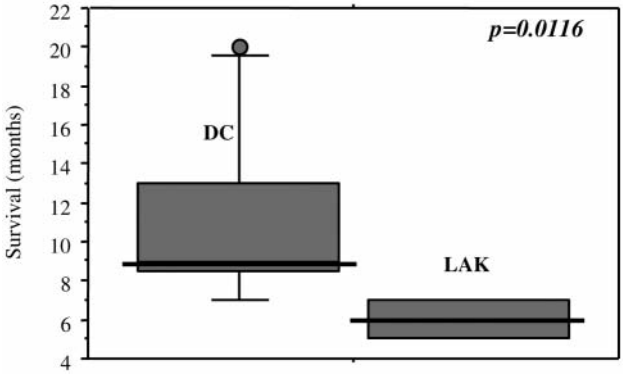

Figure 3. *DC therapy increases MST more than LAK therapy. Nine out of 13 patients, excluding 4 surviving patients, underwent DC therapy and 4 patients had LAK therapy. The MSTs of patients with DC and LAK therapy were 9 and 6 months, respectively, and were statistically different (p=0.0116). DC; DC therapy, LAK; LAK therapy.*

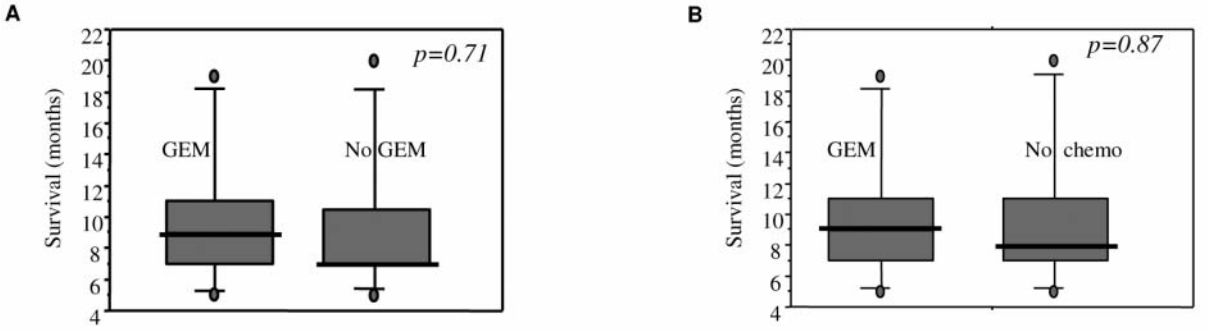

Figure 4. *MST of patients who underwent immunotherapy with or without gemcitabine. A, Six out of 13 patients underwent combination therapy of immunotherapy and gemcitabine (GEM), while seven patients underwent immunotherapy without gemcitabine (no GEM). The MSTs of GEM and no GEM patients were 9 and 7 months, respectively (p=0.71). B, The MST of GEM patients was compared with 6 patients who underwent immunotherapy without any combination of chemotherapy (No chemo). The MST of the GEM group and No chemo group were 9 and 8 months, respectively (p=0.87).*

Combined immunotherapy with intracavital injection of activated lymphocytes, monocyte-derived DCs and low-dose OK-432 improved the MST of patients with malignant effusion. Peritoneal metastasis is one of the prognostic factors in patients with gemcitabine-refractory pancreatic cancer (33). This might be the reason why PM was a statistically significant factor in MST in our series of pancreatic cancer patients undergoing immunotherapy.

Our data are the first to directly compare the influence of DC and LAK therapy on survival of cancer patients. Yamaguchi *et al*. reviewed the current status of adoptive lymphocyte therapy and mentioned that the overall response rate of tumor shrinkage was marginal (9%) (34). Kammula and Marincok reviewed clinical trials of the systemic administration of LAK cells and mentioned that LAK cells did not prove useful for the treatment of patients with metastatic melanoma and renal cancer (35). In contrast, the treatment of 86 patients with metastatic melanoma using tumor-infiltrating lymphocytes (TIL) plus IL-2 resulted in a 34% objective response rate (35). We have completed a phase I/II study of DC therapy and reported that the survival time of disseminated cancer patients responding to DC therapy was significantly prolonged compared with that of the non-responders ($p<0.0001$) (23). These published reports were consistent with our data and indicate that recognition of the tumor antigen is clinically pivotal in the immunotherapy of cancer as suggested in basic immunological reports.

Gemcitabine has been reported to mediate immunological effects relevant for tumor immunotherapy (36-38). Antitumor cytotoxic T-lymphocyte (CTL) responses can be induced by DCs cross-presenting antigens of tumor cells treated with a multidrug regime including gemcitabine (39). Enhanced cross-presentation of tumor antigens by DCs after gemcitabine treatment also leads to increased tumor recognition by CTLs *in vivo* (40). Bauer *et al*. demonstrated that gemcitabine sensitizes human pancreatic carcinoma cells to DC-induced tumor-specific CTL responses (41).

Although our data contains a small number of patients, one possible factor causing a discrepancy between our results and the published data is that most of our patients had been administered gemcitabine and became refractory to single agent therapy with gemcitabine. This possibility is generally troublesome, because currently gemcitabine is one of a few drugs revealed to be effective for pancreatic cancer. Eventually most refractory pancreatic cancer becomes resistant to gemcitabine, although there are few other drugs for pancreatic cancer.

We presented our experience and a retrospective analysis of a series of pancreatic cancer patients undergoing immunotherapy. Our data suggest that immunotherapy may confer some advantages on pancreatic cancer patients. There are a limited number of drugs for pancreatic cancer, and their efficacy on recurrent pancreatic cancer is still poor. Considering the present situation of refractory pancreatic cancer, establishment of promising treatment including immunotherapy is a task of great urgency.

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