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<https://escholarship.org/uc/item/930031n3>

Journal

Cancer, 88(6)

ISSN

0008-543X

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Publication Date

2000-03-15

DOI

10.1002/(SICI)1097-0142(20000315)88:6<1325::AID-CNCR8>3.0.CO;2-T

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Phase I Clinical Trial of Allogeneic Mixed Lymphocyte Culture (Cytoimplant) Delivered by Endoscopic Ultrasound–Guided Fine-Needle Injection in Patients with Advanced Pancreatic Carcinoma

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Presented as a plenary paper at a meeting of the American Society of Gastrointestinal Endoscopy in New Orleans, Louisiana, May 1998.

Supported in part by Meyer Pharmaceuticals, LLC (Irvine, California), and the Chao Family Comprehensive Cancer Center.

Dr. Chang served as a consultant to Applied Immunotherapeutics in 1997–1998.

The authors thank Frank L. Meyskens, Jr., M.D., for his mentoring and review of this article.

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Received June 3, 1999; revision received November 22, 1999; accepted December 10, 1999.

BACKGROUND. To the authors' knowledge, there are no other published clinical studies that have employed either systemic or local biologic response modifiers in the treatment of patients with pancreatic carcinoma. The purpose of this study was to determine the feasibility and safety of allogeneic mixed lymphocyte culture (cytoimplant) delivered by endoscopic ultrasound (EUS)–guided fine-needle injection (FNI) in patients with advanced pancreatic carcinoma.

METHODS. Eight patients with unresectable adenocarcinoma of the pancreas were enrolled: 4 patients in Stage II, 3 in Stage III, and 1 in Stage IV. Cytoimplants were delivered locally into the tumor using a novel EUS-guided FNI technique. Escalating doses of 3, 6, or 9 billion cells were implanted into the pancreatic tumor by a single EUS-guided FNI. Toxicity (modified National Cancer Institute criteria) was assessed at Day 1, Week 1, and Months 1 and 3. Clinical endpoints included Karnofsky performance status (KPS), CA 19-9, tumor response (computed tomography and/or EUS), and survival with follow-up examinations and imaging tests on months 3, 6, 9, 12, and 24.

RESULTS. There were no bone marrow, hemorrhagic, infectious, renal, cardiac, or pulmonary toxicities. There were 3 transient Grade 3 gastrointestinal toxicities, and 3 patients had transient episodes of hyperbilirubinemia that were reversed by replacement of biliary stents. Seven of 8 patients (86%) experienced low grade fever that responded to acetaminophen, and all fever was resolved within the first 4 weeks. There were no procedure-related complications. There were 2 partial responses and 1 minor response, with a median survival of 13.2 months.

CONCLUSIONS. A single injection of cytoimplant immunotherapy by EUS-guided FNI appears to be feasible and is not associated with substantial toxicity. *Cancer* 2000;88:1325–35. © 2000 American Cancer Society.

KEYWORDS: pancreatic neoplasms, adenocarcinoma, drug therapy, antineoplastic agents, immunotherapy, survival analysis, adult.

Pancreatic carcinoma is the fifth leading cause of cancer-related deaths in the United States, with nearly equal annual incidence and death rates.¹ The disease is associated with a high mortality rate, with the median survival for untreated patients estimated at approximately 4 months. In three clinical trials in which patients with locally advanced (Stage II, III, or IV)² pancreatic carcinoma were randomized to either observation or combination chemotherapy, the median survival periods averaged 3.5 months in the observation group and 4.5 months in the chemotherapy group.^{3–5} The combination of chemotherapy with external beam radiation therapy in randomized clinical

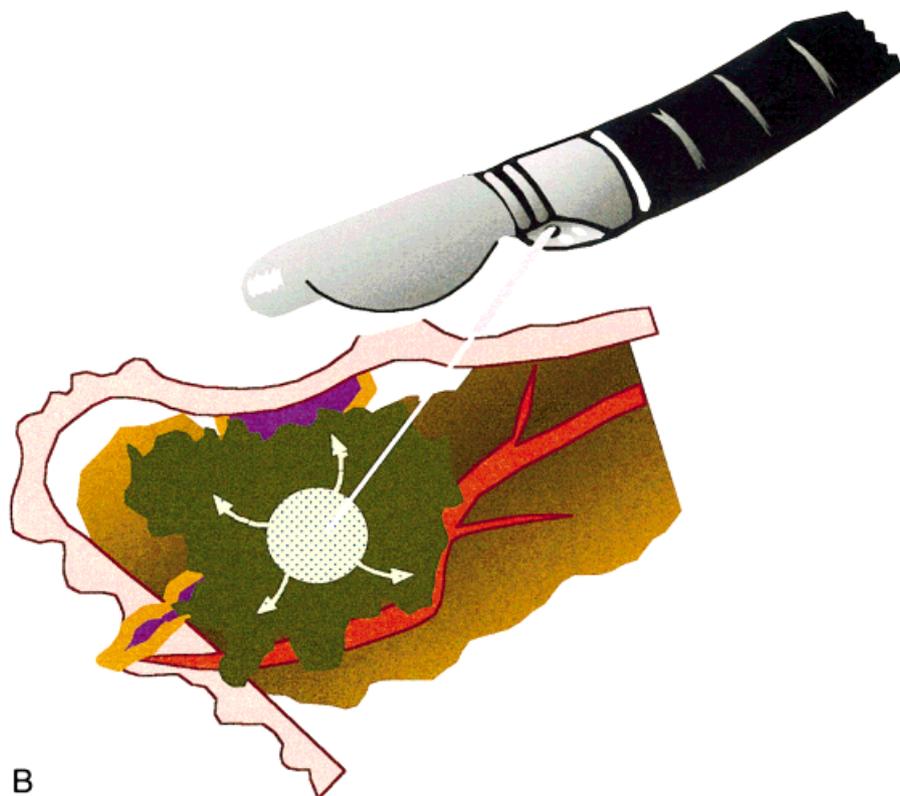
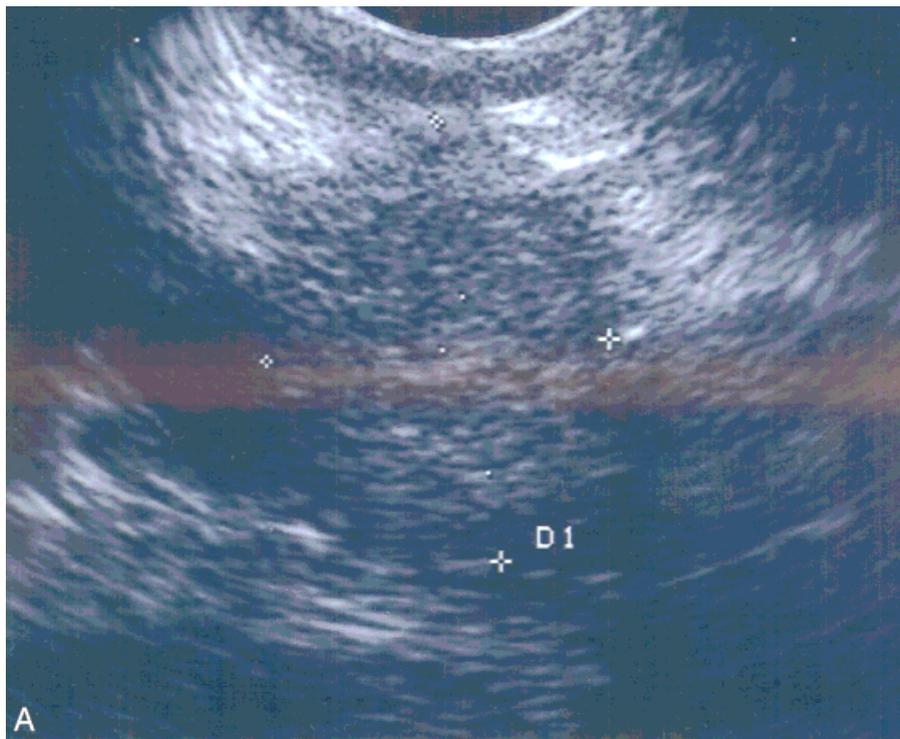


FIGURE 1. (A) An endoscopic ultrasound (EUS) image (7.5 MHz) shows a 2.5×2.0 cm adenocarcinoma in the head of the pancreas with portal vein invasion. (B) A diagram illustrates the EUS-guided fine-needle injection (FNI) technique. (C) An EUS image demonstrates the technique of EUS-guided FNI of cytoimplant into the pancreas tumor. Arrows indicate the needle tip during injection.

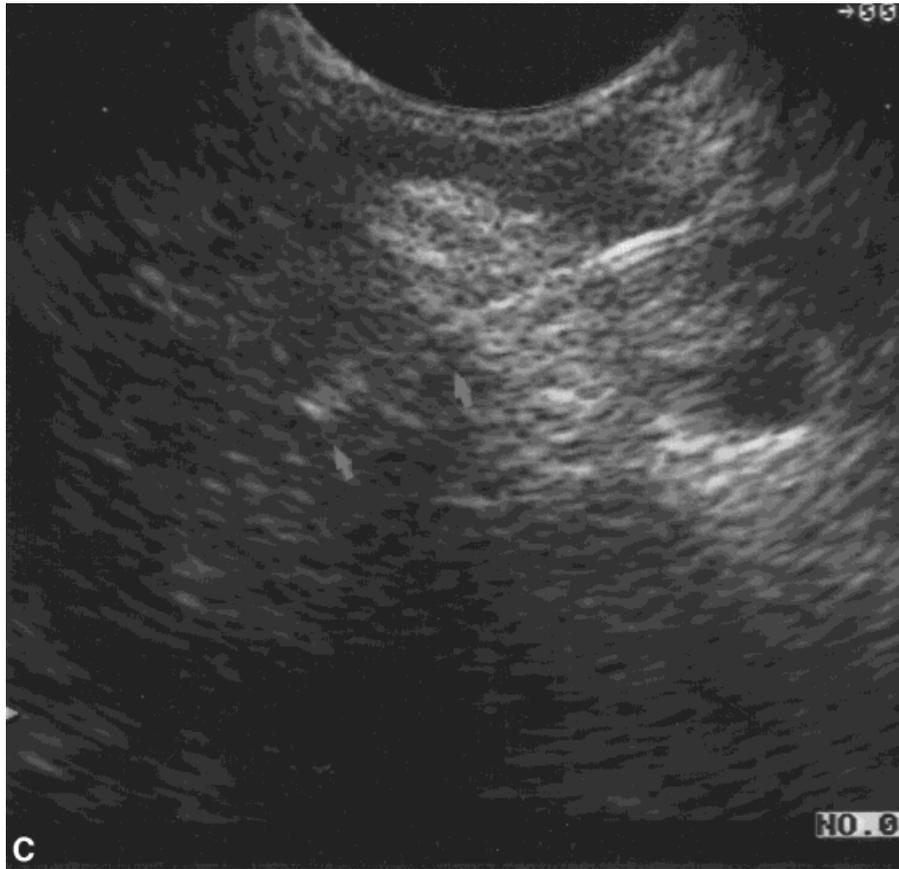


FIGURE 1. (continued)

trials have yielded a median survival of up to 11.5 months⁶⁻⁹ with associated toxicities. More recently, gemcitabine as a single agent showed a median survival of 5.7 months; by contrast, 5-fluorouracil showed a median survival of 4.4 months.¹⁰ Clearly, new therapies are needed.

To our knowledge, no published clinical studies have employed either systemic or local biologic response modifiers (BRM) or cellular-based immune therapies, such as lymphokine-activated killer cells or tumor-infiltrating T lymphocytes, for patients with pancreatic carcinoma. It is well documented that cytokine production directly within a tumor can induce its regression by host antitumor effector mechanisms.¹¹⁻¹³ The mixed lymphocyte reaction (MLR) is generated by coincubation of host and allogeneic donor peripheral blood mononuclear cells (PBMC) and results in the release of cytokines and the activation of immune effector cells.¹⁴⁻¹⁸ Based on these well-recognized principles, a novel form of local immunotherapy that recreates the MLR directly in the center of malignant pancreas tumors has been developed. A preliminary animal study showed prolonged survival in the mixed lymphocyte culture (MLC)-treated group.¹⁹

One of the difficulties of local BRM production in patients with advanced pancreatic carcinoma is the necessity for invasive surgical procedures to deliver the BRM that would otherwise not be indicated. In this regard, a new technique, endoscopic ultrasound (EUS)-guided fine-needle injection (FNI), has overcome this limitation. EUS is a procedure in which an endoscope with an ultrasound transducer mounted on the tip can be guided into the stomach and duodenum. Because of the ultrasound probe, the device can image through the wall of the gastrointestinal tract, allowing for high-resolution visualization of adjacent structures, such as the pancreas.²⁰⁻²² Recently, EUS has been combined with the ability to perform fine-needle aspiration (FNA).²³⁻²⁹ This EUS-guided FNA technique has more recently been modified to be used as an injection modality to deliver therapy, such as EUS-guided celiac neurolysis.³⁰ To our knowledge, this technique, known as EUS-guided FNI, has not been previously described for injecting antitumor agents directly into a local cancer. Therefore, this treatment involves the combination of a novel immunologic therapy and a new delivery technique.

TABLE 1
Patient Demographics

Parameters	No. of patients (n = 8)
Age	
Median (range) 62.4 (53-89)	
< 50	0
50-65	7
> 65	1
Gender	
Male	4
Female	4
Race	
White	6
Asian	1
Hispanic	1
Stage	
I	0
II	4
III	3
IV	1
Prior therapy	
None	5
Radiation	1
Combination ^a	2

^a One patient received radiation and 5-fluorouracil (5-FU); a second patient received 5-FU, radiation, and gemcitabine.

METHODS

Patient Eligibility

Patients were eligible for enrollment if they were older than 18 years and had histologically proven adenocarcinoma of the pancreas bidimensionally measurable by computed tomography (CT) or EUS. Tumors were deemed unresectable based on vascular invasion or metastasis to the lymph nodes or liver. All patients had a Karnofsky performance score (KPS) of 60 or better and an expected survival of longer than 2 months. Written informed consent (Institutional Review Board [IRB]-approved protocol and consent University of California, Irvine [UCI] #95-013) was obtained before study entry. This study was conducted under Food and Drug Administration IND #6288.

Patients were excluded from the study if they had received chemotherapy, radiation therapy, or therapy with biologic response modifiers (interferons or interleukins) within 28 days of study enrollment. Patients with a history of previous myocardial infarction within 3 months preceding enrollment or previous myocardial infarction with left ventricular ejection fraction < 40% (or < 50% with clinical symptoms of congestive heart failure) were also excluded. A concurrent medical condition requiring systemic steroid therapy, documented human immunodeficiency virus (HIV) infection, or primary malignancy (present or remote) of

sites other than the pancreas were reasons for exclusion. Also excluded were patients who had undergone prior surgery within 30 days of execution of informed consent or had had persistent fever higher than 39 °C (unless clinically assessed to be caused by tumor or use of investigational drugs) within 30 days of enrollment. Laboratory requirements included a white blood cell count greater than 3000/ μ L, a platelet count greater than 100,000/ μ L, hematocrit greater than 33%, hemoglobin greater than 10.5 gm/dL, prothrombin time (protime) within 3 seconds of control, and serum creatinine less than or equal to 1.5 mg/dL. Patients must have recovered from previous chemotherapy with eligible hemoglobin, platelet, and white blood cell count. Pretreatment evaluation within 14 days of enrollment included a complete medical history and physical examination (including weight and KPS assessment), as well as a pathologic review of the primary pancreatic carcinoma. Patients had a baseline laboratory panel, including complete blood count, differential white blood cell count, liver function tests, amylase, lipase, protime, partial thrombin time (PTT), urinalysis, carcinoembryonic antigen (CEA), and CA19-9 tumor markers. A CT scan of the abdomen and EUS were obtained for all patients. EUS-guided FNA was performed if there was no prior histologic diagnosis of pancreatic adenocarcinoma. A pregnancy test was performed if applicable.

Cytoimplant (Mixed Lymphocyte Culture)

A genetically unrelated, healthy adult donor was identified in the University of California-Irvine Medical Center (UCIMC) Blood Donor Center donor list. Informed consent was obtained (IRB-approved) and the donor was tested for hepatitis A, B, and C serologies; anti-human T-cell lymphotropic virus types 1 and 2; anti-HIV; HIV antigen; anti-cytomegalovirus (anti-CMV); and Venereal Disease Research Laboratory test. Any donor who screened positive for any of these markers (except anti-CMV) was excluded from the study. Donor and patient peripheral blood mononuclear cells (PBMC) were collected during a single modified leukapheresis procedure (peripheral stem cell collection). Cytoimplant was established by coculturing PBMC from patient and donor.

EUS-Guided FNI Technique

Patients were first evaluated by EUS using a radial scanning echoendoscope, GF-UM3 or GF-UM20 (Olympus America, Melville, NY). EUS-guided FNA (along with color Doppler ultrasound) and EUS-guided FNI of the cytoimplant were both performed using a curved linear array echoendoscope (FG32UA or FG36UX; Pentax Precision Instruments, Orange-

TABLE 2
Summary of Toxicities (Grades 0–4)

Toxicity	Patient							
	1	2	3	4	5	6	7	8
Dose × 10 ⁹	3	3	6	6	6	9	9	9
Alopecia	0	0	0	0	0	0	0	0
Lung	0	0	0	0	0	0	0	0
Heart	0	0	0	0	0	0	0	0
Kidney	0	0	0	0	0	0	0	0
Fever	0	2	1	1	0	2	1	1
Liver	4	0	3	1	4	3	3	0
Gastrointestinal tract	0	2	0	3	3	1	3	2
Infection	0	0	0	0	0	0	0	0
Hemorrhage	0	0	0	0	0	0	0	0
Bone marrow	0	0	0	0	0	0	0	0

TABLE 3
Serious Adverse Events (Grade 3 and 4 Toxicities up to Month 3)

Patient	Event	Grade	Time of occurrence	Comment
1	Elevated bilirubin	4	Week 4	Reversed with stent change
3	Elevated SGPT	3	Day 1	Reversed
	Elevated bilirubin	3	Month 2	Reversed with stent change
4	Vomiting	3	Week 2	Reversed after hospitalization, i.v. hydration
	Nausea	3	Week 4	Reversed
5	Elevated bilirubin	4	Day 1	Reversed with stent change
	Elevated SGPT	3	Day 1	Reversed
	Elevated Alkaline phosphatase	3	Day 1	Reversed
	Elevated bilirubin	4	Week 1	Reversed with stent change
	Elevated alkaline phosphatase	3	Week 1	Reversed
	Nausea and vomiting	3	Week 3	Reversed after hospitalization, i.v. hydration
	Elevated amylase	3	Week 4	Reversed
6	Elevated SGOT	3	Month 3	Reversed
7	Nausea and vomiting	3	Week 1	Reversed after hospitalization, i.v. hydration
	Elevated SGOT/SGPT	3	Week 4	Reversed

SGPT: serum glutamic-pyruvic transaminase; SGOT: serum glutamic-oxaloacetic transaminase.

burg, NY). FNA was done using the GIP/Medi-Globe (Tempe, AZ) 22-gauge, 10-cm needle as previously described,²³ and FNI was performed under direct real-time ultrasound guidance into the pancreatic tumor using a modified technique (Fig. 1). After localizing the tumor on EUS, the needle was advanced through the bulk of the tumor by real-time ultrasound guidance. After a “well” was created with the needle, the needle was slowly withdrawn while the cytoimplant was simultaneously injected in a slow, steady fashion.

Study Design

Escalating doses of 3, 6, and 9 billion cytoimplant cells were implanted into the pancreatic tumor by a single EUS-guided FNI on Day 0. Toxicity were assessed at Day 1, Week 1, and Months 1 and 3. Toxicities were

monitored according to National Cancer Institute (NCI) criteria. Liver tests, including transaminase, alkaline phosphatase, and especially bilirubin, are commonly elevated in patients with pancreatic carcinoma due to obstruction of the common bile duct. This is particularly true for patients with prior biliary obstruction requiring insertion of plastic biliary stents. These stents often obstruct spontaneously, causing hyperbilirubinemia, which is readily corrected and reversed with a simple stent change. Therefore, we modified the NCI toxicity criteria in this category to reflect changes above baseline values instead of normal values. Dose-limiting toxicity was defined as irreversible Grade 3, irreversible biliary Grade 4, and any nonbiliary Grade 4 toxicity.

Serious adverse events were defined as follows:

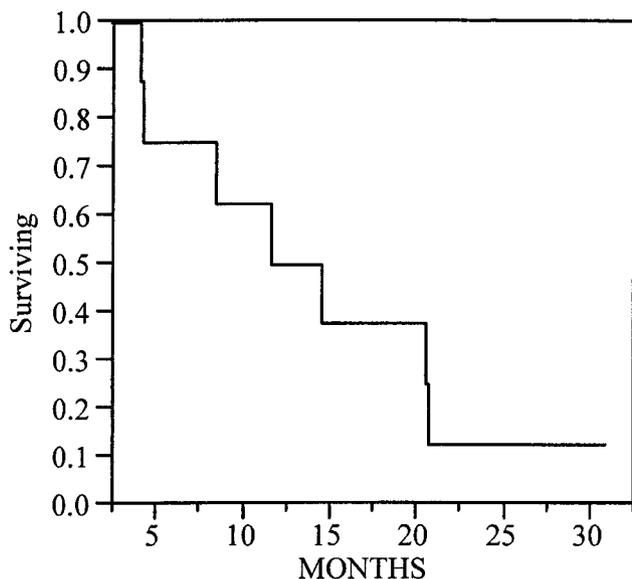


FIGURE 2. A Kaplan-Meier survival curve for the study patients is shown.

any deaths, life-threatening events, events that required or prolonged in-patient hospitalization, any new cancer, any laboratory abnormality assessed as Grade 4, or any other laboratory abnormalities that the investigator felt were major clinical concerns (especially when associated with relevant signs or symptoms).

Clinical endpoints monitored included survival, tumor response (CT and/or EUS), CA19-9, and Karnofsky performance status assessed at Months 3, 6, 9, 12, and 24. Survival was measured from the date of first treatment to the date of death. Tumor response based on CT and/or EUS were defined as follows: Complete response was defined as total disappearance of all tumor manifestations initially observed, with no evidence of new areas of malignant disease. A partial response was a greater than 50% reduction in the product of the 2 dimensions measured on CT scan or EUS. Tumor reduction smaller than 50% for more than 2 months was designated "minor response" and tumor stabilization longer than 2 months as "no change." "Progressive disease" was defined as a greater than 25% increase in known malignant disease. Tumor marker (CEA and CA19-9) regression of more than 20% was designated "decrease in tumor marker." When both tumor markers were elevated, only the parallel reduction of CEA plus CA19-9 was considered a "decrease." A rise of 20 points in KPS from baseline within the first 3 months was defined as improved performance, whereas a decrease of 20 points from baseline was defined as decreased performance.

Statistics

All demographic and background variables, efficacy variables, and toxicity variables were descriptively summarized. Categorical variables were summarized by the number and percentage of patients in each category. Continuous variables were summarized by the number, mean, median, standard error, minimum, and maximum.

The primary objective of this study was to assess the feasibility of intratumoral injections of cytoimplant via EUS-guided FNI and to assess the toxicity associated with three dose levels of such therapy when given as an outpatient regimen. Toxicity rates were descriptively summarized.

In addition, patient survival and tumor response (using imaging studies and serum tumor markers) were also recorded. A Kaplan-Meier survival curve was plotted for the entire cohort. Tumor response, CA19-9 serum marker response, and KPS were descriptively summarized.

RESULTS

From May 1995 to March 1997, 8 patients were entered into this Phase I study and treated with cytoimplant. The characteristics of all patients entered into this study, including clinical and pathologic staging and prior therapy, are listed in Table 1.

Toxicities

Toxicities were monitored according to the NCI common toxicity grading system and are presented in Table 2. All Grade 3 and 4 events are summarized in Table 3. As this therapy represented only a single injection of cytoimplant, the toxicities and adverse events were reported for a 3-month interval (from implant to Month 3). A dose-limiting toxicity was not reached during this study. The most common side effect was a low grade fever, which occurred in all but two patients. Patients 2, 6, 7, and 8 developed low grade fever (between 37.4 °C and 38.2 °C) on Day 1; this persisted through Month 1 but subsequently normalized prior to the next follow-up. Patients 3 and 4 had only transient Grade 1 fever. These low grade fevers were not associated with leukocytosis and were treated successfully with acetaminophen. Elevated bilirubin, liver enzymes, and nausea/vomiting with dehydration were the most common Grade 3 and 4 events (Table 3). Three patients developed hyperbilirubinemia. All three of these patients had preexisting biliary stents prior to treatment, and all three had normalization of bilirubin to baseline with replacement of new biliary stents. Three patients experienced Grade 3 nausea/vomiting during the first 3 months

TABLE 4
Summary of Clinical Response

Patient	Dose (10 ⁹ cells)	KPS	CA19-9 response	Tumor response	Survival (mos)
1	3.0	Stable	Inc	PR	4.2
2	3.0	Stable	Dec	PR	20.8
3	6.0	Stable	Dec	NC	20.7
4	6.0	Stable	Stable	PD	4.3
5	6.0	Stable	Inc	MR	14.6
6	9.0	Stable	Stable	NC	36.0+
7	9.0	Stable	Inc	NC	11.7
8	9.0	Stable	Stable	PD	8.5

KPS: Karnovsky performance status; Inc: increase; Dec: decrease; PR: partial response; MR: minor response; NC: no change; PD: progressive disease.

TABLE 5
Tumor Response Determined by Imaging Studies

Patient	Tumor response	Imaging	Baseline tumor size (cm ²)	Follow-up size (cm ²)	Follow-up interval (mos)
1	Partial	CT	22.5	6.0	4
2	Partial	EUS	6.8	2.4	6
5	Minor	CT	30.0	22.4	5
		EUS	20.6	15.6	6

CT: computed tomography; EUS: endoscopic ultrasound.

and required hospitalization. All three of these patients had reversal of symptoms after several days of intravenous hydration. Serum amylase at Day 1, Week 1, and Month 1 remained normal in all patients except 1 with Grade 3 hyperamylasemia at Month 1 (the patient had a history of recurrent chronic pancreatitis as well as adenocarcinoma of the pancreas). Therefore, there were no immediate postprocedural complications in this series. There were no bone marrow, cardiac, pulmonary, neurologic, or dermatologic toxicities.

With respect to serious adverse events, within the first 3 months there were no deaths or life-threatening events. Events that required hospitalizations in the first 3 months included intravenous hydration for nausea and vomiting (3), biliary stent change (2), and pain management (1).

Survival

Survival was measured from the time of cytoimplant to the time of death. Kaplan–Meier survival analysis is shown in Figure 2. The overall median survival was 13.2 months, with a range of 4.2 to 36+ months (Table 4). Patient 6 was still alive at the time this article was submitted for publication, with a KPS of 80 (Month 33). This patient had a 2.7 × 2.6 cm pancreatic adenocarcinoma in the head of the pancreas, with evidence of portal vein invasion on EUS and a 1.2 × 1.1

cm lesion in the left lobe of the liver, which was confirmed on cytology to be metastatic on EUS-guided FNA prior to therapy. At 6 months of follow-up, the pancreatic tumor and liver lesion were not significantly changed in size. Both the tumor and liver lesion were rebiopsied by EUS-guided FNA. There were no cancer cells found. An ultrasound at Month 22 showed the liver lesion to have increased in size, and the patient was determined to have disease progression. The patient subsequently received chemotherapy with gemcitabine and continues to have slow disease progression.

Tumor Response

Tumor response demonstrated by CT and EUS are described in Tables 4 and 5. There were two partial responses, one minor response, three described as no change, and two described as progressive disease. The partial responders (Patients 1 and 2) showed a greater than 50% decrease in the largest cross-sectional area (bidimensional product) of the pancreatic tumor at Months 4 and 6. Patient 1, with known pancreatic ascites documented at baseline, had an increase in her ascites on the Month 4 CT. Neither paracentesis nor autopsy revealed any peritoneal metastasis. Of note, the microscopic examination of the pancreatic tumor at autopsy showed many foci of inflammatory cells surrounding tumor cells, consisting of lymphocytes

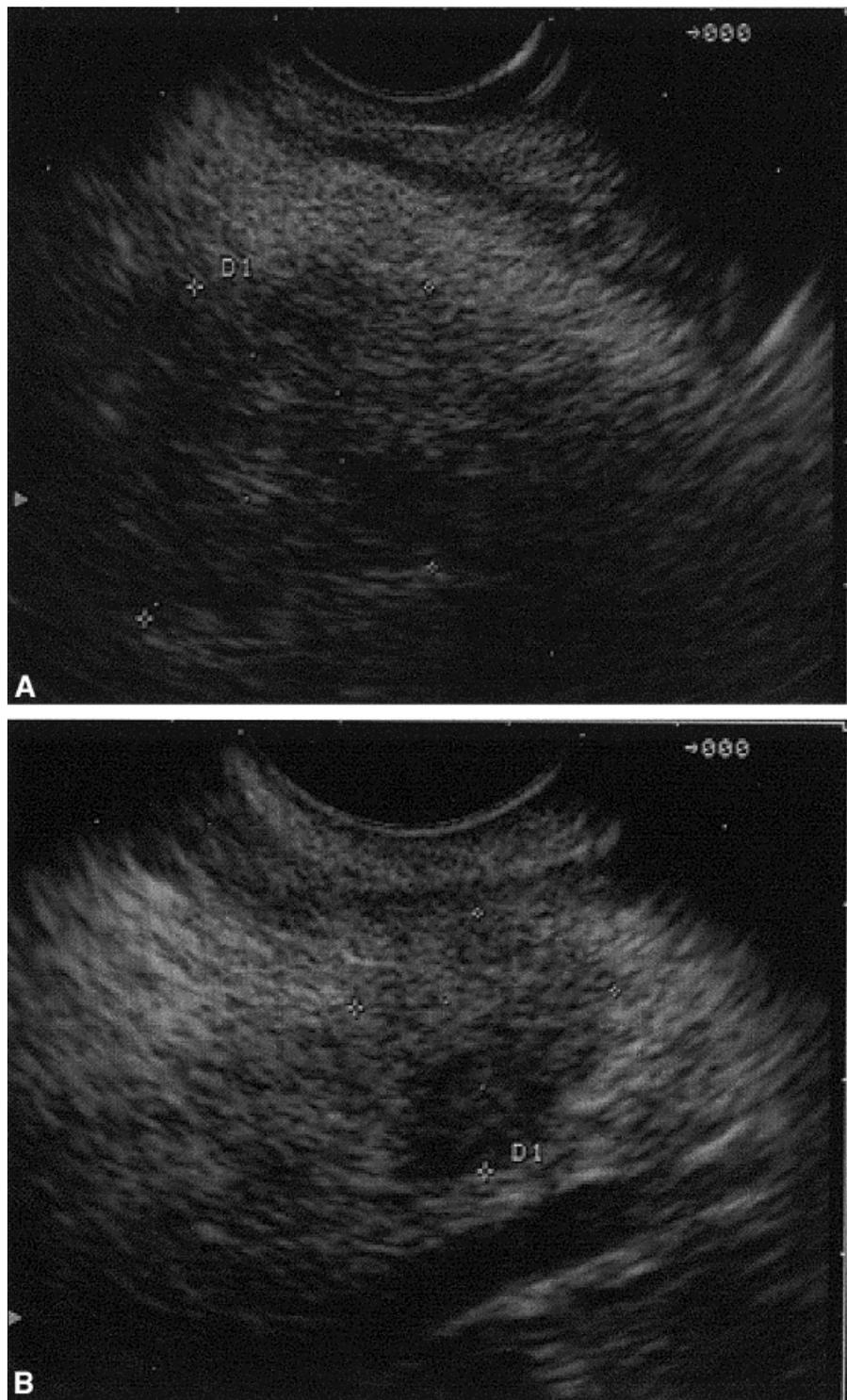


FIGURE 3. (A) An endoscopic ultrasound (EUS) image shows pancreatic adenocarcinoma at baseline, measuring 3.1×2.2 cm. (B) An EUS image shows that the same patient has decreased tumor size at Month 6; the tumor measures 1.5×1.6 cm.

and neutrophils as well as eosinophils and mast cells. Patient 2 had a baseline EUS showing a 3.1×2.2 cm tumor, which was deemed unresectable based on portal vein invasion. Subsequent EUS examination at Month 6 showed tumor shrinkage to 1.5×1.6 cm (Fig. 3). Patient 5 had a minor response on both CT and

EUS at Month 6.

CA19-9 tumor marker response is depicted in Table 4 and Figure 4. Three patients had increasing CA19-9, three remained stable, and two showed a decrease in CA19-9. The two patients with decreases in CA19-9 were Patients 2 and 3. Patient 2 showed a

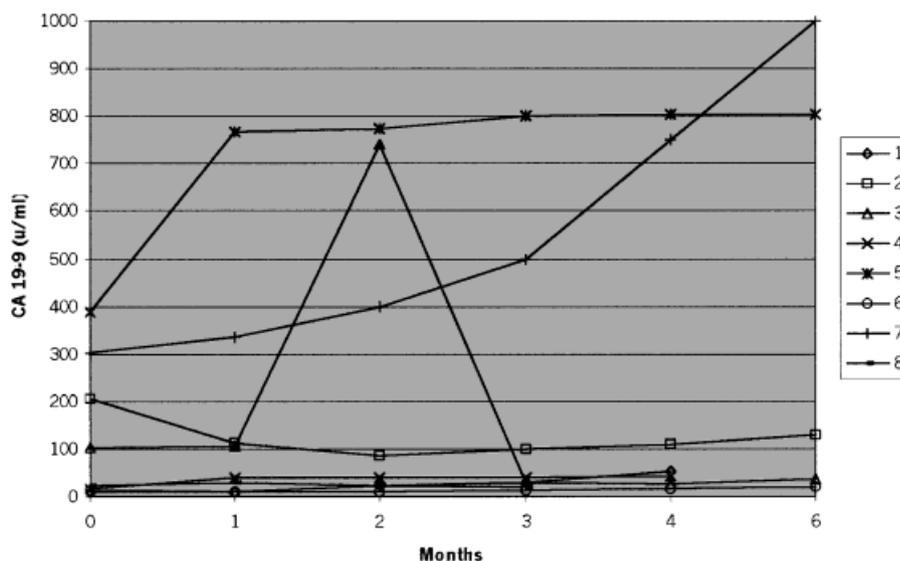


FIGURE 4. CA19-9 tumor marker response is shown by individual patient.

gradual decrease from a baseline of 206 μmL to 86 μmL at Month 2, which appeared to plateau with a gradual rise to 422 at Month 9. Patient 3 had a baseline of 103 μmL , which then flared to 740 μmL at Month 2 and normalized by Month 4.

KPS remained stable in all 8 patients during the first 3 months of therapy (Table 4).

DISCUSSION

Allogeneic mixed lymphocyte culture (cytoimplant) delivered by EUS-guided FNI represents both a novel immune therapy and a new delivery system for the treatment of patients with unresectable pancreatic adenocarcinoma. The rationale for such a therapy is based on preclinical and clinical data showing that cytokine production directly within a tumor can induce its regression by host antitumor effector mechanisms,^{11–13} as well as the well-established fact that mixed lymphocyte culture results in the release of cytokines and the activation of immune effector cells.^{14–18} In vitro studies of MLR have measured production of cytokines such as interleukin-2, interferon- γ , and soluble interleukin-2R. The presence of high concentrations of immune-enhancing cytokines may up-regulate tumor-associated major histocompatibility complex Class I antigens, thus facilitating recognition of the tumor by the inflammatory infiltrates.¹⁵ We conducted an animal study on experimental liver tumor (MADB106) in Fisher rats.¹⁹ Control animals all died after a mean survival of 38 days (range, 17–62 days). Animals receiving intratumoral implants of normal allogeneic Wistar lymphocytes survived significantly longer (mean, 51 days; range, 32–63), but all animals ultimately died of huge hepatic tumors. How-

ever, animals receiving intratumoral implants with allogeneic (Wistar) lymphocytes sensitized in vitro against Fisher alloantigens (MLC) survived 68 days (range, 55–300 days). Animals in the MLC group that survived had developed systemic immunity, for they were resistant to an intraperitoneal injection with a lethal dose of MADB106 cells. Based on these well-recognized principles and preclinical data, we developed a novel form of local immunotherapy that recreates the MLR directly in the center of malignant pancreatic tumors using a new EUS-guided FNI technique.

Toxicities

Toxicities were generally acceptable in this study. Dose escalation did not reach a limiting toxicity in this study. The maximal number of cytoimplant cells with a single leukaphoresis was approximately 10 billion cells. The volume of cytoimplant was limited to less than 10 mL due to the size of these tumors. The most common side effect was a low grade fever not associated with leukocytosis. The mechanism for the low grade fever was uncertain and there was no correlation to tumor response or survival. All fevers were treated successfully with acetaminophen. Elevated bilirubin, liver enzymes, and nausea/vomiting with dehydration were the most common Grade 3 and 4 events. All three of the patients who developed hyperbilirubinemia had preexisting biliary stents. This most likely represented the natural history of biliary prosthesis with plastic stent occlusion that typically occurs within 3 months of placement. This is further supported by the normalization of bilirubin to baseline with replacement of new biliary stents. Three patients

experienced Grade 3 nausea/vomiting during the first 3 months and required hospitalization; symptoms resolved after several days of intravenous hydration. Possible explanations for the nausea and vomiting included cytoimplant-induced tumor necrosis, dehydration, and transient gastric outlet obstruction. Treatment-induced pancreatitis as an explanation for the nausea and vomiting was unlikely, given that serum amylase at Day 1, Week 1, and Month 1 remained normal in all patients except the single patient with a preexisting history of recurrent chronic pancreatitis. The incidence of EUS-guided FNA causing pancreatitis is probably well below 1%.²⁹ Although we were concerned about the possibility of pancreatitis caused by the injection, our results did not show any complications of clinically significant pancreatitis, and there were no other immediate postprocedural complications in this series.

Survival and Tumor Response

The overall median survival was 13.2 months, with a range of 4.2 to 36+ months (Table 5). One patient is still alive 3 years from initial therapy. This patient had cytologic evidence of liver metastasis prior to therapy. At 6 months, both the primary tumor and the liver metastasis were negative for cancer on repeat EUS-guided FNA. Survival of longer than 12 months was seen in all 3 dose groups. There were two patients with partial tumor response and one with a minor response. There was no obvious correlation between tumor response and survival. The volume of the "tumor," however, on imaging studies may not accurately reflect the number of cancer cells, with fibrosis, necrosis, and inflammatory cells being constituents of the mass lesion. In 1 patient with no change in tumor size over 6 months, repeat EUS-guided FNA was negative for malignant cells despite a positive baseline cytology. With injection of cytoimplant and the proposed immunologic reaction, the volume of the tumor on imaging studies may hypothetically remain unchanged or increase despite reduction of malignant cells. In 1 of 2 autopsies performed in this series, an unexpected eosinophilic reaction was observed within the primary tumor 4 months after therapy.

Three patients had increasing CA19-9 levels during the study, whereas 3 remained stable and 2 showed an overall decrease. Of the 3 patients with stable CA19-9, all had normal values at baseline. These most likely represent non-CA19-9-producing tumors. Two patients had a decrease in CA19-9 from baseline. Patient 3 had an elevated baseline value of 103 μ /mL, which suddenly peaked at 740 μ /mL at Month 2 before a sustained normalization at Months 4 and 6. The cause of this peak at Month 2 was uncertain, although

one could hypothesize that the release of CA19-9 correlated with rapid tumor cell destruction prior to normalization. Both patients with decreased CA19-9 showed a nadir between Months 2 and 4. This may suggest a time interval of approximately 3 months to see the maximal effect of this therapy.

We thus conclude that a single injection of cytoimplant immunotherapy by EUS-guided FNI appears to be feasible, without substantial toxicity. A multicenter Phase II/III clinical trial is currently underway to determine the efficacy of cytoimplant compared with gemcitabine.

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