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Efficacy of Immuno-cell Therapy in Patients with Advanced Pancreatic Cancer

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Abstract. *Background: Patients with advanced pancreatic carcinoma have a risk of relapse after primary therapy, and the prognosis for these patients remains bleak. The effect of immuno-cell therapy in advanced pancreatic carcinoma, with or without other standard therapies, was examined. Patients and Methods: Forty-six patients with advanced pancreatic carcinoma, undergoing immuno-cell treatment, were evaluated. Results: Of all the patients, those who received immuno-cell therapy alone accounted for 15.4% of partial response (PR), 23.1% of long-term stable disease (SD), 46.2% of SD and 15.4% of progressive disease (PD), and had a 50% survival time of 14.5 months. The respective values for the 28 patients undergoing immuno-cell therapy with gemcitabine were 10.7% of PR, 10.7% of long-term SD, 32.1% of SD and 46.4% of PD, with a 50% survival time of 15.8 months; for 5 patients undergoing immuno-cell therapy with UFT or TS-1, the values were 0% of PR, 0% of SD, 20.0% of SD and 80.0% of PD, with a 50% survival time of 16.1 months. Conclusion: The combination of immuno-cell therapies with standard therapies may be effective in the short-term in patients with advanced pancreatic cancer. Long-term survival depends on the presence of metastases and the duration of coadministration with these standard therapies.*

Although the outcomes for many gastrointestinal cancers have improved, a nationally based investigation of pancreatic cancer in Japan in 1999 showed that the 5-year survival rate for pancreatic carcinoma remains at less than 19% (1). Because pancreatic carcinoma is often asymptomatic in its early stages, most patients have widespread disease at the time of diagnosis, often with a TNM Classification of Malignant Tumors advanced tumor staging of T3 or T4. In particular, owing to the difficulty of early diagnosis, the degree of biological malignancy and the anatomically disadvantageous location of the pancreas next to the portal vein, aortic artery and para-aortic lymph nodes, early invasive ductal carcinoma develops rapidly and shows an extremely poor prognosis (2). This poor outlook for ductal cancer is compounded by the primary and acquired resistance of tumor cells to antineoplastic agents. Unfortunately, the available treatment regimens for progressive or recurrent pancreatic carcinoma have yielded disappointing results, with responses generally of short duration. It is, thus, necessary to examine the impact of new treatment modalities on response rates and survival in pancreatic carcinoma patients with progressive or recurrent disease.

CD3-LAK, a type of autologous activated lymphocyte therapy (3), is currently the predominant immuno-cell therapy in Japan (4). In this therapy, T-lymphocytes, mostly peripheral blood mononuclear cells, are activated and proliferated through a culture process under stimulation with immobilized CD3-antibody and IL-2, and given by repeat administration to patients without the use of high-dose IL-2 administration. This therapy has few severe side-effects. Beginning in the early 1990s and continuing until the present, many university hospitals and cancer centers have actively investigated the use of autologous activated lymphocyte therapies, including LAK, TIL, CTL, CD3-LAK and dendritic cell (DC) (5). Among these, the Seta Clinic Group, which includes four private clinics providing

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Key Words: Immuno-cell therapy, CD3-LAK, dendritic cell (DC), pancreatic carcinoma, gemcitabine.

Table I. *Patient characteristics.*

Characteristic		Value
Sex M/F		32/14
Age (yr)		
Median		64.9
Range		41-88
Risk factors and past illness		
Smoking		7 (15.2%)
Diabetes mellitus		12 (26.1%)
Chronic pancreatitis		0
Gallstones		3 (6.5%)
Peptic ulcer		1 (2.2%)
Previous therapy for pancreatic cancer		
Operable cases	operation alone	2
	with chemotherapy	5
	with radiotherapy	1
Inoperable cases		26
Recurrent cases		12
Metastasis (+/–)		24 / 22
Performance status (0/1/2/3/4)		(22/20/10/2/0)
Immuno-cell therapy-related adverse effects		the entire 668 cycles
Fever		-
Fatigue		-
Eruption		-
Others		-

Table II. *Comparative data in terms of previous therapy for pancreatic carcinoma.*

	Operable cases	Inoperable cases	Recurrent cases
Patient numbers	8	26	12
Sex M/F	4/4	17/9	11/1
Age (yr)			
Median	65.5	63.9	62.0
Range	41-78	52-88	50-73
Performance status (0/1/2/3/4)	(3/3/2/0/0)	(13/9/4/0/0)	(3/5/2/2/0)
Metastasis +/–	4/4	10/16	10/2
Type of immuno-cell therapy (since beginning of therapy)			
Immuno-cell therapy alone			
total numbers	3	9	1
PD/SD/long SD/PR	1/1/1/0	2/4/2/1	0/0/0/1
Immuno-cell therapy with GEM			
total numbers	4	15	9
PD/SD/long SD/PR	2/1*/0/1*	6*/5/2/2	5/3/1/0
Immuno-cell therapy with others			
total numbers	1	2	2
PD/SD/long SD/PR	1/0/0/0	2/0/0/0	1/1/0/0

*: including one case of DC+LAK (DC vaccination and CD3-LAK injection)

specialized immuno-cell therapies with cell-processing facilities, has treated more than 2,800 cancer patients using immuno-cell treatment over the past 6 years.

Here, as part of ongoing studies to improve response rates and survival in patients with progressive or recurrent cancer, we investigated the use of immuno-cell therapy in the treatment of patients with pancreatic cancer, with or without other standard therapies.

Patients and Methods

Patients. Of a total of 197 patients with pancreatic carcinoma treated from 1 April, 1999 to 28 February, 2005, 108 patients underwent at least 6 episodes of immuno-cell treatment. Evaluation by diagnostic imaging was done in 54 patients; among these, 8 were classified as showing no evidence of disease (NED) at first or follow-up examination and were therefore excluded from the analysis. Interestingly, 7 of these 8 remain cancer-free at the time of writing, while 1 has relapsed and is now under treatment with a combination of immuno-cell therapies and gemcitabine.

The remaining 46 patients with advanced pancreatic carcinoma were eligible for inclusion and were enrolled in the study. All had histologically-proven pancreatic carcinoma. The patient characteristics are summarized in Table I and are detailed in Table II. There were 32 men and 14 women, with a median age at the beginning of immuno-cell therapy of 64.9 years (range 41 to 88 years). Among them, 7 patients (15.2%) were habitual smokers, 12 (26.1%) had Type II diabetes, 3 (6.5%) had gallstones and 1 (2.2%) had peptic ulcers. No patient had chronic pancreatitis. The patients were classified as follows: 2 patients were within 3 months of previous surgery but had received no other therapy; 5 were within 3 months of previous surgery and first-line chemotherapy; 1 was within 3 months of previous surgery and radiotherapy (6); 26 were within 3 months of previous inoperable surgery subsequent to exploratory laparotomy; and 12 were suffering from recurrent pancreatic carcinoma after previous surgery and first-line chemotherapy with a treatment-free interval of at least 6 months. Twenty-four patients (52.2%) had remote metastases. The ECOG performance status was 0 to 3 in all 46 patients.

Treatment. Activated lymphocytes were generated as described elsewhere (3). Briefly, about 22.5 ml of peripheral blood was obtained, and mononuclear cells (MNCs) were separated using Vacutainer (Becton Dickinson, NJ, USA). Following activation with immobilized anti-CD3 monoclonal antibody (Janssen-Kyowa, Tokyo, Japan) using HyMedium 930 (Kohjin Bio, Saitama, Japan) containing 1% autologous serum, the MNCs were cultured for 2 weeks with 700 IU/ml of recombinant interleukin-2 (Proleukin®, Chiron, Amsterdam, Netherlands). After culture, 3-10x10^9 cells were harvested and suspended in 100 ml of saline for intravenous injection. For generation of DCs, 45 ml of peripheral blood was collected, and MNCs were separated and allowed to adhere to the plastic culture flask to obtain adherent cells. After removal of non-adherent cells, the adherent cells were cultured in the presence of

Table III. *Results of immuno-cell therapy with or without other therapies.*

	Immuno-cell therapy alone		with GEM		with others	
	meta (–)	meta (+)	meta (–)	meta (+)	meta (–)	meta (+)
Number of patients	7	6	13	15	1	4
Immuno-cell populations						
Total cell populations during the 1st course	23.1		28.3		20.7	
(6 times DIV;billions)	18.4	27.7	23.9	32.1	28.1	19.2
Average cell populations at two-week	4.68		4.54		3.44	
intervals (billions)	3.63	5.73	3.91	5.10	4.68	3.20
Effectiveness						
PD	1	1	4	9	1	3
SD	3	3	5	4	0	1
Long-term SD	2	1	2	1	0	0
PR	1	1	2	1	0	0
CR	0	0	0	0	0	0
Effective ratio						
(PR+CR; %)	14.3	16.7	15.4	6.7	0.0	0.0
	15.4		10.7		0.0	

50 ng/ml GM-CSF (Primmune Corp., Osaka, Japan) and 50 ng/ml IL-4 (Primmune Corp.) for 6 days to obtain immature DCs. The DCs were cultured with antigens appropriate to the patient's tumor and allowed to mature for 24 hours prior to administration. Approximately 1-10x10^6 of mature DCs were then harvested and suspended in 1 ml of saline for subcutaneous vaccination. Immuno-cell therapy involves the single administration of lymphocytes, DCs or both, approximately every 2 to 4 weeks. Three patients, marked with asterisks in Table II, underwent DC+LAK therapy, while all others underwent CD3-LAK therapy.

Twenty-eight patients received gemcitabine by intravenous injection over 30 minutes either daily or bi-weekly for 3 weeks, followed by a 1-week pause, repeated every 4 weeks. The usual single dose was 1000 mg/m^2, but varied depending on the patient size, blood count and the cancer being treated (7, 8).

To prevent any adverse effect on lymphocytes while the blood level of anticancer agent was high, a treatment-free interval of at least 7 days was maintained between immuno-cell therapy and gemcitabine. Further, a treatment-free interval of at least 2 days was maintained between immuno-cell therapy and oral UFT or TS-1. In practice, the oral anticancer agent was given daily for 10 days, followed by a 4-day pause, with immuno-cells given on the third day of this pause. This 2-week cycle was then repeated.

Clinical response and assessment. Only patients with measurable lesions were included in the analysis. The responses were assessed according to the following definitions: complete remission (CR) indicated no assessable tumor disease, and normalization of tumor markers and laboratory values for at least 4 weeks; partial response (PR) indicated a decrease in the size of all measured lesions by 50% or more of the original diameter for at least 4 weeks, with no lesions increasing in size and no new lesions appearing; stable disease (SD) indicated a steady state or a response less than PR, but with no disease progression for at least 4 weeks, with no new lesions appearing and no symptoms worsening; and progressive disease (PD) indicated any increase of 25% in a single dimension or in the sum of the products of the perpendicular diameters of any measurable lesion. The time to progression and overall time were measured from the beginning of therapy to the time of disease progression and time of death, respectively. The Kaplan-Meier method was used to calculate survival probabilities for all patients.

Results

All patients were assessable for toxicity. Side-effects due to immuno-cell therapy were rare, with no treatment-related toxicity in the entire 668 cycles of immuno-cell therapy and no fever over 38.0˚C (grade 2 or greater), as described in Table I.

The overall response rate was 10.9%. No patient had a CR, while 5 (10.9%) had a PR of their measurable cancer lesions. SD was observed in 22 patients (47.8%), of whom 6 (13.0%) remained SD for more than 6 months (long-term SD). Nineteen patients (41.3%) had PD.

The patient who underwent immuno-cell therapy alone obtained a PR. On entry, this patient had multiple metastatic tumors in the liver and a marked elevation of serum levels of CA19-9 and CEA, to 203 U/ml (<35 U/ml) and 11.0 ng/ml (<2.5 ng/ml), respectively (Figure 1-A). The liver tumors disappeared after the completion of a single course of immuno-cell therapy, but the CEA and CA19-9 levels were not normalized (Figure 1-B). This patient has been treated with immuno-cell therapy alone for 20 months to the present. Repeat CT scanning of the liver at 3-month intervals has shown no reappearance of the tumor (Figure 1-C), and the CA19-9 and CEA levels have remained stable, ranging from 97 to 258 U/ml and 11.0 to 14.8 ng/ml, respectively.

Life tables of the patients were obtained and survival curves were computed using the Kaplan-Meier method. The overall 50% survival time, measured in all patients from the beginning of immuno-cell therapy, was 15.8 months (range, 3.1 to 36.7 months). When classified by performance status, 50% survival time was 22.3 months for the PS"0" group, 16.1 months for the PS"1" group and 10.7 months for the PS"2" or "3" groups (Figure 2), with no significant difference between them (Wilcoxon $p=0.6236$). Further, the 50% survival time was 10.7 months for the relapsed group, 14.5 months for the inoperable group and 17.9 months for the operable group (Figure 3), again with no significant difference between them (Wilcoxon $p=0.5228$).

The 13 patients who underwent immuno-cell therapy alone with no other therapies included 7 patients without

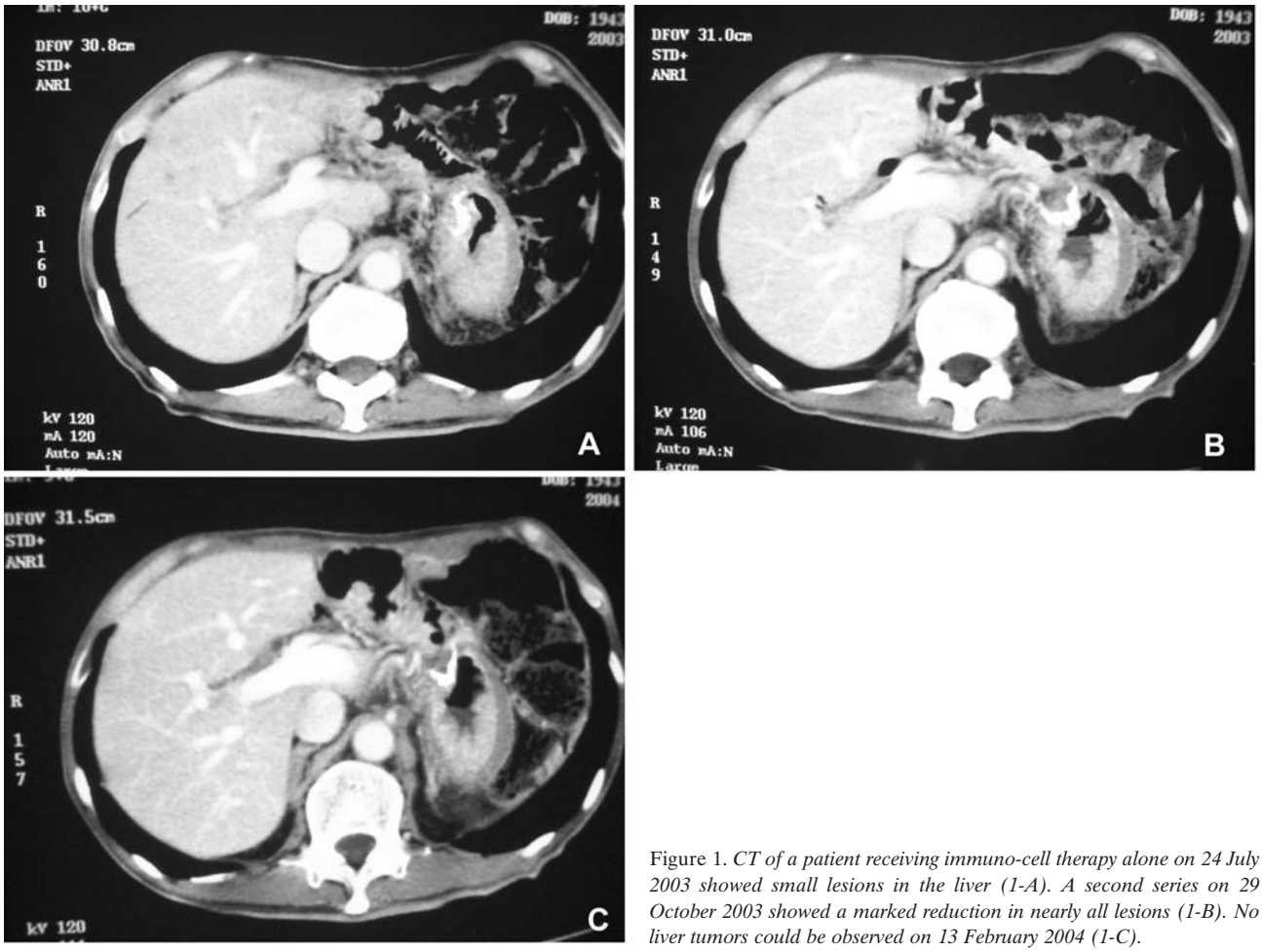

Figure 1. *CT of a patient receiving immuno-cell therapy alone on 24 July 2003 showed small lesions in the liver (1-A). A second series on 29 October 2003 showed a marked reduction in nearly all lesions (1-B). No liver tumors could be observed on 13 February 2004 (1-C).*

and 6 with metastases. The former accounted for 14.3% (1/7) of PR, 28.6% (2/7) of long-term SD, 42.9% (3/7) of SD and 14.3% (1/7) of PD, while the latter accounted for 16.7% (1/6) of PR, 16.7% (1/6) of long-term SD, 50.0% (3/6) of SD and 16.7% (1/6) of PD. The 50% survival time in this immuno-cell therapy-only group was 14.5 months (Figure 4).

The 28 patients who underwent immuno-cell therapy with gemcitabine included 13 patients without and 15 with metastases. The former accounted for 15.4% (2/13) of PR, 15.4% (2/13) of long-term SD, 38.5% (5/13) of SD and 30.8% (4/13) of PD, while the latter accounted for 6.7% (1/15) of PR, 6.7% (1/15) of long-term SD, 26.7% (4/15) of SD and 60.0% (9/15) of PD. The 50% survival time was 15.8 months in this immuno-cell therapy and gemcitabine group (Figure 4), being 10.3 months in the group with metastases and 22.3 months in the group without metastases, with no significant difference between them (Wilcoxon $p=0.1835$).

The 5 patients who underwent immuno-cell therapy in conjunction with other therapies such as oral UFT or TS-1 included 1 patient without and 4 with metastases. The former showed a PD, while the latter accounted for 25.0% (1/4) of SD and 75.0% (3/4) of PD. The 50% survival time in this immuno-cell and other therapy group was 16.1 months (Figure 4).

In total, the 50% survival time was 10.7 months in the group with metastases and 15.8 months in the group without metastases, with this difference showing slight statistical significance (Wilcoxon $p=0.4330$) (Figure 5). By response, the 50% survival time was 15.8 months for the PR group, 22.3 months for the SD group and 16.1 months for the PD group (Figure 6), with no significant difference among the 3 groups (Wilcoxon $p=0.8512$).

Discussion

The results of this investigation into the use of immuno-cell therapy in the treatment of advanced pancreatic cancer suggest that this treatment may be valuable in patients classified as PS"0" or PS"1".

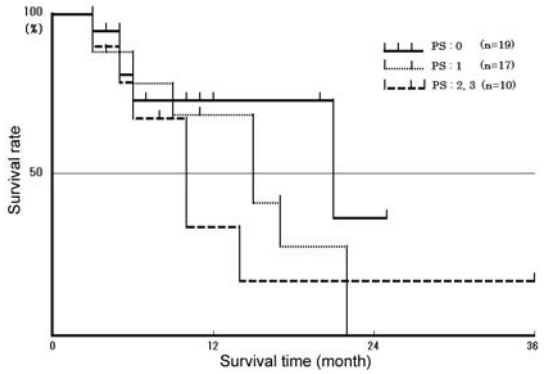

Figure 2. *Cumulative survival rates in relation to ECOG performance status.*

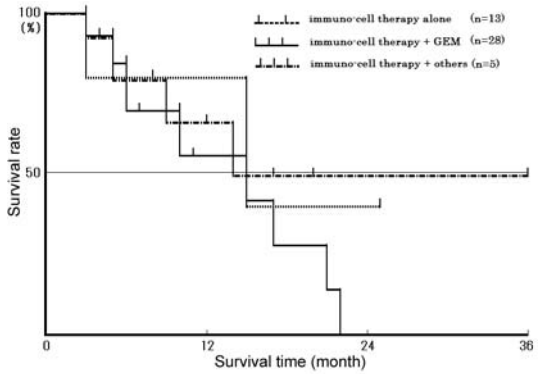

Figure 4. *Cumulative survival rates in relation to treatment method.*

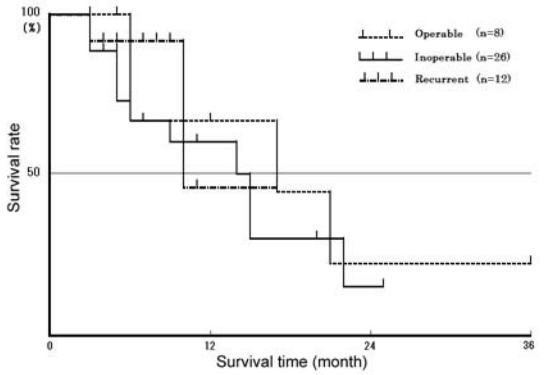

Figure 3. *Cumulative survival rates in relation to past history.*

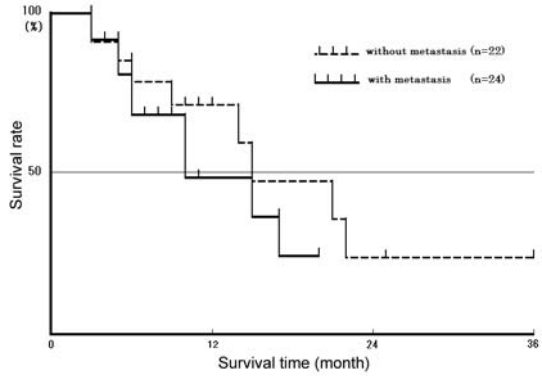

Figure 5. *Cumulative survival rates in relation to the presence of metastases.*

The treatment outcome in advanced pancreatic carcinoma largely depends on the extent to which the tumor can be surgically removed. When sufficient mass reduction is obtained, prognosis then depends on the histological malignancy of the tumor, the presence of adhesions to surrounding tissues, and dissemination or metastases (2, 6, 9). If the tumor is inoperable, the prognosis is poor. In the study group, the 50% survival time tended to decrease in the order of operable, inoperable and relapsed groups.

The short-term effect of treatment for 3 months in the immuno-cell treatment-alone group was better than that in the immuno-cell treatment plus gemcitabine group, but not significantly so. This difference was probably due to the greater number of patients with severe metastasis in the latter group.

The variation seen here in the effects of immuno-cell treatment are probably due to its different systemic effects and local immunosuppression of the cancer itself (10). This variation is probably exacerbated by combination with gemcitabine or other anticancer agents, which suppress not only tumor progression, but also the immune system.

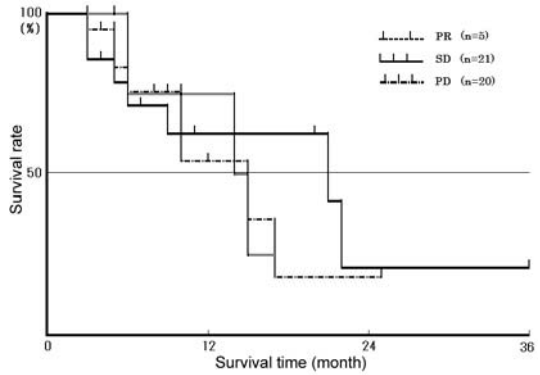

Figure 6. *Cumulative survival rates in relation to short-term response.*

Although a short-term benefit is not always reflected in the long-term prognosis, the long-term survival of the immuno-cell treatment-alone group was also better than that of the immuno-cell plus gemcitabine group.

Gemcitabine is reported to be useful in improving the general condition and in alleviating pain in pancreatic cancer, even though it does not induce a decrease in tumor size (7). In the present study, the patient condition deteriorated rapidly due to side-effects with gemcitabine to the extent that the treatment was stopped.

Given the findings that a poor prognosis is most clearly indicated by the presence of liver metastases (11), we plan to administer gemcitabine (or 5-FU) and immuno-cells by injection into the hepatic artery (12). The advantage of DC vaccinations against CD3-LAK reported by Kalady *et al.* could not be confirmed in our small patient population (13).

Under the dosing protocol used here, regular administration of gemcitabine had no adverse effect on the proliferation of lymphocytes. When immuno-cell treatment is combined with anticancer drugs, lymphocytes should not be collected until after a sufficient decrease in the blood level of the anticancer drugs, and the treatment should not be administered within several days of anticancer drugs. In a preliminary study, CD3-LAK obtained from the peripheral blood of healthy subjects was cultured in the presence of different concentrations of gemcitabine. Both cell proliferation and the cell survival rate were suppressed when gemcitabine blood levels increased above 5 μg/ml, but were hardly influenced at levels below 1 μg/ml. The gemcitabine package insert states that blood levels in patients are reported to decrease below 1 μg/ml within 1.5 hour of systemic administration of the drug at 1,000 mg.

Conclusion

This retrospective analysis of 46 patients, who primarily underwent activated autologous-lymphocyte treatment with CD3-LAK, showed encouraging results in advanced and relapsed pancreatic carcinoma. Immuno-cell therapy may allow a prolongation of the use of combination therapies and, thus, contribute to the prevention of drug resistance and an increase in the response to re-treatment with gemcitabine. Further studies are required to confirm that immuno-cell therapies can improve the response rates in patients with advanced and relapsed pancreatic carcinoma.

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