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Effect of adoptive T-cell immunotherapy on immunological parameters and prognosis in patients with advanced pancreatic cancer

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ABSTRACT

Background aims: Immunotherapy is effective for many types of cancer, but its benefits in advanced pancreatic cancer, which has a poor prognosis, are not well established. In this study, the authors examined the effects of adoptive T-cell immunotherapy (ATI) on immune cell profiles and prognosis in patients with unresectable advanced pancreatic cancer.
Methods: Seventy-seven patients with unresectable advanced pancreatic cancer were treated with six cycles of αβ T cells alone or in combination with chemotherapy or chemoradiation. Immune cell profiles in peripheral blood samples obtained before and after treatment were comprehensively evaluated by flow cytometry. Furthermore, associations between changes in immune cell frequencies and prognosis were determined.
Results: ATI prolonged survival to 18.7 months compared with previous estimates of 6.2–11.1 months for patients treated with chemotherapy alone. ATI decreased CD3+CD4+CD8− T cell frequency in peripheral blood and increased CD3+CD4−CD8+ T cell frequency. An increase in CD3+ T cells and CD3+TCRγδ− T cells in peripheral blood after treatment was associated with a good prognosis.
Conclusions: ATI altered the immune profile in peripheral blood, including CD3+CD4−CD8+ T cells, and improved prognosis in pancreatic cancer.
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Introduction

Rather than targeting the cancer itself, cancer immunotherapy activates the host immune system via cytokines, antibodies, immune checkpoint inhibitors and immune cells to treat cancer. In particular, the clinical application of immune checkpoint inhibitors has increased substantially. However, an effective cancer immunotherapy has not been established for pancreatic cancer, which ranked eighth with respect to cancer-related deaths in 2016 and accounts for increasing numbers of deaths each year [1,2]. Despite advances in chemotherapy, the 5-year survival rate for the disease is approximately 5–10% [1,2], and the prognosis of unresectable advanced pancreatic cancer remains poor.

For some cancers, the characteristics of the cancer microenvironment and relevant immune cell profiles have been elucidated. In melanoma, regulatory T cells infiltrating the cancer microenvironment express high levels of cytotoxic T lymphocyte-associated antigen 4 [3], and anti-cytotoxic T lymphocyte-associated antigen 4 antibodies exert an anti-tumor effect by suppressing these cells [4]. Additionally, repeated exposure to tumor antigens increases programmed cell death protein 1 expression on the surface of cytotoxic T cells and decreases cytotoxic activity [5]. T cells are reactivated by an anti-programmed cell death protein 1 antibody in melanoma, non-small cell lung cancer and renal cancer, thereby improving anti-tumor immunity in patients [6]. However, in advanced pancreatic cancer, the effects of immunotherapy on immune cell profiles and prognosis have not been adequately examined. In this study, immune cell profile and prognosis were evaluated in patients with unresectable advanced pancreatic cancer treated with adoptive T-cell immunotherapy (ATI) using αβ T cells.

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Table 1
Patient characteristics.

	All cases (n = 77)
Age (range)	65 years (39–86 years)
Sex, M/F	47/30
PS, 0/1/2–4	57/18/2
UICC TNM stage I/II/III/IV	0/0/17/60
Distant metastasis, yes/no	60/17
Previous treatment, yes/no	65/12
Surgery (chemotherapy after surgery)	7 (5)
Chemotherapy (chemotherapy after surgery)	50 (5)
(GEM)	(7)
(GEM + CDDP)	(1)
(GEM + TS-1)	(7)
(GEM + erlotinib)	(4)
(GEM + nab-PTX)	(20)
(TS-1)	(4)
(FOLFIRINOX)	(7)
CRT (chemoradiotherapy)	13
CRT (GEM/ GEM + TS-1/ TS-1/ SOX)	(4/1/7/1)
Concomitant treatment, yes/no	74/3
Surgery	0
Chemotherapy	69
(GEM)	(8)
(GEM + CDDP)	(2)
(GEM + TS-1)	(5)
(GEM + nab-PTX)	(23)
(TS-1)	(18)
(TS-1 + PTX)	(1)
(FOLFIRINOX)	(11)
(FOLFIRI)	(1)
CRT (chemoradiotherapy)	5
CRT (GEM/ GEM + TS-1/ TS-1)	(1/1/3)
Clinical response, CR/PR/SD/PD	0/11/28/38

CDDP, cisplatin; CR, complete response; CRT, chemoradiotherapy; F, female; FOLFIRI, irinotecan + fluorouracil + levofolinate calcium; FOLFIRINOX, oxaliplatin + irinotecan + fluorouracil + levofolinate calcium; GEM, gemcitabine; M, male; nab-PTX, nab-paclitaxel; PD, progressive disease; PR, partial response; PTX, paclitaxel; SD, stable disease; SOX, tegafur/gimeracil/oteracil potassium+oxaliplatin; TS-1, tegafur/gimeracil/oteracil potassium.

Table 2
Patient characteristics in PD and non-PD groups.

	PD (n = 38)	non-PD (n = 39)	*P* value
Age (range)	65 years (47–86 years)	65 years (39–80 years)	0.585
Sex, M/F	27/11	20/19	0.103
PS, 0/1/2–4	31/6/1	26/12/1	0.297
UICC TNM stage I/II/III/IV	0/0/5/33	0/0/12/27	0.098
Distant metastasis, yes/no	33/5	27/12	0.114
Previous treatment, yes/no	35/3	30/9	0.114
Surgery (received chemotherapy after surgery)	5 (4)	2 (1)	0.452
Chemotherapy (chemotherapy after surgery)	29 (4)	21 (1)	0.543
Chemoradiotherapy	5	8	0.165
Concomitant treatment, yes/no	37/1	37/2	1.00
Surgery	0	0	–
Chemotherapy	36	33	0.358
Chemoradiotherapy	1	4	0.358

F, female; M, male.

Methods

Patient population

The study included 77 patients (median age 65 years [39–86 years], 47 men and 30 women) with stage III or IV advanced pancreatic cancer based on the Union for International Cancer Control (UICC) staging system (eighth edition) who consulted the Kanazawa Advanced Medical Center between October 2010 and December 2018 and received six cycles of αβ T cells as ATI (see supplementary Figure 1 for consort diagram). This study was conducted in accordance with the Declaration of Helsinki after obtaining approval from the ethics committee of the Kanazawa Advanced Medical Center. All patients provided their written informed consent prior to the start of ATI.

Laboratory and imaging tests

Patient sera were used to measure tumor markers—carcinoembryonic antigen (CEA), carbohydrate antigen 19-9 (CA19-9) and Duke pancreatic monoclonal antigen type 2 (DUPAN-2)—before and after ATI administration. Serum CEA, CA19-9 and DUPAN-2 levels were measured by chemiluminescent immunoassay, electro chemiluminescence immunoassay and enzyme immunoassay methods, respectively.

A positron emission tomography-computed tomography (PET-CT) scan was performed before and after treatment. The tumor size at fluorodeoxyglucose (FDG) accumulation sites was measured by CT and evaluated based on revised (version 1.1) response evaluation criteria in solid tumors. Tumors without FDG accumulation prior to the start of treatment were excluded from the analysis. The following definitions were applied: complete response (CR), or disappearance of all target lesions; partial response (PR), or ≥30% reduction in the sum of target lesion diameters compared with baseline; progressive disease (PD), or ≥20% increase in the diameter of the smallest target lesion during the clinical course and ≥5 mm increase in the absolute value of the sum of

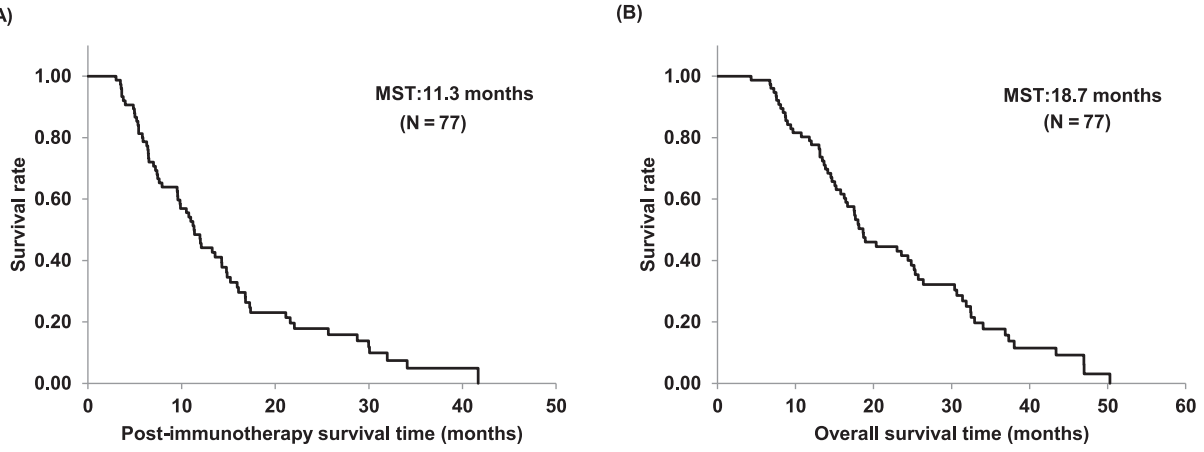

Figure 1. Kaplan-Meier survival curves. (A) Post-immunotherapy survival time. (B) Overall survival time.

diameters; and stable disease (SD), or neither PR nor PD. In addition, CR, PR and SD were defined as non-progressive disease (NPD).

Adoptive T-cell immunotherapy

Lymphocytes were isolated from peripheral blood (24 mL) drawn from each patient and activated by culturing with anti-CD3 monoclonal antibody (Jansen-Kyowa, Tokyo, Japan). These were then cultured for 14 days with IL-2 until the cell count was $\geq 0.3 \times 10^9$. The cultured lymphocytes consisted mainly of CD3+CD4+CD8− or CD3+CD4−CD8+ T cells (αβ T cells), with small percentages of natural killer cells and γδ T cells. The αβ T cells with a viable cell rate of ≥80% and endotoxin test results <0.25 EU/mL that passed sterility testing were returned to the patient's body intravenously. This was performed six times at 2-week intervals to complete one course. Fresh αβ T cells were repeatedly expanded from patients at every injection. The authors analyzed immune cell profiles in the administered T cells at least once in one course.

Analysis of peripheral immune cell profiles

Heparinized peripheral blood was collected from patients. Peripheral blood mononuclear cells (PBMCs) were analyzed using whole-blood staining with OptiLyse C lysis solution. Absolute cell counts were determined using Flow-Count fluorospheres. OptiLyse C, Flow-Count beads and monoclonal antibodies against CD3, CD4, CD8, CD25, CD45, CD56 and TCR pan γδ were purchased from Beckman Coulter (Brea, CA, USA). Lymphoprep (Axis-Shield PoC AS, Oslo, Norway) was used with gradient centrifugation to isolate the PBMCs. The PBMCs were fixed and permeabilized using a fixation/permeabilization kit (BioLegend, San Diego, CA, USA) according to the manufacturer's protocol, followed by staining with anti-Foxp3 monoclonal antibodies (clone 259D; BioLegend). The PBMCs were suspended in RPMI 1640 medium (Invitrogen, Grand Island, NY, USA) supplemented with 10% heat-inactivated fetal bovine serum (Invitrogen) containing 20 ng/mL phorbol 12-myristate 13-acetate (Sigma-Aldrich, St Louis, MO, USA), 2 μg/mL ionomycin (Sigma-Aldrich) and 20 μg/mL brefeldin A (Sigma-Aldrich) at 1×10^6 cells/mL. Cells were incubated at 37°C in a humidified atmosphere with 5% carbon dioxide for 4 h. Activated cells were fixed and permeabilized using IntraPrep, followed by staining with anti-interferon gamma (IFN-γ) or anti-IL-4 antibodies (Beckman Coulter). Isotype controls were stained with anti-human IgG1 (BioLegend), IgG1 (Beckman Coulter), MsIgG1 (Beckman Coulter) or MsIgG2a (Beckman Coulter). A Cytomics FC500 flow cytometer (Beckman Coulter) was used for data acquisition, and data were analyzed using CXP (Beckman Coulter).

Statistical analysis

Chi-square or Fisher's exact tests were used to compare categorical variables (e.g., sex) between the PD and NPD groups. The Wilcoxon signed-rank test was used to compare the immune cell profiles before and after ATI treatment. Mann-Whitney U tests were used to compare the immune cell profiles between the PD and NPD groups before and after treatment. Survival was evaluated using the Kaplan-Meier method and compared using the log-rank test and generalized Wilcoxon test. Binomial logistic regression analysis was used to compare the association between the T-cell subsets of the administered αβ T cells and progressive disease. $P < 0.05$ was considered statistically significant. The statistical analysis was conducted using Bell Curve for Excel (Social Survey Research Information Co, Ltd).

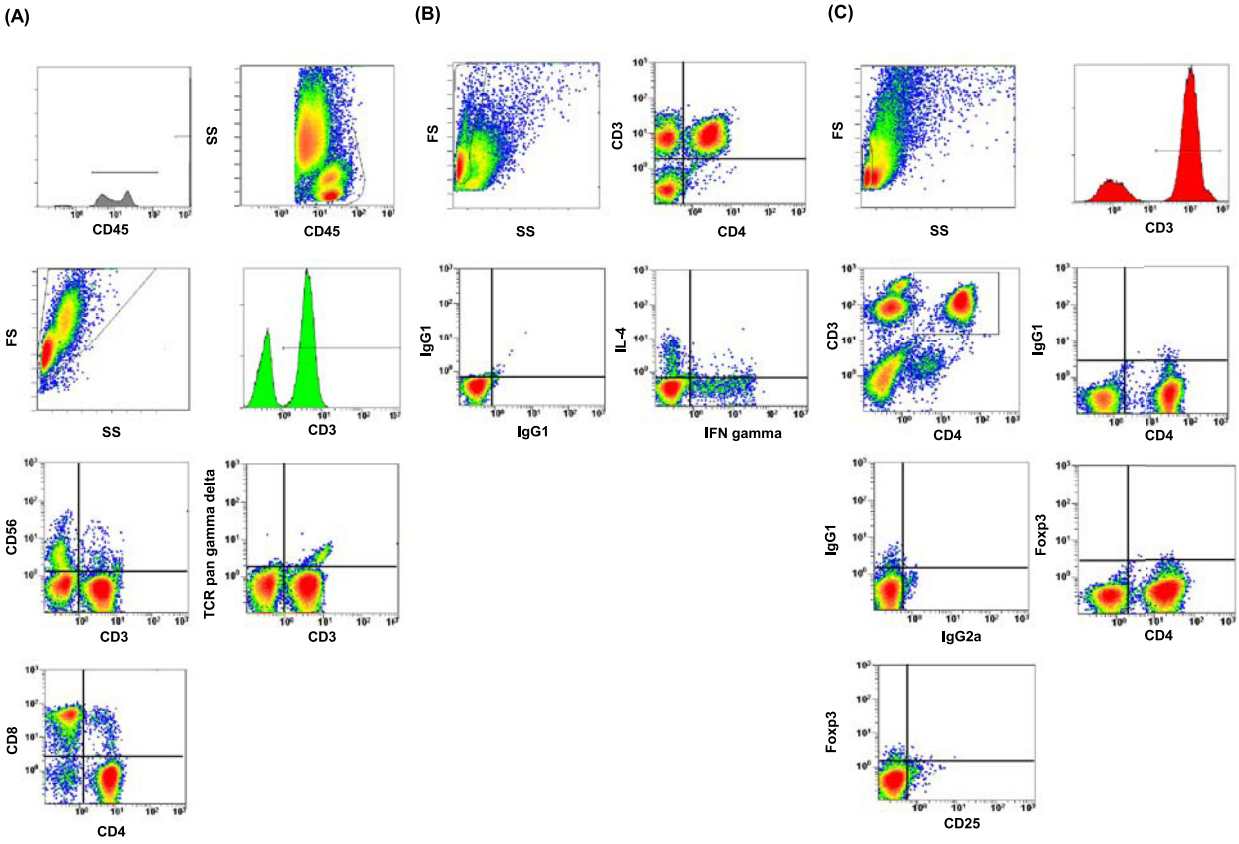

Figure 2. Peripheral blood immune cell profiles. (A) Frequencies of various cell populations were measured by multicolor FACS using the following antibodies: anti-CD3, CD4, CD8, CD45, CD56 and TCR pan γδ. (B) Frequencies of CD3+CD4+CD8−(IFN-γ+ IL-4−) T cells and CD3+CD4+CD8−(IFN-γ− IL-4+) T cells were measured by multicolor FACS using anti-CD3, CD4 and CD8 antibodies and cytokine production assays (IFN-γ and IL-4). Isotype controls were stained with anti-human IgG1 and IgG1. (C) Frequencies of CD3+CD4+CD25+Foxp3+ T cells were measured by multicolor FACS using anti-CD3, CD4 and CD25 antibodies and Foxp3 staining. Isotype controls were stained with MsIgG1 and MsIgG2a. FACS, fluorescence-activated cell sorting.

Results

Patient profile

Table 1 shows the clinical characteristics of patients with pancreatic cancer (n = 77). The median age was 65 (39–86) years, and there were 47 men and 30 women. In total, 57 patients had a performance status (PS) of 0, 18 patients had a PS of 1 and two patients had a PS of 2–4. According to the UICC staging system, 17 patients were classified as stage III, and 60 patients were classified as stage IV. There were 65 patients with a history of previous treatments, including two cases of surgery alone, five cases of chemotherapy after surgery, 45 cases of chemotherapy alone and 13 cases of chemoradiation therapy. Concomitant treatments were administered to 74 patients, where none of the patients underwent surgery; 69 patients received chemotherapy alone; and five patients underwent chemoradiation therapy.

Outcomes of ATI

After ATI, CR was not observed. PR was observed in 11 patients, SD in 28 patients and PD in 38 patients (38 patients in the PD group and 39 patients in the NPD group) (Table 1). Overall survival was defined as the time from the date of diagnosis to the date of death or final confirmation of survival, and post-immunotherapy survival was defined as the time from the start of ATI to the date of death or final confirmation of survival. The post-immunotherapy median survival time (MST) was 11.3 months (Figure 1A). MST was 18.7 months (Figure 1B). Clinical factors did not differ significantly between the PD and NPD groups (Table 2). The post-immunotherapy MST was 7.6 months in the PD group and 14.8 months in the NPD group ($P < 0.01$) (see supplementary Figure 2A). MST was 17.5 months in the PD group and 24.4 months in the NPD group ($P = 0.04$) (see supplementary Figure 2B).

With respect to treatment-induced adverse events, according to the common terminology criteria for adverse events (version 4.0), six patients (7.8%) exhibited fever (grade 1), one patient (1.3%) had malaise (grade 1–2), and 70 patients (90.9%) had no remarkable adverse events.

Immune cell profiles in the peripheral blood

Figure 2 shows an example of the immune cell profile in peripheral blood. In the CD45 histogram, the white blood cell region was set at the CD45+ peak. The mononuclear cell region was set in the CD45/SS cytogram gated with the CD45+ region, excluding granulocytes. Dead cells were removed from the SS/FS cytogram gated with the mononuclear cell region. In the CD3 histogram gated in this region (FS/SS), a CD3+ T-cell region was set at the CD3+ peak, and CD3+ T cells were separated. From the CD3/CD56 cytogram gated with the region (FS/SS) set as the analysis target, a four-split region was set, and CD3−CD56+ cells were isolated. From the CD3/TCR pan $\gamma\delta$ cytogram gated with the region (FS/SS) set as the analysis target, a four-split region was set, and CD3+TCR$\gamma\delta$− T cells and CD3+TCR$\gamma\delta$+ T cells were isolated. From the CD3 histogram gated in the region (FS/SS) set as the analysis target, a four-split region was set from the CD4/CD8 cytogram gated in the CD3+ T-cell region, through which CD3+CD4+CD8− T cells and CD3+CD4−CD8+ T cells were isolated (Figure 2A).

In SS/FS cytograms stained with PBMCs for intracellular cytokine staining, regions were set in the lymphocyte cell population with dead cells removed. A four-split region was set in the CD4/CD3 cytogram gated with this region (FS/SS). In the IgG1/IgG1 cytogram gated with the CD3+CD4+ region, the authors set a four-split region in the negative region as an isotype control. This region was also applied to the IFN-γ/IL4 cytogram to isolate CD3+CD4+CD8−(IFN-γ+ IL-4−) T cells and CD3+CD4+CD8−(IFN-γ− IL-4+) T cells (Figure 2B).

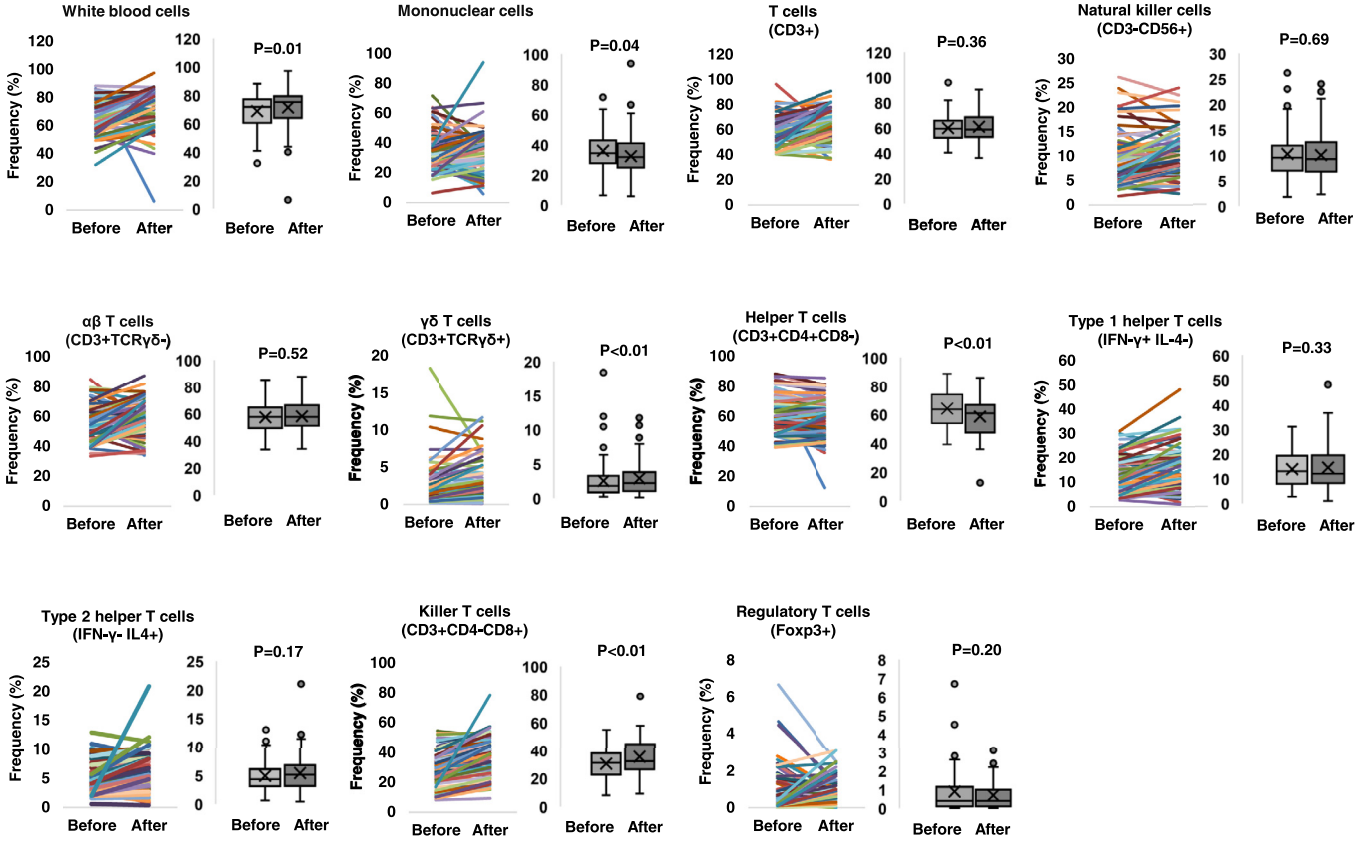

Figure 3. Differences in the immune cell profile before and after ATI. The line graph shows changes in immune cell frequencies in each patient after ATI. Box plots show the median (25%, 75%), mean, minimum and maximum frequencies before and after ATI.

Dead cells were removed from SS/FS cytograms with PBMCs, and a region was set for analysis of the lymphocyte cell population. In the CD3 histogram gated in this region (FS/SS), a region was set in the CD3+ peak. On the CD4/IgG1 cytogram gated in the CD3+ region, a four-split region was established in the negative region as an isotype control. This region was also applied to the CD4/Foxp3 cytogram. On the CD4/CD3 cytogram gated in the region (FS/SS), the CD4+CD3+ region was gated. On the IgG2a/IgG1 cytogram gated in this CD3+CD4+ region, a four-split region was established in the negative region as an isotype control. This region was also applied to the CD25/Foxp3 cytogram, and CD3+CD4+CD25+Foxp3+ T cells were isolated (Figure 2C).

The authors defined CD3−CD56+ cells as natural killer (NK) cells, CD3+TCR$\gamma\delta$− T cells as αβ T cells, CD3+TCR$\gamma\delta$+ T cells as $\gamma\delta$ T cells, CD3+CD4+CD8− T cells as helper T cells, CD3+CD4+CD8−(IFN-γ+ IL-4−) T cells as type 1 helper T cells, CD3+CD4+CD8−(IFN-γ− IL-4+) T cells as type 2 helper T cells, CD3+CD4−CD8+ T cells as killer T cells and CD3+CD4+CD25+Foxp3+ T cells as regulatory T cells.

Change in immune cell profile in response to treatment

Figure 3 shows differences in frequencies of each cell population before and after ATI. There was a significant increase in the frequencies of white blood cells from 71.6% (60.4–77.1%) to 75.1% (63.9–79.4%) (P = 0.01), of $\gamma\delta$ T cells (among PBMCs) from 1.8% (0.9–3.3%) to 2.2% (1.0–3.8%) (P < 0.01) and of killer T cells (among CD3+ T cells) from 31.0% (22.6–37.9%) to 32.2% (26.4–44.6%) (P < 0.01). There was a significant decrease in mononuclear cells (in white blood cells) from 34.4% (27.6–42.9%) to 31.7% (24.7–40.9%) (P = 0.04) and helper T cells (among CD3+ T cells) from 63.7% (54.0–74.1%) to 60.9% (47.5–66.8%) (P < 0.01). The frequencies of CD3+ T cells, NK cells, αβ T cells, type 1 helper T cells, type 2 helper T cells and regulatory T cells did not differ significantly before or after treatment.

Three patients received ATI monotherapy. Case 1 received chemotherapy (gemcitabine [GEM] + TS-1) as previous treatment. The therapeutic effect of ATI was PD. After ATI, the frequency of regulatory T cells declined from 1.8% to 0.8%, but there were no changes in helper T cells (59.6% vs 60.2%) or killer T cells (31.3% vs 30.2%) (see supplementary Figure 3A). Case 2 underwent surgery as previous treatment. The therapeutic effect of ATI was non-PD. After ATI, helper T cells (57.6% vs 52.5%) and regulatory T cells (1.0% vs 0.1%) decreased, and killer T cells (37.9% vs 41.9%) increased (see supplementary Figure 3B). Case 3 underwent chemotherapy (GEM + erlotinib) as previous treatment. The therapeutic effect of ATI was non-PD. After ATI, helper T cells (56.6% vs 52.1%) and regulatory T cells (2.2% vs 0%) decreased, and killer T cells (37.9% vs 42.6%) increased (see supplementary Figure 3C).

Relationship between immune cell profile and prognosis

Figure 4 shows the Kaplan-Meier survival curve and MST for each immune cell profile. MST was significantly longer in the group that

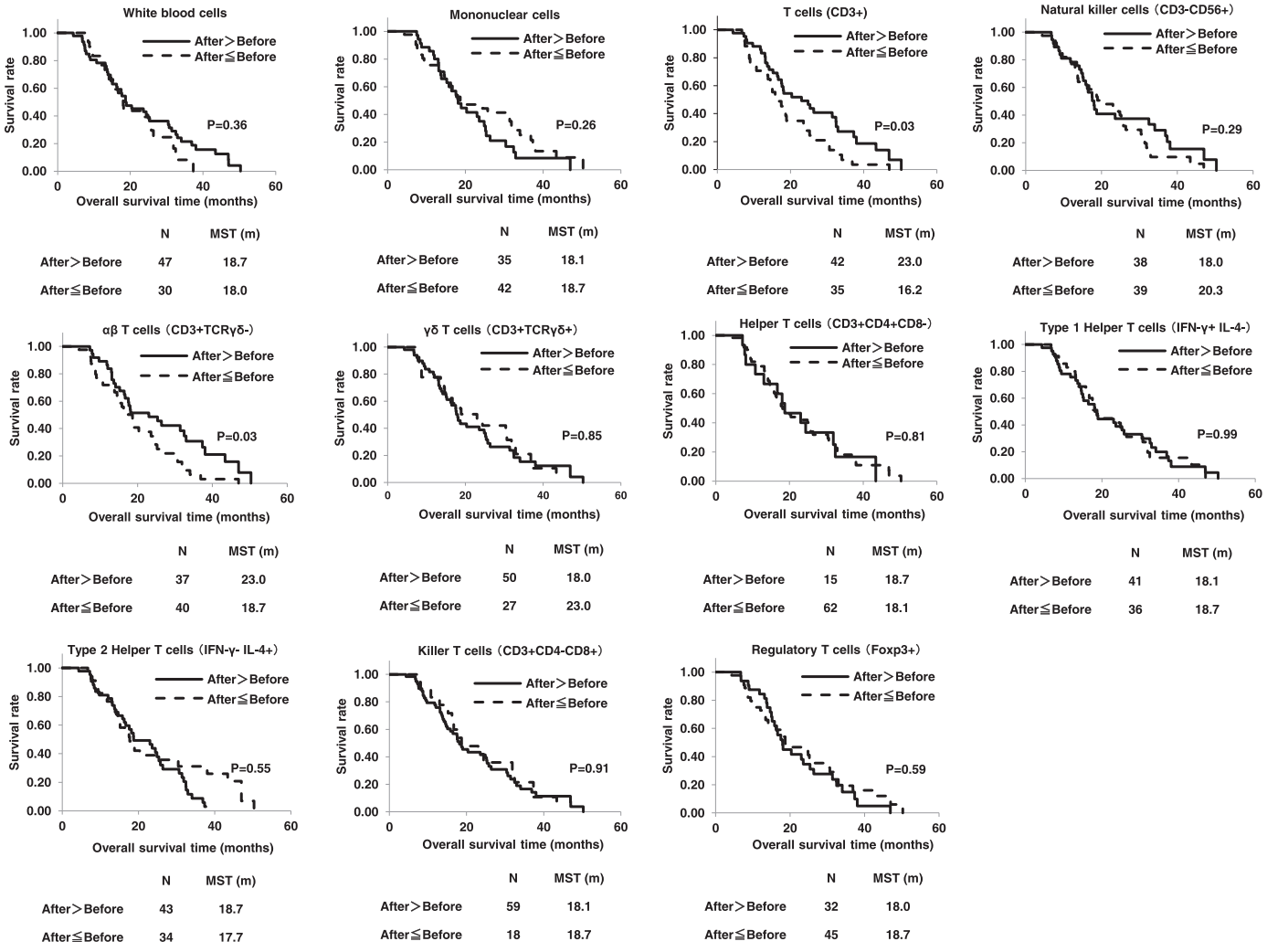

Figure 4. Comparison of survival by Kaplan-Meier survival curves between the groups with an increase or decrease in immune cell populations in response to ATI. Data are presented as medians (months). m, months.

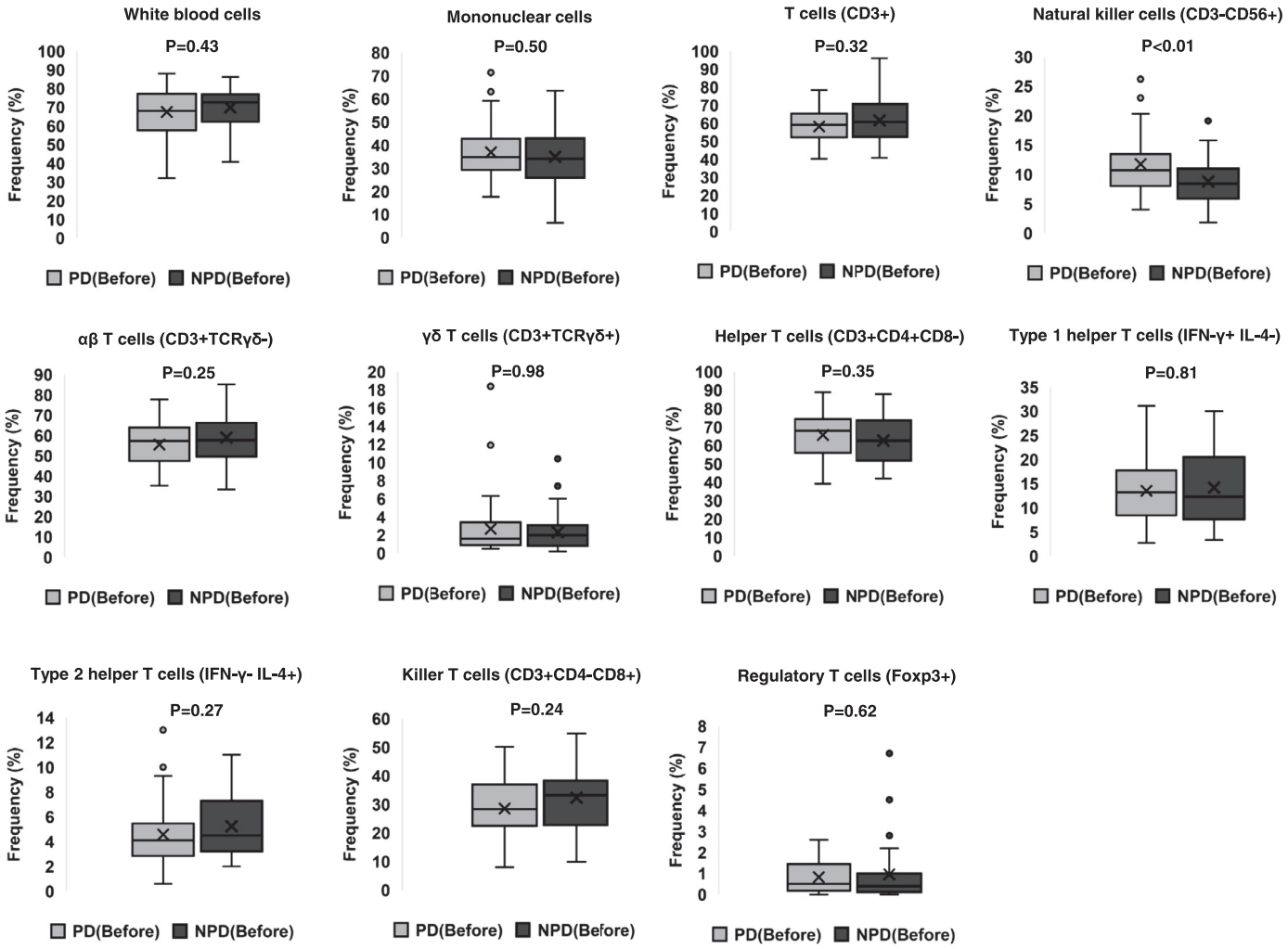

Figure 5. Comparison of immune cell profiles between the PD and NPD groups before the start of ATI. Box plots show median (25%, 75%), mean, minimum and maximum frequencies of immune cell types in the PD and NPD groups before ATI.

showed an increase in the frequency of CD3+ T cells after treatment (23.0 months) than in the group that showed a decrease in these cells (16.2 months) ($P = 0.03$). MST was also significantly longer in the group showing an increase in αβ T cells (23.0 months) than in the group showing a decrease (18.7 months) ($P = 0.03$). There were no differences in survival with respect to increases or decreases in white blood cells, mononuclear cells, NK cells, $\gamma\delta$ T cells, helper T cells, type 1 helper T cells, type 2 helper T cells, killer T cells or regulatory T cells.

Immune cell profile differences between PD and non-PD groups before immunotherapy

Figure 5 summarizes the immune cell profiles in the PD and NPD groups before ATI treatment. The frequency of NK cells prior to treatment was significantly lower in the PD group than in the NPD group (1.07% vs 8.4%, $P < 0.01$). Before treatment, the PD and NPD groups did not differ significantly with respect to frequencies of other examined cell types.

Immune cell profile differences between PD and non-PD groups after immunotherapy

Figure 6 summarizes the immune cell profiles in the PD and NPD groups after treatment. The frequencies of CD3+ T cells (63.4% vs 57.4%, $P = 0.04$), αβ T cells (60.9% vs 55.0%, $P = 0.03$) and type 2 helper T cells (5.4% vs 4.2%, $P = 0.04$) were significantly higher in the NPD group than in the PD group. The NK cell frequency was significantly lower in the NPD group (7.9%) than in the PD group (10.2%) after treatment ($P < 0.01$). There were no differences in other cell types between the two groups after treatment. There was no significant association between the T-cell subsets of the administered αβ T cells and progressive disease (see supplementary Table 1).

Clinical course and changes in immune cell profiles in two patients who responded to ATI

Figure 7 shows the clinical course and immune cell profiles of two representative patients with a PR to ATI. Let us consider case 1 (Figure 7A–C). A 50-year-old male was diagnosed with pancreatic ductal cancer based on serological and diagnostic imaging findings. In this case, the frequency of αβ T cells increased after ATI, and PET-CT images showed that the sizes of the primary and metastatic lesions were significantly reduced and the FDG accumulations of the lesions were significantly decreased after ATI. The primary lesion was pancreatic tail cancer, with para-aortic lymph node metastasis, multifocal liver metastasis and lung metastasis, clinically classified as T3N1M1 stage IV per the UICC staging system. There were no previous treatments, and oxaliplatin + irinotecan + fluorouracil + levofolinate calcium (FOLFIRINOX) was started as first-line chemotherapy. ATI was added from the third course of chemotherapy. After ATI, the tumor marker CEA decreased from 1392.2 ng/mL to 21.8 ng/mL, and CA19-9 decreased significantly from 27 100 U/mL to 2422 U/mL

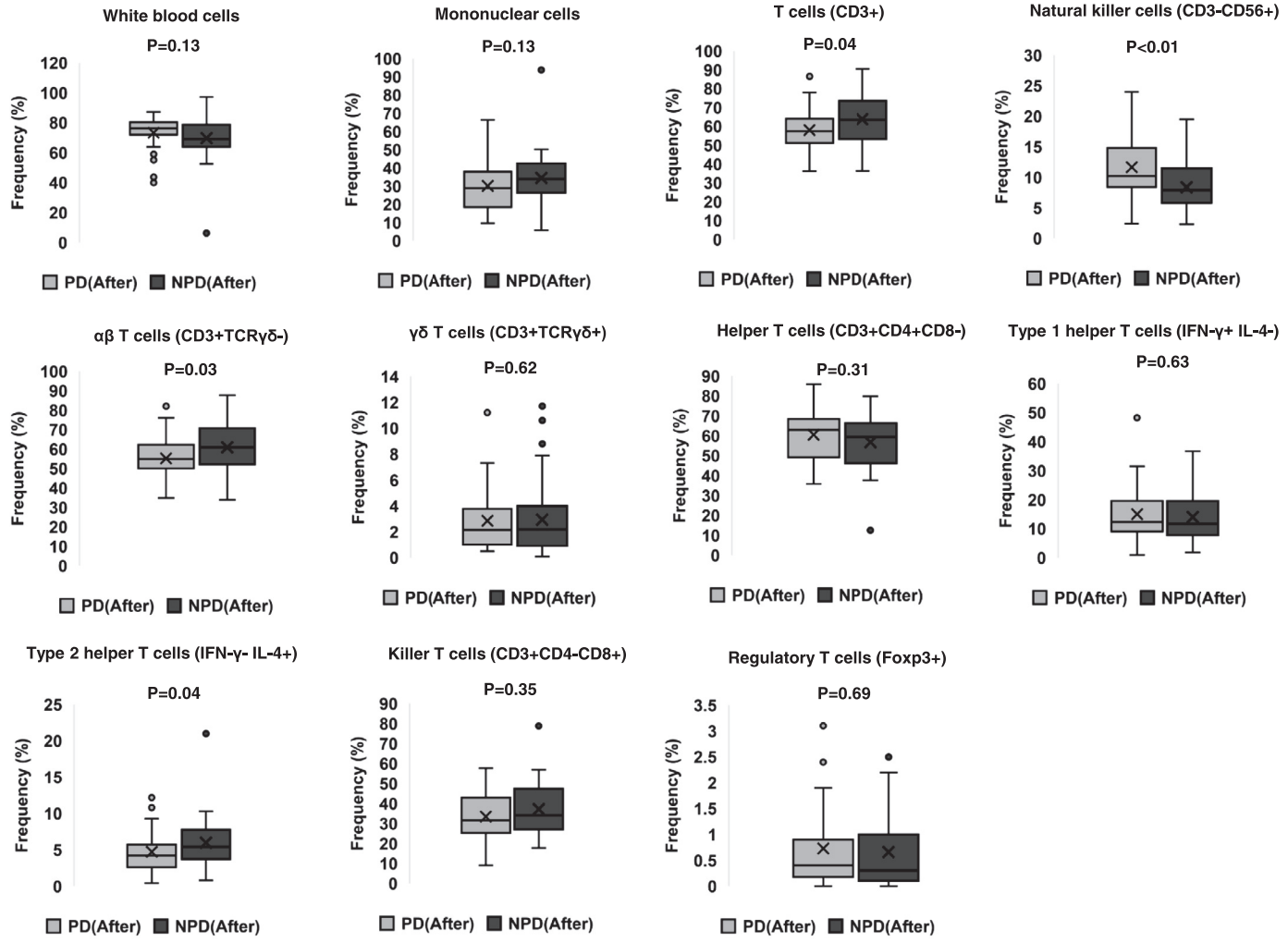

Figure 6. Comparison of immune cell profiles in the PD and NPD groups at the end of ATI. Box plots show median (25%, 75%), mean, minimum and maximum frequencies in the PD and NPD groups after ATI.

(Figure 7A). PET-CT images showed a standardized uptake value (SUV) of 4.2 in the pancreatic tail mass before treatment, but this decreased to an SUV of 3.0 after ATI with a reduction in tumor size. Prior to ATI, an SUV of 3.4 was observed in the lymph node metastasis around the para-aorta, with an SUV of 1.4 at the right lung nodule, but both disappeared after ATI. The multifocal liver metastasis showed a substantial decrease in accumulation after ATI (Figure 7B). As shown in Figure 7C, the frequencies of CD3+ T cells (65.9% vs 71.9%), αβ T cells (64.5% vs 69.8%) and killer T cells (21.6% vs 29.8%) increased after ATI, and the frequency of helper T cells (74.7% vs 66.0%) decreased.

Now let us consider case 2 (Figure 7D–F). A 49-year-old female received a histological diagnosis of adenocarcinoma based on endoscopic ultrasound and fine-needle aspiration. In this case, the frequency of αβ T cells increased the most after ATI in PR cases, and PET-CT images showed the FDG accumulations of the metastatic lymph node metastasis had disappeared after ATI. The primary lesion was pancreatic body cancer, with multifocal lymph node metastasis and celiac artery infiltration around the pancreas, clinically classified as T4N1M0 stage III per the UICC staging system. Because of celiac artery infiltration, surgery was not possible. There was no previous treatment, and GEM + nab-paclitaxel was started as first-line chemotherapy. ATI was added from the first course of chemotherapy. After ATI, there were no substantial changes in the tumor markers CEA and CA19-9, but there was a decrease in DUPAN-2 from 1540 U/mL to 1200 U/mL (Figure 7D). PET-CT images showed an SUV of 5.9 for both the pancreatic body primary lesion and the surrounding lymph nodes prior to ATI, but the lymph node accumulation disappeared after ATI, and the lesion in the pancreatic body became SUV 2.2, which was similar to the level in non-lesion areas (Figure 7E). As shown in Figure 7F, the frequencies of CD3+ T cells (51.6% vs 74.6%) and αβ T cells (48.9% vs 69.4%) increased after ATI, whereas the frequency of killer T cells (29.2% vs 29.7%) increased slightly. After ATI treatment, the frequency of helper T cells (63.2% vs 60.9%) decreased.

Discussion

Chemotherapy for pancreatic cancer has resulted in an MST of 6.2–11.1 months, depending on the agent or combination of agents [7–10]. MST of patients treated in this study with a combination of ATI and chemotherapy or chemoradiation was 18.7 months. Survival was particularly improved in the NPD group after ATI (i.e., 24.4 months). These results suggest that the combination of ATI and chemotherapy or chemoradiation might improve the prognosis of patients.

Studies have indicated that immune cells infiltrating pancreatic cancer tissues show higher frequencies of CD4+ T cells, CD8+ T cells and regulatory T cells than those in normal pancreatic tissues, with a positive correlation between CD4+ T cells and regulatory T cells and a

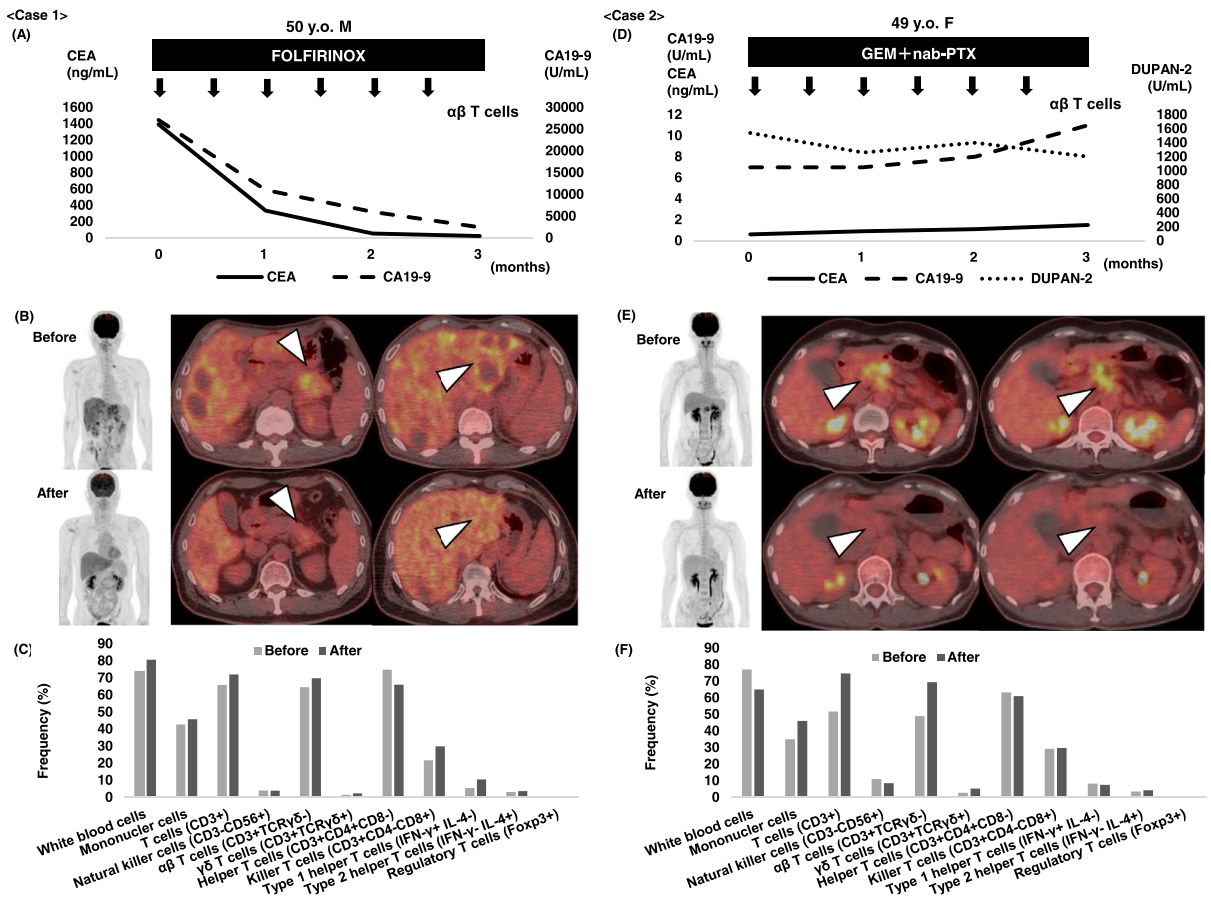

Figure 7. Clinical course and changes in the immune cell profile in two patients who responded to ATI. (A) Clinical course for case 1. (B) PET-CT images of the primary lesion and metastatic lesion before and after ATI in case 1. (C) Change in immune cell profiles before and after ATI in case 1. (D) Clinical course for case 2. (E) PET-CT images of the primary lesion and metastatic lesion before and after ATI in case 2. (F) Change in immune cell profiles before and after ATI in case 2. F, female; FOLFIRINOX, oxaliplatin + irinotecan + fluorouracil + levofolinate calcium; GEM, gemcitabine; M, male; nab-PTX, nab-paclitaxel; y.o., year-old.

negative correlation between CD8+ T cells and regulatory T cells [11]. In this study, the frequency of CD4+ T cells in pancreatic cancer tissues did not affect prognosis after surgery, but prognosis was good in the group with a high frequency of CD8+ T cells and low frequency of regulatory T cells. Furthermore, in a comparison of chemoradiation after pancreatic cancer surgery and chemotherapy (CapRI study, a phase 3 trial of post-operative cisplatin, interferon alpha-2b and fluorouracil combined with external radiation treatment versus fluorouracil alone for patients with resected pancreatic adenocarcinoma), prognosis was reportedly better when the frequency of CD8+ T cells in peripheral blood was high prior to treatment [12]. In the present study, the frequency of killer T cells was not related to therapeutic efficacy and prognosis but increased significantly in the peripheral blood after ATI. This suggests that the increase in killer T cells in response to ATI improves prognosis compared with that of chemotherapy alone.

Cancer is often associated with an increase in regulatory T cells and type 2 helper T cells and a decrease in CD3+ T cells, αβ T cells and type 1 helper T cells in the peripheral blood compared with those of healthy individuals, with no differences in NK cells, helper T cells or killer T cells. In a previous study, the frequency of killer T cells increased after ATI, and the frequencies of helper T cells and regulatory T cells decreased [13]. In the authors' study, there were no changes in regulatory T cells after ATI. Chemotherapy before or in combination with ATI reportedly does not affect the change in regulatory T cells in response to ATI [14]. This can be explained, in part, by the reduction in regulatory T cells and myeloid-derived suppressor cells with chemotherapy [15], thereby influencing the immune cell profile prior to ATI. Therefore, ATI did not lead to further changes in the frequency of these cells. Many patients in the present study underwent chemotherapy or combined therapy with chemotherapy, and the frequencies of regulatory T cells may have been reduced prior to the start of ATI, without subsequent alterations in response to ATI. Furthermore, the authors' results confirmed the increase in killer T cells and decrease in helper T cells after ATI.

In the present study, a good prognosis was related to an increase in CD3+ T cells and αβ T cells after ATI. The frequency of these cells increased more substantially in the NPD group than in the PD group. This suggests that the prognosis was favorable in patients who received sufficient adoptive T cells to exert an anti-tumor effect.

The CapRI trial previously indicated that prognosis is poor in patients with a high frequency of NK cells in the peripheral blood prior to treatment [12]. In the present study, the frequency of NK cells in the peripheral blood prior to ATI was significantly lower in the NPD group than in the PD group, and the same result was obtained after treatment. The authors' results suggest that the frequency of NK cells before ATI may be a predictor of its therapeutic effect.

Fever, malaise, itching and injection site reactions are the main adverse events associated with ATI. In a previous report (n = 484), aside from one patient with itchiness classified as grade 3, all adverse events were grade 1–2 [16]. In the present study, adverse events related to ATI included fever (grade 1) and malaise (grade 1–2), and 90.9% of patients did not show any adverse events. These results support the safety of ATI for advanced cancer.

As a limitation of this study, the majority of patients underwent chemotherapy or chemoradiation therapy in combination with

immunotherapy, and it is conceivable that chemotherapy affected the immune cell profiles. However, a study has shown that the immune cell profile changes in response to ATI in patients not receiving chemotherapy or chemoradiation therapy within 60 days prior to ATI [13]. In the present study, all three patients (one in the PD group and two in the NPD group) who received only ATI showed a decreased frequency of regulatory T cells after treatment, and the two patients in the NPD group showed decreased helper T cells and increased killer T cells after ATI treatment. These results suggest that ATI had a direct effect on the immune cell profile. Another limitation of this study is that since only a small number of people achieved the desired outcome, it did not allow for a detailed survival analysis. In conclusion, the results of the present study indicate that ATI in combination with standard treatments alters immune cell profiles, including CD3+CD4−CD8+ T cells (killer T cells) in the peripheral blood, and improves prognosis by promoting host anti-tumor activity.

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Declaration of Competing Interest

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Author Contributions

Conception and design of the study: TK and EM. Acquisition of data: TK, TH, HN and TM. Analysis and interpretation of data: TK and EM. Drafting or revising the manuscript: TK, EM, MK, TM, SG, TK, RT, TY, YS, TY, MH, KT and SK. All authors have approved the final article.

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Supplementary materials

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References

[1] Global Burden of Disease Cancer Collaboration Fitzmaurice C, Akinyemiju TF, et al. Global, regional, and national cancer incidence, mortality, years of life lost, years lived with disability, and disability-adjusted life-years for 29 cancer groups, 1990 to 2016: a systematic analysis for the global burden of disease study. JAMA Oncol 2018;4:1553–68.
[2] GBD 2017 Pancreatic Cancer Collaborators. The global, regional, and national burden of pancreatic cancer and its attributable risk factors in 195 countries and territories, 1990-2017: a systematic analysis for the Global Burden of Disease Study 2017. Lancet Gastroenterol Hepatol 2019;4:934–47.
[3] Nishikawa H, Sakaguchi S. Regulatory T cells in cancer immunotherapy. Curr Opin Immunol 2014;27:1–7.
[4] Hodi FS, O'Day SJ, McDermott DF, et al. Improved survival with ipilimumab in patients with metastatic melanoma. N Engl J Med 2010;363:711–23.
[5] Iwai Y, Ishida M, Tanaka Y, et al. Involvement of PD-L1 on tumor cells in the escape from host immune system and tumor immunotherapy by PD-L1 blockade. Proc Natl Acad Sci USA 2002;99:12293–7.
[6] Topalian SL, Hodi FS, Brahmer JR, et al. Safety, activity, and immune correlates of anti-PD-1 antibody in cancer. N Engl J Med 2012;366:2443–54.
[7] Ueno H, Ioka T, Ikeda M. Randomized phase III study of gemcitabine plus S-1, S-1 alone, or gemcitabine alone in patients with locally advanced and metastatic pancreatic cancer in Japan and Taiwan: GEST study. J Clin Oncol 2013;31:1640–8.
[8] Moore MJ, Goldstein D, Hamm J, et al. Erlotinib plus gemcitabine compared with gemcitabine alone in patients with advanced pancreatic cancer: a phase III trial of the National Cancer Institute of Canada Clinical Trials Group. J Clin Oncol 2007;25:1960–6.
[9] Conroy T, Desseigne F, Ychou M, et al. FOLFIRINOX versus gemcitabine for metastatic pancreatic cancer. N Engl J Med 2011;364:1817–25.
[10] Von Hoff DD, Ervin T, Arena FP, et al. Increased survival in pancreatic cancer with nab-paclitaxel plus gemcitabine. N Engl J Med 2013;369:1691–703.
[11] Tang Y, Xu X, Guo S, et al. An increased abundance of tumor-infiltrating regulatory T cells is correlated with the progression and prognosis of pancreatic ductal adenocarcinoma. PLoS One 2014;9:e91551.
[12] Karakhanova S, Ryschich E, Mosl B, et al. Prognostic and predictive value of immunological parameters for chemoradioimmunotherapy in patients with pancreatic adenocarcinoma. Br J Cancer 2015;112:1027–36.
[13] Noguchi A, Kaneko T, Naitoh K, et al. Impaired and imbalanced cellular immunological status assessed in advanced cancer patients and restoration of the T cell immune status by adoptive T-cell immunotherapy. Int Immunopharmacol 2014;18:90–7.
[14] Ishikawa T, Kokura S, Sakamoto N, et al. Whole blood interferon-γ levels predict the therapeutic effects of adoptive T-cell therapy in patients with advanced pancreatic cancer. Int J Cancer 2013;133:1119–25.
[15] Weir GM, Liwski RS, Mansour M. Immune modulation by chemotherapy or immunotherapy to enhance cancer vaccines. Cancers 2011;3:3114–42.
[16] Kamigaki T, Matsuda E, Okada S, et al. Prospective evaluation of safety of immune-cell therapy for patients with various types of advanced cancer. Anticancer Res 2014;34:4601–7.