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Changes in Immunological Status in Patients With Metastatic Colorectal Cancer Treated With First-line Chemoimmunotherapy

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Abstract. *Background/Aim: Chemoimmunotherapy is a promising treatment for various malignant diseases. In this study, we examined whether first-line chemoimmunotherapy using adoptive immune-cell therapy was effective for metastatic colorectal cancer (mCRC). Patients and Methods: The therapeutic efficacy and safety of the standard first-line chemoimmunotherapy with adoptive αβ T cell therapy and bevacizumab were assessed using thirty-two patients with mCRC in our hospital. Immunological status after this chemoimmunotherapy was also evaluated. Results: The response and disease control rates were 68.8% and 87.5%, respectively. Further, median progression-free and overall survival were 14.2 and 35.3 months. Immunotherapy-associated toxicity was minimal. Significant decrease in the change of monocyte number (p=0.006) and increase in the change of rate of lymphocyte-to-monocyte ratio (p=0.039) were seen in the complete response group. Conclusion: First-line chemoimmunotherapy with adoptive αβ T cell therapy may be useful for mCRC.*

Colorectal cancer (CRC) is one of the major malignant diseases in Japan (1). Chemotherapy is one of the effective treatments for CRC, and various novel chemotherapeutic agents including oxaliplatin, irinotecan and targeted therapies have contributed to the improvement of the prognosis (2). However, the prognosis of metastatic CRC (mCRC) remains poor (3-5). Therefore, the development of a novel therapy overcoming mCRC is urgent.

Recently, immunotherapy is considered as a promising therapeutic strategy for advanced cancers. In particular, blockade of T cell inhibitory checkpoint molecules can unleash anti-tumour T cell activity and lead to long-term clinical responses in patients with advanced malignant diseases including lung cancer, melanoma, and Hodgkin's disease (6-10). However, patients in mCRC rarely enjoy the benefits of the current immune checkpoint blockade regimens due to inherent tumour cell resistance or extrinsic factors restraining anti-tumour immunity (11). Therefore, establishment of further improved immunotherapy for mCRC is a pivotal challenge.

Adoptive immune-cell therapy is a novel cancer immunotherapy characterized as the re-infusion of *ex vivo*-activated and expanded T cells, such as αβ T cells, γδ T cells and natural killer cells. It has been proposed that adoptive immune-cell therapy stimulates and restores anti-tumour immunity because it enables immune cells to recognize and terminate tumour cells (12). Additionally, this therapy has a limited toxicity profile as opposed to established chemotherapy and checkpoint blockade (13-15). Among them, *ex vivo*-expanded αβ T cells have been studied for their antitumor effects (16, 17) and applied to treatment of some cancers including hepatocellular carcinoma (18) and lung cancer (19) in the clinical setting. Recently, chemoimmunotherapy, defined

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as a combination between chemotherapy and immunotherapy, has been developed as a more powerful anticancer therapy. Accumulating evidence sheds light on chemoimmunotherapy using adoptive T cell transfusions as a useful mCRC therapy (19-22). Previously, we showed that chemoimmunotherapy using αβ T cells was feasible and safe in stage IV CRC (22, 23). In addition, inhibition of angiogenic factors including vascular endothelial growth factor (VEGF) is considered effective for prohibition of tumor progression by regulating tumor immunity. For example, while bevacizumab, anti-human VEGF-A monoclonal antibody, is known as an agent which prevents tumor angiogenesis (24), some groups showed that blockade of the VEGF-A – VEGF receptor (VEGFR) pathway prevented tumour-induced regulatory T cell (Treg) proliferation in CRC or glioblastoma (25, 26).

Based on these findings, we have promoted chemoimmunotherapy using αβ T cell therapy and bevacizumab for the improvement of survival in mCRC. In this study, we examined the efficacy and safety of the standard first-line chemoimmunotherapy with adoptive αβ T cells and bevacizumab for mCRC. Immunological status after this treatment was also evaluated.

Patients and Methods

Patients. The medical records of patients, aged ≥20 y, histologically diagnosed as metastatic or unresectable CRC without prior chemotherapy or with completion of adjuvant chemotherapy at least 6 months before and enrolled from December 2012 to September 2019, were retrospectively reviewed. They also met the following criteria: Eastern Cooperative Oncology Group (ECOG) performance status 0-2; life expectancy ≥12 weeks; white blood cell count ≥3,000/mm^3; neutrophil count ≥1,500/mm^3; platelet count ≥75,000/mm^3; hemoglobin ≥8.5 g/dl; total bilirubin ≤2.0 times the upper limit of normal value; aspartate aminotransferase and alanine aminotransferase ≤3.0 times the upper limit of normal value; serum creatinine ≤2.0 mg/dl (22, 23).

Treatment. Patients received systemic therapies including XELOX (130 mg/m^2 of oxaliplatin on Day 1 plus 1,000 mg/m^2 of capecitabine twice daily on Days 1-14), XELIRI (200 mg/m^2 of irinotecan on Day 1, plus 800 mg/m^2 of capecitabine twice daily on Days 1-14) plus bevacizumab therapy (7.5 mg/kg on Day 1) or FOLFIRI (150 mg/m^2 of irinotecan plus 200 mg/m^2 of racemic folic acid and 400 mg/m^2 of fluorouracil as an intravenous bolus with 46-h continuous injection of 2,400 mg/m^2 of fluorouracil on Day 1) plus bevacizumab therapy (5 mg/kg on Day 1); these are the standard first-line treatments for mCRC. Dose-reduction, delay and cancelation of the treatment were decided by occurrences of adverse events including leukopenia and thrombopenia (neutrophil count <1,000/mm^3, platelet count <75,000/mm^3), active infection (body temperature ≥38.0˚C) and grade 2 or worse peripheral sensory and non-hematological toxicity, as described in our previous studies (22, 23). Regarding αβ T cell therapy, peripheral blood mononuclear cells (PBMCs) were isolated from approximately 22.5 ml of peripheral blood by centrifugation using a Vacutainer (Becton, Dickinson and Company, Franklin Lakes, NJ, USA). Over 1×10^6 harvested PBMCs were cultured with an immobilized anti-CD3 antibody and interleukin (IL)-2 for 14 days. As a result, over 5×10^9 lymphocytes on average were obtained. The cultured lymphocytes were composed of 61±15% of CD8$^+$, 30±15% of CD4$^+$ (CD4$^+$:CD8$^+$ ratio, 0.8 on average) and a small percentage of natural killer cells and natural killer T cells. This indicated that the proliferation of CD8$^+$ T lymphocytes was more prominent than that of CD4$^+$ cells during the 2-week culture period (27). Among them, αβT lymphocytes were isolated, cultured and expanded *ex vivo*. Over 4.5×10^9 αβT lymphocytes were injected intravenously into patients on day 17 or 18, once every 3 weeks (22, 23).

Evaluation of therapeutic efficacy and toxicities of chemoimmunotherapy. All patients underwent physical examination, chest radiography, and computed tomographic (CT) scans of the chest, abdomen and pelvis before the treatment. Sizes of the tumors were measured at 6- to 8-week intervals using CT scans after starting the treatment and the responses to the treatment were evaluated every time by radiologists according to the response evaluation criteria for solid tumours (RECIST), version 1.1. Decision of complete and partial responses (CR and PR) was required subsequent confirmation of response after an interval of at least 4 weeks. The severity of adverse effects was evaluated using the National Cancer Institute Common Toxicity Criteria (NCI-CTC), version 4.0.

Assessment of Immunological status using flow cytometry. For the assessment of the immunological status of patients, we examined immune cell populations and their rates in peripheral blood samples obtained before the first and after their 6th chemoimmunotherapy using flow cytometry (FCM) (17). PBMCs were isolated by gradient centrifugation using Lymphoprep™ (Axis-Shield PoC AS, Oslo, Norway). Absolute cell number was determined using Flow-Count™ (Beckman Coulter, Brea, CA, USA) fluorosphere internal standard beads. The OptiLyse C, Flow-Count™ beads and monoclonal antibodies (mAbs) against CD3, CD4, CD8, CD45, CD56, TCR pan αβ, TCR pan γδ, and TCR Vγ9 were purchased from Beckman Coulter. We utilized the isolated PBMCs for Foxp3 staining and cytokine production assay. For Foxp3 staining, the PBMCs were fixed and permeabilized using a fixation/permeabilization kit (BioLegend, San Diego, CA, USA) and stained with anti-Foxp3 mAb (clone 259D, BioLegend).

Statistical analyses. Statistical analyses were performed using SPSS version 19.0 statistical software (IBM, Armonk, NY, USA). Progression-free and overall survival (PFS and OS) of patients received chemoimmunotherapy were analysed using the Kaplan–Meier curves of univariate analysis in all enrolled patients. The comparisons of survival were performed by log-rank test. The initial point of follow-up was the date of the initial administration of adoptive αβ T cell therapy. Regarding the change in the immunological status through chemoimmunotherapy, the data obtained from FCM were analysed using one-way analysis of variance (ANOVA) and the Bonferroni-correction was applied for multiple comparisons. A p-value of <0.05 was considered as indicating statistically significant differences.

Results

Baseline patient characteristics. In our hospital, forty-four patients with mCRC received chemoimmunotherapy using adoptive αβ T cells. Among them, twelve patients were

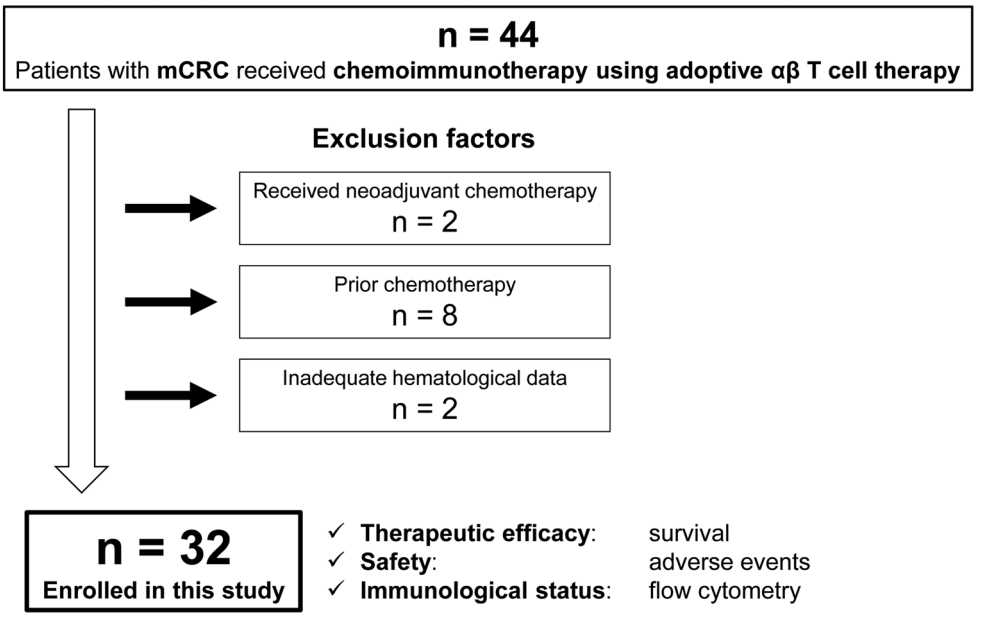

Figure 1. *Study design*.

Table I. *Baseline patient characteristics*.

Age (years)	67 (37-80)
Gender (n)	Male: 16, Female: 16
Primary site (n)	Appendix: 1, Cecum: 3, Ascending: 6, Transverse: 2 Descending: 2, Sigmoid: 7, Rectum: 11
Histological differentiation (n)	tub1: 18, tub2: 7, por: 3, muc: 1, endocrine cell carcinoma: 1, unknown: 2
KRAS status (n)	Wild-type: 13, G12D: 10, G13D: 3, G12V: 1, Unknown: 5
Synchronous *vs*. Metachronous (n)	Synchronous: 20, Metachronous: 12
Surgery of the primary CRC (n)	Yes: 10, No: 22
Chemotherapy (plus bevacizumab) (n)	XELOX: 30, FOLFIRI: 1, XELIRI: 1
Numbers of chemotherapy cycles (cycles)	11 (1-39)
Numbers of immunotherapy cycles (cycles)	8 (1-44)
Numbers of αβ T lymphocytes (cells)	5.9×10^9 (4.5×10^9-7.9×10^9)

tub1: Well differentiated type of tubular adenocarcinoma; tub2: moderately differentiated type of tubular adenocarcinoma; por: poorly differentiated adenocarcinoma; muc: mucinous adenocarcinoma; KRAS: v-Ki-ras3 Kirsten rat sarcoma viral oncogene homolog; CRC: colorectal cancer.

excluded due to receiving neoadjuvant chemotherapy (n=2), prior chemotherapy (n=8) and hematological, renal and hepatic dysfunction (n=2). Therefore, the remaining 32 patients were enrolled (Figure 1). The median age of the 32 patients (16 male and 16 female) was 67 y (range=37-80 y). ECOG performance status scores were zero for all patients. Other patient characteristics are summarized in Table I.

Treatment. Regimens of first-line chemotherapy were XELOX (n=30), FOLFIRI (n=1) or XELIRI (n=1). All the patients were administered bevacizumab. The median

Table II. *Efficacy of chemoimmunotherapy using adoptive αβ T cell therapy.*

RECIST	N	%
CR	4	12.5
PR	18	56.3
SD	6	18.7
PD	4	12.5

RECIST: Response Evaluation Criteria in Solid Tumors; CR: complete response; PR: partial response; SD: stable disease; PD: progressive disease.

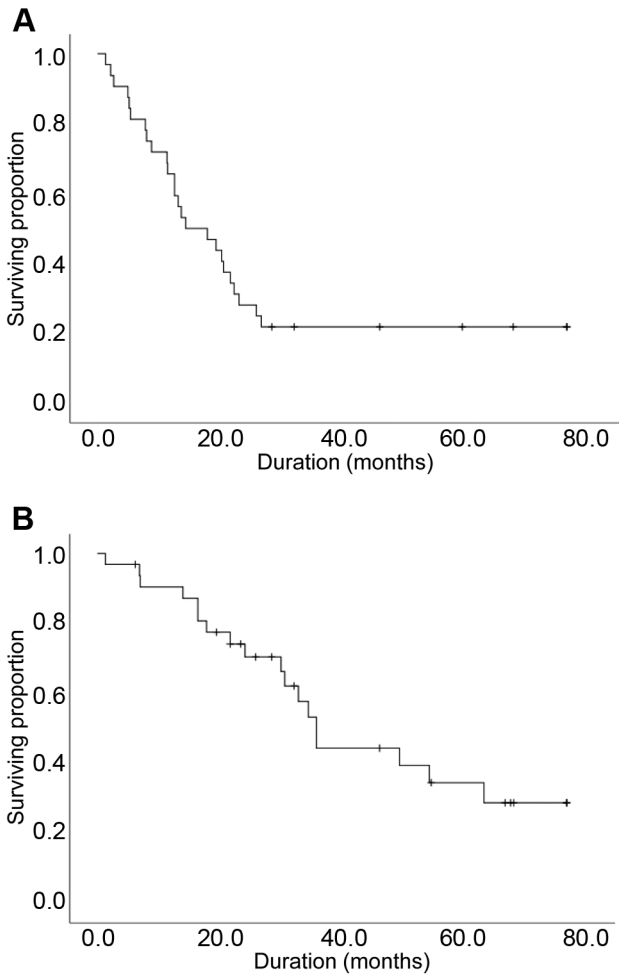

Figure 2. *Progression-free and overall survivals of the 32 patients. Progression-free (A) and overall (B) survivals were analyzed using the Kaplan–Meier curves of univariate analysis.*

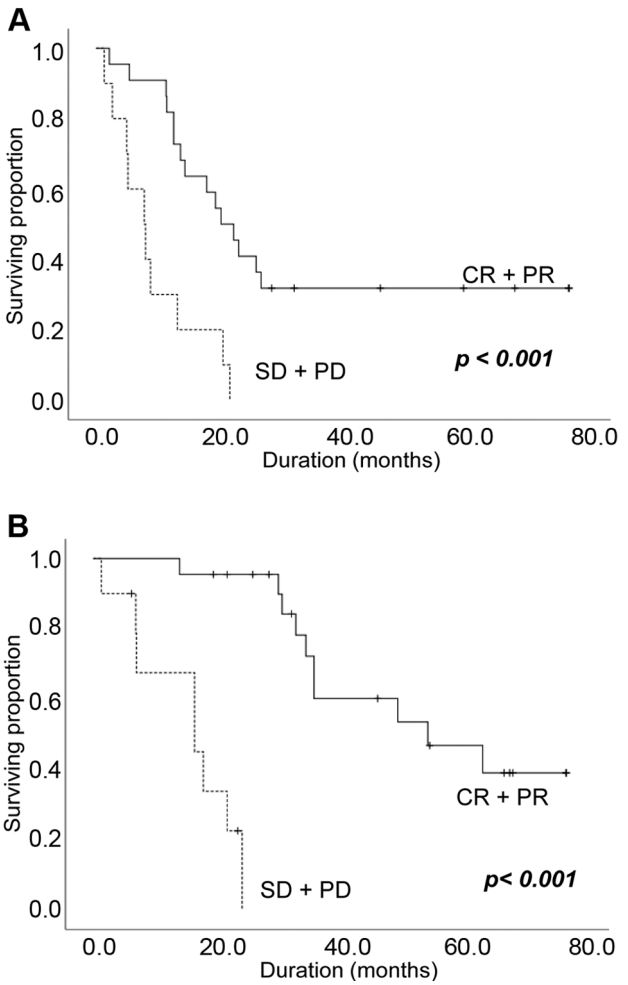

Figure 3. *Kaplan–Meier survival curve showing progression-free (A) and overall (B) survival in relation to the clinical response. CR: Complete response; PR: partial response; SD: stable disease; PD: progressive disease. The comparisons in the survivals were done by log-rank test and a p-value of <0.05 was considered as statistically significance.*

number of chemotherapy cycles was 11 (range=1-39). Twenty patients (62.5%) continued treatment through eight cycles, while the reasons for discontinuing treatment were adverse events in one patient and personal reasons in another. Treatment was delayed in 14 patients (43.8%) due to neutropenia in two, thrombocytopenia in six, hand-foot syndrome (HFS) in four, fatigue in one, and diarrhea in one. Seven patients (21.9%) required dose reduction at least once within the eight cycles because of neutropenia in one, thrombocytopenia in four, fatigue in one, and diarrhea in one. Regarding αβ T cell therapy, the median number of treatment cycles was eight (range=1-44). The average cell number was 5.9×10^9 (range=4.5×10^9-7.9×10^9) for each infusion (Table I).

Chemoimmunotherapy using adoptive αβ T cells improved the prognosis of mCRC. In our study, the confirmed response rate [CR+PR] and disease control rate [CR+PR+SD] of the mCRC patients were 68.8% and 87.5%, respectively. In detail, 12.5% showed CR (n=4), 56.3% PR (n=18), 18.7% stable disease (SD, n=6) and 12.5 % in progressive disease (PD, n=4) (Table II). Median PFS (Figure 2A) and OS (Figure 2B) were 14.2 and 35.3 months, respectively. It is considered that chemoimmunotherapy using adoptive αβ T cells improved the prognosis of mCRC, because PFS and OS in this study were longer than those in previous phase III studies (3-5) (Table III). Figure 3 shows the comparisons in PFS (Figure 3A) and OS (Figure 3B) between CR+PR and SD+PD, and the significant

Table III. *Comparison of our response rate, disease control rate, progression-free survivals and overall survivals with other groups.*

Authors	Treatment regimen	RR	DCR	PFS	OS	References no.
Saltz LB *et al.*	XELOX/FOLFOX-4+Bev	47.0%	No data	9.4 months	21.3 months	(3)
Heinemann V *et al.*	FOLFIRI+Bev	58.0%	95.0%	10.3 months	25.0 months	(4)
Pectasides D *et al.*	XELIRI+Bev	38.5%	59.5%	10.2 months	20.0 months	(5)
Teppei *et al.*	Our regimen	68.8%	87.5%	14.2 months	35.3 months	

RR: Response rate; DCR: disease control rate; PFS: progression-free survival; OS: overall survival.

improvements seen in CR+PR. The median PFS and OS of the patients in the CR+PR group and those in SD+PD group were 20.0 and 7.7 months in PFS ($p<0.001$; Figure 3A) and 53.5 and 16.2 months in OS, respectively ($p<0.001$; Figure 3B). While there were no patients who could survive for 5 years from the initiation of this chemoimmunotherapy in the SD+PD group, 31.8% of the patients was progression-free and 46.8% survived for five years in the CR+PR group. These results indicated that the clinical response of this chemoimmunotherapy using adoptive αβT cells strongly enhanced the prognosis of mCRC.

Safety of chemoimmunotherapy using adoptive αβ T cells was equivalent to conventional chemotherapy. Adverse events for the 32 patients are summarized in Table IV. Grade 3 or higher hematological and non-hematological toxicities were noted in 9.4% (n=3) and in 12.5% (n=4) of patients, respectively. Grade 3 hemotological toxicities were thrombocytopenia, leukopenia and neutropenia. Grade 3 non-hematological toxicities were HFS, allergic reaction, malaise and hypertension. Eight patients developed Grade 2 thrombocytopenia. There were no other severe treatment-related adverse events and deaths during the treatments.

Table IV. *Adverse events during chemoimmunotherapy using adoptive αβ T cell therapy.*

	Hematological	Non-hematological
Grade 2	Thrombocytepenia: 8	HFS: 4
	Leukopenia: 1	Allergic reaction: 3
	Neutropenia: 1	PN: 2
	Anemia: 1	Anorexia: 1
	Raised AST: 1	Diarrhea: 1
	Raised ALT: 1	Ileus: 1
Grade 3	Thrombocytopenia: 2	HFS: 1
	Leukopenia: 1	Allergic reaction: 1
	Neutropenia: 2	Malaise: 1
		Hypertension: 1

AST: Aspartate transaminase; ALT: alanine transaminase; WBC: white blood cell; HFS: hand–foot syndrome; PN: peripheral neuropathy.

Fluorescence-activated cell sorting analysis showed chemoimmunotherapy using adoptive αβ T cells enabled to increase the number of effector cells and reduce the rate of Treg cells. The immunological status before and after the chemoimmunotherapy using adoptive αβ T cells was assessed in 20 out of 32 patients who completed at least six or more αβT cell therapies. The evaluation of the immunological status was performed using FACS and cell number or rate of PBMC, lymphocytes, T cells, NK cells, Vγ9γδT cells, αβT cells, γδT cells, Helper T cells, Killer T cells, B cells and Tregs were calculated. Regarding the number of lymphocytes, increases of T cells, NK cells and killer T cells after the treatment were seen in 80% (16/20), 85% (17/20), 70% (14/20) and 80% (16/20) of these patients, respectively. Furthermore, increases in the rate of T cells, NK cells and killer T cells were detected in 80% (16/20), 70% (14/20), 55% (11/20) and 80% (16/20) of them. Regarding the number of Tregs and the CD4-to-CD8 ratio, decreases were observed in 50% (10/20) and 80% (16/20) of them. The rate of Tregs and the CD4-to-CD8 ratio also decreased in 75% (15/20) and 80% (16/20), respectively. These results indicated that chemoimmunotherapy using adoptive αβ T cells enhanced the expression of antitumor T cells and compromised the immune tolerance for tumor. Furthermore, we also evaluated the change in immunological status before and after this chemoimmunotherapy among the four groups related to clinical response [CR (n=4), PR (n=10), SD (n=4), PD (n=2)]. There were significant differences in the change of monocyte number ($p=0.009$; Table V) and rate of monocytes ($p=0.036$) and lymphocyte-to-monocyte ratio ($p=0.045$; Table VI) among the four groups as was indicated by one-way ANOVA. We employed Bonferroni-correction for multiple comparisons to examine the correlation between the changes of cell number or rate of immune cells and the clinical response, and found a significant decrease in the change of monocyte number ($p=0.006$) and a significant increase in the change of rate of lymphocyte-to-monocyte ratio ($p=0.039$) in comparisons between the CR and PD groups.

Table V. *Change in immunological status (cell number) before and after chemoimmunotherapy using adoptive αβ T cell therapy among the four groups.*

	CR	PR	SD	PD	*p*-Value
PBMC	–248.50±751.587	318.60±402.000	501.75±229.832	538.00±1380.272	0.255
Lymphocytes	–136.50±653.990	297.00±348.026	473.00±153.590	243.50±1170.260	0.362
T cells	–20.00±421.636	251.30±273.973	409.00±144.395	256.50±668.216	0,344
NK cells	–41.75±110.663	51.40±94.581	57.75±41.331	99.50±102.530	0,263
Vγ9γδT cells	11.75±12.447	14.10±23.844	26.25±11.295	–21.50±20.506	0,089
αβT cells	–22.25±402.523	230.20±259.942	378.50±136.561	270.50±679.530	0,368
γδT cells	11.00±24.536	22.90±32.926	35.00±15.122	–13.50±2.121	0,242
Helper T cells	–68.25±244.685	94.40±174.132	159.75±39.601	125.50±468.812	0.444
Killer T cells	46.50±160.787	136.80±141.191	215.00±108.943	144.00±207.889	0,459
CD4 cells/CD8 cells	–0.41±0.283	–0.54±0.981	–0.541±0.187	–0.80±0.210	0.946
B cells	–64.75±71.537	–22.70±29.863	–2.00±81.613	–22.50±139.300	0.557
Treg cells	–2.75±15.756	3.30±18.056	7.50±10.344	–16.50±0.707	0,346
Monocytes	*–112.0±119.496	21.60±105.446	28.75±103.284	*294.50±210.011	0.009
Lymphocytes/Monocytes	2.23±3.443	0.56±1.781	1.63±2.300	–2.60±0.475	0.115

One-way ANOVA was used to obtain *p*-value. *Statistically significant using multiple comparisons (Bonferroni-correction). CR: Complete response; PR: partial response; SD: stable disease; PD: progressive disease.

Table VI. *Change in immunological status (cell rate) before and after chemoimmunotherapy using adoptive αβ T cell therapy among the four groups.*

	CR	PR	SD	PD	*p*-Value
PBMC (%)	17.53±3.674	9.35±9.240	7.04±14.310	2.13±6.390	0.274
Lymphocytes (%)	21.14±3.581	12.12±11.135	12.12±15.616	–8.54±9.708	0.053
T cells (%)	7.41±3.838	3.69±6.165	9.45±7.658	–1.39±2.283	0.172
NK cells (%)	–1.71±3.699	0.40±3.010	–0.61±2.202	0.88±5.720	0.688
Vγ9γδT cells (%)	0.77±0.855	0.71±1.154	1.05±0.492	–1.95±2.906	0.052
αβT cells (%)	7.01±2.659	2.79±5.735	8.26±7.407	0.17±0.332	0.219
γδT cells (%)	0.93±1.264	0.96±1.572	1.38±0.543	–1.69±2.340	0.121
Helper T cells (%)	–5.49±3.315	–5.89±8.574	–8.61±4.147	–4.62±3.620	0.882
Killer T cells (%)	5.74±3.563	5.56±7.366	7.37±3.950	7.73±1.612	0.934
CD4 cells (%)/CD8 cells (%)	–0.40±0.284	–0.53±0.976	–0.54±0.189	–0.80±0.211	0.943
B cells (%)	–2.24±1.145	–2.19±2.226	–3.68±3.613	–4.33±0.700	0.531
Treg cells (%)	–0.40±0.905	–0.73±1.148	–1.16±2.445	–2.19±1.965	0.556
Monocytes (%)	–3.61±6.570	–2.77±6.186	–5.09±4.807	10.68±3.309	0.036
Lymophocytes (%)/Monocytes (%)	*1.93±1.102	0.77±0.977	0.96±0.957	*-0.69±0.382	0.045

One-way ANOVA was used to obtain *p*-value. *Statistically significance using multiple comparisons (Bonferroni-correction). CR: Complete response; PR: partial response; SD: stable disease; PD: progressive disease.

Discussion

According to the current data published from National Cancer Center, the five-year survival rate of CRC is approximately 70% in Japan (28). While CRC is recognized as a curative disease in general, the prognosis of mCRC, which is advanced stage of this disease due to relapse and metastasis, is still poor (3-5). Though some of them are curable by intensive treatment including surgery and chemoradiotherapy, the conventional treatment can induce a variety of adverse effects and impair antitumor immunity, failing to eliminate the tumor cells and relapse of disease.

In this study, we examined the therapeutic effect of the standard first-line chemoimmunotherapy with adoptive αβ T cells and bevacizumab in 32 mCRC patients and two findings were obtained. One was the usefulness of the chemoimmunotherapy for managing mCRC, which contributed to prolong the survival. We revealed that management of the progression was achieved to approximately 90% of the patients, and 70% of them acquired elimination and remission of the disease with an acceptable toxicity profile. In addition, the PFS and OS in this study were longer than those in previous phase III studies as shown in Table III (3-5). The accuracy of our findings was guaranteed by a previous

systematic review and meta-analysis by Zhou et al. It showed that chemotherapy combined with dendritic cell vaccine and cytokine-induced killer cells enhances OS and disease-free survival (DFS) with no severe adverse events in patients with CRC (29). Our study also demonstrated that it prolongs in PFS and OS in the CR+PR group compared with the SD+PD group, similar to other first-line chemotherapies in phase III trials for patients with advanced CRC (30). The other was that the chemoimmunotherapy enhanced antitumor immunity. The assessment of the number and rate of lymphocytes revealed that T cells and killer T cells increased in more than 70% of patients after this combination therapy. Additionally, a decrease in the rate of Tregs and the CD4-to-CD8 ratio were observed in more than 75% of patients after this combination therapy. These results indicated that this chemoimmunotherapy restored impaired and imbalanced T cell immune status.

The therapeutic effect of bevacizumab was unclear in this study, but we predicted it might contribute to the regulation of tumor progression by anti-angiogenesis and anti-immunologic functions. According to a previous study, an anti-VEGF-A monoclonal antibody might enhance the anti-tumour activity of adoptively transferred anti-tumour T cells through the augmentation of lymphocyte infiltration into tumors (31).

As for the change in immunological status before and after this chemoimmunotherapy among the four groups related to clinical response, a significant decrease in the change of monocyte number and a significant increase in the change of rate of lymphocyte-to-monocyte ratio were found in the CR group compared with the PD group under multiple comparisons. In many types of cancer, tumor-associated macrophages (TAMs) are mainly derived from monocytes, and they are known to promote tumor growth by releasing growth factors, inhibiting immune surveillance and enhancing angiogenesis, among other mechanisms (32-34). TAMs infiltration is also positively correlated with cancer metastasis and poor clinical prognosis in a variety of human cancers (34, 35). Therefore, our data which showed the increase in the change of monocyte number and the decrease in the change of rate of lymphocyte-to-monocyte ratio might be a predictive factor for poor clinical response and prognosis of mCRC.

This study has some limitations including the small sample size, and being a single-institution and single arm retrospective study. In the future, a prospective multicenter randomized trial is recommended to establish the general effects of this chemoimmunotherapy.

In conclusion, this study found that standard first-line chemoimmunotherapy with adoptive αβ T cells and bevacizumab is a feasible and safe treatment for mCRC. This chemoimmunotherapy enables to increase the number of effector cells and reduce the rate of Treg cells. These results strongly support the recommendation of future phase III prospective studies for confirming the safety and efficacy of chemoimmunotherapy for mCRC.

Conflicts of Interest

The Authors declare that they have no conflicts of interest related to this study.

Authors' Contributions

T.Y., Y.Y., N.S., S.K. and S.H. designed research; T.Y., Y.Y., G.Y., N.A., A.K., R.K., Y.M., H.N., K.N., K.Y., R.T., T.K., S.G., and F.Y. performed research; T.M. and K.Y. analyzed data; T.Y. wrote the paper; and Y.Y., N.S., S.K., and S.H. revised the manuscript.

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