Prognostic Factors for Colorectal Cancer Patients Treated With Combination of Immune-cell Therapy and First-line Chemotherapy: A Retrospective Study

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Abstract. *Background/Aim:* In this retrospective study, we aimed to investigate the efficacy of immune-cell therapy using T lymphocytes activated in vitro with or without dendritic cells vaccination (DCs), in combination with 1st-line chemotherapies in terms of the survival of patients with advanced colorectal cancer (CRC). *Patients and Methods:* A total of 198 patients who were diagnosed with advanced CRC and administered 1st-line chemotherapies were enrolled in this study. The correlation between overall survival (OS) and various clinical factors was examined by univariate and multivariate analyses. *Results:* Univariate analyses revealed that the prognosis was improved in CRC patients who received immune-cell therapy with PS 0, bevacizumab (BV), and capecitabine-including regimens (Cap). Finally, multivariate analysis demonstrated that PS=0, and the combination of immune-cell therapy and Cap provided a survival benefit in patients with advanced CRC. *Conclusion:* The survival benefit could be potentially obtained with better PS by the combination of immune-cell therapy and Cap as a 1st-line setting in patients with CRC.

Colorectal cancer (CRC) is one of the most prevalent cancers and remains one of the leading causes of cancer-related mortality worldwide (1). The evolution of chemotherapy for patients with CRC has involved a series of landmark advances, such as the discovery of 5-fluorouracil (5-FU) (2), the identification of reduced folate leucovorin as a clinical potentiator of 5-FU cytotoxicity (3), and the advent of novel

cytotoxic agents, which led to the development of combination chemotherapies such as FOLFOX and FOLFIRI (4-6). Furthermore, the introduction of molecular targeting drugs, such as anti-vascular endothelial growth factor (VEGF) and anti-epidermal growth factor receptor (EGFR) antibodies, has further improved the prognosis of patients with metastatic CRC. Although the development of combination chemotherapies has extended the median survival period of patients with advanced CRC to more than 25 months, half of the patients eventually die from the disease (7). Thus, it is imperative to develop novel therapies for patients with CRC.

The immune system can protect the host from tumorigenesis through immune surveillance mechanisms (8, 9). One of the mechanisms attributed to the occurrence or development of cancer is the deficiency of the immune system. Various strategies have been developed to improve the immune function of cancer patients, which include the use of cytokines, cancer vaccines, checkpoint inhibitors, and adoptive cell transfer (ACT). ACT using tumor-infiltrating lymphocytes (TILs) for CRC patients was first applied in the 1990s (10); however, there was no significant difference in disease-free survival between the TIL-treated group and the conventional chemotherapy-treated group. ACT utilizing cytokine-induced killer cells (CIK) has also been shown to be safe for CRC patients, decrease the recurrence of CRC, and improve OS (11, 12).

Recently, the combination of CIK and dendritic cells (DCs) (CIK/DCs) has been actively pursued in the immunotherapy research field. It has been reported that ACT using CIK/DCs could inhibit disease progression and improve OS in patients with CRC (13-15). A combination of immune-cell therapy and conventional chemotherapy was also tested and showed a significant improvement in OS of patients with CRC (16). Furthermore, several studies have demonstrated that CRC patients with liver metastasis could undergo surgical resection after a combination of

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Figure 1. Selection procedure for patients enrolled in this study.

chemotherapy and immune-cell therapy (17-19). However, the efficacy and prognostic factors of immune-cell therapy combined with 1st-line chemotherapy in a large number of patients with CRC remain unclear. In this study, we retrospectively analyzed patients with advanced CRC who had been administered immune-cell therapy as the 1st-line setting at the clinics of the Seta Clinic Group.

Patients and Methods

Patients. The database of patients administered immune-cell therapy at the clinics of the Seta Clinic Group was searched to identify patients with CRC. As a result, 1,331 patients were identified and enrolled in this study, and we retrospectively reviewed the medical records of those administered $\alpha\beta$ T cell therapy, DC vaccine therapy, or a combination of both between 1999 and 2015. The study protocol was approved by the Research Ethics Committee of the Seta Clinic Group. Available data on age, sex, performance status (PS) score on the Eastern Cooperative Oncology Group (ECOG) scale, metastasis sites, tumor location, clinical stage, treatments, and vital status were extracted from the medical records of the patients.

Treatment. For $\alpha\beta$ T cell therapy, activated lymphocytes were generated as previously described (20). In brief, peripheral blood mononuclear cells (PBMCs) were isolated from a patient's peripheral blood using Vacutainer (Becton, Dickinson and Company, Franklin Lakes, NJ, USA). The PBMCs were activated in a culture flask with an immobilized monoclonal antibody to CD3 (Jansen-Kyowa, Tokyo, Japan) in Hymedium 930 (Kohjin Bio, Saitama, Japan) containing 1% autologous serum. The PBMCs were then cultured for 14 days with 700 IU/ml recombinant interleukin-2 (IL-2) (Proleukin®; Chiron, Amsterdam, the Netherlands), after which, $3\text{--}10 \times 10^9$ cells were harvested and suspended in 100 ml of normal saline for intravenous injection. To prepare a DC vaccine, PBMCs were collected from the patients by leukapheresis and allowed to adhere to a plastic culture flask. The adherent cell fraction was used for DC culture for 6 days using a medium supplemented with 50 ng/ml IL4 (Primmune Corp., Osaka, Japan) and 50 ng/ml granulocyte macrophage colony-stimulating factor (GM-CSF) (Primmune Corp.) to generate

immature DCs. The DCs were pulsed with antigenic tumor-specific peptides or an autologous tumor lysate and allowed to mature for 24 h. After the culture, $1\text{--}10 \times 10^6$ mature DCs were harvested and suspended in 1 ml of normal saline for subcutaneous injection, and then cryopreserved until the day of administration. Immune-cell therapy consists of $\alpha\beta$ T cell therapy, DC vaccine therapy, or both and is commonly administered 6 times, that is, every 2 weeks for 3 months, as one course.

Assessment. OS was defined as the length of time from the initial administration of immune-cell therapy to death from any cause and calculated for every patient. The Kaplan–Meier method was used to calculate survival probabilities for all patients.

Statistical analyses. OS was examined by the Kaplan–Meier analysis with the Wilcoxon test, and the hazard ratio was obtained by Cox regression methods in univariate and multivariate analyses. All statistical analyses were two-sided and performed using JMP, version 11.2.0 for Microsoft Windows 7 (SAS, Cary, NC, USA). Differences were considered statistically significant when $p < 0.05$.

Results

Patient selection. A total of 1,331 patients with CRC were enrolled in this study (Figure 1). Among them, 1,325 patients were confirmed to have CRC by biopsy or suspected to have adenocarcinoma on the basis of diagnostic imaging findings, and six patients had malignant solid tumors other than adenocarcinoma. Of the 1,325 patients, 1,109 had advanced or recurrent CRC (216 patients were excluded because immune-cell therapy was performed as an adjuvant therapy after surgery). Finally, to examine the efficacy of immune-cell therapy combined with a 1st-line chemotherapy, we selected 198 patients with CRC (Figure 1).

The patients' characteristics are summarized in Table I. In this study, the correlation between OS and various factors including sex, age, PS, clinical stage, tumor location, chemotherapy, radiation therapy, and immune-cell therapy were evaluated by univariate analysis and multivariate Cox regression analysis.

Table I. Patient characteristics.

Characteristic	Number of cases (%)
Total, n	198
Male	100 (50.5)
Female	98 (49.5)
Median age (range), years	62 (29-84)
≥ 62	99 (50.0)
< 62	99 (50.0)
Performance status	
0	144 (72.7)
1-4	54 (27.3)
Clinical Stage	
II	8 (4.0)
III	50 (25.3)
IV	115 (58.1)
Unknown	25 (12.6)
Location	
Colon	125 (63.1)
Rectum	73 (36.9)
Left/Right side	
Left (Rectum/Sigmoid/Descending)	123 (62.1)
Right (Transverse/Ascending/Ileum)	67 (33.8)
Treatment	
Operation	
Yes	176 (88.9)
No	22 (11.1)
Chemotherapy	
Yes	198 (100)
No	0 (0)
Radiation therapy	
Yes	26 (13.1)
No	172 (86.9)
Immunotherapy	
$\alpha\beta$ T	156 (78.8)
$\alpha\beta$ T + DC	42 (21.2)

$\alpha\beta$ T: Activated lymphocyte therapy; DC: dendritic cell vaccine therapy.

N	MST (month)	95%CI	3-year survival rate	5-year survival rate
198	21.2	15.2 – 25.7	25.9%	9.4%

Figure 2. Kaplan–Meier estimates of overall survival in the full analysis set. OS: Overall survival (months); MST: median survival time.

Table II. Items of chemotherapeutic agents.

Classification	Number of cases
BV	
Yes	64
No	130
1st-line agents	
Capecitabine-including regimens	29
FOLFOX/FOLFIRI-including regimens	83
TS1	13

BV: Bevacizumab; FOLFOX: fluorouracil+folinic acid+oxaliplatin; FOLFIRI: fluorouracil+folinic acid+irinotecan; TS1: tegafur/gimeracil/oteracil.

Overall survival. The median age of the 198 patients (99 males and 99 females) with advanced or recurrent CRC was 62 years (range=29-84 years, Table I). The median survival time (MST) of patients with advanced or recurrent CRC was 21.2 months since the administration of immune-cell therapy has started, and the 3- and 5-year OS rates were 25.9% and 9.4%, respectively (Figure 2). There was no significant difference in survival time in relation to sex, age, clinical stage, and tumor location; however, a significant difference was observed in relation to PS (Figure 3). The analysis of survival time demonstrated that the MSTs of the patients with a PS score of 0 and those with PS scores of 1-4 were 26.0 and 12.7 months, respectively ($p=0.0003$; Figure 3C).

We then examined the effect of treatment strategy on the survival time of patients with CRC (Tables I and II). There was no significant difference in survival time in relation to

the combination of immune-cell therapy and surgical operation or radiation therapy (Figure 4A and 4B). Of the 198 CRC patients treated with immune-cell therapy, 156 received $\alpha\beta$ T cell therapy alone and 42 received both DC vaccine therapy and $\alpha\beta$ T cell therapy (DC+ $\alpha\beta$ T). Regarding survival analysis by the type of immune-cell therapy administered, there was a significant difference in MST between patients treated with DCs+ $\alpha\beta$ T cells and those treated with $\alpha\beta$ T cells only (26.5 and 20.0 months, respectively; $p=0.0253$, Figure 4C). We then examined whether the type of 1st-line chemotherapy combined with immune-cell therapy affected OS in patients with CRC (Table II, Figure 5). Kaplan–Meier analysis by the Wilcoxon test showed that the MST of bevacizumab (BV)-treated patients was longer than that of non-BV-treated patients (26.5 vs. 14.3 months; $p=0.0002$, Figure 5A). Furthermore,

Figure 3. Correlation of Kaplan-Meier estimates of overall survival with sex (A), age (B), performance status (C), clinical stage (D), and tumor location (E, colon and rectum; F, right and left). OS: Overall survival; MST: median survival time.

there was a significant difference in MST between patients treated with capecitabine-including regimens and those treated with FOLFOX/FOLFIRI or TS1-including regimens (32.5 vs. 20.0 vs. 13.7 months, $p=0.0023$, Figure 5B).

Univariate and multivariate analyses. The univariate analyses of patients' characteristics demonstrated that PS was one of the independent prognostic factors for CRC patients (HR=0.410; 95%CI=0.271-0.631; $p<0.0001$, Table III). Regarding treatments with chemotherapeutic agents, the univariate analyses revealed that the prognosis was improved in the patients with CRC who received immune-cell therapy with BV (HR=0.553; 95%CI=0.368-0.816; $p=0.0026$, Table IV). The prognosis was also improved in the patients who received immune-cell therapy combined with capecitabine-including regimens compared with those who received FOLFOX/FOLFIRI (HR=2.256; 95%CI=1.285-4.197; $p=0.0040$) or TS1 (HR=3.684; 95%CI=1.578-8.264; $p=0.0034$) (Table IV).

Finally, multivariate analysis demonstrated that PS=0 indicated a better prognosis in CRC patients treated with immune-cell therapy (HR=0.334; 95%CI=0.194-0.589; $p=0.0002$, Table V). Additionally, capecitabine-including

regimens with immune-cell therapy improved survival in advanced CRC patients compared with FOLFOX/FOLFIRI (HR=2.174; 95%CI=1.226-4.078; $p=0.0071$) and TS1 (HR=3.138; 95%CI=1.305-7.289; $p=0.0117$). Thus, it was indicated that PS and capecitabine-including regimens were independent prognostic factors for patients with CRC treated with 1st-line chemotherapy and immune-cell therapy.

Discussion

Although the development of combination chemotherapies has prolonged the median survival length of patients with advanced CRC to more than 25 months, many CRC patients suffer from poor prognosis owing to relapse or metastasis, especially for advanced tumors (1). Conventional treatments, including surgery, chemotherapy, and radiotherapy, may induce various adverse effects and impair the patients' antitumor immunity, resulting in residual tumor. In this retrospective study, we extracted 198 patients with CRC from 1,331 patients who have visited our clinic and diagnosed as having colorectal tumor, and we analyzed the

Figure 4. Correlation of Kaplan–Meier estimates of overall survival with surgical operation (A), radiation therapy (B), and immune-cell therapy (C). OS: Overall survival; MST: median survival time; RT: radiation therapy; $\alpha\beta T$: $\alpha\beta T$ immune-cell therapy; DC: dendritic cell vaccine therapy.

Figure 5. Kaplan–Meier estimates of overall survival according to treatment with or without bevacizumab (A) and type of chemotherapy (B). OS: Overall survival; MST: median survival time; BV: bevacizumab; FOLFOX: fluorouracil+folinic acid+oxaliplatin; FOLFIRI: fluorouracil+folinic acid+irinotecan; TS1: tegafur/gimeracil/oteracil.

efficacy of immune-cell therapy combined with a 1st-line chemotherapy. As a result, we observed an increased efficacy of immune-cell therapy as a 1st-line setting for patients with metastatic CRC.

In the comparison between immune-cell therapy alone and that combined with other treatments, several studies have demonstrated that a combination therapy shows better therapeutic effects than immune-cell therapy alone (Figures 4 and 5 and Table V) (17–19). In our study, the combination of

immune-cell therapy and chemotherapy, especially BV or capecitabine chemotherapy, provided a survival benefit in the advanced or recurrent CRC patients (Figure 5). This benefit can be explained by the reinforcement of the immune system *via* the actions of anticancer agents; for example, suppressive regulatory T cells were found to be depleted by several chemotherapeutic agents, resulting in enhanced T-cell reactivity (21, 22). Oxaliplatin induces the immunogenic death of colorectal cancer cells, and this effect determines its therapeutic

Table III. Univariate analyses of overall survival in relation to clinical background.

Characteristics	HR	95%CI	p-Value
Gender			
M	1		
F	0.969	0.665-1.404	0.8673
Age, years			
<62	1		
≥62	0.869	0.602-1.253	0.4497
PS			
≥1	1		
0	0.410	0.271-0.631	<0.0001
Clinical stage			
II	1		
III	1.332	0.503-4.623	0.5910
IV	1.704	0.690-5.690	0.2733
Location			
Rectum	1		
Colon	1.009	0.692-1.482	0.9632
Left/Right			
Right	1		
Left	0.881	0.596-1.324	0.5336

PS: Performance status.

Table IV. Univariate analyses of overall survival in relation to therapy.

Characteristics	HR	95%CI	p-Value
Immune-cell therapy			
αβT	1		
αβT+DC	0.752	0.482-1.140	0.1839
Radiation therapy			
No	1		
Yes	1.572	0.916-2.549	0.0970
Surgical operation			
No	1		
Yes	0.823	0.478-1.542	0.5203
BV			
No	1		
Yes	0.553	0.368-0.816	0.0026
1st-line agents			
Capecitabine	1		
FOLFOX/FOLFIRI	2.256	1.285-4.197	0.0040
TS1	3.684	1.578-8.264	0.0034

αβT: Activated lymphocyte therapy; DC: dendritic cell vaccine therapy; BV: bevacizumab; FOLFOX: fluorouracil+folinic acid+oxaliplatin; FOLFIRI: fluorouracil+folinic acid+irinotecan; TS1: tegafur/gimeracil/oteracil.

efficacy in patients with CRC (23). BV, which is an antibody against VEGF, can enhance the antitumor activity of adoptively transferred antitumor T-cells (24). A previous study has shown that 5-FU can up-regulate tumor antigen expression and MHC class I expression in CRC and breast cancer cells (25).

Table V. Multivariable analyses of overall survival in relation to therapy.

Characteristics	HR	95%CI	p-Value
PS			
≥1	1		
0	0.334	0.194-0.589	0.0002
BV			
No	1		
Yes	0.740	0.443-1.238	0.2501
1st-line agents			
Capecitabine	1		
FOLFOX/FOLFIRI	2.174	1.226-4.078	0.0071
TS1	3.138	1.305-7.289	0.0117

PS: Performance status; BV: bevacizumab; FOLFOX: fluorouracil+folinic acid+oxaliplatin; FOLFIRI: fluorouracil+folinic acid+irinotecan; TS1: tegafur/gimeracil/oteracil.

Furthermore, it has been demonstrated that 5-FU selectively kills tumor-associated myeloid-derived suppressor cells (MDSCs) resulting in the up-regulation of T-cell-dependent antitumor immunity (26-28). Capecitabine, a prodrug of 5-FU, has also been reported to reverse tumor escape by inhibiting MDSCs more effectively than 5-FU could (29). Thus, these findings suggest that chemotherapeutics can provide several beneficial effects on the immune system, resulting in the improvement of the patient's prognosis (30).

Recently, it has been shown that patients with microsatellite instability-high (MSI-H) CRC showed better response to anti-PD-1 antibodies (*e.g.*, nivolumab and pembrolizumab) than those with MSI-low (MSI-L) CRC (31, 32). MSI is caused by mutations in a mismatch repair (MMR) gene with the consequent inability to correct DNA errors during cell replication (33). It is now recommended that the MSI status should be evaluated in all newly diagnosed CRC cases, because the MSI-H status increases the expression levels of neoantigens owing to the high genomic instability in such cases (34), leading to the enhancement of local immune responses (35). Note that pembrolizumab is now approved in Japan for patients with advanced cancers with MSI-H including CRC. Since immune-cell therapy potentiates the immune reaction against cancer cells, the combination of check-point inhibitors (*e.g.*, pembrolizumab and nivolumab) and immune-cell therapy could be a promising immunotherapy for MSI-H CRC patients.

In conclusion, a better prognosis could be obtained with better PS by the combination of immune-cell therapy and chemotherapy with the normal immune-cell function preserved. However, to establish a comprehensive immunotherapy for CRC, it is necessary to conduct a randomized trial to further elucidate the benefits of the combination of immune-cell therapy and various other treatments, such as chemotherapy, radiotherapy, or therapy with immune check-point inhibitors.

Conflicts of Interest

The Authors declare that there are no potential conflicts of interest in relation to this study.

Authors' Contributions

Conception and design: R. Takimoto, T. Kamigaki, and S. Goto; Administrative support: S. Okada, E. Matsuda, H. Ibe, and E. Oguma; Collection and assembly of data: S. Okada, E. Matsuda, H. Ibe, E. Oguma, K. Naitoh, K. Makita and K. Yasumoto; Data analysis and interpretation: R. Takimoto, S. Okada, T. Kamigaki, and S. Goto; Final approval of manuscript: All Authors.

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