Pretreatment Immune Status Correlates with Progression-Free Survival in Chemotherapy-Treated Metastatic Colorectal Cancer Patients

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Abstract

It remains unclear whether the immunologic status of cells in peripheral blood can be used as a prognostic indicator of response to treatment for patients with unresectable metastatic colorectal cancer (MCRC). We therefore investigated the relationship between the pretreatment immunologic status of 40 patients with MCRC who planned to receive the first-line chemotherapy and their progression-free survival. Twenty-five immune cell subsets, including monocytic myeloid-derived suppressor cells (M-MDSC), low CD4+ T cells (TEM) and effector memory T cells (T EM), were measured by multicolor-flow cytometry. We divided patients into high and low (above and below the median, respectively) groups based on the median value for each immune cell subset and compared progression-free survival of the two groups. Patients with high M-MDSC, low CD4+ TEM or low CD8+ TEM quantities had significantly shorter progression-free survival (P = 0.004, 0.005, and 0.002, respectively). Patients were classified into two prognostic groups based on numbers of adverse factors; having two or three adverse factors (n = 21, 52.5%) was correlated with significantly shorter progression-free survival compared with none or one (n = 19, 47.5%; P < 0.001). The presence of two or three adverse factors was an independent poor prognostic factor for progression-free survival (HR, 9.2; 95% confidence interval, 2.5–34.2; P < 0.001). These results provide evidence that pretreatment peripheral immune status can inform the outcome of patients with MCRC treated with first-line chemotherapy. Cancer Immunol Res; 4(7); 592–9. ©2016 AACR.

Introduction

Effector T cells in cancer patients respond to tumor cells. Cytotoxic CD4+ or CD8+ T cells recognize tumor-specific antigens or tumor-associated antigens and exert direct cytotoxic actions against tumor cells. Colorectal cancer was the first neoplasm found to be under immune surveillance (1, 2). An increased quantity of tumor-infiltrating lymphocytes (TIL), CD3+ cells, CD8+ cells, and Th1 cells in surgically resected colorectal tumor specimens is associated with an improved prognosis (1, 3–11). In a more detailed analysis of the infiltrating T cells, the quantity of CD45RO+ cells (memory T cells) or effector memory (TEM) cells in a colorectal tumor was shown to have strong prognostic significance after surgery (12, 13).

However, it has not been determined whether the quantity of T cells or memory T cells correlates with the outcome of unresectable metastatic colorectal cancer (MCRC) patients treated with systemic chemotherapy. Because of the limited opportunities for obtaining resected specimens in MCRC patients, TIL analyses are difficult to conduct, and thus alternative immunologic parameters for determination of prognosis are needed. Peripheral blood is easily obtained with little burden on patients. Our preliminary study, which quantified the immune cells in peripheral blood of patients with gastrointestinal cancer treated with a peptide vaccine, suggested that patients with a high quantity of memory T cells in peripheral blood at pretreatment had durable stable disease, whereas patients with a low quantity had early progressed disease (unpublished data). Memory T cells are subdivided into effector memory (TEM) and central memory (T CM) cells. The former play an important role in antitumor immunity (12, 14, 15). Therefore, we hypothesized that the quantity of TEM cells in peripheral blood might correlate with prognosis in patients with MCRC.

Immune-suppressive cells, including myeloid-derived suppressor cells (MDSC), inhibit the activation and proliferation of effector T cells, such that the tumor can evade the host immune response. Large numbers of MDSCs are a poor prognostic factor for colorectal cancer. The infiltrating MDSCs in colorectal tumors have been shown to influence tumor progression (16–19). Tumor-associated M-MDSCs and low CD8+ TEM cells might be poor prognostic factors for colorectal cancer (20–23). In this study, we tested whether the quantity of M-MDSC, low CD4+ TEM, or low CD8+ TEM cells in peripheral blood correlated with progression in colorectal cancer patients treated with systemic chemotherapy.
for various cancer patients (16–20). Therefore, we also hypothesized that the quantity of MDSCs might affect the prognosis of patients with MCRC.

In the current prospective study, the primary objective was to investigate the relationship between the quantity of TEM cells or MDSCs in peripheral blood and the progression-free survival (PFS) of patients with unresectable MCRC who had received first-line chemotherapy. We also carried out an exploratory investigation of the relationship between the quantity of other immune cells and PFS of those patients.

Materials and Methods

Patients
Patients (N = 40) were prospectively enrolled to this study if they met the following criteria: age at least 20 years; histologically proven adenocarcinoma of the colorectum; curatively unresectable, metastatic, or recurrent disease; treatment plan to receive oxaliplatin-based first-line chemotherapy; and no active viral infection such as with the human immunodeficiency virus or hepatitis B or hepatitis C. Attending physicians chose either the 5-fluorouracil, folinic acid, and oxaliplatin (FOLFOX) regimen or the capecitabine plus oxaliplatin (XELOX) regimen combined with bevacizumab (BV). The FOLFOX regimen consisted of a simultaneous intravenous infusion of 85 mg/m² of oxaliplatin (2 hours), 200 mg/m² 1-leucovorin (2 hours), and 400 mg/m² bolus 5-fluorouracil on day 1, followed by a continuous infusion of 2,400 mg/m² of 5-fluorouracil (46 hours), repeated every 2 weeks (21). The XELOX regimen consisted of 130 mg/m² of oxaliplatin (2 hours) on day 1 plus oral capecitabine, 1,000 mg/m² twice daily for 2 weeks, repeated every 3 weeks (22). BV was administered before oxaliplatin at a dose of 5 mg/kg (FOLFOX) or 7.5 mg/kg (XELOX) on day 1 of each cycle. These treatments were repeated until disease progression, unacceptable toxicities, or patient’s refusal. The FOLFOX-without-BV regimen was used only when a patient had a history of vascular complications. To evaluate treatment efficacy, systemic CT was repeated every 2 months. All of the patients provided fully informed consent for study registration and blood collection. The study protocol was reviewed and approved by the institutional ethics committee of the National Cancer Center.

Staining of peripheral blood mononuclear cells
Peripheral blood was collected within 5 days before initial chemotherapy administration. The second and third blood collection was performed just before the fifth cycle (ninth week) in the FOLFOX + BV cohort or the third cycle (seventh week) in the XELOX + BV cohort, and at 6 months after initial chemotherapy, respectively. If chemotherapy was terminated because of disease progression or an adverse event before the second or third blood collection, blood was collected at that time.

Blood samples were centrifuged, and the separated plasma was cryopreserved. Peripheral blood mononuclear cells (PBMC) were isolated from peripheral blood by density gradient centrifugation. MDSCs were measured in fresh PBMC samples, because a previous study demonstrated that the MDSC fraction is decreased by cryopreservation (23). Dendritic cells (DC) were measured at the same time as measurement of the MDSC because a common flow cytometry panel was used for both cell subsets. The remaining PBMCs were cryopreserved and used for measurement of T cells, regulatory T cells (Treg), and natural killer (NK) cells.

Aliquots containing 2.5 × 10⁵ to 5 × 10⁵ PBMCs were suspended in 100 μL staining buffer (PBS containing 2% FBS). The antibodies for surface markers were then added followed by a 30-minute incubation at 4°C. For staining of intracellular proteins (Ki-67, FOXP3, perforin, and granzyme B), FOXP3/Transcription Factor for Staining Buffer Set (eBioscience) was used according to the manufacturer’s protocol. The antibodies used were as follows: Lineage (Lin, CD3/CD16/CD19/CD20/CD56) cocktail FITC, CD14–PerCP (peridinin chlorophyll protein)-Cy5.5, CD11b–APC (allophycocyanin)-Cy7, CD33–PE (phycoerythrin)-Cy7, CD11c–Alexa Fluor700, CD123–Brilliant Violet 421, CD15–V500, CD3–APC, Ki-67–Alexa Fluor700, CD8–APC-Cy7, granzyme B–FITC, CD56–PE-CF594 (BD Pharmingen), CD4–Brilliant Violet 650, CD16–PerCP-Cy5.5, CCR7–PerCP-Cy5.5 (Biolegend), FOXP3–PE, CD66b–APC (eBioscience), CD45RA–FITC, HLA-DR–EDC (Beckman Coulter), and perforin–PE (Cell Sciences). Isotype controls included the appropriate fluorochrome-conjugated mouse IgG1, IgG1/k, or IgG2a/k. The stained cells were detected using an LSR II Fortessa with FACS Diva software (BD Biosciences). All analyses were carried out using FlowJo software (TreeStar).

Definition and analysis of immune cell subsets
We analyzed 25 immune cell subsets in this study. The immune cell subsets were defined as follows: monocytic MDSC (M-MDSC), Lin−CD14+CD33-CD11b+ HLA-DRlow/, granulocytic MDSC (Gr-MDSC), CD33dimCD15CD66+CD11b+, naive Treg, CD3+CD4+CD45RA−FOXP3high, effector Treg, CD3+CD4+CD45RA+FOXP3high, plasmacytoid DC, Lin−CD14+CD123+HLA-DRlow, myeloid DC, and Lin−CD14+CD11c+HLA-DRhigh T cells were classified as naive (CD45RA+CCR7−), central memory (CD45RA+CCR7+), effector memory (CD45RA+CCR7+), and terminally differentiated effector cells (CD45RA+CCR7−) in CD4+ or CD8+ T cells. Expression of granzyme B, perforin, and Ki-67 was also assessed in CD4+ or CD8+ T cells. Appropriate isotype controls served as the cutoff levels between positivity and negativity. A positive gate was set to include less than 0.1% cells in each specimen with a matched isotype control.

The proportion of lymphoid subsets was obtained by dividing the cell number of each subset by the cell number of the lymphocyte fraction based on the results of flow cytometric analysis. Absolute counts of lymphoid subsets (μL) were estimated using the following formula: (Proportion of each lymphoid subset) × (absolute count of lymphocytes (μL)). The M-MDSC proportion in the monocytes was obtained by dividing the cell number of HLA-DRlow/− by the cell number of Lin−CD14+CD33+CD11b+. The absolute count of M-MDSCs (μL) in peripheral blood was estimated using this formula: (Proportion of M-MDSCs) × (absolute count of monocytes/μL; ref. 19).

Measurement of cytokines in plasma
Cryopreserved plasma was used for measuring VEGF-A, VEGF-C, VEGF-D, IL2, IL6, IL8, IL10, IFNγ, TNFα, and GM-CSF. These cytokines were simultaneously measured on an MSD SECTOR Imager 2400 instrument (Meso Scale Discovery, Inc.) according to the manufacturer’s protocol.

Statistical analysis
The primary objective of the analysis was to describe the relationship between pretreatment peripheral immune status and
PFS. Based on the median values for proportion of or absolute counts for each immune cell subset or cytokine value, the patients were divided into high (above median) and low (below median) groups. PFS was defined as the time period from the day of initial chemotherapy until disease progression or death from any cause. Disease progression was judged according to the RECIST guidelines (Version 1.1).

Cox proportional hazards models were used to estimate the effect of immune status. The proportional hazards assumption was confirmed by a score test for proportional hazards (24); no relevant violations of the assumption were found. The following model building process was done. Step 1: Variables included 25 immune cell subsets. Variables with a \( P \) value < 0.05 on univariate analysis were considered candidate variables. Finally, the candidate variables were combined into a single variable of immune status, because the absolute values of these coefficients were similar. Step 2: As the final analysis, a multivariate Cox proportional hazards model was used after adjustment for important covariates. An important covariate is defined as a characteristic that meets any of the following criteria: It is a known prognostic factor (e.g., use of BV) if removal of the covariate has produced an important change (more than 10%) in the coefficient of the immune status. This final model was chosen based on a clinical and statistical perspective by reference to a previously published approach (25).

For the patient characteristics, summary statistics comprise frequencies and proportions for categorical variables, and median and range for a continuous variable. Between-group differences in patient characteristics were analyzed with the Fisher exact test. The Kaplan–Meier method was used to estimate the PFS, and the differences were compared using the log-rank test. The Student t test was used for comparisons of measured values of cytokines. Pearson correlation was used to evaluate for relationships between two immune cell subsets. All \( P \) values are two-sided. \( P \) values of < 0.05 were considered to indicate statistical significance, and 95% confidence intervals (CI) were calculated. All statistical analyses were performed using SAS software version 9.4 (SAS Institute).

### Results

#### Patient characteristics

Patient characteristics are presented in Table 1: 21 (52.5%) and 15 (37.5%) patients were treated with FOLFOX+BV and XELOX+BV, respectively; 4 (10%) patients received FOLFOX without BV because they had past history of vascular complication; and 39 (97.5%) patients had good performance status (0–1).

### Associations between the quantity of each immune cell subset and PFS

The gating strategy and representative dot plots for M-MDSCs and effector memory cells in CD4\(^+\) or CD8\(^+\) T-cell fractions (CD4\(^+\) TEM, or CD8\(^+\) TEM) are shown in Fig. 1A and B. The median values of the proportion and absolute count for each immune cell subset were 13.3\% (range, 0.7–59.0) and 40.5/\mu L (range, 1.5–536.3) for M-MDSC; 7.1\% (range, 2.8–21.5) and 86.5/\mu L (range, 36.9–326.8) for CD4\(^+\) TEM, and 9.9\% (range, 1.5–14.3); and 75.1/\mu L (range, 13.9–274.0) for CD8\(^+\) TEM, respectively. The gating strategy and representative dot plots for other immune cell subsets (Gr-MDSCs, Tregs, DCs, NK cells, granzyme B, perforin\(^\), or Ki-67\(^+\) cells) are shown in Supplementary Fig. S1.

Patients were divided into high (>median) and low (<median) groups based on the median value of the proportion for each immune cell subset, and PFS was then compared between each pair of groups (Table 2). A high proportion of M-MDSCs, low proportion of CD4\(^+\) TEM, and low proportion of CD8\(^+\) TEM were associated with significantly shorter PFS (median PFS, not reached vs. 8.5, not reached vs. 8.5, not reached vs. 9.0 months; \( P = 0.004, 0.005, \) and 0.002, respectively; Fig. 1C). A high absolute count of M-MDSCs, low absolute count of CD4\(^+\) TEM, and low absolute count of CD8\(^+\) TEM were also associated with significantly shorter PFS (\( P = 0.005, 0.03, \) and 0.046, respectively).

### Correlations between quantities of M-MDSCs, CD4\(^+\) TEM, and CD8\(^+\) TEM

Because the quantities of M-MDSCs, CD4\(^+\) TEM, or CD8\(^+\) TEM each correlated with PFS, correlations between each pair among the proportions of M-MDSC, CD4\(^+\) TEM, and CD8\(^+\) TEM were investigated (Fig. 2A). A weak inverse correlation between M-MDSC and CD4\(^+\) TEM was observed \( (r = –0.33, P = 0.03); 14 \) (35\%) patients presented with high M-MDSC and low CD4\(^+\) TEM whereas 14 (35\%) patients had low M-MDSC and high CD4\(^+\) TEM. This inverse correlation is reasonable because MDSCs are immune-suppressive cells that inhibit the proliferation and activation of T cells. However, the quantities of M-MDSCs and CD4\(^+\) TEM were somewhat discrepant: 6 (15\%) patients presented with low M-MDSCs with low CD4\(^+\) TEM whereas 6 (15\%) had high M-MDSC with high CD4\(^+\) TEM. Similarly, there was a trend toward an inverse correlation between M-MDSC and CD8\(^+\) TEM, although the difference did not reach statistical significance \( (r = –0.28, P = 0.08); 13 \) (32\%) patients each presented with high M-MDSC and low CD8\(^+\) TEM, or low M-MDSC and CD8\(^+\) TEM. Whereas 7 (17\%) each had low M-MDSC with low CD8\(^+\) TEM, and high M-MDSC with high CD8\(^+\) TEM. In addition, a weak positive correlation between CD4\(^+\) TEM and CD8\(^+\) TEM was observed \( (r = 0.32, P = 0.045); 14 \) (35\%) patients each presented with high CD4\(^+\) TEM and high CD8\(^+\) TEM or low CD4\(^+\) TEM and low CD8\(^+\) TEM whereas 6 (15\%) each had low CD4\(^+\) TEM with high CD8\(^+\) TEM and high CD4\(^+\) TEM with low CD8\(^+\) TEM.

### Table 1. Patient characteristics

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<td>2</td>
<td>12</td>
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<td>3</td>
<td>5</td>
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Figure 1.
A and B, gating strategy and representative dot plots for M-MDSCs (A) and naive/memory cells in T-cell fractions (B). Two independent dot plots are shown as high and low M-MDSCs (A), and high and low effector memory T cells (B). C, the Kaplan–Meier curves for PFS are shown according to the pretreatment quantities of M-MDSCs, effector memory CD4⁺ T cells, and CD8⁺ T cells. CM, central memory; EM, effector memory; N, naive; TE, terminally differentiated effector.
NOTE: Patients were divided into high (median) and low (<median) groups based on the median value of the proportion of each immune cell subset, and univariate analyses for PFS were performed by using Cox proportional hazards models. The reference for the HR is a low group of each immune cell subset.

Based on this grouping, univariate analyses were performed to estimate the HR of the each immunologically distinct subgroup (Supplementary Table S1). Patients with high M-MDSC and low CD4+ TEm values had significantly higher HR than those with low M-MDSC and high CD4+ TEm (HR, 6.9; 95% CI, 2.1–22.9; P = 0.001). Similarly, patients with high M-MDSC and low CD8+ TEM values had significantly higher HR than those with low M-MDSC and high CD8+ TEM values (HR, 9.1; 95% CI, 2.4–34.7; P = 0.001). In addition, patients with low CD4+ TEM and low CD8+ TEM values had significantly higher HR than those with high CD4+ TEM and high CD8+ TEM values (HR, 7.2; 95% CI, 2.2–23.8; P = 0.001).

Combined M-MDSC, CD4+ TEM and CD8+ TEM assessments improved PFS prediction

High M-MDSC, low CD4+ TEM or low CD8+ TEM values were adverse immunologic factors. We also noted that approximately 70% of patients presented with concordance between the quantity of M-MDSCs and that of CD4+ TEM or CD8+ TEM, whereas approximately 30% showed discrepant results for these quantities. Therefore, we investigated whether a combined assessment of the immune-suppressive cells (M-MDSCs) and effector memory cells (CD4+ TEM and CD8+ TEM) would increase the accuracy of predicting clinical outcomes for the different patient groups. Patients were divided into four groups based on the numbers of adverse immunologic factors: Group 1, no adverse factors (n = 11, 27.5%); Group 2, one adverse factor (n = 8, 20%); Group 3, two adverse factors (n = 11, 27.5%); and Group 4, three adverse factors (n = 10, 25%; Fig. 2B). The Kaplan–Meier curves for the PFS of each group are shown in Fig. 2C. The curve of Group 1 overlapped that of Group 2, and the curve of Group 3 overlapped that of Group 4. These findings suggest that Groups 1 and 2 have an equivalent prognosis, as do Groups 3 and 4. Therefore, Groups 1 and 2 were combined, as were Groups 3 and 4. The Kaplan–Meier curves of Groups 1 and 2 and Groups 3 and 4 are shown in Fig. 2D. Groups 3/4 had significantly shorter PFS than Groups 1 and 2 (median PFS, not reached vs. 8.0 months, P < 0.001).

Next, patient characteristics were compared between Groups 1 and 2 and Groups 3 and 4 (Supplementary Table S2). Groups 3 and 4 included a significantly higher number of patients with elevated alkaline phosphatase values (> normal range; P = 0.02), but the differences between Groups 1 and 2 and Groups 3 and 4 with regard to other characteristics such as age, sex, history of adjuvant chemotherapy, use of BV, Eastern Cooperative Oncology Group (ECOG) PS, primary lesion, and number of metastatic lesions, were not statistically significant.

Finally, the univariate and multivariate analyses for PFS were performed on different groups, patient characteristics, and cytokine values (Table 3 and Supplementary Table S3). The multivariate analysis showed Groups 3 and 4 to have a significantly higher HR than Groups 1 and 2 (HR, 9.2; 95% CI, 2.5–34.2; P < 0.001) after adjusting for important covariates, which were chosen based on the criteria as described in Materials and Methods.

Comparison of plasma cytokine concentrations between Groups 1/2 and Groups 3/4

We measured the concentration of 10 plasma cytokines to assess any differences between Groups 1 and 2 and Groups 3 and 4 (Supplementary Table S4). Concentrations of VEGF-A in Groups 3 and 4 were significantly higher than those in Groups 1 and 2 (mean ± SD, 87.0 ± 62.5 vs. 35.6 ± 21.4; P = 0.002; Fig. 2E), and IL6 was also significantly higher in Groups 3 and 4 than in Groups 1 and 2 (mean ± SD, 5.2 ± 4.7 vs. 1.9 ± 1.9; P = 0.007; Fig. 2E). The two groups had no statistically significant differences in other cytokines, such as VEGF-C, VEGF-D, IL2, IL8, IL10, IFNγ, TNFα, and GM-CSF.

Quantitative changes in M-MDSC and TEM after chemotherapy and their impact on PFS

We also analyzed the quantity of M-MDSC and TEM cells after chemotherapy. Although a second blood collection was performed in all patients (N = 40), a third blood collection was done only in 31 patients because of discontinuance of chemotherapy or patients’ withdrawal. The means of %M-MDSC or %TEM were compared between before (first blood collection) and after chemotherapy (second or third blood collection) using a paired t test; however, no tendency for an increase or decrease of any immune cell subset was noted (Supplementary Fig. S2A). Regarding changes in immune cell subsets from before chemotherapy to the second blood sample, M-MDSC increased in 18 (45%) patients and decreased in 22 (55%), CD4+ TEM increased in 18 (45%) and decreased in 22 (55%), and CD8+ TEM increased in 25 (62.5%) and decreased in 15 (37.5%). PFS was then compared between patients who showed an increase or decrease in those cells; however, no statistically significant differences were found (patients with
increased vs. decreased M-MDSC, CD4⁺ TTEM, and CD8⁺ TTEM; 
P = 0.3, 0.9, and 0.3, respectively; Supplementary Fig. S2B). Comparison of PFS between before chemotherapy and the third blood sample was not performed because patients who underwent a third blood collection were selected patients who could continue initial chemotherapy for up to 6 months.

**Discussion**

The present study demonstrated that pretreatment immune status correlates with the PFS of patients with unresectable MCRC given first-line chemotherapy. We analyzed 25 immune cell subsets and identified high M-MDSC, low CD4⁺ TTEM, and low CD8⁺ TTEM values as adverse prognostic factors for PFS. In addition, combined assessment of all three adverse factors showed the outcomes of patients who had two or three of these factors (Groups 3 and 4) to be significantly poorer than those of patients who had zero or one adverse factor (Groups 1 and 2). This negative impact remained statistically significant in multivariate analysis. Although many retrospective studies have already shown that the quantity of TILs in surgically resected specimens correlates with the outcomes of patients with resectable colorectal cancer (1, 3–13, 26), this prospective study has demonstrated that the quantity of immune cells in peripheral blood correlates with the outcomes of those with unresectable tumors.

Approximately 27.5% of the patients in this study had low M-MDSC, high CD4⁺ TTEM, and high CD8⁺ TTEM values (Group 1), whereas 25% of patients had high M-MDSC, low CD4⁺ TTEM, and low CD8⁺ TTEM values (Group 4). This inverse correlation...
between M-MDSCs and effector memory T cells is reasonable because MDSCs are immune-suppressive cells that inhibit the proliferation and activation of T cells. However, the remaining 47.5% of patients (Groups 2 and 3) showed discrepant results for the quantities of M-MDSCs, CD4<sup>+</sup>TEM, and CD8<sup>+</sup>TEM. These results suggest that the quantities of M-MDSCs, CD4<sup>+</sup>TEM, and CD8<sup>+</sup>TEM are specific for each patient (Fig. 2B and Supplementary Fig. S2). Therefore, combined assessment of the immune-suppressive cells (M-MDSC) and the cytotoxic effector cells (CD4<sup>+</sup>TEM and CD8<sup>+</sup>TEM) may provide a more appropriate reflection of the immune status of each patient and would also, presumably, illustrate the correlation between immune status and prognosis more accurately than would individual assessments of these cell subsets. For example, a patient with a high quantity of M-MDSCs would generally have a short PFS, but the negative impact might be canceled out in the presence of high quantities of CD4<sup>+</sup>TEM. Similarly, a patient with a low quantity of CD8<sup>+</sup>TEM might be expected to have a short PFS, the negative impact could be canceled out in the presence of a low M-MDSC and a high CD4<sup>+</sup>TEM. In fact, we demonstrated that PFS in Group 2, which consisted of such patients, is equivalent to that in Group 1, comprised of patients with low M-MDSC, high CD4<sup>+</sup>TEM, and high CD8<sup>+</sup>TEM values (Fig. 2C).

Our present prospective study included a rather small number of patients. Nevertheless, we identified statistically significant prognostic factors for PFS. Assessment of the impact on overall survival requires an additional follow-up period because only 8 of our patients did not survive. Despite this limitation, our results have meaningful clinical implications: Antitumor immunity may be helpful for the effects of chemotherapy and thus provide a rationale for developing a regimen combining chemotherapy with immunotherapy. An immunotherapeutic approach that reduces M-MDSCs or increases effector memory T cells might overcome immunologically mediated adverse impacts on prognosis.

In conclusion, we analyzed 25 immune cell subsets in peripheral blood from patients with unresectable MCRC before first-line chemotherapy and identified high M-MDSC, low CD4<sup>+</sup>TEM, and CD8<sup>+</sup>TEM quantities as significant adverse factors for PFS. Combining the assessment of these three adverse factors gave greater accuracy of PFS prediction for the immunologically different patient subgroups. These results suggest that pretreatment peripheral immune status correlates with the outcomes of patients with unresectable MCRCs receiving first-line chemotherapy. Further studies involving patients with other types of cancer are warranted to assess our results.

**Disclosure of Potential Conflicts of Interest**

No potential conflicts of interest were disclosed.

**Authors’ Contributions**

Conception and design: K. Tada, S. Kitano, Y. Shimada, K. Aoki, N. Okita, Y. Yamada, Y. Heike, T. Hamaguchi

Development of methodology: K. Tada, S. Kitano, Y. Heike

Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): K. Tada, H. Shoji, T. Nishimura, N. Hiraoka, S. Iwasa, A. Takashima, Y. Heike, T. Hamaguchi

Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): K. Tada, K. Nagashima, K. Aoki, Y. Yamada, Y. Heike, T. Hamaguchi

Writing, review, and/or revision of the manuscript: K. Tada, S. Kitano, Y. Shimada, K. Nagashima, Y. Horima, N. Okita, Y. Yamada, N. Katayama, N. Boku, Y. Heike, T. Hamaguchi

Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): K. Tada, S. Kitano, K. Kato, Y. Heike, T. Hamaguchi

Study supervision: S. Kitano, Y. Shimada, N. Katayama, Y. Heike, T. Hamaguchi

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Relationship between Immune Status and PFS of MCRC Patients

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