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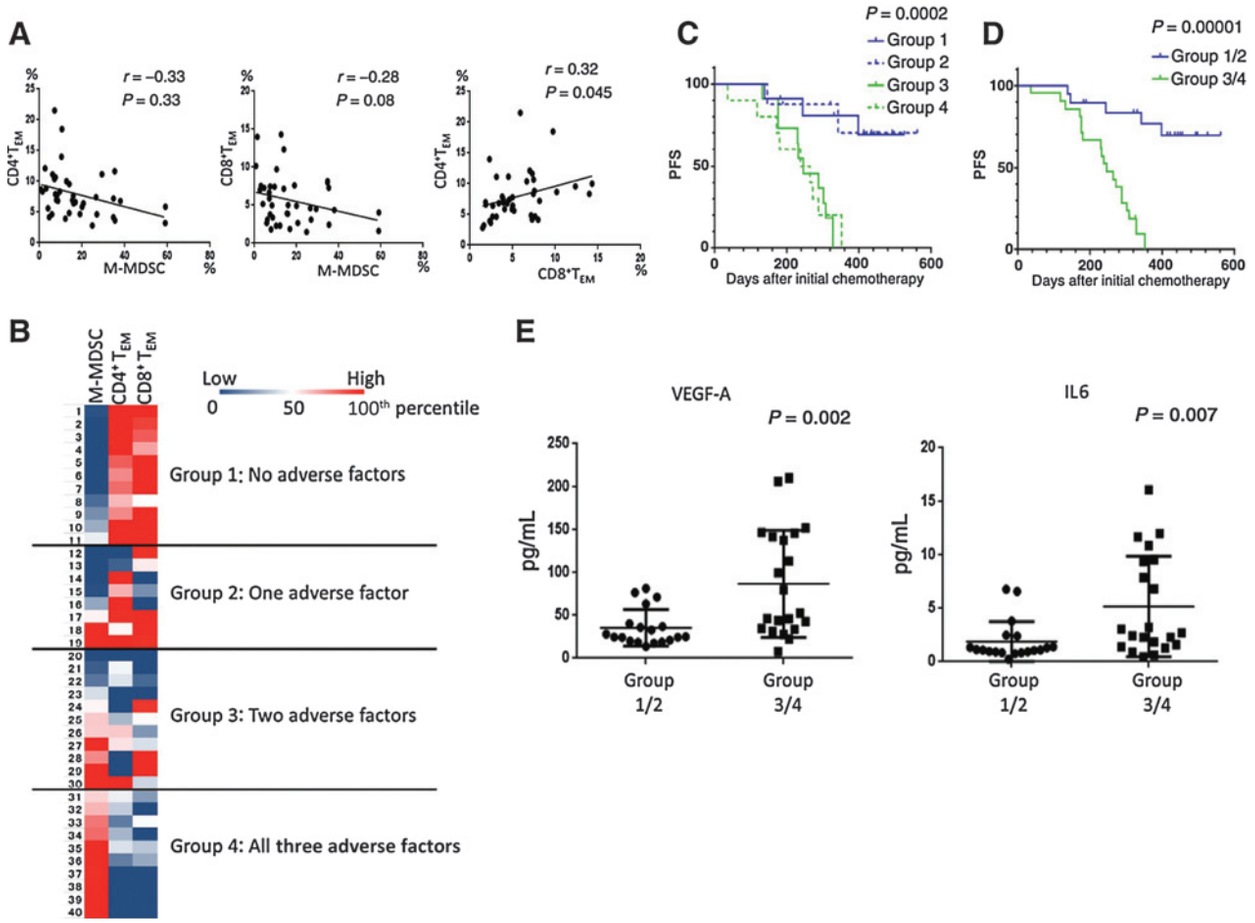

Figure 2.

Combined M-MDSC, CD4$^+$ T_{EM}, and CD8$^+$ T_{EM} assessments. A, correlations between each pair of the proportions of M-MDSCs, CD4$^+$ T_{EM}, and CD8$^+$ T_{EM} cells are shown in dot plots. Pearson correlation coefficient is indicated as "r." B, analysis of patient groups with different numbers of the adverse factors of high M-MDSC, low CD4$^+$ T_{EM}, and low CD8$^+$ T_{EM}, which we identified as adverse immunologic factors as shown in Fig. 1C and Table 2. Patients were divided into four groups based on the number of adverse factors of each patient: Group 1, no adverse factor ($n = 11$); Group 2, one ($n = 8$); Group 3, two ($n = 11$); Group 4, three ($n = 10$). Blue and red colors in the heat map indicate that a patient has low (<median value) or high (>median value) quantities, respectively, of the corresponding immune cell. C and D, Kaplan–Meier curves for PFS of the four different immunologic groups are shown in C. Because the curve of Group 1 overlapped with that of Group 2, and the curve of Group 3 overlapped with that of Group 4, 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 D. *P* values were calculated by the log-rank test. E, values of VEGF-A and IL6 were compared between Groups 1 and 2 and 3 and 4 with a *t* test. The long and short horizontal lines in the figure indicate the means and SDs, respectively. Comparison of other cytokine values are shown in Supplementary Table S4.

increased vs. decreased M-MDSC, CD4$^+$$T_{EM}$, and CD8$^+$$T_{EM}$; $P =$ 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$^+$ T_{EM}, and low CD8$^+$ T_{EM} 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$^+$$T_{EM}$, and high CD8$^+$$T_{EM}$ values (Group 1), whereas 25% of patients had high M-MDSC, low CD4$^+$$T_{EM}$, and low CD8$^+$$T_{EM}$ values (Group 4). This inverse correlation

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Table 3. Multivariate analysis for PFS

	Multivariate analysis ($N = 40$)	
Covariates	**HR (95% CI)**	***P* value**
Group		
1/2	Reference	
3/4	9.2 (2.5–34.2)	<0.001
Use of bevacizumab		
Used	Reference	
Not used	2.5 (0.7–9.3)	0.2
Primary lesion		
Right hemicolon	Reference	
Left hemicolon	0.5 (0.1–1.7)	0.3
Rectum	0.5 (0.2–1.7)	0.3
IFNγ		
Low	Reference	
High	1.6 (0.6–4.6)	0.4
IL8		
Low	Reference	
High	2.7 (0.9–8.7)	0.09

NOTE: Multivariate analysis for PFS was performed on different immune groups, patient characteristics, and cytokine values by using a Cox proportional hazards model. Covariates were chosen based on the criteria described in Materials and Methods.

between M-MDSCs and effecter 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, $CD4^+T_{EM}$, and $CD8^+T_{EM}$. These results suggest that the quantities of M-MDSCs, $CD4^+T_{EM}$, and $CD8^+T_{EM}$ 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 ($CD4^+T_{EM}$ and $CD8^+T_{EM}$) 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 $CD4^+T_{EM}$ and/or $CD8^+T_{EM}$. Similarly, although a patient with a low quantity of $CD8^+T_{EM}$ 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 $CD4^+T_{EM}$ value. 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 $CD4^+T_{EM}$, and high $CD8^+T_{EM}$ values (Fig. 2C). Our results demonstrate that individual assessments of M-MDSC and effector memory T cells have potential prognostic value for PFS and that the combined assessment of these cell subsets predicts PFS with greater accuracy than that of any one cell subset alone.

We demonstrated that the immune status at pretreatment correlated with PFS; however, changes of those cells after chemotherapy did not correlate with PFS. It is very likely that change of immune status after chemotherapy is influenced by several factors, such as direct cytotoxicity from therapeutic agents, disease progression or regression, incidence of adverse event, and so on. These various factors may make it difficult to interpret the correlation between change of immune status and PFS.

We also analyzed plasma cytokines that affect the formation of immune cell subsets. We found that VEGF-A and IL6 were significantly higher in Groups 3 and 4 than in Groups 1 and 2. VEGF-A contributes not only to tumor angiogenesis but also to formation of the immunosuppressive microenvironment in tumors (27). VEGF-A augments MDSCs (28, 29) and inhibits DC maturation (30, 31), directly inhibits the activation and proliferation of T cells (32), and upregulates expression of the programmed death-1 molecule on T cells (33). IL6 is a multifunctional cytokine with pro- and anti-inflammatory activity. Under certain pathologic circumstances, IL6 augments MDSCs. Based on these findings, increased VEGF-A and IL6 concentrations in our cohort may have contributed to the adverse immune status, which resulted in shorter PFS.

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 $CD4^+T_{EM}$, and $CD8^+T_{EM}$ 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

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