Cancer Immunology, Immunotherapy (2019) 68:1341–1350
https://doi.org/10.1007/s00262-019-02366-0

ORIGINAL ARTICLE

Peri-operative monocyte count is a marker of poor prognosis in gastric cancer: increased monocytes are a characteristic of myeloid-derived suppressor cells

Shinya Urakawa[1,2] · Makoto Yamasaki[2] · Kumiko Goto[1,3] · Miya Haruna[1,3] · Michinari Hirata[1,3] · Akiko Morimoto-Okazawa[1] · Atsunari Kawashima[1] · Kota Iwahori[1] · Tomoki Makino[2] · Yukinori Kurokawa[2] · Tomomi Yamada[4] · Masaki Mori[5] · Yuichiro Doki[2] · Hisashi Wada[1]

Received: 21 October 2018 / Accepted: 5 July 2019 / Published online: 19 July 2019

Abstract

Gastric cancer (GC) is the most common malignant tumor in digestive organs, and the prognosis of GC patients who have undergone surgery remains poor because of frequent recurrence. Therefore, the identification of new markers to predict the outcome of these patients is needed. Monocyte count is a negative prognostic factor associated with inflammation. We investigated the relationship between peripheral monocytes in the peri-operative period and prognosis in GC patients. A high pre-operative monocyte count was identified as a prognostic factor in a retrospective analysis of 278 stage II and III GC patients who underwent curative gastrectomy. In contrast, an increased post-operative monocyte count compared to the pre-operative monocyte count was a marker of poor prognosis, particularly for early relapse. In a prospective analysis of 75 GC patients, a subset of the increased post-operative monocytes was similar to $CD14^+$ HLA-DR^- $CD11b^+$ $CD33^+$ cells by flow cytometry, and these monocytes produced IDO and arginase and suppressed T cell functions; therefore, we classified these cells as monocytic myeloid-derived suppressive cells (M-MDSCs). Peri-operative neutrophils and C-reactive protein (CRP), which are also related to inflammation, did not affect the prognosis of GC patients, and a neutrophil immunosuppressive function was not observed. These results suggest that peripheral monocytes in the peri-operative period in GC patients are a useful marker for the prognosis of GC patients, and a subset of increased post-operative monocytes may be characterized as M-MDSCs.

Keywords Biomarker · Post-operative monocyte count · Prognosis · Immunosuppression

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s00262-019-02366-0) contains supplementary material, which is available to authorized users.

✉ Hisashi Wada
hwada@gesurg.med.osaka-u.ac.jp

1 Department of Clinical Research in Tumor Immunology, Graduate School of Medicine, Osaka University, 2-2 Yamadaoka, Suita, Osaka 565-0871, Japan

2 Department of Gastroenterological Surgery, Graduate School of Medicine, Osaka University, Osaka, Japan

3 Drug Discovery and Disease Research Laboratory, Shionogi and Co., Ltd., Toyonaka, Osaka, Japan

4 Department of Medical Innovation, Osaka University Hospital, Osaka, Japan

5 Department of Surgery and Science, Graduate School of Medical Sciences, Kyushu University, Fukuoka, Japan

Abbreviations

CCL17	C–C motif chemokine ligand 17
CI	Confidence intervals
CRP	C-reactive protein
GC	Gastric cancer
G-MDSC	Granulocyte-like myeloid-derived suppressor cell
HR	Hazard ratio
ICAM-1	Intercellular adhesion molecule-1
ICI	Immune checkpoint inhibitor
iNOS	Inducible nitric oxide synthase
LMR	Lymphocyte–monocyte ratio
M-MDSC	Monocytic myeloid-derived suppressor cell
NLR	Neutrophil–lymphocyte ratio
OS	Overall survival
PBL	Peripheral blood leukocytes
POD	Post-operative day
RFS	Recurrence-free survival

ROS	Reactive oxygen species
TAM	Tumor-associated macrophage
Treg	Regulatory T cell

Introduction

Gastric cancer (GC) is the most common malignant tumor in digestive organs, and surgical resection is the main curative treatment. In spite of recent advances in adjuvant and neoadjuvant chemotherapies, the prognosis of GC patients who have undergone surgery remains poor because of recurrence and metastasis, which leads to high cancer-related death rates worldwide [1, 2]. The identification of new surrogate factors involved in the tumor microenvironment in GC for predicting patient outcomes, besides the profiles of tumor cells, is needed.

Evidence from successful cancer immunotherapy suggests that host tumor immunity is involved in tumor progression [3, 4]. Long-term survivors, even among advanced cancer patients, treated with immune checkpoint inhibitors (ICIs) reveal that immunosuppressive factors in tumor immunity impact the prognosis of cancer patients. There are several subpopulations of immunosuppressive cells including $CD14^+$ monocytes, $CD15^+$ neutrophils in myeloid cells, and $Foxp3^+$ $CD4^+$ regulatory T cells (Tregs) in lymphoid cells [5].

$CD15^+$ cells are associated with inflammation that induces tumor progression and metastasis in cancer patients [6]. In combination with lymphocytes, $CD15^+$ neutrophil count, particularly the neutrophil–lymphocyte ratio (NLR), is a biomarker of prognosis and therapy outcomes in patients with various types of cancers [7]. Furthermore, granulocyte-like myeloid-derived suppressor cells (G-MDSCs) among neutrophils suppress T cells by producing IDO, arginase, reactive oxygen species (ROS), inducible nitric oxide synthase (iNOS), and C–C motif chemokine ligand 17 (CCL17), which induce Tregs, and by expressing PD-L1, which results in tumor progression [8–10].

$CD14^+$ cells are antigen-presenting cells, such as DCs and macrophages, that enhance anti-tumor immunity, and a subpopulation of these cells function as immune suppressors such as monocytic myeloid-derived suppressor cells (M-MDSCs) and tumor-associated macrophages (TAMs) [8, 11]. $CD14^+$ $CD15^-$ $HLA\text{-}DR^-$ $CD11b^+$ $CD33^+$ M-MDSCs and $CD14^-$ $CD15^+$ $HLA\text{-}DR^-$ $CD11b^+$ G-MDSCs proliferate as immature myeloid cells in peripheral blood during cancer progression and chronic infection and markedly suppress T cells and DCs [8–10]. Therefore, M-MDSCs are also a useful marker of poor prognosis in cancer patients [12–14]. ICI therapy is clinically beneficial for melanoma patients with only a few M-MDSCs in peripheral blood [15–17]. After migration into the tumor microenvironment, some M-MDSCs differentiate into TAMs [8–10].

In GC, Tregs in tumor tissues and neutrophils, G-MDSCs, NLR, and the lymphocyte–monocyte ratio (LMR) in peripheral blood are strongly associated with immune suppression and prognosis of patients, and TAMs in tumor tissues may contribute to the epithelial–mesenchymal transition of cancer cells [18–26].

In cancer patients with post-operative inflammation and stress, myeloid cells are recruited from the bone marrow and peak in number within a few days; these monocytes and neutrophils increase rapidly after an operation and are immature cells. Here, we examined the profile and function of circulating immature monocytes after standard curative gastrectomy both retrospectively and prospectively and analyzed these factors in anti-tumor immunity and GC patient prognosis.

Materials and methods

Patients and blood samples

For prognostic analyses of overall survival (OS) and recurrence-free survival (RFS), the clinical and pathological data of 278 GC patients who underwent curative gastrectomy and were diagnosed with pathological stages II and III between January 2007 and December 2014 at Osaka University Hospital were retrospectively analyzed. For the prospective analyses, peripheral blood and clinical and pathological data were obtained from 75 GC patients who underwent curative gastrectomy with clinical stages I, II, and III between April 2016 and December 2017 at Osaka University Hospital. Peripheral blood was obtained on post-operative days (POD) 0, 1, 3, and 7. As a control, peripheral blood was collected from 11 healthy donors.

Antibodies

The fluorescently labeled antibodies used for flow cytometry were as follows: CD3-Alexa Fluor 700 (clone UCTH1; BioLegend), CD4-Brilliant Violet 711 (clone RPA-T4; BioLegend), CD8-Brilliant Violet 510 (clone RPA-T8; BioLegend), CD45RA-FITC (clone H100; BioLegend), CD11b-Brilliant Violet 605 (clone M1/70; BioLegend), CD14-Alexa Fluor 700 (clone HCD14; BioLegend), CD15-Brilliant Violet 510 (clone W6D3; BioLegend), HLA-DR-V450 (clone G46-6; BD Biosciences, Franklin Lakes, NJ, USA), CD33-Brilliant Violet 711 (clone WM53; BioLegend), CD25-PE/Cy7 (clone BC96; BioLegend), Foxp3-APC (clone PCH101; ThermoFisher Scientific, Waltham, MA, USA), IDO-FITC (clone 700838; R&D Systems, Minneapolis, MN, USA), and arginase 1-APC (clone 658922; R&D Systems).

Flow cytometry

Fresh peripheral blood was treated with BD Pharm Lyse buffer (BD Biosciences) to eliminate red blood cells. The remaining peripheral blood leukocytes (PBL) were stained with fluorophore-conjugated antibodies at 4 °C for 30 min after FcR block (Human TruStain FcX Fc Receptor blocking solution; BioLegend). The BD Pharmingen™ Transcription Factor Buffer Set (BD Biosciences) was used for intracellular staining according to the manufacturer's protocol. Stained cells were analyzed by LSR Fortessa (BD Biosciences), and the frequencies of cell populations were obtained in an analysis using DiVA software (BD Biosciences). To determine positive staining, an isotype control of the primary antibody conjugated with each fluorophore was used.

Measurement and calculation of cell counts

Pre-operative and post-operative monocyte, neutrophil, and white blood cell counts were obtained from general blood tests in medical reports. Increases in the post-operative cell count on post-operative day one (POD1) or day three (POD3) compared to the pre-operative cell count were calculated for monocytes, neutrophils, and $CD14^+$ $HLA\text{-}DR^-$ cells as follows: (peak post-operative cell count on POD1 or POD3) − (pre-operative cell count).

Cell numbers were calculated using the frequency in the PBL obtained by flow cytometry (Supplementary Fig. 1) and the absolute white blood cell count.

Suppression assay

A total of 1×10^4/well CD8 T cells co-cultured with autologous suppressor cells were incubated in 20 IU/mL IL-2 (Takeda Chemical Industries, Osaka, Japan) in 96-well culture plates at 37 °C for 48 h and then stimulated by anti-CD3/anti-CD28 mAb-coated (CD3/CD28) beads (Dynabeads Human T-Activator CD3/CD28; ThermoFisher Scientific) for 4 h. To detect IFN-γ, T cells were harvested, washed, and labeled with IFN-γ Catch Reagent (Miltenyi Biotec, Bergisch Gladbach, Germany) on ice for 5 min and then incubated at 37 °C for 45 min. Cells were washed and stained with IFN-γ Detection Antibody (Miltenyi Biotec) and anti-CD8 antibody [27]. CD8, CD14, and CD15 cells were purified with antibody-coated magnetic beads (Invitrogen, Carlsbad, CA, USA). $CD14^+$ $HLA\text{-}DR^-$ and $CD14^+$ $HLA\text{-}DR^+$ cells were sorted and purified by FACS Aria II (BD Biosciences) for use in the suppression assay (Supplementary Fig. 1).

Statistical analysis

The significance of differences in each experimental dataset between two groups was assessed using the Student's two-tailed paired *t* test. The Kruskal–Wallis test, Mann–Whitney U test, and Chi squared test were used for univariate analysis. Survival curves were estimated using the Kaplan–Meier method and compared by the log-rank test. A multivariate Cox proportional-hazard regression model was used to identify independent prognostic markers; variables for which the *p* value in the univariate analysis was < 0.1 were used in the multivariate model. Hazard ratios (HR) were reported with 95% confidence intervals (CI). *p* values < 0.05 were considered significant. Bonferroni's correction was applied for multiple comparisons of pre-, post- and increased monocyte, and the *p* values indicated were rough-*p* values. All statistical analyses were performed using JMP Pro 14 statistical Discovery™ (SAS Institute Inc., Cary NC, USA).

Results

Impact of the peri-operative monocyte count on the prognosis of GC patients

We retrospectively analyzed the time course of monocyte counts of 278 GC patients in the peri-operative period from data obtained from routine blood tests in medical reports. Rapid increases in the post-operative monocyte count were observed in most patients and peaked on POD1 or POD3 (Supplementary Fig. 2a). We then investigated the impact of the pre-operative monocyte count (mean ± SD; 422 ± 144/μL, median; 401/μL) and peak post-operative monocyte count (645 ± 281, 590) on POD1 or POD3 for the prognosis of each patient. Patients were divided into high and low monocyte count groups based on median count. The OS and RFS in patients with a high pre-operative monocyte count were longer than in those with a low pre-operative monocyte count, but the OS and RFS in patients with a high post-operative monocyte count were shorter than in those with a low post-operative monocyte count (Fig. 1a, b). We then evaluated the increase in the post-operative monocyte count compared to the pre-operative monocyte count, which we termed the increased monocyte count, in each patient (223 ± 256, 176) and analyzed its relationship with prognosis. When patients were divided based on the median increased monocyte count, RFS, and OS were shorter in patients with a high increased monocyte count than in those with a low increased monocyte count (rough-$p = 0.035$ and $p = 0.070$, respectively, Fig. 1c; Table 1; Supplementary Table 1), but no statistically significant difference was found with Bonferroni's correction. On the other hand, pre- and post-operative neutrophil counts and the increase

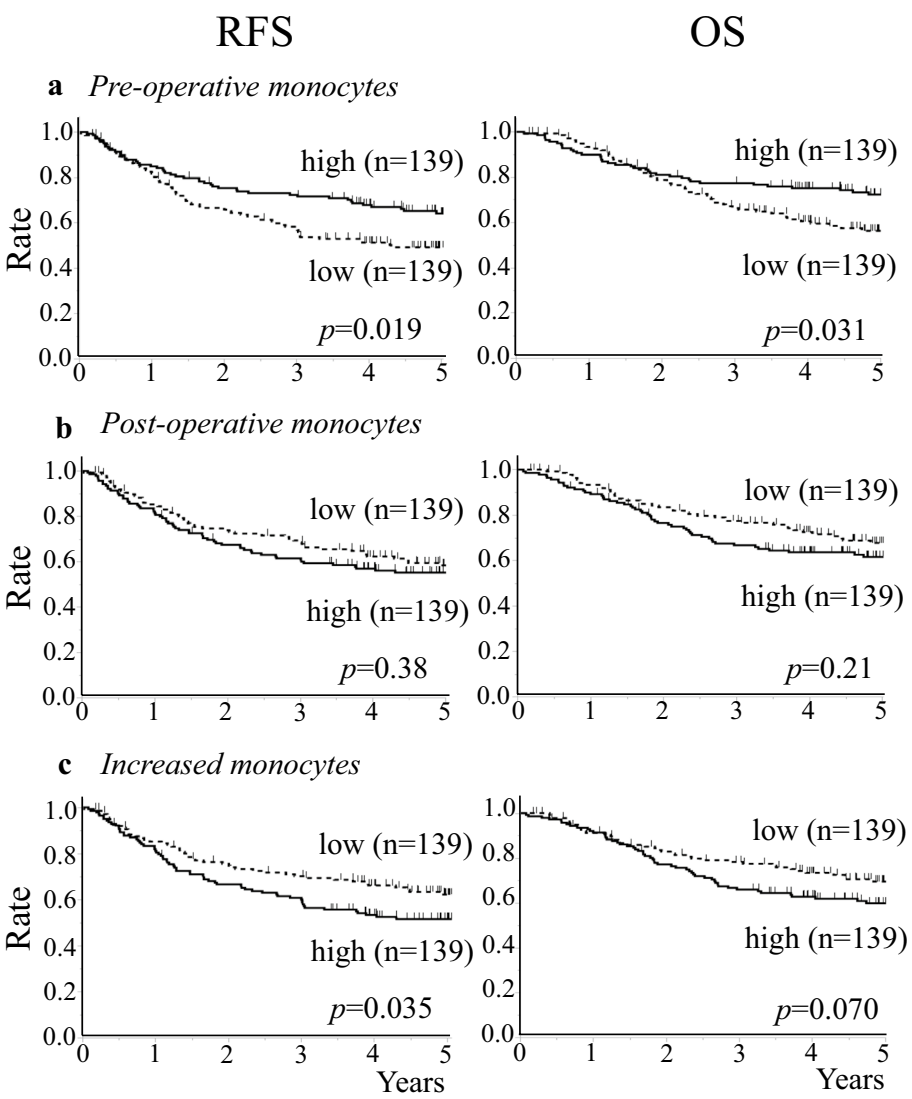

Fig. 1 Recurrence-free survival and overall survival curves of 278 GC patients. Patients were divided into high (solid line) and low (dotted line) groups by the median values of the pre-operative monocyte count (**a**), peak post-operative monocyte count on POD1 or POD3 (**b**), and the increase in post-operative monocyte count compared to the pre-operative monocyte count, which was called the increased monocyte count (**c**), and recurrence-free survival and overall survival curves were compared by the Kaplan–Meier method and log-rank test, respectively. *p* values indicated are rough-*p*. None of them showed statistically significant after Bonferroni's correction

in the post-operative neutrophil count compared to the pre-operative neutrophil count, which we termed the increased neutrophil count, were not associated with the prognosis of GC patients (Supplementary Fig. 2b).

Therefore, we investigated the increased monocyte count in GC patients in more detail.

$CD14^+$ $HLA\text{-}DR^-$ $CD33^+$ cells increased rapidly after gastrectomy

A prospective analysis by flow cytometry was performed on fresh peripheral blood obtained from 75 GC patients in the peri-operative period, and cell numbers were calculated. $CD14^+$ cell numbers rapidly increased after surgery and peaked on POD1 or POD3 (Supplementary Fig. 3), and the time course of this change was consistent with that of monocytes analyzed retrospectively. $CD14^+$ cell subpopulations were analyzed using HLA-DR as a marker of maturation and the monocytic marker CD33 (Supplementary Fig. 1). $CD14^+$ $HLA\text{-}DR^-$ $CD33^+$ cells that were $CD11b^+$ (data not shown) were considered immature monocytic immunosuppressive cells and detected even in healthy donors, but at low numbers, and were abundant and increased as cancer stage worsened in pre-operative GC patients (Supplementary Fig. 4a). After gastrectomy, a marked increase in $CD14^+$ $HLA\text{-}DR^-$ cells was observed in GC patients and peaked on POD1 or POD3, but $CD14^+$ $HLA\text{-}DR^+$ cell numbers remained constant (Fig. 2). The number of $CD4^+$ $CD25^+$ $Foxp3^+$ Tregs did not markedly increase after gastrectomy (Supplementary Fig. 3).

Increases in post-operative monocyte counts were similar to those in $CD14^+$ $HLA\text{-}DR^-$ cells

Since the peri-operative time course of monocytes was consistent with that of $CD14^+$ cells and $CD14^+$ $HLA\text{-}DR^-$ cells,

Table 1 Univariate and multivariate analyses between peri-operative variants and recurrence-free survival

	Univariate analysis		Multivariate analysis	
	HR (95% CI)	*p*	HR (95% CI)	*p*
Age				
≥ 68 vs < 68 years	1.10 (0.77–1.59)	0.60		
Sex				
Male vs female	0.87 (0.59–1.29)	0.47		
BMI				
≥ 21.8 vs < 21.8 kg/m^2	0.88 (0.61–1.26)	0.47		
Alb				
≥ 3.9 vs < 3.9 g/dL	0.61 (0.43–0.89)	0.0060	0.70 (0.47–1.02)	0.065
Pre-NLR				
≥ 2.20 vs < 2.20	1.12 (0.79–1.63)	0.51		
Pre-LMR				
≥ 3.75 vs < 3.75	1.12 (0.78–1.61)	0.55		
Histology				
Un- vs well-differentiated	1.31 (0.91–1.92)	0.15		
Surgical approach				
Open vs laparoscopy	1.41 (0.97–2.13)	0.074	1.32 (0.86–2.05)	0.21
Type of gastrectomy				
Total vs distal	1.65 (1.09–2.39)	0.0068	1.05 (0.69–1.59)	0.83
pT				
3–4 vs 1–2	1.58 (0.97–2.73)	0.068	1.84 (1.04–3.39)	0.034
pN				
1–3 vs 0	2.75 (1.71–4.69)	< 0.0001	2.83 (1.71–4.92)	< 0.0001
CRP_{max}				
≥ 11.4 vs < 11.4 mg/dL	1.30 (0.90–1.86)	0.16		
Pre-monocyte count				
≥ 401 vs < 401/μL	0.64 (0.45–0.93)	0.019	0.66 (0.45–0.99)	0.033
Post-monocyte count				
≥ 590 vs < 590/μL	1.17 (0.81–1.70)	0.38		
Increased monocyte count				
≥ 176 vs < 176/μL	1.48 (1.03–2.14)	0.035	1.48 (1.01–2.19)	0.046
Post-complications				
Yes vs no	2.03 (1.26–3.13)	0.0041	1.83 (1.12–2.88)	0.016
Adjuvant chemotherapy				
Yes vs no	0.73 (0.50–1.07)	0.11		

TNM categories were based on the 7th edition of the International Union Against Cancer (UICC) TNM classification

HR hazard ratio, *CI* confidence interval, *BMI* body mass index, *Alb* albumin, *NLR* neutrophil–lymphocyte ratio, *LMR* lymphocyte–monocyte ratio, CRP_{max} maximum CRP, *Pre* pre-operative, *Post* post-operative

whereas that of CD14$^+$ HLA-DR$^+$ cells remained constant, we compared the increased monocyte count to the increase in CD14$^+$ HLA-DR$^-$ cells after gastrectomy compared to pre-operative CD14$^+$ HLA-DR$^-$ cells, which we termed the increased CD14$^+$ HLA-DR$^-$ cell count. The increased monocyte count correlated with the increased CD14$^+$ HLA-DR$^-$ cell count ($r^2 = 0.57$, $p < 0.001$, $Y = 12.7 + 1.1X$), which suggests that the increased monocyte count was similar to the increased CD14$^+$ HLA-DR$^-$ cell count in most of the individuals examined (Fig. 3). GC patients with a high increased monocyte count had a short RFS in the retrospective analysis, which suggested that CD14$^+$ HLA-DR$^-$ cells affect tumor immunity and the prognosis of GC patients.

Immunosuppressive function of CD14$^+$ HLA-DR$^-$ cells

To analyze the immunosuppressive function of myeloid cells, we used whole CD14$^+$ cells in peripheral blood obtained from GC patients on POD1. The production of

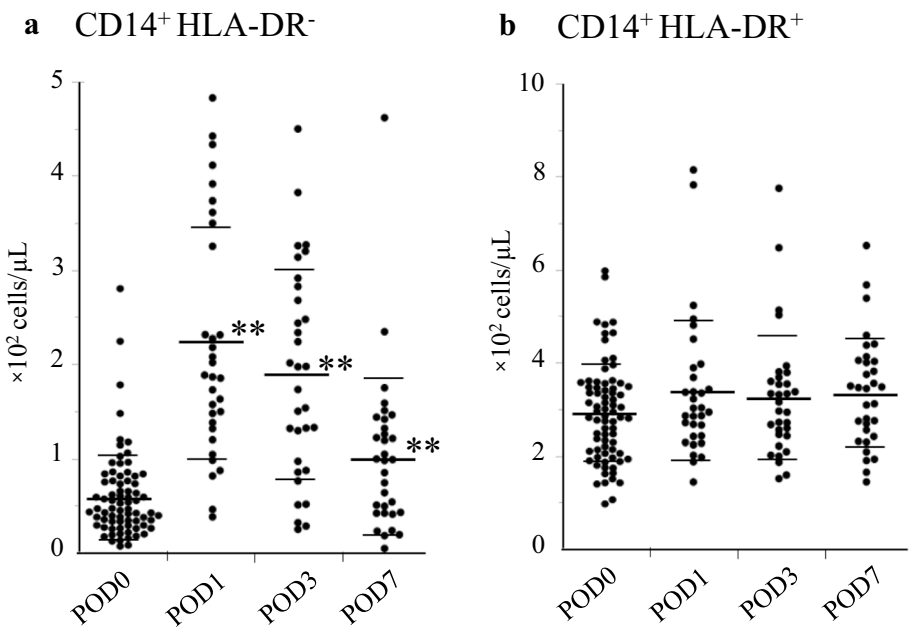

Fig. 2 Number of $CD14^+$ $HLA\text{-}DR^-$ cells and $CD14^+$ $HLA\text{-}DR^+$ cells in 75 GC patients in the peri-operative period. The numbers of $CD14^+$ $HLA\text{-}DR^-$ cells (**a**) and $CD14^+$ $HLA\text{-}DR^+$ cells (**b**) on POD 0, 1, 3, and 7 were calculated using frequencies obtained by flow cytometry and absolute white blood cell counts from routine blood tests in medical reports. Each dot indicates an individual patient. Bars indicate the mean ± SD. $^*p < 0.05$, $^{**}p < 0.01$ to POD0

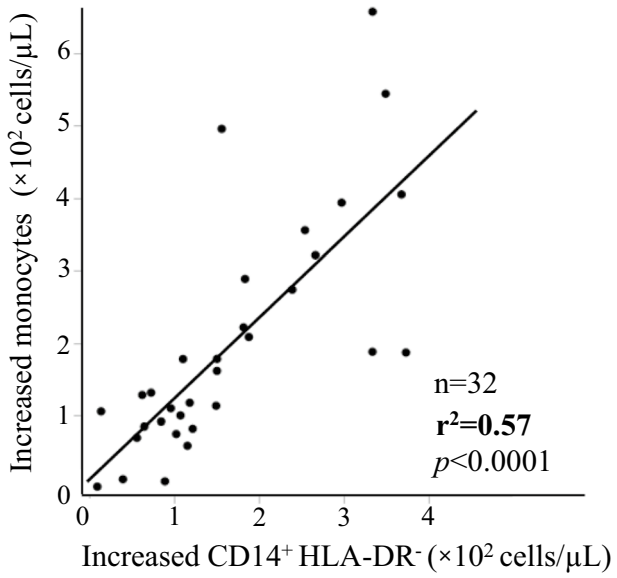

Fig. 3 Increased monocyte count and $CD14^+$ $HLA\text{-}DR^-$ cells. An increased monocyte count and increases in $CD14^+$ $HLA\text{-}DR^-$ cells after surgery were plotted individually, and an approximately straight line was depicted ($n = 32$, $p < 0.0001$, $r^2 = 0.57$, $Y = 12.7 + 1.1X$). The relationship was analyzed by Pearson's correlation coefficient (r)

IFNγ from purified CD8 T cells was markedly suppressed when cells were co-cultured with purified $CD14^+$ cells and stimulated, but not when they were co-cultured with $CD15^+$ cells (Fig. 4a) nor pre-operative $CD14^+$ cells (Supplementary Fig. 4b). The suppressive function of $CD14^+$ $HLA\text{-}DR^-$ cells purified from peripheral blood obtained on POD1 was then investigated. When purified CD8 T cells were co-cultured with $CD14^+$ $HLA\text{-}DR^-$ cells stimulated by CD3/CD28 beads, IFNγ-producing T cells decreased significantly by 50%. This decrease was smaller when T cells were co-cultured with $CD14^+$ $HLA\text{-}DR^+$ cells (Fig. 4b). The production of IDO and arginase, which suppress T cell function and induce other suppressive cells, was analyzed. Stronger cytosolic expression of IDO and arginase was observed in $CD14^+$ $HLA\text{-}DR^-$ cells compared to that in $CD14^+$ $HLA\text{-}DR^+$ cells purified from peripheral blood obtained on POD1 (Fig. 4c).

Increased monocyte count was an independent marker for the prognosis of GC patients

We retrospectively investigated whether the increased monocyte count was an independent immune-related factor for the prognosis of patients among several immunosuppressive and clinicopathological variants in the peri-operative period. Univariate and multivariate analyses were performed on RFS and OS of the 278 GC patients retrospectively. Univariate analysis revealed that high albumin level, distal gastrectomy, a laparoscopic approach, low TN stages, post-operative complications, a high pre-operative monocyte count and a low increased monocyte count were roughly associated with RFS ($p < 0.1$, Table 1). Multivariate analysis between these factors showed that a high pre-operative monocyte count and a low increased monocyte count were independent variants for RFS (Table 1). In these analyses, relationships were not observed among high pre-operative monocytes, low increased monocyte counts, and OS (Supplementary Tables 2, 3). In addition, RFS analysis using the increased monocyte count combined with the pre-operative monocyte count indicated poor prognosis among stage 2 and

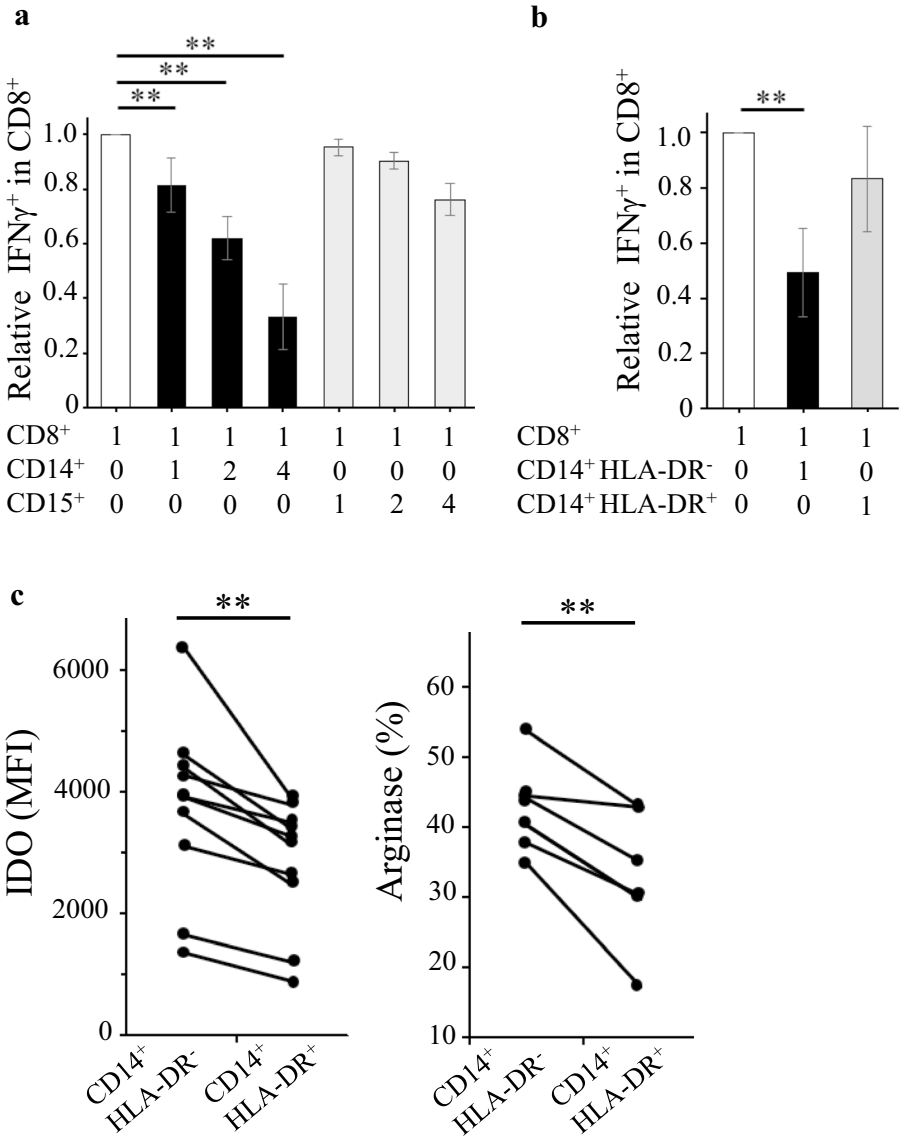

Fig. 4 Immunosuppressive function of myeloid cells. A suppression assay was performed with $CD14^+$, $CD15^+$ cells (**a**) and $CD14^+$ $HLA\text{-}DR^-$, $CD14^+$ $HLA\text{-}DR^+$ cells (**b**) obtained on POD1 and purified. A total of 1×10^4 cells/well of purified CD8 T cells were co-cultured with purified autologous suppressor cells at the ratio indicated below each bar and then stimulated. The relative rate of IFNγ-producing CD8 T cells is shown. Assays were performed on three patients using triplicate wells, and representative data are shown. Bars indicate the mean ± SD. $^{**}p < 0.01$ to the control. The production of IDO and arginase by $CD14^+$ $HLA\text{-}DR^-$ and $CD14^+$ $HLA\text{-}DR^+$ cells obtained on POD1 was detected by intracellular staining and flow cytometry (**c**). The mean fluorescence intensity (MFI) of IDO and frequency of $arginase^+$ cells were compared individually in 10 and 6 patients, respectively. $^{**}p < 0.01$

3 GC patients ($p = 0.0093$ and 0.063, respectively, Supplementary Fig. 5).

Discussion

After curative surgical resection, relapse may result from residual cancer locally or in other organs. In general, smaller cancers are more markedly eliminated by the immune system in both mice and humans [28], which is supported by recent studies of tumor burden on biomarkers for immune therapies with ICIs [29, 30]. The body of patients after surgery is in an inflammatory state, which fosters tumor cells and may weaken tumor immunity against residual cancer. Although pre- and post-operative C-reactive protein (CRP) was not associated with the prognosis of GC patients, monocyte count in peripheral blood was a prognostic factor of relapse in GC patients. Strong immunosuppressive functions were observed in $CD14^+$ monocytes in peripheral blood obtained on POD1, and $CD14^+$ $HLA\text{-}DR^-$ cells suppressed T cell activation more effectively than $CD14^+$ $HLA\text{-}DR^+$ cells. Since the increased monocyte count was similar to the increased $CD14^+$ $HLA\text{-}DR^-$ $CD11b^+$ $CD33^+$ cell count with immunosuppressive functions, we characterized the increased monocytes as M-MDSCs. MDSCs in peripheral blood are associated with various cancers at a higher stage and poorer prognosis [12–14, 31–33]. In GC, a few reports have examined MDSCs that were G-MDSCs, but not M-MDSCs, in peripheral blood [21, 22, 34]. In a mouse model, surgical stress induces MDSCs, which is followed by the cancellation of anti-tumor immunity of antigen-specific T cells elicited by a cancer vaccine [35]. The transfer of inflammatory blood monocytes

accelerates tumor development in tumor-bearing mice [36]. However, in humans, there is evidence of a close relationship between M-MDSCs and anti-tumor immunity. In melanoma patients, circulating M-MDSCs have a negative impact on prognosis and are negatively associated with the induction of NY-ESO-1 and Melan-A-specific T cells [37]. Furthermore, biomarker studies of ICIs support the relationship between the prognosis of patients and M-MDSCs, which suggests that M-MDSCs are an immunosuppressive factor in tumor immunity [15–17]. We found that M-MDSCs were associated with GC progression and patient prognosis.

MDSCs contribute to the formation of a pre-metastatic niche to recruit tumor cells [38]. In a tumor-bearing mouse model, MDSCs are detected in the liver before metastasis, and many types of chemokines and chemokine receptors recruit MDSCs before tumor metastasis [39]. TAMs are recruited from peripheral immature monocytes and also have a role in niche formation [40]. We observed a close relationship between M-MDSCs and $CD206^+$ TAMs in tumor tissue and HLA-DR^- $CD14^+$ cells in peripheral blood (data not shown). The contribution of post-operative M-MDSCs to the formation of a pre-metastatic niche may result in shorter RFS in GC patients.

In contrast to the post-operative increased monocyte count, the high pre-operative monocyte count in peripheral blood was an independent prognostic factor for GC patients with limited tumor stages of II and III. Krieg et al. reported that patients with a high frequency of $CD14^+$ $CD16^-$ classical monocytes in baseline peripheral blood have a longer OS in response to anti-PD-1 immunotherapy, and that these monocytes strongly express activation markers such as HLA-DR and intercellular adhesion molecule-1 (ICAM-1) [41]. $CD14^+$ cells before surgery had no effect on cytokine production by T cells, possibly because helper functions for anti-tumor immunity by classical monocytes, such as macrophages and DCs, may have overcome the immunosuppressive functions of the small number of M-MDSCs in the patients analyzed. A high neutrophil count was also not associated with an advanced tumor stage or poor prognosis in GC patients. Since neutrophils not only increase as a result of inflammation but also suppress tumor immunity, $CD15^+$ cells, which account for most neutrophils, were collected from GC patients before and after surgery. However, an immunosuppressive effect of $CD15^+$ cells on T cells was not observed. In addition, tumor cell factors, post-operative complications, and the indicators of physical condition may also serve as candidate biomarkers [42].

In conclusion, we demonstrated that the monocyte count in the peri-operative period may be a biomarker for the prognosis of GC patients. Increased monocytes, which were similar to immunosuppressive M-MDSCs, negatively impacted tumor immunity and correlated with a poor prognosis. Therefore, an increased monocyte count has potential as a novel marker to select GC patients who might relapse after standard surgery.

Acknowledgements We thank all the patients who contributed to this study. We thank Kayoko Maekawa and Junko Yamagishi for their help.

Author contributions Conception of the work: SU, MM, YD, and HW. Data collection and analysis: SU, KG, MH, MH, TM, YK and TY. Manuscript writing/editing: AK, KI, and HW. Preparation of figures: MY and SU. Critical revision of the manuscript: AM, AK, KI, and HW. Final approval: all the authors.

Funding This study was performed as a research program of the Project for the Development of Innovative Research on Cancer Therapeutics (P-Direct) and The Japan Agency for Medical Research and Development (AMED), and was also supported by the Practice Research for Innovative Cancer Control (15ck0106159 h) from AMED.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval and ethical standards The present study was approved by the Institutional Ethics Committee of Osaka University Hospital (#13266-13, #8226-6).

Informed consent The patients in the retrospective study provided written general consent (#8226-6) to the use of their medical data and publication at the time of their first hospitalization according to the Declaration of Helsinki. The healthy donors and the patients in the prospective study provided written informed consent (#13266-13) to the use of samples and to the use of their medical data and publication before sampling.

References

1. Van Cutsem E, Sagaert X, Topal B, Haustermans K, Prenen H (2016) Gastric cancer. Lancet 388(10060):2654–2664. https://doi.org/10.1016/S0140-6736(16)30354-3
2. Nashimoto A, Akazawa K, Isobe Y, Miyashiro I, Katai H, Kodera Y, Tsujitani S, Seto Y, Furukawa H, Oda I, Ono H, Tanabe S, Kaminishi M (2013) Gastric cancer treated in 2002 in Japan: 2009 annual report of the JGCA nationwide registry. Gastric Cancer 16(1):1–27. https://doi.org/10.1007/s10120-012-0163-4
3. Kang YK, Boku N, Satoh T, Ryu MH, Chao Y, Kato K, Chung HC, Chen JS, Muro K, Kang WK, Yeh KH, Yoshikawa T, Oh SC, Bai LY, Tamura T, Lee KW, Hamamoto Y, Kim JG, Chin K, Oh DY, Minashi K, Cho JY, Tsuda M, Chen LT (2017) Nivolumab in patients with advanced gastric or gastro-oesophageal junction cancer refractory to, or intolerant of, at least two previous chemotherapy regimens (ONO-4538-12, ATTRACTION-2): a randomised, double-blind, placebo-controlled, phase 3 trial. Lancet 390(10111):2461–2471. https://doi.org/10.1016/S0140-6736(17)31827-5
4. Kudo T, Hamamoto Y, Kato K, Ura T, Kojima T, Tsushima T, Hironaka S, Hara H, Satoh T, Iwasa S, Muro K, Yasui H, Minashi K, Yamaguchi K, Ohtsu A, Doki Y, Kitagawa Y (2017) Nivolumab treatment for oesophageal squamous-cell carcinoma:

an open-label, multicentre, phase 2 trial. Lancet Oncol 18:631–639. https://doi.org/10.1016/S1470-2045(17)30181-X
5. Saito T, Nishikawa H, Wada H, Nagano Y, Sugiyama D, Atarashi K, Maeda Y, Hamaguchi M, Ohkura N, Sato E, Nagase H, Nishimura J, Yamamoto H, Takiguchi S, Tanoue T, Suda W, Morita H, Hattori M, Honda K, Mori M, Doki Y, Sakaguchi S (2016) Two FOXP3(+)CD4(+) T cell subpopulations distinctly control the prognosis of colorectal cancers. Nat Med 22(6):679–684. https://doi.org/10.1038/nm.4086
6. McGarry Houghton A, Rzymkiewicz Danuta M, Ji Hongbin, Gregory Alyssa D, Egea Eduardo E, Metz Heather E, Stolz Donna B, Land Stephanie R, Marconcini Luiz A, Kliment Corrine R, Jenkins Kimberly M, Beaulieu Keith A, Mouded Majd, Frank Stuart J, Wong Kwok K, Shapiro Steven D (2010) Neutrophil elastase-mediated degradation of IRS-1 accelerates lung tumor growth. Nat Med 16(2):219–223. https://doi.org/10.1038/nm.2084
7. Zhou XL, Li YQ, Zhu WG, Yu CH, Song YQ, Wang WW, He DC, Tao GZ, Tong YS (2017) Neutrophil-to-lymphocyte ratio as a prognostic biomarker for patients with locally advanced esophageal squamous cell carcinoma treated with definitive chemoradiotherapy. Sci Rep 7:42581. https://doi.org/10.1038/srep42581
8. Gabrilovich DI, Ostrand-Rosenberg S, Bronte V (2012) Coordinated regulation of myeloid cells by tumours. Nat Rev Immunol 12(4):253–268. https://doi.org/10.1038/nri3175
9. Kumar V, Patel S, Tcyganov E, Gabrilovich DI (2016) The nature of myeloid-derived suppressor cells in the tumor microenvironment. Trends Immunol 37(3):208–220. https://doi.org/10.1016/j.it.2016.01.004
10. Tcyganov E, Mastio J, Chen E, Gabrilovich DI (2018) Plasticity of myeloid-derived suppressor cells in cancer. Curr Opin Immunol 51:76–82. https://doi.org/10.1016/j.coi.2018.03.009
11. Chan JC, Chan DL, Diakos C, Engel A, Pavlakis N, Gill A, Clarke SJ (2017) The lymphocyte-to-monocyte ratio is a superior predictor of overall survival in comparison to established biomarkers of resectable colorectal cancer. Ann Surg 265(3):539–546. https://doi.org/10.1097/SLA.0000000000001743
12. Vetsika EK, Koinis F, Gioulbasani M, Aggouraki D, Koutoulaki A, Skalidaki E, Mavroudis D, Georgoulias V, Kotsakis A (2014) A circulating subpopulation of monocytic myeloid-derived suppressor cells as an independent prognostic/predictive factor in untreated non-small lung cancer patients. J Immunol Res. 2014:659294. https://doi.org/10.1155/2014/659294
13. Idorn M, Kollgaard T, Kongsted P, Sengelov L, Thor Straten P (2014) Correlation between frequencies of blood monocytic myeloid-derived suppressor cells, regulatory T cells and negative prognostic markers in patients with castration-resistant metastatic prostate cancer. Cancer Immunol Immunother 63:1177–1187. https://doi.org/10.1007/s00262-014-1591-2
14. Arihara F, Mizukoshi E, Kitahara M, Takata Y, Arai K, Yamashita T, Nakamoto Y, Kaneko S (2013) Increase in CD14+HLA-DR-/low myeloid-derived suppressor cells in hepatocellular carcinoma patients and its impact on prognosis. Cancer Immunol Immunother 62:1421–1430. https://doi.org/10.1007/s00262-013-1447-1
15. Kitano S, Postow MA, Ziegler CG, Kuk D, Panageas KS, Cortez C, Rasalan T, Adamow M, Yuan J, Wong P, Altan-Bonnet G, Wolchok JD, Lesokhin AM (2014) Computational algorithm-driven evaluation of monocytic myeloid-derived suppressor cell frequency for prediction of clinical outcomes. Cancer Immunol Res 2(8):812–821. https://doi.org/10.1158/2326-6066.CIR-14-0013
16. Weber R, Fleming V, Hu X, Nagibin V, Groth C, Altevogt P, Utikal J, Umansky V (2018) Myeloid-derived suppressor cells hinder the anti-cancer activity of immune checkpoint inhibitors. Front Immunol 9:1310. https://doi.org/10.3389/fimmu.2018.01310
17. Martens A, Wistuba-Hamprecht K, Geukes Foppen M, Yuan J, Postow MA, Wong P, Romano E, Khammari A, Dreno B, Capone M, Ascierto PA, Di Giacomo AM, Maio M, Schilling B, Sucker A, Schadendorf D, Hassel JC, Eigentler TK, Martus P, Wolchok JD, Blank C, Pawelec G, Garbe C, Weide B (2016) Baseline peripheral blood biomarkers associated with clinical outcome of advanced melanoma patients treated with Ipilimumab. Clin Cancer Res 22(12):2908–2918. https://doi.org/10.1158/1078-0432.CCR-15-2412
18. Nagase H, Takeoka T, Urakawa S, Morimoto-Okazawa A, Kawashima A, Iwahori K, Takiguchi S, Nishikawa H, Sato E, Sakaguchi S, Mori M, Doki Y, Wada H (2017) ICOS+Foxp3+TILs in gastric cancer are prognostic markers and effector regulatory T cells associated with *Helicobacter pylori*. Int J Cancer 140(3):686–695. https://doi.org/10.1002/ijc.30475
19. Zhang X, Xu W (2017) Neutrophils diminish T-cell immunity to foster gastric cancer progression: the role of GM-CSF/PD-L1/PD-1 signalling pathway. Gut 66(11):1878–1880. https://doi.org/10.1136/gutjnl-2017-313923
20. Wang TT, Zhao YL, Peng LS, Chen N, Chen W, Lv YP, Mao FY, Zhang JY, Cheng P, Teng YS, Fu XL, Yu PW, Guo G, Luo P, Zhuang Y, Zou QM (2017) Tumour-activated neutrophils in gastric cancer foster immune suppression and disease progression through GM-CSF-PD-L1 pathway. Gut 66(11):1900–1911. https://doi.org/10.1136/gutjnl-2016-313075
21. Wang L, Chang EW, Wong SC, Ong SM, Chong DQ, Ling KL (2013) Increased myeloid-derived suppressor cells in gastric cancer correlate with cancer stage and plasma S100A8/A9 proinflammatory proteins. J Immunol 190(2):794–804. https://doi.org/10.4049/jimmunol.1202088
22. Shoji H, Tada K, Kitano S, Nishimura T, Shimada Y, Nagashima K, Aoki K, Hiraoka N, Honma Y, Iwasa S, Takashima A, Kato K, Boku N, Honda K, Yamada T, Heike Y, Hamaguchi T (2017) The peripheral immune status of granulocytic myeloid-derived suppressor cells correlates the survival in advanced gastric cancer patients receiving cisplatin-based chemotherapy. Oncotarget 8(56):95083–95094. https://doi.org/10.18632/oncotarget.18297
23. Lieto E, Galizia G, Auricchio A, Cardella F, Mabilia A, Basile N, Del Sorbo G, Castellano P, Romano C, Orditura M, Napolitano V (2017) Preoperative neutrophil to lymphocyte ratio and lymphocyte to monocyte ratio are prognostic factors in gastric cancers undergoing surgery. J Gastrointest Surg 21(11):1764–1774. https://doi.org/10.1007/s11605-017-3515-x
24. Lin JP, Lin JX, Cao LL, Zheng CH, Li P, Xie JW, Wang JB, Lu J, Chen QY, Lin M, Tu RH, Huang CM (2017) Preoperative lymphocyte-to-monocyte ratio as a strong predictor of survival and recurrence for gastric cancer after radical-intent surgery. Oncotarget 8(45):79234–79247. https://doi.org/10.18632/oncotarget.17058
25. Yan Y, Zhang J, Li JH, Liu X, Wang JZ, Qu HY, Wang JS, Duan XY (2016) High tumor-associated macrophages infiltration is associated with poor prognosis and may contribute to the phenomenon of epithelial-mesenchymal transition in gastric cancer. Onco Targets Ther 9:3975–3983. https://doi.org/10.2147/OTT.S103112
26. Yamaguchi T, Fushida S, Yamamoto Y, Tsukada T, Kinoshita J, Oyama K, Miyashita T, Tajima H, Ninomiya I, Munesue S, Harashima A, Harada S, Yamamoto H, Ohta T (2016) Tumor-associated macrophages of the M2 phenotype contribute to progression in gastric cancer with peritoneal dissemination. Gastric Cancer 19(4):1052–1065. https://doi.org/10.1007/s10120-015-0579-8
27. Becker C, Pohla H, Frankenberger B, Schüler T, Assenmacher M, Schendel DJ, Blankenstein T (2001) Adoptive tumor therapy with T lymphocytes enriched through an IFN-gamma capture assay. Nat Med 7(10):1159–1162. https://doi.org/10.1038/nm1001-1159
28. Moore AE, Rhoads CP, Southam CM (1957) homotransplantation of human cell lines. Science 125:158–160. https://doi.org/10.1126/science.125.3239.158

29. Joseph RW, Elassaiss-Schaap J, Kefford R, Hwu WJ, Wolchok JD, Joshua AM, Ribas A, Hodi FS, Hamid O, Robert C, Daud A, Dronca R, Hersey P, Weber JS, Patnaik A, de Alwis DP, Perrone A, Zhang J, Kang SP, Ebbinghaus S, Anderson KM, Gangadhar TC (2018) Baseline tumor size is an independent prognostic factor for overall survival in patients with melanoma treated with pembrolizumab. Clin Cancer Res. https://doi.org/10.1158/1078-0432.CCR-17-2386 **in press**
30. Kato R, Yamasaki M, Urakawa S, Nishida K, Makino T, Morimoto-Okazawa A, Kawashima A, Iwahori K, Suzuki S, Ueda R, Mori M, Satoh T, Doki Y, Wada H (2018) Increased Tim-3+T cells in PBMCs during nivolumab therapy correlate with responses and prognosis of advanced esophageal squamous cell carcinoma patients. Cancer Immunol Immunother. https://doi.org/10.1007/s00262-018-2225-x **in press**
31. Chun E, Lavoie S, Michaud M, Gallini CA, Kim J, Soucy G, Odze R, Glickman JN, Garrett WS (2015) CCL2 promotes colorectal carcinogenesis by enhancing polymorphonuclear myeloid-derived suppressor cell population and function. Cell Rep 12(2):244–257. https://doi.org/10.1016/j.celrep.2015.06.024
32. Barrera L, Montes-Servín E, Hernandez-Martinez JM, Orozco-Morales M, Montes-Servín E, Michel-Tello D, Morales-Flores RA, Flores-Estrada D, Arrieta O (2018) Levels of peripheral blood polymorphonuclear myeloid-derived suppressor cells and selected cytokines are potentially prognostic of disease progression for patients with non-small cell lung cancer. Cancer Immunol Immunother 67(9):1393–1406. https://doi.org/10.1007/s00262-018-2196-y
33. Jordan KR, Amaria RN, Ramirez O, Callihan EB, Gao D, Borakove M, Manthey E, Borges VF, McCarter MD (2013) Myeloid-derived suppressor cells are associated with disease progression and decreased overall survival in advanced-stage melanoma patients. Cancer Immunol Immunother 62:1711–1722. https://doi.org/10.1007/s00262-013-1475-x
34. Li N, Han D, Sun J, Li Y, Zhang J, Zhang Y, Liu M, Peng R, Wang H, Zhang Z, Wang J, Liu Z, Ma J (2018) Subtypes of MDSCs in mechanisms and prognosis of gastric cancer and are inhibited by epirubicin and paclitaxel. Discov Med 25(137):99–112
35. Ananth AA, Tai LH, Lansdell C, Alkayyal AA, Baxter KE, Angka L, Zhang J, Tanese de Souza C, Stephenson KB, Parato K, Bramson JL, Bell JC, Lichty BD, Auer RC (2016) Surgical stress abrogates pre-existing protective T cell mediated anti-tumor immunity leading to postoperative cancer recurrence. PLoS One 11(5):e0155947. https://doi.org/10.1371/journal.pone.0155947
36. Augier S, Ciucci T, Luci C, Carle GF, Blin-Wakkach C, Wakkach A (2010) Inflammatory blood monocytes contribute to tumor development and represent a privileged target to improve host immunosurveillance. J Immunol 185(12):7165–7173. https://doi.org/10.4049/jimmunol.0902583
37. Weide B, Martens A, Zelba H, Stutz C, Derhovanessian E, Di Giacomo AM, Maio M, Sucker A, Schilling B, Schadendorf D, Büttner P, Garbe C, Pawelec G (2014) Myeloid-derived suppressor cells predict survival of patients with advanced melanoma: comparison with regulatory T cells and NY-ESO-1- or melan-A-specific T cells. Clin Cancer Res 20(6):1601–1609. https://doi.org/10.1158/1078-0432.CCR-13-2508
38. Safarzadeh E, Orangi M, Mohammadi H, Babaie F, Baradaran B (2017) Myeloid-derived suppressor cells: important contributors to tumor progression and metastasis. J Cell Physiol 233(4):3024–3036. https://doi.org/10.1002/jcp.26075
39. Wang D, Sun H, Wei J, Cen B, DuBois RN (2017) CXCL1 Is critical for premetastatic niche formation and metastasis in colorectal cancer. Cancer Res 77(13):3655–3665. https://doi.org/10.1158/0008-5472.CAN-16-3199
40. Chen XW, Yu TJ, Zhang J, Li Y, Chen HL, Yang GF, Yu W, Liu YZ, Liu XX, Duan CF, Tang HL, Qiu M, Wang CL, Zheng H, Yue J, Guo AM, Yang J (2017) CYP4A in tumor-associated macrophages promotes pre-metastatic niche formation and metastasis. Oncogene 36(35):5045–5057. https://doi.org/10.1038/onc.2017.118
41. Krieg C, Nowicka M, Guglietta S, Schindler S, Hartmann FJ, Weber LM, Dummer R, Robinson MD, Levesque MP, Becher B (2018) High-dimensional single-cell analysis predicts response to anti-PD-1 immunotherapy. Nat Med 24(2):144–153. https://doi.org/10.1038/nm.4466
42. Kubota T, Hiki N, Sano T, Nomura S, Nunobe S, Kumagai K, Aikou S, Watanabe R, Kosuga T, Yamaguchi T (2013) Prognostic significance of complications after curative surgery for gastric cancer. Ann Surg Oncol 21(3):891–898. https://doi.org/10.1245/s10434-013-3384-9