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Myeloid Cells and Related Chronic Inflammatory Factors as Novel Predictive Markers in Melanoma Treatment with Ipilimumab

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Abstract

Purpose: Immunotherapy with ipilimumab improves the survival of patients with metastatic melanoma. Because only around 20% of patients experience long-term benefit, reliable markers are needed to predict a clinical response. Therefore, we sought to determine if some myeloid cells and related inflammatory mediators could serve as predictive factors for the patients' response to ipilimumab.

Experimental Design: We performed an analysis of myeloid cells in the peripheral blood of 59 stage IV melanoma patients before the treatment and at different time points upon the therapy using a clinical laboratory analysis and multicolor flow cytometry. In addition, the production of related inflammatory factors was evaluated by ELISA or Bio-Plex assays.

Results: An early increase in eosinophil count during the treatment with ipilimumab was associated with an improved clinical response. In contrast, elevated amounts of monocytic myeloid-derived suppressor cells (moMDSC), neutrophils, and monocytes were found in nonresponders ($n = 36$) as compared with basal levels and with responding patients ($n = 23$). Moreover, in nonresponders, moMDSCs produced significantly more nitric oxide, and granulocytic MDSCs expressed higher levels of PD-L1 than these parameters at baseline and in responders, suggesting their enhanced immunosuppressive capacity. Upon the first ipilimumab infusion, nonresponders displayed elevated serum concentrations of S100A8/A9 and HMGB1 that attract and activate MDSCs.

Conclusions: These findings highlight additional mechanisms of ipilimumab effects and suggest levels of eosinophils, MDSCs, as well as related inflammatory factors S100A8/A9 and HMGB1 as novel complex predictive markers for patients who may benefit from the ipilimumab therapy. *Clin Cancer Res; 21(24); 5453–9. ©2015 AACR.*

Introduction

Despite an observed immunogenicity, malignant melanoma is characterized by its fast progression and poor response to the treatment (1). This was shown to be due to a strong immunosuppressive network in the melanoma microenvironment represented by immunosuppressive leukocytes and soluble factors (2, 3). Ipilimumab (Ipi), a fully monoclonal antibody against human anti-cytotoxic T lymphocyte-associated antigen (CTLA)-4, has been recently shown to be one of the most successful immunotherapeutic drugs for melanoma therapy, resulting in the improved overall survival (OS) in patients with metastatic melanoma (4). The underlying mechanism of the treatment is a blockade of inhibitory signaling between CTLA-4 upregulated on activated T cells and CD80 and CD86 on antigen-presenting cells, leading to the activation and accumulation of tumor-reactive T cells (5, 6). However, the clinical response rate is only around 10%, and about 20% of treated patients achieve a long-term clinical benefit with the survival up to 10 years (7). Low responder frequencies indicate that other immunosuppressive mechanisms might be important under such treatment, including (i) an upregulation of another inhibitory pathway mediated by an interaction of program death (PD)-1 receptor and PD-1 ligand (PD-L1; ref. 8), (ii) an accumulation and activation of myeloid-derived suppressor cells (MDSC; refs. 9–12), and (iii) an enhancement of chronic inflammation, inducing a strong immunosuppression (3, 13).

To address this question, we analyzed the peripheral blood and serum of Ipi-treated metastatic melanoma patients who were divided in two groups (responding and nonresponding to the treatment). We found that responders were characterized by an early increase in eosinophil count in the peripheral blood after Ipi infusion. In contrast, elevated MDSC frequencies and activity,

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Translational Relevance

In this study, we present evidence for novel predictive markers for ipilimumab treatment of metastatic stage IV melanoma patients. Clinical response to the therapy was associated with an early increase in eosinophil count in the peripheral blood. In contrast, nonresponders showed elevated amounts of circulating monocytic myeloid-derived suppressor cells (moMDSC) and increased serum levels of S100A8/A9 and HMGB1 that attract and activate MDSCs. We found also in nonresponders a significantly stronger production of nitric oxide by moMDSCs and higher expression of PD-L1 by granulocytic MDSCs, indicating their enhanced immunosuppressive capacity. Our findings provide new insight into complex mechanisms of the therapeutic effect of ipilimumab in advanced melanoma patients. We suggest that the measurement of eosinophils and MDSCs as well as S100A8/A9 and HMGB1 should be performed before and during ipilimumab treatment to predict a clinical response to this treatment.

reflected here by higher nitric oxide (NO) production and PD-L1 expression, increased neutrophil and monocyte counts as well as elevated concentrations of chronic inflammatory factors S100A8/A9 and HMGB1 were associated with a poor clinical response. We suggest that the combination of these markers has a predictive value and will help to detect the group of metastatic melanoma patients that benefit from Ipi treatment.

Materials and Methods

Patients, treatment, and clinical evaluation

This multicenter retrospective immunomonitoring study included 46 metastatic melanoma patients receiving Ipi (Bristol-Myers Squibb) at the Skin Cancer Center, University Medical Center Mannheim, Germany, and 13 patients receiving Ipi at the Department of Dermatology, University Hospital Essen, Germany. Patients were included if they (i) had a confirmed diagnosis of stage IV melanoma according to the 2009 AJCC melanoma staging and classification, (ii) were alive 12 weeks after the first Ipi perfusion, and (iii) were receiving at least four courses of Ipi over 90 minutes at a dose of 3 mg/kg of body weight every 3 weeks. Other inclusion criteria were: at least 18 years of age and no specific melanoma therapy during the previous 28 days. All histologic types of melanoma, including mucosal and uveal melanoma, were eligible for inclusion. Exclusion criteria were the presence of an autoimmune disease, HIV, hepatitis B or C, pregnancy, symptomatic brain metastases, or concomitant systemic therapy for melanoma. Asymptomatic or pretreated brain metastases were allowed to be included (Table 1).

Treatment efficacy was assessed using contrast-enhanced CT/MRI/PET-CT at around week 12 after the first Ipi infusion and clinical response defined based on immune-related response criteria (irRC; ref. 14; Table 2). A clinical response was defined as complete response, partial response, and stable disease.

Analysis of peripheral blood samples

The peripheral blood was taken 2 to 5 days before the first Ipi infusion (point 0—baseline) as well as 2 to 3 days before the second (point 1—after the first infusion), before the third (point 2—after the second infusion), before the fourth infusion (point 3—after the third infusion), and within 3 to 6 weeks after the fourth infusion (point 4). Counts for leukocytes (white blood count, WBC), eosinophils, monocytes, and neutrophils were measured in the peripheral blood by routine clinical laboratory analysis using a Sysmex XE-5000 analyzer (Sysmex). The following counts were considered as normal: leukocytes (4,200–10,200/μL), eosinophils (0–400/μL), monocytes (300–800 μL), and neutrophils (2,200–6,300/μL). Peripheral blood mononuclear cells (PBMC) were obtained from heparinized venous blood by density gradient centrifugation using Biocoll (Biochrom). Isolated cells were cryopreserved in RPMI supplemented with 30% human serum and 10% DMSO at −80°C. To collect serum, blood samples were centrifuged at 3,000 rpm for 10 minutes, aliquoted, and stored at −80°C.

Table 1. Characteristics of melanoma patients treated with ipilimumab

Variables	*n* (%)
Patients	59
Median age (range)	65.2 (32–84)
Sex	
Male	36 (61)
Female	23 (39)
Primary melanoma site	
Cutaneous	40 (68)
Mucosal	1 (2)
Uveal	6 (10)
Occult	7 (12)
Unclassified	5 (8)
AJCC stage	
IV	59 (100)
M1a	1 (2)
M1b	13 (22)
M1c	45 (76)
CNS metastases at baseline	18 (30)
Prior surgery for CNS metastases	5 (8)
Prior radiotherapy for CNS metastases	11 (18)
Lactate dehydrogenase level above the ULN	19 (32)
Prior therapy	
$N = 0$	11
$N = 1$	24
$N = 2$	15
$N = 3$	5
$N \geq 4$	4
Cytotoxic chemotherapy	39 (66)
Radiotherapy	28 (47)
Adjuvant therapy	29 (49)
Other therapies	
BRAF + MEK inhibitor	8 (14)
Imatinib (tyrosine kinase inhibitor)	1 (2)

Abbreviations: CNS, central nervous system; ULN, upper limit of normal.

Table 2. Clinical response and OS after therapy with ipilimumab

Tumor response after Ipi therapy according to irRC	*n* (%)
Best overall response	
Complete response	1 (2)
Partial response	5 (8)
Stable disease	17 (29)
Progressive disease	36 (61)
Disease control rate	23 (39)
OS (mo)	
Median OS (95% CI)	9.8 (5.7–14.1)
6 mo OS (%)	45.2
12 mo OS (%)	35.7
24 mo OS (%)	18.9

Abbreviation: mo, months.

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Flow cytometry

The following fluorescent-labeled monoclonal antibodies were used for the surface staining: HLA-DR-APC-Cy7, CD14-PerCP, CD15-PE, CD11b-APC, and PD-L1 (CD274)-PE-Cy7 (all from BD Biosciences). Staining with 4,5-Diaminofluorescein Diacetat (DAF-2DA; Cell Technology) was performed for intracellular NO measurement according to the manufacturer's recommendation. Acquisition was performed by six-color flow cytometry using FACSCanto II with FACSDiva software (both from BD Biosciences) with dead cell exclusion based on scatter profile or 7-AAD (Biolegend). The compensation control was performed with BD CompBeads set (BD Biosciences) using the manufacturer's instruction. FlowJo software (Tree Star) was used to analyze at least 100,000 events. Data were expressed as dot plots.

ELISA

Serum levels of S100A8/A9 and HMGB1 were determined by ELISA assays for S100A8/A9 (Bühlmann Laboratories) and for HMGB1 (IBL International) according to the manufacturers' protocols.

Bio-Plex assay

Concentrations of eotaxin-1 (CCL11) in serum of treated patients were measured by the multiplex technology (Millipore) according to the manufacturer's protocol.

Statistical analysis

All data are shown as mean ± SE for the indicated time points. Results were assessed with a nonparametric two-sided Mann–Whitney *U* test, a two-sided Fisher exact test, a one-way ANOVA with Dunn's multiple comparison test using Prism software (GraphPad), and a multivariate logistic regression for all cell markers using SAS software (Version 9.2). Results of the multivariate analysis were described by mean of ORs together with 95% confidence intervals (CI) and *P* values. The linear relationship between the moMDSC frequencies and NO production in these cells was analyzed using a Pearson coefficient, with a statistical validation by a two-tailed *P* test, 95% CI (Prism software, GraphPad). Throughout the analyses, *P* values less than 0.05 were considered statistically significant. Survival was defined as the time from inclusion to death due to any cause. OS was estimated by the Kaplan–Meier method.

Results

Fifty-nine melanoma patients who received Ipi were retrospectively included in this study (Table 1). The group contained 36 males (61%) and 23 females (39%). The median age of patients was 65.2 years (ranging from 32 to 84 years). Patients received Ipi treatment intravenously at a dose of 3 mg/kg of body weight every 3 weeks. The median OS was 9.8 months (95% CI, 5.7–14.1) from the date of Ipi initiation (Table 2). For the evaluation of different biomarkers, patients were divided in two groups (responding and nonresponding to Ipi treatment).

First, we analyzed the total leukocyte count in the peripheral blood of Ipi-treated patients. The amount of leukocytes in nonresponder patients at baseline (point 0) was significantly higher than in responders ($P < 0.05$; Fig. 1A; Table 3). Moreover, analyzing different subpopulations of myeloid leukocytes, we

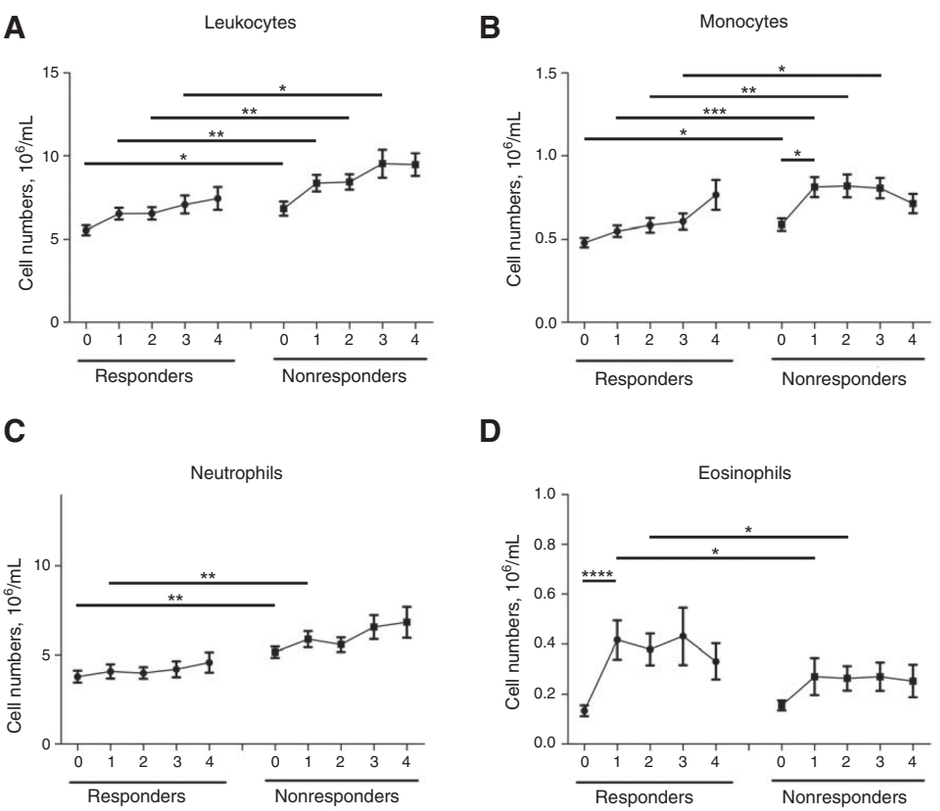

Figure 1.
Number of total leukocytes and different myeloid cell subsets in advanced melanoma patients under the course of Ipi treatment. Counts for leukocytes (A), monocytes (B), neutrophils (C), and eosinophils (D) were measured in the peripheral blood using routine laboratory tests. Samples were taken before each Ipi infusion (point 0—before the treatment; point 1—after the first infusion; point 2—after the second infusion; point 3—after the third infusion) and 3 to 6 weeks after the fourth infusion (point 4). Data from 59 patients (responders vs. nonresponders) are expressed as 10^6 cells/mL (mean ± SD). *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$; ****, $P < 0.0001$.

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Table 3. Univariate analysis of possible predictive markers for response to ipilimumab

Variables	Responders/nonresponders	*P*
Baseline (0)		
Leukocyte count	23/36	0.0487
Lymphocyte count	23/36	0.2832
Monocyte count	23/36	0.0403
Eosinophil count	23/36	0.4542
Neutrophil count	23/36	0.0030
Change between baseline (0) to time point after 1st infusion (1)		
Leukocyte count	23/36	0.6691
Lymphocyte count	23/36	0.2663
Monocyte count	23/36	0.6975
Eosinophil count	23/36	<0.0001
Neutrophil count	23/36	0.9566
LDH		
Normal	16/25	0.0043
Elevated (>250 U/L)	7/11	
BRAF status		
Wild-type	19/23	
Mutation	4/13	0.1876

observed elevated monocyte and neutrophil counts at baseline in nonresponders as compared with responders ($P = 0.04$ and $P = 0.003$ respectively, Fig. 1B and C, Table 3). Upon the first Ipi infusion (point 1), we found that eosinophil counts were significantly higher than at baseline that was associated with an improved clinical response ($P < 0.0001$; Fig. 1D). Furthermore, using a univariate analysis, we demonstrated a strong increase in eosinophil counts from baseline to the point 1 in responders as compared with such change in nonresponders ($P < 0.0001$; Table 3). In contrast, monocyte counts in nonresponders were significantly higher after the first Ipi infusion as compared with baseline and with counts in responders at the same time points ($P < 0.05$; Fig. 1B).

To investigate possible confounding effects between different markers, we also performed a multivariate logistic regression analysis including eight potential markers such as lymphocyte, monocyte, eosinophil, and neutrophil counts as well as change in these markers between baseline and point 1 (Supplementary Table S1). Because leukocyte counts highly correlated with neutrophil counts, leukocytes were excluded from the multivariate model. The analysis confirmed the results of univariate analysis, indicating a significant increase in the eosinophil count between baseline and point 1 as the only marker to predict a response to Ipi ($P = 0.017$; OR of 23.2).

To address the question if the treatment with Ipi could influence MDSCs that are reported to be the most powerful immunosuppressive myeloid cells in metastatic melanoma (3, 9, 15, 16), we analyzed MDSCs in the peripheral blood of treated patients. These cells consist of monocytic and granulocytic subsets, which are defined as $CD14^+$ $CD11b^+HLA\text{-}DR^{lo/neg}$ SSC^{low} (moMDSCs) and $CD15^+CD11b^+HLA\text{-}DR^{lo/neg}SSC^{low}$ (grMDSCs; refs. 9, 15, 16; Fig. 2A). We demonstrated that before the treatment, nonresponders displayed a tendency for an increase in the frequency of moMDSCs before the treatment as compared with responders ($P > 0.05$; Fig. 2B–D). Upon the first Ipi infusion, moMDSC levels in nonresponders were significantly higher than in responders ($P < 0.05$; Fig. 2D). Moreover, moMDSCs in responders were strongly reduced already after the first infusion as compared with baseline levels, whereas in nonresponders, these values showed a pronounced elevation upon the second Ipi infusion ($P < 0.05$ and $P < 0.01$, respectively; Fig. 2B–D). In contrast, we failed to find any changes in frequencies of grMDSCs both in responding and nonresponding melanoma patients upon the Ipi treatment (Fig. 2E).

Next, we investigated the suppressive potential of MDSCs in treated patients. To address this question, we analyzed NO production and PD-L1 (CD274) expression in these cells (Fig. 2F and G). Upon the second Ipi infusion, the level of intracellular NO was significantly elevated in moMDSCs from nonresponders as compared with that in responders ($P < 0.05$; Fig. 2F). Furthermore, we analyzed a possible correlation between the frequency of moMDSC and NO production by these cells measured simultaneously after the treatment. It was found that upon the first infusion, higher levels of moMDSC in nonresponders significantly correlated with an elevated intensity of NO production in these cells (Supplementary Fig. S1). Measuring the production of NO by grMDSCs under the treatment with Ipi, we observed that it was at the similar level in both groups of patients (data not shown). However, the PD-L1 expression on grMDSCs from responders measured by mean fluorescence intensity (MFI) was demonstrated to be downregulated already after the first Ipi infusion as compared with the pretreatment values ($P < 0.01$; Fig. 2G). Moreover, at this time point, the intensity of PD-L1 expression on grMDSCs in responders was significantly reduced as compared with this parameter in nonresponders ($P < 0.05$; Fig. 2G). In contrast, the expression of PD-L1 on the surface of moMDSCs of all treated patients remained mostly at the same level (data not shown).

It is known that melanoma is strongly associated with chronic inflammation, which also supports MDSC generation, expansion, and functions (3, 9). Therefore, we studied in the course of Ipi therapy soluble inflammatory factors, such as S100A8/A9 and HMGB1, that are known to activate and attract MDSC to the tumor site (17–19) as well as eotaxin-1 (CCL11) that was reported to play a critical role in the recruitment of eosinophils (20). We detected a pronounced upregulation of serum levels of both S100A8/A9 and HMGB1 in nonresponding patients already after the first Ipi infusion ($P < 0.05$; Fig. 3A and B). Furthermore, in responders, S100A8/A9 concentrations were decreased after the first infusion as compared with baseline levels (Fig. 3A). In addition, the content of eotaxin-1 in serum from nonresponding melanoma patients was significantly lower than before the therapy ($P < 0.05$; Supplementary Fig. S2).

Discussion

In this retrospective immunomonitoring study, we aimed to find predictive immune-related markers of the responsiveness to the Ipi treatment. The median OS of our patient cohort amounted to 9.8 months, which is in line with previous publications (4).

It has been demonstrated that Ipi can block CTLA-4–mediated suppression of effector tumor-specific T cells (5, 6). However, only a relatively small number of metastatic melanoma patients treated with Ipi demonstrate a clinical response over an extended period of follow-up (2, 4, 7). An explanation might be the activation of other immunosuppressive mechanisms, including the recruitment, accumulation, and stimulation of innate immune cells such as MDSCs that represent immature cells of myeloid origin exhibiting a high immunosuppressive potential (9). Tumor microenvironment biomarkers have been successfully linked to clinical activity of Ipi in patients with advanced melanoma (5, 6)

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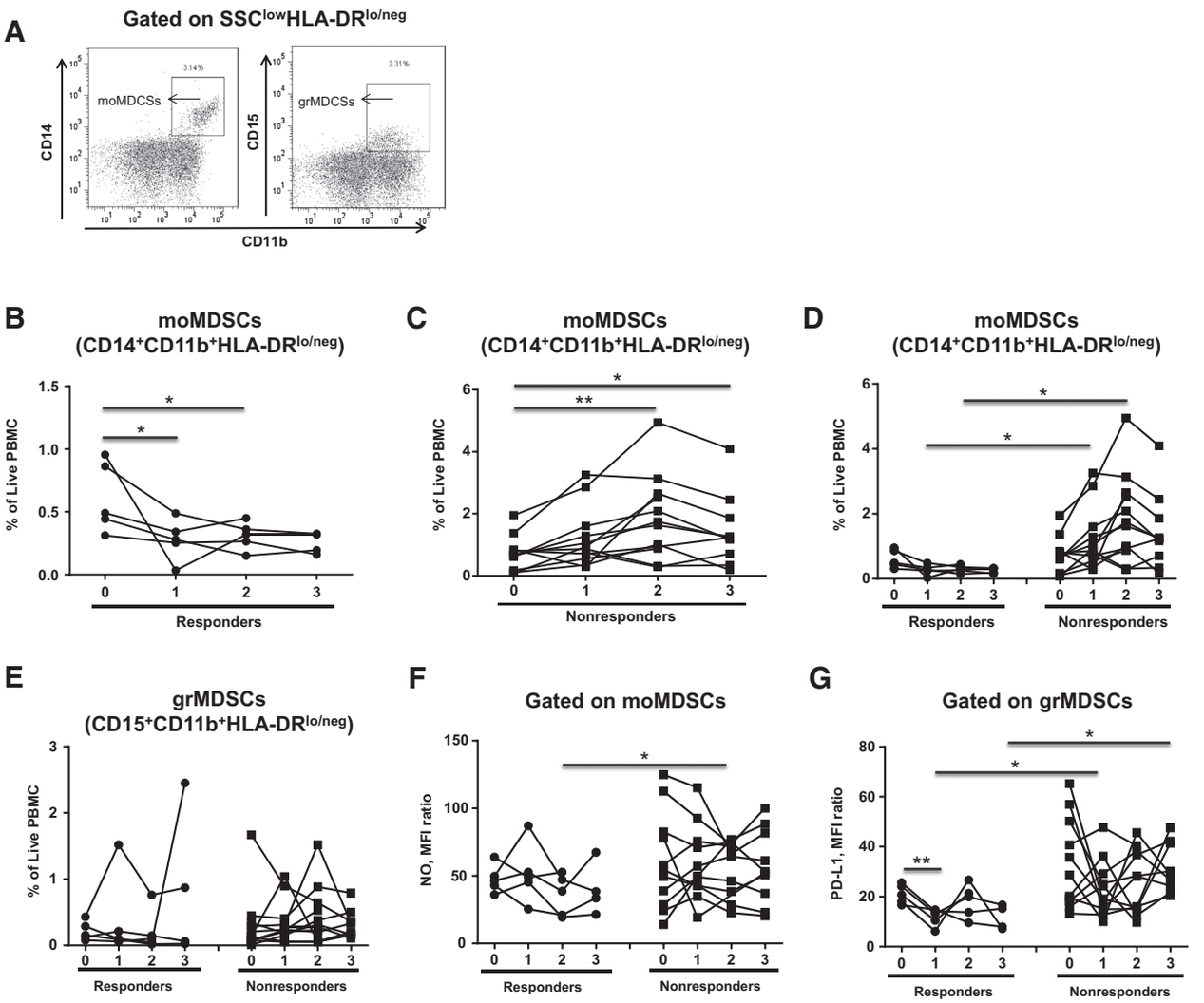

Figure 2.

Analysis of MDSCs in melanoma patients upon Ipi therapy. PBMCs obtained from the peripheral blood of 17 melanoma patients before each Ipi infusion (point 0—before the treatment; point 1—after the first infusion; point 2—after the second infusion; point 3—after the third infusion) were assessed by flow cytometry. A, representative dot plots with the gating strategy identifying moMDSCs ($SSC^{low}HLA\text{-}DR^{lo/neg}CD11b^{+}CD14^{+}$ cells) and grMDSCs ($SSC^{low}HLA\text{-}DR^{lo/neg}CD11b^{+}CD15^{+}$ cells). B–D, the frequency of moMDSCs in 17 melanoma patients responding (B and D) or nonresponding (C and D) to the Ipi treatment is presented as the percentage of these cells within live PBMCs. E, the frequency of grMDSCs in 17 melanoma patients is shown as the percentage of these cells among live PBMCs. F, the intracellular concentration of NO in moMDSCs is expressed as the MFI ratio (MFI of experimental samples/MFI of respective negative controls). G, the level of PD-L1 expression on grMDSCs is shown as the MFI ratio. *, $P < 0.05$; **, $P < 0.01$.

but have not been validated so far. Here, we focused on the evaluation of myeloid cells (eosinophils, neutrophils, monocytes, and MDSCs) and related circulating inflammatory factors as possible predictive markers of the treatment efficiency of Ipi in advanced melanoma patients.

First, we observed an early significant increase in eosinophil counts (already after first Ipi infusion) in the peripheral blood of responding patients as compared with their numbers before the beginning of Ipi therapy, which is in line with a previous report (21). Moreover, in nonresponding patients, we observed a significant reduction in the concentration of eotaxin-1 as compared with baseline levels. Because this chemokine is considered to play a critical role in the eosinophil recruitment (20, 22), such changes indicate poor conditions for eosinophil accumulation. Eosinophils have been reported to infiltrate tumors that were associated with a better prognosis in most cases (22). In the B16 melanoma mouse model, eosinophil accumulation in solid tumors was considered as an early and persistent inflammatory host response (23). In addition, it has been recently reported that in this melanoma model, tumor-infiltrating eosinophils can guide T cells into the tumor, which resulted in tumor eradication and improved survival (24). However, the exact mechanism of beneficial effects of eosinophils in tumor-bearing hosts remains elusive and needs further investigation.

In contrast with eosinophils, we demonstrated a significant elevation of monocyte and neutrophil counts at baseline in

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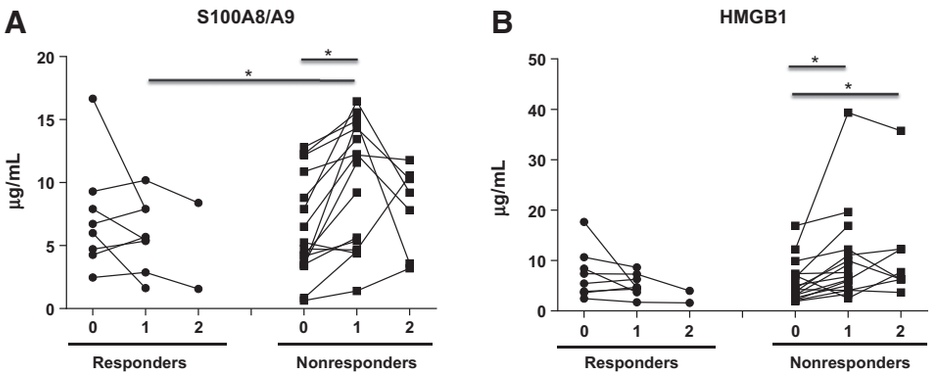

Figure 3.
Increased concentrations of S100A8/A9 and HMGB1 in melanoma patients are associated with the poor response to Ipi therapy. Inflammatory factors were measured in serum from 24 melanoma patients before each Ipi infusion (point 0—prior to treatment; point 1—after the first infusion; point 2—after the second infusion) by ELISA. A, S100A8/A9 concentrations from responders or nonresponders are presented as µg/mL. B, levels of HMGB1 are presented as ng/mL. *, $P < 0.05$.

nonresponders as compared with responders. Tumor-associated monocytosis or neutrophilia and/or tumor infiltration by these myeloid cells were reported to represent an adverse prognostic feature in metastatic melanoma, and a high baseline neutrophil count was demonstrated to be a strong, independent risk factor indicating a poor clinical outcome (25).

Since MDSCs represent immature myeloid cells containing monocytic (moMDSCs) and granulocytic (grMDSCs) subsets with a high immunosuppressive potential (9), we analyzed these cells in the peripheral blood of Ipi-treated patients. MoMDSCs were reported to be strongly associated with a poor prognosis in stage IV melanoma patients (14, 15, 26, 27). In our cohort of patients, we found that a pretreatment moMDSC frequency in nonresponders was slightly higher than in responders. However, in contrast with recent publications (11, 28), this elevation was not statistically significant. Furthermore, we observed a significant increase of moMDSC frequencies in nonresponders after the first and second Ipi infusion, whereas in responders, the moMDSC level showed a strong reduction upon the therapy as compared with basal values. In addition, in responders, the frequencies of moMDSC upon the first and second infusion were significantly higher than in nonresponders. This observation is in accordance to results of Kitano and colleagues (28) who showed that lower moMDSC frequencies at week 6 after Ipi treatment are associated with improved OS. However, Meyer and colleagues (11) detected only a nonsignificant elevation of moMDSC frequencies in nonresponders as compared with responders, which might be due a low number of patients by whom these cells were measured.

We also measured the intracellular NO production as a marker of the MDSC immunosuppressive potential (9) and found that its production by moMDSCs from patients responding to Ipi was decreased as compared with nonresponders, suggesting an Ipi-related downregulation of the moMDSC activity. Analyzing a correlation between the frequencies of moMDSC and NO production by these cells measured simultaneously in the same patients, we demonstrated that after the first Ipi infusion, higher frequencies of moMDSC in nonresponders significantly correlated with an elevated intensity of NO production in these cells.

Measuring the frequency of grMDSC subpopulation during Ipi therapy, we failed to detect its reduction in responders in contrast with a recent report (10). This discrepancy might be explained by differences in markers applied for the detection of grMDSCs in this report and in our study as well as by a poor survival of grMDSCs in our frozen PBMC samples. However, we demonstrated that grMDSCs from responders displayed an early significant downregulation of the PD-L1 expression as compared with the baseline and with this parameter in responders. This molecule has been shown to be involved in MDSC-mediated inhibition of T-cell reactivity through the binding to PD-1 expressed on effector T cells (8), suggesting the role of this pathway in the preservation of immunosuppression in patients resistant to Ipi therapy. GrMDSCs were also shown to produce NO-like moMDSCs, although we found no differences in NO levels in terms of the responsiveness to the Ipi treatment.

To elucidate the mechanism of the changes in MDSC frequencies and immunosuppressive phenotype upon CTLA-4 blockade with Ipi, we measured serum levels of S100A8/A9 and HMGB1. A significant elevation of both soluble markers after the first infusion of Ipi was evident in melanoma patients who were defined as nonresponders. Both proteins are members of the damage-associated molecular pattern (DAMP) molecules (also known as alarmins) that are released upon cell stress or damage promoting thereby an inflammation via receptors, such as receptor for advanced glycation end-products (RAGE) or toll-like receptor 4 (TLR4; refs. 29–32). They have been described as critical factors for MDSC recruitment and stimulation of their immunosuppressive functions in the tumor microenvironment (16–18). Because both alarmins are produced by melanoma-associated immune cells and relate to the tumor aggressiveness and progression (29, 30), changes in their levels in melanoma patients over the clinical course might reflect individual immune responses and could therefore be useful as novel biomarkers predicting the responsiveness to Ipi treatment.

Taken together, we demonstrated an early increase in eosinophil counts as well as a reduction in moMDSCs, S100A8/A9, and HMGB1 in melanoma patients responding to Ipi therapy. Moreover, in these patients, MDSCs displayed a decreased NO production and PD-L1 expression, suggesting their decreased activity. In contrast, higher neutrophil and monocyte counts at baseline as well as an early elevation of moMDSC frequencies and serum levels of S100A8/A9 and HMGB1 indicated a lack of response to Ipi therapy. Our data highlight additional important mechanisms of Ipi effects and suggest the measurement of eosinophils, MDSCs, as well as related chronic inflammatory factors S100A8/A9 and HMGB1 as new biomarkers detecting the group of patients who may benefit from such therapy.

Disclosure of Potential Conflicts of Interest

J. Utikal reports receiving speakers bureau honoraria from and is a consultant/advisory board member for Roche. No potential conflicts of interest were disclosed by the other authors.

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Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): C. Gebhardt, A. Sevko, H. Jiang, R. Lichtenberger, T. Holland-Letz, L. Umansky, D. Schadendorf, J. Utikal, V. Umansky
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