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Research Article

The Ratio between Monocytic Myeloid Derived Suppressor Cells/Monocytes and Dendritic Cells is A Prognostic Intratumoral and Blood Biomarker for Survival of Epithelial Ovarian Cancer Patients

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Abstract

Predictive biomarkers are essential for tailoring therapy of patients with epithelial ovarian cancer (EOC). In this study, various immune populations in both tumor tissue and blood samples from EOC patients were analyzed to identify an easy accessible biomarker.

The frequencies of circulating immune cells were determined by flow cytometry and subsequent analysis by opt SNE and FLOWSOM using OMIQ in 36 EOC patients. Primary tumor material (n = 21) was analyzed by multiplex immunofluorescence using Vectra imaging and InForm software. Data were related to overall survival using Kaplan–Meier curves and log-rank testing.

A low ratio between mMDSC to DC ($p = 0.0004$), total monocyte to DC ($p = 0.0026$), mMDSC to CD19hi B cells ($p = 0.0013$) and monocyte to CD19hi B cells ($p = 0.003$) in blood was associated with prolonged survival in EOC patients. Importantly, low mMDSC to DC ($p = 0.006$) and CD14+HLA-DR+ monocyte to DC ratios ($p = 0.006$) in tumor tissue were also strongly prognostic for better survival.

This is the first study showing that both the intratumoral and systemic balance between two cell types functions as prognostic biomarkers for EOC survival. Validation of their predictive power is ongoing in a prospective study and clinical immunotherapy trial.

Keywords: epithelial ovarian cancer, mMDSC to DC ratio, monocyte to DC ratio, prognostic, biomarker, overall survival

Introduction

The majority of patients with epithelial ovarian cancer, fallopian tube cancer, and primary peritoneal cancer (all abbreviated as EOC) present with advanced disease. Treatment is mainly based on chemotherapy in combination with surgery and maintenance PARP inhibition [1]. Although most patients have an initial good response, around 70-80% of the patients will relapse with subsequent short survival [2,3]. Therefore, there is a need for clinical biomarkers to identify the poor responding patients as they may prefer treatment by experimental strategies.

The tumor microenvironment (TME) is composed of a wide variety of immune cells, and is well studied since subsets of immune cells have been shown to be prognostic and/or predictive for clinical outcome in the response to (chemo)therapy [4,5]. Generally, EOC hosts an abundancy of immunosuppressive cell subsets in the TME, such as tumor-associated macrophages (TAMs), myeloid-derived suppressor cells (MDSCs) and regulatory T cells (Tregs). In addition, the TME can contain CD8+ cytotoxic T cells and dendritic cells (DCs) which are associated with better clinical outcome of patients after standard of care therapy [5-9].

To identify and monitor biomarkers, either as a prognostic tool or for prediction of therapy response, tumor sampling is often used. However, liquid biopsies have proven to be an attractive alternative since it is less invasive, inexpensive and easy to repeat [10,11]. Yet, it is essential to use blood biomarkers that are a good reflection of the TME [12-14]. Earlier we showed that the presence of high numbers of monocytes/macrophages and monocytic MDSCs (mMDSCs) in baseline blood samples was associated with reduced OS, whereas high levels of DCs were associated with an OS benefit. Importantly, the ratio of monocyte/macrophage to DC, and in particular of mMDSC to DC, formed an independent prognostic factor for OS [15]. In the current study, we analyzed these myeloid immune populations in tumor tissue to investigate if they have the same prognostic capacity and thus, if the blood can be considered a reflection of the TME. Furthermore, we aimed to identify additional blood biomarkers. Here, we report that relatively low numbers of monocytes and mMDSCs as well as higher numbers of DCs were predictive for longer survival either when measured in the tumor or in the blood. Since these cells are also key to suppress or activate, respectively, tumor specific T-cell reactivity [15,16], they may also predict the response to immunotherapy.

Materials and methods

Phenotyping of PBMC

Immunostaining of PBMC and acquisition by multiparameter flow cytometry was previously performed [17, 18]. Data obtained with the monocyte/macrophage panel consisting of the live/dead marker yellow amine reactive dye, CD3, CD1a, CD11b, CD11c, CD16, CD19, CD45, HLA-DR, CD163 and CD206 antibodies, was used to re-analyze the monocyte/macrophage, mMDSC and other immune cell subsets by high-dimensional single cell data analysis using the OMIQ data science platform (n = 36). To this end, CD45+ cells were manually gated using FlowJo software version 10.8, after which newly generated FCS files were used for analysis by automated optimized parameters for T-distributed stochastic neighbor embedding (optSNE) followed by clustering in a self-organizing map (FlowSOM) using OMIQ data analysis software (www.omiq.ai).

Patients and tumor material

Formalin-fixed paraffin-embedded (FFPE) samples from primary tumors from EOC patients treated within the PITCH and/or CHIP study were collected (Table S1). From 21 out of 36 patients of whom PBMC phenotyping was performed, tumor material was available for analyses.

Eligibility criteria for these two trials have been described previously [17,18]. In summary, the PITCH study was a phase I dose-escalation study where patients with recurrent platinum sensitive EOC received standard chemotherapy (carboplatin/(pegylated liposomal)doxorubicin) with or without tocilizumab. The highest dose of tocilizumab was combined with interferon alpha. The CHIP study was a phase I/II study in patients with recurrent platinum resistant EOC in which patients received standard chemotherapy (gemcitabine), whether or not in combination with interferon alpha with or without a vaccine against p53. Both studies were conducted in accordance with the Declaration of Helsinki and approved by the Medical Ethics Committee Leiden, in agreement with the Dutch law for medical research involving humans, and registered in the clinical trial register (PITCH study: NCT01637532 and CHIP study: NTC01639885).

Multiplex immunofluorescence imaging of tumor tissue

A seven-color multispectral immunofluorescence panel was used for identification of mMDSCs,

DCs, macrophages and monocytes in tissue, consisting of antibodies against CD1c, CD163, CD11c, CD14, CD68, HLA-DR and DAPI. The panel is a combination of indirect (CD14, CD163) and direct (CD11c, CD68, HLA-DR) detection, and tyramide signal amplification with Opal (CD1c) (PerkinElmer, Waltham, Massachusetts). An overview of the panel can be found in Table S2.

Immunofluorescent stains were performed on 4μm tissue sections from FFPE blocks with tumor material from debulking procedures. Tissue sections were deparaffinized and endogenous peroxidase was blocked with hydrogen peroxide, after which epitope retrieval was performed with tris-EDTA (10 mM/1 mM, pH 9.0). Non-specific binding sites were blocked with SuperBlock (ThermoFisher Scientific). Antibodies were applied following the recommended protocols in the following order: CD1c, unconjugated antibodies (CD14 and CD163 with overnight incubation) and conjugated antibodies (CD11c, HLA-DR, CD68 with 5h incubation). Finally, DAPI was used for nuclear staining. Slides that underwent the complete immunofluorescent staining procedure without primary antibodies served as negative controls, human tonsil slides served as positive control.

Enumeration of immune cells in tumor sections

The numbers of mMDSCs, DCs, macrophages and monocytes were counted using the Vectra 3.0.5, an automated quantitative pathology imaging system, with InForm 2.4 software (PerkinElmer, Waltham, Massachusetts). mMDSCs were characterized as CD68-CD14+HLA-DRCD11c-CD1c-, DCs as CD68-CD14-HLA-DR+CD11c+, M1-like macrophages as CD68+CD163-, M2like macrophages as CD68+CD163+, inflammatory macrophages as CD68+CD14+HLA-DR+, total macrophages as CD68+ and monocytes as CD68-CD14+HLA-DR+. In brief, slides were scanned for image acquisition (×4 magnification) after which five multispectral images per slide on average were selected manually (×20 magnification, one image representing roughly 0.33 mm2). Using the semi-supervised deep learning based InForm software, the software was trained to segment stroma, tumor epithelium, and empty tissue regions, and to distinguish between mMDSCs, DCs, macrophages, monocytes and other cell phenotypes. To improve the accuracy of the training process, the training was divided in three sub-analyses (HLA-DR and CD14, CD68 and CD163, CD68 and CD163) after which the phenotypes of the individual sub-analyses were merged per cell based on their X,Y-positions with an R script to obtain the complete expression profile of each individual cell. Finally, the number of immune cells were calculated as number per mm2 stroma, mm2 tumor and mm2 stroma plus tumor.

Statistics

Statistical analyses were performed using SPSS (v.25.0 for Windows, IBM SPSS statistics). Patients from whom tumor material appeared non-evaluable after staining were excluded from analyses. Survival analyses were performed using Kaplan–Meier curves and log-rank testing. A variable for each individual immune cell population (low/high) was calculated using the median as cut-off value, since immune cell counts were not normally distributed. For the ratios, first an individual ratio per patient was calculated, after which the median ratio was used to differentiate between a low or high ratio. The clinical endpoint examined was OS, defined as time from surgery to death due to any cause. Correlations between different populations were tested using non-parametric Spearman r correlation analysis.

Results

Low blood mMDSC to DC, total monocyte to DC, mMDSC to CD19hi B cell and monocyte to CD19hi B cell ratios are associated with prolonged survival

In the past, we analyzed pre-defined cell populations on manually gated PBMC samples from EOC patients [15]. Newly developed state-of-the-art techniques such as opt SNE [19] and FLOWSOM [20] using OMIQ allow true objective and automatic analyses of the immune cell composition of PBMC, and these were utilized to re-analyze the data sets from 36 EOC patients of two phase I trials [17,18]. In addition, survival data were updated (cutoff date 2022-05-19). A total of thirteen individual immune cell clusters were identified, with CD14+HLA-DR+CD16- monocytes (population 2) and T-cells (population 7) being the two most substantial populations. CD14+HLA-DR- mMDSCs (population 1), CD14+CD16+ monocytes (population 3), B cells (populations 8 and 9), NK and NK/T cells (populations 11 and 12) were also prominently present (Figure 1A-C, Figure S1A). When patients were grouped into low or high according to the median frequency of each population, high frequencies of circulating CD14+HLA-DR- mMDSCs (population 1), CD14+HLA-DR+CD16- monocytes (population 2) and CD14+HLA-DR+ total monocytes (populations 2, 3 and 5) were associated with worse overall survival (OS), while high frequencies of circulating DCs (population 4) and strongly CD19 expressing B cells (population 9) were associated with OS benefit (Figure 1D, Figure S1B).

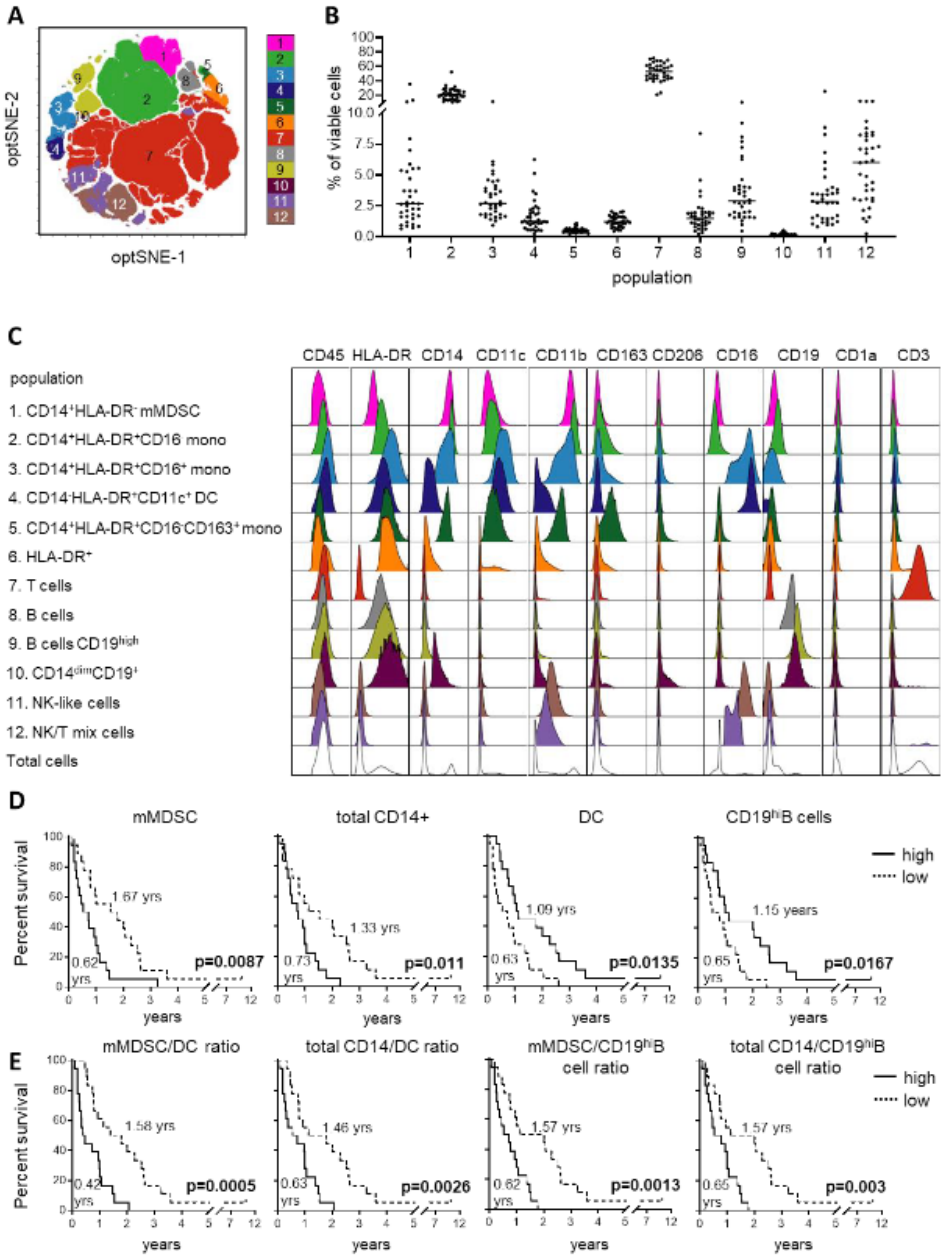

Figure 1: Circulating immune cell subsets associated with short or prolonged survival in EOC. Baseline frequencies of immune cells were determined in platinum-sensitive and –resistant. Epithelial Ovarian Cancer (EOC) patients using flow cytometry data acquired earlier [15]. Data was analyzed by subsequent optSNE and FLOWSOM using OMIQ. A) optSNE plot visualizing the FLOWSOM cluster for all EOC patients. B) Graph displaying the frequencies of the identified FLOWSOM clusters as percentage of viable cells for each patient. C) Phenotypic plots visualizing the expression of the indicated markers for the different immune cell clusters. Kaplan-Meier survival plots of 36 EOC patients for D) mMDSC (left), total CD14+ monocytes (center left), DC (center right) and CD19hi B cell (right) and E) mMDSC to DC ratio (left), total monocytes to DC ratio (center left), mMDSC to CD19hi B cell ratio (center right) and total monocytes to CD19hi B cell ratio (right). Patients were grouped into high or low groups according to the median frequency of the indicated myeloid cell subpopulations. The solid line depicts patients with frequencies above the median and the dotted line depicts patients with frequencies below the median. Statistical significance of the survival distribution was analyzed by log-rank testing.

Since these immune cell populations were associated with opposite clinical outcome, the ratios between mMDSCs and DCs or CD19hi B cells (population 9) as well as total monocytes (having a better separation of the survival curves than CD14+CD16- monocytes) and DCs or CD19hi B cells (population 9) were calculated. The differences in median OS were even more prominent when a low mMDSC to DC ($p = 0.0004$), a low total monocyte to DC ($p = 0.0026$), a low mMDSC to CD19hi B cell ($p = 0.0013$) or a low monocyte to CD19hi B cell ratio ($p = 0.003$) was used as biomarker (Figure 1D). Thus, the mMDSC to DC, monocyte to DC, mMDSC to CD19hi B cell and monocyte to CD19hi B cell ratio in the blood displayed the best prognostic capacity for survival in EOC patients.

A variety of myeloid immune cell populations infiltrate EOC tumors

To investigate whether the prognostic immune subsets in PBMCs have the same prognostic impact when measured in tumor tissue and thus may reflect what is locally going on, primary tumor material from 21 of the 36 patients from which PBMCs were analyzed could be collected, stained with a multispectral myeloid cell panel consisting of antibodies against CD1c, CD163, CD11c, CD14, CD68, HLA-DR and DAPI and analyzed. Material from the other patients was not available or not enough tumor was left for analysis. Patient and tumor characteristics are described in Table S1. Representative images of immunofluorescent stains are shown in Figure 2 and Figure S2.

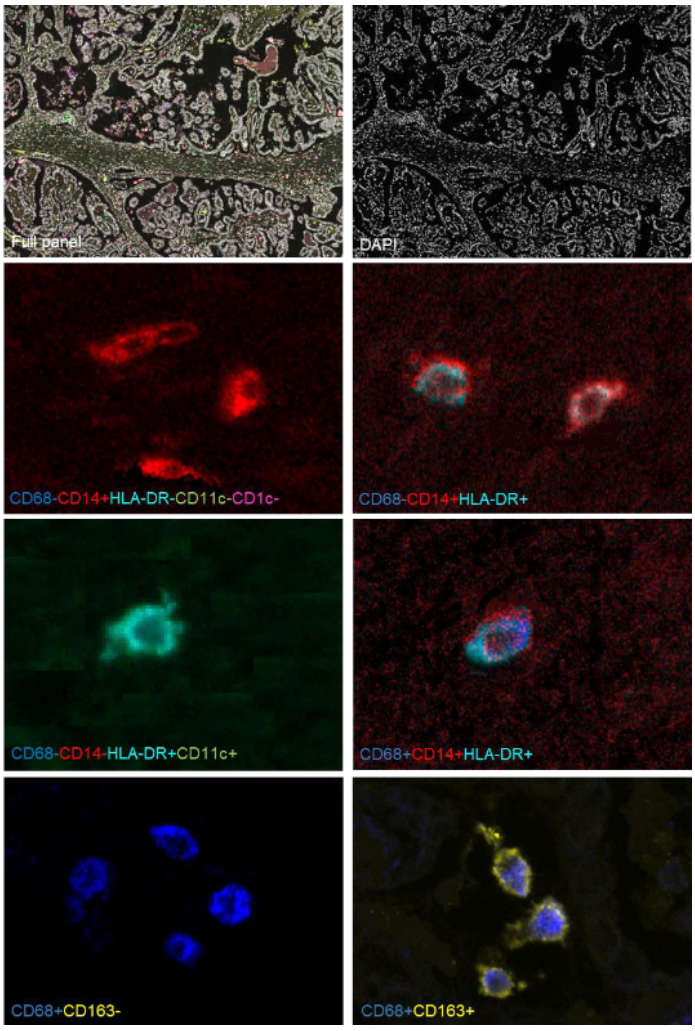

Figure 2: Detection of different myeloid cell types by multiplex immunofluorescence. Representative images of immunofluorescent staining to detect mMDSCs (CD68-CD14+HLA-DR-CD11c CD1c-), DCs (CD68-CD14-HLA-DR+CD11c+), macrophages (CD68+CD163- (M1-like), CD68+CD163+ (M2-like), CD68+CD14+HLA-DR+ (inflammatory macrophages) and monocytes (CD68-CD14+HLA-DR+). CD68 = blue, CD14 = red, HLA-DR = turquoise, CD11c = green, CD163 = yellow, CD1c = pink, DAPI = white. Images were taken at ×20 magnification.

For each of the defined immune cell subsets (Table 1) the counts per mm2 stroma, mm2 tumor and mm2 stroma and tumor combined were analyzed (Figure 3; Table S3). In general, tumor tissue was strongly infiltrated with CD68+CD163+ (M2-like) macrophages, CD68+CD14+HLA-DR+ (inflammatory) macrophages and CD68-CD14+HLA-DR+ monocytes (Figure 3A), and to a lesser extent with mMDSCs, CD68+CD163- (M1-like) macrophages and DCs (Figure 3B).

Population	Markers
mMDSCs	CD68 - CD14+ HLA-DR- CD11c- CD1c-
DCs	CD68 - CD14- HLA-DR+ CD11c+
M1 -like macrophages	CD68+ CD163-
M2 -like macrophages	CD68+ CD163+
Inflammatory macrophages	CD68+ CD14+ HLA -DR+
Total macrophages	CD68+
Monocytes	CD68- CD14+ HLA-DR+

Table 1: Definitions of the different myeloid cell populations in tumor tissue from EOC patients

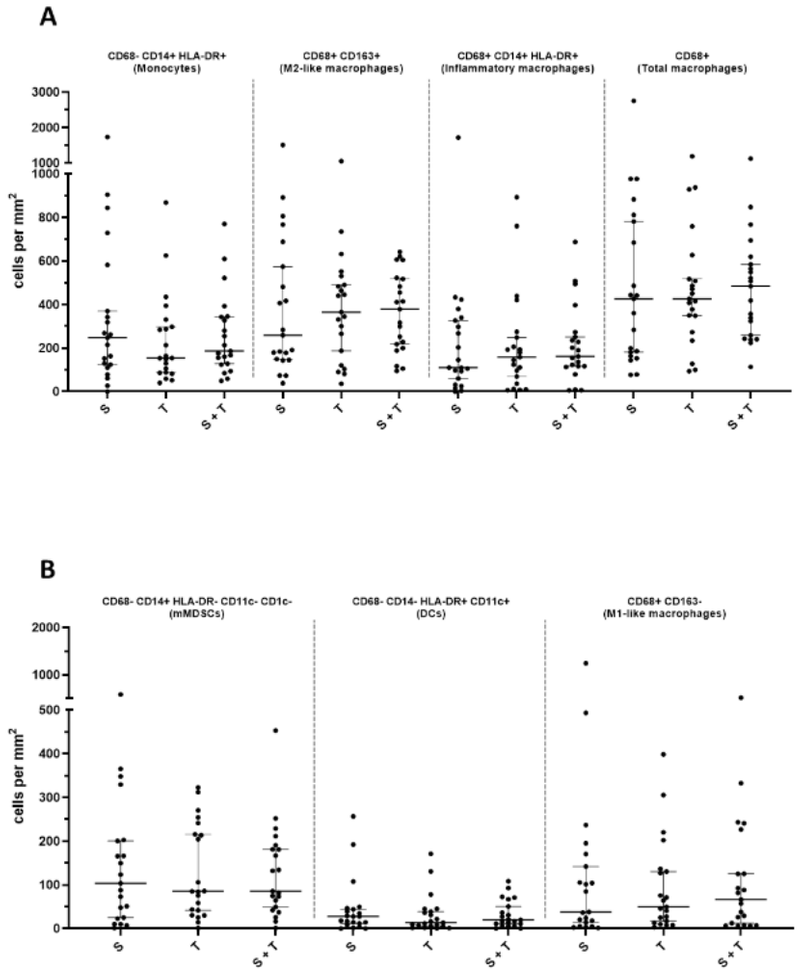

Figure 3: Tumor tissue from EOC patients is mainly infiltrated with CD68+CD163^{+}Macrophages. Immune cell counts in tumor tissue from EOC patients. **A)** mMDSC counts (CD68-CD14+HLA-DR-CD11c-CD1c-), DC counts (CD68-CD14-HLA-DR+CD11c+), Monocyte counts (CD68CD14+HLA-DR+) **B)** Macrophage counts; M1-like (CD68+CD163-), M2-like (CD68+CD163+), inflammatory macrophages (CD68+CD14+HLA-DR+), total macrophages (CD68+). The number of stromal and tumoral myeloid cell population counts are presented as cells per mm2 tissue with the median and the 95% confidence interval (CI). S stroma, T tumor, S + T stroma and tumor.

Stromal and intra-tumoral counts for mMDSCs, DCs and CD68+CD163- macrophages are correlated

To gain insight in the coordination of the infiltrating immune cell populations in EOC patients, immune cell counts in both stroma and tumor were analyzed with Spearman r correlation and data were visualized with a heatmap (Figure 4).

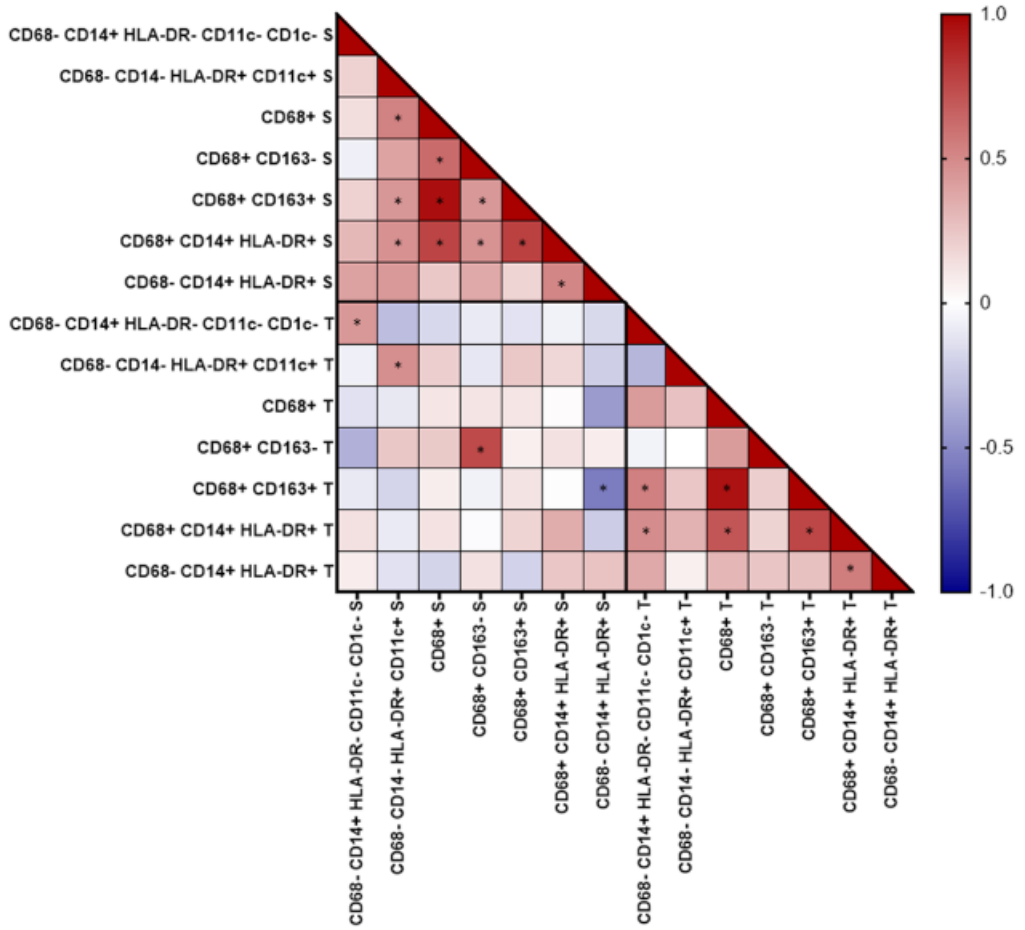

Figure 4: **Correlation between the counts of different myeloid cell populations in tumor tissue from patients with EOC**. Correlation between the numbers of different tissue infiltrating myeloid cell populations in primary tumor tissue from patients with EOC. CD68-CD14+HLA-DRCD11c-CD1c-= mMDSCs, CD68-CD14-HLA-DR+CD11c+ = DCs, CD68+ = total macrophages, CD68+ CD163- = M1-like macrophages, CD68+ CD163+ M2-like macrophages, CD68+ CD14+ HLA-DR+ = inflammatory macrophages, CD68-CD14+ HLA-DR+ = monocytes. Non-parametric Spearman r correlation analysis was performed to analyze the co-infiltration of these populations and is displayed as a heatmap. T tumor, S stroma, * represents $p < 0.05$.

For mMDSCs, DCs, and CD68+CD163- (M1-like) macrophages, we found a strong positive correlation between stromal and intratumoral immune cell counts. A negative correlation was observed between CD68+CD163+ (M2-like) macrophages in the tumor and CD14+HLA-DR+ monocytes in the stroma. No other correlations between immune cells in the two locations were found. Within each of the two locations (tumor or stroma), several positive correlations between the numbers of different subsets of myeloid cell populations were found (Figure 4).

The mMDSC to DC and CD14+HLA-DR+ monocyte to DC ratio in tumor tissue are prognostic biomarkers for OS. Next we assessed whether individual myeloid cell populations in the tumor tissue would be prognostic for survival. Therefore, patients were grouped (low vs high) according to the median cell count per mm2 tissue (stroma and tumor combined, as they are positively correlated). Only

CD14+HLA-DR+ monocytes displayed prognostic value for survival, with patients with low numbers of infiltrating CD14+HLA-DR+ monocytes displaying a significant survival benefit (p= 0.04) (Figure S3). All the other individual myeloid cell populations' showed no or only prognostic trends for survival (Figure S3).

Subsequently, we determined if the balance between immune stimulating (e.g. DCs) and immune suppressing subsets (e.g. mMDSCs and CD14+HLA-DR+ monocytes) would form stronger prognostic markers, similar to what was observed within the blood (Figure S4). The cohort was then grouped according to the median of the ratio. The low mMDSC to DC and CD14+HLA-DR+ monocyte to DC ratios were shown to be strongly prognostic for a better survival as compared to high mMDSC to DC and CD14+HLA-DR+ monocyte to DC ratios (Figure 5). The other ratios did not have prognostic value (Figure S5). These data demonstrate that different subsets of myeloid cells infiltrating the EOC microenvironment bear a negative or positive impact on OS, and that the balance between these tumor-infiltrating myeloid cell subsets is of importance. Moreover, both the intratumoral and systemic measurement of mMDSC to DC and CD14+HLA-DR+ monocyte to DC ratio can function as prognostic biomarkers for OS in EOC patients, suggesting that the measurement of these subsets in the blood is a good reflection of what occurs within the tumor tissue of EOC patients.

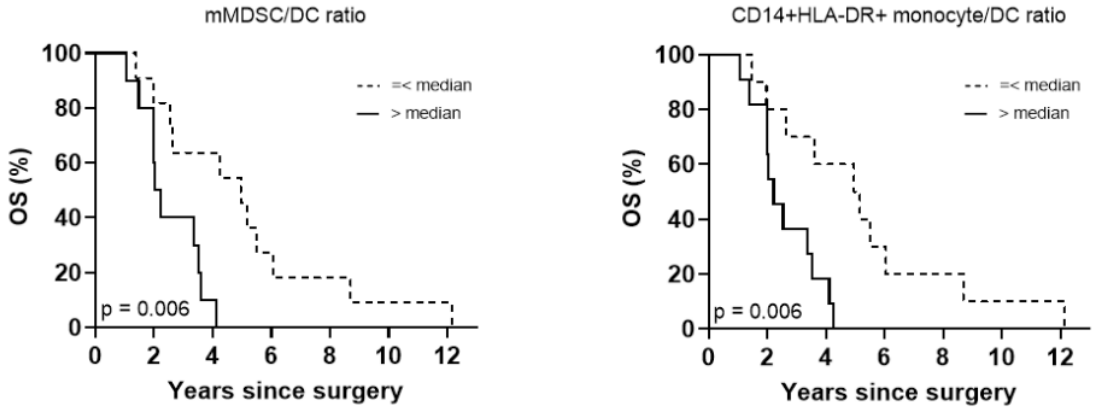

Figure 5: Low mMDSC or CD14+HLA-DR+ monocyte to DC ratios in the tumor tissue are associated with better survival. Kaplan–Meier survival estimates of myeloid immune cell subsets ratio's. Patients were grouped according to the median ratio. P values represent log-rank survival test.

Discussion

Our data reveal that the intratumoral mMDSC to DC and monocyte to DC ratios are prognostic biomarkers for survival of patients with EOC. Furthermore, when assessed in the blood of EOC patients they display similar prognostic value. Even though the tumor material was taken during debulking of the patients, while the blood samples were drawn at a later point in time, when patients had developed recurrent disease [17,18], our data show that these two biomarkers are prognostic relevant at both time points.

Our study shows that high frequencies of circulating CD14+MDSCs, CD14+HLA-DR+ CD16- monocytes and total CD14+HLA-DR+ monocytes in EOC patients were associated with worse OS. In cervical cancer, high frequencies of circulating CD14+ cells were directly involved in the suppression of T-cell reactivity [21] and the same was shown to apply to advanced EOC [15]. In contrast, high frequencies of circulating DCs and CD19hi B cells were associated with OS benefit.

Like DCs, B cells can function as antigen presenting cells, able to stimulate anti-tumor immunity [22]. It is therefore logical that the balance between the suppressing and activating immune cell subsets forms an even stronger prognostic marker for survival. In particular, a low mMDSC to DC, low mMDSC to CD19hi B cell, low total monocyte to DC and low total monocyte to CD19hi B cell ratio were associated with better OS. In line with these findings, a low mMDSC to DC and monocyte to DC ratio in the tumor tissue displayed a similar strong survival benefit. Studies on biomarkers in tumor tissue have already shown DCs to be prognostic for outcome [23, 24]. Also the role of M1-like and M2-like macrophages (TAMs) and their effect on outcome and prognosis has been studied profoundly [25, 26]. Less is known about mMDSCs in the TME, but Okla et al. suggested low numbers of tumor-infiltrating mMDSCs to be associated with a prolonged survival [27], as we now confirm in this study.

The objective and automatic analysis of immune cells in the blood not only confirmed previous findings [15] but in addition identified CD19hi B cells as well as the mMDSC to CD19hi B cell and monocyte to CD19hi B cell ratios as prognostic biomarkers in the blood. In a study by Henriksen et al., high numbers of circulating B cells were also shown to give a survival advantage in chemotherapy treated EOC patients [28]. This fits with the emerging positive role of tumorinfiltrating B cells to stimulate T cells and to foster a "hot" TME via the coordinated infiltration of different immune cells [22, 29, 30]. While the difference in surface expression levels of CD19 between circulating B cells in population 8 and population 9 is clear by flowcytometry, one can't expect to see that difference when tumors are studied using immunohistochemistry or immunofluorescence as performed in the current study. Therefore, we did not include CD19 into our immunofluorescence panel and are thus not able to study whether tumor-infiltrating B cells had a prognostic impact as well. CD19 is part of the cell surface receptor complex that regulates B cell reactivity to transmembrane signals [31]. Potentially, the superior prognostic value of CD19hi B cells when compared to the other B cell population relies in the fitness of this B cell population [32].

An effect of chemotherapy on myeloid cells has been shown before, including in the two trials the studied patients participated in. In the PITCH trial, an increase in circulating M1macrophages was demonstrated [18] which was also seen in the CHIP trial [17]. Furthermore, whereas a reduction of cells with a particular MDSC phenotype as consequence of chemotherapy in this trial was earlier reported [17], it became clear in later studies that this particular MDSC subpopulation was not functionally active [15] . In patients with cervical cancer treated with carboplatin and paclitaxel, several myeloid cells populations were transiently reduced [21] and this observation was recently confirmed in our trial (NCT04077263) in EOC patients treated with the same chemotherapeutic agents [33]. Moreover, a first application of the new biomarkers in this trial, in which recurrent platinum sensitive EOC were treated with adoptive cell transfer using autologous TILs, confirms its usefulness with respect to survival prediction after TIL treatment [33]. Although different chemotherapeutic agents alter myeloid cell populations, our blood biomarkers were not affected since analyzed samples were taken at baseline.

The limitations of our study are that the study group is fairly small, analyses were done in an explorative setting, and there was no PBMC sample obtained at time of surgery/debulking. Therefore, we have recently started the prospective IMPrOVE study (NCT03862677), which focuses on determining prognostic immune markers in both blood and tumor at different time points during treatment in both patients with primary and recurrent EOC and will also allow us to examine the effects of chemotherapy on these intratumoral cell types. In addition, the phenotype of the circulating CD19hi and the other B cell population should be studied in more detail in order to identify potential functional differences but also to identify markers that can be used for imaging studies in EOC.

Conclusion

To our knowledge this is the first study showing not only that intratumoral mMDSC to DC and monocyte to DC ratios are prognostic biomarkers for survival of patients with EOC, but also that these biomarkers can be assessed in the blood, strongly increasing the clinical applicability of these biomarkers. It would be important to study if these biomarkers display predictive power in randomized trials with/without immunotherapy.

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Authors Contributions

Conceptualization, AG, SS, JK and SB; Methodology, AG, SS, JK and SB; Software, AG and ZA;

Validation, AG, SS and ZA; Formal Analysis, AG, SS, JK and SB; Investigation, AG, SS; Resources,

AG, SS and ZA; Data Curation, AG, ED and SS; Writing – Original Draft Preparation, AG, SS, JK and SB; Writing – Review & Editing, AG, SS, ZA, ED, JK, SB; Visualization, AG and SS; Supervision, JK and SB; Project Administration, AG, ED and SS; Funding Acquisition, JK and SB.

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Conflict of interest

The authors declare no conflict of interest. The sponsors had no role in the design, execution, interpretation, or writing of the study.

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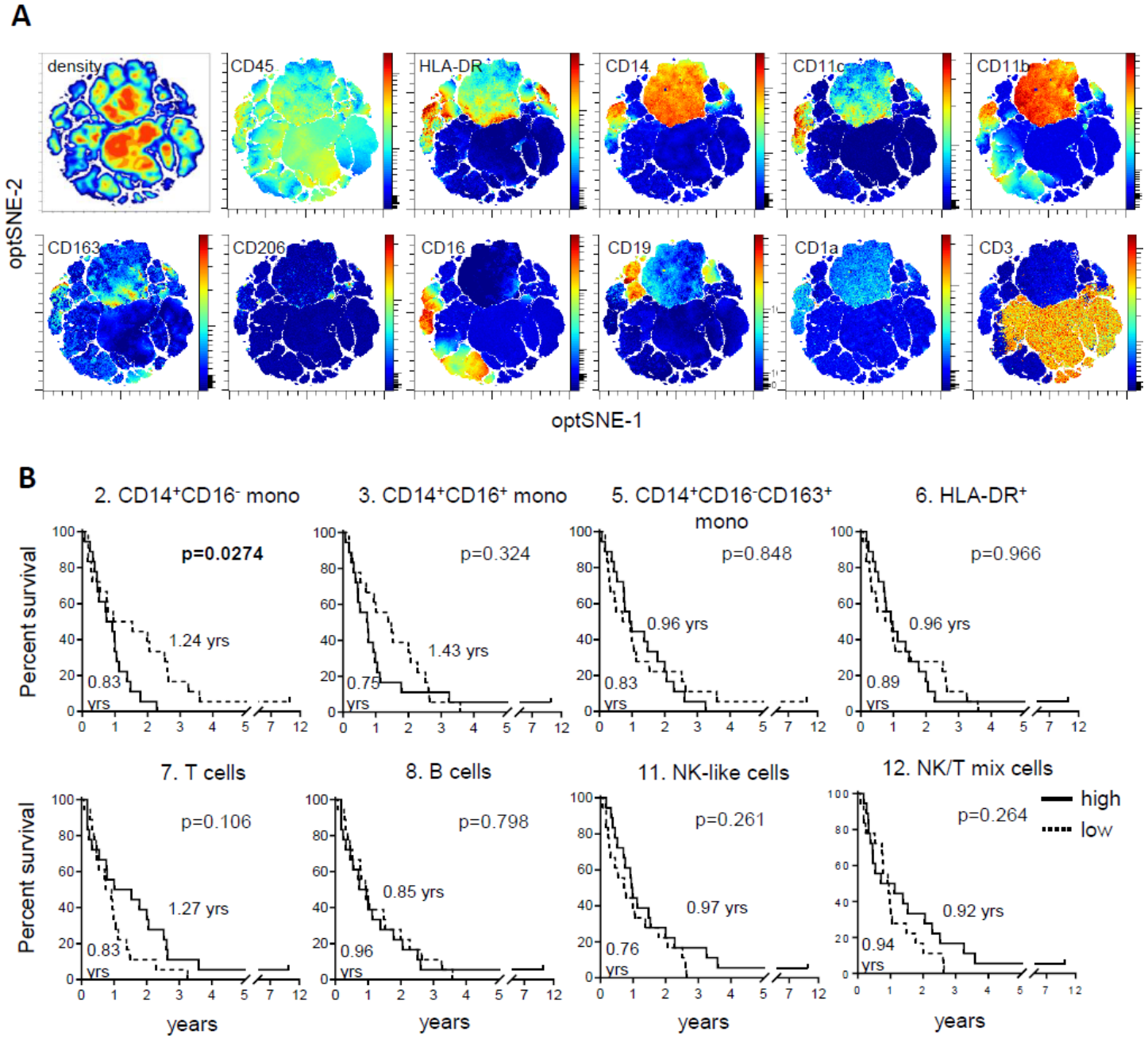
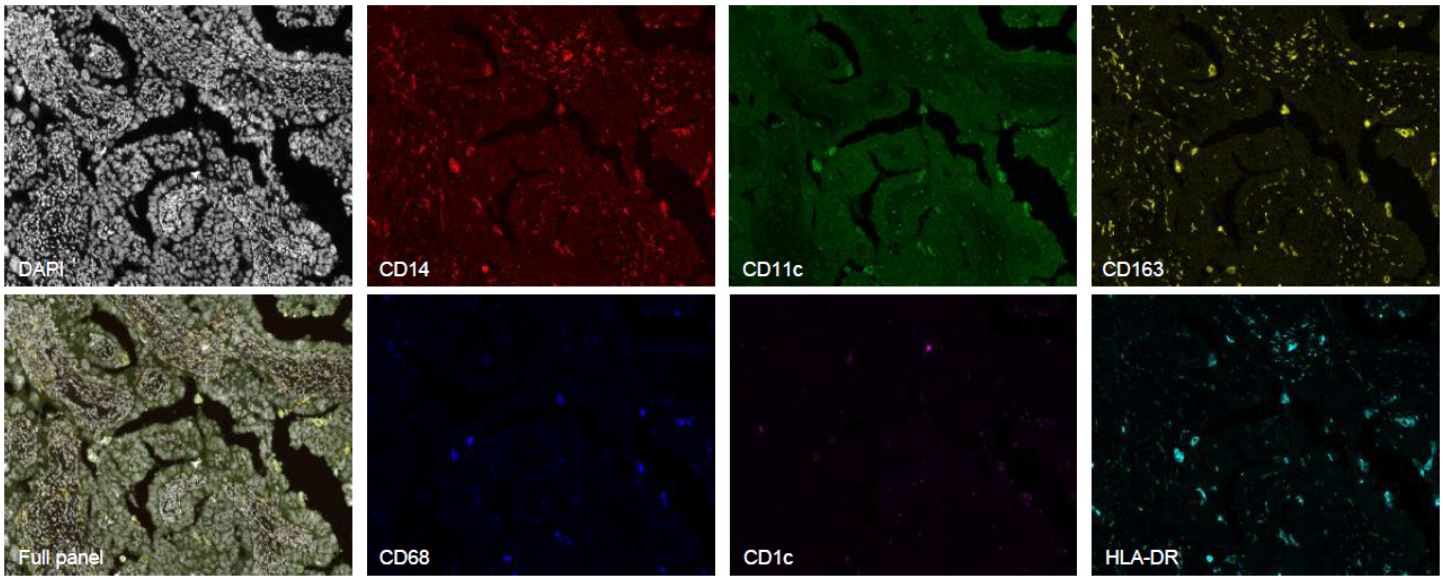
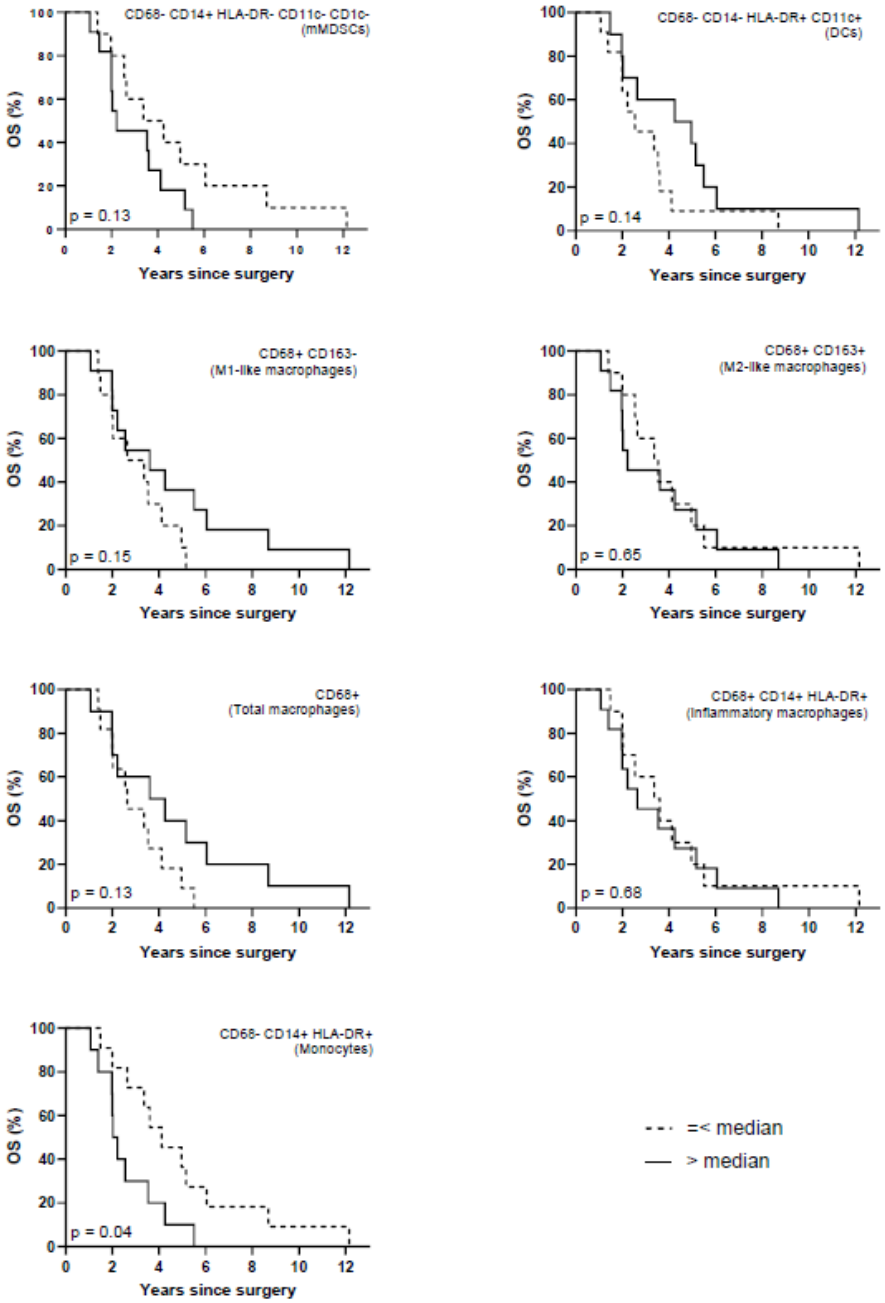
Supplementary Figures and Tables



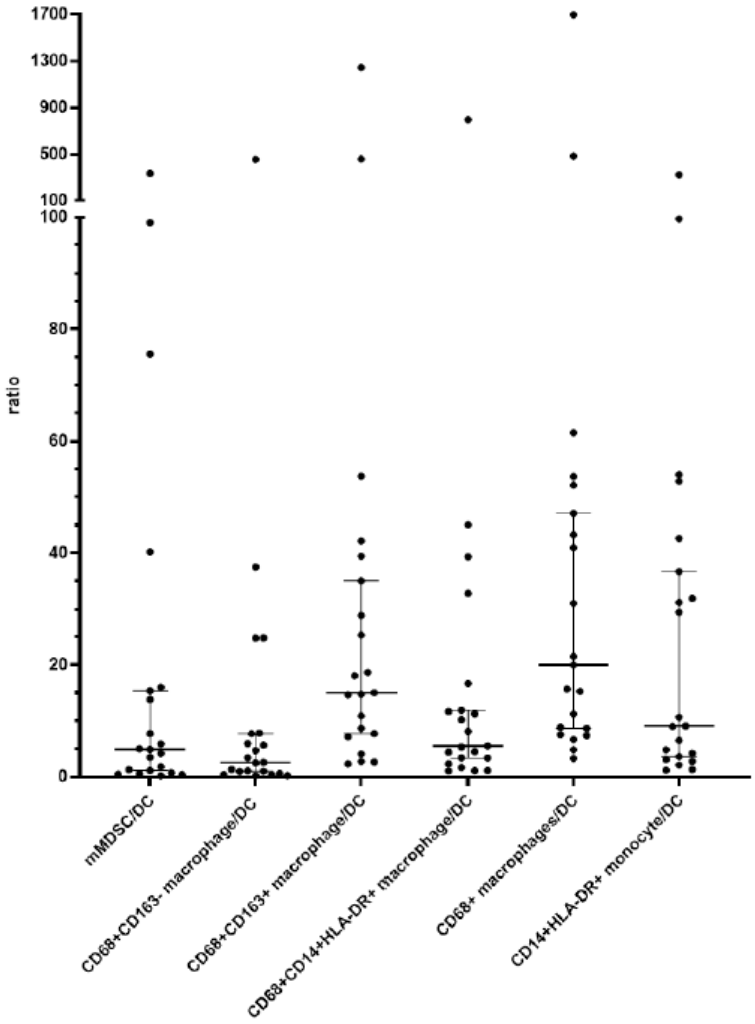
Supplementary Figure 1 – Baseline frequencies of immune cells were determined in platinumsensitive and –resistant Epithelial Ovarian Cancer (EOC) patients by flow cytometry analysis as described [15]. Data was analyzed by subsequent optSNE and FLOWSOM analysis using OMIQ. **A)** optSNE plots visualizing visualizing the expression of the indicated markers. **B)** Kaplan-Meier survival plots of 36 EOC patients for the indicated immune cell subsets. Patients were grouped into to high or low groups according to the median frequency of the indicated myeloid cell subpopulations. The solid line depicts patients with frequencies above the median and the dotted line depicts patients with frequencies below the median. Statistical significance of the survival distribution was analyzed by log-rank testing.



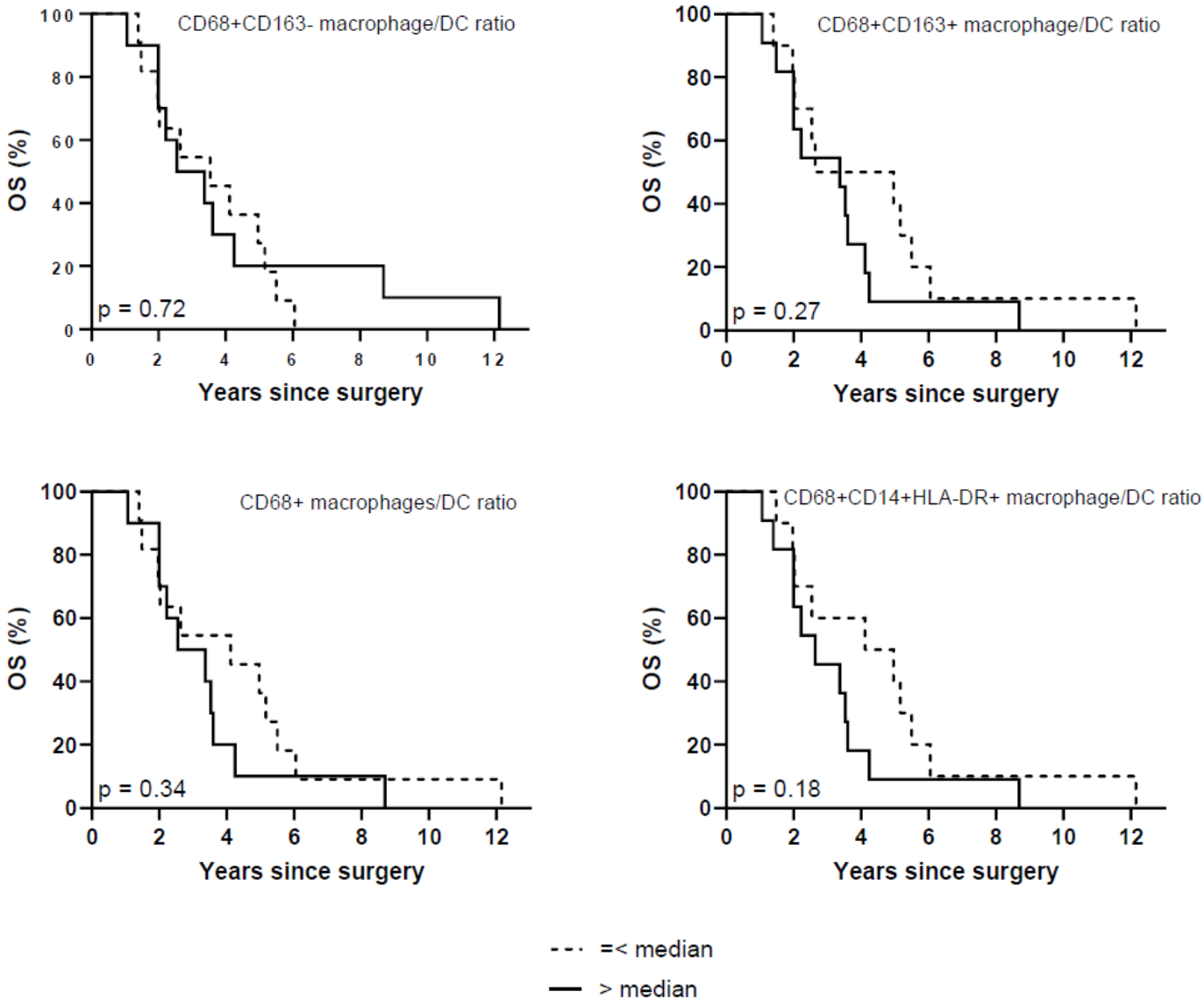
Supplementary Figure 2 Representative images of an immunofluorescent staining to detect mMDSCs , DCs, macrophages and monocytes. Markers are shown individually (DAPI, CD14, CD11c, CD163, CD68, CD1c and HLA DR) as well as in a composite image including all m arkers (full panel). Images were taken at × 20 magnification.



Supplementary Figure 3 – Kaplan–Meier survival estimates of individual myeloid immune cell subsets. Patients were grouped according to the median cell count per mm2 tissue (stroma and tumor combined). P values represent log-rank survival test.



Supplementary Figure 4- Ratios of different myeloid cell populations in tumor tissue from EOC patients



Supplementary Figure 5 – Kaplan–Meier survival estimates of myeloid immune cell subsets ratios. Patients were grouped according to the median ratio. P values represent log-rank survival test.

Patient #	Age	Histology	OS (years)
1	54	Serous	1.39
2	52	Serous	2.54
3	60	Serous	3.36
4	47	Endometrial	4.11
5	53	Serous	1.97
6	62	Serous	8.69
7	55	Serous	2.63
8	45	Serous	1.99
9	66	Serous	6.05
10	51	Serous	2.21
11	61	Clear cell	1.48
12	57	Serous	5.16
13	68	Serous	1.06
14	64	Serous	3.53
15	43	Endometrial	4.96
16	62	Serous	5.50
17	62	Serous	3.60
18	48	Serous	12.15
19	56	Serous	2.02
20	68	Serous	1.99
21	64	Serous	4.24

Age: age at surgery. OS: overall survival, time form surgery till death (any cause) in years

Supplementary Table 1- Patient and tumor characteristics of patients of whom tumor material was analyzed

Marker	Primary antibody (clone, company)	Species and isotype	Secondary antibody
CD1c	OTI2F4 (Abcam)	Mouse IgG1	Poly-HRP and Opal 520
CD14	D7A2T (Cell Signaling Technology)	Rabbit IgG	Alexa goat anti rabbit IgG 680
CD163	10D6 (Invitrogen ThermoFisher)	Mouse IgG1	CF goat anti mouse IgG1 633
CD11c	EP1347Y (Abcam) directly labelled with Alexa 546	Rabbit IgG	N.A.
HLA-DR	TAL1B5 (Abcam) directly labelled with Alexa 594	Mouse IgG1	N.A.
CD68	D4B9C (XP Cell Signaling Technology) directly labelled with Alexa 647	Rabbit IgG	N.A.
DAPI	Fluorescent probe (ThermoFisher)	N.A.	N.A.

N.A. Not applicable

Supplementary Table 2- Immunofluorescence panel

	DCs			mMDSCs			M1-like macrophages			M2-like macrophages			Inflammatory macrophages			Total macrophages			Monocytes		
	S	T	S + T	S	T	S + T	S	T	S + T	S	T	S + T	S	T	S + T	S	T	S + T	S	T	S + T
Median	27.8	13.6	20.0	103.6	85.2	85.6	38.1	49.8	66.7	259.6	365.5	379.2	110.6	158.6	162.5	426.1	426.4	484.7	247.3	154.8	186.7
Minimum	0.0	0.8	0.5	1.2	1.3	1.3	1.6	3.4	6.4	39.0	36.2	95.4	0.0	6.9	6.0	77.3	93.9	113.9	0.8	40.6	49.9
Maximum	256.9	171.1	108.5	587.0	322.9	453.1	1247.1	398.5	521.2	1509.6	1054.1	642.4	1722.3	893.9	687.8	2756.7	1190.7	1126.4	1734.3	868.9	770.5

Scores represent number of immune cells per mm² area. S stroma, T tumor, S + T stroma and tumor

Supplementary Table 3- Overview of general statistics for individual immune cell population counts in tumor tissue from EOC patients