Frequency of Immune Cell Subtypes in Peripheral Blood Correlates With Outcome for Patients With Metastatic Breast Cancer Treated With High-Dose Chemotherapy

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Abstract

The frequencies of circulating myeloid and T-cell populations were correlated with clinical outcome for 88 patients with metastatic breast cancer treated with high-dose chemotherapy. The ability to predict outcome depended on the chemotherapy regimen. The frequency of monocytes indicated outcome for patients treated with cyclophosphamide-based chemotherapy, while the frequency of T cells indicated outcome for patients treated with paclitaxel-based chemotherapy.

Background: The frequency of circulating leukocytes has been shown to be a prognostic factor in patients being treated for different types of cancer. In breast cancer, tumor-infiltrating leukocytes may predict patient outcome, but few studies have investigated such associations for circulating leukocytes. **Patients and Methods:** Multiparametric flow cytometry was used to examine the immunophenotypes of circulating peripheral blood mononuclear cells for 88 patients with metastatic breast cancer, which was then correlated to breast cancer–specific survival. Patients had been treated either with high-dose cyclophosphamide-containing regimens (group 1, n = 51 patients) or high-dose paclitaxel-containing regimens (group 2, n = 37 patients). **Results:** The frequency of peripheral blood $CD14^+$ monocytes indicated prognosis for patients in group 1 (but not group 2), while higher levels of $CD11c^+$ dendritic cells indicated a better prognosis for patients in group 2 (but not group 1). The frequency of a number of different $CD4^+$ or $CD8^+$ T cell subtypes also predicted prognosis for patients in group 2. For example, patients in group 2 with a higher frequency of circulating $CD4^+$ or $CD8^+$ naive T cells ($CD45RA^+CD95-CD27^+CD28^+$) showed a poorer prognosis. In contrast, T cells were not associated with prognosis for patients in group 1. **Conclusion:** Circulating leukocytes can predict clinical outcome for patients with breast cancer. Prediction of clinical outcome in this cohort of metastatic breast cancer patients was specific to the type of chemotherapy, and this finding is likely to apply to other therapies.

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Introduction

The level of immune cell populations both in the tumor and in circulation has been shown to be clinically informative for patients with cancer. For example, the frequency of intratumoral macrophages and $CD4^+$ and/or $CD8^+$ T immune cells has been associated with clinical outcome for patients with cancer, including breast cancer and advanced melanoma.[1-4] The absolute number of circulating $CD14^+$ monocytes[5,6] and the level of inflammatory

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monocytes[7] or dendritic cells[8] (DCs) correlates with a poorer prognosis in several cancers. However, in patients with breast cancer, a higher frequency of circulating plasmacytoid DCs (pDCs; $CD11c^+CD123^+$) was associated with an improved 5-year survival.[9] The presence of a high level of circulating myeloid-derived suppressor cells (MDSC, $CD14^+CD11b^+HLA\text{-}DR-/low$) is associated with grade, stage, and tumor burden and can indicate a relatively poor prognosis for patients with cancer,[10-12] including breast cancer[13,14] or advanced melanoma.[15]

A higher density of T cells in tumor tissue predicts a better clinical outcome for patients with breast cancer or melanoma.[16-18] In patients with melanoma, the presence of circulating $CD4^+$ and $CD8^+$ T cells was also strongly associated with improved prognosis for patients treated with ipilimumab.[19,20] The frequencies of different effector and central memory $CD4^+CD45RA-$[21] or $CD8^+CD45RA-$[22] T cells (designated EM1-EM4 on the basis of the presence or absence of CD27 or CD28 expression) are also linked to prognosis for patients with some cancers.[23,24] The presence of circulating T cells that recognize shared tumor-associated antigens also correlates with clinical outcome. Patients with malignant melanoma that possessed peripheral T cells that can respond to the melanoma-associated antigens NY-ESO-1 and melan-A (but not survivin or MAGE-A3) experienced a better clinical outcome.[25,26] Patients who possessed $CD4^+$ and/or $CD8^+$ cells that respond to NY-ESO-1 or who had only $CD8^+$ cells that respond to melan-A showed improved outcome.[27] Patients with breast cancer that possessed circulating $CD4^+$ and $CD8^+$ T cells that can respond to the HER2 tumor antigen showed an improved clinical outcome and had a lower frequency of monocytes, natural killer cells, DCs, and T-regulatory cells (Tregs).[16,28,29] However, patients with an elevated level of circulating MDSCs and Tregs did not show an improved prognosis associated with the presence of T cells responsive to tumor-associated antigens for breast cancer[28] or advanced melanoma.[13,27]

While the prognostic impact of tumor-infiltrating cells in breast cancer is well established, there are few studies examining the role of blood-based immune cell signatures in relation to clinical outcome. One study showed that a high baseline frequency of late-stage differentiated effector memory $CD8^+$ cells ($CD8^+CD45RA^+CCR7-CD27-CD28-$) was correlated with poorer survival.[30] Patients with breast cancer have a higher frequency of early differentiated T cells and a lower level of more differentiated T cells than healthy individuals without cancer,[20] although the impact of these differences on patient survival remains unknown. As such, the present study is likely the first to perform a detailed characterization of circulating immune cells in association with patient outcome in breast cancer. To achieve this we examined a large number of circulating mononuclear immune cell populations (myeloid and lymphoid) and determined associations with clinical outcome for a group of metastatic breast cancer patients treated with either cyclophosphamide (CTX)- or paclitaxel-based high-dose chemotherapy. CTX and paclitaxel are commonly used as part of standard treatment for patients with metastatic breast cancer.[31] Considering the mounting evidence implicating the functionality of the immune system in patient outcome we sought to determine whether pretreatment immune profiles could be used to improve patient selection for these drugs. This information may be valuable for individualization of patient management.

Patients and Methods

Patients

All procedures performed in studies involving human participants were in accordance with the ethical standards of Laurentian Hospital, Sudbury, and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. Informed consent was obtained from all individual participants included in this study.

Cryopreserved peripheral blood mononuclear cells were derived from a group of patients enrolled in clinical trials to test the efficacy of high dose chemotherapy and autologous stem cell transplantation for the treatment of women with metastatic breast cancer between 1991 and 1997 at Sudbury, Ontario, Canada. Patients were enrolled in 5 separate studies; 4 studies included phase 2 clinical trials to study treatment with high dose CTX, mitoxantrone, and vinblastine or carboplatin (patient outcomes for these studies were indistinguishable and are combined into group 1), and one study included a phase 1/2 clinical trial to study treatment with high dose paclitaxel, CTX, and mitoxantrone (group 2).[32,33] Eligible patients were histologically diagnosed with metastatic breast cancer (stage IV), had a Karnofsky performance status of $\geq$ 60%, did not have central nervous system metastases, and had not received chemotherapy for metastatic breast cancer or adjuvant chemotherapy for at least 6 months before enrollment. Patients were hormone receptor-negative, had progressed on hormone therapy, or had rapidly progressing disease where a response to hormone treatment could not be awaited.

Patient characteristics and high dose chemotherapy treatment regimens are listed in Table 1. Informed consent was obtained from all of the patients enrolled in these studies according to the Laurentian Hospital Research Ethics Board. The clinical outcome for these patients has been published previously.[33-35] Blood samples were taken from the patients at enrollment. The peripheral blood mononuclear cells were collected, isolated from fresh blood by Ficoll-Hypaque density gradient centrifugation, and cryopreserved in 5% dimethyl sulfoxide. Vials of cryopreserved cells were removed from liquid nitrogen storage and packaged on dry ice for shipping to Tübingen, Germany. On receipt, they were once again placed in liquid nitrogen until the day of analysis. Profiles were obtained from 88 patients (51 in group 1 and 37 in group 2).

Flow Cytometry

Peripheral blood mononuclear cells were thawed and immediately analyzed by flow cytometry. For phenotyping of blood myeloid cells (including monocytes, MDSCs, and DCs) and lymphoid populations (including the differentiation stages of $CD4^+$ and $CD8^+$ T cells) we used a previously described approach.[13] The antibody panels employed are provided in Appendix A in the online version. To identify DCs, lineage-negative events were gated, followed by CD14−and $HLA\text{-}DR^+$ cells. From this population, myeloid DCs were identified on the basis of CD11c expression, while pDCs were identified as CD123-expressing cells. To identify mMDSCs, lineage-negative events were selected before the

exclusion of CD14− cells. This population was then gated for HLA-DR$^+$ or HLA-DR− events using an HLA-DR− internal reference population. The gating steps for both myeloid and T cells are shown in Appendixes B and C in the online version. For the establishment of antibody panels, we employed fluorescence minus one controls. Due to limited availability of patient material, we could not perform multiple testing of the same sample, but consistency in machine performance was verified using cytometer setup and tracking beads before and after each sample measurement. Furthermore, repeated measurements of a biological control donor were used in each measurement to confirm consistency in measurement conditions. An overview of the immune cell phenotypes investigated is provided in Appendix D in the online version.

Statistical Analysis

Breast cancer−specific survival (BCSS) was defined as the time from study enrollment until death from breast cancer. Progression-free survival was defined as the time from study enrollment until documented progression of metastatic disease or censorship. Potential cut points to define positive versus negative marker levels were determined by quantile analysis. Flow cytometry data were sorted by ascending percentage of positive cells, and the patients were divided into groups, each with approximately 10 subjects, and the values for percentage of positive cells that distinguished each group were used as the potential quantile cut points. The quantiles were then compared for differences in BCSS based on pairwise comparisons by log-rank statistics for all patients or those in group 1 or group 2 separately. Only a single cut point was chosen, usually near the median value of positive cells (Appendix E in the online version). The cancer-specific survival likelihood was estimated by the Kaplan-Meier method and compared by log-rank tests. Cox proportional hazards regression models were used to verify single factors. The results of the Cox regression analysis are described by the means, hazard ratios, and P values (Wald test) for each immunophenotype for each group of patients. All reported P values for these analyses were unadjusted. To account for multiple comparisons (for 8 tests per panel), a Bonferroni type I error level of 0.00625 could be used. Correlations between immunophenotypes and clinicopathologic features were examined by Spearman correlation and were subjected to Bonferroni correction to account for multiple testing.

Results

Patient Response to Therapy

The patients in this study were treated with two different high-dose chemotherapy regimens. Patients in group 1 were treated with high-dose CTX-containing chemotherapy, and patients in group 2 were treated with high-dose paclitaxel-containing chemotherapy. All data were analyzed for the combined set of patients, then separately for those in group 1 or group 2. Clinicopathologic characteristics (Table 1) and clinical outcome (Figure 1) indicate that the patients in groups 1 and 2 were similar with regard to age, hormone receptor status, sites of metastasis, and clinical outcome, thus suggesting that the different therapies had equivalent efficacy.

Frequency of Circulating Monocytes and DCs Is Prognostic for Patients With Metastatic Breast Cancer

Patients with a higher frequency of circulating mature monocytes (CD14$^+$HLA-DR$^+$) showed a significant difference in BCSS. Patients in group 1, with a high frequency of mature monocytes within the CD14$^+$ population (ie, at a cutoff of > 88.8% of gated cells), showed a better BCSS than patients with a low frequency of these cells (14.0 vs. 25.2 months; hazard ratio [HR] = 1.78 (1.01−3.17), P = .049) which was also seen for the whole cohort (16.6 vs. 24.7 months, HR = 1.58 (1.01−2.47), P = .043) but not for patients in group 2 (Figure 2A; Appendix E in the online version). In contrast, patients in group 1 with a high frequency of mature monocytes (CD14$^+$HLA-DR$^+$ cells) within the CD45$^+$leukocyte population (> 38% of gated cells) had a shorter BCSS (21.4 vs. 10.5 months; HR = 0.43 (0.23−0.89), P = .021) (Figure 2B). Further, an increased frequency of cells with the MDSC phenotype (CD14$^+$HLA-DR− cells) was associated with a longer BCSS but only for patients in group 1 (15.0 vs. 26.6 months, HR = 1.83 (1.03−3.26), P = .04) (Figure 2C).

On the other hand, patients in group 2 with a higher frequency of myeloid DCs (CD11c$^+$CD123−/CD16$^+$ cells), had a longer BCSS (13.4 vs. 25.3 months, HR = 4.60 (1.23−17.1), P = .023) as did the entire cohort of patients (17.7 vs. 25.5 months, HR = 1.63 (1.04−3.33), P = .019), but not patients in group 1 (Figure 2D). Similarly, a higher frequency of pDCs (CD11c-CD123$^+$ cells) also indicated a longer BCSS for patients in group 2 (16.3 vs. 25.7 months, HR = 2.09 (1.05−4.16), P = .036) (Figure 2E).

Frequency of Circulating CD8$^+$ T Cells Is Prognostic for Patients With Metastatic Breast Cancer

The frequency of several different cytotoxic CD8+ T-cell phenotypes correlated with prognosis for patients with metastatic breast cancer treated with high-dose paclitaxel-containing chemotherapy (group 2), but not for patients treated with high-dose CTX (group 1). In general, patients with higher frequencies of early differentiated CD8$^+$CD45RA$^+$ cytotoxic T cells were associated with poorer prognosis, while patients with higher frequencies of CD8$^+$CD45RA− later differentiated cytotoxic T cells were associated with a better prognosis. In particular, we found that patients in group 2 with a higher frequency of T cells, including CD8$^+$CD45RA$^+$ (Figure 3A) or CD8$^+$CD45RA$^+$CD95− T cells (Figure 3B) had a shorter BCSS (27.5 vs. 12.6 months, HR = 0.43 (0.21−0.88), P = .021) or (28.7 vs. 12.6 months, HR = 0.42 (0.20−0.87), P = .012), respectively. Similarly, patients in group 2 with a higher frequency of the naive CD8$^+$CD45RA$^+$CD95−CD27$^+$CD28$^+$ phenotype had a shorter BCSS (28.7 vs. 12.6 months, HR = 0.32 (0.15−0.67), P = .0028) (Figure 3C). Patients with a higher frequency of CD8$^+$CD45RA$^+$CD95$^+$CD27$^+$CD28− cells also had a shorter BCSS both in the entire cohort (20.7 vs. 15.0 months, HR = 0.55 (0.30−0.95), P = .033) and in group 2 (8.0 vs. 25.9 months, HR = 6.13 (1.97−19.1), P = .0017) (Figure 3D).

In contrast, patients in group 2 with a higher frequency of later differentiated CD8$^+$ cell types, such as CD8$^+$CD45RA−CD95$^+$CD27−CD28$^+$ cells, experienced a longer BCSS (12.6 vs. 29.4 months, HR = 2.17 (1.05−4.48), P = .037)

Table 1 Clinicopathologic Characteristics of Patients With Metastatic Breast Cancer

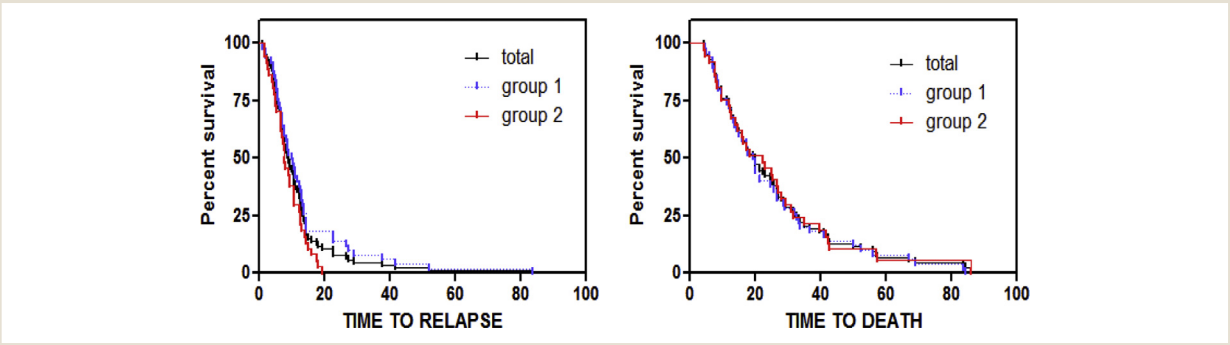
Clinical Characteristic	Total	Group 1	Group 2
Total no. of patients	88	51	37
Age			
Mean (standard deviation) (years)	43.6 (7.7)	42.7 (7.7)	44.8 (7.7)
< 40 years	22	15	7
40–49 years	45	26	19
50–59 years	21	10	11
Estrogen Receptor (N = 76)			
Negative	29	17	12
Positive	47	28	20
Progesterone Receptor (N = 75)			
Negative	33	16	17
Positive	42	28	14
HER2 (N = 76)			
Negative	36	20	16
Positive	40	24	16
No. of Metastatic Sites			
ID, 1	50	34	16
≥ 2	38	17	21
Metastatic Sites (No/Yes)			
Bone	43/45	16/25	17/20
Lung	60/28	38/13	22/15
Lymph node	65/23	39/12	26/11
Liver	72/16	42/9	30/7
Other	69/19	40/11	29/8
Adjuvant Endocrine Therapy			
No	59	35	24
Yes	29	16	13
High-Dose Chemotherapy Regimen			
Mitoxantrone, cyclophosphamide, vinblastine	29	29	
Mitoxantrone, cyclophosphamide, carboplatin	16	16	
Mitoxantrone, cyclophosphamide, paclitaxel	37		37
Thiotepa, cyclophosphamide, carboplatin	5	5	
Mitoxantrone, cyclophosphamide	1	1	

(Figure 3E). Group 2 patients and patients in the entire cohort with a higher frequency of $CD8^+CD45RA^-CD95^+CD27^+CD28^+$ cells showed a longer BCSS (12.3 vs. 25.3 months, HR = 3.16 (1.30–7.66), $P = .011$) and (14.3 vs. 21.4 months, HR = 1.74 (1.03–2.94), $P = .041$), respectively (Figure 3F). A higher frequency of $CD8^+CD95^+$ cells indicated a longer BCSS for patients in group 2 (12.5 vs. 29.4 months, HR = 5.33 (2.24–12.7), $P = .002$) and for the entire cohort (14.0 vs. 25.4 months, HR = 2.03 (1.28–3.21), $P = .0025$) (Figure 3G). A higher frequency of $CD8^+CD45RA^-CD95^+$ cells also indicated a significantly longer BCSS for patients in group 2 (15.1 vs. 31.1 months, HR = 2.61 (1.22–5.57), $P = .013$) (Figure 3H).

Frequency of Circulating $CD4^+$ T Cells Is Prognostic for Patients With Metastatic Breast Cancer

The frequency of several $CD4^+$ T helper cell subtypes also correlated with prognosis for patients with metastatic breast cancer treated with high-dose paclitaxel-containing chemotherapy (group 2). An increase in the frequency of circulating $CD4^+$ cells was associated with an improved prognosis for patients in group 2 (15.5 vs. 25.3 months, HR = 2.35 (1.10–5.02), $P = .028$), but not for the entire cohort or for patients in group 1 (Figure 4A). In addition, a higher frequency of $CD4^+CD95^+$ cells was also associated with a longer BCSS for patients in group 2 (12.5 vs. 31.1 months, HR = 3.98 (1.79–8.83), $P = .007$) (Figure 4B).

Figure 1 Clinical Outcome of Patients Treated With High-Dose Chemotherapy Including Cyclophosphamide (Group 1) or Paclitaxel (Group 2)



A higher frequency of some early differentiated T helper cell subtypes that express the $CD4^+$ and $CD45RA^+$ markers was associated with a poorer prognosis. For example, there was a shorter BCSS for patients in group 2 with a higher frequency of $CD4^+CD45RA^+CD95-$ cells (28.0 vs. 16.0 months, HR = 0.30 (0.13−0.69), $P = .0046$) (Figure 4C), a higher frequency of $CD4^+CD45RA^+$ cells (25.1 vs. 14.1 months; HR = 0.25 (0.09−0.072); $P = .011$) (Figure 4D), and a higher frequency of $CD4^+CD45RA^+CD95-CD27^+CD28^+$ cells (29.4 vs. 15.1 months, HR = 0.45 (0.22−0.91), $P = .027$) (Figure 4E).

In contrast, there was a longer BCSS for patients in group 2 with a higher frequency of later differentiated T helper cells, $CD4^+CD45RA-CD95^+$ (13.4 vs. 31.1 months, HR = 3.71 (1.68−8.21), $P = .0012$) (Figure 4F); a higher frequency of central memory T helper cells, $CD4^+CD45RA-CD95^+CD27^+CD28^+$ cells (15.1 vs. 25.3 months, HR = 2.33 (1.11−4.87), $P = .025$) (Figure 4G); and a higher frequency of effector T helper cells, $CD4^+CD45RA-CD95^+CD27-CD28^+$ cells (13.4 vs. 31.1 months, HR = 4.53 (2.01−10.21), $P = .0003$) (Figure 4H).

Associations Between Clinicopathologic Characteristics and Immune Cell Frequencies in Patients With Metastatic Breast Cancer

Because previous studies had shown correlations between the frequencies of specific cell populations and hormone receptor status,[3,36,37] we tested for interactions between the frequency of different immunophenotypes and clinicopathologic characteristics. We found no association between patient age and any of the immune cell populations. There were a few associations between estrogen receptor and progesterone receptor status and immunophenotype, but none remained significant after Bonferroni correction for multiple testing. However, there were several $CD4^+$ (but not monocyte, DC, or $CD8^+$) cell immunophenotypes that were associated with differences in HER2 status. $HER2^+$ patients in the entire cohort were associated with a higher frequency of T helper cell types including $CD4^+$ ($P = .009$), $CD4^+CD45RA^+$ ($P = .0018$), $(CD4^+)CD45RA^+CD95^+CD27-CD28^+$ ($P = .0019$), and $(CD3^+)CD4^+CD45RA^+CD95^+$ ($P = .0046$) cells and a lower frequency of effector $(CD4^+)CD45RA-CD95^+CD27-CD28^+$ ($P = .0025$) T cells compared to HER2− patients (data not shown).

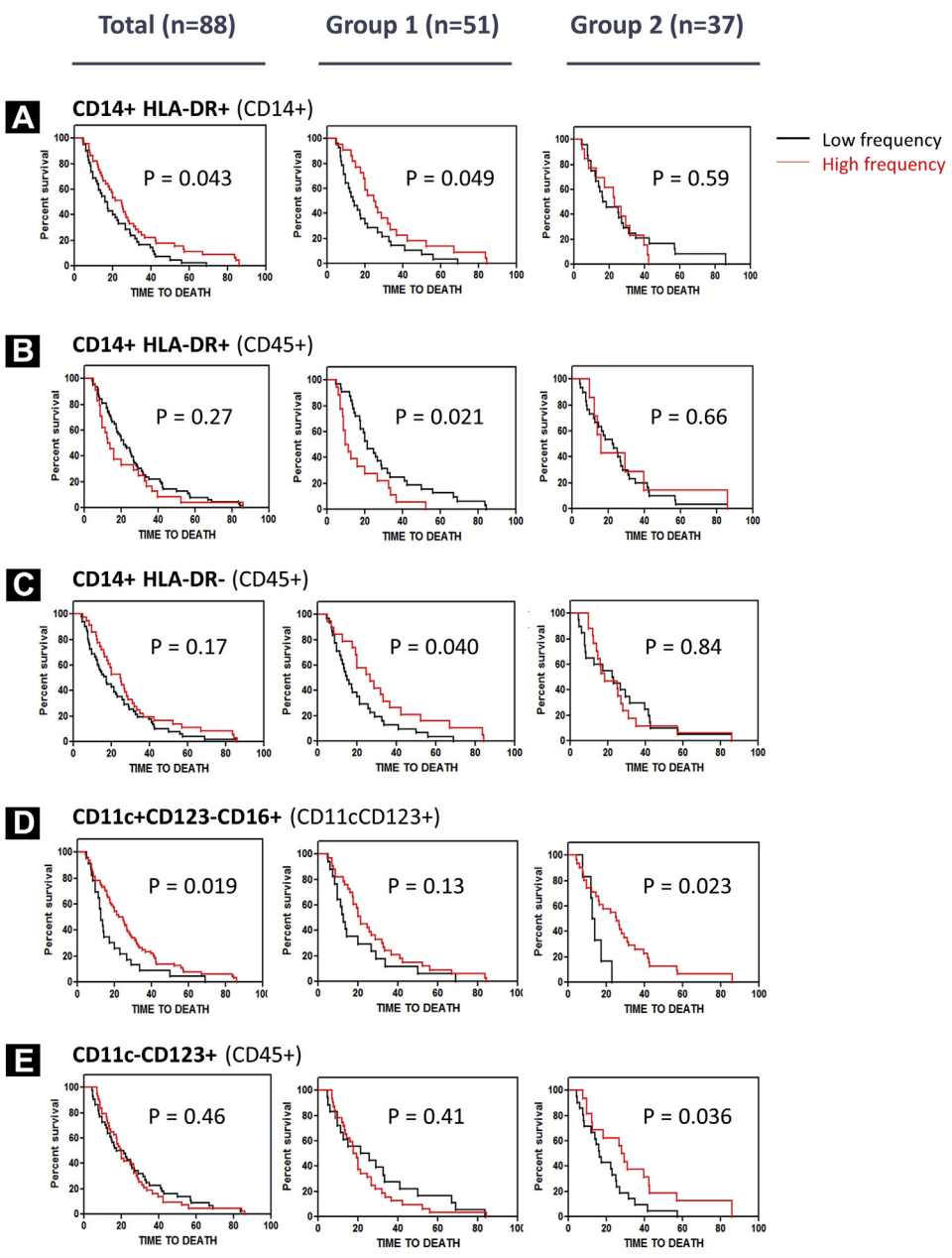
Discussion

In this study, the relative frequencies of certain monocyte, DC, and/or helper ($CD4^+$) and cytotoxic ($CD8^+$) T-cell subtypes were shown to correlate with BCSS for patients with metastatic breast cancer treated with high-dose chemotherapy. The ability of the frequency of an immune cell type to indicate differences in BCSS was dependent upon the chemotherapy regimen used to treat the patients. Differences in the frequency of some monocyte subtypes could predict prognosis for patients treated with high-dose CTX-based chemotherapy (group 1) while differences in some $CD4^+$ and $CD8^+$ T-cell subtypes could predict prognosis for patients treated with high-dose paclitaxel-based chemotherapy (group 2). Except for their treatment regimens, the patients in group 1 and group 2 were very similar in age, hormone receptor status, and clinical outcome following treatment. In addition, the frequencies of the different immune cell types were similar between the two groups of patients. Since the samples tested for this study were obtained before treatment with chemotherapy the difference in the ability of distinct baseline immune subsets to predict BCSS must be selected by outcome in response to the particular chemotherapy.

Different chemotherapy drugs have differential effects on specific immunophenotypes. For example, treatment with doxorubicin or paclitaxel can eliminate MDSCs in a mouse model[38,39] and treatment of patients with paclitaxel can decrease the number of peripheral MDSC[40] and enhance the immune response.[41] In contrast, treatment with CTX can increase the number of MDSC[42,43] and decrease the function and number of $CD4^+$ and $CD8^+$ Tregs[44,45] and cytotoxic T cells and T helper cells.[46] Therefore, treatment with different chemotherapies can alter immune survival to ablate the effects of the baseline immunophenotype on clinical outcome. For example, paclitaxel treatment eliminates MDSC, which could eliminate their impact after treatment, but has minimal effects on T cells, which allows the effects of baseline T-cell levels to remain prognostic.

Patients treated with CTX-based high-dose chemotherapy (group 1) and a higher frequency of functional monocytes ($CD14^+HLA-DR^+$) within the $CD14^+$ population (frequency range 47%−100%) correlated with a better outcome while patients with a higher frequency of $CD14^+HLA-DR^+$ as a proportion of all $CD45^+$ leukocytes (frequency range 5.4%−62.9%) correlated with a poorer

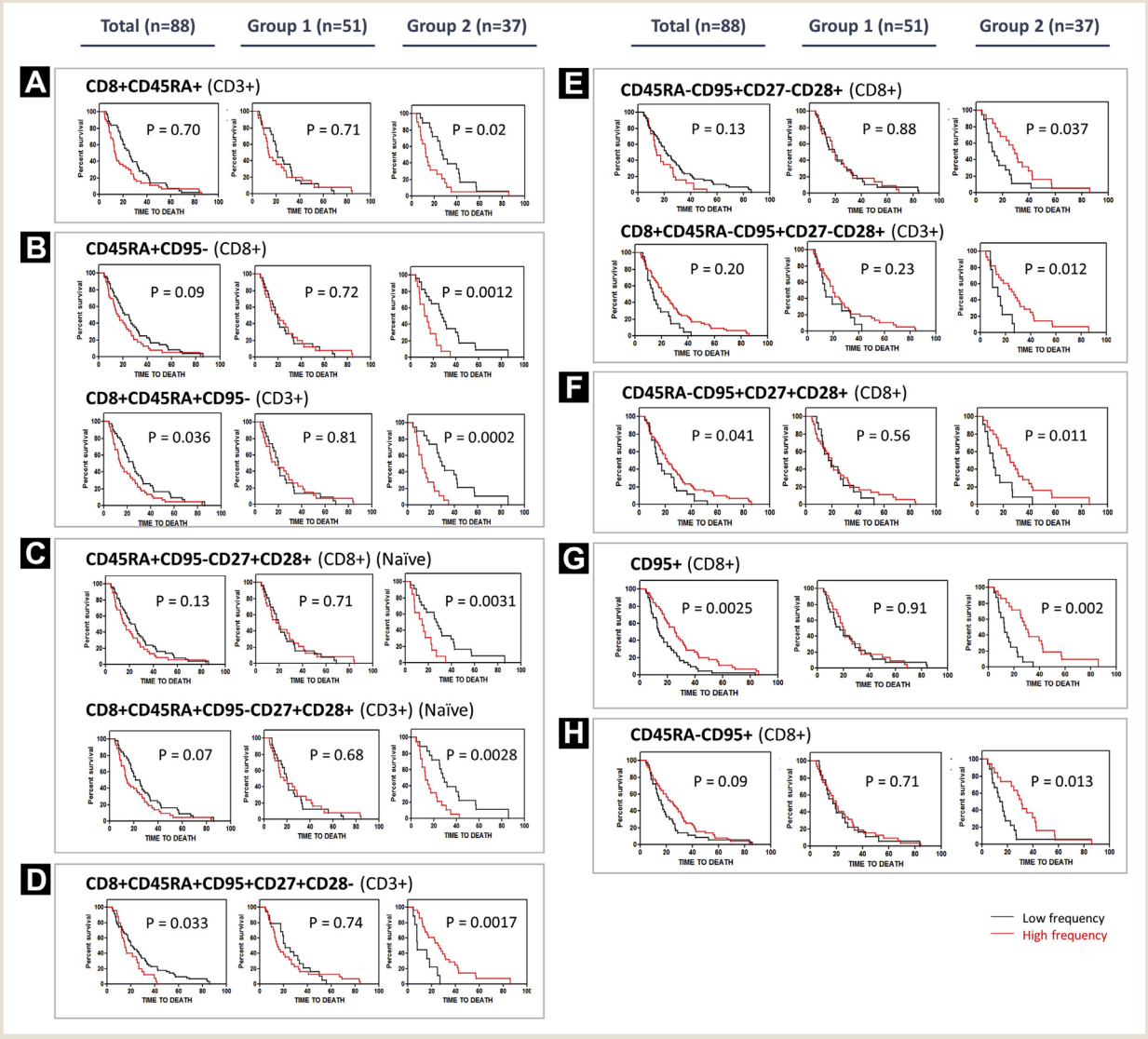
Figure 2 Impact of Myeloid Immunophenotypes on Breast Cancer–Specific Survival for Patients Treated With Cyclophosphamide-Based (Group 1) or Paclitaxel-Based (Group 2) High-Dose Chemotherapy. Total Cohort (Left, n = 88), Group 1 (Center, n = 51), and Group 2 (Right, n = 37) Were Characterized as High-Frequency (Red) or Low-Frequency (Black) Levels of Indicated Cell Type. (A-C) Indicate Monocytic Populations, Whereas (D and E) Refers to Dendritic Cell Populations. Percentage Survival Was Analyzed Using the Kaplan-Meier Survival Method. Indicated *P* Values Were Determined by Log-Rank Statistics for Comparison. Populations Shown in Brackets After Each Immune Cell Phenotype Indicate the Reference Population Used to Determine the Frequency of Cells in Each Patient



prognosis. Selection of $CD14^+HLA\text{-}DR^+$ cells from the $CD14^+$-gated population identifies the majority of the monocyte population while selection from the $CD45^+$ gated common leukocyte population is likely to include cells in addition to $CD14^+$ monocytes, such as DCs, which could have a significant impact on the relative frequency for each patient.

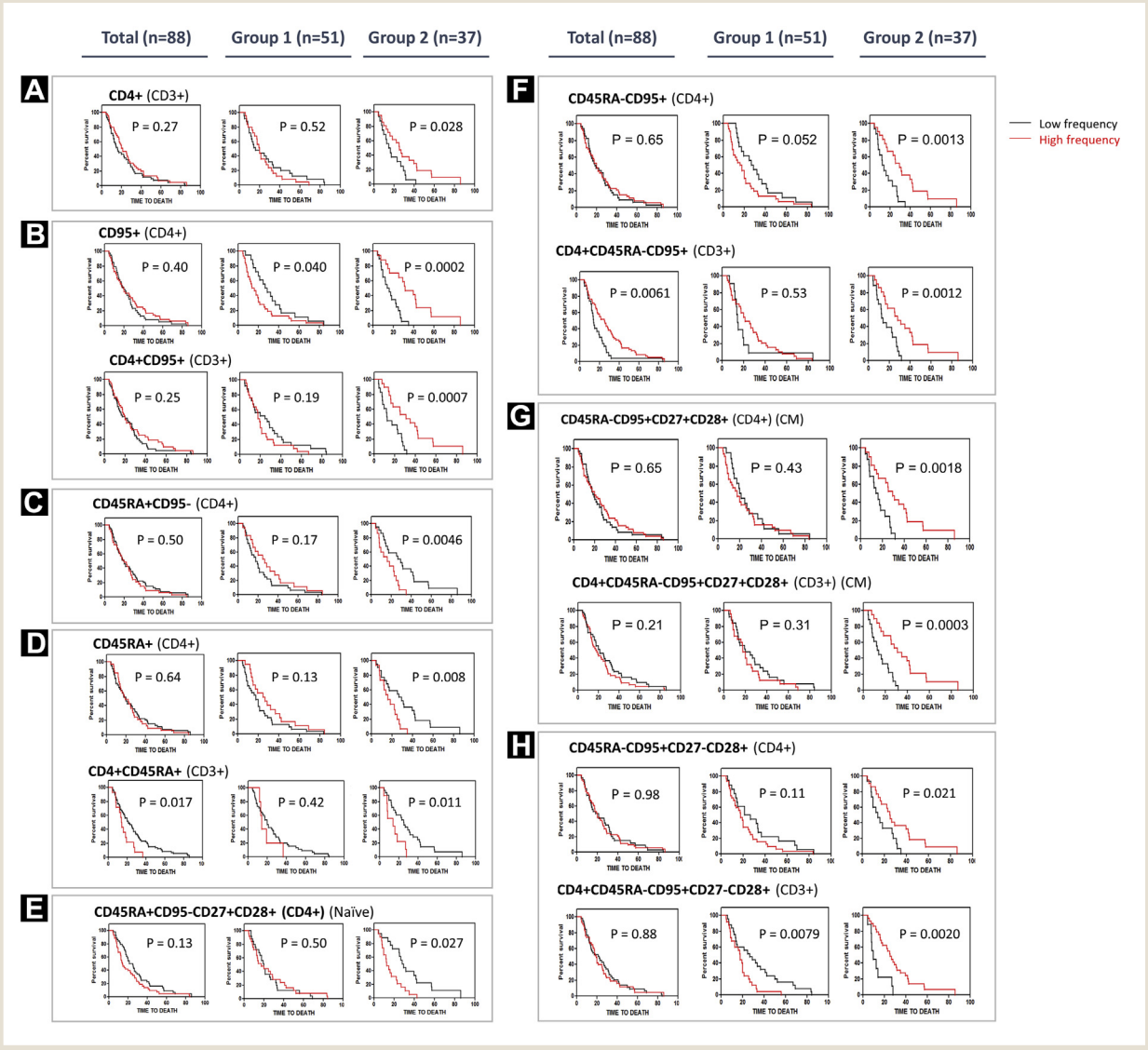
A higher frequency of cells with the MDSC phenotype $CD14^+HLA\text{-}DR-$ within the $CD14^+$ gated population (frequency range 0.01%–24.7%) was unexpectedly associated with a superior survival for patients treated with high-dose CTX. This is different from some previously published results, under other treatment conditions, where higher baseline levels of MDSC (eg, > 1% of

Figure 3 Impact of CD8+ Cytotoxic T Cell Immunophenotypes on Breast Cancer–Specific Survival for Patients Treated With High-Dose Chemotherapy Including Cyclophosphamide (Group 1) or Paclitaxel (Group 2). Total Cohort (Left, n = 88), Group 1 (Center, n = 51), and Group 2 (Right, n = 37) Were Characterized as High-Frequency (Red) or Low-Frequency (Black) Levels of Indicated Cell Type. (A-D) Refer to CD45RA+ Populations, (E, F and H) Indicate CD45RA− Phenotypes, While (G) Shows a CD95+ Population. Percentage Survival Was Analyzed Using the Kaplan-Meier Survival Method. Indicated *P* Values Were Determined by Log-Rank Statistics for Comparison. Populations Shown in Brackets After Each Immune Cell Phenotype Indicate the Reference Population Used to Determine the Frequency of Cells in Each Patient



total peripheral blood mononuclear cells) in patients with breast cancer are associated with a poorer prognosis.[10,14,47] One reason for such differences is that MDSC have been defined in previous studies using different phenotype markers than those employed here such as CD13, CD15, and CD33 which could impact the ability to compare results across studies.[18,48-50] Some reports that suggest that the levels of MDSC in breast cancer tumors do not correlate with the level of MDSC in the circulation.[51] Further, cryopreservation also depletes granulocytic MDSC which can alter the interpretation of some previous observations.[51] MDSC are thought to interfere with normal activation of adaptive immune responses against the tumor and promote the production of angiogenic growth factors to support tumor growth and spread.[52] The observation that CTX can enhance MDSC levels[42] suggests that it may be able to accelerate the expected inhibitory effect of MDSC cells in patients with lower baseline MDSC frequencies. In this study, the frequency of MDSC did not correlate with outcome for patients treated with high-dose paclitaxel-containing chemotherapy. Treatment with paclitaxel can strongly inhibit MDSC[41] and decrease MDSC number which could ablate the expected inhibitory effect of MDSC in patients with high baseline levels of MDSC.

Figure 4 Impact of CD4+ Helper T Cell Immunophenotypes on Breast Cancer–Specific Survival for Patients Treated With High-Dose Chemotherapy Including Cyclophosphamide (Group 1) or Paclitaxel (Group 2). Total Cohort (Left, n = 88), Group 1 (Center, n = 51), and Group 2 (Right, n = 37) Were Characterized as High-Frequency (Red) or Low-Frequency (Black) Levels of Indicated Cell Type. (A) Refers to CD4+ T Cells, (B) Indicates CD95+ T Cell Populations, (C-E) Indicate CD45RA+ Phenotypes, (F-H) Show CD45RA− Populations. Percentage Survival Was Analyzed Using the Kaplan-Meier Survival Method. Indicated P Values Were Determined by Log-Rank Statistics for Comparison. Populations Shown in Brackets After Each Immune Cell Phenotype Indicate the Reference Population Used to Determine the Frequency of Cells in Each Patient



Abbreviation: CM, central memory.

Increased levels of DCs, including pDCs ($CD123^+$) and myeloid DCs ($CD11c^+$), were associated with an improved BCSS for patients treated with paclitaxel-based high-dose chemotherapy (group 2) but not for patients treated with CTX-based chemotherapy (group 1). A previous study has shown that an increased frequency of pDCs was associated with a better prognosis for patients with breast cancer[9] which supports the idea that an increased level of antigen-presenting DCs could be associated with an improved antitumor immune response, regardless of the type of therapy given.

We showed that a high frequency of $CD45RA^+$ early differentiated $CD4^+$ and/or $CD8^+$ T cells was associated with a relatively poor prognosis for patients with metastatic breast cancer treated with high-dose paclitaxel-containing chemotherapy. This is consistent with the idea that most $CD45RA^+$ T cells, especially $CD4^+$ T cells, are early stage T cells that have not been previously activated[53] and should not be involved in antitumor activity. In contrast, we showed that a high frequency of CD45RA− later differentiated $CD4^+$ and/or $CD8^+$ T cells was associated with an improved

prognosis which supports the idea that previously activated $CD4^+$ or $CD8^+$ CD45RA− T cells[53] may contribute to antitumor activity. Studies in melanoma also showed that a higher baseline frequency of $CD8^+$ effector memory 1 T cells ($CD8^+CD45RA-CD27^+CD28^+$) was associated with an improved outcome.[20] Our observation that CD45RA− T cells are associated with improved prognosis makes it unlikely that the level of Tregs makes a significant contribution to prognosis in this group of patients. It appears that the level of later differentiated $CD4^+$ subtypes makes the biggest contribution to prognosis for patients in group 2 since the level of $CD4^+$ cells converted to later stages of differentiation is larger than the level of converted $CD8^+$ cells. The median frequency of helper T cells ($CD3^+CD4^+$) in the entire cohort of patients was > 50% (range 14%−78%) and the median frequency of $CD3^+CD4^+CD45RA^+$ T cells was only 13% (range 2%−47%) while the median frequency of cytotoxic T cells ($CD3^+CD8^+$) was 35% (range 14%−57%) and the median frequency of $CD3^+CD8^+CD45RA^+$ T cells was 22% (range 6%−40%).

The increased frequency of cells expressing CD95 in both the $CD4^+$ and $CD8^+$ T cell populations also seems to be associated with an improved prognosis for the patients in group 2, consistent with previous reports.[54,55] Further, the association of $CD95^+$ T cells with an improved prognosis is related to its expression of CD45RA−: T cells that are CD45RA− and $CD95^+$ indicate an improved prognosis while T cells that express CD45RA but not CD95 indicate a poorer prognosis (whereas an increased frequency of $CD45RA^+CD95^+$ cells or CD45RA−CD95− T cells did not indicate a difference in prognosis). In addition, the frequency of $CD45RA^+$ cells that also express $CD95^+$ is different between $CD4^+$ and $CD8^+$ cells. A significantly higher frequency of $CD3^+CD4^+CD45RA^+$ do not express CD95 (median 9.2%) compared to $CD3^+CD4^+CD45RA^+$ cells that are also $CD95^+$ (median 2.7%) while both $CD3^+CD8^+CD45RA^+CD95-$ cells (10%) and $CD3^+CD8^+CD45RA^+CD95^+$ cells (11%) are similar. This is consistent with the observation that a greater number of $CD4^+$ subtypes contribute to prognosis for the patients in group 2. In addition, HER2 status correlates with the frequency of a subgroup of the $CD4^+$ cells (but not $CD8^+$ cells) suggesting the possibility that a group of patients can express anti−HER2−reactive $CD4^+$ cells which may contribute to antitumor activity. However, $HER2^+$ patients with a higher frequency of effector T helper cells that were CD45RA− did not show a significant difference in outcome. Some previous reports have indicated that tumor subtype (or hormone receptor status) can impact the ability of different immune subtypes to predict prognosis in breast cancer patients.[3,36,37] For example, patients with luminal (estrogen receptor positive) breast cancer subtypes had a lower level of T-cell infiltration than patients with nonluminal (estrogen receptor negative) breast cancer and patients with triple-negative breast tumors, which have the poorest prognosis, had higher levels of T-cell infiltration. In this group of patients, the breast cancer type (luminal A/normal, luminal B, HER2 positive, and triple negative) did not correlate with differences in the frequency of the circulating $CD4^+$ or $CD8^+$ immunotypes. This suggests that there is no difference in immunophenotype specific for hormone receptor status in our metastatic breast cancer patients although it is possible that the reported differences in infiltrating T cells are not reflected in circulation.

Conclusion

Immunophenotypes can be used as biomarkers of prognosis for patients with metastatic breast cancer, although this is highly dependent on the chemotherapy used for treatment. The present study shows that a higher frequency of certain circulating $CD4^+$ immunophenotypes, and to a lesser extent of $CD8^+$ immunophenotypes, in particular those expressing CD95 but not CD45RA, were associated with a better clinical outcome for metastatic breast cancer patients treated with paclitaxel-based high-dose chemotherapy. The frequency of circulating $CD11c^+CD123-$ DCs was also associated with prognosis for this group of patients. In contrast, patients treated with CTX-based high-dose chemotherapy showed that some monocytic cells, but not T cells or DCs, could be correlated with outcome. It will be interesting to see if differences in chemotherapy regimen will identify different prognostic biomarkers in other studies of breast cancer patients.

Clinical Practice Points

- The frequency of circulating myeloid or T cell populations can indicate clinical outcome for some groups of patients with metastatic breast cancer being treated with chemotherapy.
- The utility of using immunophenotype as a biomarker for clinical outcome for patients with metastatic breast cancer depends on the chemotherapy regimen used for treatment.
- Different chemotherapy regimens can differentially affect the ability of immunophenotype to predict outcome in patients with metastatic breast cancer and therefore may also affect the potential success of immune therapies.

Disclosure

The authors have stated that they have no conflict of interest.

Supplemental Data

Supplemental appendixes accompanying this article can be found in the online version at https://doi.org/10.1016/j.clbc.2019.05.002.

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