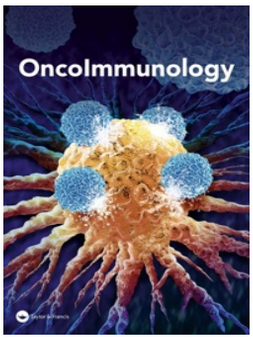

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Blood immune profiling of Ethiopian patients with breast cancer highlights different forms of immune escape

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ABSTRACT

Breast cancer (BC) is a leading cause of death worldwide, particularly also among African woman. In order to better stratify patients for the most effective (immuno-) therapy, an in depth characterization of the immune status of BC patients is required. In this study, a cohort of 65 Ethiopian patients with primary BC underwent immune profiling by multicolor flow cytometry on peripheral blood samples collected prior to surgery and to any other therapy. Comparison with peripheral blood samples from healthy donors highlighted a general activation of the immune system, accompanied by the presence of exhausted $CD4^+$ T cells and senescent $CD8^+$ T cells with an inverted CD4/CD8 ratio in approximately 50% of BC cases. Enhanced frequencies of γδ T cells, myeloid-derived suppressor cells and regulatory T cells were also found. Correlation with clinical parameters demonstrated a progressive reduction in T cell frequencies with increasing histopathological grading of the tumor. Differences in $CD8^+$ T cells and B cells were also noted among luminal and non-luminal BC subtypes. In conclusion, Ethiopian BC patients showed several alterations in the composition and activation status of the blood immune cell repertoire, which were phenotypically associated with immune suppression. The role of these immunological changes in the clinical outcome of patients with BC will have to be determined in follow-up studies and confirmed in additional patients' cohorts.

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Biomarker; blood; breast cancer; immune escape

Introduction

Breast cancer (BC) is the most commonly diagnosed cancer worldwide with an increasing incidence and mortality in sub-Saharan Africa.[1] It is a heterogeneous disease classified into three subtypes based on hormone receptors (HR) expression and HER2/neu status.[2] Targeted therapies for the HR^+ and $HER2^+$ tumors have been developed, but the existence of intrinsic or acquired resistance to these therapies resulted in in-depth genetic studies leading to the identification of different intrinsic molecular subtypes of BC.[3] HR^+ tumors are now divided into luminal A and B tumors based on the proliferation rate of the tumor cells, while immunohistochemically defined triple-negative breast cancer (TNBC) are divided into different basal-like subtypes based on their genetic characteristics.[4,5] However, there exists increasing evidence that BC classification has to be refined by including not only the molecular and biologic features of the cancer cells, but also information on the composition and distribution of the immune cell infiltrate.[6] Indeed, immune cells can directly or indirectly eliminate neoplastic cells, which might then developed various strategies to escape from ongoing anti-tumor immune response.[7–9] The frequency of infiltrating innate and adaptive immune cells significantly varied in the different breast cancer subtypes and played an important role in disease progression and responsiveness to therapies.[10,11]

Despite immunohistochemical evaluations of tumor tissues provide the most precise insights into tumor-immune cell interactions, limitations associated with the invasive procedures and possible bias of small biopsy samples[12] promoted the use of blood samples to assess, also longitudinally the

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systemic immune characteristics of cancer patients,[13] which were found to be correlated with therapy response and patients' outcome.[14–16]

To advance the understanding of BC immunology in Ethiopian patients, the immune cell repertoire of patients with newly diagnosed BC was evaluated by multicolor flow cytometry and compared to that of healthy controls (HC). The immunological characteristics identified in this study reflect general attributes of BC immunology, but also some novel features, which may be specific for Ethiopian patients and might help to identify immunotherapeutic targets specific to these patients.

Materials and methods

Patients and control cohorts

This prospective cross-sectional study was conducted on 65 newly diagnosed BC patients recruited from three public hospitals in Addis Ababa, Ethiopia, between 2018 and 2021. Clinico-pathologic data were retrieved from patients' medical card. Tumor subtypes had been identified both by immunohistochemistry and using the PAM50 gene set.[17] Females without known disease ($n = 10$) were included as healthy controls (HC). This study was approved by the institutional review board of the College of Health Sciences of Addis Ababa University (protocol 092/17/17) and the National Research Ethics Review Committee of Ethiopia (protocol MOSHE//RD). Written informed consent was obtained from participants prior to sample and data collection.

Blood collection and processing

Peripheral blood mononuclear cells (PBMC) were collected from BC patients naïve to any therapy prior to surgery and from HCs. Cells were separated by density gradient centrifugation on Histopaque®-1077 (Sigma-Aldrich, Taufkirchen, Germany) using the standard procedure and then directly cryopreserved in 10% v/v dimethyl sulfoxide (DMSO, Sigma-Aldrich) and 90% v/v fetal bovine serum (FBS, Thermo Fisher Scientific, Waltham, MA USA) until use.

PBMC characterization by flow cytometry

Flow cytometry was performed on cryopreserved PBMC using a panel of antibodies (Ab, Supplementary Table S1) as recently described.[18] In brief, after thawing, 1–4 × 10^6 PBMC cells were incubated with a Fixable Viability stain (FVS 700; BD Bioscience, Heidelberg, Germany) followed by incubation with the respective Ab for 15 min in the dark. For intracellular markers, cells were permeabilized with Fix/Perm buffer (BD Bioscience) and then incubated with the Ab in the dark for 40–50 min. Stained cells were measured on the LSR Fortessa II (BD Bioscience) flow cytometer and data were analyzed using BD FACSdiva software (BD Bioscience). Gating strategies for the different (sub) populations are provided in Supplementary Figures. Since PBMCs were employed, no absolute cell counts were determined, and data are presented as cell frequencies within the PBMC or the indicated immune cell subsets.

Statistical analysis

The unpaired t test with or without Welch's correction was implemented to compare the means of two independent groups with Gaussian distribution. Mann Whitney test was used for non-parametric tests. For evaluation of three or more groups, ordinary one-way ANOVA or Brown-Forsythe and Welch ANOVA tests were applied for normally distributed data, whereas the Kruskal–Wallis test was implemented for groups with non-Gaussian distribution. Spearman test was done for continuous variables. All statistical analyses were performed in GraphPad Prism v9 (GraphPad, San Diego, CA, USA) and p values <0.05 were considered statistically significant and are shown as * ($p < 0.05$), ** ($p < 0.01$), *** ($p < 0.001$) or **** ($p < 0.0001$).

Results

Characteristics of the BC patients and HC

In total, 65 Ethiopian BC patients with a median age of 41 years (22–83) were recruited for the study, while the control cohort consisted of 10 healthy females (median age 36 years). The BC cases were equally distributed between stage 2 and stage 3 tumors according to the American Joint Committee on Cancer (AJCC) TNM system, with only a few stage 1 cases ($n = 5$, 8%) (Table 1). On the contrary, more than half of the lesions were grade 2. With respect to the histological type, most of the tumors were invasive ductal carcinoma (IDC) (80%), whereas only 3 cases (4.6%) were invasive lobular carcinoma (ILC) and the rest of unknown, mixed or other histology (Table 1). The majority of the patients were positive for HR (78.5%) and negative for HER2 overexpression (63%). Classification of the PAM50 intrinsic subtype was performed for 45 out of 65 patients, with the luminal A subtype being the most frequent, observed in 21 cases (Table 1).

T cells and their subpopulations

BC patients exhibited higher frequencies of total $CD3^+$ T cells within the PBMCs compared to HCs, primarily due to significantly elevated levels of $CD8^+$ T cells, whereas $CD4^+$ T cells had a non-significant trend toward reduced numbers (Figure 1a). Evaluation of the memory phenotype by staining of CCR7 and CD45RA (gating strategy in Supplementary Figure S1) demonstrated no significant differences among $CD4^+$ T cells (Figure 1b), whereas $CD8^+$ T cells had fewer naïve and more central memory (Tcm) cells (Figure 1c). Moreover, higher frequencies of HLA-DR^+ and lower of $CD38^+$ cells were found in BC patients for both $CD4^+$ and $CD8^+$ T cells (Figure 1d–e). In contrast, an enhanced expression of PD1 and PD-L1 was only found in $CD4^+$ T cells of BC patients (Figure 1f). $CD8^+$ T cells had less CD28, but more perforin expression (Figure 1g) and also a non-statistically significant trend toward more $CD57^+$ cells in BC patients compared to HC (data not shown).

The frequencies of γδ T cells were significantly increased in PBMC of BC patients compared to that of HC (Figure 2a). Interestingly, the Ethiopian cohort of HC had higher

Table 1. Clinico-pathologic characteristics of breast cancer patients.

Features	Patient number (%)
Age	
median	41
min	22
max	83
Pathologic Stage	
stage 1	5 (8%)
stage 2	28 (43%)
stage 3	28 (43%)
unknown	4 (6%)
Histological grade	
G1	4 (6%)
G2	37 (57%)
G3	22 (33.9%)
unknown	2 (3.1%)
Histological type	
Invasive ductal	52 (80%)
Invasive lobular	3 (4.6%)
Mixed (ductal & lobular)	2 (3.1%)
Others	6 (9.2%)
unknown	2 (3.1%)
HR status	
HR^+	51 (78.5%)
HR^-	8 (12.3%)
unknown	6 (9.2%)
HER2 status	
$HER2^+$	16 (24,7%)
$HER2^-$	41 (63%)
unknown	8 (12.3%)
Ki-67	
low (<20%)	19 (29.2%)
high (≥20%)	39 (60%)
Unknown	7 (10.8%)
Intrinsic subtype (PAM50)	
luminal A	21 (32.3%)
luminal B	8 (12.3%)
HER2-enriched	6 (9.2%)
basal-like	10 (15.4%)
unknown	20 (30.8%)

frequencies of γδ T cells (3.2–16.3% of all T lymphocytes) than the Caucasian population (0.5–5% of T cells[19]).

Regulatory T cells (Tregs) identified as $CD25^+$ $FoxP3^+$ $CD127^{low}$ $CD4^+$ cells (gating strategy in Supplementary Figure S2) showed a non-statistically significant trend toward enhanced frequencies both among PBMC and $CD4^+$ T cells in BC patients compared to HC (Figure 2b and data not shown). Since Tregs display a functional heterogeneity, discrimination of different functional subsets based on the staining for the chemokine receptor CCR4 and the activation marker CD45RO[20] highlighted an increase of the highly suppressive double positive subpopulation, which reached significance only as percentage within the Treg but not total PBMC (Figure 2c–d).

CD4/CD8 T cell ratio alterations in breast cancer

The expansion of $CD8^+$ T cells caused 50–70% of the BC patients ($n = 33$) to have inverted CD4/CD8 T cell ratio (Figure 3a). Comparison of BC patients with inverted (BC^{IR}, CD4/CD8 < 1) or normal ratio (BC^{NR}, CD4/CD8 > 1) indicated no correlation between the CD4/CD8 ratio and patients´ age ($p = 0.0928$; data not shown). An increase in central memory $CD8^+$ T cells was associated with the BC^{NR} sub-cohort, whereas the loss of naïve and increase of effector $CD8^+$ T cells was restricted to the BC^{IR} patients (Figure 3b), who also had a more substantial loss of CD28 and an increase in perforin and CD57 expression by $CD8^+$ T cells (Figure 3c). With respect to $CD4^+$ T cells, less naïve and more effector memory cells were present in the BC^{IR} patients than in BC^{NR} and HC (Figure 3d). The expression of PD1, PD-L1, CD28 and CD57 on $CD4^+$ T cells was higher in BC^{IR} patients, but not statistically significantly different from BC^{NR} patients (data not shown).

B cell frequencies and phenotypes

The total frequency of B lymphocytes was comparable between BC patients and HC (data not shown). Further gating based on IgD and CD27 staining (Supplementary Figure S3) showed statistically significant lower levels of naïve B cells and higher levels of plasma blasts in BC patients (Figure 4a). A non-statistically significant trend toward higher frequencies of switch memory B cells in BC patients was found (Figure 4a). Interestingly, PD1, PD-L1 and CTLA4 were significantly elevated in B cells from BC patients when compared to HC (Figure 4b).

Natural killer (NK) cells

The frequencies of NK cells, evaluated as a total population or upon subdivision into the $CD56^{bright}$ and $CD56^{dim}CD16^{bright}$ subsets were comparable in the patients' and HC cohort (data not shown). In contrast, NK cells from BC patients displayed higher expression levels of the CD3ζ chain and perforin than HC, resulting in higher frequencies of double positive cells (Supplementary Figure S4).

Myeloid cells

Dendritic cells (DC), identified among lineage-negative cells (i.e. CD3, CD19, CD16 and CD56 negative) as $HLA\text{-}DR^{high}$ cells (Supplementary Figure S5), were significantly reduced in BC patients compared to HC, with a reduction in both $CD11c^+$ myeloid (mDC) and $CD123^+$ plasmacytoid DC (pDC; Figure 5a).

The number of total monocytes tended to be non-statistically significant lower in BC patients (Figure 5b) but subdivision into classical, intermediate and inflammatory subsets based on the CD14 and CD16 expression (Supplementary Figure S5) did not highlight significant differences between BC patients and HC (data not shown).

Myeloid-derived suppressor cells (MDSC) are a heterogeneous population which can be subdivided into $CD11b^+$ $CD14^-$ $CD15^+$ polymorphonuclear MDSC (PMN-MDSC), $CD11b^+$ $CD14^+$ $HLA\text{-}DR^{-/low}$ $CD15^-$ monocytic MDSC (M-MDSC) and $HLA\text{-}DR^{-/low}$ $CD33^+$ early-stage MDSC (e-MDSC) (gating strategy in Supplementary Figure S6). The frequencies of the highly immune suppressive PMN-MDSC subpopulation were significantly higher in BC patients than in HC (p value < 0.0001) (Figure 5c), whereas only a trend toward expansion in BC patients was found for the M-MDSC. Unexpectedly, e-MDSC were significantly lower in BC patients than in HC.

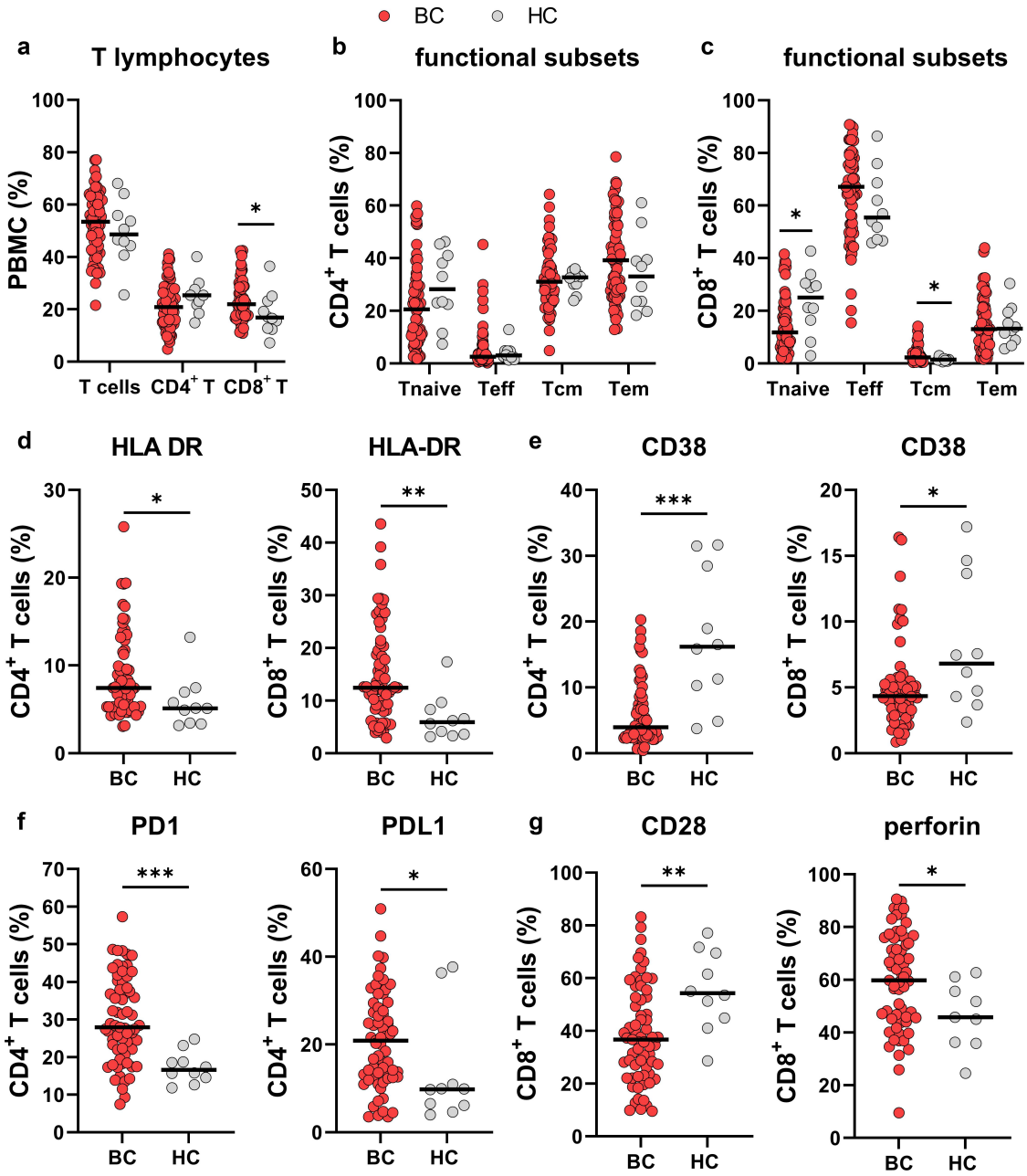

Figure 1. Frequency and phenotype of the major T cell subpopulations. The percentages of total T cells, $CD4^+$ and $CD8^+$ T cells within the PBMC (a), of the different memory subsets of $CD4^+$ (b) and $CD8^+$ T cells (c) and of $CD4^+$ or $CD8^+$ T cells expressing the indicated markers (d-g) are shown for BC patients and HC as individual values together with their median values. Tnaive; naïve T cells, Teff; effector memory T cells, Tcm; central memory T cells, Tem: effector T cells.

Clinical relevance of the systemic immune cell composition of BC patients

Next, the clinical relevance of the systemic immune characteristics of BC patients was determined. Immune parameters neither correlated with the pathologic stage nor with the tumor HR or HER2 status. Reduced T cell frequencies were detected in tumors with increased grading with a statistical significance reached between the slowly proliferating G1 and the highly aggressive G3 tumor (Figure 6a), but the number of G1 breast cancer cases was very low.

No significant differences among the four intrinsic subtypes were found but in comparison to HC, patients with luminal A tumors displayed significantly higher frequencies of $CD4^+$ T cells expressing PD1 (Figure 6b). Division of the BC patients into luminal ($n = 29$) and non-luminal cases ($n = 16$), showed that luminal tumor associated with higher frequencies of $CD8^+$ T cell, and thus also lower CD4/CD8 T cell ratio (Figure 6c–d) whereas the opposite tendency was found for B cells with enhanced frequencies in non-luminal tumors (Figure 6e).

Despite the limited number of ILC cases, some statistically significant differences were also found with respect to the histologic subtypes. Among the major populations within the PBMC, ILC cases had reduced frequencies of $CD56^{br}$ NK cells (Figure 6f). In contrast, within the $CD4^+$ T cells, ILC had higher frequencies of CD57 expressing cells as well as effector $CD4^+$ T cells whereas central memory $CD4^+$ T cells were significantly reduced (Figure 6g–i).

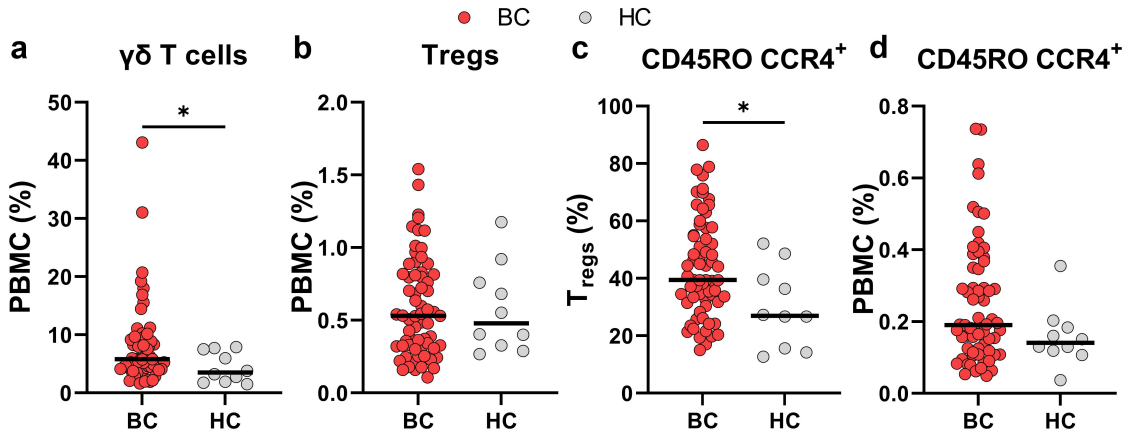

Figure 2. Frequencies of minor T cell subpopulations. The frequencies of γδ T cells (a) and Treg (b) within total PBMC as well as of the CD45RO CCR4 double positive Treg within the Treg (c) or total PBMC (d) are shown for BC patients and HC as individual values together with their median values.

Discussion

To best of our knowledge, this study is the first comprehensive assessment of the systemic immune landscape of BC patients in Ethiopia and one of the few conducted in Africa. Given the high prevalence of malnutrition and endemic infectious diseases, particularly helminths, the host immune response in this context may differ significantly from that of other populations and thus, in order to identify possible therapeutic opportunities, a direct comparison with a matched healthy cohort is required. We are aware that both the BC patients and even more the healthy cohort of the study are relatively small in size, but also other high-impact studies have successfully generated significant insights using similar or even smaller sample sizes.[21–23] While the sample size may limit detailed stratification of parameters such as intrinsic subtypes, this stratification was not the primary objective of our study. Instead, the study provides valuable insights into systemic immune characteristics, which can serve as a rational for future, larger-scale investigations. The principal finding of the study was an expansion of $CD8^+$ T cells in the BC patients, which resulted in an inverted CD4/CD8 ratio (i.e. ratio < 1) in 50% of these patients. Interestingly, BC patients with a normal CD4/CD8 ratio demonstrated an expansion of $CD8^+$ T cells with a central memory phenotype, while effector memory $CD8^+$ T cells were expanded in the patients with inverted ratios. This was further associated with high expression levels of perforin, loss of CD28 and a more senescent rather than exhausted phenotype, characterized by enhanced CD57, but equal PD1 or Tim3 expression. Inverted CD4/CD8 ratios have been associated with immune suppression and unfavorable outcomes in different cancers. In cervical carcinoma, the altered CD4/CD8 ratio has been correlated with tumor lymph node metastasis, increased tumor growth and a poorer prognosis,[24] while in early BC patients, lower CD4/CD8 ratios were associated with increased risk of distant recurrence and decreased survival.[25] In TNBC patients, higher CD4/CD8 ratios were associated with a better response to chemotherapy.[26]

In our cohort, the expansion of $CD8^+$ T cells coupled with a CD4/CD8 ratio inversion was prevalent among patients with luminal tumors, which is in contrast to the results obtained within the tumor tissue, where the non-luminal tumors (i.e. basal-like/TNBC and $HER2^+$) showed higher frequencies of $CD8^+$ T cell infiltrates.[27] Despite luminal tumors are considered the "coldest" among the BC subtypes, a predictive role of TIL for response to chemotherapy has been described.[28] Consequently, many other immunotherapeutic (combination) strategies are currently studied in luminal BC (reviewed in Kearney et al., 2021).[29]

Despite the focus of immunotherapy was mainly on effector $CD8^+$ T cells, the importance of $CD4^+$ T cell not only as helper cells, but also as direct anti-tumor mediators has been reevaluated.[30] Despite the frequencies of the $CD4^+$ T cells within the PBMC in our cohort were reduced due to the expansion of $CD8^+$ T cells, they displayed an activated phenotype characterized by a higher HLA-DR expression. They also expressed high levels of PD1, but the missing co-upregulation of other immune check point (ICP) molecules indicates that these cells were not terminally exhausted[31] and suggests a possible responsiveness to reactivation by immunotherapeutic strategies. This is in line with the reported association of enhanced frequency of $PD1^+$ $CD4^+$ T cells with an improved clinical response to ICP inhibitors in metastatic HR^+ breast cancer patients.[32]

Increased PD1, PD-L1 and CTLA4 expression levels were also found on B cells from BC patients. Non-malignant human B cells can express CTLA4 (reviewed in[33]), but their role in anti-/pro-tumor immunity remains to be elucidated. On the contrary, it has been demonstrated that B cells expressing PD1 produce IL-10 upon engagement with PD-L1,[34] whereas $PD\text{-}L1^+$ B cells inhibit NK and $CD8^+$ T cell cytotoxicity.[35] All these reports are reminiscent of regulatory B cells (Breg; reviewed in[36]), normally identified as $CD24^+$ $CD38^+$ cells. In our cohort, no differences in the frequencies of translational $CD24^+$ $CD38^+$ B cells among the naïve B cells were found between BC patients and HC, which argues against an expansion of Breg, although functional evaluations were lacking. An increased presence of "total" B cells was detected in patients with non-luminal cancer, which is in line with a report of higher B cell infiltrates in TNBC patients.[37]

An increased frequency of γδ T cells was found in BC patients, which is already higher in the Ethiopian HC than in the Caucasian population. γδ T cells can exhibit both pro- and anti-tumor

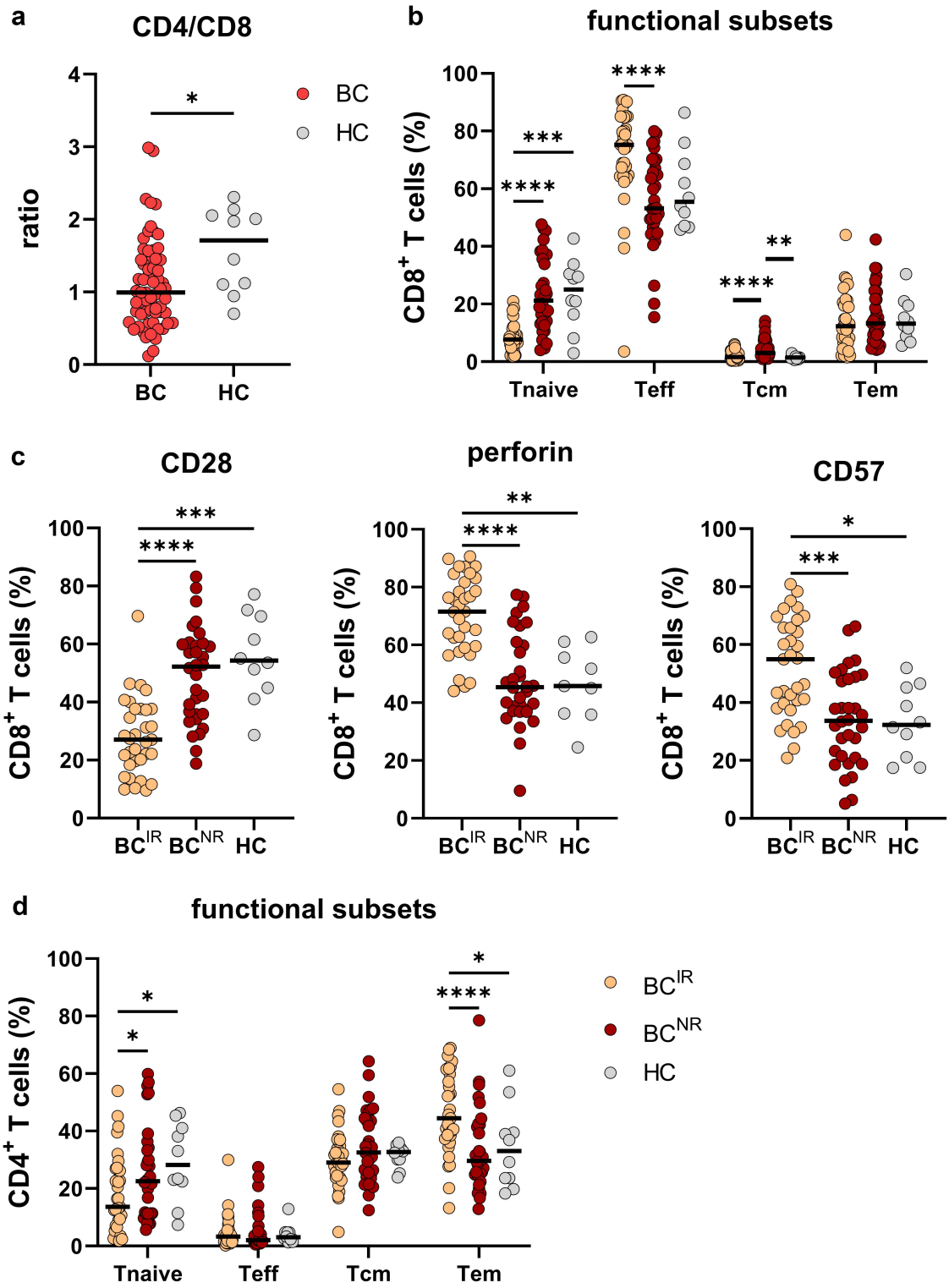

Figure 3. Immune phenotype of BC patients based on their CD4/CD8 ratio. The ratio between $CD4^+$ and $CD8^+$ T cells in BC patients and HC was calculated (a) and used to distinguish patients with an inverted (BC^{IR}) or a normal (BC^{NR}) CD4/CD8 ratio. The frequencies of the different memory subsets and marker positive cells among $CD8^+$ (b-c) and $CD4^+$ T cells (d) are shown for the BC^{IR} and BC^{NR} patients and HC.

activities[38] and could serve as prognostic and/or predictive markers positively or negatively associated with the patients' outcome. In all BC subtypes, but in particular in the $HER2^+$ subtype tumor infiltrating γδ T cells were of prognostic value.[39] In TNBC, the role of γδ T cells is more complex, since an improved survival was found for TNBC patients without a PI3K mutation in the tumor[40] or when only $V\delta1^+$ γδ T cells were taken into consideration,[31] but not in other settings.[41] In addition, expansion of γδ T cells is associated with an enhanced risk of metastasis in BC patients in presence of high levels of cholesterol.[42] Since our study did not investigate deeper the functionality of the γδ T cells, follow-up studies are crucial to correlate γδ T cell frequencies with the

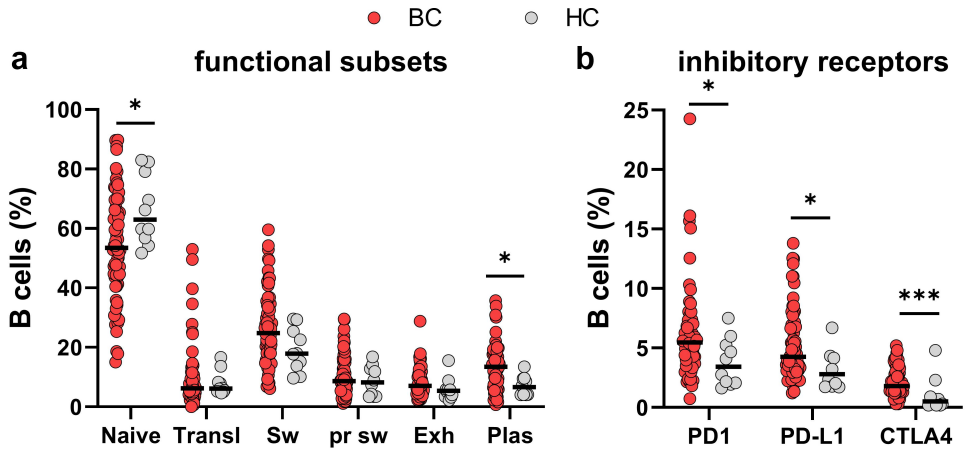

Figure 4. B cell frequencies. The frequencies of the different functional subsets of B cells (a) as well as of the cells expressing the indicated markers (b) are shown for BC patients and HC as individual values together with their median values. The B cell subsets were identified as following: Naïve (IgD^+ $CD27^-$), translational (Transl, IgD^+ $CD27^-$ $CD24^+$ $CD38^+$); switch memory (Sw, IgD^- $CD27^+$); pre switch memory (pr sw, IgD^+ $CD27^+$); exhausted memory (Exh, IgD^- $CD27^-$) and plasmablast (Plas, IgD^- $CD27^+$ $CD24^+$ $CD38^-$).

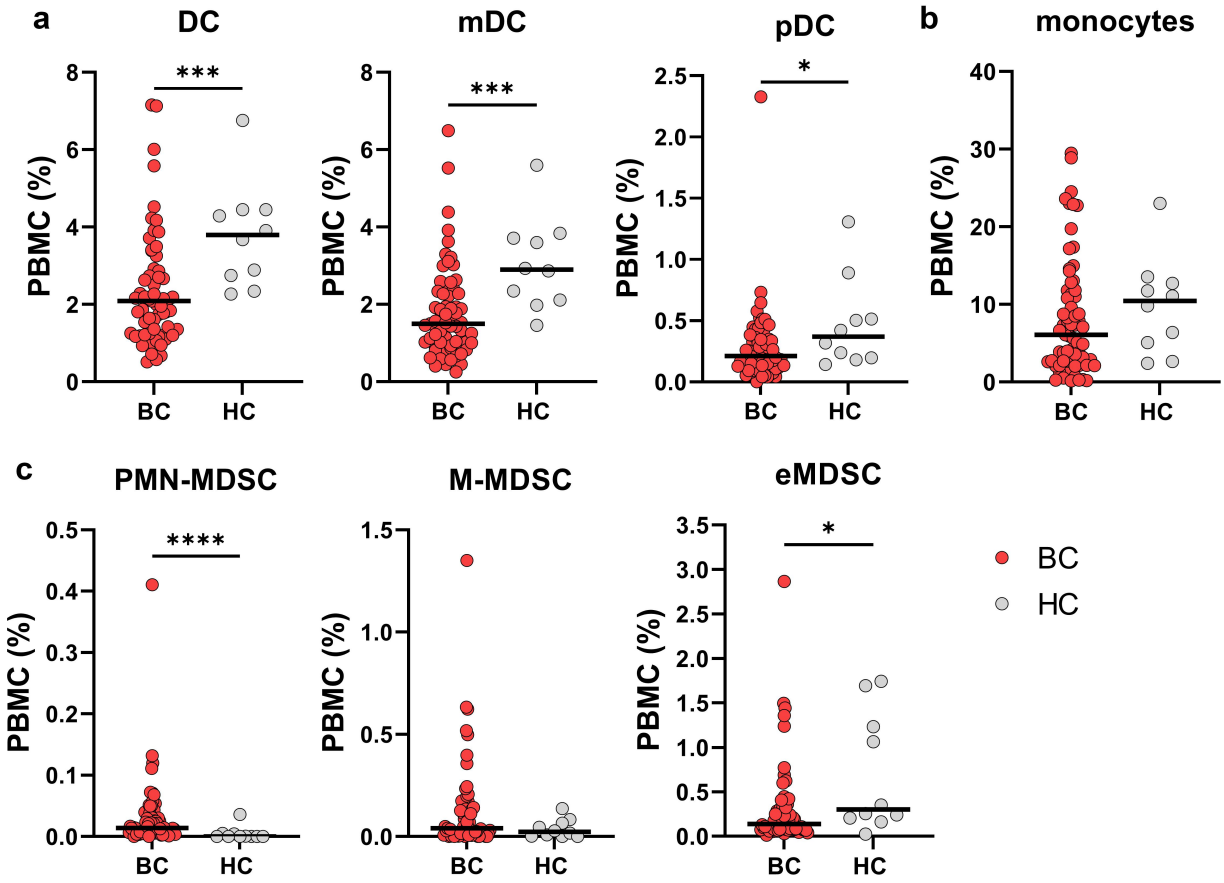

Figure 5. Frequency of myeloid cells. The frequencies of DC and their subpopulations (a), monocytes (b) and of the different MDSC subpopulations (c) are shown for BC patients and HC as individual values together with their median values.

patients' outcomes in order to enhance our understanding of their role in Ethiopian BC patients.

Next to the expansion of effector populations, BC patients revealed an increase in different suppressive populations. Treg and particularly their highly suppressive CD45RO $CCR4^+$ subpopulation was expanded in our BC cohort. More statistically significant were the differences between BC patients and HC for various MDSC subtypes, with an elevation of PMN-MDSC, but a reduction of eMDSC in BC patients.[43] However, follow-up data on patients´ overall or progression-free survival are needed to evaluate the clinical relevance of Treg and/or MDSC expansion, as reported in other BC cohorts.[44]

In conclusion, this study demonstrates for the first time a systemic (tumor-specific) activation of the immune

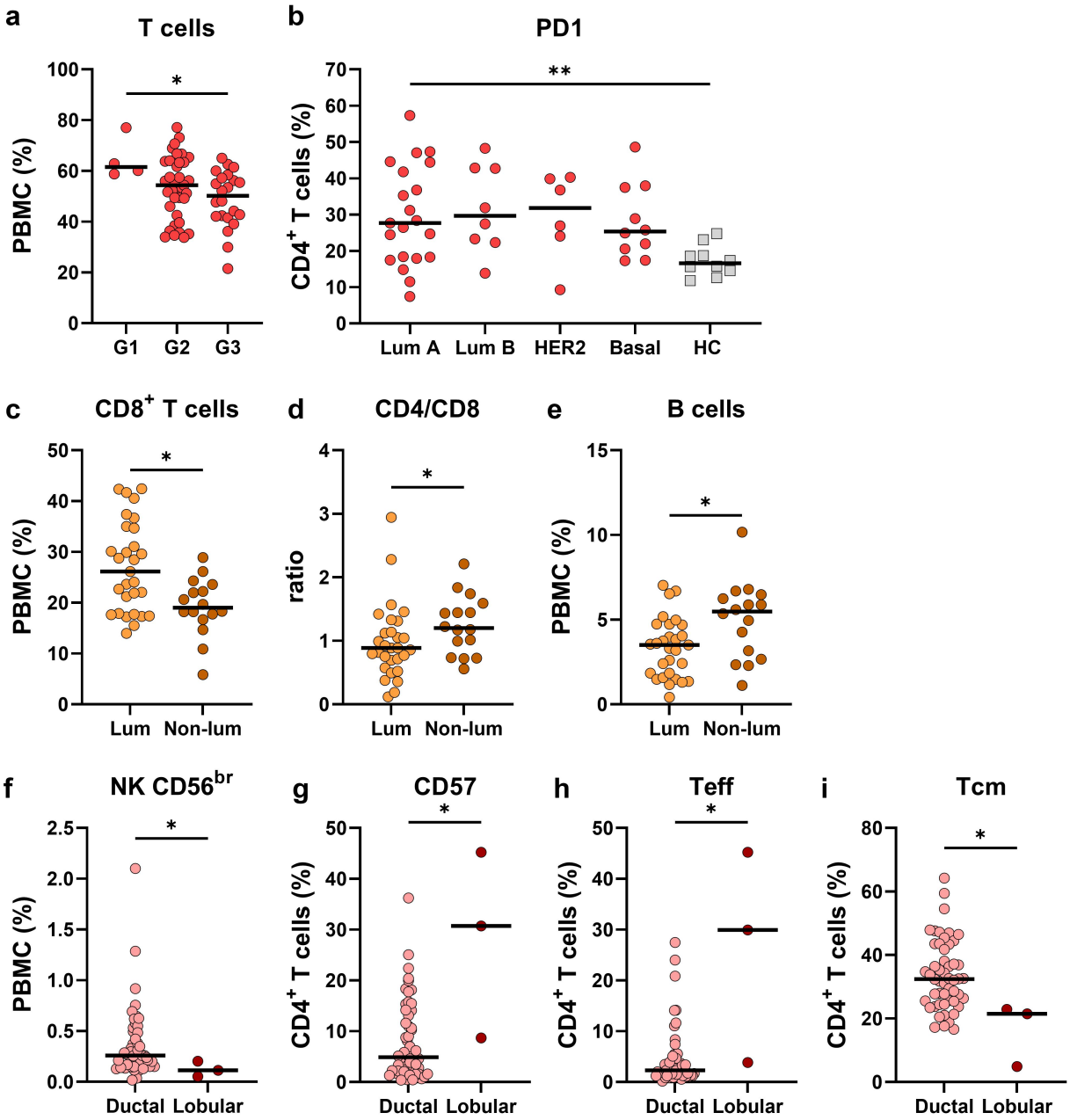

Figure 6. Correlation of immune parameters with breast cancer patients´ clinical characteristics. (a) Frequencies of T cells within PBMC are shown for the BC patients based on their tumor grading. (b) Frequencies of $PD1^+$ cells among $CD4^+$ T cells in HC and BC patients subdivided by their molecular intrinsic subtypes. (c-e) BC patients were grouped into luminal and non-luminal cases. Shown are the frequencies of $CD8^+$ T cells (c), the CD4/CD8 T cell ratios (d) and the B cell frequencies (e). (f-i) BC patients were grouped into invasive ductal and lobular cases. Shown are the frequencies of $CD56^{br}$ NK cells within the PBMC (f) as well as the frequency of $CD57^+$ (g), effector (h) and central memory (i) cells within $CD4^+$ T cells. Individual values are presented together with their median values.

system in Ethiopian BC patients, which is paralleled by the induction of different immune escape mechanisms ranging from the induction of $CD4^+$ T cell exhaustion and $CD8^+$ T cell immune senescence to the expansion of immune suppressive populations. Further functional characterization of the effector and suppressive populations will highlight the most promising strategies to revert the immune evasion and poor prognosis of Ethiopian BC patients.

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Disclosure statement

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Author contributions

MY contributed to study design, sample and data acquisition, experimental analysis, data interpretation, statistics and writing of the original and final draft. CM has done the data analysis, data interpretation, statistics and writing of the original and final draft. AA, EJK, TA, LT and BS contributed to study concept and design. ZD, EA, YB and MA contributed to sample and data acquisition. AM, ZD, KS, MB and MV carried out the experimental work. BS, CW, EJK, MV, LT, PS and TA contributed to the study design, data acquisition, data analysis, data interpretation and writing/editing of the manuscript. AM and PS contributed to data analysis and plotting. All authors reviewed and approved the final version of the manuscript.

Data availability statement

The data generated in this study are available upon request from the corresponding author.

Ethics approval

This study was approved by the institutional review board of the College of Health Sciences of Addis Ababa University (protocol 092/17/17) and the National Research Ethics Review Committee of Ethiopia (protocol MOSHE//RD).

Abbreviations

Ab	antibody
BC	breast cancer
BCIR	breast cancer patients with inverted CD4/CD8 ratio
BCNR	breast cancer patients with normal CD4/CD8 ratio
DC	dendritic cell
DMSO	dimethyl sulfoxide
EGFR	epidermal growth factor receptor
e-MDSC	early-stage MDSC
FBS	fetal bovine serum
γδ	gamma-delta
HC	healthy controls
HR	hormone receptor
ICP	immune checkpoint
IDC	invasive ductal carcinoma
IHC	immunohistochemistry
ILC	invasive lobular carcinoma
IR	inverted ratio
mDC	myeloid DC
MDSC	myeloid-derived suppressor cell
M-MDSC	monocytic MDSC
NK	natural killer
NR	normal ratio
PBMC	peripheral blood mononuclear cell
pDC	plasmacytoid DC
PMN-MDSC	polymorphonuclear MDSC
OS	overall survival
Tcm	central memory T cell
TCR	T cell receptor
Teff	effector T cell
Tem	effector memory T cell
TNBC	triple negative breast cancer
Treg	regulatory T cell

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