The Immune Microenvironment in Breast Carcinoma: Predictive and Prognostic Role in the Neoadjuvant Setting

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Established Facts

- The immune system is responsible for the maintenance of tissue homeostasis by initiating inflammatory reactions that involve the activation of innate and adaptive immune cells.
- In cancer, immune responses that aim to eliminate the tumour are elicited. However, the neoplastic cells may escape the immune control through immune-editing which depends on both tumour and immune cell interactions.
- New therapies have the potential to reactivate the immune response against cancer, e.g., in melanomas and lung cancer, and this is associated with improved outcomes.

Novel Insights

- Immune cell subpopulations, especially tumour-infiltrating lymphocytes (TILs) in the breast carcinoma microenvironment, can influence the tumour response to various neoadjuvant chemotherapy regimens, particularly in triple-negative and HER2-positive breast cancers.
- Recognition of TIL subtypes and locations in relation to tumour cells in breast carcinoma are promising for the development of more effective options of targeted therapy.
- The International TILs Working Group recommends the assessment of TILs on H&E sections. Stromal TILs should be analysed excluding necrotic and crushed areas. Immunohistochemical and digital assessments of TILs are currently limited to the research setting.

Keywords

Breast carcinoma · Tumour-infiltrating lymphocytes · Neoadjuvant chemotherapy · Tumour-infiltrating macrophages · Tumour-infiltrating neutrophils · Tumour-infiltrating dendritic cells · Pathological complete response

Abstract

The prognostic value of the immune cell infiltrate in the breast carcinoma microenvironment is still uncertain. We reviewed published articles analysing the infiltration of inflammatory cells in the microenvironment of breast carcinoma. Data revealed the importance of infiltration of these immune cells in the prognosis of breast carcinoma, particularly

the triple-negative and HER2-positive phenotypes. Tumour-infiltrating lymphocytes and their subtypes play a fundamental role in predicting the pathological complete response (pCR) to neoadjuvant chemotherapy. More research aiming to dissect a complex network of communication between cancer cells and other cellular components of the tumour microenvironment is necessary to develop more effective therapeutic approaches.

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Introduction

It has long been recognised that the tumour microenvironment is likely to play an important role in tumour development and progression. Significant progress has been made in the last few decades in describing the cellular composition of this microenvironment and its function in different types of cancer, including breast carcinoma. There is growing interest in exploring its prognostic and predictive roles in response to various types of therapy, especially chemotherapy, which can be more efficient after successful reactivation of the host's immune surveillance [1]. In the adjuvant setting, a high infiltration by lymphocytes, either into the tumour tissue or its surrounding stroma, has been significantly associated with a good prognosis in patients with estrogen receptor (ER)-/HER2-negative breast cancer treated with anthracycline only versus a combination of doxorubicin + docetaxel chemotherapy [2]. An improved clinical benefit of adjuvant anthracycline-based therapy has been seen in patients with high infiltration of the tumour by CD8+ lymphocytes [3]. It has been suggested that interferon (IFN)- γ -producing CD8 T lymphocytes play a role in local primary immunity against tumours [4, 5].

Neoadjuvant chemotherapy (NACT) can stimulate antitumour immunity, thereby increasing the pathological complete response (pCR) to the treatment [6]. Indeed, in the neoadjuvant setting, studies have highlighted the importance of assessing tumour-infiltrating lymphocytes (TILs) for predicting pCR, and this is strongly associated with a better prognosis in triple-negative breast cancer (TNBC) and HER2-positive breast cancer [7]. Many known predictive factors of NACT response are related to the phenotypic character of tumour cells (review [8]). The role of the tumour microenvironment in the neoadjuvant setting is now emerging. This review aims to summarise the current knowledge about immune cells infiltrating the tumour microenvironment in breast carcinoma, in an effort to shed light on their predictive and

prognostic role and the implications for the response to neoadjuvant chemotherapy. The various cellular components of the immune microenvironments, the methods of assessing them, and their relevance in the neoadjuvant setting are discussed below.

Evaluation of Immune Cells in the Tumour Microenvironment of Breast Cancer

Immune cells constitute an important component of the tumour microenvironment and are found either in the stroma surrounding the tumour nests (stromal) or are in direct contact with the tumour cells (intratumoural).

Several methods have been developed to evaluate immune cell infiltration, particularly that of the lymphocytic infiltrate. Hematoxylin and eosin (H&E)-stained slides of tumour tissue are used to assess the percentage of infiltrating lymphocytes. The International Immuno-Oncology Biomarker Working Group on Breast Cancer recommended the use of microscopic magnification of $\times 200$ – 400 (ocular $\times 10$, with an objective of $\times 20$ – 40) of H&E-stained FFPE tissue sections (4–5 μm) to assess TILs. All mononuclear cells including lymphocytes and plasma cells should be scored. Polymorphonuclear leukocytes and other granulocytes (i.e., eosinophils and basophils) are excluded. Quantitative assessment of other mononuclear cells such as dendritic cells and macrophages is currently not recommended, although there is increasing evidence that they may be functionally important. The Working Group recommended the evaluation of stromal TILs that are not in direct contact with invasive carcinoma cells rather than the intratumoural TILs in the tumour nests, because the stromal compartment is not affected by the density and growth pattern of the nests (Fig. 1a). Only TILs within the borders of the invasive tumours are evaluated (with the invasive edge being included in the evaluation but not reported separately). Immune infiltrates outside of the tumour borders, e.g., in the adjacent normal tissue or a ductal carcinoma in situ (DCIS) should not be included in the assessment [9]. Areas with crush artefacts, necrosis, and inflammation around biopsy sites, or extensive central regressive hyalinization should be ignored. Various cut-off values to define positivity are used. A phenotype of breast tumours with a particularly high lymphocytic infiltration, constituting >50 – 60% of the tumour microenvironment is classified as lymphocyte-predominant breast carcinoma (LPBC) [6, 10–12].

The assessment of TILs in residual disease following NACT remains a challenge. While the recommendations

Fig. 1. Methods of assessment of the inflammatory microenvironment of invasive breast carcinoma. **a** Section of invasive breast carcinoma showing stromal (long arrow) and intratumoural (short arrow) tumour-infiltrating lymphocytes (TILs). H&E. **b** Immunohistochemical staining of CD3+ T cells showing stromal and intratumoural TILs. **c** Immunohistochemical staining including a

CD20-positive lymphoid aggregate at the tumour-stromal interface. **d** Example of multiplex immunofluorescence showing concomitant expression of various cellular components. Green, CD4+ TILs; yellow, CD8+ TILs; magenta, CD68+ TIMs; cyan, CD20+ TILs; orange, FOXP3+ TILs. Blue shows nuclear counterstaining (DAPI).

are similar to those for pretreated samples, it has been suggested that the area for assessment should be confined to the borders of the residual tumour bed, defined as the largest cross-sectional area between residual invasive tumour cells. Stromal TILs in residual disease are defined according to the percentage of stromal areas occupied by mononuclear inflammatory cells within the boundaries of the residual tumour bed [13]. Immunohistochemical staining has been used to differentiate the subtypes of immune cells (Fig. 1b, c). Authors have adapted different semiquantitative grading methods, i.e., from absent to high infiltration levels, according to the percentage of infiltrating lymphocytes by eyeballing [14, 15].

Tumour-Infiltrating Lymphocytes

A strong association between lymphocytic infiltrate and chemotherapy response has been described [6] and specific lineage subsets subsequently evaluated. CD4+ T helper 1 (Th1) cells have been shown to protect against tumour growth [16] and cytotoxic CD8+ lymphocytes are considered the main mediators of the immune surveillance directed against tumours [17]. CD4+ Th2 [16] and CD4+ forkhead box P3 (FOXP3+) lymphocytes are thought to promote tumour growth [18]. CD4+ effector lymphocytes are associated with suppression of the anti-tumour immune response [17].

TILs and the Response to Different Chemotherapy Regimens

An increased number of TILs is associated with good outcomes for breast cancer patients after NACT. This was first demonstrated by Denkert et al. [6] in their analysis of 1,058 patients' core biopsies. A pCR rate of 40–42% of the patients was achieved with high TIL infiltration (>10%) following neoadjuvant anthracycline-/taxane-based chemotherapy, whereas tumours with low infiltration achieved pCR in only 3–7% of patients. Similar findings were described in hormone receptor-negative, HER2-positive breast cancer patients treated with only anthracycline-based NACT [19]. Issa-Nummer et al. [11] showed that increased infiltration by stromal lymphocytes, especially in LPBC, was an independent predictor for pCR in the multivariate analysis of a cohort of 313 breast cancer patients. Furthermore, the increased number of intratumoural lymphocytes was predictive for pCR in the univariate analysis. The authors did not comment on an association of TILs and patient survival as no survival analysis was performed in this study. In inflammatory breast carcinoma (IBC), stromal TIL infiltration of >10% was considered a significant predictor of improved overall survival (OS) in patients who received neoadjuvant therapy with taxane [20].

TILs and the Neoadjuvant Response in Breast Cancer Molecular Subtypes

In one of the earlier studies, high TIL infiltration in TNBC was positively correlated with pCR after neoadjuvant anthracycline chemotherapy alone, taxane-based regimens alone, and anthracycline and taxane sequentially or concurrently [21]. Similarly, Oda et al. [22] showed an association between high infiltration and pCR in a group of TNBC patients treated with paclitaxel followed by a combination of fluorouracil, epirubicin, and cyclophosphamide (FEC).

TNBC subtyping can be a predictor of the pCR to neoadjuvant chemotherapy [23]. Six molecular subtypes, namely basal-like (BL)1, BL2, mesenchymal, mesenchymal stem-like, immunomodulatory, and luminal androgen receptor were identified [24]. They were subsequently reduced to 4 subtypes (BL1, BL2, mesenchymal, and luminal androgen receptor) [25]. The immunomodulatory gene expression signature was described as an indicator of the presence of TILs [25]. The BL1 subtype had both a high rate of the immunomodulatory positive signature (48%) with a pCR rate of 52 %, while the BL2 (30%) and luminal androgen receptor (18%) subtypes had lower rates of the immunomodulatory positive signature as well as the lowest pCR rates (0 and 10%, respectively) [24].

High TIL infiltration has been correlated to pCR in HER2-positive breast cancer following neoadjuvant anthracycline treatment [3]. A prospective study of 498 HER2-positive breast cancer samples of the neoadjuvant GeparQuattro (G4) and GeparQuinto trials (G5) showed that the LPBC phenotype was strongly associated with pCR in the whole cohort as well as in the G4 group. Additionally, patients who were treated with trastuzumab showed higher pCR rates than those treated with lapatinib-based therapy. The LPBC phenotype was significantly associated with pCR in the trastuzumab-treated subgroup. Although disease-free survival (DFS) was significantly associated with a high pCR rate in the whole cohort, no significant association was seen between LPBC and DFS. By combining both pCR and LPBC, 2 prognostic subgroups of patients were identified. Patients with pCR and LPBC were defined as a low-risk group with a long DFS and those with no pCR/LPBC were considered a high-risk group with an increased rate of recurrence. In the whole cohort, the LPBC without pCR group represented 42.3% of patients and the non-LPBC with pCR group comprised 40.4%. It has been proposed that this subgrouping may help identify patients who would benefit from the addition of new therapeutic strategies that target the immune response [26].

In the PROMIX phase 2 trial of 150 patients with locally advanced hormone receptor-positive/HER2-negative breast cancer, a low abundance of FOXP3+ TILs (<10%) in cycle 2 correlated with an improved DFS following neoadjuvant docetaxel and epirubicin [27].

TILs in the Post-NACT Residual Disease Setting

Residual cancer burden (RCB) was developed as a method to quantify residual disease after NACT in breast carcinoma [28]. The RCB index depends on histopathological variables such as the number of nodes involved, the size of the largest nodal metastasis, and the size and percentage of cellularity of the primary tumour bed. It can be calculated through a web-based calculator, both as a numerical score and as a category [29]. RCB has been shown to have a useful predictive value for long-term survival after NACT [28, 30].

Heterogeneous immune response to NACT in the residual setting has been observed, with 48% of patients showing an increase in the level of TILs in residual disease and 47% experiencing a decrease [31]. The presence of >60% TILs in residual disease after NACT was associated with a better prognosis in patients with TNBC [10]. A significant positive correlation was found between high TIL

infiltration in post-NACT residual breast tumours and both metastasis-free survival and OS, with a relative decrease in the risk of metastasis and death and increase of 5-year metastasis-free survival and OS rates [10].

TIL infiltration in TNBC residual tumours was found to be predictive of recurrence-free survival (RFS) and OS in a study on 111 patients who received neoadjuvant doxorubicin and cyclophosphamide (half of them also received paclitaxel). The residual tumours with a low level of TILs were enriched with activating genomic alterations in the RAS-MAPK pathway (amplifications in KRAS, BRAF, RAF1, and truncations in NF1), which cause the suppression of MHC-I/II expression. These results suggested that the activation of the RAS-MAPK pathway might represent a possible mechanism by which tumour cells bypass the antigen presentation processes, thereby reducing immune recognition and lymphocyte infiltration [32]. By contrast, the extent of lymphocyte infiltration into primary HER-positive tumours was not correlated with pCR and a high level of TILs in residual disease following neoadjuvant trastuzumab therapy was associated with a poor prognosis [33].

Asano et al. [34] developed an indicator termed RCB-TILs by combining both RCB and TIL evaluations. They assessed its clinical application following neoadjuvant FEC100 and paclitaxel, with the addition of trastuzumab in the HER2-positive breast cancer group. Patients who were RCB class I and positive for TILs were considered RCB-TILs-positive, and all other combinations were RCB-TILs-negative. RCB-TILs-positive breast cancer was shown to be associated with longer survival and a lower rate of recurrence. In posttreated excisional samples, high TIL infiltration (>20%) correlated with a better RFS and OS in patients with RCB class II than in those with RCB class III [35].

TIL Subsets and Neoadjuvant Response

CD3+ TILs

In one of the earlier studies, the infiltration of CD3+ T cells could predict the response to NACT in breast cancer (Table 1). This was shown in a cohort of 25 breast cancer patients treated with neoadjuvant paclitaxel therapy. High CD3+ TIL infiltration in the tumour bed correlated significantly with the response to therapy [14]. Similarly, a subgroup of ER-negative breast cancer patients with high CD3+ TIL infiltration showed a remarkably high (74%) pCR rate to neoadjuvant anthracycline-based therapy. A high CD3+ TIL level was significantly associated with increased DFS, both HER2- and progesterone receptor (PR)-negative status and strongly associated with a

high tumour grade [3]. Increased levels of CD3+ TILs also predicted the pCR to a neoadjuvant anthracycline + taxane combination; their high infiltration was associated with an increased response rate [36].

CD4+, CD8+, and FOXP3+ TILs

High CD4+, CD8+, and FOXP3+ TIL infiltration was correlated with pCR in TNBC patients treated with neoadjuvant anthracycline-/anthracycline + taxane-based therapy [37]. High infiltration of both FOXP3+ and CD8+ TILs in pretreated biopsies featured high pCR rates in patients who received neoadjuvant paclitaxel followed by FEC [22]. A high CD8/FOXP3+ TIL ratio in pretreatment biopsies was significantly associated with pCR in TNBC and HER2-positive breast cancer, following FEC100 and paclitaxel + trastuzumab in HER2-positive breast cancer patients. Following NACT, TIL evaluation revealed a decreased CD8/FOXP3+ TIL ratio in recurrent TNBC and HER2-positive breast cancer but remained unchanged in hormone responsive breast cancer, possibly reflecting an association between tumour recurrence and a reduction in tumour immunity in the cancer microenvironment [38]. A high level of CD8+ TILs and a high CD8/FOXP3+ TIL ratio in a residual tumour could predict an improvement in RFS and breast cancer-specific survival (BCSS) in TNBC patients with non-pCR following neoadjuvant anthracycline + taxane chemotherapy [39]. Recently, Goto et al. [40] noticed an increased CD8/FOXP3+ TIL ratio following treatment with FEC100 followed by paclitaxel. This increase was significantly associated with improved clinical outcomes in TNBC and hormone-responsive breast cancer.

CD20+TIL and PDL-1 Expression in Breast Cancer

Only a few studies have examined the prognostic value of tumour-infiltrating B cells (CD20+ TILs) in breast cancer patients receiving NACT. Denkert et al. [6] found a significant correlation between the CD20+ TIL and pCR. A highly responsive group of breast carcinoma patients was characterized by higher CD20+ TIL infiltration in their pretreatment biopsies, with a statistically significant decrease of CD20+ cell counts after anthracycline-/anthracycline + taxane-based therapy only, and with the addition of trastuzumab to HER2-positive breast cancer patients [41]. In a study of pretreatment 580 breast cancer biopsies, high CD20 expression was associated with a 5.5 times likelihood of a pCR to a neoadjuvant anthracycline + taxane combination [36]. Coexistence of CD20+ TILs and PD-L1+ tumour cells were significantly associated with better

Table 1. Summary of studies investigating the relationship between various types of immune cells and response to neoadjuvant chemotherapy

First author [Ref.], year	N	Molecular phenotype	Time point	Assay	Marker	Scoring/cutoff	Correlation
Demaria [14], 2001	25	all	pre- and post-NACT	H&E IHC	CD8 CD3	score >0	positive correlation with pCR
Ladoire [15], 2008	56	all	pre-NACT	IHC	CD3 CD8 FOXP3	score >0	high CD8+ TILs positively correlated with pCR
Hornychova [80], 2008	73	all	pre- and post-NACT	IHC	CD3, CD68 CD63 CD56 CD1aS100	mean numbers	high CD3+ TILs and CD83+ dendritic cells positively correlated with pCR
Denkert [6], 2010	1,058	all	pre-NACT	H&E	TILs	60%	positive correlation with pCR
West [3], 2011	255	ER-negative BC	post-NACT	IHC	CD3 CD8 CD4	median value	TILs can predict anthracycline sensitivity in HER2-positive and TNBC subtypes
Ono [21], 2012	180	TNBC	post-NACT	H&E	TILs	score >0	positive correlation with pCR
Yamaguchi [19], 2012	68	HER2-positive/HER2-negative BC	pre-NACT	H&E	TILs	score >0	positive correlation with pCR
Oda [22], 2012	180	all	pre- and post-NACT	IHC	FOXP3 CD8	median value	positive correlation with pCR before NACT negative correlation with pCR after NACT
Issa-Nummer [11], 2013	313	TNBC luminal	pre-NACT	H&E	TILs	60%	positive correlation with pCR
Lee [81], 2013	175	all	pre-NACT	IHC	CD3 CD8 FOXP3	10% in 10 increments	positive correlation with pCR
Seo [37], 2013	153	all	pre-NACT	IHC	CD4 CD8 FOXP3	median value	positive correlation with pCR
Dieci [10], 2014		TNBC	post-NACT	H&E	TILs	60%	positive correlation with OS
García-Martínez [41], 2014	121	all	pre-NACT	IHC	CD3 CD8 CD4 CD20 CD68 FOXP3	median value	high CD4+ TILs positively correlated with pCR high infiltration of CD3+ TILs and CD68+ cells negatively correlated with DFS
Brown [36], 2014		all	pre-NACT	H&E IHC	CD3 CD8 CD20	determined by Join-point software	positive correlation with pCR
Miyashita [82], 2014	110	TNBC	pre-NACT	IHC	CD8 FOXP3	median value	high CD8+/FOXP3+ TILs ratios have a positive correlation with pCR
Bianchini [83], 2015	243	HER2-positive LABC	pre-NACT	TILs IHC	PDL-1	TILs: 50% PDL-1: score >0	no correlation with pCR
Miyashita [39], 2015	101	TNBC	pre- and post-NACT	IHC	CD8 FOXP3	near the median value	high CD8+ TIL and CD8/FOXP3 ratios in residual tumors correlated with improved RFS and BCSS
Denkert [12], 2015	580	HER2-positive TNBC	pre-NACT	H&E	TILs	60%	positive correlation with pCR

Table 1 (continued)

First author [Ref.], year	N	Molecular phenotype	Time point	Assay	Marker	Scoring/cutoff	Correlation
Ali [84], 2016	768	HER2-positive BC	pre-NACT	H&E	TILs	scores 0 and 1 according to TIL abundance	positive correlation with relative resistance to chemotherapy
Asano [38], 2016	177	all	pre-NACT	H&E IHC	CD8 FOXP3	mean numbers/ per field	high CD8+/FOXP3+ TILs ratio positively correlated with pCR in TNBC and HER2-positive BC
Ingold [26], 2016	498	HER2-positive BC	post-NACT	H&E	TILs	60%	positive correlation with pCR
Asano [34], 2017		all	pre-NACT	H&E	TILs	score >10%	RCB-TILs is a significant predictor for BC recurrence after NACT
Ibrahiem [85], 2017	100	all	pre-NACT	IHC	CD3 CD20 CD4 CD8	50%	positive correlation of CD8+ TILs
Al-Saleh [86], 2017	31	luminal B/ HER2-negative	pre-NACT	IHC	CD8	median value	positive correlation with pCR and OS
Mitrofanova [56], 2017	50	all	pre-NACT	IHC	CD68 Stabillin-1	digital pathology (Aperio Image Scope)	negative correlation with high CD68+ cells
Kaewkangsadan [57], 2017	33	all	pre-NACT	IHC	CD68 CD163 CD1a CD66b	score >0	high CD163+ TIM infiltration positively correlated with pCR
Siregar [87], 2017	51	all	post-NACT	H&E	TILs	50%	no correlation with pCR
Hamy [33], 2017	175	HER2-positive BC	pre- and post-NACT	H&E	TILs	the value that yields the lowest <i>p</i> value concerning the association with DFS	no correlation with pCR in post-treated cases
Peleanou [88], 2017	58	all	pre- and post-NACT	H&E IHC	TILs PDL-1	1% (AQUA, digital pathology)	positive correlation with recurrence-free survival in residual tumour
Cha [89], 2018	220	all	pre- and post-NACT	H&E	TILs	60%	>5 cores may accurately predict the TIL score of the entire tumour
Hou [43], 2018	123	HER2-positive BC	pre-NACT	IHC	PDL-1 CD163 CD8	PDL-1: score $\geq$ 1% of tumour cells. CD8 and CD163: score >1%	high CD8+ cells and PD-L1 expression could predict response to anti-HER2 targeted therapy
Arias-Pulido [42], 2018	221	all	pre-NACT	H&E IHC	CD20 PDL-1	PDL-1: score $\geq$ 5% CD20: score $\geq$ 1%	high CD20+ TILs positively correlated with pCR and survival coexistence of CD20+ and PD-L1+ TILs positively correlated with better outcome
Asano [90], 2018	177	all	pre-NACT	H&E	TILs	score >0	positive correlation with pCR in TNBC and HER2-positive BC
Elsayed [91], 2018	45	all	pre-NACT	IHC	CD8	50%	high CD8+ TILs positively correlated with DFS but not with pCR
Goto [40], 2018	136	all	pre- and post-NACT	H&E IHC	TILs CD8 FOXP3	TILs: score >10%	positive correlation of high CD8+/FOXP3+ TILs ratio in TNBC and hormone-responsive BC

Table 1 (continued)

First author [Ref.], year	N	Molecular phenotype	Time point	Assay	Marker	Scoring/cutoff	Correlation
Luen [35], 2019	375	TNBC	pre- and post-NACT	IHC	CD8 FOXP3	20%	positive correlation with patient survival in residual disease
Eryilmaz [92], 2018	62	all in (LABC)	pre- and post-NACT	H&E	TILs	mean number of positive cells/mm ² of tissue	positive correlation with pCR
Matikas [27], 2018	150	HER2-negative BC	pre- and post-NACT	H&E IHC	FOXP3 CD163	score >0	low FOXP3+ TILs were prognostic for long-term outcomes
Denkert [93], 2018	3,771	all	pre-NACT	H&E	TILs	score >0	positive correlation with pCR and survival in TNBC and HER2-positive BC
Ruan [94], 2018	166	TNBC	pre-NACT	H&E	TILs	score >5% in 10 increments	positive correlation with pCR
Muntasell [49], 2018	42, 71	HER2-positive BC	pre-NACT	IHC	CD3 CD56	TILs: 50% TI-NKs/50 × HPF	positive correlation with pCR TI-NK (≥1/50 × HPF) correlated with improved DFS
Miyoshi [95], 2019	639	all	pre-NACT	H&E	TILs	cutoff points for TIL proportions (10, 30, and 50%)	positive correlation with aggressive phenotypes of BC and NACT response
de Groot [96], 2019	196	all	pre-NACT	IHC	CD8 FOXP3 CD68 HLA class 1	median value	high CD8+ TILs in the presence of tumoral HLA class 1 expression positively correlated with pCR and DFS in HER2-negative BC
Kurozumi [97], 2019	128	HER2-positive BC	post-NACT	H&E	TILs	score >0	positive correlation with better prognosis in residual tumours
Van Berckelaer [20], 2019	143	IBC	pre-NACT	IHC	PDL-1	TILs: score >10% PDL-1: score >0	PD-L1-expression is a characteristic of the IBC microenvironment
Russo [98], 2019	187	all	pre-NACT	H&E	TILs	30%	positive correlation with pCR in HER2-positive BC and TNBC

BC, Breast cancer; TNBC, triple-negative breast carcinoma; DFS, disease-free survival; RCB, residual cancer burden; TIMs, tumour-infiltrating macrophages; IHF, immunofluorescence; LABC, locally advanced breast carcinoma; IBC, inflammatory breast carcinoma; TI-NK, tumour-infiltrating natural killer cells; HPF, high power field; BCSS, breast cancer-specific survival; RFS, recurrence-free survival; H&E, haematoxylin and eosin.

survival, a high pCR rate, and high infiltration of TILs in triple-negative IBC. High CD20+ and PD-L1+ TIL infiltration correlated with fewer relapses, a high pCR, and higher rates of DFS and BCSS [42]. PD-L1 expression in tumour cells was positively associated with a high infiltration of intratumoural/peritumoural CD8+ TILs and CD163+ macrophages in HER2-positive breast cancer [43].

Natural Killer Cells

Natural killer (NK) cells contribute to tumour surveillance, the inhibition of tumour growth, and the defence against metastatic processes [44]. Their role in breast

cancer and a correlation with pCR following chemotherapeutic treatment remain poorly documented in the literature. Verma et al. [44] observed a significant reduction in cytotoxicity mediated by circulating NK cells in tumours with a poor NACT response. Conversely, there was a significant increase in NK cells in the peritumoural environment in tumours that achieved a good pathological response. However, intratumoural NK cells did not show the same correlation. Increased activity of NK cells in the peripheral blood following neoadjuvant therapy correlated with the disappearance of lymph node metastasis in breast cancer patients [45]. A significant increase in the number of activated NK cells in the peripheral blood was observed in HER2-positive breast cancer in

patients with pCR [46]. The expression of 6 NK-specific genes (*NCR3* (NKp30), *NCR1* (NKp46), *CD96*, *CRTAM*, *DNAM1*, and *NKG2D*) was predictive of an improved outcome in patients with breast cancer [47]. In another study, the upregulation of selected effector molecules related to an NK cell-mediated cytotoxicity pathway (GZMB, PRF1, NCR1, KIRs, and KLRCs) correlated with better OS and DFS in breast cancer patients [48]. A notable association was described between the increase in the number of tumour-infiltrating NK cells (TI-NKs) in pretreated biopsies and the pCR to anti-HER2 antibody-based neoadjuvant treatment combined with standard neoadjuvant chemotherapy in HER2-positive breast cancer. The tumours showing infiltration by ≥ 3 TI-NKs/50 $\times$ HPF were associated with a higher pCR rate and the presence of ≥ 1 TI-NKs/50 $\times$ HPF correlated with improved DFS [49].

Tumour-Infiltrating Macrophages

Activated M1 macrophages release proinflammatory cytokines and toxic intermediates and stimulate a cytotoxic effect on tumour cells through the activation of a type I T cell response [50]. However, tumour-infiltrating macrophages (TIMs) have been shown to impart a poor prognostic outcome in breast carcinoma. A decreased relapse-free survival and OS in breast cancer were found to be associated with the infiltration of CD68+ macrophages [51]. This was explained by the growth- and invasion-promoting effects of M2 macrophages on tumour cells via the release of proteolytic enzymes, their immunosuppressive activity, and their contribution to hypoxia-induced angiogenesis [52]. M2 macrophages have also been recognized as key players in the metastatic progression of breast cancer [53]. Only a few studies have assessed the prognostic value of the different locations of TIMs as an effector on their functions. Elevated stromal macrophages were positively correlated with large-sized and high-grade tumours and a poor OS and BCSS and were inversely correlated with luminal A breast cancer [54]. Other studies show that an aggressive outcome is associated with a high infiltration of TIMs irrespective of histological location [55].

TIMs and Response to Neoadjuvant Chemotherapy

High CD68+ TIM infiltration has been associated with worse DFS and OS in breast cancer patients following neoadjuvant taxane and anthracycline (+ trastuzumab) administration to patients with HER2 overexpression [41]. CD68+ TIMs located within parenchymal tissues

correlated negatively with lymphatic metastasis following neoadjuvant cyclophosphamide methotrexate fluorouracil (CMF) or fluorouracil, adriamycin, and cytoxan (FAC) [56]. A trial involving 108 breast cancer patients evaluated the effect of the addition of capecitabine to docetaxel preceded by adriamycin and cyclophosphamide in a neoadjuvant setting [57]. It reported that only CD163+ TIMs (a M2 macrophage phenotype) correlated with pCR. Although this finding was unexpected, the authors attributed it to a possible suppressive effect on the tumour caused by the interaction of parenchymal macrophages with tumour cells following therapy. Baseline abundance of CD163+ macrophages had neither a predictive nor a prognostic role in the PROMIX phase 2 trial that included 150 patients with locally advanced HER2-negative breast cancer treated with neoadjuvant epirubicin and docetaxel (+ bevacizumab) [27].

Tumour-Infiltrating Neutrophils

Polymorphonuclear leukocytes (neutrophils) constitute 50–60% of the total circulating leukocytes in the human body; they are considered the first line of defence against different types of microbes and also target tumour cells [58]. Tumour-infiltrating neutrophils (TINs) can stimulate tumour growth by releasing growth factors. These, together with the growth factors secreted by the tumour itself, may elicit a wound-healing-like response that, in turn, promotes tumour progression [59]. TINs can also facilitate tumour progression by stimulating angiogenesis and vascular invasion [60]. Increased levels of blood neutrophils have been described in various tumours including breast cancer [61]. A high neutrophil-to-lymphocyte ratio in blood samples was associated with a poor outcome in breast cancer [62, 63].

TINs and Response to Neoadjuvant Chemotherapy

It was reported that, in patients with locally advanced breast cancer, pre-NACT high levels of circulating neutrophils were significantly associated with pCR in metastatic axillary lymph nodes following 8 cycles of capecitabine, docetaxel, adriamycin, and cyclophosphamide neoadjuvant regimens [57]. The decrease in circulating neutrophils following chemotherapy may be a reflection of drug toxicity and bone marrow suppression or a higher degree of overall cytotoxicity, including tumour-cell toxicity, and can lead to neutropenia. Therefore, the observed association with a pCR may have been in parallel and not necessarily causal. In the same study, CD66b+

neutrophils infiltrating breast tissue were few in number in the tumour microenvironment and showed no correlation with pathological response [57].

Tumour-Infiltrating Dendritic Cells

Dendritic cells are antigen-presenting cells that can initiate and direct variable adaptive immune responses [64]. Their deficient function may lead to an ineffective antitumour immune response in cancer [65]. Their capacity to present antigens may depend on their maturity, location, and likely also the regional differences around and within breast cancer [66, 67]. Very few studies have investigated the prognostic role of tumour-infiltrating dendritic cells (TIDCs) in breast carcinoma patients. CD1a+ TIDCs have been found within breast tumours but with no statistical correlation between their density and tumour grade [66, 68]. S100+ TIDC infiltration correlated positively with tumour grade, with a higher density in grade III tumours followed by grade II tumours; the lowest level of TIDCs were noted in grade I tumours [66, 69]. CD83+ mature TIDCs were strongly associated with better patient survival in node-positive tumours compared to TIDCs expressing either S100 or CD1a (which showed no significant association with the prognostic parameters), indicating that TIDCs exhibit various phenotypes and functions in breast cancers depending on their level of maturation [70]. CD11c+ dendritic cells were significantly correlated with a high CD4+ CD8+ TILs count in surgical specimens of patients with TNBC. A positive correlation was also found between CD11c positivity and the formation of tertiary lymphocytic structures. Patients with moderate or abundant tertiary lymphoid structures had better survival than those with few or no tertiary lymphoid structures at more advanced pathological stages. Greater CD11c expression was associated with a better OS in TNBC patients with positive lymph node metastasis [71].

TIDCs and Response to Neoadjuvant Chemotherapy

A significant elevation in the level of dendritic cells in the blood was noted in breast cancer patients whose tumours showed a good pathological response following neoadjuvant capecitabine and docetaxel preceded by adriamycin and cyclophosphamide regimens. However, a significant reduction in the level of intratumoural CD1a+ TIDCs was reported, with no significant association with response to therapy, in both primary breast tumours and metastatic axillary lymph nodes [57].

Conclusions and Future Directions

The profile of immune cell subpopulations in the breast carcinoma microenvironment can identify a group of patients with tumours that are more responsive to various regimens of neoadjuvant chemotherapy. TILs, specifically, are important for predicting the neoadjuvant chemotherapy efficacy pre-, post-, or during treatment settings, mainly in TNBC. Recently, the international TILs Working Group, renamed the “International Immuno-Oncology Biomarker Working Group on Breast Cancer” developed an integrated survival prediction model for patients with early-stage TNBC, based on standard clinicopathological factors and stromal TILs. They developed an application which was designed to facilitate the use of this model [72]. An emerging role of TILs in DCIS has been described in recent studies. High TIL infiltration in DCIS correlates with adverse histopathological features [73–75]. Dense lymphocytes that touch the DCIS basement membrane or are located within a distance of 1-lymphocyte-cell-thickness from the basement membrane have been shown to be an independent prognostic factor for DCIS recurrence in patients treated with breast-conserving surgery regardless of other known determinants of tumour behaviour [76]. Recently, a 20% rate of TIL infiltration was described as the cutoff associated with the highest interobserver agreement [77].

Our study had several limitations. First, the sample size in the papers cited is highly variable. Second, there was variation across studies in the neoadjuvant chemotherapy regimens used. However, most of the studies showed significant correlation between TIL infiltration and pCR regardless of the type of therapy. Third, numerous methods with different cutoffs were used in the assessment of TILs. The recommended method remains the examination of H&E-stained slides. And finally, there are only a limited number of studies investigating the role of TIMs, TINs, and TIDCs in breast carcinoma following NACT; clearly, more studies are needed to highlight their predictive role in the response to therapy. Further investigations into the subtypes and localisation of TILs in relation to the molecular phenotype of breast cancer are required to better understand the significance of the observed correlation and to develop algorithms that predict response. Quantitative digital analysis is likely to play an important role in this evolving field. Digital pathology can also be used to profile the immune cell infiltration in breast cancer and assess counts and densities with the aid of a variety of currently available software for image analysis such as UTH-SCSA ImageTool [37], ImageJ [41], automated quantita-

tive (AQUA) immunofluorescence multiplex analysis [36], and Aperio ImageScope [56]. Recently, we used Inform v2.2.1 software in the analysis of multiplex immunofluorescence stained breast cancer tissue to evaluate immune cell phenotypes in both the intratumoural and stromal areas of the tumour microenvironment in core biopsies of IBC following NACT [78] (Fig. 1d). The Working Group does not currently recommend machine-scoring approaches. The standardization of methodology and scoring in large series is required before large-scale digital scoring can be implemented. Recent promising results of the use of anti-PDL-1 immunotherapy in metastatic breast cancer have created an exciting new dimension for the management of breast cancer [79]. The study showed the significant survival advantage of adding anti-PDL-1 therapy to chemotherapy for metastatic TNBC. This paves the way for targeted therapies based not only on the molecular profile of the tumour epithelial cells but also on the composition and localisation of the components of the immune microenvironment.

Disclosure Statement

The authors have no conflicts of interest to declare.

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Author Contributions

A.M.S. conceived the idea, reviewed the data, and contributed to writing it up. N.M.B. did the literature search, writing up, and produced the first draft. F.B. contributed to reviewing data and writing up. All authors approved the final manuscript.

Search Strategy and Study Selection

A literature search was performed on the PubMed and Google Scholar databases for studies that investigated the role of infiltrating immune cells in the microenvironment of breast carcinoma. The relevant studies that included data on tumour-infiltrating immune cells in the neoadjuvant setting were highlighted. The search strategy was limited to the English language and the deadline for the date of publication was June 2019. We included relevant literature reviews and the reference lists of the studies selected were also examined for additional eligible studies.

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