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The prognostic significance of TILs as a biomarker in triple-negative breast cancer: what is the role of TILs in TME of TNBC?

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Abstract. **Triple-negative breast cancer (TNBC) accounts for approximately 15-20% of all breast cancers, and its poor response to treatment has been a major problem in the field of breast cancer. In recent years, the application of immune checkpoint inhibitors has introduced a new era of treatment. The IMpassion 130 trial used PD-L1 as a mature biomarker for immunotherapy in metastatic TNBC, but the population screened, which was only patients with positive PD-L1 expression, was too narrow. Otherwise, it could not be determined whether the PD-L1-positive group benefited from immunotherapy in early TNBC, but this was confirmed in the KEYNOTE-522 and IMpassion 031 studies, in which there was no significant difference in the benefit, whether patients were PD-L1-positive or not. Therefore, how to screen more suitable biomarkers for accurate immunotherapy has become a burning and persistent problem to be solved. In fact, immune infiltration has always been our focus in the process of exploring immunotherapy in TNBC, and tumor infiltrating lymphocytes (TILs) have been well-known for decades as a prognostic factor in early TNBC. Furthermore, TILs are positively correlated with both patient survival and pathological complete response (pCR) after neoadjuvant chemotherapy. Recently, increasingly more foundational experiments and clinical trial verifications suggest that TILs could contain more biomarkers. Thus, in this review, we will assess the composition and heterogeneity of TILs, their evaluation standards, their relationship with TNBC prognosis and the prediction ability of different treatment options in TNBC, and their correlation with other biomarkers in the clinical application. We also summarize new studies that show the future potential of TILs.**

Key Words:
Tumour infiltrating lymphocytes, Biomarker, Immunotherapy, Prognosis of triple-negative breast cancer.

Introduction

Triple-negative breast cancer (TNBC), which is defined by a lack of estrogen receptor (ER) and progesterone receptor (PR) expression and by no HER2 amplification, accounts for approximately 15-20% of all breast carcinomas and is associated with earlier age of onset, aggressive clinical course, and dismal prognosis compared to hormone receptor- and HER2-positive breast carcinomas[1-3]. According to Lehmann et al[4], TNBC can be further subclassified into four molecular subtypes using gene expression analyses: basal-like 1, basal-like 2, luminal androgen receptor subtype, and subtype M, which is characterized by tumor-infiltrating lymphocytes (TILs) and tumor-associated mesenchymal cells. TNBC displays higher early recurrence rates than other breast cancer subtypes, resulting in decreased disease-free survival (DFS), and approximately 30% of patients experiencing recurrence within 5 years of diagnosis[5]; therefore, the selection of populations suitable for different treatment in the early stage is crucial. Some studies[6-8] have shown that the presence of high levels of lymphocytic infiltration has been consistently associated with a more-favorable prognosis in patients with early-stage TNBC. In contrast, a few studies[9-11] support TILs as the source of other biomarkers, such as recently reported PD-L1. As such, TILs must be mentioned as an important biomarker for immunotherapy and chemotherapy in early TNBC. It is of great significance to discuss the value of TILs as a biomarker for predicting prognosis and treatment outcome in TNBC.

TILs are described as the mononuclear immune cells that leave the blood and enter into the tumor, a population of cells comprising a mixture of cytotoxic T cells and helper T cells, as well as B cells, macrophages, natural killer cells, and dendritic cells, which are a significant part of the tumor microenvironment (TME)[12].

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This review summarizes in detail the relationship between cells in the TME and prognosis of TNBC based on the latest studies, the role of TILs in immunotherapy for TNBC according to the latest clinical studies, the correlation with other biomarkers in clinical application, and additional aspects of TILs that can be explored in the future.

The Role and the Evaluation of TILs in the TME

Heterogeneity of TILs and Its Role in the TME and Prognosis of Breast Cancer

The composition of TILs is currently thought to be 60% T cells (20% cytotoxic CD8+ T cells and 40% helper CD4+ T cells, CTL and Th cells), 5% NK cells, and 20% B cells, while the remaining cells are 5% macrophages and <1% dendritic cells[6]; T-cell markers (CD3, CD8, and FOXP3), B-cell marker (CD20) and histiocytic marker (CD68) have been used to identify the cells[13]. Their role in the TME can be explained by immunoediting theory, including three stages, elimination, equilibrium, and escape phase[14].

In breast cancer, extensive tumor infiltration by cytotoxic CD8+ T cells (CTL) was strongly associated with patient survival and response to therapy[15]. Recently, Yam et al[16] showed that a more clonal T-cell population is associated with pCR to NAC (neoadjuvant-chemotherapy) and an immunologically active TME for TNBC, and additional predictors of pCR were a higher ratio of CD3+/CD68+ and CD3+CD8+/CD68+ cells, as well as physical proximity of T-cells to malignant cells. Among the other CD4+ T cell subpopulations, Th1 cells (the principal cellular source of interferon-γ) have been associated with favorable clinical outcomes, whereas Th2 cells have been reported to be associated with dampening of the antitumor response[17]. Th17 cells appear to have variable effects depending on the surrounding cytokine milieu, which may in part be linked with the organ site and tumor type. The presence of follicular helper (Tfh) cells is positively associated with the patient outcome both in the adjuvant and neoadjuvant settings[18]. The presence of CD4+ regulatory T cells (Tregs) has been associated with both good and bad prognosis. In addition to T cells, low levels of NK cells have been reported to be apparently related to more unfavorable clinical outcomes, and high expression of intra-tumoral CD56 is associated with improved outcome in patients with early TNBC[19,20]. The precise role of tumor-infiltrating B cells in breast cancer is not currently well-defined and remains controversial, but B cell differentiation secretory type secretes IL-21, which stimulates the immune system[21]. All of the cells mentioned above in TMEs express markers of immune checkpoints except B cells.

The two kinds of cells that have been classified into TILs in recent years are dendritic cells and macrophages. Dendritic cells (DC), in the role of priming of T cells, are crucial in generating CD8+ T cell-mediated antitumor immunity[22], all subsets of which commonly showed enrichment for the interferon pathway in TNBC[23]. Macrophages of different subtypes (M1 and M2) point to different prognostic outcomes, and clinical evidence has shown that increased TAMs (tumor-associated macrophages) are positively correlated with poor prognosis in breast cancer patients[24]. Heterogeneity of TILs and its role in TNBC are summarized in Figure 1 (Heterogeneity of TILs and its role in TNBC).

The Controversial Treg and Its Role in TNBC

As mentioned above, Tregs are controversial and have good and bad prognostic research results, respectively. However, there is an evident correlation together with CD8+T cells, which points to a good prognosis in TNBC[14]. Most scholars believe that Tregs in TILs are beneficial for breast cancer, which usually presents a CD4 CD25+++/Hi surface phenotype expressing CTLA-4 and LAG3, and the expressions of TGF-β and IL-10 in CD4 CD25 Foxp3 TILs were significantly increased after TCR (T cell receptor) stimulation[25]. TNBC and HER2-overexpressed breast cancers are associated with more CTL and Tregs, and the Treg/Th2 cell ratio is higher than that of other subtypes[26]. Costa et al[27] analyzed the role of carcinoma-associated fibroblasts (CAF) in the TME and also verified the role of Tregs in TNBC immunosuppression. CAF-S1 fibroblasts promote an immunosuppressive environment through a multi-step mechanism by secreting CXCL12 and attracting and retaining CD4 CD25 T lymphocytes.

The Standardized Evaluation of TILs

The TIL evaluation of the 2014 TIL Evaluation Criteria is expressed as a percentage, and TILs should be reported for the stromal component (=% stromal TILs and sTILs) because of isolation and observation difficulty and a higher biological importance that can be reflected by stromal TILs than intra-tumoral TILs (iTILs). In addition, both sTILs and iTILs comprise a complex mixture of different lymphocyte subtypes, dominated

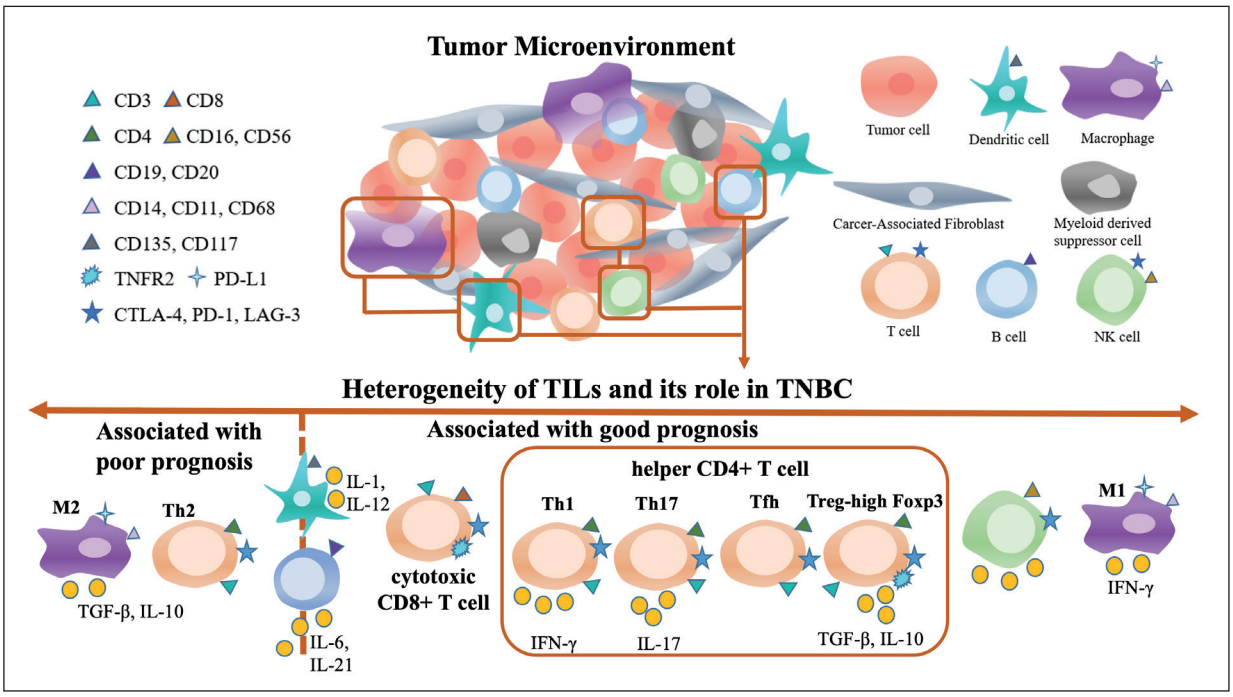

Figure 1. Heterogeneity of TILs and its role in TNBC.

by T cells, with smaller proportions of B cells, NK cells, follicular dendritic cells, and macrophages[28]. Using the 2014 TIL standard, a large retrospective study[29], including 897 TNBC patients, found that the survival [DFS (disease-free survival), D-DFS (distant disease-free survival), and OS (overall survival)] of patients were improved with every 10% increase in sTILs. Denkert et al[30] investigated TILs which were conducted by the International Immuno-oncology Biomarker Working Group in 2016, which provides standardized reports of tumor immunological parameters in clinical studies and diagnostic practice. The standardized method for TILs evaluation in breast cancer and the integrated clinicopathologic prognostic model combining standard prognostic factors and sTIL quantity are freely accessible at www.tilsinbreastcancer.org.

Clinical Studies that Have Assessed TILs and Prognosis After Treatment in TNBC

Denkert et al[31] in 2010 first reported the correlation between TILs and the prognosis of breast cancer, especially CD8+T cells and the clinical response of anthracycline/taxane-based NAC regimens that are linearly related. In terms of adjuvant therapy, Loi et al[32] in 2013 demonstrated that the presence of TILs at the time of diagnosis is clearly associated with better clinical outcomes in TNBC patients, regardless of the type of adjuvant chemotherapy (anthracyclines *versus* Adriamycin plus docetaxel), and higher TILs are linearly associated with reduced risk of recurrence and death.

Adjuvant and Neoadjuvant Studies That Have Assessed TILs and Prognosis After Chemotherapy in TNBC

The Quantity of TILs in Pooled Analyses of Adjuvant Chemotherapy Prognosis

Recently, a series of pooled data analyses confirmed the strong prognostic role of sTILs in early-stage TNBC and excellent survival with high sTILs after adjuvant chemotherapy. Loi et al[33] collected individual data from 2,148 patients from nine studies and built a multivariable model in which each 10% increment in sTILs corresponded to iDFS (non-invasive disease-free survival), D-DFS (distant disease-free survival), and OS (overall survival). In node-negative patients with sTILs > 30%, 3-year iDFS was 92%, D-DFS was 97%, and OS was 99%. For LPBC (lymphocyte-predominant BC, TILs ≥50%), a pooled analysis of four prospective adjuvant trials

showed that 3.7% of LPBC patients relapsed without death *vs.* recurrence rate and mortality rate of 28.3% for non-LPBC[34]. In addition, Park et al[35] found that sTILs can identify a subset of stage I TNBC patients with an excellent prognosis without adjuvant chemotherapy.

The Quantity Of Tils In Pooled Analyses Of Neoadjuvant Chemotherapy Prognosis

TILs are also a predictive and prognostic biomarker in neoadjuvant chemotherapy in TNBC. In GeparSixto, which investigated the effect of adding carboplatin (Cb) to an anthracycline-plus-taxane combination (PM), the pCR rate was 59.9% in LPBC and 33.8% for non-LPBC ($p < 0.001$)[36]. Moreover, a pooled analysis of 3771 patients treated with neoadjuvant combination chemotherapy in TNBC included from six randomized trials conducted by the German Breast Cancer Group showed that 10% increase in TILs was associated with longer DFS and OS in TNBC, and pCR was achieved in 31% with low TILs, 31% with intermediate TILs and 50% with high TILs[37].

Studies That Have Assessed TILs and Prognosis After Immunotherapy in Early and Advanced TNBC

The results from IMpassion 130[38] demonstrated a substantial OS benefit in patients with PD-L1+ metastatic or inoperable locally advanced TNBC through atezolizumab to first-line chemotherapy with nab-paclitaxel (25.0 months with atezolizumab versus 18.0 months with placebo), which brought breast cancer into the immunotherapy era. Moreover, they found that CD8+TILs and PD-L1 are both related to PFS (progression-free survival) and OS benefits, while sTILs are only related to PFS. In addition, TILs ≥5% have been shown to be predictive of response to pembrolizumab in the exploratory analysis of the randomized phase III KEYNOTE-119 clinical trial[39]. In the phase II KEYNOTE-086 study[40], it was confirmed that there were fewer TILs at metastasis foci than there were at the primary foci. Recently, the FUTURE trial[41] showed that immunotherapy (arm C), compared to the patients who were classified as immunomodulatory group (TP53 mutation, KDM5A expression, CD8+TILs, and PD-L1 high expression), achieved the highest ORR in the ITT (intention-to-treat) population.

In neoadjuvant therapy in early TNBC, the results from the KEYNOTE-173 study[42] showed that high expression levels of pre-treated interstitial TILs and PD-L1 in TNBC patients treated primarily with pembrolizumab plus chemotherapy were significantly associated with higher pCR and ORR. Clinical studies[31,32,36,38,39,42-48] on TILs and the prognosis of TNBC that are not detailed above and clinical studies of immunotherapy in TNBC not mentioned are summarized in Table I (studies that have assessed TILs and prognosis of TNBC are included).

Relationship with Other Biomarkers

As early as 2012, Liu et al[49] found that the favorable prognostic effect of CD8+TILs was significant only in the TNBC patients who expressed markers associated with the basal-like subtype but not in TNBC patients who lacked expression of those markers or in the other intrinsic subtypes. Thus, we question whether markers directly expressed in TILs can also be correlated with prognosis and whether the detection of these markers combined with the detection of TILs will have a stronger prognostic effect.

Relationship with PD-L1 Expression

Positive expression of PD-L1 exists as an independent predictor of advanced TNBC[38,46-48]. Recently, the International Immuno-Oncology Biomarker Working Group proposed PD-L1 and TILs as the composite biomarkers of BC, which may help reduce the risk of suboptimal patients who choose immunotherapy in clinical trials and daily practice. In contrast, the sample with high TILs is highly likely to be PD-L1+, which was confirmed in the population that could be evaluated for the biomarker explored by IMpassion130. Among them, almost all the cases[50] with TILs ≥20% are PD-L1+. Tumor-associated macrophages (TAMs) have been shown to modulate, directly and indirectly, PD-1/PD-L1 expression in the TME[51].

In fact, PD-L1 is mostly expressed in immune cells (interstitial TILs), and only a few are expressed in tumor cells in breast cancer, which is reflected in the application of CPS (combined positive score) in clinic. For instance, the ITT population was divided into three groups according to CPS ≥20, CPS ≥10 and CPS ≥1 in KEYNOTE-355, and the higher the CPS was, the more evident were the observed PFS and OS benefits[46]. In addition, the results from KEYNOTE-119[38] also showed a similar relation to ORR, PFS, and OS in TNBC patients with CPS.

Table I. Studies that have assessed TILs and prognosis of TNBC are included.

Study		Sample size	Regimen	Tumor tissue assay	Correlation with outcome	Reference
CHEMOTHERAPY						
Adjuvant chemotherapy	IG 02-98	2009total 256TNBC	A→CMF or AC→CMF	Full section H&E	in TNBC, Stromal TILs (sTIL) (continuous, per10% increase) univariate: HR0.84 (p =0.02, DFS) HR0.82 (p =0.02, OS); multivariate: HR0.85 (p =0.02, DFS) HR0.83 (p =0.02, OS).	[32]
	ECOG 1199 ECOG 2197	481TNBC	AC-Doc /T (ECOG 1199) AT/AC (ECOG 2197)	Full section H&E	in TNBC, for every 10% increase in sTILs, 14% reduction of risk of recurrence or death (p = .02), 18% reduction of risk of distant recurrence (p = .04), and 19% reduction of risk of death (p = .01); multivariable analysis: sTILs is an independent prognostic marker of DFS, DRFI, and OS.	[43]
	IBSCG 22-00	647TNBC	CM	Full section H&E	in TNBC, for every 10 % increase of TILs, BCFI risk reduction was 13 % (HR 0.87, p = 0.003). DFS, DRFI, and OS risk reductions were 11 % (p = 0.005), 16 % (p = 0.003), and 17 % (p <0.001); patients with LPBC receiving CM had a greater breast cancer risk reduction (HR 0.64) than those with non-LPBC (TILs<50 %) (HR 0.96).	[44]
Neoadjuvant chemotherapy	GeparDuo	218 total 47TNBC	AC-Doc	TILs in H&E core biopsy	in total, the pCR rate for the patients with increased iTu-Ly (> 10%) was 31% (p < .0005); LPBC had a pCR rate of 41.7% *vs* tumors without any infiltrating lymphocyte of 2.8% (p < .0005).	[31]
	GeparTrio	840 total 219TNBC	TAC±Vinorelbine/ Capecitabine	TILs in H&E core biopsy	in total, the OR for pCR increased with the extent of iTu-Ly and str-Ly, with a maximal OR of 13.39 (p < .0005) for tumors with more than 60% of iTu-Ly; LPBC had a pCR rate of 40% *vs* tumors without any infiltrating lymphocyte of 7.2% (p < .0005).	[31]
	GeparSixto	580 total 314TNBC	PM or PM+Cb	TILs in H&E core biopsy	in TNBC, stromal TILs significantly linked to pCR, with an OR of 1.22 (95% CI, 1.14 to 1.31) per 10% increase in lymphocytes (p < .001); pCR rate in PMCb therapy LPBC *vs* Non-LPBC is 73.8 vs 45.7 (p = .002).	[36]

Continued

Table 1 (Continued). Studies that have assessed TILs and prognosis of TNBC are included.

Study		Sample size	Regimen	Tumor tissue assay	Correlation with outcome	Reference
IMMUNOTHERAPY						
Immunotherapy in advanced TNBC	IMpassion 130	902TNBC	atezolizumab or placebo plus nab-paclitaxel using the PD-L1	TILs in H&E core biopsy; PD-L1 expression assessed IHC SP142 pharmDx	in TNBC, CD8+TILs and PD-L1 are both related to PFS and OS benefits, while sTILs is only related to PFS.	[38]
	KEYNOTE 086	193TNBC	pembrolizumab	TILs in H&E core biopsy; PD-L1 expression assessed using the PD-L1 IHC 22C3 pharmDx	in TNBC, higher TIL levels were associated with significantly improved ORR (OR 1.26, p = .01) and DCR (OR 1.22, p = .01); PD-L1 expression significantly correlated with TIL levels ($\rho = 0.4962$, $p < .001$).	[39]
	KEYNOTE 119	600TNBC	pembrolizumab	PD-L1 expression assessed using the PD-L1 IHC 22C3 pharmDx	in TNBC, the higher PD-L1 CPS, the more obvious ORR, PFS and OS benefit were observed.	[45]
	KEYNOTE 355	847TNBC	pembrolizumab or placebo plus nab-paclitaxel/ carboplatin/ gemcitabine	PD-L1 expression assessed using the PD-L1 IHC 22C3 pharmDx	in TNBC, the higher PD-L1 CPS, the more obvious PFS and OS benefit were observed.	[47]
Immunotherapy in early TNBC	KEYNOTE 173	60TNBC (cohorts A-F)	pembrolizumab with ±nab-paclitaxel carboplatin, followed by AC	TILs in H&E core biopsy; PD-L1 expression assessed using the PD-L1 IHC 22C3 pharmDx	in TNBC, patients with high expression levels of pretreated interstitial TILs and PD-L1 were significantly associated with higher pCR and ORR.	[42]
	GeparNUEVO	174TNBC	±durvalumab AC→nab-paclitaxel	TILs in H&E core biopsy	in TNBC, predefined sTIL levels at baseline predicted pCR in the complete cohort: OR (intermediate (10%-59%) versus low (<10%)) 1.79 (95% CI 0.93– 3.47), OR (high (>60%) versus intermediate) 3.09 (95% CI 1.12–8.52), p =0.005.	[46]
	IMpassion 031	333TNBC	atezolizumab or placebo plus nab-paclitaxel/ doxorubicin	PD-L1 expression assessed using the PD-L1 IHC SP142 pharmDx	in TNBC, regardless of PD-L1 status pCR rate benefited with a 16.5% increase (57.6% vs 41.1%, p =0.0044) in ITT population who accepted atezolizumab.	[48]
	KEYNOTE 522	1174TNBC	pembrolizumab or placebo plus nab-paclitaxel carboplatin, followed by AC	PD-L1 expression assessed using the PD-L1 IHC 22C3 pharmDx	in TNBC, regardless of PD-L1 status pCR rate benefited with a 13.6% increase (64.8% vs 51.2%, p =0.00055) in ITT population who accepted pembrolizumab.	[49]

AC, doxorubin/cyclophosphamide; CMF, cyclophosphamide, methotrexate, 5-Flourouracil; AC-Doc, doxorubicin/cyclophosphamide followed by docetaxel; TAC, docetaxel, doxorubicin and cyclophosphamide; Cb, carboplatin; PM, anthracycline, taxane; AT, doxorubicin plus paclitaxel; AC-T, doxorubicin/cyclophosphamide followed by paclitaxel; CM, cyclophosphamide, methotrexate; iTu-Ly, intratumoral lymphocytes; str-Ly, stromal lymphocytes; LPBC, lymphocyte-predominant breast cancer; DFS, disease free survival; HR, hazard ratio; NACT, neoadjuvant chemotherapy; OS, Overall survival; pCR, pathological complete remission; BCFI, breast cancer-free interval; DRFI, distant recurrence-free interval; OR, odds ratio; PFS, progression free survival; ORR, objective response rate; DCR, disease control rate; CPS, combined positive score; ITT, intention-to-treat.

Relationship with the Regulatory Molecules Is Associated with Immune Exhaustion

CTLA-4 (cytotoxic T lymphocyte-associated antigen), LAG3 (lymphocyte activation gene 3), and Tim-3 (T cell immunoglobulin mucin 3), through their binding with corresponding ligands, prevent T cells from normal recognition of tumor cells, regulate CD8+T cells from exhaustion and induce the immunosuppressant environment; all of these factors are expressed in TILs[9]. Solinas et al[52] detected LAG3 expression in a small portion of CD4+ and CD8+TILs but rarely in stromal cells and non-lymphoid cells of the TME, and tumors expressing LAG3+ also contain PD-1+CD4+ and/or CD8+TILs. In addition, the discovery of LAG3 upregulation on TIL in MHC II tumors resistant to anti-PD-1 drugs supports the correlation between LAG3 expression in BC and extensive immune infiltration. Burugu et al[53] evaluated Tim-3 IHC expression in all subtype samples of 3992 breast cancer patients and found that TIM-3 intraepithelial TIL infiltration was associated with a better prognosis. The novel inhibitory IRs, such as LAG-3, TIM-3, and TIGIT, and their relationship with the tumor immune microenvironment is a popular topic for us.

Relationship with Gene Expression

Gene expression profiles, as significant prognostic factors and biomarkers of TNBC, have also been found to be related to immunity and TILs in recent years. Criscitiello et al[54] found a four-gene signature combining the expression levels of HLF, CXCL13, SULT1E1, and GBP1, which was developed in baseline samples to predict the extent of TILs after NAC in TNBC patients, was significantly associated with DRFS (distant relapse free survival) and pCR. Romero-Cordoba et al[55] identified three TNBC clusters (ImA, B, and C) displaying unique immune gene features. Therein, an immune-active subtype (ImA) that was characterized by "T cell-inflamed" phenotype presented the lowest aggressive score and a diminished number of progression events compared to the other Im-Clus, as shown by high CD8+/CD4+ T and NK cell infiltration, which also presented an enriched expression of immune inhibitory pathways (PD-L1/PD-1/CTLA4 axis) induced by the inflammatory process. Recently, Katherine et al[56] profiled a 14-gene Th1 response-activating score, an 18-gene T cell inflamed score, a 28-gene IFNγ score, and a 7-gene immune-activating score, and they observed a concomitant trending decrease in the immune-related gene signature scores following therapy and a positive correlation of each of these scores with percent sTILs.

The Future Potential of TILs

TILs in Residual Disease and Ras/MAPK Signaling

TIL expression in the residual disease (RD) after NAC in TNBC has been found to be associated with a good prognosis in recent years. Dieci et al[57] reported that the presence of >60% TILs in RD after NAC is associated with better prognosis in patients with TNBC. In another study[58], 10% of patients had TILs>60% in their RD after NAC, and the 5-year OS rate was 91% in the high-TIL *vs.* 55% in the low-TIL groups. In addition, Luen et al[59] evaluated the added prognostic value of RD TILs to Residual Cancer Burden (RCB I, II, III) in predicting survival post-NAC for primary TNBC and suggested that if there is residual tumor load or residual lesions with high pathological grade, although CD8+TILs is highly expressed, then the immune response is inhibited, and the prognosis is poor. Loi et al[58] suggested that the mechanism may depend on the activation of the RAS/MAPK pathway in which genetic or transcriptomic alterations in RAS/MAPK signaling were significantly correlated with lower TILs.

Complement of PD-1+ TILs-TNFR2+ TILs

Similar to the different roles of Tregs in TNBC and other tumors, the levels of TNFR2 [one of the two receptors of tumor necrosis factor α (TNFα), expressed on Teffs and Tregs] also points to good prognosis in TNBC. In other tumors, TNFR2 is a target for therapy because TNFα itself was strongly and causatively connected to poor prognosis in many malignancies including TNBC[60]. However, Dadiani et al[61] proposed that the TNFR2+ TIL subset should be kept intact in TNBC for the reason that presence of TNFR2+ TILs and PD-1+ TILs was independently associated with a good prognosis, and the combination of both parameters demonstrated superior outcome relative to their lower levels. Thus, it is suggested that the TNFR2+ TIL subset should not be targeted in the course of TNBC therapy; rather, its beneficial impacts may come into power with anti-PD-1 regimens, which may potentiate immune activities to TNBC patients.

Qualitative Heterogeneity of TIL Subgroups -T_{RM}, Controversial Resident Cells

The standard view is that TILs in BC consist of tumor-specific T cells that are chronically exposed to antigen, rather than merely being resident memory lymphocytes[62]. However, Savas et al[63] found that tissue-resident memory T cell (T_{RM}) can better define patient outcomes in TNBC. As a specialized subpopulation of TILs, T_{RM} can reside indefinitely and respond to their homologous antigens with a rapid immune response and express high levels of immune checkpoint molecules and effector proteins, such as PD-1 and CTLA-4. In addition, CD8+T_{RM} gene markers obtained from the scRNA-seq data were significantly associated with improved survival in patients with early TNBC and provided a better prognosis than CD8 expression alone. KEYNOTE-086 assessed the efficacy of pembrolizumab in patients with metastatic TNBC and demonstrated the characteristic enrichment of CD8+T_{RM} cells as an effective marker for the prediction of treatment response[64]. We need to determine whether there is an immune pathway linking neoantigen-activated and resident T cells.

Construction for TILs of TME In Vitro-PDO Model

PDX (patient-derived xenograft) mice with human hematopoietic and immune systems have been used in many sorts of experiments *in vivo* as powerful tools for the analysis of tumor-immune system interaction and evaluation of immunotherapy response[65]. From the beginning, our attempts at immunotherapy in translational experiments have failed to break through the construction of simulated TME *in vitro*. Recently, Neal et al[66] cultured >100 biopsy samples from tumor patients or mice tumor-derived organoids (PDO) by an air-liquid interface (ALI) cultural method, which can be propagated from primary tumor fragments with immune and fibroblastic components for several weeks, and these ALI cultures display T cell clonal diversity that mirrors the T cell diversity in the patient's peripheral blood. Although the successful simulation of TILs in PDO has not been realized in breast cancer, the existing construction of TME *in vitro* still provides us with a new platform for TIL-related molecular pathways, TIL-related immune checkpoints and immunotherapy, as well as the idea to continue making efforts to construct the TME in the PDO model of breast cancer.

Discussion

Whether the prognosis of patients with TNBC is relevant to high or low TILs expressed in primary and metastasis sites needs to be pondered. Moreover, whether the heterogeneity of TILs in primary sites, RD after NAC and metastasis sites can guide the selection of follow-up treatment options in TNBC is another problem. Important limitations on the use of TILs currently include dependence on manual quantification with potential human error, although the development of a training website (www.tilsinbreastcancer.org) has provided a useful tool for those wanting to upskill[67]. Surprisingly, there are ripe opportunities to employ computational methods that extract spatial-morphologic predictive features that are now enabling computer-aided diagnostics[68].

Since TILs has been identified as a clear biomarker in early TNBC, how to evaluate TILs in combination with other biomarkers to guide a more specific treatment is our work to do next. In addition, the current opinion that TILs are the starting point for the expression of other biomarkers is still worth discussing.

In terms of immunomodulatory mechanisms, it deserves attention whether negative immunomodulatory regulation as part of a normal feedback loop has a positive and persistent effect on the tumor immune response. And whether the possible mechanism above potentially defines a more immunogenic tumor will need more work to be done to explore further. Moreover, we should continue to focus on the heterogeneity of TILs, the subpopulation classification of T cells, and how their respective molecular pathways regulate immunity.

Recently, TMB (tumor mutation burden) dynamic tests in the ctDNA of patients with non-small cell lung cancer and the finding of exosomal PD-L1 has advanced the field of dynamic monitoring of the changes of biomarkers. Therefore, the exploration of dynamic monitoring method of TILs in breast cancer patients could be an emerging topic for us. Simulation of TIL infiltration of the TME *in vitro* can be achieved using a PDO model, which enables us to evaluate curative effect and readjust treatment in tandem with dynamic changes.

Conclusions

The role of TILs in the TME and clinical trials related to TILs in TNBC was summarized in this review. In addition, we also summarized the

relationship between common biomarkers and TILs and the potential future research directions of TILs. As immunotherapy will be part of routine treatment in TNBC, future studies need to explore the above TIL-related issues further and to raise more new questions in combination with the clinical situation.

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Conflict of Interests

The authors declare no conflicts of interest.

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