Immunological characteristics of the tumor microenvironment of breast cancer and their utilization as prognostic factor and treatment: a review

Características imunológicas do microambiente tumoral do câncer de mama e sua utilização como fator prognóstico e tratamento: uma revisão

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ABSTRACT
Globally, breast cancer is the most diagnosed neoplasm in women. In some countries, it's consolidated as the type of neoplasm that causes the most deaths, being responsible for premature deaths. The molecular classification of cancer allows specific treatment for each patient; however, some types have resistance that results in an unfavorable prognosis. For this reason, researches are being developed in order to use the complexity of the immune system as a tool to combat the heterogeneity of breast cancer. Lately, studies have evaluated the leukocyte infiltrates present in the tumor microenvironment have become a promising field in therapeutic innovation, which is based on the cellular composition of the neoplastic environment of cancers with poor prognosis, whose immunotherapeutic action is specific to each molecular subtype. The expression of immune cells in the tumor microenvironment has potential for oncological parameters that can help in the treatment and also to define the prognosis.

Keywords: Breast Neoplasm, Immune System, Tumor Microenvironment, Prognosis, Immunotherapy.

RESUMO
O câncer de mama é a neoplasia mais diagnosticada em mulheres, a nível global. Em alguns países, consolida-se como o tipo de neoplasia que mais causa óbitos, sendo responsável por inúmeras mortes prematuras. A classificação molecular do câncer permite o tratamento específico de cada paciente, contudo, alguns tipos apresentam resistência terapêutica que resulta em prognóstico não favorável. Por esse motivo, pesquisas vêm sendo desenvolvidas no intuito de utilizar a complexidade do sistema imunológico como ferramenta de combate a heterogeneidade do câncer de mama. Ultimamente, estudos que avaliam os infiltrados leucocitários presente no microambiente tumoral tem se tornado um campo promitente na inovação terapêutica, a qual é baseada na composição celular do ambiente neoplásico de cânceres de mau prognóstico, cuja atuação imunoterapêutica é específica a cada subtipo molecular. A expressão de células imunológicas no microambiente tumoral tem potencial de fornecer parâmetros oncológicos que possam auxiliar no tratamento e também na determinação prognóstica.

Palavras-chave: câncer de mama, sistema imunológico, microambiente tumoral, prognóstico, imunoterapia.

1 INTRODUCTION

Worldwide, breast cancer is the most frequent type of neoplasm in the female population, excluding non-melanoma skin, which occupies 24.2% of all tumors, totaling more than 2 million new cases per year[1]. Breast carcinogenesis is linked to the permanence of noxious stimulus, immunosuppression and mutations in specific genes, such as BRCA1 and BRCA2, which are associated with heredity. Consequently, they are

clinically expressed with a high adversity[2,3] and, even with great possibility of remission, it is still a leader of lethality among neoplasms in the female gender[2,4].

Currently, there is the perspective of analyzing the cells of the immune system present in the tumor microenvironment as way to aid in prognostic determination, besides becoming key points in the target intervention against cancer evolution[5]. The relationship between the molecular subtype of breast cancer and the tumor microenvironment reveals important factors regarding the staging and clinical management of health professionals[6]. Therefore, the knowledge of the immune system is of extreme relevance in carcinogenesis; its cell-cell interaction has become the subject of study to understand the disease and also a specific treatment implementation[7].

Because of this, we will discuss how the tumor microenvironment adhering to molecular subtypes of breast cancer can be used as prognostic indicators, as well as addressing the importance of cellular infiltrates and their use as a parameter for a better therapeutic option in poor prognostic cancers.

2 METHODS

This is a literature review based on randomized scientific articles and bibliographic reviews. The following databases were used: Pubmed Central (NCBI), ScienceDirect and Scielo, with the Medical Subject Headings (MeSH): "Breast Neoplasm". "Immune System", "Tumor Microenvironment", "Prognosis" and "Immunotherapy" published in the last 10 years, with exclusion criteria for articles in pre-print format and with impact factor (IF) less than 0.7. The search resulted in 662 articles, after inclusion criteria, coherence with the objective of this study and reading the title and abstract of the journals, fifty six (56) articles were selected to guide how the composition of the tumor microenvironment and the presence of lymphocytic infiltrates can be used as target treatment parameters for cancers with bad prognosis.

3 DEVELOPMENT

3.1 PROFILES OF CANCERS WITH POOR PROGNOSIS

Breast cancer occupies a complex group of diseases that reflects both genetic and morphological. Molecularly, its classification is represented by the expression of hormonal receptors, such as estrogen receptor (ER) and progesterone (PR), tyrosine kinase receptor (HER2) and proliferative marker Ki-67, as shown in table 1[4,8].

Table 1. Breast cancer classification molecular.

MOLECULAR CLASSIFICATION	Ki-67	ER	PR	HER2
Luminal A	<14%	Positive	Positive	Negative
Luminal B	≥14%	Positive	Positive /Negative	Negative
Luminal HER2	≥14%	Positive	Positive	Positive
HER2	>14%	Negative	Negative	Positive
Basal-like (TNBC)	>14%	Negative	Negative	Negative

Ki-67 = Proliferative marker; ER = Estrogen Receptor; PR = Progesterone Receptor; HER2 = Human Epidermal Growth Factor Receptor 2, TNBC = Triple Negative Breast Cancer. Adapted from HARBECK, *et al*. 2019; PROVENZANO, *et al*. 2017 and IGNATIADIS; SOTIRIOU, 2013.

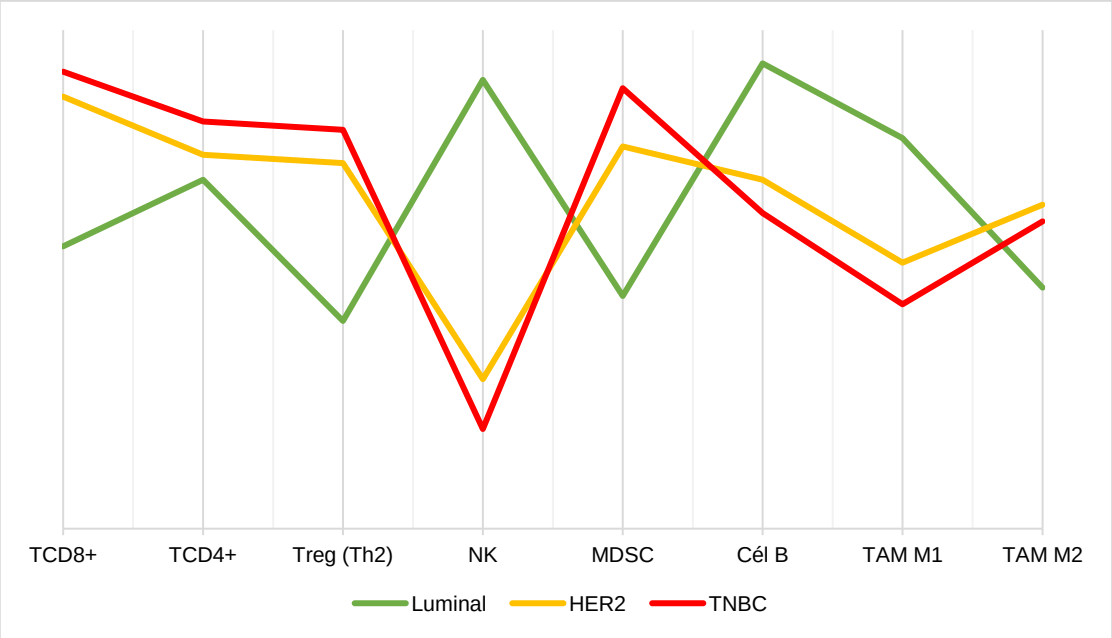
The main causes of lethality, called cancer with poor prognosis, are: HER2 positive and triple negative breast cancer (TNBC) and, one of the main reasons for this chance, is due to the composition of its unfavorable tumor microenvironment added to the absence of specific therapy[9,10]. These types of tumors present higher mutational load when compared to luminal types, due to the function of high-grade carcinogenic pathways, which have high expression of HER2 and genes that are involved in the cell cycle and proliferation, showing an adverse prognosis; contrary to low-grade pathways, with less aggressive phenotype, being associated with luminal subtypes[4,11].

The intense expression of HER2 is associated with high cell proliferation, commonly presenting changes in pathways and genes such as: cytokeratin (CK), PIK3CA, mTOR and MAPK/ERK[12], resulting in non-linear behaviors. Usually, HER2-positive tumors are submitted to therapy with the use of monoclonal antibodies[13], but sometimes do not have the expected effects, causing them to obtain an aggressive prognosis[9].

As briefly stated, the TNBC does not have specific treatment[10], being susceptible only to chemotherapy and presents greater therapeutic resistance among molecular types, classified itself as the most aggressive subtype[14]. Genetic, morphological and clinical aspects present higher heterogeneity[12], which may reflect in an earlier carcinogenesis, composed of non-uniform cells[15].

In addition to these mechanisms, the inflammatory process can negatively contribute to the clinical condition of these patients[16]. The recruitment of immunological cells has ambiguous activity, and may be immunosuppressive or antitumor, which is why the understanding of tumor-infiltrating lymphocytes (TIL) is very useful in the pathological course[17]. The composition of TIL, according to the molecular subtype of breast cancer, can be used as a prognostic parameter and immunotherapeutic treatment[18]. The main immunological signatures that are associated with the prognostic value are represented in graph 1[4,19,20].

Graph 1. Immunological cells associated to breast cancer molecular types and prognosis.



This graphic illustration demonstrates the presence of immune cells to the molecular type of breast cancer. It is noticeable that in luminal tumors the presence of immunosuppressive cells or cells correlated with chronic inflammation are lower when compared to HER2 and TNBC. TCD8 lymphocytes, which are associated with positive prognosis, have a higher prevalence in tumors with poor prognosis, which leads to the composition of their MAT having great immunogenic activity and, consequently, high presence of cancer cells. The line that represents the luminal tumor is presented in the opposite way to that of tumors with a poor prognosis.

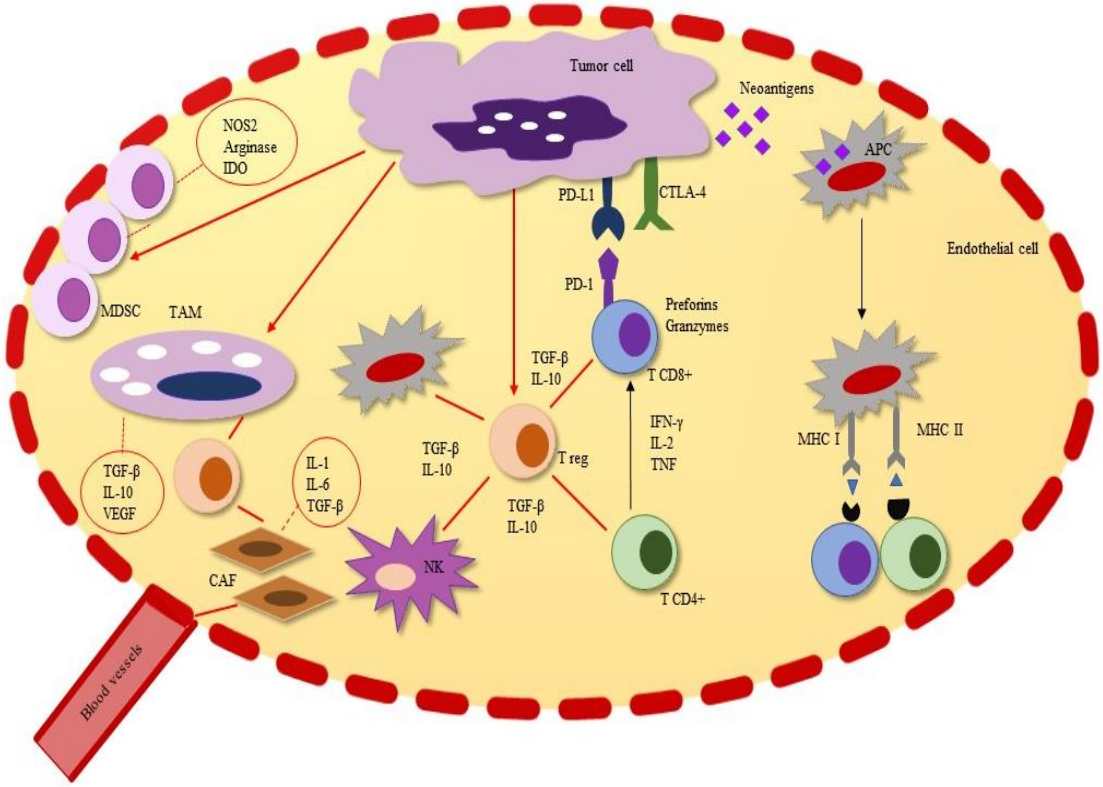
Adapted from FRIDMAN et al., 2017; JULIÁ; MORDOH; LEVY, 2020 and NOSKE et al., 2019

3.2 IMPORTANCE OF THE TUMOR MICROENVIRONMENT IN BREAST CANCER

As the pathogenesis of breast cancer is not fully comprehend, it is necessary to understand the neoplastic development from the composition of the tumor microenvironment (TME), which consists not only of cancer cells, but also by stromal cells and immunological infiltrates (see figure 1). This cellular set promotes angiogenesis, tumor growth, tissue remodeling, suppression of adaptive activity and metastasis[20]. Consequently, the recognition of TME adjunct to the molecular type of breast cancer

presumes in a potential prognosis[6]. Graph 1 demonstrates the main components of the neoplastic microenvironment associated with molecular types of breast cancer.

Figure 1. Breast cancer tumor microenvironment.



Cancer cells, fibroblasts, endothelial cells and immunological cells composed tumor microenvironment. These constituents may present effector (black arrow) and immunosuppressive functions (red arrow), thus contributing or hindering the growth of the breast tumor. TCD8+ cells are fundamental for the antitumor response adaptive through the release of their cytoplasmic granules, perforins and granzymes, responsible to do pores in the membrane of the malignant cell and induct cellular apoptosis, respectively. This response occurs through the presentation of tumor neoantigens by antigen-presenting cells (APCs) to T cells through histocompatibility complexes (MHC) 1 and 2, thus activating TCD8+ and TCD4+. The secretion of cytokines from TCD4+ as TNF, IL-2 and IFN-γ, can enhance the antitumor activity of TCD8 cells. For its growth to occur without interference of the antitumoral mechanism, cancer cells use several mechanisms to escape the immune system. Recruitment of Treg immunosuppressive cells, MDSC and phenotype 2 TAMs, aid tumoral progress by inactivation of CD8+ T and CD4 T through the release of TGF-β and IL-10 cytokines, which also inhibit APCs

and NKs. MDSCs block T lymphocyte traffic to the tumor, in addition to releasing arginase, Indoleamine 2, 3-dioxygenase (IDO) and Nitrogen Dioxide (NO2), responsible for promoting T-cell depletion. TGF-β, secreted by TAMs and cancer-associated fibroblasts (CAF), has as its function the recruitment of Reg T cells. In addition, TAMs can produce vascular endothelial growth factor (VEGF) and CAFs are able to reprogram endothelial cells favoring angiogenesis. CAFs can also produce Il-1 and IL-6 immunosuppressive cytokines. Tumor cells can stimulate T-cell exhaustion by expression of immune checkpoint regulators (PD-L1 and CTLA-4).

Adapted from HARBECK, *et al*., 2019; SOYSAL, TZANKOV, MUENST, 2015 and HAMMERL, *et al.,* 2018

TME is indispensable for initiation and progression of breast cancer, especially those with unfavorable prognosis[14]. Depending on their oncogenic pathway, disturbances in the microenvironment may be intense, besides being able to induce immunosuppressive mechanisms[7], as in chronic inflammation[16]. It is common for cancer cells to stimulate a pro-neoplastic environment from the production of cytokines and suppressive molecules. The transformation growth factor beta (TGF-β), one of the main cytokines present in the cancer environment, has the activity of contributing to tumor growth and converting TCD4+ into regulatory T lymphocytes (Treg), having an immunosuppressive character[21]. The presence of myeloid-derived suppressor cells (MDSC), tumor-associated macrophages (TAM) M2 and lymphocytes T helper 2 (Th2) also assist tumor development, as well as interleukins (IL) 1, 6, 8 and 10, and cytokines: tumor necrosis factor alpha (TNF-α), interferon (IFN) and vascular endothelial growth factor (VEGF)[20,21].

Another component that suffers the impacts of participation neoplastic cells on the stromal cells is the extracellular matrix (ECM)[22], whose change reflected in its biochemical composition, corroborating the hallmark of the TME, leading to: hypoxia, angiogenesis, resistance to apoptosis, immune evasion and metastasis[20–22].

The use of TME to induce anti-tumor responses based on the diversity of immune cells against neoplastic cells has become one of the main therapeutic strategies researched[14]. The development of anti-cancer drugs presents promising responses when based on cytotoxicity, reaching rates of 95% efficacy *in vitro* studies (preclinical stage), but which fall to 7.5% when submitted to *in vivo* studies[22], thus making it necessary to understand the specific behavior of the microenvironment to the molecular subtype of cancer[10].

The microenvironment of tumors with poor prognosis, especially TNBC, presents a large amount of TIL, which are also composed for: B cells, Natural Killers (NK), macrophages and dendritic cells (DCs)[18], being also present to the ligation of the programmed cell death protein 1 (PD-L1)[14], which results in some immunotherapeutic and favorable prognostic possibilities[23]. The presence of TIL in TME is considered as an immunogenic marker and immunological treatment, however, despite only as therapeutic potential, these immunological signatures in the neoplastic microenvironment present a possibility of a new oncological classification, which is based on immunological score, quantifiable cell density and location of infiltrates, whose parameters are measured by immunohistochemistry (IHQ) and/or flow cytometry[14,22–24].

4 IMMUNOLOGICAL CHARACTERISTICS OF BREAST CANCER AND ITS USE AS A SPECIFIC THERAPY

Since the 20th century, the idea that immune system controls cancer development has been analyzed[25]. In the oncological context, immunovigilance has the activity of recognizing the self and non-self, however, neoplastic cells are able to evade this mechanism by inhibiting immune checkpoint, expressing programmed cell death protein 1 (PD-1) and protein associated with cytotoxic T lymphocytes 4 (CTLA-4), which causes low immunogenicity[26], making this expression one of the main targets of immunotherapy[27].

The new oncological objective is the target treatment based on the interaction of the immune system with neoplastic cells, added to the cellular infiltrate in TME[7,23] and molecular characteristics, so each molecular type of breast cancer has its own amount and composition of cellular infiltrate, which reflects in the complexity of treatment[10,18,28].

As a result, immunotherapeutic techniques have gained prestige in the interface of genetic alterations and host anticancer activity. This type of therapy focuses on the evaluation of leukocyte infiltrates, which are also used as immunological markers for tumors with unfavorable prognosis[19,28]. The main immunotherapeutic techniques are: administration of monoclonal antibodies (immunogenic cell death), cellular activation transfer (CAT), immune checkpoint and tyrosine kinase's inhibitors, cancer vaccines, oncolytic virotherapy and T-cell chimeric antigen receptor therapy (CAR)[7,11,29]. Table 2 depicts the main immunotherapeutic targets associated with the molecular subtypes of breast cancer.

Table 2. Immunotherapies based on molecular characteristics breast cancer.

Immunotherapy	Target	Molecular subtype	Favorable environment for immunotherapy	References
Anti-checkpoint immune	PD-1, PD-L1, CTLA-4, TIM-3, LAG-3, OX40	TNBC, HER2, Luminals	When immune cells express immune regulatory proteins and cancer cells synthesize their ligands to evade the antitumor response.	4, 10, 14, 18, 23, 26, 28, 30, 37, 39
Antibodies against receptors	HER2, RA, TK, CDK	HER2	Favorable when receptors are overexpressed and inducing cell proliferation.	4, 11, 13, 18, 19, 26, 28, 30, 31, 35
Oncological vaccines	EGFR	HER2	Main action on antigens, when they are masked, presence of neoantigens or TAAs. The use of APCs, especially DC, is very promising.	6, 28
Cellular immunotherapies ACT CAR	TIL (TCD8+ and Antigen-recognition domain T cell)	TNBC, HER2	When immune cells do not recognize cancer cells and are enhanced to recognize them, synthesizing antigens on tumor cells and expanding a population of immune effectors. Performed by genetic engineering.	5, 19, 26, 28, 40
Immunosuppressors' inhibitors	Th2, Treg, FOXP3, MDSC, TGF-β	TNBC, HER2, Luminals	When the immunosuppressant population is high and preventing an optimal immune attack from occurring.	7, 23
Oncogenic pathways' inhibitors	PI3K/Akt/mTOR, ERK-MAPK, JAK/STAT	TNBC, HER2, Luminais	When tumor cells modify the activity of oncogenic pathways favoring tumor growth, angiogenesis, cellular disorder, immunosuppression, induction of chronic inflammation, resistance to apoptosis and metastasis.	11, 19, 23
PARP's inhibitors	BRCA	TNBC	When there is mutation or overexpression of the BRCA gene.	4, 15, 26, 39

ACT = Adoptive Cell Transfer; APCs = Antigens Presenting Cells; AR = Androgen Receptor; CAR = Chimera Antigens Receptor; CDK = Cyclin Dependent Kinase; DC = Dendritic Cells; EGFR = Epidermal Growth Factor Receptor; FOXP3 = Forkhead Box P3;; LAG3 = Lymphocyte Activating 3; OX40 = TNF receptor superfamily member 4; PARP = Poly ADP-ribose Polymerase; TAAs = Tumor associated antigens; TIM-3 = T cell Immunoglobulin and Mucin domain-containing 3; TK = Tyrosine Kinase

5 IMMUNOTHERAPY AIMED AT CANCERS WITH POOR PROGNOSIS

About 20% of breast cancers are HER2 positive[11], which has aggressive behavior[14]. Its target therapy consists in the administration of trastuzumab (monoclonal

antibody for HER2), however, its standard treatment consists in the application of the antibody in association with chemotherapy[30], being it adjuvant or neoadjuvant, depending on the stage of the lesion[31]. Lately, second-generation drugs such as tyrosine kinase inhibitors (TKI), monoclonal antibodies and drug-conjugated antibodies have been developed[32], turning into very promising in cases of HER2 overexpression. Some antibodies are also used to block cyclin-dependent kinases (CDK), which causes the production of IFN and increases cytotoxic T-cell (CTL) activity[7,11,33]. In type HER2, the evaluation of lymphocytic infiltrate is used to predict therapeutic choice and prognostic potential. High levels of TCD8+ is used as a favorable marker in the treatment with trastuzumab[31].

Some target drugs for HER2 stimulate cells of the innate and adaptive system, which causes antigenic recognition of cancer cells, defense against oncogenic pathways and induction of neoplastic cell death[32]. Immune checkpoint block (PD-1 and CTLA-4) is also used in HER2-positive tumors. This inhibition stimulates cytotoxic activity, as it reduces the amount of Tregs and allows the attack of CTLs to be continuous, correlated with a favorable prognosis[26,28].

TNBC cancer, on the other hand, is expressed in about 15% of breast carcinomas[34]. Its great genomic instability reflects in complex phenotypes with therapeutic resistance[9,19,35]. Due to the absence of markers, chemotherapy is the only systemic treatment administered, although, the use of TIL as biomarkers enables the implementation of target therapies and increase the potency of cytotoxic effect from neoadjuvant chemotherapy drugs (especially with anthracycline), in addition to recruiting TCD8+ to TME[35–37].

Numerous clinical trials had shown that checkpoint inhibitors (anti-PD1, anti-PD-L1 and anti-CTLA-4) and antibodies against CDK 4/6 are efficient immunotherapeutic strategies in TNBC[37,38]. The use of poly ADP-ribose polymerase inhibitor enzymes (PARP) demonstrate promising preclinical and clinical results in DNA repair, being effective in the environment where the BRCA gene is mutated[35] and in patients expressing PD-L1[39]. Another immunotherapeutic medium is the blockade of regulatory and proliferative pathways, such as inhibition of PI3K/AKT/mTOR, having beneficial potential in luminal, HER2 positive and TNBC tumors[8,19,35], and MAPK block, which is correlated with increased sensitivity to chemotherapy[11].

In addition to the treatments mentioned above, the use of cellular immunotherapy (ACT and CAR) for HER2 and TNBC consists of a unique treatment that leads to the

effective potentiation of T cells. , which can be optimized to perform their antitumor function and expand their volume, assisting for lysis of neoplastic cells, as well as in the recognition of antigens without presentation of the main histocompatibility complex (MHC), tumor-associated antigens (TAAs) and neoantigens synthesized by tumor cells[6,7,39,40].

Breast cancers that express neoantigens could recruit lymphocytes, nevertheless, it's necessary the activity of antigen-presenting cells (APCs) to perform the presentation by MHC I and/or MHC II[7,17], and thus, get more adept antitumor response. The CDs are trained for this activity, which can be configured in alternatives for cancer vaccines[6] aimed at cancers that expose protein receptors, as in positive HER2[24,29–31].

6 RESULTS

The search resulted in sixteen (16) research articles that analyzed immunotherapeutic strategies against breast cancer and possible immunological markers, represented in table 3.

Table 3. Researches which used immunotherapies, immune cells and cytokines as prognostic marker in breast cancer patients.

Authors/years	**Objective and samples**	**Immunotherapeutic applicability**	**Results (prognosis)**
Liu et al., 2021	Propose a mechanism to regulate MDSC-mediated immunogenicity of B cells in order to promote a new immunotherapeutic strategy. Samples: Female BALB/C mice aged 6–8 weeks and 4T1 mammary cells (mice) were used in the research.	The use of immune anti-checkpoint may be feasible in the presence of immunosuppressive cells, such as in Breg and MDSCs. Blocking PD-1 / PD-L1 in B cells and MDSC activates antitumor activity and secretes TCD8 in the TME.	Combination therapy between AKT signaling inhibitor and anti-PD-1 has been shown to decrease carcinogenesis, tumor expansion and immunosuppressive effect (mainly in Breg) being more effective associated (conjugated therapy) than individual.
Noske et al., 2019	Investigate the presence of TIL and its association with immune checkpoint (PD-1 and PD-L1) and associate it with the prognostic factor and biomarkers. Samples: 1371 FFPE samples by TMA and IHC technique to detect breast cancer subtypes and assess immune checkpoints.	The expression of PD-1 and PD-L1 is usually accompanied by high expression of TIL, which keeps the TME of patients with breast cancer in monotony, which leads to the need for the use of immunotherapeutics against these immunocheckpoints, especially in TNBC tumors.	Increased TIL was associated with negative hormone receptor. Immune checkpoints were detected in both immune and cancer cells, however, in cancer samples that had more TIL, they consequently had more expressions of PD-1 and PD-L1 in immune cells. PD-1 expression in immune cells was associated with poor tumor differentiation, high levels of Ki-67 and presence of TNBC, but not HER2.

Peng et al., 2020	To analyze the expression of the CTLA-4 gene in different types of breast cancer, focusing on TNBC and associating its expression with cell infiltrate and other checkpoint immune systems. Public domain databases and bioinformatics software (Weighted correlation network analysis – WGCNA) were used to compare gene expression.	CTLA-4 expression reflects on the clinicopathological aspects of patients with breast cancer, which may provide an immunotherapeutic target against this immune suppressor, especially in TNBC tumors.	The search resulted in 903 breast cancer patients who had mRNA sequencing and molecular information in line with the purpose of the study. CTLA-4 expression is greater in TNBC than in other subtypes. We analyzed miRNAs that down-regulate CTLA-4 and found that hsa-mir-92a has significance in TNBC survival.
Juliá; Mordoh; Levy, 2020	To assess whether cetuximab-mediated cytotoxicity against TNBC cells promotes NK cell activation and DC maturation after tumor antigen uptake, leading to an adaptive antitumor response. Samples: IIB-BR-G and MDA-MB-231 cell line, both TNBC cell line with K-RAS mutation and EGFR overexpress.	TNBC cells can be refuted using Cetuximab. The presence of NK cells is essential in the use of cetuximab.	NK increases the lysis of IIB-BR-G cells when associated with cetuximab, in addition, its association also contributes to the activation of DC and the increase in INF-γ and TNF-α, however, in MDA-MB-231 cells it just increased the NK level. NK promotes IL-12p70 secretion by DC, inducing Th1 cell production and also promotes IL-15 release.
Li et al., 2020	Investigate CD155 expression and assess its association with prognosis and possible target for immunotherapy. 126 patients recruited for the study and CD155 measurement by IHC.	The CD155 protein can be measured in an environment where PD-L1 is not highly expressed, which may increase the scope of immunotherapy. Its expression is associated with DNA damage and the presence of PD-1.	CD155 has been associated with cell proliferation, TME dysfunction and its overexpression has been associated with worse relapse-free and overall survival and may be an immunotherapeutic target.
Zheng et al., 2012	To evaluate the effect of doxorubicin (ADM) on DC activation and maturation in MCF-7 breast cancer cell line *in vitro*.	The induction of CD allows for greater antitumor activity, its maturation and its stimulation has been associated with the development of oncological vaccines and therapies that prosper its activity.	ADM induces apoptosis in MCF-7 cells proportionally to concentration and also induces DC maturation. In addition, it stimulates lymphocyte proliferation and effector cytokine (IFN-γ) secretion, which also stimulates DC production.
Song et al., 2016	To analyze the therapeutic efficacy of T cells in CAR specific for folate receptor alpha (FRα) in murine models with human TNBC cell lineage of ovarian (control) and breast cancer.	FRα is a TAA expressed in cancer epithelial cells and is associated with poor prognosis in breast cancer.	CAR-FRα T cells have antitumor activity on MDA-231 cells *in vitro* and *in vivo*, its expression level is related to the efficacy of immunotherapy, but its presence has not been very quantified.
Strack et al., 2020	Evaluate the prognostic activity of macrophages by molecular analysis, mRNA sequencing and CD206 transcriptomics in the TME. Forty-eight (48) fresh samples and FFPE subjected to TMA, macrophages were isolated and cultured in RPMI 1640 cell line from AB+ human serum.	The macrophages' phenotype (marked by CD206+ or CD206-) has distinct activities and this uniqueness reflects on the prognosis of patients with breast cancer.	In non-cancerous tissues, the highest expression of CD206+ macrophages was observed, which are associated with lymphocyte infiltration and improved OS, while CD206- macrophages were associated with cancer

			tissue, negative prognosis and poor survival.
Su et al., 2017	Evaluate the formation of Treg cell infiltrate and TCD4+ and associate the CCL18 chemokine produced by TAM with the prognostic factor. Samples: human breast and blood tissue for cell isolation and culture and xenotransplantation in mouse models.	Inhibition of TCD4+ recruitment may be an immunotherapeutic target, as some phenotypes have immunosuppressive activity in the TME.	It was observed that in cancerous tissues it has more Treg infiltrate than in healthy tissues. Tregs in breast tissue are converted from TCD4+ in peripheral blood, which are recruited by TAM-derived CCL18. Recruitment inhibition of Treg and CCL18 are promising immunotherapeutic strategies.
Zhao et al., 2020	To evaluate the functions of CD8+ and FOXP3+ infiltrates and PD-L1 expression in response to neoadjuvant therapy in HER2 positive cancer. Samples: 173 patients treated with neoadjuvant chemotherapy with a +3 score by IHC or FISH amplified gene, FFPE samples.	TIL and FOXP3+ can be used as tumor response markers in HER2 positive cancer.	50.3% of patients had pCR, PD-L1 expression was low in HER2 positive. FOXP3+ expression was associated with patients who had positive pCR, low ER and PR expression, high Ki—67, HER2 IHC score +3 and high FOXP3+ count was associated with pCR rate.
Bailur; Gueckel; Pawelec, 2016	To investigate the prognostic relevance of circulating antigen-presenting cells and immunosuppressive cells (MDSCs and Treg) in relation to T cell response in HER2 positive patients. Samples: blood sample from 75 breast cancer patients.	DC expression can be used as a marker of OS and therapeutic monitoring. The addition of pDC in the prognostic evaluation of breast cancer patients reveals the effectiveness of the treatment and determines the presence of effector and immunosuppressive cells.	The presence of pDC was correlated with early stages (T 0 and 1 by TNM staging), whereas MDSC was associated with T2, 3 and 4. The 5-year OS was associated with a higher concentration of pDC and is also higher in the presence of associated TCD8+ at high pDC (93%) than TCD8+ with low pDC (70%), as well as the low presence of Treg also has a positive value; however, it still has favorable results. The absence of TCD8+ is associated with the expression of immunosuppressant MDSC.
Núñez et al., 2020	Analyze TCR and Treg by mRNA sequencing to characterize their phenotype, also measure the expression of CD80 which is expressed by Treg subsets and associate with the prognostic factor. Samples: histological tumor and lymph node samples from 54 patients with luminal câncer.	Knowledge of lymph node composition is important for patients with breast cancer, as it is the first location, usually in cases of metastasis. The presence of immune and suppressor effectors reflects on the immunological activity and can be used as a prognostic factor, regardless of the molecular type.	The cell composition in lymph nodes is quite heterogeneous, with Treg subsets being more abundant. Furthermore, several immune checkpoints in the lymph node have been shown. CD80 was associated with negative prognosis
Ma; Huang; Kong, 2018	Evaluate the role of IL-17 on MDSC as a proliferative mediator and apoptotic function in breast cancer cells. Samples: Blood samples from breast cancer	IL-17 activity is also correlated with MDSC differentiation. The function of IL-17 can be modulated to	IL-17 stimulates MDSC differentiation by inducing STAT3 (high expression of STAT3 mRNA), which is found at high levels in the

	patients and control donors, Balb/c and MCF-7 cell lines were also used.	fight cytokines and immunosuppressive cells.	peripheral blood of patients with advanced stage breast cancer (T3 and 4, compared to T1 and two). Cells subjected to IL-17 activity had reduced levels of IL-10 and TGF-β and increased apoptosis.
Song et al., 2017	Evaluate the prognostic value of T CD8+CD28- suppressor T lymphocytes (OS and PFS) of patients with metastatic breast cancer undergoing adoptive cell immunotherapy and associate the findings with the chemotherapy interaction performed by the same patients previously. Samples: 232 blood samples from patients with metastatic breast cancer.	The expression of suppressors acts in complex ways, depending on the treatment given to the patient. Some subpopulations of effector cells can counteract its antitumor function, as observed in the cases of TCD8+CD28-, which inhibits TCD4+ helper. Blocking this immunosuppressant may have a positive aptitude for immunotherapy.	The mean PFS was 11.8 months for patients with suppressor T lymphocytes ≤24.2% compared to 7.1 months for patients with a proportion of suppressor T lymphocytes > 24.2%, whereas the mean OS was 36. 2 and 26.9 months for suppressor T lymphocytes ≤24.2% and >24.2%, respectively. High levels of suppressor cells were associated with first cycle of chemotherapy, while high levels of TCD8+CD28- was associated with low OS in second cycle of chemotherapy.
Wei et al., 2020	Evaluate the association of IDO, PD-L1 and TIL of different phenotypes by IHC in the TME. The clinicopathological relationship with the microenvironment was also analyzed to assess the potential prognosis. Samples: 77 FFPE samples from breast cancer patients.	The immune regulatory activity of IDO is associated with reduced anticancer activity in solid tumors, making it an immunotherapeutic target. It is possible that its inhibition associated with an immune ani-checkpoint is a therapeutic strategy.	IDO was expressed in the cytoplasm of tumor cells and in some TIL, but not expressed in normal adjacent tissues, PD-L1 was expressed in tumor cells and only 16 (20.78%) obtained co-expression of IDO and PD-L1. All infiltrates were found in negative hormone receptor samples, some TIL were associated with metastatic and advanced stage patients, such as TCD4+ and NK CD56+. High IDO expression has also been associated with advanced metastasis and lesion.
Q. Liu et al., 2021	Evaluate JAK2 expression as a prognostic factor and inflammatory activity in breast cancer. Public domain databases for RNA sequence (by mRNA) and genes related to breast cancer were used.	JAK2 expression can be used as a prognostic and immune effector marker. The presence of JAK2 has the potential to be used as an associate of immune checkpoints and inflammatory activity.	JAK2 expression is quite fickle, has low expression in breast cancer, low association with T staging (TNM) and has no difference between TNBC and non-TNBC, but it is associated with RP and negative HER2. As for the prognostic factor, it has better OS and RFS in luminal B, but not in luminal A and HER2. JAK2 may have an immunoregulatory function, as its expression is

			associated with the presence of some immune effectors, and this composition changes according to the molecular type.

AKT = AKT Serine/Threonine Kinase; Breg = B cells regulatory; FFPE = Formalin-fixed paraffin-embedded ; FISH = Fluorescence in situ hybridization; DC = Dendritic cells; IDO = Indoleamine 2 3-dioxygenase; IFN-γ = Interferon Gamma; IHC = Immunohistochemistry; IL= Interleukin; JAK = Janus Kinase; mRNA = Messenger RNA; miRNA= Micro RNA; OS = Overall Survival; pCR = Pathological Complete Response; pDC = Plasmacytoid Dendritic Cells; PFS = Progression Free Survival; STAT = Signal Transducer and Activator of Transcription; TCR = T Cell Receptor; Th Cell = T helper Cell; TMA = Tissue Microarray; TNF-α = Tumor Necrosis Factor Alpha.

Studies based on the use of antibodies against checkpoint immue[41–43] and receptors[44,45] were observed, in addition to the use of vaccines[46], cellular immunotherapy[47], immunosuppressive inhibitors[48–55] and oncogenic pathways[56]. The analysis of TME was fundamental in most studies, which is designated as an independent factor of the molecular type and in the immunotherapeutic option administered[41,42,45,49,55], however, the association of infiltrates with the molecular subtype of breast cancer is also necessary, as can be seen in the expression of TCD4, which can be expressed as a potential effector or immunosuppressant, depending on its phenotype[52], which reflects the uniqueness of the treatment[42,50,55]. It was also observed that MDSCs express PD-L1 not only to evade CD8 T lymphocytes, but effector B cells too, which presupposes that blocking PD-L1 in MDSCs has anticancer activity[41], in addition, was investigated the antitumor function of antibody-mediated immune cells, such as NK, which aids in the differentiation and maturation of DC and in the production of IL-12 and IL-15, which are growing as potential immunotherapeutic[44]. It was also observed that conjugated immunotherapy shows promising results when compared to individual immunotherapy[41,44].

7 CONCLUSION

The HER2 e TNBC tumors have great leukocytic infiltrate concentration[44], being lymphocytes as major, but the luminal tumors, on the other hand, do not show as many infiltrates[19], although, they have a better prognosis[32]. The molecular classification add to the compositional knowledge of the carcinogenic microenvironment can become extremely important for the prognosis and even in the treatment of breast cancer, as it allows the realization of a new oncological classification and implementation of personalized therapies, which have great potential for remission in the subtypes of worst prognosis. The interaction between cancer and immune cells provides principles that

could be used as new therapeutic means in clinical practice, that is, the heterogeneity of the disease would be countered by the diversity of the immune system. Research shows that the use of antagonists of immunosuppressants, oncogenic pathways, proliferative receptors and immune anti-checkpoint have positive clinical responses that control cancer progression, making the treatment more specific and covering the extension of therapies for cancer patients.

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