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REVIEW ARTICLE

Immune modulation and epigenetic therapies for enhanced outcome of treatment in triple-negative breast cancer

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Abstract

Triple-negative breast cancer (TNBC) is a subtype of breast cancer characterized by the absence of human epidermal growth factor receptor 2, estrogen receptor, and progesterone receptor expression. While traditional TNBC treatment primarily relies on systemic chemotherapy, epigenetic modification has an important role in immune evasion and tumor progression. Recent classifications of TNBC into "hot" and "cold" tumors, based on immune activity and mutation burden, have revealed that poor response to immune checkpoint inhibitors (ICIs) in these tumors is due to low tumor-infiltrating lymphocytes and the presence of immunosuppressive cells. Emerging treatments such as ICIs and poly(ADP-ribose) polymerase inhibitors offer promising alternatives. The tumor microenvironment (TME) shapes immune responses in TNBC, and a limited subset of patients has shown encouraging responses to ICIs in treating TNBC at both early and advanced stages. However, combination therapies face challenges, including medication interactions and side effects. Epigenetic modulators, such as histone deacetylase and DNA methyltransferase inhibitors, also show promise in reversing epigenetic changes by enhancing anti-tumor immunity in TNBC. The frequent expression of indoleamine 2,3-dioxygenase 1 (IDO1) in TNBC, which catalyzes the conversion of tryptophan to kynurenine, contributes to immune suppression in TNBC patients. High IDO1 levels impair the tumoricidal activity of natural killer cells and correlate with poor responses to anti-PD-L1 therapy, which can be improved using IDO1 inhibitors. Targeting myeloid-derived suppressor cells, regulatory T-cells, and tumor-associated macrophage, along with utilizing epigenetic mechanisms, show promise in modulating the immunosuppressive TME in TNBC. Combining these approaches with standard therapies, along with biomarker-guided patient selection, can enhance treatment efficacy. Clinical trials and preclinical models are essential for optimizing these strategies. This study emphasizes the importance of understanding the mechanisms of modulation, tumor-immune interactions, and the potential of epigenetic therapies in improving treatment outcomes in TNBC.

Keywords: Triple-negative breast cancer; Epigenetic modifications; Immune modulation; Combination therapies

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1. Introduction

Triple-negative breast cancer (TNBC) is a breast cancer (BC) subtype defined by the absence of human epidermal growth factor receptor 2 (HER2), estrogen receptor (ER), and progesterone receptor (PR) expression.[1,2] As the BC with the most aggressive trait and presenting with the worst prognosis, TNBC is distinguished from amplified tumors expressing HER2 and hormone receptor-positive (HR^+) cancers. According to Lin *et al*.,[3] it is characterized by a poor prognosis coupled with early death rates and recurrence in Stages I–III (which is often operable), as well as lower overall survival (OS) in Stage IV (in an inoperable setting).[4,5]

By identifying and killing cancer cells based on tumor-specific antigens or stress-induced chemical expression, the immune system's role in cancer surveillance is critical, thus preventing potential injury to the body.[6] Even with this defense against carcinogenesis and preservation of cellular homeostasis,[7] immune evasion causes poor tumor antigen presentation, limiting immunotherapy's efficacy in TNBC. Genetic and epigenetic changes, such as aberrant RNA splicing, contribute to this evasion strategy.[8] Due to TNBC's low genetic mutation frequency, it differs from other solid tumors. This emphasizes the significance of epigenetic modifications in the formation of aggressive phenotypes and the progression of cancer.[9]

Cancer can result from the accumulation of mutations and epigenetic changes in tumor-suppressor genes and oncogenes.[10] Growing research indicates that epigenetic modifications are involved in several biological functions of tumor cells, such as invasion, metastasis, proliferation, and metabolic reprogramming.[11,12] These functions are influenced by the interactions between cancerous cells and their surrounding environment.[10] Treatment for TNBC mostly consists of systemic chemotherapy instead of targeted therapies because it lacks HER2 and HRs. Consequently, regulatory agencies such as the European Medicines Agency and the United States Food and Drug Administration have approved various treatment regimens combining chemotherapy and immunotherapy for TNBC patients.

According to recent research,[13,14] neoadjuvant immunotherapy in combination with chemotherapy increases pathological complete response rates in early-stage TNBC. The effectiveness of immunotherapy may be impacted by the role that epigenetics plays in controlling the infiltration and activation of immune cells into the tumor microenvironment (TME).[15,16] Thus, focusing on epigenetic modifications offers a potential strategy for TNBC immunotherapy, especially when paired with therapies such as immune checkpoint inhibitors (ICIs), to enhance patients' quality of life, and survival. This review aims to investigate how the regulation of immune checkpoint molecules, tumor-associated antigens, and immune cell infiltration through epigenetics influences the anti-tumor immune response in the microenvironment of TNBC.

2. The TNBC microenvironment and immune modulation

The TME is an intricate network of different cell types, extracellular matrix elements, and signaling chemicals. The TME plays a crucial role in controlling the immune system and influencing the progression of TNBC, displaying both immunosuppressive and immunoreactive properties.[17] According to Zheng *et al*.,[17] the subclassification of TNBCs into "tumor-immune microenvironment (TIME) subtypes" based on TME constituents aids in the prediction of outcomes and suggests personalized treatments based on unique TNBC characteristics.

Myeloid-derived suppressor cells (MDSCs), tumor-infiltrating lymphocytes (TILs), and regulatory T-cells (Tregs) are the biological constituents of the TNBC microenvironment, with recent studies employ multi-

omics methods to stratify the immune subtypes of TNBC, distinguishing between "hot" and "cold" phenotypes. A "hot" tumor is characterized by lower clonal heterogeneity, high levels of TILs, macrophages, checkpoint molecules, and type 1 interferon (IFN) signaling. These features make it sensitive to ICIs and are associated with a good prognosis. Stromal signatures and quiescent immune activity, in addition to a higher mutation burden (including MYC and PTEN/PI3K mutations) and neoantigen (B7-H4), are the main characteristics of a "cold" tumor. A poor response to ICIs is caused by low numbers of TILs and an enrichment of immunosuppressive neutrophils or MDSCs.[18]

Research offers a model to identify possible targets that can turn "cold" TNBC tumors into "hot" ones, in addition to helping identify a subset of TNBCs with superior therapeutic outcomes from ICIs. Wang *et al.* developed a unique technique to enhance the effectiveness of ICI on "cold" tumors by identifying AURKA inhibitors that might stimulate T-cell infiltration by reprogramming the tumor immunological phenotype of TNBC.[19] Since lymphocyte infiltration and IFN-γ status characterize the "T-cell inflamed" phenotype (also known as "hot tumors"), they are essential for the efficacious administration of anti-programmed cell death-1 (PD-1)/programmed cell death ligand 1 (PD-L1) therapy. As opposed to this, "non-inflamed" or "cold tumors" do not have T-cell infiltration, while other myeloid or immune cells may be present. Immunotherapy treatment of cold tumors is extremely difficult since no developed or sustained adaptive immune response has occurred.[20]

2.1. TILs

TILs are present in the BC microenvironment among several cell types and are essential for carcinogenesis and cancer progression, according to growing evidence.[21] Interestingly, as compared to HR-positive subtypes, TNBC tumors show comparatively higher TIL numbers, making them the most immunogenic BC subtype.[22] TILs are mainly made up of immune cells that have a significant impact on patient prognosis by either stimulating or inhibiting tumor growth. According to studies, patients with elevated TIL levels in metastatic BC frequently have better therapy outcomes compared to those without.[23] Furthermore, higher TIL levels in BC patients are associated with a better response to chemotherapy.[24-26]

A greater rate of pathologic complete response (pCR) was observed in TNBC patients with high TIL levels compared to those with low TIL levels, as reported by Gao *et al.*[27] The study also added that for every 10% rise in the level of TIL, TNBC patients have a higher pCR rate (OR 1.09, 95% CI 1.02 – 1.16).[27] Furthermore, TILs in TNBC patients are a favorable prognostic factor since prior studies have demonstrated that these individuals typically had higher disease-free survival (DFS) and OS than patients with low levels of TIL.[27] The influence of TILs on tumor immunosurveillance and immunosuppressive pathways may contribute to this phenomenon.[28] Evaluation of the clinical connections between the overall TIL count and specific TIL subtype levels in TNBC can enhance the ability to forecast treatment outcomes and prognostic trends for this BC subtype, given the distinct clinicopathological and immunohistochemical features of TNBC.

2.2. MDSCs

The TME regulates the differentiation and transformation of normal myeloid cells by secreting different soluble factors that convert them into immunosuppressive cells, resulting in the establishment of a tumor-promoting "macroenvironment" that significantly inhibits the effectiveness of cancer immunotherapy.[29] MDSCs are a diverse group of cells with strong immunosuppressive properties, found in the tumor-bearing mice's spleen and tumor tissues, as well as in cancer patients' peripheral blood and tumor locations.[30] MDSCs are distinguished by their diverse makeup and potent immunosuppressive abilities, which support immunosuppressive environment development through several strategies, such as nutrition depletion and regulatory Tregs recruitment.[31]

Myeloid cells responsible for inhibiting the immune system significantly contribute to tumor growth and spread by helping tumors evade immune surveillance. They accumulate in tumor and inflammatory tissues, affecting B-cells and other cell populations within the inflammatory response, as well as immune cell function.[32] Furthermore, Lu *et al.*[33] have reported that MDSCs play a role in creating a premetastatic niche, thereby accelerating the spread of tumors. Under normal circumstances, immature myeloid cells rapidly differentiate into three types of immune cells – macrophages, mature granulocytes, or dendritic cells – each with distinct roles in the body's defense mechanisms. However, in the presence of inflammation, cancer, and infection, immature myeloid cells become dysfunctional, including becoming MDSCs, when they are unable to differentiate appropriately. This dysfunction, such as reduced T-cell and L-selectin expression, generally contributes to MDSC-mediated immunosuppression.[34] According to Xu *et al.*,[35] while MDSCs primarily impact T-cell tolerance, they have also been reported to suppress a variety of other cells.

In addition, myeloid stem cells are activated by interleukin-34 (IL-34), which, during differentiation into monocytic MDSCs (M-MDSCs), recruits Tregs while

hindering differentiation into polymorphonuclear MDSCs (PMN-MDSCs). Although a decline in PMN-MDSCs inhibits angiogenesis, the increase in M-MDSCs aids in the formation of an immunosuppressive TME, leading to chemotherapy resistance. According to Kajihara *et al.*,[36] blocking the differentiation of M-MDSC prevents the accumulation of M-MDSC using an ER inhibitor or an anti-IL-34 monoclonal antibody, thereby slowing tumor growth and restoring chemosensitivity by promoting the accumulation of PMN-MDSC.

Recent trials have shown that deactivating MDSCs can significantly improve immune surveillance against tumors.[37] TNBC patients have been found to have more MDSCs than non-TNBC patients. Studies have also revealed that the recruitment of MDSCs to metastatic sites and primary tumors is directly facilitated by CXCL2 and CCL22 chemokines through ΔNp63-dependent activation. The elevated number of MDSCs in basal TNBC patients has been linked to high levels of the transcription factor ΔNp63, which, in turn, promotes tumor growth, progression, and metastasis in TNBC cells in both human and mouse models.[38] Furthermore, MDSCs have been found to release prometastatic factors such as chitinase 3-like 1 and MMP-9, suggesting their contribution to the advancement of TNBC in a non-immunologic manner. Nevertheless, CXCR2/CCR4 inhibitors have shown promise in lowering angiogenesis, metastasis, and MDSC recruitment, indicating a potential new approach for treating this subgroup of TNBC patients.[39]

The table below (Table 1) presents an overview of various inhibitors that target MDSCs in TNBC. The table includes details on the mechanism of action, clinical data, and significant findings related to each inhibitor.

2.3. Tregs

A significant proportion of solid tumors contain Tregs, which are associated with poor outcomes primarily due to their role in inhibiting the immune response against tumor progression and fostering immunosuppression.[45] BC is one of the several solid tumor types that includes high frequencies of T-cells. In TNBC, as well as other subtypes of BC and the majority of solid tumors with poorer prognoses, a high ratio of Forkhead box P3 (FOXP3$^+$) Treg to CD8$^+$ T-cells, or high Tregs frequencies among CD4$^+$ T-cells within TILs, has been linked to worse outcomes. Studies have revealed that increased tumor-infiltrating Tregs are correlated with reduced DFS, greater tumor sizes, and higher rates of local recurrence, according to the association between Tregs and the clinicopathological characteristics of TNBC.[45]

Tregs have been found to be more common in TNBC, with this higher frequency of Tregs associated with higher-grade lesions across all BC subtypes that have been investigated.[46] A study by Liu *et al.*[47] observed an increased proportion of CD4$^+$CD25$^+$Foxp3$^+$ Treg infiltration in BC tissues, which was associated with decreased OS and progression-free survival, as well as excessive expression of HER2, negative ER and PR status, and high histology grade. In contrast, Yeong *et al.*[48] observed that TNBC patients with a high proportion of tumor-infiltrating CD4$^+$CD25$^+$Foxp3$^+$ Tregs had a better prognosis but also a higher lymph node status and tumor grade. However, the accumulation of tumor-infiltrating Tregs could increase the immunosuppressive capabilities of local cells, thereby subduing anti-tumor immunity and promoting tumor growth and invasion. Tregs, CD8/Treg ratio, and cancer stem cell frequencies have been associated with disease progression, including metastasis to the lymph nodes.

Table 2 summarizes various inhibitors targeting Tregs that have been studied or have potential relevance for treating TNBC, along with their mechanisms of action and their clinical development.

Furthermore, cytotoxic properties of Tregs have been established by previous reviews, which may destroy effector T-cells directly, which could account for the correlation between Foxp3$^+$ Tregs infiltration and a low chance of recurrence-free survival in BC patients.[58] ^{Dec}rease in T helper 1 cell infiltration and increased tumor infiltration of M2 macrophages have been linked with Tregs, along with elevated *TP53* gene mutation rate and alterations of copy number. Immediately after neoadjuvant chemotherapy, the pCR obtained was associated significantly with Tregs in TNBC in low abundance; this correlation was absent in Her2-negative/ER-positive subgroups of the disease. On the other hand, in contrast, increased tumor expression of several ICI genes was substantially connected with high Treg abundance.[59] Several therapeutic approaches that target Tregs suppressive function, including the cytotoxic T-lymphocyte-associated protein 4 (CTLA-4), transforming growth factor beta, and CD39/CD73/Ado pathways, are presently being developed for use in clinical settings. Nevertheless, the therapeutic advantages of these approaches are probably going to be restricted to situations because Tregs use a variety of pathways to carry out their suppressive role.[60]

2.4. TAMs

TAMs are leukocytes that infiltrate tumors, originating from mononuclear cells in the blood, which differentiate into macrophages on entering body tissues. TAMs

Table 1. Inhibitors targeting myeloid-derived suppressor cells in triple-negative breast cancer

Inhibitor	Mechanism of action	Clinical data available	Notes	References
ATRA	Induces differentiation of MDSCs into mature myeloid cells	ATRA+DAPT (γ-secretase inhibitor) together sensitize the cell lines with NOTCH1 ICD, which blocks the internal aberration and constituent expression of NOTCH1, thus making the TNBC cells render sensitivity to ATRA	Demonstrated reduction in MDSCs and enhanced immune response in TNBC	40,41
Pexidartinib (PLX3397)	CSF1R inhibitor, reduces MDSC accumulation	Demonstrate that the PTX and PLX3397 combination provides consistent therapeutic improvement across several TNBC models	Reduces MDSCs and tumor-associated macrophages in TNBC	42
Entinostat	HDAC inhibitor, reduces MDSC function and proliferation	Phase II trial in TNBC NCT02708680	Terminated early due to low accrual NCT01234532 Primary endpoint not met NCT01349959	43
PI3Kγ inhibitors (e.g., IPI-549)	Inhibits PI3Kγ signaling in MDSCs, reducing their immunosuppressive activity	Phase II trials in TNBC (Mario-3) NCT03961698	These effects allowed for decreasing immune suppression and increasing immune activation, leading to the activation and proliferation of T-cells that can attack cancer cells	44

Abbreviations: ATRA: All-trans retinoic acid; CSF1R: Colony-stimulating factor 1 receptor; HDAC: Histone deacetylase; IMCs: Immature myeloid cells; MDSCs: Myeloid-derived suppressor cells; NOTCH1 ICD: NOTCH1 intracellular domain; PI3Kγ: Phosphoinositide 3-kinase gamma; PTX: Paclitaxel; TNBC: Triple-negative breast cancer.

Table 2. Inhibitors targeting regulatory T-cells in triple-negative breast cancer

Inhibitor name	Mechanism of action	Target pathway	Clinical trials	References
CTLA-4 monoclonal antibody (Ipilimumab)	CTLA-4 blockade	CTLA-4	NCT01928394 NCT02536794	49,50
PD-L1 inhibitor (Pembrolizumab and Atezolizumab)	PD-1 blockade	PD-1	KEYNOTE-522 NCT02425891	51,52
Anti-CD25 antibody	Depletes Tregs inhibiting antitumor immunity	IL-2 receptor (CD25)	NCT01521676	53
TGF-β inhibitor (Galunisertib)	Blocks TGF-β signaling preventing the development of drug-resistant cancer stem cells	TGF-β pathway	Clinical trials still recruiting MSB0011359C	54,55
DNMT inhibitor (Decitabine)	Epigenetic modification, increasing the antitumor T-cell response	DNA methylation	-	56
HDAC inhibitor	Epigenetic modification by promoting T-cell infiltration in the tumor	Histone deacetylation	-	57

Abbreviations: CTLA-4: Cytotoxic T-lymphocyte associated protein 4; DNA; Deoxyribonucleic acid; DNMT: DNA methyltransferase; HDAC: Histone deacetylase; IL-2: Interleukin 2; PD-1: Programmed cell death protein 1; TGF-β: Transforming growth factor-beta; Tregs: Regulatory T-cells.

influence the progression of TNBC throughout its course, from initiation to metastasis, and may also be useful in determining TNBC patients' DFS and OS.[61] In TNBC, a high TAM density correlates with a worse prognosis and a higher likelihood of tumor metastasis.[62] Recent studies have revealed that macrophages are not homogeneous; rather, they can be classified into numerous subgroups based on phenotype, function, and the need for polarization. Based on chemokines and cytokines receptor expression, the classification of TAMs is either alternatively activated M2 subtype or classically activated M1 subtype.[63]

Immunohistochemistry staining of tumor tissues with TAM markers has shown that individuals with higher pathological grades frequently have higher TAM levels. In addition, patients with higher TAM infiltration have significantly shorter OS and disease-free intervals compared to those with lower TAM infiltration.[64] For TAM identification, $CD163^+$ and $CD68^+$ markers are most commonly used.[64] Prior research has demonstrated that $CD163^+$ TAM infiltration, rather than $CD68^+$, is linked to a poor prognosis in TNBC patients, as indicated by multivariate analysis.[65] Because $CD204^+$ and $CD163^+$

TAMs are associated with fast proliferation and poor differentiation, their infiltration into BC often correlates with a poor prognosis.

TAMs can be triggered by common TNBC chemotherapeutic agents, and these activated TAMs encourage the healing of damaged tumor tissues, leading to tumor resistance to chemotherapy.[61] In addition, TAMs produce significant amounts of IL-10, which inhibits the production of IL-12 by dendritic cells, thereby limiting immune-mediated tumor killing by $CD8^+$ T-cells.[66] According to the previous studies, the phenotype of TAMs is a better indicator of the effectiveness of anthracycline chemotherapeutics in treating TNBC than their absolute quantity.[67]

An important approach to preventing TAM recruitment is to block the action of chemokines by targeting the CCL2–CCR2 axis, thereby reducing the mobilization of mononuclear cells from the bone marrow and lowering macrophage infiltration in the breast.[61] Although apoptosis inducers by bisphosphonate-based macrophage have been broadly utilized to deplete TAMs,[68] the immune status changes from anti-tumor to immunosuppressive when TAMs undergo a phenotypic transition within the TME. This dynamic modification highlights the critical function of TAMs in controlling tumor behavior and offers insights for assessing therapeutic efficacy.

Since TAMs play a part in the growth of tumors, TAM-targeting therapeutic approaches have been developed. Yang *et al.*[69] reported the advantages of TAM-targeting therapy, which includes more precise drug delivery through nanomedicines – such as nanospheres, nanocapsules, nanomicelles, and nanoliposomes – modified biologically or physiochemically, and fewer harmful effects on healthy tissues, such as myelosuppression, gastrointestinal distress, organ toxicity, and other typical side effects of chemotherapy drugs. Recent TAMs-targeting therapeutic research has focused on the following areas: inhibition of TAM survival, repolarization of the M2-like phenotype into the M1-like phenotype, and suppression of macrophage recruitment.[69] Niu *et al.*[67] also explored a strategy to eliminate TAMs in TNBC by utilizing nanoparticles targeted at the CD206 receptor, which delivered doxorubicin effectively in a pH-sensitive manner to enhance therapeutic outcomes. However, because TNBC is highly heterogeneous, targeted therapies concentrating on a specific TAM-related pathway frequently face obstacles that result in failure.

3. Epigenetic modifications and effect of alteration on immune recognition in TNBC

Although limited to a small patient population, there has been encouraging effectiveness using ICIs in treating TNBC in an early and advanced stage. According to Llinàs-Arias *et al.*,[9] there is growing evidence that epigenetic modifications – such as altered chromatin architecture and gene regulatory elements (GREs) – are important means of immune evasion. These modifications are particularly interesting because they are reversible through the inhibition of epigenetic regulators. The low frequency of genetic mutations observed in TNBC, in contrast to many other solid tumors, emphasizes the significance of epigenetic modifications in the development of aggressive phenotypes and cancer progression.

DNA methylation and histone modifications are two important types of epigenetic alterations that are well-established. For instance, Table 3 examines various epigenetic mechanisms involved in TNBC. These included DNA methylation of genes such as breast cancer gene 1 (*BRCA1*) and Ras association domain family member 1A (*RASSF1A*), histone modifications involving overexpression of enhancer of zeste 2 polycomb repressive complex 2 subunit and histone deacetylase 1 or 2 (HDAC1/2), non-coding RNAs like the miR-200 family and miR-221/222, and chromatin remodeling factors including the SWI/SNF complex (AT-rich interactive domain-containing protein 1A) and chromodomain helicase DNA binding protein 1.

DNA methylation primarily occurs at the fifth position of cytosines that precede guanosines (5mC). On the other hand, the catalog of histone modifications is considerably more complex, involving a variety of chemical groups and locations. These chemical modifications, which are finely tuned, are regulated by a group of enzymes that have gained increasing importance in cancer research: "writers" impose chemical traces, "erasers" remove them, and "readers" identify the epigenetic code, enlisting additional proteins.[86] Repressive histone marks or promoter hypermethylation are usually associated with the silencing of neighboring genes, including tumor suppressor genes like *BRCA1*. However, GREs, such as enhancers and insulators, which control the activation of gene expression programs linked to cancer, can also be affected by epigenetic modifications, in addition to gene promoters. Global hypomethylation of DNA has the potential to cause oncogene reactivation and genomic instability.[87]

Furthermore, immunogenic antigens production stimulation and reactivation of transposable elements through the use of small molecule inhibitors to chemically eliminate aberrant epigenetic markers may strengthen the immunological response in TNBC.[9] The immune response may be impaired, allowing tumor cells to avoid immune surveillance due to the downregulation of antigen processing and presentation and loss of MHC Class I

Table 3. Epigenetic mechanisms in triple-negative breast cancer

Epigenetic mechanism	Gene/Region	Role	Notes	References
DNA methylation	*BRCA1*	Promoter hypermethylation leading to gene silencing	Common in sporadic cases of TNBC	70
	RASSF1A	Hypermethylation causing tumor suppressor inactivation	Frequently methylated in TNBC	71
	APC	Promoter hypermethylation resulting in gene silencing	Linked to poor prognosis	72
Histone modification	EZH2	Overexpression leading to H3K27 trimethylation and gene repression	Increased invasive activity of TNBC cells	73
	HDAC1/2	Overexpression contributing to global histone deacetylation	Overexpression is directly linked to accelerated cell proliferation and survival	74
Non-coding RNAs	miR-200 family	Downregulation leading to increased epithelial-mesenchymal transition	miR-200 targets ZEB1 and ZEB2	75
	miR-221/222	Upregulation resulting in downregulation of p27 and increased cell proliferation	Commonly upregulated in TNBC	76
Chromatin remodeling	SWI/SNF complex (ARID1A)	Mutations cause loss of function, affecting chromatin accessibility	Mutations found in a subset of TNBC	77
	CHD1	Loss of function mutations leading to disrupted chromatin structure	Contributes to genomic instability	

Abbreviations: APC: Adenomatous polyposis coli; ARID1A: AT-rich interactive domain-containing protein 1A; BRCA1: Breast cancer gene 1; CHD1: Chromodomain helicase DNA-binding protein 1; DNA: Deoxyribonucleic acid; EZH2: Enhancer of zeste 2 polycomb repressive complex 2 subunit; HDAC1/2: Histone deacetylase 1 or 2; miR-200: MicroRNA-200; miR-221/222: MicroRNA-221/222; RASSF1A: Ras association domain family member 1A; SWI/SNF: Switch/sucrose non-fermentable; TNBC: triple-negative breast cancer; ZEB1: Zinc finger E-box binding homeobox 1; ZEB2: Zinc finger E-box binding homeobox 2.

expression. Downregulating MHC Class I is more common than total removal because total depletion, through non-classic MHC molecules, exposes tumor cells to the effects of natural killer (NK) cells. Moreover, cancer cells have been shown to exhibit epigenetic dysregulation of genes linked to the machinery involved in antigen processing and presentation. These changes include the inhibition, in different forms of cancer, of the MHC Class I transactivator NLRC5 and the HLA Class II-chaperone CD74 by DNA methylation.[88,89]

One mechanism of immunotherapeutic resistance that has been identified is the impairment of MHC Class I, evident, especially in the apocrine subtype and metastatic TNBC. High transmembrane protein MAL2 expression in metastatic TNBC lowers the stability and quantity of antigen-loaded MHC Class I molecules on cell membranes, impairing antigen presentation and limiting $CD8^+$ T-cell recognition.[9] MHC Class I-based research has discovered the presentation of melanoma-associated antigen-A11 (MAGE-A11) peptides by HLA Class I molecules. The cancer-testis antigen MAGE-A11 is also linked to a poor prognosis, which is frequently detected in BC.[90,91] Therefore, the induction of MAGE-A11 is critical for the detection and destruction of BC cells by cytotoxic T lymphocytes (CTLs). Furthermore, MAGE-A11 antigens induced cell toxicity in MAGE-A11-positive TNBC cells through effector CTLs.[92]

In TNBC, abnormal upregulation of the MHC Class II pathway is associated with prolonged progression-free survival and increased T-cell infiltration.[93] Impairment of MHC Class I has been identified as a mechanism of immunotherapeutic resistance. Moreover, increased expression of CD8A, CD3D, and CD4, as well as the presence of TILs, has also been linked to MHC Class II molecule expression.[94] The regulation of MHC Class I and II expression occurs through the methylation of their coding HLA genes by promoter DNA. The relationship between mRNA expression level and DNA methylation in the transcription start site of HLA genes in BC is inverse. Moreover, there is a correlation between lower levels of CD8A mRNA and hypermethylation of HLA promoters.[95]

It has been demonstrated that epigenetic modulation with the next-generation hypomethylating agent, DNA methyltransferase inhibitor (DNMTi) guadecitabine, increases expression of MHC Class I, increases secretion of IFN-γ, and attracts CTLs into the TME. Therefore, tumor and immune systems interaction may be affected by DNMTi therapy in a variety of ways, such as by activating the immune response and demethylating MHC

Class I.[9] Table 4 summarizes various immunotherapy treatments for TNBC, focusing on their mechanisms of action and associated epigenetic mutations or targets. These treatments include monoclonal antibodies such as pembrolizumab, atezolizumab, and nivolumab, which target PD-L1 expression, as well as chimeric antigen receptor T-cell therapies targeting tumor-specific antigens.

3.1. Epigenetic mechanisms underlying the dysregulation of immune checkpoint molecules

Immune checkpoints can impede CTL immune response, which can eliminate tumor cells once identified. The strength and duration of the immune response are regulated by a variety of inhibitory mechanisms that are part of ICs. By activating IC pathways, cancer cells can evade T-cell cytotoxicity within the TME.[96] The most well-documented mechanisms of T-cells deactivation entail the binding of CTLA-4 on T-cells to CD80/86 on antigen-presenting cells (APCs) and the binding of PD-1 on T-cells to PD-L1 on cancer cells and APCs.[9]

In primary BC, various immune checkpoint proteins, including CTLA-4, TIM-3, and LAG-3, are upregulated through mechanisms such as DNA hypomethylation and decreased repressive histone marks (H3K27me3 and H3K9me3) in promoter regions.[97] Regarding regulation of PD-L1, while DNA methylation influences its expression in other malignancies, such as gastric cancer and melanoma, BC appears to exhibit full demethylation of PD-L1, with histone modifications serving as the primary mechanism of control. To re-establish antitumor immunity, aberrant epigenetic processes could potentially be rectified using epigenetic drugs such as HDAC inhibitors (HDACis) and DNMTis. These treatments contribute to the overexpression of several immunological checkpoint proteins.

Apart from PD-1/PD-L1 and CTLA-4, there is increasing popularity of inhibitory receptors as possible targets in immunotherapy. The impact of ICIs combined with HDACis to improve immunotherapy responses in TNBC *in vivo* was studied by Fattori *et al.*[53] It was discovered that HDACi was responsible for increased HLA-DR and PD-L1 expression in TNBC cells. This enhanced the inhibition response of PD-1/CTLA-4 in a mouse model of TNBC, leading to tumor development reduction and an improvement in survival. This effect was linked to $CD4^+$ and $FOXP3^+$ T-cell's downregulation as well as enhanced T-cell tumor infiltration.[53]

The tumor cell surface-expressed proteins CD155 and CD112 interact with the T-cell immunoglobulin and ITIM domains (TIGIT) of the T-cell immunoreceptor on the membranes of NK, $CD8^+$ T-cells, and $CD4^+$ T-cells. Following FOXP3 binding and promoter hypomethylation, TIGIT is overexpressed, and metastatic BC has been shown to have higher levels of CD155 expression compared to normal tissue. CD155 expression is associated with a poorer prognosis in BC, highlighting its significance in novel therapeutics and outcome prediction.[98]

Table 4. Immune therapy approaches and targets in triple-negative breast cancer

Immunotherapy	Mechanism of action	Targets	Notes	References
Pembrolizumab (Keytruda)	Anti-PD-1 monoclonal antibody	PD-L1 expression	Approved for TNBC with high PD-L1 expression	13,78
Atezolizumab (Tecentriq)	Anti-PD-L1 monoclonal antibody	PD-L1 expression	Approved for use in combination with nab-paclitaxel and in metastatic PD-L1-positive TNBC	79
Nivolumab (Opdivo)	Anti-PD-1 monoclonal antibody	PD-L1 expression, TMB	Investigational use in TNBC, associated with high TMB and a subset of patients with previously treated mTNBC	80
Durvalumab (Imfinzi)	Anti-PD-L1 monoclonal antibody	PD-L1 expression	Investigational use, in combination with chemotherapy and other ICIs	81,82
Ipilimumab (Yervoy)	Anti-CTLA-4 monoclonal antibody	Enhanced T-cell activation	Often combined with anti-PD-1/PD-L1 therapies, ongoing trials with tremelimumab	83
Avelumab (Bavencio)	Anti-PD-L1 monoclonal antibody	PD-L1 expression	Investigational use in combination therapies in metastatic, heavily pre-treated breast cancer	84
CAR-T-cell therapy	CAR-T-cell therapy	EGFR, HER2, and eradication of other tumor-specific antigens	Early-phase clinical trials in TNBC	85

Abbreviations: CAR-T-cell: Chimeric antigen receptor T-cell; CTLA-4: Cytotoxic T-lymphocyte-associated protein 4; EGFR: Epidermal growth factor receptor; HER2: Human epidermal growth factor receptor 2; ICIs: Immune checkpoint inhibitors; mTNBC: Metastatic triple-negative breast cancer; PD-1: Programmed cell death protein 1; PD-L1: Programmed cell death ligand 1; TMB: Tumor mutational burden; TNBC: Triple-negative breast cancer.

Another potential target in TNBC is B7-H3, which promotes a pro-vascularization state due to the increased expression on TAMs and cancer cells, resulting in a poorer prognosis. In TNBC mouse models, blocking PD-1 was found to be more effective when combined with the inhibition of this immunological receptor. Knockdown of B7-H3 *in vitro* made cells more susceptible to AKT/mTOR inhibitors and reduced glycolytic activity. Disrupted epigenetic processes that promote elevated B7-H3 expression levels have been linked to cancer development in several studies.[9]

3.2. Epigenetic implication to immune response and anti-tumor immunity enhancement in TNBC

HDACs, a class of epigenetic enzymes, play a crucial role in regulating the equilibrium of acetylation in proteins and histones through lysine acetylation and deacetylation processes, which are essential for carcinogenesis. Carcinogenesis is aided by the dysregulation of HDAC expression and activity in cancer, which results in chromatin remodeling and changed expression of tumor suppressor genes. Inhibiting HDACs can induce apoptosis from cell cycle arrest by releasing oncogenic transcription suppressors. Research has demonstrated that HDACis decrease the number of tumor-associated cells, such as MDSCs, while increasing the production of T-cell chemokines, thereby supporting enhanced T-cell infiltration into tumors. In addition, HDACis increase the expression of immune checkpoint proteins PD-L1 and PD-L2 and enhance the efficacy of immune checkpoint blockade therapies, such as PD-1 blockade, which suggests the potential of combining HDACis with immunotherapy to amplify the immunotherapeutic response.[99]

However, the interaction between DNA methylation and histone deacetylation might result in a self-reinforcing silencing mechanism, potentially compromising the effectiveness of monotherapy and leading to resistance. In humans, HDACis have been demonstrated to downregulate the expression of DNMT1, a DNA methyltransferase, in BC cells. Furthermore, blocking HDAC3 can increase DNMT1's acetylation level, reducing its stability. Combining HDAC3 inhibition with DNMT1 inhibition in multiple myeloma cell lines and patients has shown synergistic effects in generating DNMT1 downregulation, growth inhibition, and death. This shows that there may be synergistic benefits and improved BC outcomes when DNMTis and HDACis are combined.[100] Research indicates that HDACis can improve immune checkpoint blockade therapies by increasing T-cell infiltration and upregulating PD-L1 and PD-L2 expression, suggesting a synergistic potential when combined with immunotherapy.[99] In addition, as illustrated in Table 5, various epigenetic factors, such as indoleamine 2,3-dioxygenase 1 (*IDO1*) promoter hypomethylation and *CTLA-4* DNA methylation changes, play crucial roles in modulating immune responses, highlighting the importance of integrating epigenetic therapies to optimize immunotherapeutic outcomes in TNBC.

However, combination treatments present certain difficulties, including drug interactions, adverse side effects, and patient adherence. Furthermore, according to Huang *et al.*,[99] HDAC1 in the HDAC family largely acts as a pro-cancer factor, whereas HDAC6 may have anticancer properties. To determine the best dosage regimens and combinations of HDACis to maximize their therapeutic advantages in cancer treatment, more investigation and clinical studies are required.

4. The role of IDO1 in TNBC

Breast carcinomas frequently express IDO1, particularly in triple-negative, basal-like breast tumors.[109] IDO1 is a key player in cancer immunotherapy, responsible for catalyzing the conversion of tryptophan (Trp) to kynurenine (Kyn). According to Dill *et al.*,[110] IDO1 expression is highest in TNBC. Approximately 70% of PD-L1$^+$ breast neoplasms express IDO1, while the majority of low PD-L1-expressing breast neoplasms are negative for IDO1. Moreover, Asghar *et al.*[111] found that elevated IDO1 levels in BC are correlated with high COX-2 expression, with around 40% of HER2$^+$ breast tumors overexpress IDO1, suggesting the possibility of using synergistic therapeutic techniques that are comparable to those employed in TNBC.[112]

The role of IDO1 and its immune-suppressive effect in TNBC cells was investigated by Jing *et al.*,[113] who showed that high IDO1 levels impair the tumoricidal potential of local NK cells. Silencing or inhibiting IDO1 in TNBC cells significantly boosts IFN-γ production and enhances the cytolytic activity of co-cultured NK cells. Furthermore, early-stage TNBC patients who test positive for PD-L1 have significantly higher levels of IDO1 than PD-L1, indicating that IDO1 plays an important role in the low response to anti-PD-L1 therapy and that it is an important target for therapeutic modification of the immunosuppressive TME.[114,115]

IDO1 activation is one mechanism by which tumors evade antitumor immunity. Higher IDO1 mRNA expression has been linked to basal-like BC in earlier research.[116] IDO1 is more commonly expressed in ER$^-$ tumors, particularly in the basal-like subtype, as opposed to the luminal subtype, as demonstrated by Noonepalle *et al.*[117] Using matched 450K-methylation array data, they discovered that differential IDO1 expression is linked to its promoter methylation status, demonstrating an inverse relationship

Table 5. Epigenetic modifications in triple-negative breast cancer and their implications for immune response and anti-tumor immunity

Epigenetic factor	Type of modification	Role in TNBC	Impact on immune response	References
IDO1	Promoter hypomethylation	Enhances *IDO1* expression	Increases immune suppression through non-enzymic effects; potential target for IDO1 inhibitors to enhance immune response	101,102
CTLA-4	DNA methylation changes	Modulates immune checkpoints	Alters T-cell activity and immune evasion	103
PD-1/PD-L1	Histone acetylation	Regulates immune checkpoint expression	Affects T-cell exhaustion and tumor immune evasion	
FOXP3	Histone deacetylation	Regulates Treg function	Enhances Treg-mediated immunosuppression	104
EZH2	Histone methylation	Silences tumor suppressor genes	Promotes immune evasion and cell proliferation, and cell invasion and driving cancer progression	105
DNMT1	DNA methylation	Silence genes involved in immune responses	Reduces T-cell infiltration and activation	106
HDACs	Histone deacetylation	Alters chromatin structure and gene expression	Can promote or inhibit immune cell infiltration depending on context	107
BRCA1/2	DNA methylation	Loss of function mutations	Impacts DNA repair mechanisms and may affect immune response indirectly	108

Abbreviations: BRCA1/2: Breast cancer gene 1/2; CTLA-4: Cytotoxic T-lymphocyte associated protein 2; DNA: Deoxyribonucleic acid; DNMT1: DNA methyltransferase 1; EZH2: Enhancer of zeste 2 polycomp repressive complex 2 subunit; FOXP3: Forkhead box P3; HDACs: Histone deacetylases; IDO1: Indoleamine 2,3-dioxygenase 1; PD-1/PD-L1: Programmed cell death protein 1/programmed cell death ligand 1; TNBC: Triple-negative breast cancer; Treg: Regulatory T-cell.

between IDO1 promoter methylation and gene expression. On activation with IFN-γ, only cells with a hypomethylated promoter, like MDA-MB-231, produce enzymatically active IDO1. The methylation pattern found in primary BC samples and BC cell lines indicates that IDO1 promoter methylation can predict IDO1's inducibility *in vivo* by IFN-γ. This is further supported by the Kyn levels analysis within tumor samples, as the relative abundance of Kyn strongly correlates with IDO1 expression and promoter methylation in primary BC samples.

IDO1 is a novel immunological checkpoint, as demonstrated by the potential anticancer activity of pembrolizumab combined with the highly selective IDO1 inhibitor epacadostat in various advanced solid tumors.[113] Jing *et al.*[113] discovered that TNBC cells expressing high levels of IDO1 display extensive immune cell infiltration and a suppressive immunological milieu. This implies that IDO1 could potentially impact the prognosis by either stimulating immune cell dysfunction or conditioning them to promote tumor growth.

TNBC is frequently associated with high antigenic tumors and a high mutational burden. Higher T-cell infiltration is correlated with better survival outcomes for TNBC patients, which is consistent with this. On the other hand, TNBC cells also show upregulated IDO1, marked by demethylated promoters and functional IDO1 synthesis in response to IFN-γ generated from T-cells. Consequently, Noonepalle *et al.*[117] suggest that TNBC patients may be good candidates for IDO1 inhibitor therapy. Blocking the mechanisms that downregulate or counteract the suppression caused by IDO1 could greatly improve the overall prognosis for TNBC patients, especially those with an inflammatory TME caused by T-cells, as many already exhibit a strong immunological response.[118] IDO1 promoter hypomethylation may be a potential genetic characteristic of TNBCs that indicate a T-cell-inflamed tumor phenotype. Therefore, immunotherapy based on IDO1 inhibitors could be very beneficial for this subtype of BC.

4.1. Importance of heme insertion and nitric oxide (NO) regulation immune suppression

Encoded by the *INDO* gene (located on human chromosome 8p22), the IDO1 protein consists of 403 amino acids and functions as an intracellular heme-containing dioxygenase or metalloprotein. The prosthetic group heme is necessary for its catalytic activity. IDO1 catalyzes the oxidative cleavage of Trp to yield the intermediate product N-formylkynurenine, which is subsequently hydrolyzed to Kyn, in addition to the reduction of inactive heme-Fe^{3+} into active heme-Fe^{2+}. IDO1 is thought to be an immunomodulatory enzyme

involved in anti-inflammation, tumor immune escape, and immunoregulation. It promotes maternal tolerance toward the allogeneic fetus, suppresses transplant rejection, regulates autoimmune disorders, and more. This is achieved through Trp depletion and Kyn production.[119]

Over the past two decades, there has been significant effort in drug discovery aimed at developing anticancer small molecules that target both TDO and IDO1 enzymes based on this hypothesis. Numerous IDO1 catalytic inhibitors have been developed, each with unique mechanisms of inhibition (competitive, noncompetitive, and heme-displacing compounds) and desirable qualities for therapeutic candidates, such as better selectivity, potency, oral bioavailability, and a favorable safety profile.[120,121] Targeting IDO1 in cancer immunotherapy has garnered considerable attention due to encouraging preclinical research showing the antitumor effect of IDO1 blockade, either in combination with other ICIs or as monotherapy, in several animal models.[122] The study by Sono *et al*. explored the inhibitory mechanisms of 4-phenyl-imidazole (4-PI) on IDO1. The binding of 4-PI to IDO1's heme iron was confirmed through a co-crystal structure (PDB code: 2D0T). Subsequent optimizations led to the development of navoximod, a potent IDO1 inhibitor. When combined with atezolizumab, navoximod showed limited efficacy in clinical trials. Structural analyses (PDB code: 6O3I) revealed that the imidazoisoindole ring of navoximod coordinates with heme iron and engages in hydrophobic and hydrogen bonding interactions, enhancing its inhibitory activity and stability.[123]

NO levels significantly influence the heme insertion into IDO1. Studies have shown that NO can modulate the availability and insertion of heme into IDO1, thereby regulating its activity. NO produced by inducible NO synthase can inhibit IDO1 activity by preventing heme incorporation, which is crucial for IDO1's catalytic function. This regulation is particularly relevant in the context of cancer, where the balance between NO levels and IDO1 activity can affect the tumor's ability to evade immune detection and destruction.[124]

By targeting this regulatory mechanism, there is potential to develop therapeutic strategies aimed at reducing IDO1-mediated immunosuppression. This could enhance the efficacy of existing cancer treatments and improve anti-tumor immune responses, particularly in TNBC, where IDO1 expression is often upregulated.

5. Key findings

This review of the TNBC microenvironment and immune modulation highlights several key findings regarding the relationship between the immune system and tumor cells in TNBC. The role of the TME in shaping TNBC disease progression and immune responses is very crucial, exhibiting both immunosuppressive and immunoreactive properties. Subclassification of TNBC based on TIME subtypes aids in predicting outcomes and personalized treatments.

In TNBC, TILs are significantly elevated, impacting patient prognosis and response to therapy, including chemotherapy. Higher TIL levels correlate with better therapy outcomes and a greater pCR rate in TNBC patients.

MDSCs play an important role in creating an immunosuppressive TME, promoting tumor growth, progression, and metastasis. Approaches targeting MDSCs have shown promise in enhancing immune surveillance against tumors and restoring chemosensitivity. Tregs are found in high frequencies in TNBC tumors and are associated with unfavorable prognoses, although their precise role remains complex- and context-dependent.

Dysregulation of immune checkpoint molecules, such as PD-1/PD-L1 and CTLA-4, through epigenetic mechanisms, contributes to immune evasion in TNBC. The role of epigenetic modifications, such as altered chromatin architecture and DNA methylation, is significant in immune evasion and disease progression.

DNMTis and HDACis have shown potential in reversing epigenetic modifications and enhancing anti-tumor immunity in TNBC. However, the interaction between histone deacetylation and DNA methylation may lead to self-reinforcing silencing mechanisms, potentially compromising the effectiveness of monotherapy. Combining HDACis with DNMTis may offer synergistic benefits and improved outcomes in TNBC treatment. Nonetheless, challenges such as drug interactions, side effects, and patient adherence need to be addressed in combination therapies.

6. Recommendations and future directions

First, we recommend further exploration and refinement of the subclassification of TNBC based on TIME subtypes. This characterization can provide valuable insights into the heterogeneity of TNBC and its implications for treatment response and patient outcomes. Utilizing advanced technologies such as single-cell sequencing and spatial transcriptomics can enhance our understanding of the diverse cellular compositions within the TME. Clinically, integrating TIME subtyping into routine pathology assessments can help predict treatment responses and guide personalized therapeutic strategies for TNBC patients.

Second, given the significant impact of TILs on TNBC prognosis and therapy outcomes, efforts should be made

to enhance the assessment of TIL levels in clinical practice. Standardized methodologies for TIL assessment, including histopathological evaluation and immune profiling techniques, should be established to ensure consistency and reliability across different health-care settings. Moreover, incorporating TIL assessment into TNBC clinical trials can facilitate the evaluation of novel immunotherapeutic approaches and the stratification of patient populations based on TIL status.

Furthermore, strategies targeting MDSCs hold promise for modulating the immunosuppressive TME in TNBC. Preclinical and clinical studies investigating MDSC-targeting agents, such as inhibitors of MDSC recruitment or function, should be prioritized. In addition, combination therapies integrating MDSC-targeting approaches with standard treatments such as chemotherapy or immunotherapy warrant exploration to enhance treatment efficacy and overcome resistance mechanisms. Biomarker-guided patient selection and monitoring are essential for optimizing MDSC-targeted therapies in TNBC.

Moreover, due to the complex role of Tregs in TNBC progression, strategies to modulate Treg function should be pursued to restore anti-tumor immunity. Research efforts should focus on identifying specific molecular targets and signaling pathways involved in Treg-mediated immunosuppression within the TME. Therapeutic approaches targeting Treg accumulation or activity, such as Treg depletion or inhibition of Treg recruitment, should be investigated in preclinical models and clinical trials. Furthermore, combination therapies that include Treg-targeting agents with ICIs or other immunomodulators may offer synergistic benefits in TNBC treatment.

In addition, TAMs play a critical role in shaping the immunosuppressive TME and promoting TNBC progression. Therefore, targeting TAMs through various strategies, including inhibition of TAM recruitment or repolarization of TAMs toward an anti-tumor phenotype, holds therapeutic potential. Clinical trials evaluating TAM-targeted therapies, such as CCL2–CCR2 axis inhibitors or bisphosphonate-based macrophage apoptosis inducers, should be expanded to assess their efficacy and safety in TNBC patients. In addition, biomarker-driven approaches for patient selection and monitoring of treatment response are essential for the successful implementation of TAM-targeted therapies.

Finally, strategies to harness epigenetic mechanisms, such as HDACis and DNMTis, should be explored to enhance immune recognition and restore anti-tumor immunity. Clinical trials evaluating the efficacy of epigenetic-modifying agents alone or in combination with immunotherapies should be conducted to assess their therapeutic potential in TNBC patients. In addition, mechanistic studies elucidating the interplay between epigenetic alterations and immune checkpoint regulation can provide valuable insights into novel therapeutic targets and strategies for TNBC treatment.

7. Conclusion

The immune system plays an essential part in cancer surveillance, influencing tumor progression and response to therapy. TILs are linked to a better prognosis in TNBC patients, while immunosuppressive agents such as TAMs, Tregs, and MDSCs aid in immune evasion and tumor growth. In TNBC, epigenetic changes – such as DNA methylation and histone modifications – play a crucial role in controlling gene expression by silencing tumor suppressor genes, activating oncogenes, and affecting the immune recognition of cancer cells. ICIs and epigenetic medications together provide promise for improving TNBC outcomes, but there are still issues, including toxicity and drug interactions to be resolved. Novel therapeutic approaches, such as combining immunotherapy with epigenetic modulators to boost anti-tumor immunity and improve patient outcomes, may result from an understanding of the epigenetic control of immunological checkpoints and tumor-associated antigens in TNBC.

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References

1. Wolff AC, Hammond MEH, Hicks DG, Dowsett M, McShane LM, Allison KH. Recommendations for human epidermal growth factor receptor 2 testing in breast cancer. *J Clin Oncol*. 2013;31(31):3997-4013.

   doi: 10.5858/arpa.2018-0902-SA

2. Yin L, Duan JJ, Bian XW, Yu SC. Triple-negative breast cancer molecular subtyping and treatment progress. *Breast Cancer Res*. 2020;22:61.

   doi: 10.1186/s13058-020-01296-5

3. Lin NU, Vanderplas A, Hughes ME, Theriault RL, Edge SB, Wong YN. Clinicopathologic features, patterns of recurrence, and survival among women with triple-negative breast cancer in the National Comprehensive Cancer Network. *Cancer*. 2012;118(22):5463-5472.

   doi: 10.1002/cncr.27581

4. Cortes J, Rugo HS, Cescon DW, Im SA, Yusof MM, Gallardo C. Pembrolizumab plus chemotherapy in advanced triple-negative breast cancer. *N Engl J Med*. 2022;387(3):217-226.

   doi: 10.1056/NEJMoa2202809

5. Bardia A, Hurvitz SA, Tolaney SM, Loirat D, Punie K, Oliveira M. Sacituzumab govitecan in metastatic triple-negative breast cancer. *N Engl J Med*. 2021;384(16):1529-1541.

   doi: 10.1056/NEJMoa2028485

6. Swann JB, Smyth MJ. Immune surveillance of tumors. *J Clin Invest*. 2007;117(4):1137-1146.

   doi: 10.1172/JCI31405

7. Kim R. Cancer immunoediting: From immune surveillance to immune escape. *Cancer Immunother*. 2007;1:1-279.

   doi: 10.1111/j.1365-2567.2007.02587.x

8. Ignacio J, Manughian-Peter A, Matthew P, O'Day S, Hoon DSB, Marzese DM. Mechanisms of immune evasion in triple-negative breast cancer patients. *J Clin Oncol*. 2018;36:1096.

   doi: 10.1200/JCO.2018.36.15_suppl.1096

9. Llinàs-Arias P, Íñiguez-Muñoz S, McCann K, Voorwerk L, Orozco JIJ, Ensenyat-Mendez M. Epigenetic regulation of immunotherapy response in triple-negative breast cancer. *Cancers* (*Basel*). 2021;13(16):4139.

   doi: 10.3390/cancers13164139

10. Yang J, Xu J, Wang W, Zhang B, Yu X, Shi S. Epigenetic regulation in the tumor microenvironment: Molecular mechanisms and therapeutic targets. *Signal Transduct Target Ther*. 2023;8:210.

    doi: 10.1038/s41392-023-01480-x

11. Jung G, Hernández-Illán E, Moreira L, Balaguer F, Goel A. Epigenetics of colorectal cancer: Biomarker and therapeutic potential. *Nat Rev Gastroenterol Hepatol*. 2020;17:111-130.

    doi: 10.1038/s41575-019-0230-y

12. Dawson MA, Kouzarides T. Cancer epigenetics: From mechanism to therapy. *Cell*. 2012;150:12-27.

    doi: 10.1016/j.cell.2012.06.013

13. Schmid P, Cortes J, Pusztai L, McArthur H, Kümmel S, Bergh J. Pembrolizumab for early triple-negative breast cancer. *N Engl J Med*. 2020;382(9):810-821.

    doi: 10.1056/NEJMoa1910549

14. Mittendorf EA, Zhang H, Barrios CH, Saji S, Jung KH, Hegg R. Neoadjuvant atezolizumab in combination with sequential nab-paclitaxel and anthracycline-based chemotherapy versus placebo and chemotherapy in patients with early-stage triple-negative breast cancer (IMpassion031): A randomized, double-blind, phase 3 trial. *Lancet*. 2020;396(10257):1090-1100.

    doi: 10.1016/S0140-6736(20)31953-X

15. Zhang B, Wu Q, Li B, Wang D, Wang L, Zhou YL. M6A regulator-mediated methylation modification patterns and tumor microenvironment infiltration characterization in gastric cancer. *Mol Cancer*. 2020;19(1):53.

    doi: 10.1186/s12943-020-01170-0

16. Duan Q, Zhang H, Zheng J, Zhang L. Turning cold into hot: Firing up the tumor microenvironment. *Trends Cancer*. 2020;6:605-618.

    doi: 10.1016/j.trecan.2020.03.002

17. Zheng H, Siddharth S, Parida S, Wu X, Sharma D. Tumor microenvironment: Key players in triple-negative breast cancer immunomodulation. *Cancers* (*Basel*). 2021;13:3357.

    doi: 10.3390/cancers13163457

18. Bai X, Ni J, Beretov J, Graham P, Li Y. Immunotherapy for triple-negative breast cancer: A molecular insight into the microenvironment, treatment, and resistance. *J Natl Cancer Cent*. 2021;1(3):75-87.

    doi: 10.1016/j.jncc.2021.06.001

19. Wang H, Li S, Wang Q, *et al.* Tumor immunological phenotype signature-based high-throughput screening for the discovery of combination immunotherapy compounds. *Sci Adv*. 2021;7(4):eabd7851.

    doi: 10.1126/sciadv.abd7851

20. Petrosyan V, Dobrolecki LE, LaPlante EL, *et al.* Immunologically "cold" triple negative breast cancers engraft at a higher rate in patient derived xenografts. *NPJ Breast Cancer*. 2022;8:104.

    doi: 10.1038/s41523-022-00476-0

21. Perdiguero EG, Geissmann F. Identifying the infiltrators. *Science*. 2014;344:801-802.

doi: 10.1126/science.1255117

22. Huertas-Caro CA, Ramírez MA, Rey-Vargas L, *et al*. Tumor infiltrating lymphocytes (TILs) are a prognosis biomarker in Colombian patients with triple negative breast cancer. *Sci Rep.* 2023;13(1):21324.

doi: 10.1038/s41598-023- 48300-4

23. Dieci MV, Radosevic-Robin N, Fineberg S, *et al*. Update on tumor-infiltrating lymphocytes (TILs) in breast cancer, including recommendations to assess TILs in residual disease after neoadjuvant therapy and in carcinoma *in situ*: A report of the International Immuno-Oncology Biomarker Working Group on Breast Cancer. *Semin Cancer Biol.* 2018;52:16-25.

doi: 10.1016/j.semcancer.2017.10.003

24. Demaria S, Volm MD, Shapiro RL, *et al*. Development of tumor-infiltrating lymphocytes in breast cancer after neoadjuvant paclitaxel chemotherapy. *Clin Cancer Res*. 2001;7(10):3025-3032.

doi: 10.1158/1078-0432.CCR-07-1004

25. Liu S, Duan X, Xu L, *et al*. Optimal threshold for stromal tumor-infiltrating lymphocytes: Its predictive and prognostic value in HER2-positive breast cancer treated with trastuzumab-based neoadjuvant chemotherapy. *Breast Cancer Res Treat*. 2015;154(2):239-249.

doi: 10.1007/s10549-015-3617-7

26. West NR, Milne K, Truong PT, Macpherson N, Nelson BH, Watson PH. Tumor-infiltrating lymphocytes predict response to anthracycline-based chemotherapy in estrogen receptor-negative breast cancer. *Breast Cancer Res*. 2011;13(6):R125.

doi: 10.1186/bcr3072

27. Gao G, Wang Z, Qu X, Zhang Z. Prognostic value of tumor-infiltrating lymphocytes in patients with triple-negative breast cancer: A systematic review and meta-analysis. *BMC Cancer*. 2020;20(1):179.

doi: 10.1186/s12885-020-6668-z

28. Mao Y, Qu Q, Chen X, Huang O, Wu J, Shen K. The prognostic value of tumor-infiltrating lymphocytes in breast cancer: A systematic review and meta-analysis. *PLoS One*. 2016;11(4):e0152500.

doi: 10.1371/journal.pone.0152500

29. Wu Y, Yi M, Niu M, Mei Q, Wu K. Myeloid-derived suppressor cells: An emerging target for anticancer immunotherapy. *Mol Cancer*. 2022;21:184.

doi: 10.1186/s12943-022-01657-y

30. Ostrand-Rosenberg S, Sinha P. Myeloid-derived suppressor cells: Linking inflammation and cancer. *J Immunol*. 2009;182(8):4499-4506.

doi: 10.4049/jimmunol.0802740

31. Gomez S, Tabernacki T, Kobyra J, Roberts P, Chiappinelli KB. Combining epigenetic and immune therapy to overcome cancer resistance. *Semin Cancer Biol*. 2020;65:99-113.

doi: 10.1016/j.semcancer.2019.05.008

32. Bird L. MDSC metabolite stuns T cells. *Nat Rev Immunol*. 2020;20:352-353.

doi: 10.1038/s41577-020-0336-z

33. Lu Z, Zou J, Li S, *et al*. Epigenetic therapy inhibits metastases by disrupting premetastatic niches. *Nature*. 2020;579(7798):284-290.

doi: 10.1038/s41586-020-2054-x

34. Ge Y, Cheng D, Jia Q, Xiong H, Zhang J. Mechanisms underlying the role of myeloid-derived suppressor cells in clinical diseases: Good or bad. *Immune Netw.* 2021;21:e21

doi: 10.4110/in.2021.21.e21

35. Xu Z, Ji J, Xu J, *et al*. MiR-30a increases MDSC differentiation and immunosuppressive function by targeting SOCS3 in mice with B-cell lymphoma. *FEBS J*. 2017;284(15):2410-2424.

doi: 10.1111/febs.14133

36. Kajihara N, Kobayashi T, Otsuka R, *et al*. Tumor-derived interleukin-34 creates an immunosuppressive and chemoresistant tumor microenvironment by modulating myeloid-derived suppressor cells in triple-negative breast cancer. *Cancer Immunol Immunother*. 2023;72(4):851-864.

doi: 10.1007/s00262-022-03293-3

37. Mehdizadeh R, Shariatpanahi SP, Goliaei B, Rüegg C. Targeting myeloid-derived suppressor cells in combination with tumor cell vaccination predicts anti-tumor immunity and breast cancer dormancy: An *in silico* experiment. *Sci Rep.* 2023;13(1):5875.

doi: 10.1038/s41598-023-32554-z

38. Hao Z, Li R, Wang Y, Li S, Hong Z, Han Z. Landscape of myeloid-derived suppressor cell in tumor immunotherapy. *Biomark Res*. 2021;9(1):77.

doi: 10.1186/s40364-021-00333-5

39. Kumar S, Wilkes DW, Samuel N, *et al*. Δnp63-driven recruitment of myeloid-derived suppressor cells promotes metastasis in triple-negative breast cancer. *J Clin Invest*. 2018;128(11):5095-5109.

doi: 10.1172/JCI99673

40. Nefedova Y, Fishman M, Sherman S, Wang X, Beg AA, Gabrilovich DI. Mechanism of all-trans retinoic acid effect on tumor-associated myeloid-derived suppressor cells. *Cancer Res*. 2007;67(22):11021-11028.

doi: 10.1158/0008-5472.CAN-07-2593

41. Paroni G, Zanetti A, Barzago MM, *et al*. Retinoic acid sensitivity of triple-negative breast cancer cells characterized by constitutive activation of the notch1 pathway: The role of rarβ. *Cancers* (*Basel*). 2020;12(10):3027.

doi: 10.3390/cancers12103027

42. Lim C, Hwang D, Yazdimamaghani M, *et al*. High-dose paclitaxel and its combination with CSF1R inhibitor in polymeric micelles for chemoimmunotherapy of triple-negative breast cancer. *Nano Today*. 2023;51:101884.

doi: 10.1016/j.nantod.2023.101884

43. Connolly RM, Rudek MA, Piekarz R. Entinostat: A promising treatment option for patients with advanced breast cancer. *Future Oncol*. 2017;13(13):1137-1148.

doi: 10.2217/fon-2016-0526

44. Fuso P, Muratore M, D'angelo T, *et al*. PI3K inhibitors in advanced breast cancer: The past, the present, new challenges and future perspectives. *Cancers* (*Basel*). 2022;14:2161.

doi: 10.3390/cancers14092161

45. Cinier J, Hubert M, Besson L, *et al*. Recruitment and expansion of Tregs cells in the tumor environment-how to target them? *Cancers* (*Basel*). 2021;13(8):1850.

doi: 10.3390/cancers13081850

46. Plitas G, Konopacki C, Wu K, *et al*. Regulatory T cells exhibit distinct features in human breast cancer. *Immunity*. 2016;45(5):1122-1134.

doi: 10.1016/j.immuni.2016.10.032

47. Liu F, Lang R, Zhao J, *et al*. CD8+ cytotoxic T cell and FOXP3+ regulatory T cell infiltration in relation to breast cancer survival and molecular subtypes. *Breast Cancer Res Treat*. 2011;130(2):645-655.

doi: 10.1007/s10549-011-1647-3

48. Yeong J, Thike AA, Lim JCT, *et al*. Higher densities of Foxp3+ regulatory T cells are associated with better prognosis in triple-negative breast cancer. *Breast Cancer Res Treat*. 2017;163(1):21-35.

doi: 10.1007/s10549-017-4161-4

49. Letendre P, Monga V, Milhem M, Zakharia Y. Ipilimumab: From preclinical development to future clinical perspectives in melanoma. *Future Oncol*. 2017;13:625-636.

doi: 10.2217/fon-2016-0385

50. Sobhani N, Tardiel-Cyril DR, Davtyan A, Generali D, Roudi R, Li Y. CTLA-4 in regulatory T cells for cancer immunotherapy. *Cancers* (*Basel*). 2021;13:1440.

doi: 10.3390/cancers13061440

51. Howard FM, Pearson AT, Nanda R. Clinical trials of immunotherapy in triple-negative breast cancer. *Breast Cancer Res Treat*. 2022;195:1-15.

doi: 10.1007/s10549-022-06665-6

52. Takahashi M, Cortés J, Dent R, *et al*. Pembrolizumab plus chemotherapy followed by pembrolizumab in patients with early triple-negative breast cancer: A secondary analysis of a randomized clinical trial. *JAMA Netw Open*. 2023;6(11):E2342107.

doi: 10.1001/jamanetworkopen.2023.42107.

53. Fattori S, Roy A Le, Houacine J, *et al*. CD25high effector regulatory T cells hamper responses to PD-1 blockade in triple-negative breast cancer. *Cancer Res*. 2023;83(18):3026-3044.

doi: 10.1158/0008-5472.CAN-23-0613

54. Strauss J, Heery CR, Schlom J, *et al*. Phase I trial of M7824 (MSB0011359C), a bifunctional fusion protein targeting PD-L1 and TGFβ, in advanced solid tumors. *Clin Cancer Res*. 2018;24(6):1287-1295.

doi: 10.1158/1078-0432.CCR-17-2653

55. Bhola NE, Balko JM, Dugger TC, *et al*. TGF-β inhibition enhances chemotherapy action against triple-negative breast cancer. *J Clin Invest*. 2013;123(3):1348-1358.

doi: 10.1172/JCI65416

56. Hu C, Liu X, Zeng Y, Liu J, Wu F. DNA methyltransferase inhibitors combination therapy for the treatment of solid tumors: mechanism and clinical application. *Clin Epigenetics*. 2021;13:166.

doi: 10.1186/s13148-021-01154-x

57. Terranova-Barberio M, Thomas S, Ali N, *et al*. HDAC inhibition potentiates immunotherapy in triple-negative breast cancer. *Oncotarget*. 2017;8(29):48223-48235.

doi: 10.18632/oncotarget.23169

58. Zahran AM, El-Badawy O, Kamel LM, Rayan A, Rezk K, Abdel-Rahim MH. Accumulation of regulatory T cells in triple-negative breast cancer can boost immune disruption. *Cancer Manag Res*. 2021;13:6019-6029.

doi: 10.2147/CMAR.S285128

59. Oshi M, Asaoka M, Tokumaru Y, *et al*. Abundance of regulatory T cell (Treg) as a predictive biomarker for neoadjuvant chemotherapy in triple-negative breast cancer. *Cancers* (*Basel*). 2020;12(10):3038.

doi: 10.3390/cancers12103038

60. Qin D, Zhang Y, Shu P, Lei Y, Li X, Wang Y. Targeting tumor-infiltrating Tregs for improved antitumor responses. *Front Immunol*. 2024;15:1325946.

doi: 10.3389/fimmu.2024.1325946

61. Qiu X, Zhao T, Luo R, Qiu R, Li Z. Tumor-associated macrophages: Key players in triple-negative breast cancer. *Front Oncol*. 2022;12:772615.

doi: 10.3389/fonc.2022.772615

62. Bottai G, Raschioni C, Székely B, *et al*. AXL-associated tumor inflammation as a poor prognostic signature in

chemotherapy-treated triple-negative breast cancer patients. *NPJ Breast Cancer*. 2016;2(1):16033.

doi: 10.1038/npjbcancer.2016.33

63. Noy R, Pollard JW. Tumor-associated macrophages: From mechanisms to therapy. *Immunity*. 2014;41:49-61.

doi: 10.1016/j.immuni.2014.06.010

64. Hollmén M, Karaman S, Schwager S, *et al*. G-CSF regulates macrophage phenotype and associates with poor overall survival in human triple-negative breast cancer. *Oncoimmunology*. 2016;5(3):e1115177.

doi: 10.1080/2162402X.2015.1115177

65. Jamiyan T, Kuroda H, Yamaguchi R, Abe A, Hayashi M. CD68- and CD163-positive tumor-associated macrophages in triple negative cancer of the breast. *Virchows Arch*. 2020;477:767-775.

doi: 10.1007/s00428-020-02855-z

66. Wang J, Chen H, Chen X, Lin H. Expression of tumor-related macrophages and cytokines after surgery of triple-negative breast cancer patients and its implications. *Med Sci Monit*. 2016;22:115-120.

doi: 10.12659/msm.895386

67. Niu M, Valdes S, Naguib YW, Hursting SD, Cui Z. Tumor-associated macrophage-mediated targeted therapy of triple-negative breast cancer. *Mol Pharm*. 2016;13(6):1833-1842.

doi: 10.1021/acs.molpharmaceut.5b00987

68. Rogers TL, Holen I. Tumour macrophages as potential targets of bisphosphonates. *J Transl Med*. 2011;9:177.

doi: 10.1186/1479-5876-9-177

69. Yang Q, Guo N, Zhou Y, Chen J, Wei Q, Han M. The role of tumor-associated macrophages (TAMs) in tumor progression and relevant advance in targeted therapy. *Acta Pharm Sin B*. 2020;10:2156-2170.

doi: 10.1016/j.apsb.2020.04.004

70. Panjarian S, Issa JPJ. The roles of DNA demethylases in triple-negative breast cancer. *Pharmaceuticals* (*Basel*). 2021;14:628.

doi: 10.3390/ph14070628

71. Malpeli G, Innamorati G, Decimo I, *et al*. Methylation dynamics of RASSF1A and its impact on cancer. *Cancers* (*Basel*). 2019;11:959.

doi: 10.3390/cancers11070959

72. Saelee P, Pongtheerat T. APC promoter hypermethylation as a prognostic marker in breast cancer patients. *Asian Pac J Cancer Prev*. 2020;21(12):3627-3632.

doi: 10.31557/APJCP.2020.21.12.3627

73. Chien YC, Liu LC, Ye HY, Wu JY, Yu YL. EZH2 promotes migration and invasion of triple-negative breast cancer cells via regulating TIMP2-MMP-2/-9 pathway. *Am J Cancer Res*. 2018;8:422-434.

74. Yang H, Salz T, Zajac-Kaye M, Liao D, Huang S, Qiu Y. Overexpression of histone deacetylases in cancer cells is controlled by interplay of transcription factors and epigenetic modulators. *FASEB J.* 2014;28(10):4265-4279.

doi: 10.1096/fj.14-250654

75. Title AC, Silva PN, Godbersen S, Hasenöhrl L, Stoffel M. The miR-200-Zeb1 axis regulates key aspects of β-cell function and survival *in vivo*. *Mol Metab*. 2021;53:101267.

doi: 10.1016/j.molmet.2021.101267

76. Miller TE, Ghoshal K, Ramaswamy B, *et al*. MicroRNA-221/222 confers tamoxifen resistance in breast cancer by targeting p27Kip1. *J Biol Chem*. 2008;283(44):29897-29903.

doi: 10.1074/jbc.M804612200

77. Zhang Z, Zhou C, Li X, *et al*. Loss of CHD1 promotes heterogeneous mechanisms of resistance to AR-targeted therapy via chromatin dysregulation. *Cancer Cell*. 2020;37(4):584-598.e11.

doi: 10.1016/j.ccell.2020.03.001

78. Cortazar P, Zhang L, Untch M, *et al*. Pathological complete response and long-term clinical benefit in breast cancer: The CTNeoBC pooled analysis. *Lancet*. 2014;384(9938):164-172.

doi: 10.1016/S0140-6736(13)62422-8

79. Kagihara JA, Andress M, Diamond JR. Nab-paclitaxel and atezolizumab for the treatment of PD-L1-positive, metastatic triple-negative breast cancer: Review and future directions. *Expert Rev Precis Med Drug Dev*. 2020;5(2):59.

doi: 10.1080/23808993.2020.1730694

80. Adams S, Schmid P, Rugo HS, *et al*. Pembrolizumab monotherapy for previously treated metastatic triple-negative breast cancer: Cohort A of the phase II KEYNOTE-086 study. *Ann Oncol.* 2019;30(3):397-404.

doi: 10.1093/annonc/mdy517

81. Ozaki Y, Matsumoto K, Takahashi M, *et al*. Phase II study of a combination therapy of nivolumab, bevacizumab and paclitaxel in patients with HER2-negative metastatic breast cancer as a first-line treatment (WJOG9917B, NEWBEAT trial). *J Clin Oncol*. 2018;36(15_suppl):TPS1110.

doi: 10.1016/j.ejca.2022.05.014

82. Chen F, Chen N, Gao Y, Jia L, Lyu Z, Cui J. Clinical progress of PD-1/L1 inhibitors in breast cancer immunotherapy. *Front Oncol*. 2022;11:724424.

doi: 10.3389/fonc.2021.724424

83. Li Y, Zhang H, Merkher Y, *et al*. Recent advances in therapeutic strategies for triple-negative breast cancer. *J Hematol Oncol*. 2022;15:121.

doi: 10.1186/s13045-022-01341-0

84. Dirix LY, Takacs I, Jerusalem G, *et al*. Avelumab, an anti-PD-L1 antibody, in patients with locally advanced or metastatic breast cancer: A phase 1b JAVELIN solid tumor study. *Breast Cancer Res Treat*. 2018;167(3):671-686.

doi: 10.1007/s10549-017-4537-5

85. Xie Y, Hu Y, Zhou N, *et al*. CAR T-cell therapy for triple-negative breast cancer: Where we are. *Cancer Lett*. 2020;491:121-131.

doi: 10.1016/j.canlet.2020.07.044

86. Simó-Riudalbas L, Esteller M. Targeting the histone orthography of cancer: Drugs for writers, erasers and readers. *Br J Pharmacol*. 2014;172(11):2716-2732.

doi: 10.1111/bph.12844

87. Skvortsova K, Stirzaker C, Taberlay P. The DNA methylation landscape in cancer. *Essays Biochem*. 2019;63:797-811.

doi: 10.1042/EBC20190037

88. Shukla A, Cloutier M, Santharam MA, Ramanathan S, Ilangumaran S. The MHC class-I transactivator NLRC5: Implications to cancer immunology and potential applications to cancer immunotherapy. *Int J Mol Sci*. 2021;22:1964.

doi: 10.3390/ijms22041964

89. Zeiner PS, Zinke J, Kowalewski DJ, *et al*. CD74 regulates complexity of tumor cell HLA class II peptidome in brain metastasis and is a positive prognostic marker for patient survival. *Acta Neuropathol Commun*. 2018;6(1):18.

doi: 10.1186/s40478-018-0521-5

90. Park SY, Park JW, Chun YS. Jumonji histone demethylases as emerging therapeutic targets. *Pharmacol Res*. 2016;105:146-151.

doi: 10.1016/j.phrs.2016.01.026

91. Guo L, Sang M, Liu Q, Fan X, Zhang X, Shan B. The expression and clinical significance of melanoma-associated antigen-A1, -A3 and -A11 in glioma. *Oncol Lett*. 2013;6(1):55-62.

doi: 10.3892/ol.2013.1304

92. Won JK, Bakhoum SF. The cytosolic DNA-sensing cGAS-STING pathway in cancer. *Cancer Discov*. 2020;10:26-39.

doi: 10.1158/2159-8290.CD-19-0761

93. Andres F, Yufeng L, Dongquan C, *et al*. Expression of the MHC class II pathway in triple-negative breast cancer tumor cells is associated with a good prognosis and infiltrating lymphocytes. *Cancer Immunol Res*. 2016;4(5):390-399.

doi: 10.1158/2326-6066.CIR-15-0243

94. Stewart RL, Matynia AP, Factor RE, Varley KE. Spatially-resolved quantification of proteins in triple-negative breast cancers reveals differences in the immune microenvironment associated with prognosis. *Sci Rep*. 2020;10(1):6598.

doi: 10.1038/s41598-020-63539-x

95. Luo N, Nixon MJ, Gonzalez-Ericsson PI, *et al*. DNA methyltransferase inhibition upregulates MHC-I to potentiate cytotoxic T lymphocyte responses in breast cancer. *Nat Commun*. 2018;9(1):248.

doi: 10.1038/s41467-017-02630-w

96. Kakimi K, Karasaki T, Matsushita H, Sugie T. Advances in personalized cancer immunotherapy. *Breast Cancer*. 2017;24(1):16-24.

doi: 10.1007/s12282-016-0688-1

97. Sasidharan Nair V, El Salhat H, Taha RZ, John A, Ali BR, Elkord E. DNA methylation and repressive H3K9 and H3K27 trimethylation in the promoter regions of PD-1, CTLA-4, TIM-3, LAG-3, TIGIT, and PD-L1 genes in human primary breast cancer. *Clin Epigenetics*. 2018;10(1):78.

doi: 10.1186/s13148-018-0512-1

98. Li YC, Zhou Q, Song QK, *et al*. Overexpression of an immune checkpoint (CD155) in breast cancer associated with prognostic significance and exhausted tumor-infiltrating lymphocytes: A cohort study. *J Immunol Res*. 2020;2020:3948928.

doi: 10.1155/2020/3948928

99. Huang W, Zhu Q, Shi Z, *et al*. Dual inhibitors of DNMT and HDAC induce viral mimicry to induce antitumor immunity in breast cancer. *Cell Death Discov*. 2024;10(1):143.

doi: 10.1038/s41420-024-01895-7

100. Sheikh TN, Chen X, Xu X, *et al*. Growth inhibition and induction of innate immune signaling of chondrosarcomas with epigenetic inhibitors. *Mol Cancer Ther*. 2021;20(12):2362-2371.

doi: 10.1158/1535-7163.MCT-21-0066

101. Kiyozumi Y, Baba Y, Okadome K, *et al*. Indoleamine 2,3-dioxygenase 1 promoter hypomethylation is associated with poor prognosis in patients with esophageal cancer. *Cancer Sci*. 2019;110(6):1863-1871.

doi: 10.1111/cas.14028

102. Zhai L, Bell A, Ladomersky E, *et al*. Immunosuppressive IDO in cancer: Mechanisms of action, animal models, and targeting strategies. *Front Immunol*. 2020;11:1185.

doi: 10.3389/fimmu.2020.01185

103. Hosseini A, Gharibi T, Marofi F, Babaloo Z, Baradaran B. CTLA-4: From mechanism to autoimmune therapy. *Int Immunopharmacol*. 2020;80:106221.

doi: 10.1016/j.intimp.2020.106221

104. Colamatteo A, Carbone F, Bruzzaniti S, *et al*. Molecular mechanisms controlling Foxp3 expression in health and autoimmunity: From epigenetic to post-translational regulation. *Front Immunol*. 2020;10:3136.

doi: 10.3389/fimmu.2019.03136

105. Wang X, Brea LT, Yu J. Immune modulatory functions of EZH2 in the tumor microenvironment: Implications in cancer immunotherapy. *Am J Clin Exp Urol*. 2019;7:85-91.

106. Wong KK. DNMT1: A key drug target in triple-negative breast cancer. *Semin Cancer Biol*. 2021;72:198-213.

doi: 10.1016/j.semcancer.2020.05.010

107. Ropero S, Esteller M. The role of histone deacetylases (HDACs) in human cancer. *Mol Oncol*. 2007;1:19-25.

doi: 10.1016/j.molonc.2007.02.001

108. Morand S, Stanbery L, Walter A, Rocconi RP, Nemunaitis J. BRCA1/2 mutation status impact on autophagy and immune response: Unheralded target. *JNCI Cancer Spectrum*. 2021;4(6):pkaa077.

doi: 10.1093/jncics/pkaa077

109. Kim S, Park S, Cho MS, Lim W, Moon BI, Sung SH. Strong correlation of indoleamine 2,3-dioxygenase 1 expression with basal-like phenotype and increased lymphocytic infiltration in triple-negative breast cancer. *J Cancer*. 2017;8(1):124-130.

doi: 10.7150/jca.17437

110. Dill EA, Dillon PM, Bullock TN, Mills AM. IDO expression in breast cancer: An assessment of 281 primary and metastatic cases with comparison to PD-L1. *Mod Pathol*. 2018;31(10):1513-1522.

doi: 10.1038/s41379-018-0061-3

111. Asghar K, Loya A, Rana IA, *et al*. Association between cyclooxygenase-2 and indoleamine 2,3-dioxygenase expression in breast cancer patients from Pakistan. *Asian Pac J Cancer Prev*. 2019;20(11):3521-3525.

doi: 10.31557/APJCP.2019.20.11.3521

112. Costa RLB, Czerniecki BJ. Clinical development of immunotherapies for HER2+ breast cancer: A review of HER2-directed monoclonal antibodies and beyond. *NPJ Breast Cancer*. 2020;6:10.

doi: 10.1038/s41523-020-0153-3

113. Jing R, Bai S, Zhang P, *et al*. IDO-1 impairs antitumor immunity of natural killer cells in triple-negative breast cancer via up-regulation of HLA-G. *Breast Cancer*. 2024;31(1):135-147.

doi: 10.1007/s12282-023-01522-w

114. Carter JM, Polley MYC, Leon-Ferre RA, *et al.* Characteristics and spatially defined immune (micro) landscapes of early-stage PD-L1-positive triple-negative breast cancer. *Clin Cancer Res.* 2021;27(20):5628-5637.

doi: 10.1158/1078-0432.CCR-21-0343

115. Keenan TE, Tolaney SM. Role of immunotherapy in triple-negative breast cancer. *J Natl Compr Canc Netw.* 2020;18:479-489.

doi: 10.6004/jnccn.2020.7554

116. Jacquemier J, Bertucci F, Finetti P, *et al*. High expression of indoleamine 2,3-dioxygenase in the tumour is associated with medullary features and favourable outcome in basal-like breast carcinoma. *Int J Cancer*. 2012;130(1):96-104.

doi: 10.1002/ijc.25979

117. Noonepalle SK, Gu F, Lee EJ, *et al*. Promoter methylation modulates indoleamine 2,3-dioxygenase 1 induction by activated T cells in human breast cancers. *Cancer Immunol Res*. 2017;5(4):330-344.

doi: 10.1158/2326-6066.CIR-16-0182

118. Spranger S, Spaapen RM, Zha Y, *et al*. Up-regulation of PD-L1, IDO, and T(regs) in the melanoma tumor microenvironment is driven by CD8(+) T cells. *Sci Transl Med*. 2013;5:200ra116.

doi: 10.1126/scitranslmed.3006504

119. Song X, Si Q, Qi R, *et al*. Indoleamine 2,3-dioxygenase 1: A promising therapeutic target in malignant tumor. *Front Immunol*. 2021;12:800630.

doi: 10.3389/fimmu.2021.800630.

120. Zhai L, Spranger S, Binder DC, *et al*. Molecular pathways: Targeting IDO1 and other tryptophan dioxygenases for cancer immunotherapy. *Clin Cancer Res*. 2015;21:5427-5433.

doi: 10.1158/1078-0432.CCR-15-0420

121. Sun L. Advances in the discovery and development of selective heme-displacing IDO1 inhibitors. *Expert Opin Drug Discov*. 2020;15:1223-1232.

doi: 10.1080/17460441.2020.1781811

122. Pallotta MT, Rossini S, Suvieri C, *et al*. Indoleamine 2,3-dioxygenase 1 (IDO1): An up-to-date overview of an eclectic immunoregulatory enzyme. *FEBS J.* 2022;289(20):6099-6118.

doi: 10.1111/febs.16086

123. Tomek P, Palmer BD, Flanagan JU, Sun C, Raven EL, Ching LM. Discovery and evaluation of inhibitors to the immunosuppressive enzyme indoleamine 2,3-dioxygenase 1 (IDO1): Probing the active site-inhibitor interactions. *Eur J Med Chem.* 2017;126:983-996.

doi: 10.1016/j.ejmech.2016.12.029

124. Munn DH, Mellor AL. IDO in the tumor microenvironment: Inflammation, counter-regulation, and tolerance. *Trends Immunol.* 2016;37:193-207.

doi: 10.1016/j.it.2016.01.002