**Review Article**
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# Triple-Negative Breast Cancer: Emerging Biomarkers for Early Diagnosis, Prognosis, and Treatment

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## Abstract

Breast cancer (BC) is a heterogeneous disease characterized by significant global mortality and incidence rates. Annually, approximately 1 million cases of BC are diagnosed worldwide, with over 170,000 classified as triple-negative. Triple-negative breast cancer (TNBC) is a particularly aggressive subtype lacking targeted therapeutic options, which contributes to poorer outcomes compared to other BC subtypes. The five-year survival rate for patients with TNBC is roughly 30% lower than that for patients with other subtypes. TNBC treatment options are limited to surgery, radiotherapy, and chemotherapy. There is a critical need for the development of targeted therapies. Enhancing early detection through effective diagnostic and prognostic biomarkers can significantly improve survival rates. This review explores recent advancements in clinically relevant proteomic, genetic, and metabolomic biomarkers for TNBC, highlighting their potential roles as prognostic, diagnostic, and predictive markers that could facilitate personalized treatment approaches.

***Keywords:*** Triple-negative breast neoplasms, Biomarkers, Proteomics, Genetic, Metabolomics

## Introduction

Cancer is increasingly a global problem, and breast cancer (BC) remains the most common neoplasia in women.[1] Every year, BC causes 450,000 deaths worldwide. In 2018, the estimated incidence of BC was 2,088,849 new cases worldwide. BC is considered the most common cancer in women, excluding non-melanoma skin cancer.[2, 3] Triple-negative breast cancer (TNBC) comprises 15% of all invasive BCs diagnosed.[4]

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According to the St. Gallen guidelines, the American Society of Clinical Oncology, and the American College of Pathology, TNBC is defined as progesterone receptor (PR)-negative, estrogen receptor (ER)-negative, and human epidermal growth factor receptor 2 (HER2)-negative.[5] TNBC is a complex and highly heterogeneous disease at the molecular level. Pathological and clinical manifestations of TNBC occur mainly in premenopausal young women. TNBC is more prone to metastasis and relapse at visceral sites such as the central nervous system (CNS), lung, and liver.[6]

Comparing all BC subtypes, TNBC has the strongest tumor immunogenicity.[7] Immune-related gene sets including HLA, tumor-infiltrating lymphocytes (TILs), Tregs, M1 macrophages, M0 macrophages, activated dendritic cells, activated TCD4$^{+}$ memory cells, T follicular helper cells subsets, immune checkpoints, chemokine (C-C Motif) receptor (CCR), gene sets related to metastasis, proinflammatory mediators, and PI had significantly higher proportions in TNBC than in the non-TNBC.[8, 9] But resting TCD4 memory cell subsets, resting mast cells, and M2 macrophages, metastasis-inhibiting gene-set showed significantly lower expression levels.[9, 10] Interestingly, elevated expression of most of them was associated with ER and HER2 status and better survival prognosis in TNBC due to more sensitivity of TNBC to chemotherapy.[11]

Due to the absence of specific molecular targets, conventional chemotherapeutic agents, such as Anthracyclines and Taxol, are currently the preferred treatments for patients with TNBC, which sometimes results in persistent side-effects such as hair loss, diarrhea, nausea, vomiting, and the worst outcome.[12] Hence, it is urgent to explore uniform targets that can help achieve less toxic and more effective treatment for TNBC.[13] Results of clinical and preclinical studies are shown in table 1.The primary purpose of this review is to update emerging biomarkers that are currently available and to provide more effective biomarkers for the prognosis and early detection of different molecular subtyping of TNBC.

### *Molecular subtyping of BC*

According to changes in the expression of ER, PR, and HER2, BC is classified into the following four main molecular subgroups: Luminal A, B, HER2+, and TNBC.[14, 15] Luminal A and B are 65 to 80% of BCs, with a good prognosis, and are involved in cell proliferation and invasiveness of BC cells. The HER2 subtype proliferates the HER2 oncogene, and hormone therapy is ineffective. TNBC is the next subtype. TNBC patients are young people under the age of 40 of African-American descent and have shorter disease-free survival (DFS) and overall survival (OS) than non-TNBC patients.[16] Recurrence occurs 1-3 years after diagnosis with an increased risk of lung metastasis or CNS.[16]

In 2011, Vanderbilt University researchers, based on GFP and DNA microarray, classified TNBC into six subtypes with distinct molecular signatures. These were: A. Basal cell-like1 (BL-1), B. Basal cell-like2 (BL-2), C. Immunomodulatory (IM), D. Mesenchymal (M), E. Mesenchymal stem-like (MSC), F. Luminal androgen receptor (LAR) subtypes.[17, 18] Identifying these subtypes is also essential to understand the biological features and clinical behavior of TNBC for the development of targeted agents.

MSC and M subtypes have features of epithelial-mesenchymal transitions (EMT). MSC subtypes express genes involved in angiogenesis (e.g., VEGFR2) and respond vigorously to Dasatinib (tyrosine kinase inhibitors) and mTOR inhibitors.[19, 20] IM subtypes are rich in factors involved in the processing of immune cells. The LAR subtypes are the most differentiated TNBC subtype that is characterized by androgen receptor-signaling, and the expression of AR mRNA is nine times greater than the other subtypes;[21, 22] hence, it is firmly susceptible to AR antagonists like Biclutanid. This subgroup has increased DFS and OS. BL-1 subtypes are rich in cell cycle pathways and division compounds and associated with increased expression of Ki67 mRNA due to their proliferative nature. BL-2 subtypes include growth factor signaling pathways (e.g., Wnt/β-catenin, NGF, MET, EGF, and IGF1R) as well as metabolic pathways (e.g., glycolysis and glu-

coneogenesis).[19, 21] TNBC is often associated with basal BC, due to the similarity of the expression pattern of mRNA with basal cells or myoepithelial located on the basal side of normal mammary glands. Approximately 75% of TNBCs are basal-like, and another 25% are other subtypes.[22] Different studies have investigated the responses to neoadjuvant chemotherapy among different subtypes of TNBC. BL-1 subtypes have the highest rates of pathological complete response (PCR=52%), and BL-2, LAR, and MSL had the lowest response rates (0%, 10%, and 23%, respectively).[23]

*Emerging biomarkers in TNBC*

TNBC biomarkers identified in the past are summarized in table 1 and figure 1. This section introduces new biomarkers. Based on the function of each marker and their roles in different cellular processes, the biomarkers are divided into separate categories and described as follows (Figure 1).

## Genes and Proteins Related to DNA Damage Responses and Cell Cycle Control

*Treacle ribosome biogenesis factor 1 (TCOF1) gene*

TCOF1 gene, via interaction with NBS1 and MRNM, has been implicated in DNA damage response in neuroepithelium.[24]

TCOF1 is highly expressed in TNBC cell lines but not in luminal cell lines, with its expression elevation correlating with shorter OS. TCOF1depletion attenuates the growth and stemness of basal-like TNBC considerably, unlike those of mesenchymal-like cells. In this respect, the lack of TCOF1 expression in normal breast tissues suggests the potential for prognostic markers and therapeutic targets in TNBC.[25]

*Cyclin-dependent kinases (CDK)*

Altered cyclin expression of D, E, CDK2, and CDK4/6 can be seen in TNBC, and inhibitory treatment of CDK is an important strategy in TNBC.[12] More than 10 CDK inhibitors are being reviewed in clinical trials, such as Abemaciclib, Palbociclib, Ribociclib, and Dinaciclib.[26] CDK4/6 inhibitors (Palbociclib, Ribociclib) treat advanced BC with $HR^+$ and $HER2^-$.[26] Furthermore, these CDK4/6 inhibitors have synergy PI3K inhibitors in the TNBC cell line.[27] The inhibition of CDK4/6 has recently blocked breast tumor metastasis in the xenograft TNBC model.[27] The inhibitory property of Palbociclib does not affect the growth of primary tumors but significantly prevents the spread of TNBC to distant organs through SNAIL1 protein instability.[28] Ribociclib and Palbociclib, in combination with Bicalutamide (AR antagonist), have been recently used to treat advanced TNBC $AR^+$. Abemaciclib has a different toxicity pattern and is being tested as a single agent in advanced TNBC with a high expression of RB1.[29] Dinaciclib (pan CDK inhibitor) also has anti-TNBC activity in-vitro and in-vivo.[30]

*1.3-NUF2 and FAM83D*

In a study to identify differentially expressed genes (DEGs) in TNBC tissue, three independent data sets (GSE38959, GSE65194, GSE45827) were downloaded from GEO (Gene Expression Omnibus). Bioinformatics tools such as the DAVID and STRING databases were used to describe and verify hub genes, and real time-quantitative polymerase chain reaction (RT-qPCR) was used.[31] In this analysis, 161 DEGs were screened between 222 non-TNBC and 126TNBC samples. The Ndc80 kinetochore complex component (NUF2) and family with sequence similarity 83 member D (FAM83D) expression levels were significantly higher in TNBC than in the adjacent tissue. According to the Kaplan-Meier survival curve, the expression of NUF2 and FAM83D was associated with recurrence-free survival in TNBC samples. RT-qPCR also confirmed that the expression of these two in TNBC tissue was significantly regulated, and thereby, they can be considered biomarkers for prognosis and diagnosis.[31] NUF2 is the principal constituent of the kinetochore-associated complex (NDC80) and plays a regulatory role in chromosome segregation. Xu et al. showed that NUF2 is closely related to BC through cell cycle pathways.[32] FAM83D is involved in cell growth, proliferation, migration, and epithelial transition to the mesenchymal feature.[33] Yiduo Liu et al. identified 105 differential expression genes between TNBC and other BC subtypes. They suggested that FAM83B, KITLG, CFD, and RBM24 affected the prognosis of TNBC

patients.[34]

## MicroRNAs, Lnc-RNAs and CircRNAs

MicroRNAs are non-coding RNAs with 22 nucleotides that regulate gene expression by pairing specific sequence bases with target mRNAs.[35] MicroRNAs regulate tumor suppression or oncogenic pathways, including P53, RAS, BCR-ABL, and C-myc; however, oncogenes or tumor suppressors regulate the expression of microRNAs.[36] MicroRNAs in BC showed a differentiated or deregulated expression and were associated with ER and HER2 levels.[36] Many studies emphasize the role of microRNAs as regulators, so they are considered new candidates for prognosis, diagnosis, and target therapy.[35] In a recent meta-analysis, decreased miR-155 and increased miR-21 expression were associated with weaker OS.[37] The miR-34 family, including miR-34 a, b, and c, have been linked to TNBC in most microRNA studies and have multiple roles as biomarkers. The miR-34a was associated with impaired tumor growth, induced apoptosis, senescence, and cell cycle arrest in the TNBC cancer cell line. The miR-34c was associated with a worse prognosis.[38-40] TNBC patients with increased expression of miR497 have a better prognosis, which may be a new prognostic marker. Mir373 and 520c stimulate the migration and invasion of tumor cells in vivo and in vitro.[35] The expression of miR-371b-5p was found to be reduced in TNBC cells.[41]

Lnc-RNAs are transcripts of 200 nucleotides that may not be translated into proteins and, together with other proteins, regulate the transcription of genes encoding proteins. lnc-RNAs are impaired in many cancers, including TNBCs.[42] An lnc-RNA called long non-coding RNA in non-homologous end-joining pathway 1 (LINP1) is expressed in TNBC. LINP1 enhances the repair of broken double-stranded DNA, and blocking it increases the sensitivity of TNBC to radiotherapy.[43] Another lnc-RNA called highly upregulated in liver cancer (HULC) is regulated in patients' breast tissue and TNBC cell line and

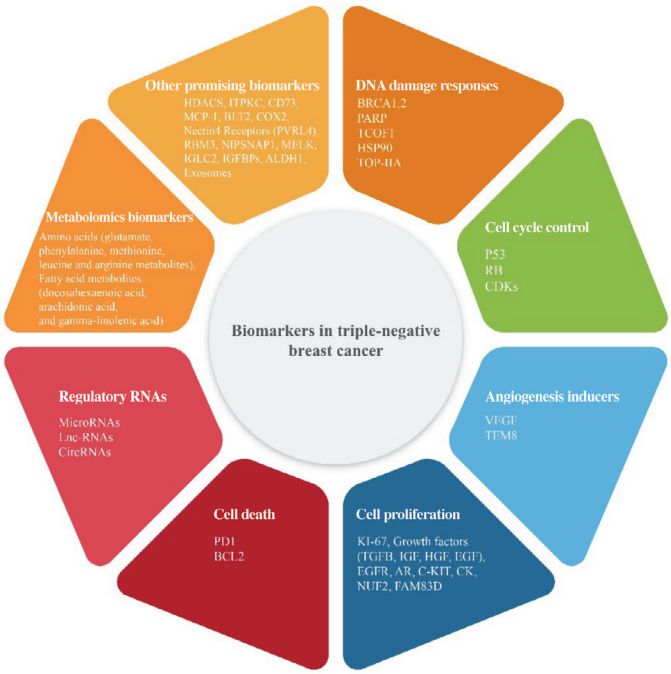


**Figure 1.** This figure summarizes the potential genetic, proteomic, and metabolomic markers in triple-negative breast cancer.

BRCA1,2: Breast cancer gene1,2; PARP: Poly adenosine diphosphate-ribose polymerase; HSP90: Heat shock protein 90; TOP-IIA: Topoisomerase IIA;TCOF1: Treacle ribosome biogenesis factor 1; RB: Retinoblastoma; CDKs: Cyclin-dependent kinases; VEGF: Vascular endothelial growth factor; TEM8: Tumor endothelial marker 8; TGFB: Transforming growth factor beta; IGF1: Insulin-like growth factor1; HGF: Hepatocyte growth factor; EGF: Epidermal growth factor; EGFR: Epidermal growth factor receptor; AR: Androgen receptor; CK: Cytokeratin; NUF2: Ndc80 kinetochore complex component; FAM83D: Family with sequence similarity 83 member D; PD1: Programmed cell death protein 1; BCL2: B-cell lymphoma 2; lnc RNA: Long non-coding RNAs; circRNA: Circular RNAs; HDACs: Histone deacetylases; ITPKC: Inositol 1,4,5-trisphosphate 3-kinase; MCP-1: Monocyte chemoattractant protein-1; BLT2: Leukotriene B4 receptor 2; COX2: Cyclooxygenase 2; RBM3: RNA binding motif protein 3; NIPSNAP1: Non-neuronal SNAP25-like protein homolog 1; MELK: Maternal embryonic leucine zipper kinase; IGFBPS: Insulin-like growth factor binding proteins; ALDH1: Aldehyde dehydrogenase 1

**Table1.** Selected phase II or III clinical trials in patients with TNBC from 2015 to 2022* (continued)

| Entry | Promising therapy | Clinical trials gov identifier | Clinical studies |
|---|---|---|---|
| 1 | PARP inhibitors | NCT01945775 | Phase III, Talazoparib (BMN 673) as monotherapy significantly increased PFS in advanced breast cancer with germline BRCA1/2 mutations, including TNBC. |
| | | NCT02000622 | Phase III, Olaparib as monotherapy significantly increased PFS and reduced the risk of progression and death in metastatic breast cancer with germline BRCA1/2 mutations, including TNBC. |
| | | NCT02032823 | Phase III, Olaparib in patients with germline BRCA-associated TNBC or high-risk hormone-positive breast cancer. |
| | | NCT02401347 | Phase III, Talazoparib (BMN 673) monotherapy in BRCA1/2 wild-type advanced TNBC with homologous recombination deficiency or germline/somatic mutation in HR pathway genes. |
| | | NCT03330847 | Phase II, Olaparib+AZD6738+AZD1775, targeted PARPin advanced or metastatic TNBC. |
| | | NCT03801369 | Phase II, Olaparib +Durvalumab targeted PARP and PD-L1 in advanced or metastatic TNBC. |
| 2 | EGFR pathway inhibitors | NCT01036087 | Panitumumab + Nab-paclitaxel and carboplatin demonstrated the highest reported pCR rates in inflammatory TNBC in phase II. |
| 3 | PI3K/AKT/mTOR/ PTEN pathway inhibitors | NCT01629615 | Phase II, BKM120 as monotherapy in metastatic TNBC. |
| | | NCT02531932 | Phase II, Everolimus + Carboplatin in advanced TNBC. |
| | | NCT02162719 | Phase II, Ipatasertib + Paclitaxel, increased PFS and OS of patients with metastatic TNBC compared with the paclitaxel alone. |
| | | NCT02457910 | Phase I/II, Enzalutamide + Taselisib (GDC-0032, PI3K inhibitor) in patients with AR+ metastatic TNBC. |
| | | NCT02301988 | Phase II, Preoperative GDC-0068 + Paclitaxel in women with stage I–III TNBC. |
| | | NCT02423603 | Phase II, AZD5363 + Paclitaxel, compared with the Paclitaxel alone, prolonged the PFS and OS of patients with metastatic TNBC. |
| | | NCT03337724 | Phase II/III. A study of Ipatasertib +Paclitaxel as a treatment for participants with PIK3CA/AKT1/PTEN-altered, locally advanced or metastatic TNBC or hormone receptor-positive, HER2-negative breast cancer. |
| | | NCT03997123 (CapItello290) | Phase III, Capivasertib(AKT inhibitor) +Paclitaxel in advanced or metastatic TNBC. |
| | | NCT03961698 (MARIO-3) | Phase II, IPI-549+Atezolizumab+Bevacizumab+Nab-paclitaxel in advanced or metastatic TNBC. |
| | | NCT02576444 (OLAPCO) | Phase II, Capivasertib + Ceralasertib +Adavosertib +Olaparibin advanced or metastatic TNBC. |
| | | NCT02162719 | Phase II, Ipatasertib (AKT inhibitor)+ Paclitaxel in locally advanced or metastatic TNBC. |
| | | NCT01042379 | Phase II, MK2206 (AKT inhibitor) in neoadjuvant stage II–III breast cancer (any subtype). |

**Table1.** Selected phase II or III clinical trials in patients with TNBC from 2015 to 2022* (continued)

| Entry | Promising therapy | Clinical trials gov identifier | Clinical studies |
|---|---|---|---|
| 4 | RAS/MAPK/ERK | NCT03394027 | Phase II, ONC 201 (ERK/AKT inhibitor) in advanced or metastatic TNBC. |
| | | NCT02322814 | Phase II, Cobimetinib + Paclitaxel (MEK1/2 inhibitor) in locally advanced or metastatic TNBC. |
| 5 | Anti-angiogenic therapy | NCT03348098 | Phase II, Apatinib + Paclitaxel as neoadjuvant treatment for locally advanced TNBC. |
| | | NCT01234337 | Phase III, Sorafenib + Capecitabine, failed to show clinical benefit compared with capecitabine alone in advanced or metastatic HER2-negative breast cancer, including TNBC. |
| 6 | Androgen targeted therapy | NCT03055312 | Phase III, Bicalutamide as a single agent compared with standard chemotherapy in metastatic AR+ TNBC. |
| | | NCT01889238 | Phase II, Enzalutamide as monotherapy was well tolerated and showed clinical benefit in patients with advanced AR+ pre-treated TNBC. |
| | | NCT02971761 | Phase II, Enobosarm + Pembrolizumab (AR, PD-1 inhibitor) in advanced or metastatic TNBC. |
| | | NCT03055312 | Phase II, Bicalutamide (AR inhibitor) in advanced or metastatic TNBC. |
| 7 | HDAC inhibitors | NCT01349959 | Phase II, Entinostat (HDAC inhibitor) + Azacitidine in advanced breast cancer. |
| 8 | Checkpoint inhibitors | NCT02425891 | Phase III, Atezolizumab(MPDL3280A) + Nab-paclitaxel prolonged both PFS and OS of patients with metastatic TNBC, especially in patients with PD-L1 expressing tumors. |
| | | NCT02926196 | Phase III, Avelumab as adjuvant treatment for high-risk TNBC. |
| | | NCT02555657 | Phase III, Study of Pembrolizumab vs. single-agent chemotherapy for metastatic TNBC. |
| | | NCT02648477 | Phase II, Pembrolizumab + Doxorubicin in metastatic TNBC. |
| | | NCT02499367 | Phase II, Nivolumab + various chemotherapy drugs in advanced TNBC. |
| | | NCT03125902 | Phase III, A study of Atezolizumab (MPDL3280A, an engineered anti–PD-L1 antibody)+ Paclitaxel in participants with previously untreated locally advanced or metastatic TNBC. |
| | | NCT03197935 | Phase III, A study to investigate Atezolizumab (MPDL3280A)+ chemotherapy compared with placebo and chemotherapy in the early stage TNBC. |
| | | NCT02819518 | Phase III, Study of Pembrolizumab (MK-3475) + chemotherapy vs. placebo plus chemotherapy for previously untreated locally recurrent inoperable or metastatic TNBC. |
| | | NCT04085276 | Phase III, Toripalimab (PD-1 inhibitor) + Nab-paclitaxel in advanced or metastatic TNBC. |
| | | NCT04129996 | Phase II, Camrelizumab + Famitinib+ Carboplatin (PD-1inhibitor) in advanced or metastatic TNBC. |

**Table1.** Selected phase II or III clinical trials in patients with TNBC from 2015 to 2022* (continued)

| Entry | Promising therapy | Clinical trials gov identifier | Clinical studies |
|---|---|---|---|
| | | NCT03971409 | Phase II, PF-04518600+Avelumab+Binimetinib+Utomilumab (Anti-OX-40, PD-L1, MEK, 4-1BB/CD137) in advanced or metastatic TNBC. |
| | | NCT03789110 | Phase II, Nivolumab + Ipilimumab (PD-1/CTLA-4inhibitor) in advanced or metastatic TNBC. |
| 9 | JAK/STAT pathway | NCT02876302 | Phase II, Ruxolitinib+ standard neoadjuvant chemotherapy in triple-negative inflammatory breast cancer. |
| 10 | CXCR1/2 (stem cell pathway) | NCT02370238 | Phase II, Double-blind study of Paclitaxel + Reparixin or placebo for metastatic TNBC. |
| 11 | Antibody-drug conjugate | NCT01997333 | Phase II, Study of Glembatumumab + Vedotin (CDX-011) in patients with metastatic, gpNMB-overexpressing TNBC. |
| | | NCT02574455 | Phase III, Sacituzumab+ Govitecan + chemotherapy in advanced or metastatic TNBC. |

TNBC: Triple-negative breast cancer; PARP: Poly adenosine diphosphate-ribose polymerase; PFS: Progression-free survival (months); BRCA1,2: Breast cancer gene1,2; HR: Hazard ratio; PDL1: Programmed cell death ligand 1; EGFR: Epidermal growth factor receptor; PI3K: Phosphatidylinositol 3-kinase; mTOR: Mechanistic target of rapamycin; OS: Overall survival; MAPK: Mitogen-activated protein kinase; ERKs: Extracellular signal-regulated kinases; HER-2: Human epidermal growth factor receptor 2; AR: Androgen receptor; HDACs: Histone deacetylases; pCR: Pathological complete response; PTEN: Phosphatase and tensin homolog; CTLA4: Cytotoxic T-lymphocyte associated protein 4; JAKs: Janus kinases; STATs: Signal transducer and activator of transcription proteins ; CXCR1,2: chemokine receptors1,2
*By searching the trial identifier number in the US National Institutes of Health Registry, details of the presented trials can be obtained (https://clinicaltrials.gov/).

has poor clinical outcomes and can be a target for TNBC therapy.[43] Other lnc-RNAs involved in TNBC include SChLAP1, MALAT1, LINK-A, HOTAIR, and LincRNA.[44, 45]

Covalently closed circular RNAs (circRNAs) are novel non-coding RNA (ncRNAs). These RNAs belong to a class with tissue/developmental stage-specificity, making circRNA a potential novel biomarker for cancer prognosis and diagnosis. Because they have no free 5' and 3' ends, circRNAs show more stability than traditional linear RNAs, facilitating circRNAs to exert their regulatory function.[46] CircRNAs can modulate miRNA activities and regulate gene expression by sequestering specific miRNAs. In this regard, Circ_0044234 is one of the most down-regulated circRNAs in TNBC and cell lines versus non-triple-negative ones and could act as an upstream sponge in the miR-135b/GATA3 axis.[47] The overexpression ofcircPSMA1 facilitates TNBC cell migration, tumorigenesis, metastasis, and proliferation in vivo and in vitro through the miR-637/Akt1/β-catenin (cyclin D1) axis.[48]

## Metabolomics Biomarkers

Metabolic reprogramming is a hallmark of cancer.[49] In cancer cells, metabolism is dysregulated to support the demands of uncontrolled proliferation.[50] Hence, understanding the metabolic adaptations dependences of cancer cells may provide new strategies for cancer diagnosis, therapy, and monitoring.[51] Metabolomic and lipidomic studies in 330 TNBC samples described the heterogeneity and metabolomic landscape of TNBCs. They classified them into three metabolomic subgroups, including C1, C2, and C3.[52] The C1 subgroup had the highest levels of ceramides and fatty acids; the LAR subtype overlapped with the metabolomic C1 subtype. The experiments indicated that targeting sphingosine-1-phosphate (S1P), an intermediate of the ceramide pathway, was a promising therapy for LAR tumors.[52] The C2 subgroup was differentiated with the upregulation of metabolites related to glycosyl transfer and oxidation reaction, while the C3 subgroup of TNBCs showed the lowest level of metabolic dysregulation. The IM, basal-like immunosuppressed (BLIS), and mesenchymal-like subtypes were mainly divided into metabolomic C2 and C3 subgroups.[52] Additionally, N-acetyl-aspartyl-glutamate (NAAG) was found to be a crucial tumor-promoting metabolite in BLIS. These data

suggested the subtype-specific metabolomic therapeutic targets for the LAR (e.g., S1P targeting) and BLIS (e.g., NAAG targeting) subtypes of TNBCs. The comparison of TNBC with triple-positive breast cancer (TPBC) using high-resolution magic angle spinning magnetic resonance spectroscopy (HR MAS MRS) methods indicated that Choline, a metabolite involved in oncogenic signaling and cell proliferation, was higher in TNBC.[53] In addition, TNBC tumors contained a higher level of glutamate and a lower level of glutamine compared with the TPBC tumors, which indicated an increase in glutaminolysis metabolism.[53]

TNBCs had increased levels of several arginine metabolites. This feature may suggest a substantial increase in proinflammatory signaling via arginine metabolism and nitric oxide production for other biofunctional needs.[54] Furthermore, an increase in extracellular matrix metabolic breakdown products (e.g., proline) in TNBC samples may reflect more excellent tissue remodeling in aggressive/advanced types of these tumors.[54]

Additionally, fatty acid metabolites, including arachidonic acid, docosahexaenoic acid, and gamma-linolenic acid, were elevated in TNBC vs. control cell lines. In contrast, branched-chain amino acids (leucine and valine) and one aromatic amino acid (tryptophan) showed decreased levels.[51] Yu Song et al. showed that a higher branched-chain amino acid transferase 1 (BCAT1) expression indicated shorter DFS and OS. They concluded that BCAT1 can be considered a prognostic biomarker in TNBC patients.[55] TNBC tumors have metabolic pathways that distinguish them from ER-positive tumors. Therefore, distinctive metabolic characteristics of these tumors may offer new targets for TNBC.

Despite the number of clinical trials conducted in the metabolomic field in various cancers, few trials have been conducted regarding TNBC.

## Other Promising Biomarkers

### *Inositol 1,4,5-trisphosphate 3-kinase (ITPKC) gene*

ITPKC is an isoenzyme of Inositol 1,4,5-trisphosphate 3-kinase that phosphorylates Inositol 1,4,5-trisphosphate (IP3) (a critical secondary messenger in many cell types), regulates the immune response. For example, it is the negative regulator of T-cell activation.[56] TNBCs with a lower expression of ITPKC have higher PCR rates after neoadjuvant chemotherapy (NAC). Lower activity of ITPKC is associated with higher cancer immunity, resulting in a better response to NAC and survival in TNBC.[57] To understand which cells express ITPKC in BC, their expressions were measured in B-cells, myeloid cells, tumor cells, stromal cells, and T-cells in single-cell tuberculosis sequencing data. It was expressed in BC cells more than in the stromal or immune cells.[58] An increased expression of ITPKC was significantly associated with the reduction of disease-specific survival (DSS), OS, and DFS in TNBC ($P < 0.001$).[58]

### *CD73*

CD73 is an ectoenzyme that is expressed on the surface of the stromal, tumor, and immune cells and induces tumor escape through the extracellular production of adenosine, an immunosuppressive metabolite, in the tumor microenvironment.[59, 60] In one study, 122 FFPE samples from primary TNBC patients were included in the phase III trial study. This study evaluated the expression of CD73 protein on tumor cells, tumor-infiltrated leukocytes, and stromal cells using image analysis and multiplex immunofluorescence. The results showed that high levels of CD73 expression on tumor epithelial cells were significantly associated with decreased OS DFS and were negatively associated with tumor-infiltrated immune cells.[61] Additionally, patients with high levels of CD73 and low levels of tumor-infiltrated leukocytes had a worse clinical outcome.[61] In another study, microarray data showed that CD73 mRNA expression levels in immune and epithelial cells were higher than in stromal cells. These features were more common in the lobular form and individuals with severe LN involvement. CD73 expression was associated with OS and increased anthracycline resistance in the TNBC subtype but was not significantly associated with tumor age and menstrual status. These studies show CD73 expression is associated

with poor prognosis and reduced antitumor immunity in human TNBC. Therefore, targeting CD73 could be a promising strategy for reprogramming the tumor microenvironment in the TNBC subtype.[59]

*Monocyte chemoattractant protein-1 (MCP-1)*

An in-vivo study indicated that increased expression and secretion of MCP-1 from TNBC cells induce macrophage invasion into the tumor environment.[62] It has been shown that basal and claudin-low cell lines had high expression of MCP-1. MCP-1 is regulated in TNBC and can be used as a diagnostic-prognostic marker for TNBC cells. MCP-1 cooperates with RANTEZ to induce angiogenesis in BC patients. Therefore, anti-MCP-1Ab (ABN912) might be a promising strategy for treating TNBC.[63]

*Leukotriene B4 receptor 2 (BLT2)*

Leukotriene B4 (LTB4) is made from arachidonic acid via the 5-lipoxygenase pathway and performs its function through GPCRS (LTB4R1,2). BLT2 is a low-affinity receiver for LTB4. Recent studies show that LTB4 is closely associated with various aspects of invasion, metastasis, and survival in TNBC.[64] One of TNBC's development mechanisms is reactive oxygen species (ROS) production. ROS is tumorigenic, and its ability to increase survival, cell proliferation, and induce DNA damage leads to genetic damage and causes tumor cell formation and subsequent tumor progression. ROS regulation plays a vital role in the invasive phenotype of cancer.[65] In TNBC, ROS is produced by the tumor from the NOX complex. BLT2 also regulates ICAM1 expression in MDA-MB-231 cells via the BLT2-ERK-NFkb cascade. Subsequently, ICAM1 promotes tumor progression and shortening of recurrence-free survival in TNBC. The BLT2-NOX1 pathway also regulates the NFKB signaling cascade in TNBC. This pathway is involved in TNBC invasion, producing proinflammatory cytokines IL-6 IL-8 and subsequent invasion and metastasis of TNBC cells by increasing the expression of these two cytokines. It was found that in patients with metastatic BC (n=545), those with higher BLT2 had lower DFS than in the other subgroups.[66]

*Cyclooxygenase-2 (COX2)*

The enzyme is a converter of arachidonic acid and prostaglandins, which is usually present in minimal amounts in most tissues in the nuclear membrane of the endothelium and reticulum and is highly inducible as the tumor progresses.[67] Negative hormone receptors with $COX2^{+}$ expression indicate a poor prognosis; its expression is associated with HER2 expression and MDR1. Patients with COX2, HER2, and MDR1 have the slightest response to chemotherapy.[68] In ER-negative breast tumors, COX2 expression is engaged in neo-angiogenesis. Therefore, its expression is considered an adverse prognostic factor.[69] In TNBC patients, COX2 expression is inversely related to capsular effraction; therefore, it is considered a favorable prognostic factor.[67] Balaji Krishnamachary et al. identified significantly higher collagen 1 (Col1) fiber density with increased cancer-associated fibroblasts (CAFs) in COX-2 over-expressing tumors derived from triple-negative SUM-149 BC cells. These higher fiber content may have contributed to the altered extracellular matrix (ECM).[70] In non-metastatic TNBC patients, using COX2 inhibitors (Celecoxib) as maintenance therapy may be associated with better DFS. So, targeting COX2 may have a role in this aggressive disease.[71]

*Nectin4 receptors (PVRL4)*

Nectin4 receptors (PVRL4) is a cell surface protein expressed explicitly in TNBC malignant cells and not present in normal breast tissue. Thus, it is a desirable surface biomarker.[72] Nektin4 adhesive molecule is a new therapeutic target in various cancers. The expression of nectin4 mRNA and its association with clinicopathological findings, including metastasis-free survival (MFS) in 5673 BC samples, were analyzed.[73] Increased mRNA expression, especially in TN and basal subtypes, has an independent adverse prognostic role for MFS in TNBC. PVRL4 was strongly expressed in 61% of TNs and 62% of basal specimens compared with 47% of non-TN and non-basal specimens. In the whole population, high expression of PVRL4 was associated with shorter MFS. Prognostic variable analysis in molecular subtypes showed that high expression

of PVRL4 was associated with a decrease in 5-year MFS in the TN subtype (47% in high PVRL4 vs. 62% in low PVRL4 groups, respectively).[73] Taken together, PVRL4/nectin4 expression might independently predict shorter MFS in TNBC.[73] Jasmin Zeindler et al. showed that high expression of nectin-4 was associated with a lower tumor stage, significantly better OS, and pN0 lymph node stage compared with a low expression of nectin4. Thus, regarding the role of nectin4 expression as a potential target in TNBC, its role in molecular-defined BC subtypes should be investigated in larger patient cohorts.[74]

*RNA binding motif protein 3 (RBM3) and non-neuronal SNAP25-like protein homolog 1 (NIPSNAP1)*

RBM3 is a cold shock protein family member that regulates mRNA metabolism and has pleiotropic effects on cellular stress oncogenesis. It rarely increases in normal tissue but in some solid tumors.[75] High levels of RBM3 are independent prognostic factors for DFS and OS in breast, gastric, colon, prostate, and melanoma cancers.[76] In TNBC models, RBM3 was necessary for maintaining the mesenchymal phenotype and migration and invasion in vitro. Loss of RBMS3 significantly impaired both spontaneous metastasis and tumor progression in vivo.[77] NIPSNAP1 is a protein commonly expressed in the CNS, liver, and kidneys, and its association with cancer is unknown.[75] A proteomics study in 136 samples of TNBC indicated that RBM3 was associated with a low risk of relapse and NIPSNAP1 with a high-risk resulting in a worse prognosis.[78] In a Pilar Zamora Aunon et al. study, 1.206 proteins were identified in a cohort of 125 TNBC tumors using high-throughput proteomics based on SWATH-MS. Of these 1,206 proteins, 29 were related to DFS. In addition, multivariate analysis was used to predict signature based on the expression of two proteins (RBM3 and NIPSNAP1). These two proteins supplied significant information to the clinical parameters.[79]

*Maternal embryonic leucine zipper kinase (MELK)*

MELK was previously considered an essential oncogenic kinase for proliferation in the basal BC.[80, 81] MELK is vital in the mitosis of glioma stem cells and protects stem cells from radiation-induced death.[82] Corey Speers et al. showed that MELK was overexpressed in TNBC.[83] This group hypothesized that MELK might affect TNBC sensitivity to radiation. A previous study showed that knocking down SiRNA MELK delayed the repair of two broken strands of ionizing radiation and reduced TNBC cell growth in xenograft mice. In addition, combining radiation with knocking down MELK inhibited tumor growth in vivo.[84] It has been reported that MELK could be used as a robust biomarker of local recurrence risk in the early stages.[85] However, the safety and efficacy of MELK inhibitors should be investigated in clinical trials.

*Insulin-like growth factor binding proteins (IGFBPS)*

IGFs are secreted by cancer cells and adipocytes, increasing the risk of BC metastasis. IGFBPS are a family of 6 receptors (IGFBP1-6) that enhance tumorigenesis by binding to IGF and subsequently increasing the half-life of IGF and sequestering it.[86] African-American women have a higher prevalence of obesity and risk of TNBC than Caucasians, which may be due to the participation of the IGF/IGFBP pathway in TNBC.[87] Preclinical evidence suggests that IGFBP2 and other biomarkers could be a potential prognostic and RFS predictor in TNBC.[88] Hernandez et al. (2016) examined the relationship between IGF and IGFBP expression and survival rates in Asia, the Pacific, and Caucasus patients. The results showed that elevated IGFBP2 expression was associated with decreased survival rates in TNBC, and elevated IGFBP2 expression varied among different ethnicities.[89]

*Aldehyde dehydrogenase 1 (ALDH1)*

ALDH1, an enzyme that catalyzes the oxidation of aldehydes to inactive species of carboxylic acid, is present in several BCs, and its expression was significantly associated with tumor metastasis and increased resistance to taxan and epirubicin-based chemotherapy. Therefore, ALDH1 may be considered a specific TNBC biomarker.[87] Ohi et al. conducted a study on 107 patients with the basal phenotype (EGFR and

cytokeratin 5/6 positive) of TNBC, and the results showed that RFS was lower in ALDH1$^+$ tumors.[90] In another study of some African patients with invasive breast tumors, a significant association was found between the prevalence of ALDH1 and AR expression in TNBC. TNBC AR$^+$ cell lines are more sensitive to AR antagonists, so ALDH1 is a potential predictive biomarker for AR-targeted therapy and prognosis in TNBC.[91]

## Conclusion

TNBC represents one of the most formidable challenges in oncology. However, recent advancements offer new hope for patients afflicted with this aggressive disease. Numerous biomarkers have been identified that hold promise for improving the diagnosis and treatment of BC. Clinical and preclinical studies indicate that immune checkpoint inhibitors significantly enhance treatment outcomes for patients with PD-1/PD-L1-positive TNBC. Additionally, PARP inhibitors have demonstrated considerable potential in patients with BRCA1/2 mutations. The targeting of VEGF/VEGFR to inhibit angiogenesis alone has shown promising efficacy. Furthermore, integrating epigenetic modifications with other therapies, such as chemotherapy or immunotherapy, mainly using epigenetic drugs like HDAC inhibitors, has proven highly effective. CDK4/6 inhibitors also offer enhanced benefits when used with other targeted treatments.

Continued clinical trials are essential to substantiate the clinical utility of metabolomic biomarkers in TNBC. Each targeted therapy presents its own set of benefits and limitations when used independently; therefore, combining various targeted treatments may provide a more effective therapeutic strategy, improving outcomes for a broader patient population and predicting responses to immunotherapy. Moreover, deeper investigations are needed to clarify the functional roles of other biomarkers discussed in this review.

In conclusion, while promising, the potential of unique and practical biomarkers for routine clinical application in the detection, management, and treatment of TNBC remains underexplored. Technological advances such as microarrays and high-throughput sequencing are poised to uncover more reliable biomarkers for survival, diagnosis, and prognosis, paving the way for more personalized and effective treatment strategies.

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## Authors' Contribution

Abbas Ghaderi.; Contributed to the conception and study design. Zahra Roshanizadeh; Contributed to review the articles, the data interpretation, and design the pictures and tables. Abbas Ghaderi, and Mohammad Reza Haghshenas; Supervised the project. Zahra Roshanizadeh Drafted the manuscript, and then it was revised by Mohammad Reza Haghshenas, and Abbas Ghaderi. All authors read and approved the final manuscript.

## Conflict of Interest

None declared.

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