Int J Cancer Manag. 2018 December; 11(12):e87024. doi: 10.5812/ijcm.87024.

Published online 2018 December 15. **Review Article**

Novel Approaches to Immunotherapy in Triple Negative Breast Cancer

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Received 2018 December 03; **Revised** 2018 December 08; **Accepted** 2018 December 08.

Abstract

Context: Triple negative breast cancers (TNBC) constitute about 15% of breast neoplasms. In contrast to estrogen receptor (ER) or human epidermal growth factor receptor (HER2) positive breast cancers, which respond to hormonal therapy (such as tamoxifen) or anti-HER2 therapy (such as trastuzumab), respectively, the main standard therapy in either early or late stage TNBC is chemotherapy. Therefore, it is necessary to find new treatment modalities for TNBC patients. We searched the literature to find published studies on immunotherapy in triple negative breast cancer and the putative biomarkers of response to these treatments.

Evidence Acquisition: We searched PubMed, Scopus, and Web of Science Core Collection with these keywords: "Triple negative breast cancer, Immunotherapy, Resistance, Response, Programmed cell death 1 receptor, CTLA-4, Tumor mutation burden, and Immune signature".

Results: TNBC is considered a heterogeneous neoplasm with regard to molecular aberrations. Analysis of genomic expression profile of TNBC has delineated 4 subtypes. TNBC tumors show high genetic instability. Tumor infiltrating lymphocytes (TILs) are detected more in TNBCs, compared to other breast cancer types. It has been shown that the amount of CD8 positive T cells in TNBCs is an independent predictor of overall survival. Up to now, two immunotherapy strategies have been used in clinical trials of TNBC, including immune checkpoint blockers and therapeutic cancer vaccines. Tumor programmed cell death ligand 1 (PDL1) expression is the most widely used immunotherapy biomarker. Tumor mutation burden (TMB) can be a promising biomarker of response to immunotherapy. The more somatic mutations a cancer cell has, the more neoantigens it probably produces. Analysis of TMB can give an estimate of tumor mutation load. Increased somatic mutation load has also been observed in tumors with impaired mismatch repair (MMR).

Conclusions: As TNBC is regarded a heterogeneous disease, the discovery of biomarkers of response to immunotherapy will increase the likelihood of response to these therapies. Further in-depth investigations are needed to find novel biomarkers of response to these immunotherapies for the better management of patients with TNBC.

Keywords: Immunotherapy, Triple Negative Breast Cancer, Immune Signature, Biomarker

1. Context

Breast cancer is the most prevalent cancer among women in the world (1). Triple negative breast cancers (TNBC), which constitute about 15% of breast neoplasms, do not express estrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor receptor (HER2) (2, 3). TNBC is considered the most fatal type of breast cancer and patients diagnosed with TNBC have worse clinical outcome in comparison to other types (2, 4-6). In contrast to ER or HER2 positive breast cancers which respond to hormonal therapy (such as tamoxifen) or anti-HER2 therapy (such as trastuzumab), respectively, the main standard therapy in either early or late stage TNBC is chemotherapy (7, 8). In addition, response to chemotherapy is usually short-lived and treatment of these patients is considered challenging (9). Therefore, it is necessary to find new treatment modalities for TNBC patients.

In recent years, cancer immunotherapy has developed considerably, including the use of checkpoint blockers such as antibodies against programmed cell death protein 1 (PD1) or cytotoxic T lymphocyte associated protein 4 (CTLA4), therapeutic cancer vaccines and adoptive cell transfer using chimeric antigen receptor (CAR) T-cell therapy (10). Multiple trials are analyzing the efficacy of immunotherapy in breast cancer. We searched the literature to find relevant published studies on triple negative breast cancer and the putative biomarkers of response to these treatments.

2. Evidence Acquisition

We searched PubMed, Scopus, and Web of Science Core Collection with these keywords: "Triple negative

breast cancer, Immunotherapy, Resistance, Response, Programmed cell death 1 receptor, CTLA-4, Tumor mutation burden, and Immune signature". We also examined the references of the selected articles.

3. Results

3.1. Molecular Biology of TNBC

TNBC is considered a heterogeneous neoplasm with regard to molecular aberrations. Analysis of genomic expression profile of TNBC has delineated 4 subtypes, including: Basal like 1 (BL1), Basal like 2 (BL2), Luminal androgen receptor (LAR), and Mesenchymal (M) (11, 12). BL1 constitutes about 35% of TNBCs and express cell cycle and DNA repair genes. BL2 constitutes 22% of TNBCs and express genes involved in the signal transduction of growth factors (epidermal growth factor (EGF), insulin-like growth factor 1 (IGF1), Wnt/β-catenin), growth factor receptors (epidermal growth factor receptor (EGFR), MET, insulin-like growth factor 1 receptor (IGF1R), EPH receptor A2 (EPHA2)), glycolysis and gluconeogenesis pathways. LAR constitutes 16% of TNBC and expresses androgen receptor and luminal genes. M constitutes 25% of TNBC and expresses genes involved in epithelial mesenchymal transition (EMT), cellular movement and differentiation, cancer stem cell regulation and growth factor signal transduction. This vast amount of heterogeneity in TNBC neoplasms necessitates personalization of treatment of TNBC based on novel discoveries in precision oncology.

About 10% to 20% of patients with TNBC carry germline mutations in *BRCA1* gene. In addition, in patients who are negative for germline *BRCA1* mutation, somatic mutations in homologous recombination pathway can create a similar phenotype named "BRCAness" (13). Increased response rate to genotoxic treatments such as platinium-based agents like carboplatin and cisplatin, have been detected both in carriers of *BRCA1* mutations and in patients with tumors showing BRCAness phenotype (14).

TNBC tumors show high genetic instability, with median mutation number of 1.7 in 1 million bases (range: 0.16 - 5.23) (15, 16). In addition, complex copy number alteration (CNAs) and structural rearrangement have been detected in TNBCs (17). There is a vast amount of variation in the genes mutated in TNBCs. Although some TNBCs have limited somatic mutations, in most TNBC neoplasms, a high rate of mutations have been detected in genes involved in signal transduction pathways (18). The most frequent mutated genes in TNBCs are *TP53* and *PIK3CA* which are mutated in 82% and 10% of these tumors, respectively (19). However, in contrast to ER positive breast cancers, somatic mutations in TP53 in TNBCs are mostly nonsense single nucleotide variants and indels (18, 19). Somatic mutations in other known cancer driver genes, including *PTEN*, *RB1*, *NF1*, *BRCA1*, *BRCA2*, *ERBB3*, *ERBB4*, and *ALK*, have also been detected in TNBC neoplasms (17).

3.2. Immune Signature in TNBC

Pathologic evaluation of breast tumors have shown the diversity and clinical significance of leukocytic infiltration in different breast neoplasms (20, 21). Tumor infiltrating lymphocytes (TILs) are detected more in TNBCs, compared to other breast cancer types. It has been shown that the amount of CD8 positive T cells in TNBCs, when completely infiltrated within tumoral tissue, not just detected in stroma around the tumor, is an independent predictor of overall survival in multivariate analysis (20, 22, 23). Infiltration of follicular CD4 positive helper T cells, which probably shows the existence of structured tertiary lymphoid structures (TLS) within tumoral tissue, is also a predictor of better overall survival in TNBC (24, 25). Detection of regulatory T cells (Tregs) is considered a negative predictor of survival in TNBC (26).

Several studies have shown the association between increased expression of genes involved in immune response pathways and decreased risk of breast cancer recurrence (27-30). There is association between tumoral immune signature and metastasis risk in breast cancer. Tumors with increased expression of B cell/plasma cell, T cell and natural killer cell, monocyte and dendritic cell associated genes show lower metastasis risk, while tumors with decreased expression of any immune cell group show higher metastasis risk (31).

3.3. Immunotherapy in TNBC

The main concept of immunotherapy is to stimulate the immune system against the tumor in order to enhance its recognition and destruction by immune cells. Up to now, 2 immunotherapy strategies have been used in completed or ongoing clinical trials in TNBC, including immune checkpoint blockers and therapeutic cancer vaccines, which will be described briefly (Table 1).

3.3.1. Immune Checkpoint Blockers

Monoclonal antibodies against suppressive immunoregulatory mechanisms of response to tumor-associated antigens has been approved for various cancers, including melanoma, Non-small cell lung cancer, head and neck cancer, bladder cancer, lymphoma, and colorectal cancer (32). The most widely used targets are PD1, PDL1, and CTLA4.

During T cell activation, T cell receptor (TCR) binds with antigen presented by major histocompatibility antigen (MHC), but additional costimulatory signals are necessary. B7-1 (CD80) and B7-2 (CD86) on the antigen presenting cell (APC) bind with CD28 on the T cell, which leads to T cell proliferation and differentiation through production of cytokines like interleukin 2 (IL2). CTLA4 is a homolog of

Table 1. Samples of Immunotherapy Clinical Trials in TNBC

Agent	Clinical Trial Id	Phase	Recruitment Status
Immune checkpoint blockers			
Tremelimumab (CTLA4 antibody)	NCT02527434	II	Active, not recruiting
Combination of Durvalumab (PDL1 antibody) and Tremelimumab (CTLA4 antibody)	NCT02658214	Ib	Recruiting
Pembrolizumab (PD1 antibody)	NCT01848834	Ib	Active, not recruiting
Pembrolizumab (PD1 antibody)	NCT02447003	II	Active, not recruiting
Pembrolizumab (PD1 antibody)	NCT02555657	III	Active, not recruiting
Nivolumab (PD1 antibody)	NCT02393794	I/II	Recruiting
Combination of Nivolumab (PD1 antibody) and Cabozantinib (Tyrosine kinase inhibitor)	NCT03316586	II	Recruiting
Atezolizumab (PDL1 antibody)	NCT03125902	III	Recruiting
Atezolizumab (PDL1 antibody)	NCT02425891	III	Active, not recruiting
Therapeutic cancer vaccines			
Personalized peptide vaccine (PPV)	UMIN000001844	II	Recruiting
Dendritic cell (DC) vaccine	NCT02018458	I/II	Unknown
MUC1 vaccine	NCT00986609	I	Completed
Folate receptor alpha peptide vaccine	NCT02593227	II	Active, not recruiting

CD28 with increased affinity to B7 (33). The relative amount of CD28:B7 versus CTLA4:B7 is determinant of alternative paths of T cell activation or anergy (34). Antibodies against CTLA4 provoke antitumor immunity through phosphorylation of Akt and inhibition of Foxp3+ Treg cells (35).

Tremelimumab is a fully human monoclonal antibody specific for CTLA4. A phase II, open label, multicenter clinical trial (NCT02527434) is going to study Tremelimumab monotherapy in patients with advanced solid tumors including triple negative breast cancers. This study will analyze the safety and efficacy of Tremelimumab in the treatment of different patients with advanced cancer. If patients develop disease progression on Tremelimumab, they will receive Durvalumab (PDL1 inhibitor) or Tremelimumab and Durvalumab combination (36). A phase Ib study (NCT02658214) will determine the tolerability and safety of Durvalumab and Tremelimumab combined with first-line chemotherapy in patients with advanced solid tumors (37).

PD1, which is a member of B7 family, binds to its ligands (PDL1 and PDL2) and inhibits T cell proliferation, and IL2, interferon γ (IFNγ) and tumor necrosis factor α (TNFα) production (38). Pembrolizumab is a humanized monoclonal antibody against PD1. KEYNOTE-012 trial (NCT01848834) investigated the safety and efficacy of pembrolizumab in advanced solid tumors (including a cohort of patients with triple negative breast cancer). This phase Ib trial studied 111 patients with TNBC, 58.6% of whom had PDL1 positive tumors (defined as PDL1 expression in stroma or in $\geq$ 1% of tumor cells). In 27 patients who were selected for treatment and subsequently were analyzed for anti-tumor activity, the overall response rate was 18.5% (39). A phase II study is examining the efficacy and safety of pembrolizumab monotherapy in patients with metastatic TNBC (KEYNOTE-086, NCT02447003) (40). KEYNOTE-119 (NCT02555657) is an ongoing phase III randomized trial which is analyzing patients with metastatic TNBC who has received single agent Pembrolizumab versus single agent chemotherapy (41).

Nivolumab is a fully human monoclonal antibody against PD1. A phase I/II non-randomized study is examining Cisplatin plus Romidepsin (histone deacetylase (HDCA) inhibitor) and Nivolumab in metastatic TNBC or *BRCA* mutation-associated locally recurrent or metastatic breast cancer (NCT02393794) (42). Another phase II study is analyzing combination of Nivolumab with Cabozantinib (a non-specific tyrosine kinase inhibitor) in metastatic TNBC (NCT03316586) (43). Atezolizumab is a fully humanized monoclonal antibody specific for PDL1. An ongoing phase III multicenter randomized, double bind, placebo controlled trial is studying Atezolizumab in combination with paclitaxel compared to placebo with paclitaxel in patients with inoperable locally advanced or metastatic TNBC (NCT03125902) (44). Another phase III multicenter randomized, double bind, placebo controlled trial is examining Atezolizumab in combination with Nab-paclitaxel compared to placebo with Nab-paclitaxel in patients with metastatic TNBC (NCT02425891) (45).

3.3.2. Therapeutic Cancer Vaccines

To date, several therapeutic cancer vaccination strategies have been used to treat TNBC. A phase II trial examined personalized peptide vaccination (PPV) in metastatic

TNBC. In this trial, vaccine antigens were selected from a pool of candidate peptides on the basis of pre-existing immunity. This PPV regimen boosted the immune response (both cytotoxic T lymphocyte (CTL) and IgG response) and resulted in possible clinical benefit (46). A phase I/II trial examined the safety of combination of preoperative chemotherapy with dendritic cell (DC) vaccination in patients with locally advanced TNBC (NCT02018458) (47). Preliminary results of this study showed that DC vaccination during preoperative chemotherapy is safe in TNBC patients (48).

A pilot study evaluated the efficacy of MUC1 peptide in boosting the immune response in stage I-III TNBC (NCT00986609). This early phase I study also analyzed the safety of MUC1 peptide-poly-ICLC adjuvant vaccine. (49). MUC1 is a member of the transmembrane mucin family, which are normally expressed on the gland-forming epithelial cells. Upon malignant transformation of these cells, hypoglycosylated mucins are produced which are recognizable by the immune system (50). The results of this study has not been published yet. A randomized multi-center phase II trial is currently examining the safety and immunogenicity of folate receptor alpha (FRα) peptide vaccine mixed with a vaccine adjuvant (granulocyte-macrophage colony stimulating factor (GM-CSF)) in stage IIb-III TNBC (NCT02593227) (51). It has been shown that 86% of TNBCs overexpress FRα (contrary to the limited expression in normal tissues) and its expression is associated with poor prognosis and increases the risk of recurrence (52, 53).

3.4. Potential Biomarkers for Immunotherapy

The ultimate goal of personalized cancer therapy is therapy selection based on individual patient characteristics. Given the high variability of breast cancers, it is important to find predictive biomarkers to select best treatment for the individual patient (54-57). Finding immunotherapy biomarkers will spare non-responding patients from ineffective expensive treatments and their adverse side effects. Tumor PDL1 expression is the most widely used immunotherapy biomarker. PDL1 is mostly expressed on dendritic cells and antigen presenting macrophages and binds to PD1 on activated T cells. Analysis of PDL1 expression by immunohistochemistry (IHC) is approved by the United States Food and Drug Administration (FDA) as companion diagnostic testing for pembrolizumab in several cancers (58, 59). However, multiple factors complicate the interpretation of PDL1 expression by IHC. Multiple PDL1 antibodies have been developed, but their comparative performance characteristics are not known. There is not a clear definition of "positive PDL1 staining", with cut-off points varying from > 1% to 50%. In addition, there are limited antibody binding sites on PDL1, because it only has two hydrophilic regions. Therefore, its immunohistochemical detection in formalin-fixed paraffin embedded (FFPE) samples is not much effective (60). PDL1 expression as a biomarker of immunotherapy response is imperfect and it is necessary to find improved biomarkers (61, 62).

Neoplastic transformation is due to the accumulation of somatic mutations in tumor cells. There is considerable variation in the frequency of somatic alteration between individual tumors (63-65). It seems that tumor mutation burden (TMB) can be a promising biomarker of response to immunotherapy (66, 67). Some of the somatic mutations can produce neoantigens, which are recognized by the immune system and trigger an immune response that destroys neoplastic cells, especially after therapies that lead to T cell activation (68, 69). It is important to note that not all somatic mutations produce new peptides presented on the surface of major histocompatibility complex (MHC) molecules and not all neopeptides presented are immunogenic (70, 71). However, the more somatic mutations a cancer cell has, the more neoantigens it probably produces. Analysis of TMB can give an estimate of tumor mutation load.

Increased somatic mutation load has also been observed in tumors with impaired mismatch repair (MMR). MMR system controls the integrity of the genome. MMR proteins repair single base mismatches (insertions or deletions) which are produced during DNA replication, thus maintains the stability of the genome (72). It has been shown that MMR deficient tumors respond to immunotherapy irrespective of histologic type or tumor anatomic location (73). Therefore, MMR status can be a potential biomarker of response to immunotherapy.

4. Conclusions

TNBC is considered the subtype of breast cancer that is most likely to benefit from immunotherapy. As TNBC is regarded a heterogeneous disease, the discovery of biomarkers of response to immunotherapy will increase the likelihood of response to these therapies. Further in-depth investigations are needed to find novel biomarkers of response to these immunotherapies for the better management of patients with TNBC.

Acknowledgments

None declared.

Footnotes

Authors' Contribution: All authors designed the study. Sanaz Tabarestani reviewed the literature and wrote the manuscript. All authors approved the final manuscript.

Conflict of Interests: None declared.

Financial Disclosure: None declared.

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