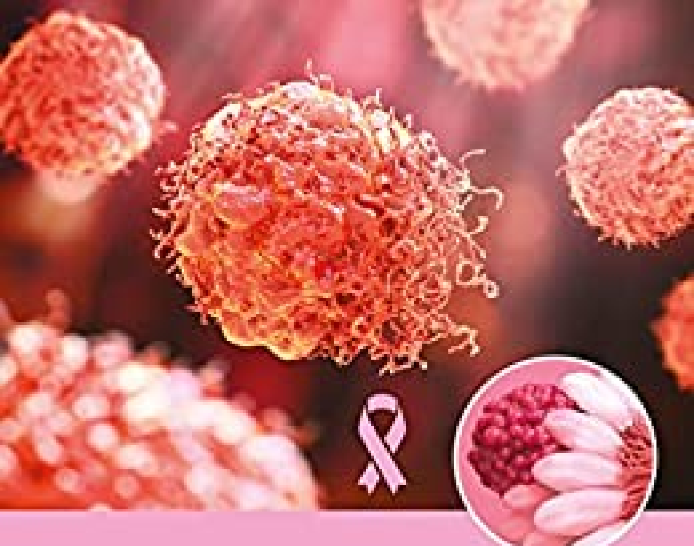

Combinational Therapy in Triple Negative Breast Cancer

Manzoor A. Mir

COMBINATIONAL THERAPY IN TRIPLE NEGATIVE BREAST CANCER

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Dr. Manzoor Ahmad Mir holds Master's Degree in Zoology from HNBG Central University and after qualifying prestigious National level CSIR-JRF-NET examination he worked jointly for his PhD at Jawaharlal Nehru University New Delhi and CSIR-Institute of Microbial Technology Chandigarh in the field of Immunology. His basic research interests include cancer biology, combination therapy in breast cancer, molecular immunology, and tuberculosis immunology. He has published more than 50 high-impact research papers and book chapters, in recognition of which he has received several awards and royalties from international publishing houses. Dr. Manzoor has authored more than 15 books with international publishers like Elsevier USA and Nova Science Publisher USA, Bentham Sciences, IGI Global, Springer-Nature. He is on the editorial board and reviewer of some prestigious journals like DOVE medical press, Springer Plus, Cancer Biomarkers, Frontiers, Plos, etc., and has been an invited speaker at various scientific meetings/conferences within India and abroad. He is member of many scientific organizations and societies like American Association of Cancer Research, Fellow of Royal British Society, International Immunology Association, Indian Cancer Society, Indian Immunology Society, Indian National Science Association, IMMUNOCON, etc. Dr. Manzoor was awarded Teachers Associate Research Excellence Fellowship (TARE) by DST Government of India. He has been awarded Summer Research Fellowship Programme (SRFP-2019) by Indian Academy of Sciences and National Science Academy. He was awarded a research project on combination therapy in breast cancer by J&K Science Technology and Innovation. Dr. Manzoor has developed massive open online course (MOOCs) in immunology and endocrinology for UG students sanctioned by UGC-Consortium for Educational Communication (CEC) SWAYAM Ministry of HRD Government of India. He has research and teaching experience of 15 years. He currently teaches cancer biology, and immunology at the Department of Bioresources, School of Biological Sciences, University of Kashmir. He is presently heading the Department of Bioresources, University of Kashmir.

Foreword

I am delighted to provide the foreword to Dr. Manzoor Ahmad Mir's' valuable book, Combinational Therapy in Triple Negative Breast Cancer. Cancer, in general, breast cancer, in particular, is a dreadful disease that causes physical and mental suffering to individuals who are affected. Despite huge investments in cancer treatment, the number of new cases and deaths continues to rise. Breast cancer is the second most prevalent cancer diagnosed in women after skin cancer. Breast cancer can strike both men and women, although it affects women significantly more frequently. TNBC is considered to be one of the most threatening types of breast cancer. Although TNBC accounts for around 15%–20% of all breast carcinoma occurrences, it is extremely metastatic, making it the most dangerous and having the worst prognosis when contrasted to other breast carcinomas.

Dr. Mir discusses critical problems about the occurrence, treatment, and prevention of triple-negative breast cancer. The book has particularly highlighted the conventional as well as the newly developed treatment approaches in breast cancer, particularly TNBC. In this regard the new innovative treatment method, especially the targeted therapies and the nanotechnology intervention approaches, has revolutionized the field of breast cancer. At present, various combination regimens are showing the positive results in TNBC patients, still the studies need to get more and more expanded and the advancements in the treatment approaches should come with the feasible innovative methods to have best out of best results in breast cancer patients, particularly TNBC.

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Preface

Breast cancer is the most widespread type of baleful neoplasms that develops in the breast tissue, mostly from the ductal epithelium and is the foremost trigger of cancer-induced death among women. More than 1 million new cases of breast cancer are reported per year. The heterogeneity and the complexity of breast cancer make it the most vulnerable. A report of 2018 reflects 18.1 million cases of cancer accounting for about 9.6 million deaths worldwide. Among this statistical count, breast cancer accounts for 2.1 million (11.6%) with 0.63 million (6.6%) deaths. Breast carcinoma is a disease with a high degree of heterogeneity. TNBC is considered to be one of the most threatening types of breast cancer. Although TNBC accounts for around 15%–20% of all breast carcinoma occurrences, it is extremely metastatic, making it the most dangerous and having the worst prognosis when contrasted to other breast carcinomas. TNBC is divided into six subtypes, each with its own molecular profile, prognosis, and likely treatment responses. Gene expression analysis shows that most TNBC possesses a basal-like molecular profile. Their pathological and clinical characteristics are similar to inherited BRCA1 breast tumors. According to epidemiological studies, TNBC is most common in premenopausal younger females below 40 years of age. Increased hip-to-waist ratios, higher parity, the shorter period of breast-feeding, young age at first-term pregnancy, and shorter period of breastfeeding have all been linked to an elevated frequency of triple-negative breast cancers among premenopausal African-American women. TNBC has specific imaging characteristics, commonly showing as a mass on mammograms and ultrasonography with generally benign characteristics and more alarming results on magnetic resonance imaging. Epigenetics is a promising area of inquiry in modern cancer research. Improvements in cancer therapy, detection, and prevention are feasible by researching the epigenetic processes driving tumorigenesis – DNA methylation, noncoding RNAs, and histone changes.

The worse OS, increased rate of recurrence, and increased occurrence of distant metastases are all characteristics associated with TNBC. Since TNBC is associated with worse outcomes and thus doesn't get benefit from hormonal therapy or therapies targeted to HER2. TNBC is a very aggressive cancer, and about 46% of TNBC females will develop distant metastases. TNBCs account for more than 80% of breast tumors in people who have the BRCA1 gene mutation.

The treatment options for BC may include radiotherapy, chemotherapy, immunotherapy, and targeted therapy. But at present, chemotherapy is the only treatment that has been approved for TNBC. Although TNBC represents the most aggressive type of BC, 20% of TNBC patients show a pathologic complete response (pCR) after being exposed to neoadjuvant chemotherapy. Chemotherapy is effective against TNBC, and it is still the standard of care (SOC). Anthracyclines (e.g., doxorubicin topoisomerase II inhibitor, and DNA intercalating agents), alkylating compounds (e.g., cyclophosphamide), an antimicrotubule drug taxane, as well as antimetabolite fluorouracil (5-FU) are all popular chemotherapeutics. In spite of the fact that chemotherapy is the better treatment option in TNBC as compared to the other forms of BC, it still shows a worse prognosis. The main reason for this is that the disease-free period between neoadjuvant and adjuvant therapy is less and a much-threatened course in the metastatic setting. CT in combination with other treatment options may prove beneficial for TNBC patients. At present there are various combinations of regimes that could benefit TNBC patients, still, various ongoing studies are in progress to find more effective regimes that may improve and lead to development in the treatment of TNBC.

Furthermore, the immune system is also getting an attention for treating the disease. The immune cells in this regard had played a significant role in regulating the protumorigenic or anti-tumorigenic functions of the immune system. Various immune cells in TNBC will impact the survival outcomes in TNBC individuals. The expression of immune-related checkpoints in TNBC decides the survival outcomes in TNBC individuals. There are various types of immune checkpoints that are associated with the TNBC subtype. Examples include LAG3, CTLA4, IDO1/2, PD-L1/2, TIGIT, and PD-1. Due to the expression of such types of immune checkpoints, the cancer cells in TNBC evade immunosurveillance. These immune checkpoints and other emerging immune-related molecules could be used in immunotherapy and thus the progression of the disease could be retarded in a much better way. Immune checkpoint inhibitors are the latest agents that show a vital role in modulating the immune system of a patient in such a way so that the significant destruction of the tumor cells takes place. One of the most targeting pathways that are to be blocked during immune checkpoint blockade is the PD-1/PDL-1 pathway. The immune checkpoint blockades also play their role in TNBC by being used with chemotherapy for advanced/metastatic TNBC or chemotherapy or RT as neoadjuvant/adjuvant therapy for early TNBC and also with other targeted drugs. With the advent of antigens that are exclusively displayed by TNBC cells and the advancements in monoclonal antibody technology, cancer vaccines, and chimeric antigen receptors, the field of immunotherapy has progressed a lot and is thus evolving as a new promising approach for TNBC. The field of immunotherapy has further succeeded with the concept of intertwining the field of chemotherapy, immunotherapy, and of course the targeted therapy.

With the advancements in the treatment field of TNBC, Various biological agents have been evaluated in this aspect. In view of this, targeted therapy has evaluated various biological molecules and signaling pathways have been targeted for having effective progress in the treatment of TNBC. A great focus has been grown in the recently developed targets for TNBC, for instance, the signaling pathways like hedgehog (Hh) pathway, notch signaling pathway, Wnt/-catenin pathway; the target molecules like (mTOR) inhibitors, EGFR inhibitors, PARP1 inhibitors, angiogenesis inhibitors, chondroitin sulfate proteoglycan 4 (CSPG4) protein targeted monoclonal antibody and TGF-inhibitors. Furthermore, various target agents are in experimental trials for assessing their therapeutic role in treating TNBC. With the advancement of nanotechnologies, Nano medicine is also developing in respect of precise and speedy diagnosis, as well as target-directed treatment in malignancies. Due to their target-specific multipurpose capabilities, nanoparticles are a crucial actor in most tumor research. Nanocarriers have recently become the focus for improved availability, tailored cellular absorption, and minimum cytotoxicity. Such smart nanovehicles are equipped with all of the required armaments (drugs, tracking probes, and ligands) and are intended to target specific TNBC cells on site. Nanosoldiers have extraordinary ability to eliminate TNBC cells due to their variety in terms of drug loading, material composition, and releasing process, capability to adjust in vivo drug distribution, multifunctional properties facilitating the identification, therapy, and monitoring, and so on.

Summing up the contention, we see that there needs to have much more evolving ways to increase the survival outcomes and to reduce the recurrence rates among TNBC patients.

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CHAPTER

1

Triple-negative breast cancer - an aggressive subtype of breast cancer

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Introduction

Cancer is among the leading causes of death around the world (Momenimovahed and Salehiniya, 2017). Malignant diseases claimed the lives of 8 million people in 2008, and this number is expected to rise to 11 million by 2030 (Benson and Jatoi, 2012). Breast carcinoma is the most frequent malignancy in females, and it is also one of the leading causes of mortality in women. BC is a multifaceted disease (Zendehdel et al., 2018), meaning that it is caused by a combination of causes. BC is defined as the uncontrollable development and multiplication of cells that begin in the breast tissue (Khuwaja and Abu-Rezq, 2004). There are two kinds of tissues in the breast: stromal (supporting) tissues and glandular tissues. Glandular tissues contain the milk-producing glands (lobules) and ducts (milk passageways), whereas stromal tissues contain the breast's fibrous and fatty connective tissues. Lymphatic tissue, an immune systems tissue that drains cellular fluids and debris, is also found in the breast (Sharma et al., 2010). There are a variety of malignancies that can grow in various locations of the breast. The majority of tumors in the breast are caused by benign (non-cancerous) alterations. For example, fibrocystic alteration is a non-cancerous disease in which females develop cysts (fluid-filled packets), fibrosis (scar-like connective tissue production), lumpiness, or thickening of areas, discomfort, or breast pain (Sharma et al., 2010; Mir et al., 2021). The cells that lining the ducts are where most breast tumors begin (ductal cancers). Some tumors develop in the cells that make up the lobules (lobular cancers), whereas others arise in adjacent tissue. BC is malignant cancer that can spread to other organs like the bone, brain, liver, and lung making it incurable (Mir et al., 2021). A favorable prognosis and a significant survival percentage are possible if the disease is detected early. Even though the malignancy is found all around the world, its occurrence, death, and survival rates varied significantly between regions, which may be related to a variety of factors including genetic factors,

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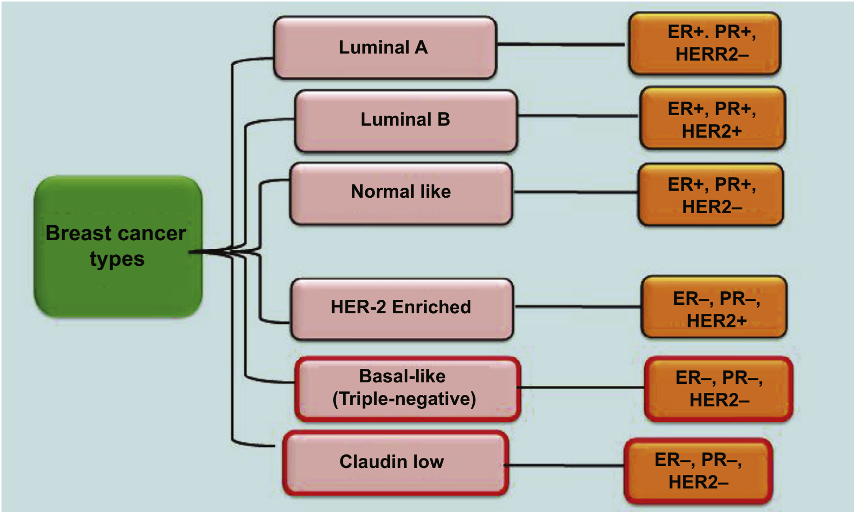

FIG 1.1 **Classification of breast cancer based on presence and absence of receptors.**

lifestyle, population structure, and environment (Hortobagyi et al., 2005). Modifications in risk factors have resulted in a rise in the incidence of BC, which continues to rise every day (Parkin and Fernández, 2006). While screening individuals for BC can lessen the impact of the disease, it has drawbacks such as adverse effects, overdiagnosis, and higher expenses. Mammography is a frequently utilized screening method for detecting BC that has been shown to substantially decrease mortality. Alternative screening modalities like MRI which is highly accurate than mammography have also been used and explored throughout the previous decade (Drukteinis et al., 2013). BC is currently divided into six molecular subtypes based on the progesterone receptor (PR), estrogen receptor (ER), and human epidermal growth factor receptor-2 (HER-2) expression (Fig. 1.1). The ER (+) and/or HER2 (+) subtypes are defined by the presence of ER/PR and/or HER2 expression, whereas triple-negative BC is defined by the lack of ER, PR, and HER2 expression. The other types include luminal A, luminal B, normal-like, Claudin-low, and basal-like (Perou et al., 2000). Targeted treatment is efficacious and commonly used to treat both ER (+) and HER2 (+) subtypes (Howlader et al., 2018). TNBCs, on the other hand, do not have targeted treatment and are generally treated with systemic chemotherapeutic medicines. Furthermore, TNBCs have more severe clinical signs (Howlader et al., 2018) and recur faster with greater frequency, making them the most malignant subtype of BC (Lin et al., 2012; Plasilova et al., 2016).

Triple-negative breast cancer (TNBC)

TNBC is defined by the absence of ER, PR, and HER2 receptor expression. Germline BRCA1 mutations (Wong-Brown et al., 2015), high mitotic numbers, and TP53 positive (Carey et al., 2010) are also features of the TNBC subtype. Most TNBCs are basal like (~70%) (Arnedos et al., 2012) exhibit basal-type cytokeratin 5 and cytokeratin 6, and also the EGFR in higher expression (Sørlie et al., 2001). The next most frequently altered gene in TNBC is

PIK3CA (10%) (Shah et al., 2012), however mutations in this gene are substantially more common in LAR TNBCs (46.2%) in comparison to other subtypes (average 4.5%) (Lehmann et al., 2014). Although TNBC accounts for around 15%–20% of all breast carcinoma occurrences, it is extremely metastatic, making it the most dangerous and having the worst prognosis when contrasted to other breast carcinomas. According to epidemiological studies, TNBC is most common in premenopausal younger females below 40 years of age (Morris et al., 2007; Mir et al., 2020). TNBC is a very aggressive cancer, and about 46% of TNBC females will develop distant metastases (Mir et al., 2021). After metastasis, the median survival duration is 13.3 months only, and the risk of recurrence following resection is as much as 25%. The brain and visceral organs are frequently involved in metastasis. The majority of distant metastases develop in the third year since diagnosis (Lin et al., 2008). In non-TNBC females, the average duration of recurrence is 35–67 months, whereas, in TNBC patient populations, the average duration of recurrence is only 19–40 months. TNBC females have a 75% death rate within three months of relapse (Gluz et al., 2009; Zhang et al., 2015). TNBC is resistant to hormonal therapy and molecular targeted therapy because of its unique molecular profile. As a result, chemotherapy is the primary systemic treatment, but traditional postoperative adjuvant chemo-radiotherapy is ineffective (Chaudhary et al., 2018).

TNBC histological classification

Most of TNBCs (95%) are histologically categorized as invasive breast carcinomas of no particular kind (or invasive ductal carcinomas) and lack distinguishing histological features; however other subtypes have also been found (Weigelt and Reis-Filho, 2009). The classically reported medullary carcinoma, which has been identified as a subtype within TNBC (Bertucci et al., 2006) by gene-expression analysis, is uncommon (0.4%–1%) and is marked by elevated lymphoplasmacytic infiltration and a favorable outcome when contrasted to other subtypes (Huober et al., 2012). However, the reliability of this histological definition has not been established, and it is uncertain whether better results may be gained by adjusting adjuvant treatment choices for individuals in this category. Other subgroups with distinct phenotypes, such as adenoid cystic carcinoma, adenosquamous carcinoma, and fibromatosis-like spindle-cell metaplastic carcinomas, are uncommon (1%), least aggressive, and generally only capable of local relapse, a factor to take into consideration when going to plan adjuvant therapy (Weigelt and Reis-Filho, 2009; Wetterskog et al., 2012). Adenoid cystic carcinoma is a genomically different subtype defined by a low incidence of copy-number abnormalities and a typical chromosomal translocation t(6;9) (q22–23; p23–24), that results in the MYB–NFIB fusion gene, that is found in 90% of instances of this TNBC type (Wetterskog et al., 2012).

TNBC molecular classification

Many research groups have made significant progress in understanding TNBC variation and linking gene expression profiles to molecular or genotypic subtypes. Lehmann and colleagues classified TNBC into six subgroups based on gene expression analysis of 587 tumor

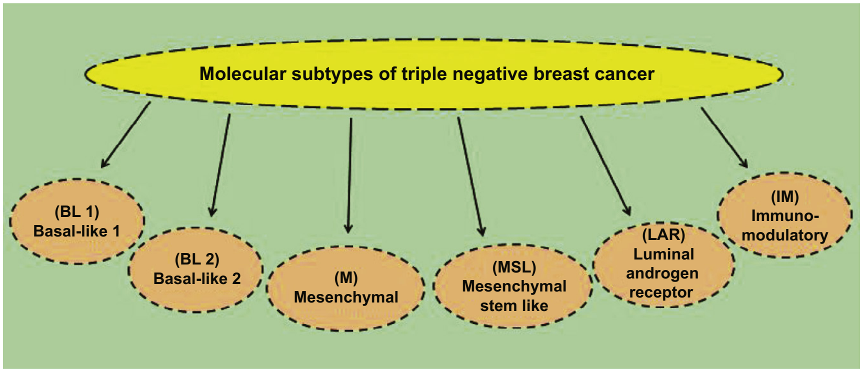

FIG 1.2 Different subtypes of triple negative breast cancer.

specimens from patients with TNBC in 2011: basal-like 1 (BL1), basal-like 2 (BL2), mesenchymal stem-like (MSL), mesenchymal (M), immunomodulatory (IM), and luminal androgen receptor (LAR) (Lehmann et al., 2011) (Fig. 1.2, Table 1.1). This subcategorization is useful not just for better comprehending the disease, but also for identifying molecular targets for therapy.

BL-1 and BL-2

TNBC tumor samples were subjected to gene expression profiling, which revealed unusual expression of cell-cycle controlling genes and DNA repair-related genes in the BL1 subtype (elevated amplification of CCNE1, AKT2, CDKN2A/B, CDK6, FGFR1, IGF1R, MYC, KRAS, and PIK3CA) as well as an elevated frequency of homozygous or heterozygous deletion of DNA repair-related genes like BRCA2, MDM2, PTEN, TP53, and RB1. On the other hand, the BL-2 subtype had distinct gene ontologies including epidermal growth factor signaling and also gluconeogenesis and glycolysis. Microarray analysis revealed increased expression of EGFR, MET, TP63, NGF, IGF-1R, and other genes (Lehmann et al., 2014).

TABLE 1.1 TNBC molecular subtypes.

S. No	TNBC subtypes	Genetic abnormalities
1	Basal-like 1	DNA damage response pathway
2	Basal-like 2	Glycolysis, gluconeogenesis, and growth factor signaling
3	Mesenchymal	Pathway of cell differentiation, extracellular receptor interactions, and cellular mobility
4	Mesenchymal stem-like	Comparable to the M subtype, but it is Claudin-low and has a strong expression of mesenchymal stem cells.
5	Immunomodulatory	Immune cell process
6	Luminal Androgen Receptor	Highly active genes related to hormonal signaling pathways

M subtype

The M subtype is also known as metaplastic BC because it has strongly activated cell migration-related signaling pathways (controlled by actin), differentiation pathways (anaplastic lymphoma kinase, TGF, Wnt-signaling pathways), and ECM–receptor interaction pathways (Lehmann et al., 2014; Mir, 2015). The M subtype contains squamous epithelial cell-like or sarcoma-like tissue and is susceptible to developing chemotherapeutic treatment resistance. As a result, M-subtype individuals may benefit from mTOR inhibitors or medications that target the epithelial-mesenchymal transition (Gibson et al., 2005).

MSL subtype

In comparison to the M subtype, cell proliferation-related genes in the MSL subtype are expressed in low levels and higher levels of stemness-related genes (ALDHA1, ABCA8, ABCB1, BCL2, BMP2, ENG, PROCR, PER1, TERT2IP, and THY), (Mehraj et al., 2021) HOX genes (MEIS1, MEIS2, MSX1, HOXA10, HOXA5, MEOX2, and MEOX1), and mesenchymal stem cell-specific genes (ENG, ITGAV, BMP2, NGFR, NT5E, KDR, THY1, PDGFR, and VCAM1). It is thought that PI3K inhibitors, antiangiogenic or Src antagonist's medicines could be used to treat MSL subtype patients. Dasatinib, an Abl/Src inhibitor, has been shown in studies to be effective in the treatment of patients with MSL and M subtype TNBCs (Lehmann et al., 2014).

IM subtype

B cell receptor signaling pathway, Th1/Th2 pathway, dendritic cell (DC) pathway, NK cell pathway, T cell receptor signaling, IL-7, and IL-12 pathways, are among the signal transduction and immune cell-associated genes pathways that are considerably enriched in the IM subtype. As a result, the IM subtype has a lot in common with breast medullary cancer (Bertucci et al., 2006). It is advised that individuals with IM subtype TNBC be treated with PDL1, PD1, CTLA-4, as well as other immune checkpoint inhibitors (Lehmann et al., 2014).

LAR subtype

The gene expression pattern of the LAR subtype differs dramatically from those of other TNBC subtypes. Although the LAR subtype lacks the ER receptor, it has highly active hormonal signaling pathways (include steroid biosynthesis, porphyrin metabolism, and estrogen/androgen metabolism). The AR is strongly expressed in the LAR subtype of TNBC, with an mRNA level nine folds higher than in other TNBC subtypes (Mir et al., 2021). In the LAR subtype, immunohistochemistry revealed significant AR expression as well as a huge number of downstream metabolic markers of AR and their supplementary activators (ALCAM, FASN, DHCR24, APOD, FKBP5, PIP, CLDN8, and SPDEF) (Hayes et al., 2008). As a result, anti-AR medication is indicated for LAR-subtype TNBC patients.

PAM50 subtyping of the 6 TNBC subtypes was conducted, and their PAM50 molecular intrinsic subtypes were compared by Lehmann & coworkers. Except for the MSL and LAR subtypes, all TNBC subtypes were shown to be primarily made up of basal-like subtypes

BL1 (99%), BL2 (95%), IM (84%), and M (97%). Luminal B (14%) and HER-2 (74%) were the most common LAR subtypes, while basal-like (50%), luminal B (14%), and normal-like (28%) were the most common MSL subtypes (Lehmann et al., 2014). Masuda and coworkers looked at the prognosis of several TNBC subtypes and discovered that the LAR subtype had a better distant metastasis-free rate of survival and overall survival rate (OS), whereas the BL2 and M subtypes had worse outcomes. The BL2 and M subtypes had considerably greater 3-year relapse rates than the LAR subtype (Masuda et al., 2013).

Burstein and coworkers studied specimens from 198 patients and classified TNBC into 4 subtypes: LAR, which expresses the cell-surface mucin MUC1 and AR; M, which expresses growth factor receptors like c-Kit receptors and platelet-derived growth factor receptors (PDGFR); BLIS (basal-like immunosuppressive), which produces the immunosuppressive protein VTCN1; BLIA (basal-like immune-activated) cells express STAT signaling molecules and releasing cytokines (Burstein et al., 2015).

TNBC and BRCA

Cancer propensity is caused by genetic instability. Patients with BRCA gene mutations are more likely to develop malignancies like breast, ovarian, prostate, and pancreatic. BRCA 1 is essential for DNA repair via homologous recombination. Inactivation of this gene owing to a BRCA mutation should result in cell cycle arrest; however, this is also blocked in TNBC by p53 mutations (Foulkes et al., 2003). In cells, the absence of a functioning BRCA1/2 causes a loss of DSB-DNA double-strand break repair. In such patients, this process increases their cancer risk. TNBCs are transcriptionally and histologically comparable to BRCA1-linked breast cancers, implying that BRCA1 malfunction is present in TNBCs (Turner et al., 2004; Lakhani et al., 2005). In terms of gene expression profiling, TNBCs are diverse. TNBC has been linked to tumors among young women who carry the BRCA1 mutation, as opposed to those who are in their late forties. There is evidence of genetic instability both in BLBCs and BRCA1-linked breast tumors. In females with germ-line BRCA1 mutations, more than 80% of breast cancers are TN, and 10% of TN breast cancers include BRCA1 mutations. The causes for these relationships are unknown, but they may eventually lead to prevention and also targeted treatment with PARP inhibitors and chemotherapy utilizing DNA-damaging drugs like platinum chemicals (Tassone et al., 2003; Rottenberg et al., 2008).

Triple-negative breast cancer risk factors and epidemiology

The risk factors and epidemiology linked with TNBC are unique, particularly when contrasted to endocrine-sensitive luminal breast cancers, in addition to having a distinct molecular and clinical profile. The population-based, case-control study- The Carolina Breast Cancer Study aimed at evaluating clinical relationships and distribution among diverse breast carcinoma subtypes, has improved our knowledge of the epidemiology and risk factors related to TNBC (Carey et al., 2006). The incidence of breast carcinoma subtypes among menopausal and racial categories was found in the initial analysis of females diagnosed with invasive breast carcinoma. In over 500 tumors, immunohistochemistry was utilized to characterize distinct subtypes, and "basal-like" cancers were classified as triple-negative (ER/PR/HER2) and cytokeratin 5/6 (+) and/or HER1 (+). According to the findings, individuals having basal-like tumors are more likely to be African American than non-African Americans (26% vs. 16%) and pre-menopausal than post-menopausal (24% vs. 15%) (Table 1.2). When contrasted to postmenopausal African American females and non–African American females of any age, premenopausal African American women had a higher incidence of basal-like tumors (39% vs. 14% and 16%; $P < 0.001$). Several further investigations have validated the finding that triple-negative breast tumors are more frequent in young African American women, while the specific cause for this relationship is still unknown (Bauer et al., 2007; Morris et al., 2007).

The Carolina Breast Cancer Study was expanded to look at frequently reported risks of breast cancer in 1424 instances of invasive and in situ breast carcinoma compared with more than 2000 controls (Millikan et al., 2008). As anticipated, increasing parity and relatively young age at first-term pregnancy was inversely related to risk in women with luminal A breast carcinoma (classified as ER (+) and/or PR (+) and HER2 (-) via immunohistochemical analysis) (Table 1.2).

For basal-like breast carcinoma, on the other hand, the risk rose with younger age and parity at first term full-term pregnancy. Furthermore, individuals who breastfed for a longer period had a greater number of children breastfed, and breastfed for a greater period of months had a lower risk of basal-like breast carcinoma. This finding did not occur in patients with luminal A breast carcinoma. A higher incidence of luminal A breast carcinoma was found in postmenopausal females with a high waist-to-hip ratio. In terms of basal-like breast carcinoma risk, this was true for both pre-and post-menopausal females (Mir et al., 2021). Surprisingly, the researchers concluded that if these correlations remain true among young African American females who had the highest number of basal-like breast carcinoma risk factors, breastfeeding and lowering abdominal obesity may avert nearly two-thirds of basal-like breast malignancies. Likewise, the Polish Breast Cancer Study found that risk factor indices differed depending on the kind of breast tumor (Yang et al., 2007). In this population-based analysis, increasing age at menarche was linked to a lower risk of basal-like malignancies but not luminal malignancies, whereas rising BMI was linked to a lower risk of luminal types of cancer but not basal-like malignancies in pre-menopausal females. These studies show that risk variables differ by subtype and should be taken into account when developing and evaluating preventative methods.

TABLE 1.2 Risk factors for triple-negative breast cancer.

S. No	Factors
1	Premenopausal status
2	African American race
3	Younger age at first-term pregnancy
4	Increasing parity
5	The brief duration of breastfeeding
6	Increased waist-to-hip ratio (both pre-and postmenopausal females)
7	Use of lactation-suppression techniques

Current diagnostic options for triple-negative breast cancer

Mammography

TNBC is distinguished by the absence of spiculated borders, uneven shape, and worrisome calcifications, which are common in other types of breast cancer. As a result, although frequently being larger than other BCs at the time of detection, TNBC could be mammographically hidden (in up to 18% of instances) (Dogan and Turnbull, 2012). On mammography, the most prevalent indication of TNBC is a mass. In about one-fourth of instances, circumscribed edges are reported, and there are usually no accompanying calcifications (Yang et al., 2008; Kojima and Tsunoda, 2011; Dogan and Turnbull, 2012). A focal asymmetry, which occurs in 10%–20% of TNBC cases, and a mass with accompanying calcifications, which occurs in about 15% of cases, are two less frequent TNBC presentations (Wang, et al., 2008; Dogan et al., 2010). Isolated calcifications are a significantly less common occurrence (Yang et al., 2008). According to Dogan and coworkers, mammography imaging may be of little utility in screening people at risk for TNBC. The low occurrence of accompanying calcifications or ductal carcinoma in situ, according to these investigators, indicates fast tumor development that leads to invasive malignancy without an in situ stages (Dogan and Turnbull, 2012).

Ultrasound

For the identification of TNBC, ultrasound shows a good sensitivity (Mir et al., 2021). TNBC is most commonly seen on ultrasound as a distinct mass that lacks worrisome sonographic characteristics, similar to how it appears on mammography (Dogan and Turnbull, 2012). TNBC is distinguished by well-circumscribed borders, which have been observed in roughly 25% of instances (Dogan et al., 2010; Kojima and Tsunoda, 2011), and posterior acoustic amplification, which is found in 25%–40% of instances. TNBC has posterior acoustic amplification, which indicates tumor necrosis instead of benignity, as other breast cancers do (Lerma et al., 2009; Du et al., 2015).

Magnetic resonance imaging (MRI)

TNBC can be detected with high sensitivity using magnetic resonance imaging (MRI), with the morphologic features of TNBC on MRI being much more suspicious than any of those found on ultrasound and mammography (Boisserie-Lacroix et al., 2013). Dogan and coworkers discovered that MRI was 100% sensitive for detecting TNBC in 44 individuals, compared to 91% and 93% for mammography and ultrasonography, respectively (Dogan et al., 2010). In that study, the most common sign of TNBC was an enlarging mass, which was observed in 34 of the patients. The most prevalent mass form was oval or round, which was recorded in 35% of instances, with dominating mass borders being uneven or spiculated in 47% and 41% of instances, respectively. Rim enhancement was the most common contrast enhancement type, appearing in 76% of instances. In 8 patients, enhanced interior septations were observed. Nonmass augmentation was seen in the other 10 participants in this investigation. Uematsu and coworkers looked at 59 patients and discovered that mass lesions, rim enhancement patterns, smooth mass borders, and prolonged enhancement kinetics were all linked to TNBC (Uematsu et al., 2009). Teifke and coworkers implies that rim enhancement was the most reliable MR result for identifying ER status among these findings (Teifke et al., 2006). Although there is a significant link between TNBC and unifocal lesions (Uematsu et al., 2009), multifocality has been observed in 21% of instances in the literature (Chen et al., 2007). TNBC tends to be greater on MRI than that of other subtypes, with just a median tumor size of 4.1 +/- 2.7 cm (Chen et al., 2007). In this investigation, prominent skin augmentation was also a common result, implying that the dermal lymphatics had been invaded. Increased intratumoral T2 signal intensity that is also linked with TNBC (Uematsu et al., 2009; Youk et al., 2012; Osman et al., 2014) has been demonstrated to be highly related with intratumoral necrosis. Elevated T2 signal on MRI was found to have a 90% association with internal necrosis on pathologic inspection by Osman and coworkers (Osman et al., 2014). Internal necrosis is linked to poorer clinical results and highly malignant biology, making this a clinically relevant finding.

Future diagnostic options for TNBC

Blood-based liquid biopsy

TNBC could be diagnosed using a blood-based liquid biopsy, which is a noninvasive testing approach. A blood sample is examined for the existence of tumor-derived extracellular vesicles (exosomes), circulating tumor nucleic acids (ctNAs), and circulating tumor cells (CTCs), which includes circulating tumor DNA (ctDNA) and miRNAs (Jia et al., 2017; Zhang et al., 2017). Song and coworkers used a similar strategy to demonstrate serum apolipoprotein C-I (apoC-I) as a possible prognostic and diagnostic marker for TNBC (Song et al., 2016).

Circulating tumor nucleic acids (ctNAs)

Circulating tumour DNA (ctDNA), cell-free RNA (cfRNA), and microRNA (miRNA) are all used to analyze ctNAs (Marrugo-Ramírez et al., 2018). CtDNAs identified in a cancer patient's blood come from the main tumor (Fiala and Diamandis, 2018; Davies and Eaby-Sandy, 2019), CTCs (Schwarzenbach et al., 2009), and necrotic and apoptotic cell deaths that occur during cancer formation and progression (Jahr et al., 2001; Stroun et al., 2001). The amount of tumor ctDNA in the bloodstream is proportional to the tumor's or metastasis' size, and a study found that increasing the ctDNA concentration increases the proportion of tumor burden (Dawson et al., 2013). As a result, detecting ctDNA in the initial stages of the tumor is difficult because only a little amount of ctDNA may be discovered. Because the levels of ctDNA detected are minimal, an ultrasensitive method is desperately required to detect the early stages of cancer. The droplet digital polymerase chain reaction (ddPCR) was successful in detecting PIK3CA mutations in blood samples from patients with early-stage breast cancer (Beaver et al., 2014). Nevertheless, if ctDNA can be used as a biomarker for initial breast cancer diagnosis, it must be validated and developed further. Evaluating ctDNAs in the plasma, on the other hand, could be utilized to monitor the tumor burden in real-time and determine therapy success (Dawson et al., 2013). This is because ctDNAs possess a shorter half-life (15 minutes to several hrs.), (Fleischhacker and Schmidt, 2007; Diehl et al., 2008) enabling for earlier detection of changes in ctDNA levels in the circulation than radiological imaging.

MicroRNAs (miRNAs) are small 22-nucleotide ribonucleic acids (RNAs) which control thousands of genes by binding to targeted mRNAs (Eulalio et al., 2008). Several biological processes, including cell formation, proliferation, chromatin structure, differentiation, metabolism, apoptosis, and morphogenesis, are influenced by miRNAs (Ambros, 2004; Bartel, 2004; Kim et al., 2009). Furthermore, miRNAs that serve as tumor suppressors or oncogenic miRNAs play an important role in carcinogenesis (Kim et al., 2009). Anti-apoptotic action was demonstrated using oncogenic miRNAs, which were reported to be overexpressed in cancerous cells (Hammond, 2006; Cho, 2007; Drakaki and Iliopoulos, 2009). Tumor suppressor miRNAs, on the other hand, are frequently proapoptotic, anti-proliferative and, and are down-regulated in cancerous cells (Zhang et al., 2007; Negrini and Calin, 2008). Thakur and coworkers found that TNBC females in India have high levels of miR-220, miR-21, and miR-221 (Thakur et al., 2016), which supports Radojici and coworkers findings (Radojicic et al., 2011). In a Hong Kong-based study, however, the expression of miR-21 and miR-221 was down-regulated, underlining the potential of miRNA expression variability in different ethnic groups or depending on the patient's geographical location (Shin et al., 2015). Furthermore, various non-TNBC investigations found that varying amounts of miR-(21, 145, 221,195) and Let-7a expression were found in various types of breast cancer categories (Heneghan et al., 2010; Bockmeyer et al., 2011; Mar-Aguilar et al., 2013). This shows that the level of miRNA expression is affected not only by the tumor type but also by the grading and stages of breast carcinoma. By building diagnostic testing based on 8 circulating miRNAs (miR-16, 107, 103, 22, 148a, 19b) and let-7(d and i), Frères and coworkers established a novel screening tool for breast carcinoma (Frères et al., 2016). The researchers were able to demonstrate that the newly created approach could diagnose breast cancer malignancy and diagnose breast cancer occurrences early.

Exosomes

Exosomes are membrane-bound, extracellular vesicles released by numerous cells in both abnormal and normal situations , as first described by Pan and Johnstone in 1983. Exosomes are largely responsible for carrying biomolecules such as RNA, DNA, lipids, and proteins to recipient cells (Raposo and Stoorvogel, 2013; He and Zeng, 2016). Exosomes are also involved in intercellular molecular interactions and cell signaling (Mathivanan et al., 2010). Exosomes from TNBC have been shown to aid in cell communications and phenotypic trait transmission to secondary cells in research by O'Brien and coworkers (O'Brien et al., 2013).

Exosomes from cancerous cells have been discovered to promote tumor cell multiplication and stage immune defense evasion during carcinogenesis, boosting cancer growth and metastasis (Iero et al., 2008; Zhang and Grizzle, 2014). Exosomes from TNBC were discovered to drive tumorigenesis and lymph node metastases through intercellular interaction with macrophages in research by Piao and coworkers (Piao et al., 2018; Mehraj et al., 2021). Exosomal proteins have been established in many studies to be useful prognostic and diagnostic markers. While CD24 can be present in a variety of cancer types, including colorectal cancer (Rupp et al., 2011), Rupp and coworkers suggested that it could be used as a circulating BC biomarker. Moon and coworkers further proposed that fibronectin and endothelial Locus-1 (Del-1) from circulating exosomes in plasma might be used as biomarker options for early diagnosis of people with breast cancer (Moon et al., 2016). Despite the fact that the discovery isn't particular to TNBC, it might act as a crucial foundation for future TNBC diagnostic research. Finally, liquid biopsy gives real-time, trustworthy data saves the cost and time it takes to diagnose a problem, and allows people to avoid surgery.

Immuno-positron emission tomography (PET)

PET scan, or positron emission tomography, is a diagnostic scanning technique that uses a radioactive element or chemical to examine the functioning of organs and tissues. It is well for its ability to identify a disease even before the other imaging techniques can identify it. The radioactive element (tracer) is made up of firmly coupled radioactive atom-transport molecules (isotopes) that bind to certain biomolecules (protein, sugar, etc.) in the body of humans and produce positrons that interact with the adjacent electrons to produce photons (Berger, 2003). The PET scanner subsequently detects the photons' electric signals and uses the information to create an image of the cell, tissue, or organ (Phelps, 2000).

Immuno-PET imaging uses a comparable strategy, combining the PET system with monoclonal antibodies (mAbs) to enhance the efficiency of tumor characterization identification and assist in the selection of appropriate targeted mAb-based treatment (Verel, Visser et al., 2005). The antibody's principal function in this strategy is to identify certain cell surface tumor markers or extracellular matrix components, which are subsequently identified by the PET monitoring device (Van Dongen et al., 2007). The development of ATL-836 fragment antigen-binding (Fab) chimeric mabs against human tissue factor (TF) provides compelling evidence for this idea (Shi et al., 2015). The discovery of ATL-836 antibody offers a hopeful framework for prospective TNBC therapeutics and diagnostics because TF also called platelet tissue factor/factor III, plays an important role in cancerous cell signaling (cell migration promotion and apoptosis inhibition) and has been discovered to be prominently expressed on TNBC cells (Zhang et al., 2017; Hu et al., 2018). In a xenograft animal model having TNBC, another prospective TNBC diagnostic imaging Ab agent targeting glycoprotein non-metastatic B (gpNMB)/osteoactivin was effectively produced (Marquez-Nostra et al., 2017). This finding is critical since gpNMB expression is elevated in TNBC individuals and more crucially, in tumor growth and recurrence (Rose et al., 2007, 2010). Furthermore, the antibody-toxin conjugate was capable of reducing the growth of gpNMB-expressed TNBC cells (Rose et al., 2010). In conclusion, immuno-PET could not just detect TNBC earlier, but it can also determine the best treatment option for patients since immuno-PET can image the expression of targeted therapies (Yardley et al., 2015).

Nanobiosensor

A biosensor is a device made up of a bio-receptor, a detector, as well as a signal transducer that can be used to identify and analyze a variety of biological specimens, such as immune components (antibodies and antigens), enzymes, nucleic acid elements (RNA, DNA, ctDNA, and miRNAs), as well as other biological constituents found in humans. The analyte (complementary DNA, enzyme-substrate, antigen) is recognized by a bioreceptor, which is an immobilized biological sensing component (DNA probe, enzyme, or antibody). In a biosensor, a transducer converts the (bio) chemical signals emitted by analyte-bioreceptor interactions into electronic signals. The generated signal's intensity is proportional to the concentration of the analyte, either directly or inversely. Biosensors frequently utilize electrochemical transducers (Sassolas et al., 2012). The basic concepts of bio-recognition elements and signal transduction and are used to classify biosensors. Biosensors are categorized as optical, electrochemical, thermal, or piezoelectric sensors based on the transducing elements. Amperometric, potentiometric, and conductometric sensors are also types of electrochemical biosensors (Thevenot et al., 1999). Despite the widespread usage of antibodies and oligonucleotides, enzymes are the most prevalent biosensing elements in biosensors.

When a bio-receptor attaches to certain biological analytes, the signal transducer produces measurable binding signals that are then recognized by the detector for data processing (Fracchiolla et al., 2013). As the name implies, a nano-biosensor is a biosensor that combines nanoparticles with transducers to increase biological signaling and transduction processes (Mohammadniaei et al., 2018) (Fig. 1.3). This is achievable because nanoparticles have a high surface area to volume ratio due to their tiny size, which increases the sensor's receptiveness and lowers the detecting cut-off point by identifying biological analytes at small concentrations.

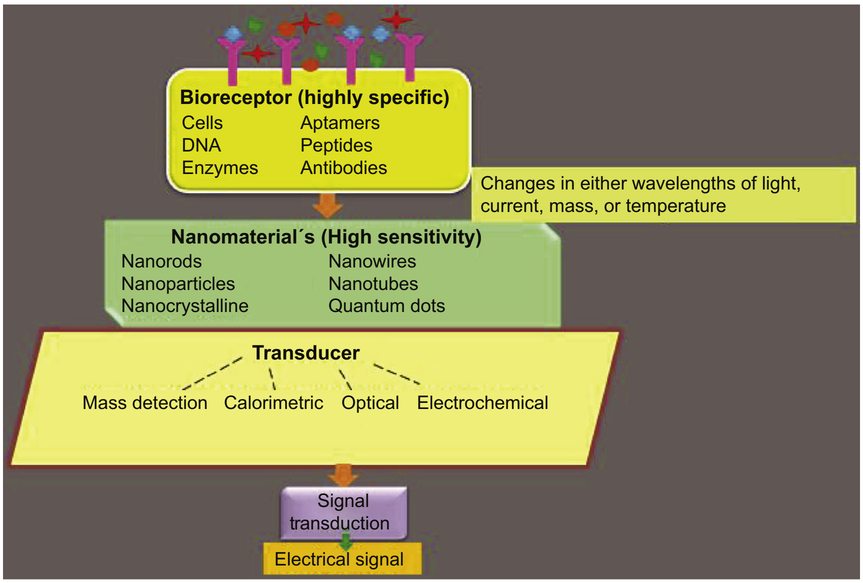

FIG 1.3 **Principles of functioning of a nanobiosensor: when bioreceptors bind to a sample analytes, they trigger a biological reaction that results in changes in wavelength, current, mass, or temperature.** The transducer will then translate the biological response into electrical impulses. Nanomaterials that combine with transducers are used to detect analytes in low concentrations.

Many nano-bio sensors for TNBC cell identification had already been created in the past. The zinc oxide (ZnO)-choline oxidase (ChOx) nano-biosensor developed in 2016 proved capable of detecting the level of choline in TNBC specimens (Thiagarajan et al., 2016; Mir et al., 2021). In another method, an electrochemical-nanosensor based on the LNA oligonucleotide probes demonstrated significant TNBC diagnostic possibilities by effectively identifying the tumor-associated miR-199a-5p markers (detection limit = 4.5f), which has been revealed to be down-regulated in TNBC cells compared to normal cells in general (Chen et al., 2016; Ebrahimi et al., 2018). As previously stated, the nano-biosensor was proven to be very responsive and specific in detecting low levels of miR-199a-5p in the blood samples of patients. Additional discoveries involve a dual-ligand co-functionalized gold nanocluster (AuNCs) that can identify and differentiate between carcinoma, normal, and metastatic breast carcinoma cells, as well as TNBC cells, demonstrating the nano-biosensor's intriguing analytical and diagnostic possibilities (Tao et al., 2017).

nCounter breast cancer 360 panel

The nCounter Breast Cancer 360TM Panel (Seattle, WA, USA) was launched in April 2018 as a research tool for analysis with about 770 genes to assist in breast cancer categorization depending on molecular subtyping. The patient's RNA specimen is isolated and integrated overnight with Breast Cancer 360TM panel test before sample and data processing are performed utilizing the Nano-string nCouter® system (Seattle, WA, USA) (Nano-String Technologies USA). The system offers a comprehensive understanding of gene expression levels, immune defensive mechanisms against breast carcinoma, and tumor microenvironment, as well as breast cancer classification based on biological signatures like prognostication assessment of microarray 50 (PAM50) and tumor inflammatory signature assays (Wallden et al., 2015). This ability of NanoString BC360 (Seattle, USA) to reveal the heterogeneity of breast carcinoma and its microenvironment was demonstrated in Phase I clinical study assessing Everolimus and Eribulin in TNBC patients (Yuan et al., 2019). Additional research used the NanoString®BC360 panel to help identify intrinsic breast cancer subtypes and then assess the efficacy of hormonal therapy for stage I luminal breast carcinoma (Schroth et al., 2019). Furthermore, the accuracy of NanoString BC360 in identifying breast carcinoma subtype (ESR1, MK167, PGR, and ERBB2 genes) has recently been demonstrated to be comparable to that of classical immunohistochemistry. Generally, the panel failed in terms of requiring a large number of specimens for data validation, and it was only useful for research purposes. The NanoString BC360 panel is planned to be used for breast carcinoma diagnoses in the future.

Digital polymerase chain reaction (dPCR)

Digital PCR, developed by Vogelstein and Kinzler in 1999, is a technology that separates materials into numerous wells prior to amplification (Fig. 1.4). When compared to a traditional qPCR, the advantages of dPCR are that it does not require a standard curve for analysis, it can endure any PCR inhibitors (Nixon et al., 2014), it can examine the presence of unusual targets in huge specimen mixtures, and it can detect minute fold changes (White et al., 2012). Furthermore, dPCR's absolute quantification and specimen segregation make it

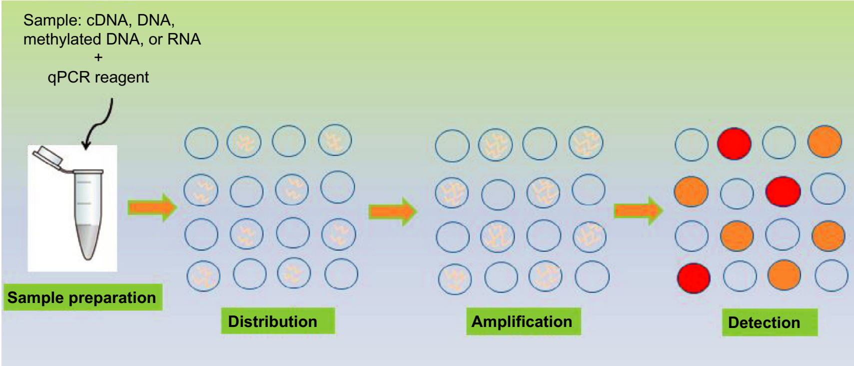

FIG 1.4 A dPCR overview: the sample is added to the qPCR reagent and mixed together. It is then spread evenly among several subvolumes (in microwells, chambers, or droplets), resulting in some partitions containing few targets and others containing none. Amplification will take place for each subdivided component. Positive columns will then be detected.

a good candidate for detecting rare alleles (Hindson et al., 2011; Castellanos-Rizaldos et al., 2015), genetic mutations like DNA deletions, variation, and replication (Chang et al., 2002; Lo et al., 2007; Whale et al., 2012), next-generation sequencing library quantification, and viral load (Laurie et al., 2013; Sedlak and Jerome, 2013; Zhou et al., 2018). Digital PCR is commonly used in cancer patients to identify circulating tumor miRNA and DNA (Laprovitera et al., 2018). To assess the carcinoma subtype, a 4-plex droplet digital PCR (ddPCR) was developed in 2019 for concurrent investigation of four carcinoma oncogenes (ESR1, PUM1, ERBB2, and PGR) (Chen et al., 2019).

Presently, many commercial dPCR systems are available, QX100 and QX200 Droplet Digital PCR System (Bio-Rad, Hercules, CA, USA), Raindrop Digital PCR System (Raindance Technologies, Billerica, MA, USA), BioMark HD System, and qdPCR 37K IFC (Fluidigm Corporation, South San Francisco, CA, USA), Clarity (JN Medsys, Singapore), and QuantStudio 3D Digital PCR System (Life Technologies, Carlsbad, CA, USA) (Demeke and Dobnik, 2018). In conclusion, digital PCR offers a potential platform with high correctness for early cancer detection.

Triple-negative breast cancer's prognostic implications

The frequencies of local and distant relapse vary significantly among breast carcinoma subtypes, with TNBC having a higher risk of relapse (Nguyen et al., 2008; Gabos et al., 2010; Voduc et al., 2010; Bae et al., 2016) than other breast carcinoma subtypes. TNBC has a relatively high rate of distant relapse than other subtypes, according to Dent and coworkers (Dent et al., 2007), with a relapse rate of 33.9% particularly in comparison to 20.4% for other subtypes. Bae and coworkers (Bae et al., 2016) looked at 398 females with initial stage (stage I or II) TNBC and discovered numerous characteristics that were linked to local relapse, including familial history of breast carcinoma, mammographically dense breasts, lymphovascular infiltration, as well as the lack of a pre-operative breast MRI. This investigation also found that variations in lymph nodal status and tumor size, both of which are key prognostic markers for other breast carcinoma subtypes were not related with substantial differences in TNBC recurrent rate (Bae et al., 2016). TNBC also has a unique recurrent pattern, with significant rates of relapse observed up to 5 years following diagnosis, resulting in a significant drop in recurrent risk after that period (Foulkes et al., 2010; Bae et al., 2016). Other breast carcinoma subtypes, on the other hand, have a consistent risk of relapse that lasts up to 17 years following diagnosis. Similarly, Dent et al. (Dent et al., 2007) discovered that TNBC patients had a reduced mean time to local relapse of 2.8 years relative to 4.2 years for other subtypes.

Patients with TNBC have a worse long-term prognosis as compared to patients who do not have TNBC (Liedtke et al., 2008; Mir, 2021). At M.D. Anderson Cancer Center, a trial of 1,118 women with breast carcinoma (including 255 with TNBC) found that participants with TNBC had lower progression-free and OS rates at three years than those with other breast tumor subtypes; survival rates remained comparable after three years (Liedtke et al., 2008). TNBC has a worse prognosis for a variety of reasons. TNBC is more prone to develop lung and visceral metastases, whereas non-TNBC is more prone to develop skin and bone metastases (Liedtke et al., 2008; Freedman et al., 2009; Kennecke et al., 2010). Brain metastasis is more common in TNBC patients (Heitz et al., 2008; Dawood et al., 2009; Brouckaert et al., 2012; Mir and Mehraj, 2019), and TNBC has a much greater nuclear grade (Brouckaert et al., 2012). Targeted therapies (hormonal therapy, trastuzumab) are available to non-TNBC patients in combination with chemotherapy, but chemotherapy is practically the only systematic treatment for TNBC (Dogan and Turnbull, 2012; Mir, et al., 2020). TNBC prognosis is thus heavily impacted by treatment response, with TNBC and non-TNBC patients achieving pCR having comparable survival. TNBC patients, on the other hand, had a much higher chance of achieving pCR than those with other breast carcinoma subtypes (Brouckaert et al., 2012; Mir et al., 2020).

Somatic genomic mutations in TNBC

Cancers contain a large number of somatic genetic changes, but only a tiny percentage of these offer a demonstrable fitness advantage, often known as "cancer drivers" (Vogelstein et al., 2013). TNBC has multiple changes in potential cancer-driver genes, according to large-scale exome and targeted sequencing investigations in primary breast cancers (Pereira et al., 2016; Weisman et al., 2016). The typical alteration rates in basal-like breast carcinoma are among the highest in breast cancers, with 1.68 mutations per mega-base (Mb); tumors with rates higher than 3 standard deviations just above average (>4.68 mutations/Mb) are classified as hyper-mutated (Cancer Genome Atlas Network 2012). Specific genome-wide classifications in breast malignancy have been put forward by grouping next-generation sequencing (NGS)–detected modifications in known cancer driver genes based on the intracellular pathways in which they are engaged like RAS/MAPK and PI3K/AKT signaling, cell-cycle, DNA-damage repair, as well as transcriptional regulation (Balko et al., 2014; Pereira et al., 2016; Mir et al., 2020) (Table 1.3).

The majority of TNBC somatic alterations arise in tumor suppressor genes (e.g., RB1, TP53, and PTEN), which have yet to be effectively targeted clinically. Oncogenic changes in the

TABLE 1.3 Exome or targeted sequencing-based classifications of potentially targetable pathways.

1	The cancer genome atlas	Genetic alteration (frequency, %)
2	PI3K/PTEN pathway	INPP4B loss, PTEN mut/loss, PIK3CA mut
3	p53 pathway	TP53 mut, the gain of MDM2
4	RB1 pathway	CCNE1 amp, RB1 mut/loss, low RB1 expression, high expression of CDKN2A

PI3K/AKT pathway were also documented in patients with basal-like breast carcinoma (PTEN mutation or deletion, 35%; AKT3 amplification, 28%; PIK3CA mutation, 7%) (Mir et al., 2020) (Cancer Genome Atlas Network 2012), possibly enrolling them for clinical trials with matching treatments. Targeted sequencing of remaining disease after neo-adjuvant treatment revealed that >90% of patients exhibited at least one mutated pathway, similar to results in untreated triple-negative cancers (Balko et al., 2014). Just three mutations, however, were shown to be substantially predictive for OS (BRCA1 truncation or alteration, JAK2 amplification: predicted poor OS; PTEN mutation: better OS). Because of the low single-agent effectiveness of drugs that block these pathways, they have largely been used in conjunction with additional therapies in TNBC clinical studies (Mir et al., 2020).

Given the complexity of TNBC's genetic landscape, single alterations in a suspected driver or proven oncogenic cascade are likely inadequate (Nik-Zainal et al., 2012). Age, carcinogenic exposures, DNA replication faults, DNA repair defects, and the APOBEC cytidine deaminases family all stamp patterns of alterations on the cancer genome, which are termed mutational signatures. The existence of five separate mutational signatures in breast carcinoma, notably localized hypermutation and APOBEC, was discovered using whole-genome sequencing of 21 breast cancers (Nik-Zainal et al., 2012). In 93 putative driver genes, somatic base substitutions, insertions and deletions, rearrangements, and copy-number changes were discovered in an enlarged investigation of 560 breast tumors (Nik-Zainal et al., 2016). MYC, TP53, PTEN, RB1, and ERBB2 appeared to be enriched in the ER-negative group, accounting for 62% of the 10 most commonly altered genes in the whole sample. Twelve base-substitution signatures (including the five earlier discovered signatures), six rearrangement signatures, and two indel signatures were discovered using mathematical methods. High tandem duplications (>100 kb) were related with rearrangement signature 1, which was predominantly detected in TP53-mutated, triple-negative cancers with significant homologous recombination–deficiency (HRD) index but no BRCA1/2 alterations or BRCA1 promoter hyper-methylation. In comparison, 91% of BRCA1 mutations or promoter hyper-methylation cases had rearrangement signature 3, which was defined primarily by short tandem duplications (10 kb). More research is needed to completely comprehend the therapeutic and prognostic implications of these signatures.

Epigenetic modifications in TNBC

Due to the variability of TNBC, the present research is focusing on developing novel techniques to combat this neoplasia; one such tactic is epigenetics. Epigenetics is becoming more widely acknowledged as a key factor in carcinogenesis in all types of cancer. This discipline is described as the study of heritable variations in gene expression that do not result from changes in DNA sequences (Kanwal et al., 2015). Several epigenetic alterations with prognostic, diagnostic, or therapeutic implications had been documented in a variety of cancers, including breast carcinoma (Basse and Arock, 2015; Mehraj et al., 2021).

Methylation of DNA and post-transcriptional changes of histones are the first and most common epigenetic changes reported and recognized by a significant number of authors (Jones and Baylin, 2007; Tammen et al., 2013; Kanwal et al., 2015). Noncoding RNAs (ncRNAs) chromatin remodeling (Jones and Baylin, 2007; Tammen et al., 2013), nucleosome placement, and chromosomal looping (Kanwal et al., 2015) are among the more recently identified and acknowledged alterations. All of these markers are intricately linked, and one epigenetic change can readily trigger another.

Methylation of DNA in triple-negative breast cancer

DNA methylation is among the well-studied epigenetic processes. DNA methyltransferases (DNMTs) perform cytosine methylation in CpG islands, which is a known marker for epigenetic silencing. DNMT1 is important for sustaining methylation sequences after replication, while DNMT3a and DNMT3b start de novo methylations (Kanwal et al., 2015).

Depending on differentially methylated regions (DMRs), one of the most extensive investigations of the TNBC methylome divided patient specimens into three methylation groups (Stirzaker et al., 2015). When compared to the more severely methylated subgroups, the hypo-methylated profile was related to improved survival during the first 5 years after diagnosis, whereas the intermediate methylated clusters were linked with the lowest survival. It also discovered 17 DMRs capable of classifying patients with TNBC into groups with favorable and worse prognoses. The gene WT1 and its antisense counterpart, WT1-AS, were among the genes studied, and elevated levels of methylation were linked to increased expression and bad survival. Although hyper-methylation of the bidirectional promoter is related to lower WT1 and WT1-AS expression and increased survival, these results need to be confirmed in a big population (Stirzaker et al., 2015). On the setting of global hypo-methylation, the research also characterized hyper-methylation events as primarily occurring in CpG islands (Fig. 1.5). The hyper-methylated areas have a high correlation with H3K27me3, an epigenetic silencing marker in human breast epithelial cells. Twelve methylation genes were found to be both mutant and down-regulated, including SEMA5A and ROBO3 (Stirzaker et al., 2015), which are important in the guidance of axon, a pathway that has recently been linked to breast cancer tumor initiation and development (Harburg and Hinck, 2011). This pathway was first discovered in brain formation ((Robichaux and Cowan, 2014), and it contains the Eph/ephrin, Netrin, Slit, and Semaphorin proteins, that had recently been discovered to govern normal breast growth and also breast carcinoma initiation, angiogenesis, and progression (Braicu et al., 2016). Seven members of this pathway had promoter hyper-methylation, which could be beneficial for future research in targeted cancer treatment (Stirzaker et al., 2015).

Earlier research examined the hyper-methylation of 110 CpG islands in 69 cancer-linked genes and also discovered a distinct methylation pattern for TNBC. The TNBC-specific pattern was characterized by the 5 genes methylation (CDKN2B, CD44, MGMT, p73, and

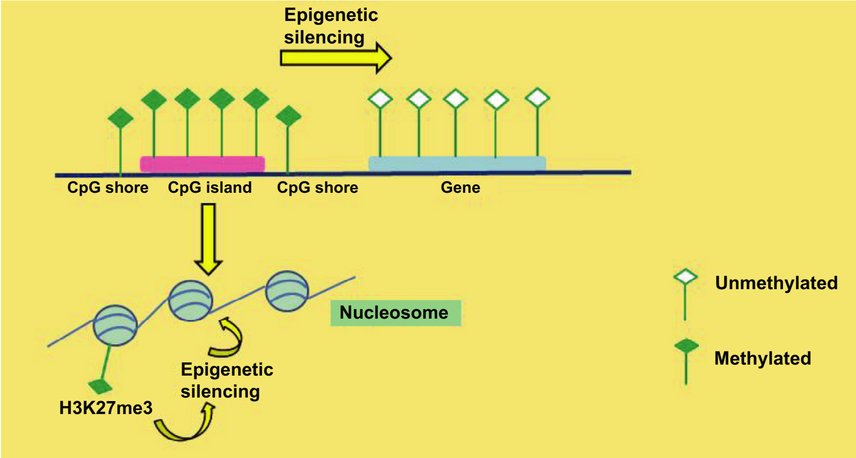

FIG 1.5 Methylation patterns in triple-negative breast cancer: epigenetic silencing is caused by hypermethylation of CpG islands and shores along with hypomethylation of intragenic regions. At the nucleosomal level, DNA methylation sequences correspond to trimethylation of lysine 27 on histone H3 (H3K27me3), additional epigenetic silencing marker.

RB) as well as the 11 genes non-methylation (PMS2, GSTP1, MSH2, CACNA1A, MLH1, MSH3, CACNA1G, MSH6, TWIST1, ID4, and DLC1), with MMR, MGMT, and ID4 having the highest connection (Mir and Agrewala, 2008; Branham et al., 2012). The methylation of promoters of both the BRCA1 and BRCA2 did not differ significantly among triple-negative and non-triple-negative cancers, which was unexpected. Nevertheless, one of the non-methylated genes, ID4, is a negative regulator of BRCA1; this could indicate a novel mechanism of BRCA silence that needs to be investigated.

Relation between DNA methylation and TNBC progression

Additional whole-genome methylation study contrasted the primary tumor to healthy nearby tissues and lymph nodal metastasis and revealed a collection of abnormalities that could explain the TNBC's progression (Mathe et al., 2016). Sixteen TNBC-specific genes were found to have differentially methylation probes, including five DMRs–COL14A1, ANKRD30B, IGF1, MEG3, and IL6ST. In lymph node metastasis, another group of genes was identified to be differently methylated. The increased methylation of EGR1, SPRY2, GREB1, LRRC17, and ITIH5, as well as the reduced methylation of AMIGO2, were associated with improved survival. According to a similar study, EGR1 down-regulation is negatively associated with its methylation (Mathe et al., 2016). Moreover, a particular gene, BRMS1, may have an epigenetically influenced effect on TNBC metastatic potential (Kong et al., 2015). In comparison to healthy breast tissue, BRMS1 expression was identified to be considerably lower in TNBC tissue specimens and cell lines; it also was discovered to be negatively connected with lymph node metastases. On breast carcinoma cell lines (HCC-1937, MDA-MB-231, and MDA-MB-435), a healthy breast tissue cell line (MCF-10A), and primary breast carcinoma tissues with comparable nonmalignant breast tissues, DNA methylation-dependent deactivation was demonstrated (Kong et al., 2015). This gene's methylation was found to be substantially associated with a bigger size and greater tumor-node-metastasis (TNM) stage, implying that it could act as a tumor suppressor.

Role of long noncoding RNAs in TNBC

Liu et al. (Liu et al., 2016) combined the profiles of mRNAs and lncRNAs to develop a unique classification strategy for TNBC. IM, LAR, MES, and BLIS are four unique clusters that are partly associated with the already described Lehmann subtypes (Lehmann et al., 2014); additionally, the BLIS subtype has been defined as the highly aggressive phenotype (Liu et al., 2016).

TNBC microarray profiling revealed a variety of lncRNAs with distinct expression profiles when compared to normal tissues (Shen et al., 2015). Nevertheless, their functions, relationships with other pathways, and significance have yet to be determined. Likewise, additional microarray profiling analysis of lncRNAs in TNBC patient's clinical specimens discovered that lncRNA LINC00993 may be linked to ER dysregulation in TNBC (Chen et al., 2015). Other lncRNA, MALAT1, has recently been discovered to play an important role in TNBC tumor progression and has been proposed as a possible predictive biomarker for lymph node-negative, TNBC, and HER2+(Jadaliha et al., 2016).

Role of MicroRNA in TNBC

MiRNAs are short non-coding RNAs (ncRNAs) with a 20-nucleotide length that can change gene expression after transcription (Palazzo and Lee, 2015). In TNBC, Gasparini et al. (Gasparini et al., 2014) discovered four-miRNA signatures that allowed patients to be classified into high- and low-risk categories. Up-regulation of miR-155 and miR-493 was linked to improved patients results, but miR-30e and miR-27a were linked to negative results (Gasparini et al., 2014).

MicroRNAs (miRNAs) have also been proposed as possible TNBC biomarkers. In breast carcinoma cell lines, miR-10b, miR-146a, miR-26a, and miR-153 were examined and found to be associated with BRCA1 expression. BRCA1 expression is suppressed in MDA-MB-231 cells by miR-26a and miR-10b. In TNBCs, miR-146a is highly expressed without impacting BRCA1 expression, however, in MDA-MB-231 cells, miR-153 can up-regulate BRCA1 expression. Kumaraswamy et al. (Kumaraswamy et al., 2015) found that BRCA1 expression is significantly correlated with miR-146a and results in EGFR down-regulation. In addition, Garcia et al. (Garcia et al., 2011) discovered that miR-146a and miR-146b-5p suppress BRCA1 expression in TNBC. MiR-590-5p and miR-4417 were discovered to be hyper-expressed in TNBC in research by Murria et al. (Murria et al., 2015). miR-590 regulates ER via interacting with the two ESR1 mRNA regions, whereas miR-4417 regulates BRCA1 mRNA (Murria et al., 2015). miRNAs are also involved in the epithelial-to-mesenchymal transition (EMT), which is a crucial step in the progression of metastases. A recent study that demonstrated the connection among two epigenetic pathways offered an understanding of the mechanisms that regulate their expression in TNBC and its association to nodal metastases. Low miR-200c expression and lymph node invasion are related to methylation of the miR-200c/miR-141 gene in TNBC, promoting metastases and changing TNBC prognosis (Damiano et al., 2017; Damiano et al., 2017). It has likewise been linked to elevated levels of the EMT-related transcription factor ZEB1, indicating that the miR-200c/ZEB1 axis could be a treatment target in aggressive TNBC. Furthermore, the mir-200 miRNA family has been demonstrated to play a crucial role in TNBC. In a murine breast xenograft cancer model, ectopic expression of miR-200b inhibited protein kinase Cα, which inhibited TNBC metastases and migration (Humphries et al., 2014). An additional member, miR-200a, had also demonstrated to modify TNBC migration by controlling the EPHA2 oncogene (Tsouko et al., 2015), while higher expression of miR-429-5p and miR-200b-3p suppresses the migration, proliferation, and invasion of TNBC cells by blocking the LIMK1/CFL1 (LIM domain kinase 1/cofilin 1) pathway (Li, Wang et al., 2017), offering up new potential for targeted therapeutics in TNBC.

Role of histone modifications in TNBC

H3K4me1, H3K4me3, H3K9ac, H3K9me3, H3K27me3, H3K36me3, H3K27ac, and H3K79me2—eight important histone modifications being examined across 13 cell lines, included four TNBC cell lines—HCC1937, MDA-MB-231, MDA-MB-468, and MDA-MB-436 (Xi et al., 2018). Histone modification patterns unique to subtypes, such as different H3K36me3 sequences in TNBC cell lines, have also been found. The said gene has not previously been associated with TNBC, but it is overexpressed and anticipates bad prognosis different cancers, such as esophageal cancer, lung malignancy, pancreatic ductal adenocarcinoma, nasopharyngeal carcinoma, hepatic melanoma, and colon cancer; it could also promote cancer cell invasion through EMT, according to the researchers. Depletion of AFAP1-AS1 by short interfering RNAs resulted in lower proliferation and colony development in MDA-MB-231 and HCC1937 cells (Xi et al., 2018).

BCL11A, a newly identified transcription factor, is abundantly expressed in TNBCs, as well as basal-like subtypes (Khaled et al., 2015), is essential for breast stem and progenitor cell types (Khaled et al., 2015), and enhances tumor growth by interacting to a common subunit (RBBP4/7) of a histone methyltransferase (PRC2) and histone deacetylase (SIN3A, NuDR) complexes to control expression and enhance tumor formation (Moody et al., 2018).

The bromodomain and extra-terminal (BET) families of protein are also implicated in the epigenetic control of gene expression; they detect lysine residues which are acetylated in nucleosomal histones (Filippakopoulos and Knapp, 2014; Ocaña et al., 2017). Inhibiting these proteins has been demonstrated to be anti-tumoral in solid tumors, as well as TNBC (Filippakopoulos and Knapp, 2014; Sahai et al., 2016; Sahni et al., 2016; Shu et al., 2016). Several BET inhibitors have demonstrated encouraging outcomes in preclinical research investigations, notably synergistic benefits with existing approved medicines (Shu et al., 2016; Nieto-Jiménez, Alcaraz-Sanabria et al., 2017; Ocaña et al., 2017; Vázquez et al., 2017), and the molecule OTX015/MK-8628 is currently being tested in clinical trials for TNBC (Ocaña et al., 2017).

According to a study employing the basal-like cell line MDA-MB-231, modifications in histones appear to play a crucial role in the EMT in TNBC. Down-regulation of histone methyltransferase G9a, H3K79 methylator DOT1L, and histone acetyltransferase KAT5 promotes E-cadherin production as well as an epithelial phenotype with reduced invasive and migratory ability (Gregoire et al., 2016). These discoveries could lead to new ways to reduce the risk of metastases by targeting epigenetic targets. EMT and the maintenance of the mesenchymal state could also be impacted by macroH2A1, a histone 2 variant. Increased expression of macroH2A1.1 was linked to claudin-low carcinoma subtype mesenchymal markers as well as a worse outcome in TNBCs (Lavigne et al., 2014).

Summary

Breast carcinoma is the most commonly detected life-threatening cancer among women today, as well as the main incidence of cancer death in females. Breast cancer research has made incredible advances in our knowledge of the illness over the previous two decades, leading to more effective and less harmful therapies. TNBC refers to a diverse spectrum of disorders characterized by genetic mutations. TNBC is extremely aggressive and also has a higher rate of early relapse in comparison to other breast carcinomas. TNBC is an immunohistochemically identified subtype with substantial subtype variability. TNBC is resistant to hormonal therapy and targeted treatments due to the negative expression of ER, PR, and HER2. TNBC has a small number of therapeutic options, all of which have low efficacy. Novel treatments are desperately needed. Clinical investigation indicates that risk factors such as age, race, pre-menopausal status, increasing parity, higher histological grade, and advanced illness were all independently linked with TNBC. On mammography and ultrasonography, TNBC can show benign but distinct characteristics. TNBC has more suspicious characteristics on MRI, which makes it the most effective screening tool for detection. There are also several interesting methods that can be used as prospective TNBC diagnostic techniques while also improving TNBC diagnostic efficacy. All carcinogenic disorders, including breast carcinoma, are currently being researched intensively for epigenetic alterations. TNBC may gain the most from breakthroughs in this area, as there is currently a lack of treatment targets for this subtype of cancer. In conclusion, triple-negative breast cancer is a unique subgroup of breast malignancies with different molecular profiles and a combination of risk factors, intrusive and rapid patterns of metastasis, a significant lack of treatment approaches, and a bad prognosis when compared to other breast carcinoma subtypes.

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CHAPTER

2

Novel biomarkers in triple-negative breast cancer - role and perspective

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Introduction

A biomarker may be defined as "any form, substance, or factor that is measurable and can influence or anticipate the consequences of a disease. According to the National Cancer Institute, the biomarker may be defined as a biological substance that is present in any body fluid including blood, or tissues that becomes an indication of normal or aberrant processes, or of any circumstance or ill-health, such as cancer (Fig. 2.1).

Biomarkers are frequently protein markers and genomic markers. TNBC's genetic and molecular profiles, which are noted for their great diversity and complexity, continue to challenge scientists all over the world. TNBC tumors are distinguished by the absence of PR, ER, and HER2 expression, as previously stated. The absence of treatment targets challenges efforts to classify TNBC using specific molecular markers in an attempt to enhance disease prognosis. Two significant investigations have been conducted to date on the genomic basis of TNBC (Koboldt et al., 2012; Shah et al., 2012). Table 2.1 summarizes genetic markers that affect prognosis and/or indicate suitable therapy (Fig. 2.2). To elucidate the mechanisms of somatic mutations, RNA-sequencing, exome-sequencing, targeted deep resequencing, and high-resolution single nucleotide polymorphism arrays were done on 104 primary TNBC specimens divided into several subgroups (Shah et al., 2012). EGFR (epidermal growth factor receptor) (5%), PTEN (phosphatase and tensin homolog) (3%), PARK2 (Parkinson disease 2) (6%), and RB1 (retinoblastoma gene 1) (5%) genes were found to have the greatest copy number abnormalities. TP53 alterations were shown to be the most frequent somatic abnormality, occurring in 53.8% of patients, whereas TNBC specimens also exhibited common mutations in the PIK3CA (10.2%), MYO3A (myosin IIIA) (9.2%), USH2A (usher syndrome 2A) (9.2%), RB1, and PTEN genes (7.7%). Nevertheless, only a small proportion of alterations (36%) were converted into mRNA (Shah et al., 2012) DNA methylation, genomic DNA copy

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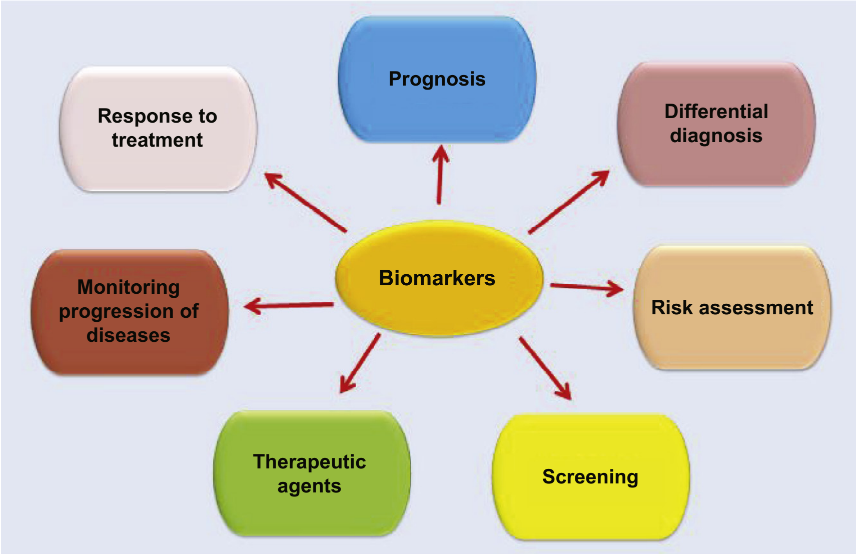

FIG 2.1 Potential uses of biomarkers.

TABLE 2.1 Important genetic markers in triple-negative breast cancer.

Gene	Type of alteration	Function	Prognostic significance	Predictive significance
TP53	Inactivating mutation	Apoptosis, DNA repair, and genome integrity	Bad prognostic factor, reduced OS, and a higher risk of metastasis	Bad response to chemotherapy
BRCA1	Inactivating mutation, epigenetic modifications	DNA double-strand break repair	Bad prognostic factor	Increased response to neoadjuvant anthracycline and taxane therapy, response to platinum-based therapy, and possible predictor of response to PARP inhibitors
PIK3CA	Activating mutation	Proliferation, differentiation, and survival	Poor prognostic factors	Possible predictors for response to PI3K/AKT/mTOR inhibitors
AR	Overexpression	Cell signaling	DFS and OS are probably better	Reduced chemotherapy sensitivity, increased sensitivity to AR inhibitors, PI3K inhibitors, and their combinations
BCL2	Overexpression	Antiapoptotic	Positive prognostic factor	Good predictor of response to CMF therapy, a poor predictor of response to neoadjuvant and adjuvant anthracycline-based chemotherapy.

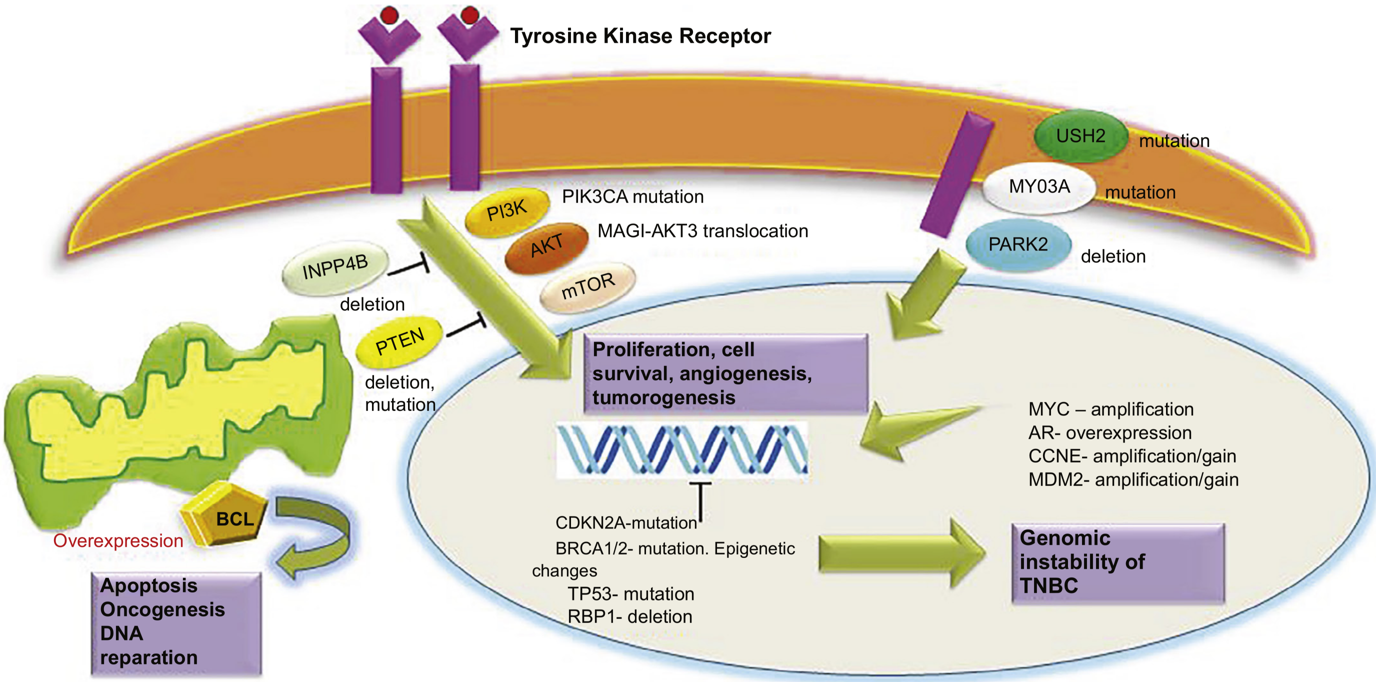

FIG 2.2 **Genetic markers with their abnormalities that affect prognosis and/or prediction of TNBC.**

number arrays, exome sequencing, microRNA sequencing, mRNA arrays, and reverse-phase protein arrays were all used by the Cancer Genome Atlas Group to examine specimens from 463 patients (Koboldt et al., 2012). The most frequently altered genes in a cohort of 93 basal-like tumors (76 TNBCs) were identified to be TP53 (80%), AFF2 (AF4/FMR2 family member 2) (4%), PIK3CA (9%), MLL3 (lysine methyltransferase 2C) (5%), RB1 (4%), and PTEN (1%). Changes in copy number were observed in a few genes or chromosomal regions, including gain or amplification of MYC (MYC protooncogene) (40%), CCNE (cyclin E1) (9%), (E3 ubiquitin-protein ligase Mdm2) (14%), and the 1q and 10p regions, and loss of RB1, PTEN, INPP4B (inositol polyphosphate-4-phosphatase type II B) (30%), and the 5q and 8p regions. Increased CDKN2A (cyclin-dependent kinase inhibitor 2A) expression, reduced RB1 expression, and elevated genomic instability also was discovered to be characteristics of the BLBC profiles (Koboldt et al., 2012; Mir et al., 2020).

The finding of the fusion gene EML4-ALK (echinoderm microtubule-associated protein-like 4-anaplastic lymphoma kinase) in NSCLC piqued researchers' interest in identifying such a structural rearrangement in breast tumors, notably in TNBC (Shaver et al., 2016). TNBC's entire exome sequencing revealed an elevation in MAGI3-AKT3 (membrane-associated guanylate kinase–AKT serine/threonine kinase 3) translocations and also rearrangements including the NOTCH1/2 (Notch 1/2) and MAST (microtubule-associated serine-threonine kinase) genes (Robinson et al., 2011; Banerji et al., 2012). Numerous biomarkers and related medicines have been identified in recent years, but just a few have proved beneficial in clinical studies. The biomarkers that helped to develop new authorized TNBC medicines are discussed in this chapter. We also talk about some of the emerging biomarkers that are demonstrating potential outcomes in clinical trials right now.

Triple-negative breast cancer: Genetic markers

TP53

TP53 is one of the key critical genes in apoptosis, cell-cycle arrest, and DNA repair for maintaining genomic integrity and homeostasis. TP53 mutations linked to abnormal p53 expression have been found in a variety of human malignancies, including all subtypes of breast carcinoma (Hussain and Harris, 2006). In node-negative breast carcinoma, the expression of mutated p53 has been linked to a higher rate of growth, early clinical relapse, and early mortality. The DNA-binding region of the TP53 gene is the most often altered region in breast t665umor, and missense mutations have been found as the cause of poor breast carcinoma results (Koboldt et al., 2012; Shah et al., 2012; Vegran et al., 2013). Although missense substitutions have been linked to the luminal subtype, frame-shift and nonsense alterations have been discovered to be common in basal-like cancers (Koboldt et al., 2012). TP53 mutations are more prevalent in ER-negative breast tumors than in ER-positive breast tumors (Langerød et al., 2007; Coates et al., 2012). Furthermore, p53 expression in ER-negative patients (HER2-positive and TNBC subtypes) has been linked to a better prognosis, whereas in ER-positive patients the p53 expression has been linked to a poor prognosis (Sakuma et al., 2011; Coates et al., 2012).

TP53 is the most commonly altered gene in TNBC, with alterations appearing in 65 to 80% (Koboldt et al., 2012; Shah et al., 2012). Alterations in TP53 were detected in 43% of non-basal TNBC and 62% of basal-like TNBC in one of the most comprehensive investigations conducted to date (Shah et al., 2012). Such alterations cause increased genomic instability and cytogenetic alterations in TNBC patients, and also a higher risk of heterozygosity loss (Mizuno et al., 2010; Olivier and Taniere, 2011). TNBC patients with impaired p53 function have been found in recent research to have a lower overall survival rate and a higher risk of metastatic disease (Kim et al., 2013; Powell et al., 2016). Other research, however, found no evidence that TP53 alterations and/or p53 expression are predictive factors; however, differences between TP53 mutation and p53 expression might be a possible predictor of bad TNBC prognosis. Mutations in TP53 have also been identified to be a predictor for chemoresistance in TNBC in other investigations (Geisler et al., 2001; Chae et al., 2009). Collectively, TP53 is mutated in the vast proportion of TNBC patients, making it a promising target for anticancer treatments.

BRCA1/2

The transcriptional control and activation of DNA damage, cell cycle regulation, and cellular differentiation and proliferation are all dependent on the gene products of BRCA1 and BRCA2 (Venkitaraman 2002). BRCA1/2 proteins, in particular, are required for DNA double-strand break repair via homologous recombination (HRR) and DNA stability control (D'Andrea and Grompe, 2003).

TNBC and/or BLBC account for over 80% of hereditary BRCA1-mutated breast tumors, with roughly 15% of TNBC patients carrying BRCA germ-line mutations (gBRCA) (Foulkes et al., 2003; Atchley et al., 2008; Chacón and Costanzo, 2010; Oakman et al., 2010; Couch et al., 2015; Engel et al., 2018). The other sporadic TNBC patients usually have similar features with BRCA1/2 mutant carriers in HRR abnormalities, which are also referred to as BRCAness (Turner et al., 2010). This BRCAness condition can be caused by the epigenetic silencing of BRCA1 by methylation of the promoter, which had been linked to a bad outcome in terms of relapse-free survival and overall survival following anthracycline- or taxane-based treatment (Sharma et al., 2014). Breast tumors with mutations in BRCA1 or BRCAness frequently display basal markers which correlate to the BL1 subtype and, as a result, respond to taxane and neoadjuvant anthracycline treatment (Sorlie et al., 2003; Masuda et al., 2013; Lehmann and Pietenpol, 2014). The POSH research, which evaluated the effects of gBRCA on breast carcinoma outcomes following standard therapy, has released some interesting observations. Overall survival over 10 years was 78% in gBRCA carriers against 69% in BRCA-negative individuals, implying that BRCA mutations gave significant survival benefit to its carriers (Copson et al., 2018). Improved gBRCA TNBC survival and most likely, BRCAness are induced by gBRCA carriers' increased sensitivity to chemotherapeutics as a consequence of HRR abnormalities or enhanced immune activation (Jiang et al., 2016; Han et al., 2018).

Furthermore, patients with BRCA1/2 deficiency should be more sensitive to DNA-damaging drugs such as PARP inhibitors and platinum compounds (Plummer 2011). In metastatic TNBC cancers with gBRCA mutations, the Treating to New Targets (TNT) trial found that carboplatin had a higher objective response rate than docetaxel (Tutt et al., 2018). Other trials have also shown that platinum-based treatment is highly effective in metastatic gBRCA TNBC (Isakoff et al., 2015). Furthermore, in the TBCR009 research, individuals with advanced TNBC who had abnormalities in the BRCA1/2 pathways (defined by higher levels of loss of heterozygosity score and large-scale state transition score) demonstrated good responsiveness to platinum treatment (Isakoff et al., 2015). Indeed, the TNT trial found that patients with higher homologous recombination deficit scores did not respond well to carboplatin (Tutt et al., 2018). Biomarkers of genetic instability that indicate a favorable response to platinum-based treatment for a subgroup of TNBC tumors should thus be verified (Anders et al., 2016). The relevance of the gBRCA mutations in platinum-based treatment response is unknown in the neoadjuvant setting. Many investigations showed that gBRCA carriers had higher responses; however, the GeparSixto research found that individuals with wild-type BRCA had higher responses (Byrski et al., 2009; Gronwald et al., 2009; Silver et al., 2010, Hahnen et al., 2017).

PI3K pathway

Modifications in cell differentiation, survival, and/or growth are typically reported in tumorigenesis when the PI3K/AKT/mTOR pathway is dysregulated (Cantley 2002). All carcinoma forms, including TNBC, have elevated signaling via the PI3K/Akt/mTOR cascade (Gonzalez-Angulo et al., 2009). INPP4B phosphatase and PTEN mutations are more prevalent in basal-like cancers than PIK3CA mutations (Cantley 2002; Shah et al., 2012). Because PIK3CA mutations are linked to ER positivity, they're more common in ER-positive breast tumors (HER2-enriched and luminal subtypes (Banerji et al., 2012; Dey et al., 2017).

PTEN is a key PI3K pathways negative regulator. PTEN loss has been linked to ER negativity and also a basal-like phenotype (Jones et al., 2013). Loss of PTEN relates to both accelerated tumorigenesis and a worse prognosis in TNBC (Beg et al., 2015). In primary ER-negative breast tumors, the INPP4B phosphatase, an additional negative regulator of the PI3K pathway, had been found to be often lost. Higher clinical grade, elevated tumor size, lack of hormone receptors, and invasive basal-like breast tumors are all linked to the loss of INPP4B (Fedele et al., 2010; Koboldt et al., 2012). Furthermore, oncogenic alterations in the PIK3CA gene, which codes for a catalytic subunit of PI3K (p110), are found in around 10% of TNBC instances and therefore could stimulate the PI3K pathway further. LAR has the maximum rate of PIK3CA mutations within TNBC subtypes, suggesting that treating AR and PIK3CA at the same time could be advantageous to patients(Lehmann et al., 2014). The new MAGI3-AKT3 translocation has been identified in addition to the recognized TNBC cancer-linked genes that regulate the PI3K pathway. About 7% of TNBC instances had this rearrangement, which causes constitutive AKT3 stimulation and PI3K pathway hyper-activation (Banerji et al., 2012).

Alterations in the PI3K/AKT/mTOR pathways are common in TNBC and are attractive therapeutic approaches. TNBC tumors are more responsive to combined therapy, according to preclinical evidence (Gordon and Banerji, 2013; De et al., 2014; Lehmann et al., 2014). PI3K, mTOR, AKT, and mTOR/PI3K inhibitors are now being evaluated in clinical trials for addressing TNBC alone or in conjunction with other medications (e.g., PARP, Cisplatin, and AR inhibitors) (Dey et al., 2017).

Androgen receptor

AR is a member of the steroid hormone nuclear receptor family, which also includes ER and PR (McGhan et al., 2014). AR controls genes implicated in metastasis, (Naderi and Hughes-Davies, 2008), FOXA1, p53, and PTEN as well as other cell-cycle regulators, and also the PI3K/AKT/mitogen-activated protein kinases signaling cascade (Peters et al., 2009). The expression of AR has been discovered in over 70% of breast tumors and is linked to ER positivity (Loibl et al., 2011; He et al., 2012). AR-positive is more frequent in older females with breast carcinoma and is linked to a nuclear grade, lower stage, and risk of lymph node involvement, and also a lower tumor size at the time of diagnosis, lower recurrence risk, and improved overall and disease-free survival (Qu et al., 2013; Vera-Badillo et al., 2014; Mina et al., 2017). AR-positive is seen in 13% to 37% of TNBC patients and is related to LAR subtype and older age at diagnosis (Mina et al., 2017). The prognostic importance of AR positivity is debatable; in earlier research, AR positivity has been linked with both good and bad prognoses (Tang et al., 2012, Qu et al., 2013; Choi et al., 2015; Aleskandarany et al., 2016). AR-positive TNBC shows a reduced Ki-67 index as compared to AR-negative TNBC and may be less responsive to chemotherapy, (Barton et al., 2015) which is consistent with results showing that the LAR subtype had poorer pCR rates than that of other TNBC subtypes (Masuda et al., 2013).

Cell line models of the LAR subtype are partly dependent upon AR signaling, according to preclinical in vitro and xenograft investigations (Cochrane et al., 2014; Lehmann and Pietenpol, 2014). Tumor growth and cell viability were significantly reduced by siRNA knockdown and pharmacological suppression of AR. Furthermore, all of the LAR cell lines studied have an activating mutation in the PIK3CA kinase domain (H1047R), making them sensitive to PI3K inhibitors (Lehmann et al., 2014). PIK3CA alterations have been identified in around 40% of AR-positive TNBC patients. Xenograft and in vitro studies had indicated that treating both non-LAR and LAR TNBC subtypes using the AR inhibitors bicalutamide and enzalutamide lowers proliferation, migration, anchorage-independent growth, and infiltration, while increasing apoptosis (Barton et al., 2015; Zhu et al., 2016). As a result, it is likely that a good response to AR antagonists is not confined to the LAR TNBC subtype. The TBCRC011 research, on the other hand, found a rather poor response; with a six-month clinical improvement rate of 19% for bicalutamide for AR-positive patients in comparison to 18% in the intention-to-treat group (Gucalp et al., 2013). Enzalutamide demonstrated better clinical efficacy in the MDV3100-11 study, with a 6-month clinical improvement rate of 28% for AR-positive patients in comparison to 20% in the intention-to-treat group (Traina et al., 2018). Alternative treatment options are currently being researched, including CYP17 (cytochrome P450 family 17 subfamily a member 1) inhibitors, AR inhibitors in association with CDK4/CDK6 (cyclin-dependent kinase) inhibitors, neoadjuvant chemotherapy, and PI3K inhibitors (Mina et al., 2017).

The clinical benefit of screening for AR-positive is that it is a readily detectable marker that really can reveal subgroups of TNBC patients who would have modest therapeutic benefits from regular treatment. AR-dependent TNBC individuals may benefit from targeted treatments based on AR antagonists alone or in conjunction with other therapeutic agents.

BCL2 gene

B-cell lymphoma 2 (BCL2) is an anti-apoptotic and carcinogenic mitochondrial protein. BCL2 inhibits cellular growth and proliferation, as well as DNA damage, causing genetic instability (Wang et al., 2008). BCL2 expression has been shown to be a potential predictive and prognostic marker, particularly in hormone receptor-positive, node-negative breast carcinoma, in a number of investigations (Paik et al., 2004; Ali et al., 2012). Because estrogens directly up-regulate BCL2 expression, ER-positive breast tumors frequently have increased levels.

The function of BCL2 in the setting of TNBC is not well understood. BCL2 positivity was discovered to be a favorable prognostic marker in TNBC, with the ER-BCL2+ group outperforming the ER+BCL2- group (Dawson et al., 2010). Furthermore, BCL2 positive was found to be a predictor of sensitivity to anthracycline-based chemotherapy in both neoadjuvant and adjuvant settings. The lack of expression of BCL2 in pre-chemotherapy TNBC specimens was linked to an increased probability of pCR after neoadjuvant doxorubicin-based chemotherapy, and it was also discovered to be an independent prognostic factor of pCR (Pusztai et al., 2004). When TNBC was administered with anthracycline-based chemotherapeutic in an adjuvant context, reduced BCL2 expression was also linked to better results (Bouchalova et al., 2015). Furthermore, increased BCL2 expression appears to predict 5-fluorouracil, cyclophosphamide, and methotrexate response (Bouchalova et al., 2014). The process underlying this response is unknown, however, it could be affected by expression changes of genes linked to BCL2 levels, such as MDM4 (Mdm2-like P53-binding protein), HER3 (human epidermal growth factor receptor 3), and p27 proteins (Abdel-Fatah et al., 2013). In clinical settings, adding BCL2 to the screening array would be easy and can give valuable predictive and prognostic knowledge about TNBC patients.

Cyclin-dependent kinases

Cyclins and CDKs are essential to cell cycle regulators and are mutated in almost all cancers. TNBC showed abnormal expression of cyclin D, cyclin E, CDK2, CDK4/6, and other proteins, suggesting that CDK inhibition therapies could be a promising treatment option (Keyomarsi et al., 2002; Velasco-Velázquez et al., 2011; Balko et al., 2014). More than ten CDK inhibitors are being studied in clinical studies, with abemaciclib, ribociclib, dinaciclib, and palbociclib, being the most effective. Palbociclib and ribociclib, CDK4/6 blockers, have previously been licensed for the treatment of metastatic breast carcinoma patients having hormone receptor-positive and HER2 negative (Walker et al., 2016). CDK4/6 inhibition (palbociclib/ribociclib) was shown to be highly responsive to the LAR subtype in TNBC. In TNBC cell lines with PIK3CA mutations, CDK4/6 inhibitors were also effective with PI3K inhibitors (Asghar et al., 2017). Inhibition of CDK4/6 was recently discovered to prevent breast cancer metastases in a TNBC xerograph model. Palbociclib inhibition had little effect on the primary tumor's development, but it did considerably slow the spread of TNBC to other parts of the body by destabilizing the SNAIL1 protein (Liu et al., 2017). Ribociclib and palbociclib, in conjunction with bicalutamide (AR antagonist), are presently being investigated as treatments for metastatic AR-positive TNBC. Abemaciclib, which has a distinct toxic potential, is being investigated as a single drug in metastatic TNBC with elevated RB1 expression (National Library of Medicine 2018). Dinaciclib (a pan-CDK inhibitor) has recently been proven to have anti-TNBC efficacy in vitro and in vivo (Rajput et al., 2016). Dinaciclib, which failed in conjunction with epirubicin due to significant toxicity, is now being investigated in conjunction with Pembrolizumab (Mitri et al., 2015).

Triple-negative breast cancer: Novel biomarkers

TNBC is distinguished by the presence of definite biomarkers. Although the occurrence of these molecules is not limited to TNBC, it does appear to be more prevalent in this subtype. The major biomarkers in TNBC are listed below.

EGFR

EGFR is among four strongly linked receptors that all play a significant role in cancer cell survival. EGFR (or ErbB-1), HER-2/neu (ErbB-2), HER-3 (ErbB-3), and HER-4 (ErbB-4) are the four receptors (Noonberg and Benz, 2000). Following ligand activation and TK, the inactive monomer receptor dimerizes, and the intracellular region of the receptor is activated via autophosphorylation, resulting in a sequence of intracellular processes. The EGFR signaling pathway is required for angiogenesis, cell proliferation, metastatic expansion, and apoptotic inhibition (Siziopikou and Cobleigh, 2007). The majority of TNBCs express EGFR, posing a significant treatment challenge (Bhargava et al., 2005). Differential EGFR expression has been identified in metaplastic breast cancer, a phenotype of BLBCs, in studies using various techniques of gene amplification (Reis-Filho et al., 2006; Gilbert et al., 2008; Gwin et al., 2011). Toyama and coworkers (Toyama et al., 2008) used real-time PCR to find that TNBCs have a higher number of copies of the EGFR gene. EGFR expression is present in 40% to 50% of women with breast carcinoma and in 80% of TNBC and is thought to substitute main breast carcinoma proliferative pathways generated by activation of ER, HER-2, and PR proteins, which are lacking in TNBC (Bidard et al., 2007).

The researchers discovered that 60% of patients having grade III cancer and more than three lymph nodes exhibited EGFR expression, implying that expression of EGFR is linked to cancer aggressiveness. Individuals with EGFR expression also exhibited lower DFS, OS, distant disease-free survival (DDFS), and cause-specific survival (Viale et al., 2009). The expression of EGFR in TNBC is linked to a poor treatment response towards chemotherapeutics (Rakha et al., 2007). According to Nogi and coworkers (Nogi et al., 2009), EGFR expression was found in 24% of TNBC individuals and was associated with worse responsiveness to chemotherapy and survival, whereas luminal groups with EGFR expression had better responsiveness to chemotherapy and survival. EGFR has recently been described in conjunction with additional markers to distinguish the BL subtype from TNBC (Mehdizadeh et al., 2012). This helps to classify TNBC into subtypes, allowing for the identification of prognostic differences and molecular targets. As a result, EGFR acts as a biomarker in TNBC and a target for the TK inhibitor cetuximab (Rydén et al., 2010). Its reaction in TNBC has been studied extensively (Carey et al., 2006; Alvarez et al., 2010; Rydén et al., 2010). EGFR expression was found to be a predictive marker for DFS in a recent study (Liu et al., 2012), not just in univariate but even in multivariate analysis.

VEGF

Angiogenesis is critical for tumor growth and expansion, particularly over a diameter of 2 mm, because nutrients and oxygen cannot travel further than this range. To facilitate neovascularization, angiogenic signals are facilitated by VEGF (vascular endothelial growth factor). Placental growth factor and VEGF A, B, C, D, E (viral factor) is a group of six proteins. Because of alternate splicing of its mRNA, the VEGF protein exists in four isoforms (Gerwins et al., 2000). The 165-amino-acid molecule is more frequent among the many VEGF165 isoforms (Ferrara et al., 2003). Several factors influence its gene expression, including NO, hypoxia, growth factors, tumor suppressor genes, oncogenes, and HER-2 (Benjamin and Keshet, 1997).

It promotes the proliferation of endothelial cells while also preserving their functional and structural integrity. It also modulates vascular permeability as well as the endothelial stem cells movement from bone marrow (Gerber et al., 1998). VEGF also regulates tumor neovascularization by boosting the production of anti-apoptotic molecules such as Bcl2, BIRC5, and XIAP. Endothelial cells die and newly produced vasculature disintegrate in the absence of it (Fox and Harris, 2004; Olsson et al., 2006). As a result, VEGF expression is required for neovascularization during tumor growth. VEGF interacts with a variety of receptor TKs, including VEGFR-1, VEGFR-2, and VEGFR-3. Angiogenesis is triggered by VEGF binding to VEGFR-2, which causes the specific stimulation of TKs, which leads to the adherence, survival, migration, proliferation, actin remodeling, and vascular permeability of endothelial cells (Iosifidou et al., 2009).

In DCIS and invasive breast carcinoma, VEGF expression is increased. It's also been used to predict prognosis in breast carcinoma (Ali et al., 2011; Chanana et al., 2014). Its measurement in tissue extracts by Immunoassay or IHC has revealed a substantial correlation with micro vessel counts and density. Because higher mean vascular density in breast carcinoma has been associated with more active tumor behavior and worse survival, intra-tumoral microvessel density is currently regarded as one of the essential determinants influencing survival (El-Arab et al., 2012). Recent research (Taha et al., 2009; Chanana et al., 2014) found a direct correlation between tissue and serum VEGF levels with grade III tumors, bigger tumor size, negative hormonal status, and positive lymph nodes, and worse survival, as well as a significant drop in levels after treatment. Elevated VEGF levels in TNBC are related to shorter DDFS, DFS, and OS. VEGF levels have also been found to be linked to tumor grade, size, and metastatic areas. Patients with increased VEGF levels had progression of the disease despite treatment, and they had a considerably poorer progression-free survival rate than those with decreased levels. When TNBC patients were given FAC, VEGF levels increased considerably from baseline to the middle of the treatment but did not increase substantially from the middle to the completion of the therapy (Linderholm et al., 2009; Taha et al., 2009, El-Arab et al., 2012). The VEGFs expression in various researches is shown in Table 2.2.

C-kit and basal cytokeratins

The cytokine receptor C-kit can be found on the hematopoietic stem cells surface as well as other cells. C-kit is a growth factor receptor that increases major physiological activities

TABLE 2.2 Expression of the VEGF receptor in triple-negative breast cancer.

S. No	Total no. of patients	No. of TNBC patients	VEGFR-2 expression	References
1	679	87	Elevated intratumor VEGF levels in TNBC	(Linderholm et al., 2009)
2	-	73	77%	(Iosifidou et al., 2009)
3	69	35	34%	(Andre et al., 2009)
4	70	27	54% in TNBC vs. 23% in non-TNBC	(Chanana et al., 2014)
5	1132	103	93.2%	(Mehdizadeh et al., 2012)

like adherence, cell survival, differentiation, proliferation, and chemotaxis by binding to stem cell factors. It promotes apoptosis and enhances cancer cell invasiveness (Andre et al., 2009). CKs are intermediate filament keratin-containing proteins present in epithelial tissue's intracytoplasmic cytoskeleton. During the time of terminal development and differentiation, distinct epithelial cells express distinct CKs. This variation in CK expression aids in the categorization of all epithelia. Distinct tumors also express distinct CKs from that epithelium. As a result, whenever an epithelium undergoes aggressive transformation, the CKs expression pattern tends to remain constant.

The use of IHC techniques to examine the CK pattern is critical for tumor pathologic categorization (Edling and Hallberg, 2007). These CKs were originally used to differentiate malignant from benign breast lesions (Schweizer et al., 2006), but their predictive value was later determined, and it had been discovered that expression of CK-14, CK-5, as well as CK-17, was associated with bad prognosis, ER negativity, short DFS, and OS and high-grade tumors (Otterbach et al., 2000; Ross and Perou, 2001; Abd El-Rehim et al., 2004). In BLBCs, it is expressed. Because BLBC and TNBC have similar traits, C-kit and basal CKs as well as other biomarkers and pathological characteristics, are utilized to distinguish TNBC from BLBCs. Several research have shown that the occurrence of CKs is greater in TNBC as compared to non-TNBC and that it is significantly greater in the BL subtype among TNBC subgroups (Table 2.3). Based on the CK and EGFR expression, the BL subclass of TNBC was discovered, and when clinicopathological aspects were examined among both the basal and non-BL, it was discovered that the BL subtype of TNBC was highly aggressive (Van De Rijn et al., 2002, Bryan et al., 2006; Kim et al., 2009; Rakha et al., 2009; Thike et al., 2010).

TOP-2A

Topoisomerase II α is encoded by the TOP-2A gene, which is important for DNA transcription. The enzyme induces the temporary breakage of double strands of DNA duplex and reunites them in a way that both strands cross over each other, affecting the structure of DNA. Cancer mutation leads to a reduction in its functioning and consequently deterioration of the condition. The gene works as an anthracycline target treatment which is a topoisomerase II inhibitor, in TNBC or breast carcinoma (Burgess et al., 2008). As a result, it serves as a marker for assessing anthracycline resistance. TOP-2A expression was shown to be greater

TABLE 2.3 Expression of C-kit in triple-negative breast cancer.

S. No	Total number of patients	No. of TNBC patients	c-kit expression	References
1	-	21	C-kit in 29% and CK 5/6 in 62%	(Nielsen et al., 2004)
2	66	4	75% of TNBC vs. 29% of non-TNBC	(Bryan et al., 2006)
3	625	147	C-kit in 11.6% and CK5/6 in 35.4%	(Kim et al., 2009)
4	7048	767	C-kit in 45%, CK 5/6 in 6%, CK-14 in 48%, CK-17 in 50%	(Thike et al., 2010)

in 2.7% to 8.8% of TNBC patients in a study (Knoop et al., 2005). Its overexpression in TNBC causes a reduction in anthracycline sensitivity and, as a result, a decline in response (Weigelt et al., 2008).

Ki67

Ki67 also called MKI67, is a cell proliferation marker. In interphase, the Ki67 antigen is found within the nucleus of the cell, and during mitosis, it is found on the chromosomal surfaces. Because it is a proliferation marker, it is present in all cells throughout the dividing stages of the cell cycle (G1, S, G2, and mitosis), but not in the resting stage (G0). It had become a marker of cellular proliferation due to its absence in resting cells and widespread existence in proliferating cells (Urruticoechea et al., 2005). Proliferation is a prominent hallmark of cancer progression and may be measured by IHC measurements of the nuclear antigen Ki67.

In healthy breast tissues, Ki67 expression is lower (around 3%). Ki67 antigen and steroid-receptor are expressed in various cells in healthy human breast epithelium, according to many types of research. Ki67 was shown to be overexpressed in ER-negative cells, and its expression in cancer cells was significantly higher (Harvey et al., 2008; Zhou et al., 2009). High Ki67 levels in breast carcinoma are linked to a bad outcome, despite the fact that these tumors respond well to combined chemotherapy. Nevertheless, its independent importance is low and will not justify measurements in ordinary clinical settings. With regard to sensitivity to treatment in breast carcinoma, Ki67 expression was discovered to be an independent prognostic marker of clinical complete response; pathologic complete response (pCR), OS, DDFS, and locoregional relapse in breast carcinoma patients. Patients without pCR also demonstrated a drop in Ki67 index after treatment (Fasching et al., 2011; Tanei et al., 2011; Selz et al., 2012). Elevated Ki67 levels were related with bad prognosis in a recent meta-analysis by de Azambuja and coworkers (De Azambuja et al., 2007), who retrieved DFS results from 29 studies and found that elevated Ki67 levels were linked with bad prognosis regardless of nodal status or whether patients received treatment or not.

Ki67 levels were observed to be substantially higher in ductal TNBC than in other histological categories of TNBC (80% in TNBC and 10%-30% in other types). Its expression was likewise connected to tumor grade and size in TNBC patients, with larger levels (> 35% staining) being associated with a greater risk of death (Munzone et al., 2011). Ki67 increase was linked to a better pCR to chemotherapy in TNBC patients, but a poorer RFS and OS. Its expression was also utilized to divide TNBC patients into two subgroups, with only 26.7% showing decreased Ki67 expression (Keam et al., 2011).

PARP

PARPs are a group of eukaryotic cell signaling enzymes that catalyze DNA binding protein poly (ADP-ribosylation). There are currently eighteen PARP enzymes known, with PARP1 being the most frequent. PARP1′s primary function is to detect DNA damage nicks. It uses NAD+ to build polymers of nicotinamide and ADP-ribose. PARP1 activation is significant in tumors for three biological causes: firstly, it is involved in DNA repair via the base excision

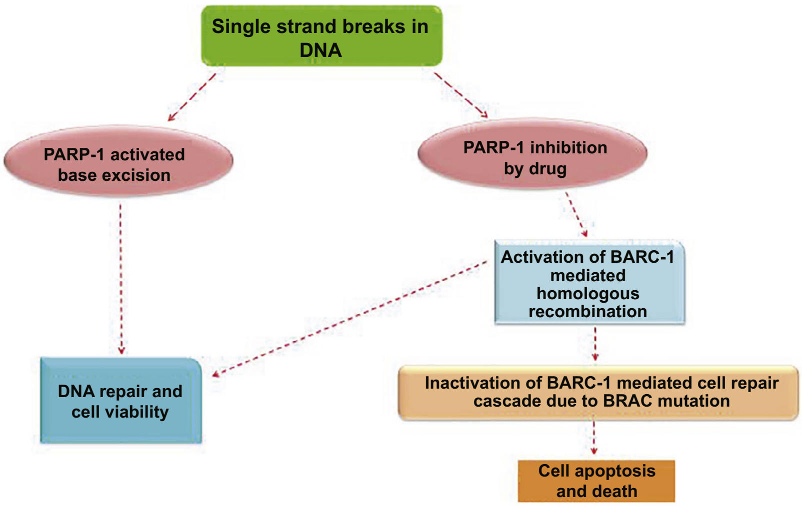

FIG 2.3 **Inhibitors of poly(ADP-ribose) polymerase-1 and their mode of action in triple negative breast cancer.**

repair mechanism; secondly, it can deplete cellular energy pools, resulting in cell necrosis and dysfunction; and finally, it can stimulate proinflammatory gene transcription. PARP enzymes play a role in cellular responses during inflammation, oxidative stress, and ischemia. Inflammation, ischemia, and oxidative stress all cause PARP enzymes to be activated. Carcinogenesis is a multi-step phenomenon that involves changes in a variety of biological functions, including genomic integrity, cell division, growth, proliferation, differentiation, and death of cells. All of these cellular activities include PARP1, suggesting a putative relationship between PARP1 functioning and carcinogenesis (Fong et al., 2009). PARP1 aids in the repair of DNA SSBs by attaching to the exposed ends of the broken DNA strand and bringing in key enzymes necessary for SSB repair (Bhattacharyya et al., 2000; Bryant et al., 2005; Farmer et al., 2005; Evers et al., 2008; Hastak et al., 2010). When PARP1 is blocked, the base excision repair mechanism fails; resulting in the buildup of SSBs. Cell division is stopped at SSBs in dividing cells entering S-phase, resulting in a DSB (Fig. 2.3).

Because the excision repair process in BRCA1-deficient cells is reliant on PARP1, PARP1 inhibition causes cell death via apoptosis (Bhattacharyya et al., 2000; Farmer et al., 2005). BRCA2, like BRCA1, works via the excision repair mechanism, and mutations in this gene make cells susceptible to PARP inhibitors (Bryant et al., 2005; Evers et al., 2008). PARP, like BRCA, is essential for DNA repair. It detects SSBs and repairs them via the base excision repair mechanism, unlike BRCA (Fong et al., 2009). Inhibitors of PARP are efficacious in TNBC because harm to one of the arms of the DNA cannot be repaired by homologous recombination owing to BRCA mutation, as well as inhibition of PARP in synergism would then generate a condition of “synthetic lethality” - a process which happens when individual genes inactivation has no impact but mutations in both genes result in cancer cell death (Bhattacharyya et al., 2000). As a result, in TNBC, the BRCA mutation is essential for the effect of several chemotherapeutic drugs. Ionizing radiation and several medicines, like DNA methylating drugs, platinum agents, and topoisomerase I inhibitors are known to increase the effects of PARP1 inhibition. The utilization of PARP inhibitors in combination with platinum drugs has been demonstrated to improve RFS and OS in mice models (Bhattacharyya et al., 2000; Fong et al., 2009) and numerous additional investigations on cell lines have found that PARP inhibitor efficacy is boosted in the presence of BRCA mutations or malfunction (Fong et al., 2009; Hastak et al., 2010). PARP1 has been directed as a therapeutic approach in TNBC with medications like olaparib, iniparib, and others. While these treatments have not proven to be beneficial on their own, their introduction to cytotoxic agents has undoubtedly increased their effectiveness and improved therapy response in TNBC patients.

Heat shock protein 90

It is a type of cellular chaperone (proteins that aid in the disassembly or assembly of many macromolecular complexes) which mediates the post-translational modifications and stabilization of many conformationally labile proteins, AKT, steroid receptors, RAF-1, cyclin-dependent kinase 4, as well as other proteins that are capable of sending cell proliferation signals (Whitesell et al., 1994). When the action of heat shock protein (HSP) 90 is inhibited, proteosomes break down the proteins that it depends on. Low HSP αB-crystalline expression is found in BLBCs and is linked to lower survival. Overexpression of this protein is linked to neoplastic alterations in breast acini and enhances invasion and metastasis in vitro. Both tanespimycin and geldnamicyn are antibiotics that also act as HSP inhibitors. Both have been proven to be clinically beneficial in patients with HER2-positive metastatic breast carcinoma. Another HSP blocker, PU-H71, demonstrated 100% responsiveness in TNBC models (Caldas-Lopes et al., 2009).

Cox-2

Cox is a prostaglandin and arachidonic acid converting enzyme. It's a 74-kilodalton protein found in the reticulum, endothelium, and nuclear membrane of cells. Stimuli like inflammatory responses and tumor promoters cause it to be expressed. Liu and coworkers (Liu et al., 2001) discovered that 85% of transgenic mice having Cox overexpression got breast carcinoma, implying that this enzyme is involved in breast cancer. Its expression has been linked to invasiveness and metastatic stimulation in breast carcinoma in several studies (Costa et al., 2002; Half et al., 2002). Overexpression of Cox-2 is found in around 40% of BC patients. Cox-2 can potentially be utilized as a biomarker to monitor breast cancer patients' responses to neoadjuvant treatment.

In women with breast cancer, lymph node status is extremely important for prognosis. The cox-2 expression has been linked to positive lymph node involvement in research. As a result, Cox-2 may play a function in lymphangiogenesis. In breast carcinoma, expression of Cox-2 has also been linked to hormonal receptors; negative HRs with Cox-2 expression has a poor prognosis. Cox-2 is linked to HER2 via the Ras/MAPK cascade and has been linked to the overexpression of HER2. MDR-1, a multidrug-resistant gene, is also linked to Cox-2 expression. Patients who show both of these are the least sensitive to treatment. As a result of its association with a number of nodes involved, tumor size, HRs, and HER2 status, Cox-2 may be a useful biomarker in breast carcinoma patients (Surowiak et al., 2005).

Epigenetic modifications in TNBC as novel biomarkers

Analysis of epigenetic alterations is one method for identifying biomarkers for TNBC. Epigenetics is the study of heritable phenotypic modifications that aren't caused by a change in the sequence of DNA. Conrad H. Waddington developed the terms "epigenesis" and "genetics" to define the "causal mechanisms" through which "the genes of the genotypes brought about phenotypic consequences" in epigenetics in 1942. It took almost 50 years for scientists to comprehend the fundamental mechanics of Waddington's findings due to a lack of experimental equipment and general understanding (Holliday, 1987). Multiple findings have been reported to date that show epigenetics can modify phenotypic without changing the DNA sequence. These include traditional epigenetic processes like histone modifications, chromatin remodeling, and DNA methylation, as well as epigenetic alterations caused by small/non-coding RNAs like miRNAs, which have only recently been found. These have been thoroughly examined in (Virani et al., 2012).

Tissue miRNAs as biomarkers in TNBC

MiRNAs that can be used as biomarkers could be found individually or as part of a set of miRNAs, called miR-signatures that are all linked to TNBC. MiR-155, miR-10b, and miR-21 are examples of independent markers that are also found in different miR-signatures. Furthermore, these molecules are dysregulated in a variety of neoplasms, including TNBC (Sempere et al., 2007; Sempere et al., 2010).

TNBC tumor and healthy breast tissues can be distinguished using an 11-miRNA signature (miR-21, miR10b, miR-31, miR-130-3p, miR-125b, miR-155, miR-181a, miR-181b, miR-183, miR-195, miR-451a) (Ouyang et al., 2014). Tissue biopsies were also taken before receiving systemic therapy in a group of 11 patients with TNBC. A signature consisting of three miRs (mir-190a, miR-200b-3p, and miR-512-5p) has been linked to complete pathologic response to various treatment methods (Kolacinska et al., 2014). Another study found an additional miR-signature (miR-16, miR-125b, miR-655, miR-374a, miR-421, miR-374b, miR-497, miR-155) that might serve as a prognostic biomarker for OS and disease-free survival in a sample of 173 TNBC patients (up to 50 years old) (116). MiR-148a and miR-629-3p have also been linked to lung metastases, whereas miR-141 has been linked to brain metastases (Debeb et al., 2016; Song et al., 2016). TNBC progression and metastases are also linked to the miR-10 family (Zhang et al., 2006).

Increased expression of miR-95-3p is associated with reduced OS and relapse-free survival in patient populations receiving anthracycline-based chemotherapy, and some other five-miRNA signatures (such as miR-30c-5p, let-7d-3p, miR-30a-3p, miR-95-3p, and miR-128-3p) has been evaluated as a novel predictive and prognostic biomarker in TNBC, forecasting patient's responsiveness to anthracycline-based chemotherapy (Turashvili et al., 2018). In addition, in a study of 173 TNBC cases, two miR-signatures were discovered. The first four miR signatures (miR-155, miR-16, miR-374a, and miR-125b) are linked to a low rate of survival. The second is composed of four miRs (miR-155, miR-27a, miR-30e, and miR-493) and has been associated with BC categorization based on ER/PR/HER2/EGFR/basal cytokeratin status, and also case classification into low- and high-risk subgroups (Usmani et al., 2015), as well as the potential to predict patient reaction to treatment with the two most frequent systemic TNBC treatments (anthracycline or anthracycline in combination with taxanes) (Gasparini et al., 2014).

Circulating miRNAs as TNBC biomarkers

Circulating miRNAs have been characterized as potential diagnostic, prognostic, or predictive biomarkers for BC in a number of recent investigations. MiRNA synthesis and maturation take place in the cytoplasm and nucleus of cells, and miRNAs can be released from the cytosol and become extracellular circulating miRNAs. MiR transport pathways within organisms include 1) wrapping in giant apoptotic bodies, 2) wrapping in HDL or LDL lipoprotein complexes, 3) wrapping in smaller exosomes or microvesicles, and 4) wrapping in an AGO protein complex (Matamala et al., 2015). In cell-cell interaction, lipid vesicles and exosomes play crucial functions. The microenvironment, and also various body fluids like plasma, saliva, serum, urine, breast milk, seminal fluid, and cerebrospinal fluid, could be used to identify and isolate cell-free circulating miRNAs (Weber et al., 2010) (Fig. 2.4).

There are advantages and disadvantages to the methods utilized to verify miRNAs as biomarkers in TNBC. In the pre-analytical phase, miRNAs have the advantage of being detectable in biological fluids, requiring a minimally invasive collection process, and being stable under a wide range of conditions (extreme pH values, repeated freeze-thaw, up to 24 h at room temp, or for lengthy time frames at 70°C) (Takahashi et al., 2013). Numerous limitations linked to patients' daily behaviors (physical exercise, smoking, food, renal pathology, and circadian rhythms), specimen collection, and handling are problems with miRNA biomarker validation (Baggish et al., 2011; Witwer 2012; Cheng et al., 2013; Takahashi et al., 2013; Lima-Oliveira et al., 2016). The primary analytical approach for assessing circulating miRNAs is real-time quantitative PCR (RT-qPCR); but, additional platforms, such as other PCR-based techniques, microarrays, and next-generation sequencing (NGS), could be employed as well. Contamination in noncirculating miRNA (skin, blood cells, and activated platelets) and hemolysis (Bustin and Nolan, 2004; Boeckel et al., 2013; Willeit et al., 2013) can have an impact on validation during the analytical phase. Finally, there is a lack of established standards and protocols in the post-analytical stage, which is cause for concern (Faraldi et al., 2018).

Novel developments in circulating miRNAs as diagnostic biomarkers for TNBC

Several studies have demonstrated the value of miRNA profiling as a noninvasive method for detecting and managing BC molecular subtypes. MiR-195-5p and miR-495 downregulation may be useful as a circulatory surrogate molecular marker for earlier diagnosis of luminal or TNBC cancers (Mishra et al., 2015). For patients having TNBC, additional seven-serum miRNA panel (miR-7-5p + let-7c-5p + miR-489-3p + miR-199a-3p + miR-195-5p + miR-15a-5p + let-7i-5p) can be used as a diagnostic marker (Qattan et al., 2017). An array of nine miRNAs (miR-18a, miR-107, miR-15a, miR-133a, miR-139-5p, miR-425, miR-143, miR-145, and miR-365) could also be used as part of a blood-based multi-marker assay for BC identification (Kodahl et al., 2014). Furthermore, additional 5 circulating miRNA

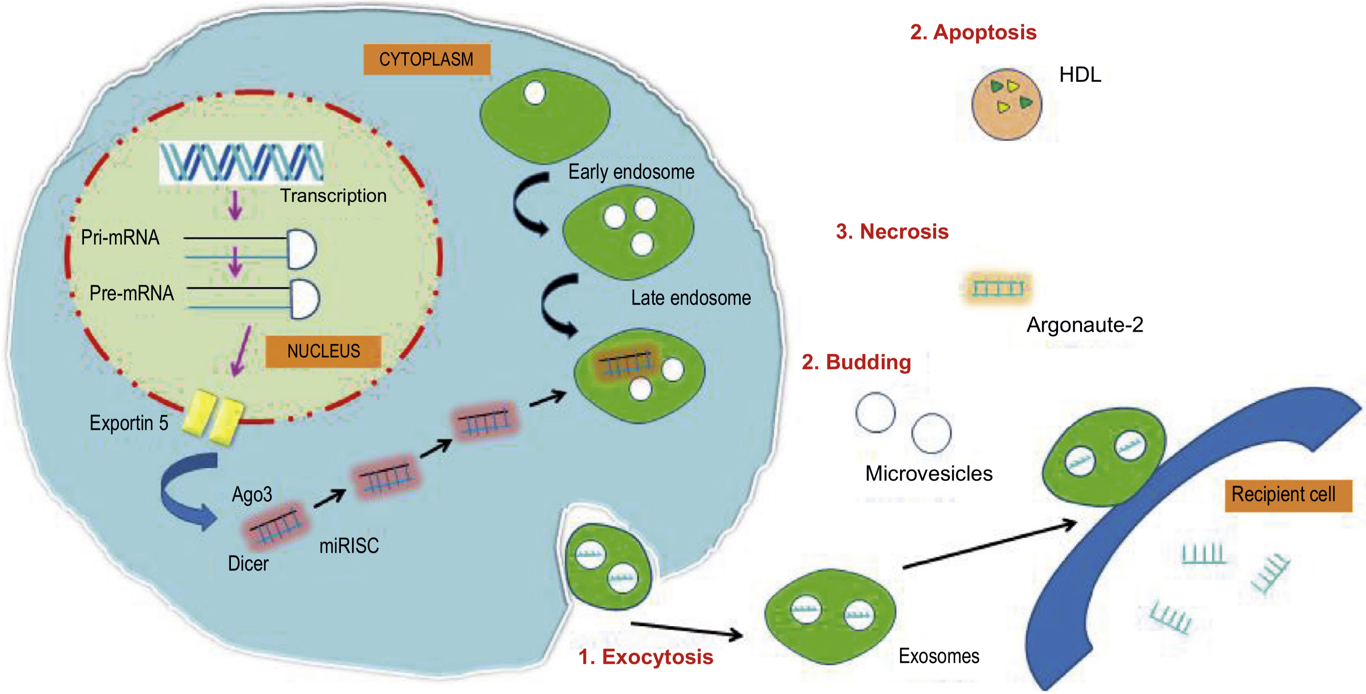

FIG 2.4 **Circulating mRNA sources and pathways 1.** Exosomal secretion-Pri-miRNA is produced, processed by Drosha, and delivered in the cytoplasm by Exportin5, where it is incorporated in the RISC complex and targets mRNA in exosomes secreted in human fluids. 2. Microvesicles develop via budding from the plasma membrane. 3. AGO-miRNA complexes are released, resulting in necrosis. 4. Apoptosis, binding of high-density lipoproteins (HDL).

combinations (miR-1246, miR-1307-3p, miR-4634, miR-6861-5p, and miR-6875-5p) can diagnose TNBC in the test group with 97.3% sensitivities, 89.7% correctness, and 82.9% specificity. In addition, this combination can identify early-stage BC (98.0% sensitivity for stage 0) (Shimomura et al., 2016).

Several circulating miRNAs have been found to be overexpressed in stage II and III TNBC patients (hsa-miRNA-188-5p, hsa-miRNA-4281, hsa-miRNA-1202, hsa-miRNA-1207-5p, hsa-miRNA-1225-5p, hsa-miRNA-4270, hsa-miRNA-642b-3p, hsa-miRNA-3141, hsa-miRNA-1290, miRNA-127-3p, miRNA-148b, miRNA-652, miRNA-409-3p, and miRNA-801) (Cuk K et al., 2013). In TNBC, the expression of serum miR-21 is linked to lymph node metastases and elevated Ki-67 expression ($p<0.01$) (Song et al., 2016). Six miRNAs were evaluated in a meta-analysis of 21 relevant types of research (2510 patients) that investigated the predictive significance of miRNAs in TNBC by evaluating miR expression levels in tumor or blood specimens: miR-155, miR-210, miR-21, miR-27a/b, miR-374a/b, and miR-454. The findings revealed that low levels of miR-155 are linked to a decreased OS. Elevated levels of miR21 expression also were related to shorter OS, and increased levels of miR-454, miR-27a/b, and miR-210 expression were linked with reduced OS, and levels of miR-374a/b and miR-454 expression were related with DFS (Lü et al., 2017).

LncRNAs as potential TNBC biomarkers

Transcriptome microarrays were used to examine 165 TNBC samples and 33 paired healthy breast tissue in a prospective observational investigation employing frozen tissue segments. Based on 8 mRNAs and 2 lncRNAs (SNRPEP4 and HIST2H2BC), an integrated mRNA-lncRNA signature was analyzed. This signature is highly accurate as compared to traditional prognostic markers in determining 2-year relapse-free survival and can effectively predict clinical results and the effectiveness of taxane treatment in TNBC patients (Fan et al., 2019). Linc00339 expression patterns in various BC cell lines were contrasted to those in healthy mammary glands epithelial cell lines, and increased expression of miR-377-3p in individuals with TNBC predicted a longer OS. MiR-377-3p modulates HOXC6 expression, impacting Linc00339-mediated TNBC growth, and hyper-expression of miR-377-3p causes a delay in TNBC cell growth through controlling cell cycle division and apoptosis. As a result, the Linc00339/miR-377-3p/HOXC6 axis plays a role in TNBC development and could be a suitable treatment target for TNBC therapy (Wang et al., 2019).

HIF1A-AS2 expression was measured in 86 TNBC samples, 30 non-TNBC samples, and 30 adjoining breast samples, revealing that it is elevated in TNBC tissues in comparison to non-TNBC tissues, implying that HIF1A-AS2 expression is linked to OS in TNBC patients (Wang et al., 2019).

Some other researchers looked at the expression of the HOTAIR which is a lncRNA in 163 instances of TNBC and discovered that its expression in cancerous tissue is highly connected with lymph node metastases and has a clear strong correlation with the expression of androgen receptor (AR). These findings point to HOTAIR's role in the control of AR-mediated processes, resulting to its proposed use as a predictive biomarker linked to novel therapeutic methods for patients with TNBC of the LAR subtype. Additionally, plasma urothelial carcinoma-associated 1 (UCA1) levels are much higher in TNBC patients, suggesting that this molecule could be used as a particular biomarker for the detection of TNBC (Liu et al., 2017).

In comparison to nearby normal breast tissues and healthy breast epithelial cell lines, a newly identified lncRNA, hepatocellular carcinoma upregulated EZH2-associated lncRNA (HEIH), is abundantly expressed in TNBC tissue and cell lines. By modulating the miR-4458/SOCS1 axis, HEIH downregulation suppresses TNBC cell growth and increases apoptosis. HEIH also has a role in therapeutic development (Li et al., 2019).

Enhanced methylation at cg06588802 in the long intergenic noncoding RNA, LINC00299, in patients with TNBC especially in comparison to controls was identified and validated by comparing genome-wide methylation patterns in peripheral blood DNA from 233 patient populations with TNBC and 231 controls, implying that hyper-methylation of LINC00299 in peripheral blood could be a beneficial circulating marker for TNBC (Bermejo et al., 2019).

Targeted antibody–drug conjugates: Protein markers in TNBC

The isolation of glycoproteins on the membrane of epithelial cancerous cells prompted the invention of antibody-drug conjugates (ADCs) to increase cytotoxic delivery of drugs to cells expressing these molecules. Most of these targets aren't essentially cancer drivers or even exclusive to breast carcinoma; instead, they necessitate differences in protein expression between cancerous and non-cancerous cells. The target antigen, which must be preferentially expressed (or highly expressed) on the desired cancerous cell, is a significant factor in the effectiveness of ADCs. As a result, the existence (or higher expression) of the target antigens could be used as a biomarker to detect patients who are possibly sensitive. TNBC cells have been found to have a number of compounds that fit these criteria. Trop-2, GPNMB, LIV-1, and the mucin 1-attached sialoglycotope CA6 are among the most hopeful. TROP2, which is expressed in over 90% of TNBCs, is targeted by sacituzumab govitecan (IMMU-132), an antibody–SN-38 conjugate (Bardia et al., 2017). IMMU-132 had a 30% ORR in patients with extensively pre-treated advanced TNBC, and PFS and OS were 6.0 and 16.6 months, correspondingly. In 68% of invasive TNBC specimens, LIV-1, a transmembrane protein with metalloprotease activity, was found. In a group of individuals with TNBC, ladiratuzumab vedotin (SGN-LIV1A) plus monomethyl-auristatin-E (MMAE) as the carrier showed a 25% ORR as well as a median PFS of 11 months (Modi et al., 2018). Substantial expression of glycoprotein-NMB (gpNMB), described as staining 25% of tumor epithelial cells, is prevalent in nearly 40% of TNBC, and in this subset, glembatumumab vedotin (CDX-011, an ADC which binds to gpNMB to administer MMAE) attained 40% ORR vs. 0% with investigator's selection of therapy (Yardley et al., 2015). In the phase II METRIC trial, however, glembatumumab vedotin failed to show better PFS, ORR, or OS as contrasted to capecitabine in preselected gpNMB-overexpressing aggressive TNBC patients, resulting to the ADC's development being halted (Celldex's METRIC Study Press Release, April 16, 2018). SGN-LIV1A is now in phase II trials, whereas IMMU-132 has progressed to phase III research (ASCENT: NCT02574455). Because several of these markers are overexpressed regularly in TNBC, IHC validation may not be required before to initiating therapy, but some proteins overexpressed less regularly may necessitate prescreening attempts to assist identify individuals who may be more likely to profit from ADC.

Immunotherapy biomarkers in TNBC

PDL1 and TILs

Immunologic evasion via different pathways is a fundamental component in the development of malignancies, and the immune system usually plays a significant role in preventing carcinogenesis. Immunotherapy has advanced dramatically in recent years to enhance results in a variety of solid tumor types. Nevertheless, researchers have only lately begun to gain a clearer understanding of its therapeutic significance in BC, which has previously not been thought to be immunogenic (Wagner et al., 2019). When compared to HR-positive BC, TNBC is much more immunogenic, as well as the existence of numerous components of the immune milieu has been connected to favorable prognostic characteristics (Desmedt et al., 2008). As a result, there is a growing interest in investigating the impact of immune-modulating medications in this BC subtype.

A transmembrane receptor protein, programmed cell death protein 1 (PD1) is present on the membrane of adaptive immunity cells like T cells that binds to a ligand called programmed death-ligand 1 (PDL1) or programmed death-ligand 2 (PDL2), which is found on tumor cells and tumor-infiltrating immune cells. This connection causes T cells to become inhibited, allowing the tumor to maintain self-tolerance and evade the immune system Fig. 2.5. PDL1 is expressed in about 20% of TNBC patients and is linked to unfavorable prognostic factors like higher grade, young age, ER-negative status, HER2-positive status, and greater tumor size (Sabatier et al., 2015; Qayoom et al., 2021).

On tumor or immune cells, PDL1 can be tested and quantified. PDL1 expression in TNBC has varied among research and institutions when measured by IHC. This range could be explained by the kind of cell examined (immune vs. tumor), TNBC stage (primary vs. progressed), metastatic disease location, antibody clonal variation, and the numerical limit utilized to determine positivity (Sabatier et al., 2015; Schmid et al., 2020a; Schmid et al., 2020b).

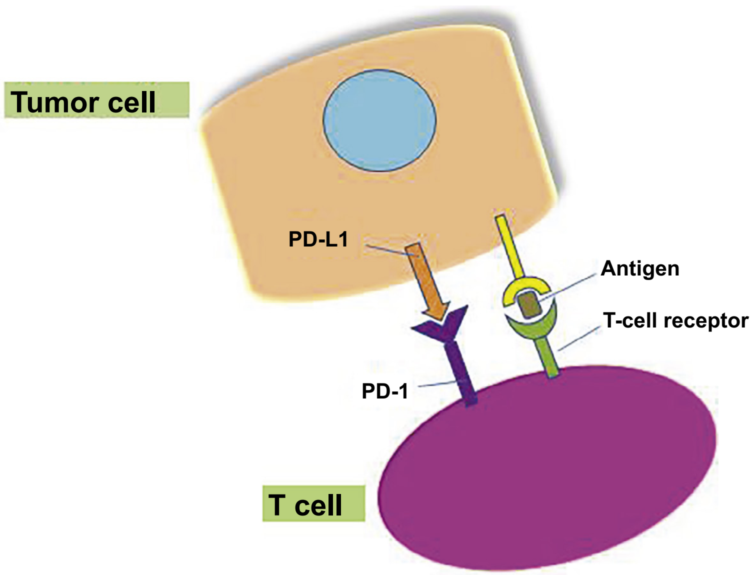

FIG 2.5 The interaction of PD-L1 with PD-1 prevents T cells from destroying tumor cells in the body.

PD-L1 positivity has been shown to have a predictive influence on IC in various therapeutic trials. Both PD-1 and PD-L1 expression is linked with positive outcomes and is linked to greater overall survival and chemotherapy responsiveness, indicating that chemotherapy's cytotoxic activity is mediated in part by the immune reaction against tumors (Bertucci and Gonçalves, 2017; Van Berckelaer et al., 2019). Monoclonal antibodies targeting PD1 and PDL1 efficiently release immune system downregulation, resulting in an immunological-mediated reaction against the tumor. PDL1 expression has also been linked to a better pCR rate (Cerbelli et al., 2017), metastatic-free survival, and overall survival (Sabatier et al., 2015). IHC was used to assess PDL1 expression by using a combined positive score (CPS) (22C3 antibody), which assesses the total of PDL1 expression on tumor and immune cells. PDL1 positive, which was characterized as a CPS≥1, was found in roughly 80% of the patients.

TNBC also has a higher mutation rate and a higher number of tumor-infiltrating lymphocytes (TILs), which are crucial adaptive immune cells in the tumor microenvironment (Mehraj et al., 2021). TILs are strongly expressed in roughly 20% of TNBC patients. TILs are found both within the tumor and in the neighboring tissue stroma, and their presence in both the tumor and the stroma has a predictive and prognostic effect. Increased TILs have been associated to increased DFS, OS, and pCR rate with NACT in early TNBC (Adams et al., 2014, Denkert et al., 2018), as measured in tumor samples from numerous large clinical trials. According to a study of two-phase III adjuvant trials, increasing TILs by 10% reduces relapse and death by 15% (Adams et al., 2014). TILS have also been discovered to have the ability to predict immunotherapy response. Increased TILs were linked to a significantly better ORR to pembrolizumab in KEYNOTE-086 (Adams et al., 2019). TILs also have been investigated as a biomarker in the treatment of metastatic TNBC, with larger levels being linked to an improved prognosis. Its ability to predict response to pembrolizumab immunotherapy in this situation was shown in KEYNOTE-119 in participants with TILs of less than 5% (Loi et al., 2020; Qayoom et al., 2021). Nevertheless, as contrasted to the primary setting, the evidence for this is less mature.

Tumor mutational burden as a biomarker

In addition to PDL1, additional prognostic/predictive biomarkers may be used to discover additional people who potentially benefit from immunotherapy. The number of somatic alterations per megabase (mut/Mb) of DNA assessed via whole-exome or gene panel sequencing is referred to as tumor mutational burden (TMB). In patients with melanoma, colorectal, and lung cancer, a higher TMB has been linked to greater T cell infiltration, increased neoantigen burden, clinical response, and enhanced survival following immunotherapy. Nevertheless, there is a scarcity of information about TMB in BC (Salmaninejad et al., 2018). There is insufficient data on TMB in breast carcinoma, and its predictive significance is debatable. Elevated TMB can be found in up to 3% of initial BC tumors, although it can be found in up to 11% of those with metastatic cancer (Bayraktar et al., 2019). TMB-high BC tumors seem to be more responsive to checkpoint inhibitors, but differences in OS were observed in BC patients with elevated TMB who received immunotherapy (Boussiotis, 2016). Pembrolizumab was authorized by the FDA in June 2020 for TMB high (>10 mut/Mb), unresectable or aggressive solid tumors that have progressed after previous therapy or have no other treatment choices, offering it a possible treatment for TNBC individuals having TMB high.

Summary

TNBC refers to a diverse set of disorders defined by a variety of genetic mutations and a scarcity of validated biomarkers. Ongoing research is focusing on discovering genes that are prevalent in all or specific TNBC subtypes and could be exploited as targeted therapies, prognostic markers, or predictors of therapy response. Although high-throughput research tools like sequencing and microarray technologies have the potential to shed light on the nature of TNBC, the findings of these techniques are rarely therapeutically useful. Clinical validation of established biomarkers requires well-defined and comprehensive sets of data. Several potential markers have been identified yet; however, they have yet to be validated using the demanding requirements of clinical trials. TNBC is treated in a variety of ways, which reflects its heterogeneity. Traditional therapeutic techniques should evaluate which subtype is being addressed until customized options become available, as the distinct subtypes vary in both proliferation activity and responsiveness to standard chemotherapy. To continue to enhance results in patients with TNBCs, there is a vital need for the discovery of additional current next generation sequencing-based biomarkers.

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CHAPTER

3

Current therapeutics and treatment options in TNBC

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Introduction

Breast tumors are divided into five intrinsic or molecular categories based on distinct gene expression patterns. Basal-like triple-negative breast cancer (TNBC) is the most common intrinsic subtype, accounting for 12–20% of all breast cancers (Wang et al., 2019). TNBC has gotten a lot of interest since it lacks expression of all three receptors (PR, ER, and Her2). As a result, anti-estrogen hormonal medications are ineffective in treating it (Slamon et al., 2011). TNBCs account for more than 80% of breast tumors in people who have the BRCA1 gene mutation (Andreopoulou et al., 2017). Even spontaneous TNBC exhibits many clinical and molecular characteristics with BRCA1-related malignancies, such as poor DNA repair, which could be caused by methylation-induced BRCA silence or alterations in other DNA-repair genes (Andreopoulou et al., 2017). Chemotherapy is effective against TNBC, and it is still the standard of care (SOC). Anthracyclines (e.g., doxorubicin topoisomerase II inhibitor, and DNA intercalating agents), alkylating compounds (e.g., cyclophosphamide), an anti-microtubule drug taxane, as well as anti-metabolite fluorouracil (5-FU) are all popular chemotherapeutics (Mir, 2021a, Fig. 3.1). Neoadjuvant chemotherapy following by surgery is the present standard of care for clinically diagnosed early TNBC. There is no conventional chemotherapy protocol for patients with recurrent or resistant TNBC. Treatment responses are typically brief, with a fast return, and visceral and brain metastases are prevalent. Anti-metabolites gemcitabine and capecitabine, DNA cross-linker platinums, and non-taxane microtubule inhibitor eribulin are among the treatments offered for people with metastatic TNBC. Following chemotherapy, the median progression-free survival (PFS) varies from 1.7 to 3.7 months, and the median overall survival (OS) from the start of metastases is 10 to 13 months. People with mTNBC who received single-agent taxane or platinum-based chemotherapeutics had a median PFS of 4 to 6 months and also an OS of 11 to 17 months in clinical studies. Novel therapy choices for advanced TNBC patients have lately become available,

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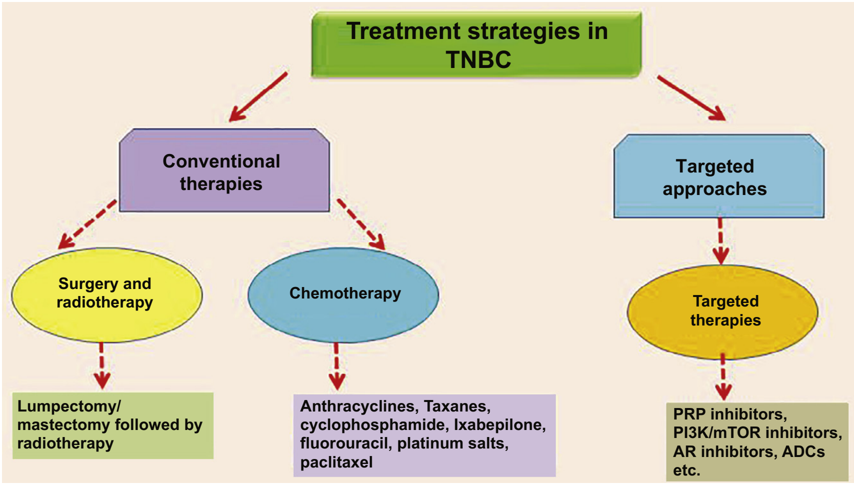

FIG. 3.1 Different types of treatment approaches in TNBC.

particularly in cases where surgery is not a possibility. Novel targets are being identified, many of which have significant therapeutic potential. The current clinical approach is evolving towards the use of molecular testing at the time of diagnosis to create a tailored tumor-specific genetic 'fingerprint' that can indicate molecular dependencies that can be treated. To comprehend and simulate the behavior of cancer cells, the translational cancer scientific community is progressively using a combination of systems biology and integrative analysis strategy. Developing therapeutic options for aggressive breast carcinoma requires a commitment to characterizing and redefining the disease's genetic signature at various points throughout its evolutionary pedigree so that treatment can be customized to a dynamic tumor microenvironment (Mehraj et al., 2021). To speed the development of breakthrough medicines for high-risk, early-stage breast carcinoma, new trial designs and re-defined outcomes as surrogates of clinical outcomes have been established.

Signaling pathways involved in triple-negative breast cancer (TNBC) treatment

Notch signaling pathway

The Notch family of transmembrane ligands and receptors was first discovered in 1917 by Thomas Hunt Morgan. This signaling route is important for cell differentiation and proliferation, and increased expression of a subset of signaling components in this pathway has been linked to the worst patient outcomes (Palomero et al., 2006). Four Notch receptors (Notch-1, 2, 3, and 4) and 5 ligands (Jagged-1, Jagged-2, Delta-like 4, Delta-like 3, and Delta-like 1) make up the pathway. Increased expression of Delta 1 and Jagged 1 has been confirmed in breast carcinoma (Soares et al., 2004; Brennan and Clarke, 2013; Speiser et al., 2013), and Notch-1, as a downstream regulator of oncogenic Ras (Weijzen et al., 2002), plays a crucial part in the genesis of human mammary tumors. Notch 1 has been linked to the Notch channel's involvement in a variety of cancers, including pancreatic carcinoma (Gao et al., 2017), hematological malignancies (Weng et al., 2004), and many others. Notch-3 and Notch-4 have been linked to tumor survival and growth in numerous researches. An increased level of Notch-2 in the MDA-MB-231 TNBC cell line, on the other hand, appears to be a protective factor (O'Neill et al., 2007).

Because overexpression of the Notch receptor and its ligands has been related to TNBC, scientists believe the receptor could be targeted using a monoclonal antibody (mAb) (Espinoza and Miele, 2013; Mir et al., 2020). Blocking of Notch-1 signaling by mAbs has been demonstrated to reduce the expression of HEY-L and HES families in the MDA-MB-231 TNBC cell lines, resulting in a reduction in cell proliferation as well as an elevation in apoptosis initiation (Sharma et al., 2012). TNBC can also be treated with DLL4 (Delta-like ligand 4 Notch ligand) mAb treatment (Benedito et al., 2009). Many transcription factors, such as the HES and HEY families, Akt, VEGF, p53, and PI3K-AKT-mTOR, use Notch signaling to code for genes involved in cancer (Chan et al., 2007; Espinoza et al., 2013, Fig. 3.2). γ-secretase inhibitors (GSIs) are medicines that disrupt the Notch signaling cascade by inhibiting the multimeric γ-secretase complex during the second proteolytic cleavage within cell cytoplasm (Chan et al., 2007).

Hedgehog signaling pathway

Recent investigations reveal that they are mutated in clinical specimens of numerous human malignancies, including breast carcinoma cell lines (Kubo et al., 2004; Nagase et al., 2008). Sonic Hedgehog (Shh) (Heussler and Suri, 2003) morphogenes network have an effect on cancer

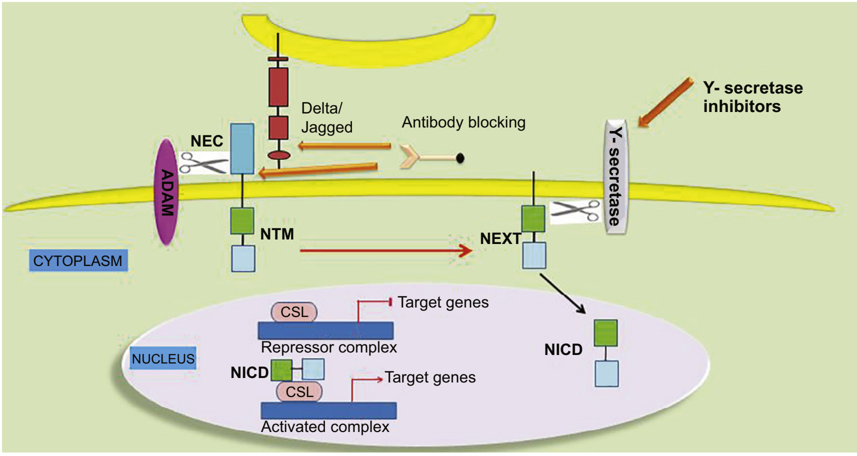

FIG. 3.2 Schematic representation showing Notch receptor activation and a clinically developed therapeutic target: Ligand interaction to the Notch receptor triggers a two-step proteolytic cleavage by ADAM family proteases and γ-secretase, which releases the Notch intracellular domain (NICD). The NICD moves into the nucleus where it binds into CSL, changing the complex's function from repressor to activator of notch target genes.

stem cell (CSC), basal cell carcinoma (Gorlin syndrome), and polydactyly syndromes. Three ligands are involved in Hedgehog signaling:

(i) Sonic hedgehog (SHH) which is strongly expressed throughout embryogenesis;
(ii) Indian hedgehog (IHH) (St-Jacques et al., 1999) typically found in hematopoietic cells, cartilage, and the endochondral skeleton;
(iii) Desert hedgehog (DHH) (Canto et al., 2004) has been shown to be expressed in the peripheral nerve systems and the testes, and alterations in the DHH gene have been linked to pure gonadal dysgenesis (PGD) (Aberger et al., 2012).

The Hedgehog signaling system is implicated in cancer cell infiltration; metastases, drug resistance, and tumor relapse after treatment (Li et al., 2012). Elevated expression of Shh is linked to poor prognosis in breast carcinoma patients, particularly TNBC patients, according to Kaplan–Meier survival analyses. Since it maintains aberrant growth and drives invasion into other tissues, SHH plays a significant function in the faulty origin of malignancies in breast carcinoma. Thiostrepton, a novel therapeutic medication that targets sonic Hedgehog signaling and decreases the number of CD44+/CD24 cancer stem cells (CSCs) in TNBC cell lines (Yang et al., 2016), has been developed by researchers. However, the significance of the Hedgehog pathway in breast CSCs (Hui et al., 2013), which has yet to be identified (Tao et al., 2011, Habib and O'Shaughnessy, 2016), must be clarified. As a consequence, the FDA has only approved a few medications to target this route, like Vismodegib, which is used to treat basal cell carcinomas (Chang et al., 2016). Further study into SHH signaling is required, which could lead to the development of novel preventative methods and molecular biomarkers for evaluating relapse, prognosis, and survival.

Wnt/β-catenin pathway

The most typically highly expressed route resulting in transcriptional factors activation essential for the activation of epithelial to mesenchymal cell (EMT) transitions in CSCs is Wnt/-catenin. In TNBC, both non-canonical and canonical components show dysregulation of Wnt signaling (Pohl et al., 2017, Mir et al., 2020). There are 10 Frizzled (FZD) and 19 Wnt receptors and coreceptors in humans, according to current knowledge (Gurney et al., 2012, Pohl et al., 2017). Wnt ligands (WNT3A, WNT3A, and WNT5A) play an important role in invasion and metastasis (Zhu et al., 2012). The FZD6 receptor is the most significant representative in TNBC because it has the ability to promote metastasis by boosting the mobility of malignant cells (Corda et al., 2017). Several new medications address Frizzled receptors; for instance, OMP-18R5, an antibody that targets Frizzled receptors, inhibits tumor cell growth in breast, lung, colon, and pancreatic malignancies (Gurney et al., 2012). Furthermore, higher expression and accumulation of the β-catenin protein enhances cell motility in TNBC cells, resulting in resistance (Pohl et al., 2017). Wnt inhibitors and modulators can eliminate CSC clonal populations and drug-resistant cells (Dean et al., 2005); however, their safety in maintaining tissue homeostasis and healing must be determined. The stimulation of the Wnt/signaling system has been linked to a poorer clinical result in TNBC (Geyer et al., 2011), which is associated with the risk of brain and lung metastases (Dey et al., 2013). Pluripotent CSCs are thought to play a major part in the genesis of primary aggressive solid tumors, according to researchers. These CSCs are also involved in the generation of drug resistance proteins in breast carcinoma (Howard and Ashworth, 2006; El Ayachi et al., 2019) and have been linked to metastasis.

Mammalian target of rapamycin (mTOR) pathway

The improper control of mTOR signaling, particularly the Phosphoinositide-3 kinase (PI3K)/Akt/mTOR cascade, is linked to cancer (Fruman and Rommel, 2014). In TNBC patients, the mTOR pathway is altered, resulting in a bad outcome (aggressiveness and tissue invasions) (Zaytseva et al., 2012).

PI3K/Akt/(mTOR)-stimulated phosphorylation events are essential for tumor growth, angiogenesis, and angiogenesis (Arcaro and Guerreiro, 2007). Increased expression of the protein kinase Akt has also been linked to tumor invasion and metastasis (Zaytseva et al., 2012; Mir, 2021b). The PI3K/Akt pathway's downstream signaling cascade is mTOR, which is found in two functionally distinct complexes (mTORC1 and mTORC2). The mTORC1 pathway increases mRNA translocation and phosphorylates a variety of substrates involved in a variety of anabolic activities (Zaytseva et al., 2012, Fig. 3.3).

Blockers of the PI3K/AKT/mTOR system are divided into six categories: 1. Pan-class I (PI3K blocker), 2. Isoform-specific (PI3K blocker), 3. Rapamycin analogues (Rapalogs: Everolimus, Deforolimus, Temsirolimus), 4. Active-site (mTOR blocker), 5. Pan-PI3K/mTOR blockers and 6. AKT blockers (Zaytseva et al., 2012). In addition, mTOR and one PI3K isoform could be addressed at the same time to improve efficacy relative to single PI3K inhibition (Zaytseva et al., 2012).

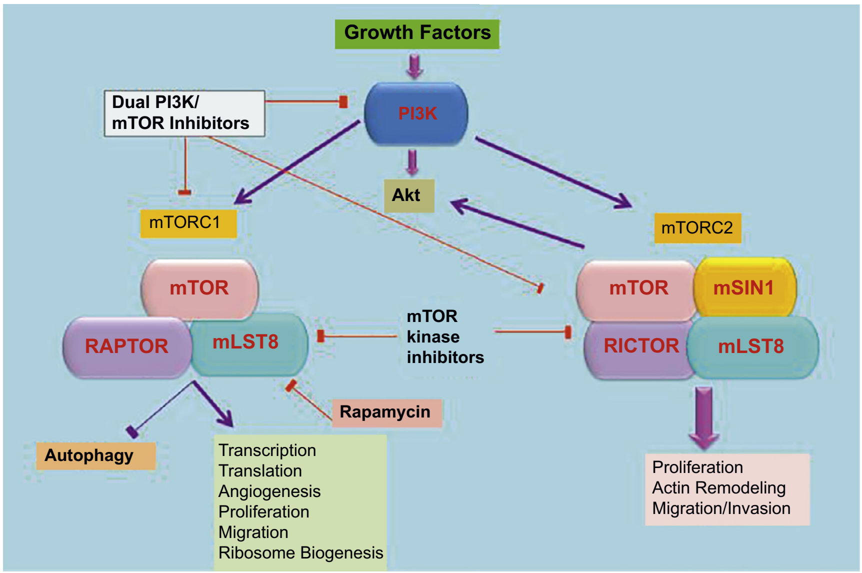

FIG. 3.3 mTOR signaling pathway: mTOR is a component of the mTORC1 and mTORC2 multi-protein complexes. mTORC1 and mTORC2 could both be activated in reaction to growth factors, but mTORC2 is the main kinase that phosphorylates and activates Akt. The relevance of mTORC1 and mTORC2 in the control of various cell processes critical for tumor progression, as well as their close contact with carcinogenic pathways, make mTOR an appealing therapeutic target. The action mechanisms of presently available mTOR inhibitors are presented.

Poly (ADP-ribose) polymerase (PARP)

The polyadenosine diphosphate-ribose polymerase (PARP), also known as poly (ADP-ribose) polymerase (PARP), is a large family of eighteen proteins that regulate all molecular mechanisms involved in cell recovering from DNA damage (take part in DNA base excision repair), apoptosis, gene transcription, and genomic stability (Park and Chen, 2012).

Approximately 70% of breast tumors that develop in BRCA1 mutation carriers and 23% of breast tumors that develop in BRCA2 carriers display a triple-negative phenotype (Mahfoudh et al., 2019). As a result, PARP inhibitors are thought to be among the most promising therapeutic medications under development for BRCA-1 and BRCA-2 mutations, and also for TNBC. The expression of PARP in TNBCs is a result of chemotherapeutic treatment. The expression of PARP in TNBCs is a result of treatment. PARP-1 and PARP-2 proteins are involved in DNA repair and are activated by DNA strand breaks. Both the BER (Base excision repair mechanism), as well as the single-strand break repair (SSBR) processes, are driven by PARP-produced ADP-ribose polymer (De Vos et al., 2012). Because suppressing PARP activity prevents the formation of the ADP-ribose complex, so PARP-dependent DNA-damage repair complex like DNA polymerase ε (Pleschke et al., 2000) are ineffective at repairing DNA damage (Helleday, 2011). PARP-DNA complexes that have been trapped are very cytotoxic, with strong anti-proliferative (and thus anticancer action) (Murai et al., 2012). Additionally, the catalytic inhibitory tendencies of Veliparib (ABT-888) and Olaparib (AZD-2281), both PARP inhibitors, varied significantly. As a result, the experimental and clinical findings of each PARP inhibitor differ in terms of inhibition (Fong et al., 2009; Gagné et al., 2012). Because PARP inhibitors vary in their ability to trap PARP-DNA complexes (Ström et al., 2011; Murai et al., 2012), variances can be noted when contrasting the two (Velipamib and Olaparib), with Velipamib being a less potent PARP1 and PARP2 repressor in comparison to Olaparib (Murai et al., 2012).

Epidermal growth factor receptor (EGFR)

RTK targets like EGFR expression are found in 89% of TNBC patients, making them a viable therapeutic option, particularly for BL2-subtype cancers with elevated EGFR gene expression (Sobande et al., 2015). The activation of this gene promotes both primary carcinogenesis and metastasis. Gefitinib (EGFR inhibitor) inhibits cancerous cells proliferation and improves carboplatin and docetaxel cytotoxicity (Eccles 2011; Sobande et al., 2015, Fig. 3.4).

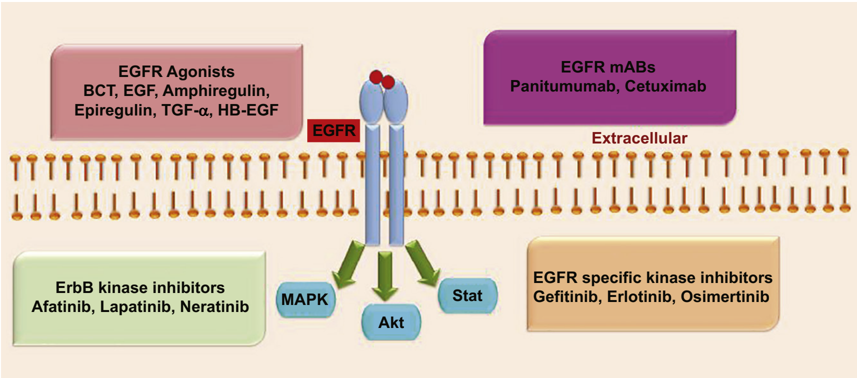

FIG. 3.4 The activators, inhibitors, and consequences of epidermal growth factor receptor (EGFR) signaling are depicted schematically.

TNBC has been treated with a variety of EGFR inhibitors, including tyrosine kinase inhibitors (TKIs) like lapatinib and erlotinib, as well as monoclonal antibodies (mAbs) like panitumumab and cetuximab (Ueno and Zhang, 2011; Layman et al., 2013; Nabholtz et al., 2014; Hsiao et al., 2015). However, failures with EGFR-TKIs and mAbs prompted researchers to develop a combined therapy that included mAbs and chemotherapy drugs and proved to be more effective. In advanced TNBC individuals, cetuximab and carboplatin, and also Cetuximab and cisplatin, exhibited twice the effectiveness of therapeutic responses (Baselga et al., 2013). Furthermore, the tri-inhibitors carboplatin, docetaxel, and gefitinib improved the cytotoxicity of TNBC cells when used in combination (Corkery et al., 2009).

Cannabidiol, another medication, inhibited breast tumor metastases by inhibiting the EGF/EGFR signaling cascades and altering the tumor environment (Velasco et al., 2016). As a result, the cannabidiol may be an effective therapeutic option for extremely aggressive TNBC (Chakravarti et al., 2014).

TGF-β signaling pathway

TGF-β1 is a member of the TGF-β superfamily of cytokines and is encoded by the TGF-β1 gene. TGF-beta 1 plays a key role in breast carcinoma stem cells, which express TGF-β1 and the TGF-β1 receptor at a rapid rate (Jamdade et al., 2015). TGF inhibitors can stop chemotherapy-resistant tumor-initiating cells (TIC) from multiplying in vivo (Jamdade et al., 2015), laying the groundwork for a combination treatment for TNBC patients. TGF-β causes breast cells to undergo an epithelial-to-mesenchymal transition (EMT), resulting in tumor-like characteristics. TGFBR1/2 inhibitors can be used to reverse EMT while also promoting mesenchymal-to-epithelial (MET) transition within breast epithelial cells (Bhola et al., 2013). TGF- is commonly abundantly expressed in the TNBC tumor milieu, particularly in tumor cells and tumor-associated immune and stromal cells. These cells also produce SMAD4 and SMAD2/3, which promote angiogenesis and metastases. This suggests that TGF-inhibitors are helpful in the treatment of metastatic patients (Bhola et al., 2013).

CSPG4 protein signaling pathway

CSPG4 also called melanoma chondroitin sulphate proteoglycan or non-glial antigen is a cell-surface proteoglycan found on basal breast cancer cells. Inhibiting CSPG4 is clinically beneficial in the treatment of breast carcinoma. This protein promotes the spread of endothelial basement membrane protein, thereby maintaining the cell-substratum connection, similar to the effects seen in TNBC. Monoclonal antibodies against CSPG4 can disrupt mitogenic, migratory, and survival signaling cascades in tumor cells, making CSPG4 a novel TNBC target (Wang et al., 2010). Furthermore, CSPG4 is overexpressed in TNBC cell types, leading to TNBC cell suppression when CSPG4 was addressed in such cells (Cooney et al., 2011).

Role of chemotherapeutic agents in TNBC

TNBC has few treatment choices, is prone to relapse and metastases, and also has a bad prognosis when compared to other kinds of breast carcinomas. The basic reason for this is that ER, PR, and HER2 expression all are negative, rendering specialized hormonal and targeted therapy ineffectual. As a result, chemotherapy has become the standard treatment for TNBC (Mir and Mehraj, 2019, Qayoom et al., 2021). A vast body of research has recently revealed that using neoadjuvant chemotherapeutic regimens in the management of TNBC seems to have a much greater pathological remission rate than hormonal receptor-positive breast carcinoma and can greatly enhance TNBC patients' prognosis. Combination treatments based on taxanes, anthracyclines, cisplatin, cyclophosphamide, and fluorouracil are recommended by the National Comprehensive Cancer Network. Currently, the adjuvants of choice include docetaxel/taxel + adriamycin + cyclophosphamide (TAC), adriamycin + cyclophosphamide (AC), docetaxel + cyclophosphamide (TC), cyclophosphamide + adriamycin + fluorouracil (CAF), cyclophosphamide + methotrexate + fluorouracil (CMF), and cyclophosphamide + epirubicin + fluorouracil + paclitaxel/docetaxel (CEF-T). As a result, selecting suitable chemotherapy medicines and optimizing chemotherapy regimens are critical for assuring satisfactory therapeutic outcomes and prognosis for TNBC patients.

Taxanes

Taxel works primarily by inhibiting microtubule depolymerization, preventing cells from forming spindles and spindle fibers in mitosis and causing them to halt in prometaphase, so limiting the division of cells. Taxel has an anticancer impact in addition to its antimitotic activity, which is mediated by activated macrophages (Mehraj et al., 2021). Taxel's antitumor toxicity is linked to its ability to induce apoptosis in cancer cells. Docetaxel has the same mode of action as taxel, but it has two times the anti-microtubule depolymerization impact and a larger anticancer spectrum at the same lethal dose. In recent years, more in-depth studies have discovered that traditional, commercially obtainable solvent-based (Sb) taxel made with polyoxyethylated castor oil (Kolliphor® EL, previously called Cremophor EL; BASF SE, Ludwigshafen, Germany) as the solvent can end up causing serious or perhaps even fatal allergic responses. Polyoxyethylated castor oil, a widely used solvent, significantly lowers taxel particle release and effectiveness. Albumin-bound paclitaxel (Nab-paclitaxel) has a greater drug delivery efficacy on endothelial cells than sb-paclitaxel (Kundranda and Niu, 2015). It also takes less time to administer and does not require pre-treatment to avert allergic responses. The BL subtype has a high expression of DNA repair genes and proliferation-related genes, implying that it may be responsive to antimitotic medications, according to gene profiling research of TNBC molecular subtypes (e.g., docetaxel or taxel) (Mir, 2021c). BL1 and BL2- the basal-like subtypes show four-fold better clinical recovery rates than the LAR and MSL subtypes following taxane-based treatment in TNBC patients (Bauer et al., 2010; Juul et al., 2010).

Anthracyclines

Streptomyces peucetius var. caesius produces anthracyclines and anthracycline antibiotics, which are a type of chemotherapy agent. They could be utilized to treat leukemia, lymphoma, breast carcinoma, endometrial cancer, ovarian cancer, and pulmonary cancer (W Edwardson et al., 2015), and they may treat more forms of cancer than any other class of chemotherapeutic medications. Researchers have discovered the appropriate dosage schedules for anthracycline adjuvant chemotherapy for breast carcinoma via a large array of clinical research: the optimal dosage of DOX is 60 mg/m^2, and the optimum dosage of epirubicin is 100 mg/m2 (Trudeau et al., 2005). Additional research revealed that increasing the dosage had no effect on survival or recurrence rates (Henderson et al., 2003). Current anthracycline-based regimens, including FEC-100 (100 mg/m2 epirubicin), can lower the risk of breast tumor recurrence and death by 25–30% (Bonneterre et al., 2005; Levine et al., 2005). According to available clinical data, after 6 months of anthracycline chemotherapeutics, the death rate in patient populations >50 years at the moment of diagnosis dropped by nearly 38%, while the death rate in individuals aged 50 to 69 years at the moment of diagnosis was reduced by roughly 20%. The effectiveness of anthracycline treatment did not differ significantly amongst breast tumor subtypes.

Nevertheless, different subtypes have different reactions to the combination of taxanes and anthracyclines. TNBC individuals having the MSL or BL1 subtypes showed a greater rate of pCR, whereas TNBC individuals having the BL2 or LAR subtypes are unaffected by the combination treatment. The BL2 subtype had a pCR rate of 0%.

Cyclophosphamide

In vitro, cyclophosphamide does not exhibit anticancer action. Upon entering the body, in the liver, the microsomal mixed-function oxidases transform cyclophosphamide to aldophosphamide. In tumor cells, cytochrome P450 activates aldophosphamide, resulting in the production of nitrogen mustard and acrolein with alkylating action. Tumor cells are killed by nitrogen mustard. TC is presently the recommended neoadjuvant chemotherapeutic regimen for HER2-negative breast carcinoma. Nakatsukasa and coworkers included 52 individuals with BC in their study. 94.2% (49/52) of participants completed four cycles of TC, with an overall pCR rate of 16.3% (8/49); women with luminal A-like breast carcinoma (ER+, Ki67 index less than 20%, HER2-) reported a pCR rate of 0% (0/12); those having luminal B-like breast carcinoma (ER+, Ki67 index more than 20%, HER2-) reported a pCR rate of 4.3% (1/23), but individuals with TNBC reported a pCR rate of 50.0% (7/14); nearly all pCR occurred in TNBC breast tumor patients (Nakatsukasa et al., 2017). The findings revealed that neoadjuvant CT in combination with TC was more effective in treating TNBC than other breast carcinomas, but had minimal effectiveness in treating other subtypes. Wu and coworkers discovered that adjuvant cyclophosphamide, fluorouracil, and methotrexate chemotherapy efficiently lowered locoregional relapse rate and sustained DFS in patient populations with node-negative TNBC, particularly in individuals with tumor diameters greater than 2 cm and those who used to have undergone partial mastectomy (Wu et al., 2014). Masuda and coworkers (Masuda et al., 2013) previously conducted a retrospective investigation of TNBC subtype-specific response rates in 130 TNBC patients administered with neoadjuvant adriamycin/Cytoxan/Taxol-containing therapy. The total pCR response was 28%, with subtype-specific responses differing significantly. The maximum pCR rate (52%) was identified in the BL1 subtype, while the lowest response rates were observed in the BL2, LAR, and MSL subtypes (0%, 10%, and 23%, respectively). A likelihood ratio analysis (Masuda et al., 2013) revealed that the TNBC subtype is an independent predictor of pCR status ($p = 0.022$). These findings highlight not just the variability of TNBC, but also the importance of aligning patients to appropriate therapy depending on their disease subtype.

Platinum agents

Most breast carcinoma patients do not profit from platinum-based drugs. Nevertheless, only a few studies have particularly looked into platinum's in TNBC to date. Owing to the coupling of platinum-induced DNA damage through double-strand cross-links and impairments in BRCA linked DNA repair, TNBC is more sensitive to platinum than other breast carcinomas as well as other cytotoxic agents.

These preclinical results are supported by retrospective studies. Platinum usage in neoadjuvant, adjuvant, or metastatic disease in TNBC vs. non-TNBC was compared using results from a single institution (Sirohi et al., 2008). TNBC had a considerably greater rate of neoadjuvant therapeutic response than non-TNBC (88% vs. 51%, correspondingly, p = 0.005). The 5-year OS after neoadjuvant/adjuvant treatment was 64% on behalf of non-TNBC vs. 85% on behalf of TNBC. Individuals with severe disease who had TNBC showed a PFS of 6 months vs. 4 months for non-TNBC patients (p = 0.05). As a result, platinum-based CT was linked to a higher pCR and a poorer OS in early breast carcinoma, but a better PFS in progressed disease.

Patients with TNBC who were administered with neoadjuvant platinum plus docetaxel and had locally progressed disease were identified retrospectively from a single organization (Leone et al., 2009). 76 individuals received neoadjuvant adriamycin and cyclophosphamide (AC) while 42 patients received adjuvant AC out of a total of 125 patients. In 42 cases, pCR was observed (34%). Among the treatment groups, neoadjuvant AC and adjuvant AC were linked to pCRs of 40% and 29%, correspondingly. In other trials, pCR was linked to a better overall survival rate (5-year OS: 73% for pCR vs. 49% for non-pCR; p 0.001). Cisplatin looked to be superior to carboplatin in terms of OS, however, the small number of patients and various factors make it difficult to draw any inferences from this trial.

Prospective evidence is restricted to a few small-scale trials focusing on TNBC individuals with BRCA mutations. In a neoadjuvant trial of four cycles of single-agent cisplatin in participants with TNBC and BRCA1 mutations, pCR was observed in 9 out of 10 (90%), with two patients receiving only two cycles. The one patient who remained showed a partial response with residual nodal illness (Byrski et al., 2008). The trial was expanded to include a total of 25 females with stage I–III breast carcinoma who had a BCRA1 mutation, irrespective of underlying molecular grouping. They were given four cycles of neoadjuvant cisplatin as a single agent (Gronwald et al., 2009). Surprisingly, pCR was reported in 18 patients (72%), implying that platinum-based chemotherapy is particularly successful in individuals with BRCA1-related breast carcinoma. The outcomes of a neoadjuvant study of single-agent cisplatin in 28 TNBC individuals have been released, with 22 patients achieving a pCR (22%) (Garber, 2006).

Cisplatin has been shown to be effective in the treatment of metastatic cancer. The introduction of weekly cisplatin to the metronomic dose of methotrexate and cyclophosphamide, after prior treatment to an anthracycline and taxane, was investigated in a single institution phase II research of 126 TNBC individuals (Staudacher et al., 2011). The cisplatin regimen looked to be safe and effective, having an ORR of 63% and a median time to progression of 13 months. Patients who did not receive cisplatin showed an ORR of 33% and a median duration to the progression of 7 months.

Zhang and coworkers performed a phase II research (NCT00601159) to assess the tolerability and effectiveness of cisplatin plus gemcitabine (GP) as a first-line therapy for mTNBC. The results demonstrated that the combined regimen was safe and effective for individuals with mTNBC, especially those with basal-like subtypes (Zhang et al., 2015). Von Minckwitz gave carboplatin-containing therapy to 269 breast carcinoma patients who were randomly chosen and non-carboplatin-containing therapy to 299 breast tumor individuals. They discovered that adding carboplatin to traditional taxel and anthracycline treatment dramatically enhanced the pCR rate in TNBC individuals, but not in HER2-positive women with breast carcinoma (Von Minckwitz et al., 2014). BL1-subtype TNBC was found to be considerably more sensitive to cisplatin treatment than other TNBC subtypes (Jovanović et al., 2017).

Fluorouracil

5-Fluorouracil (5-Fu) has no biological activity on its own. In vivo, 5-Fu can be transformed into active fluorodeoxyuridine monophosphate and fluorouridine monophosphate by orotate phosphoribosyltransferase. Capecitabine is a cytotoxic drug that targets tumor cells specifically. Capecitabine has no cytotoxicity and is particularly effective when it transforms into the cytotoxic 5-Fu in the body. The huge amount of thymidylate phosphorylase in the tumor catalyzes this process, resulting in more 5-Fu being produced in the tumor, with stronger (better than 5-Fu) antitumor effectiveness. Capecitabine can be used to treat advanced primary or metastatic breast carcinoma when PTX or anthracycline CT failed. With the extensive use of taxanes and anthracyclines in the treatment of breast carcinoma, a growing proportion of patients develop resistance to these drugs, which has become a major clinical issue. Capecitabine is a novel oral fluorouracil drug that targets tumor cells with elevated thymidine phosphorylase expression. Capecitabine is a drug with high efficacy, low toxicity, and a simple delivery method. Li and coworkers performed a phase II research on the combination of capecitabine with cisplatin in the therapy of mTNBC individuals pretreated with taxane and anthracyline and showed that the combination had considerable efficacy in mTNBC patients with tolerable side effects (Li et al., 2015).

Ixabepilone

Ixabepilone is a novel epothilone B analogue that binds to tubulin and stimulates tubulin polymerization and microtubule stabilization, effectively stopping the cell cycle and triggering death in tumor cells. A prospectively designed subgroup study of 187 TNBC individuals from a phase 3 clinical trial of capecitabine with or without ixabepilone, which included 752 participants in total, was reported. In TNBC, the inclusion of ixabepilone resulted in an improvement in response rate from 9% to 27% with PFS from 2.1 to 4.1 months (HR 0.68, 95% CI 0.50–0.93) (Rugo et al., 2007). In 161 patients, a phase II neoadjuvant trial (080 trials) with sole drug ixabepilone indicated pCR in 19% of TNBC patients as compared to 8% of non-TNBC patients (Roche et al.).

Surgery and radiotherapy

All breast tumor subtypes follow the same rules for local therapy (surgery and radiation). Several investigations have been done to see how mastectomy compares to lumpectomy in terms of prognosis (Frasci et al., 2009). The surgical therapy of choice in TNBC is breast preservation; it is because the option of surgical therapy does not enhance prognosis or local tumor relapse, so sufferers remain appropriate candidates for breast conservation (Freedman et al., 2009). A lumpectomy followed by radiotherapy can be a possibility. Provided contradictory retrospective research on whether females with TNBC are at a greater risk of local relapse after breast-conserving therapy (BCT) or whether they would be served better by a modified radical mastectomy (MRM), (Haffty et al., 2006; Nguyen et al., 2008; Adkins et al., 2011) it became acknowledged that either treatment paradigm is acceptable in the management of early-stage TNBC. Several studies have revealed that early-stage TNBC patients could be at an increased risk for tumor recurrence if managed with MRM alone, omitting postmastectomy radiation (RT) (ie, in T1-T2N0 patients lacking conventional indications for postmastectomy RT), which requires further consideration (Mir, 2021d).

McGill University researchers discovered a substantial difference in locoregional recurrent rates (LRRs) among treated patients with BCT, MRM, or MRM + RT in a large single retrospective assessment of 768 females with T1-T2N0 TNBC (Abdulkarim et al., 2011). BCT and MRM patients had 5-year LRR-free survival rates of 96% and 90%, respectively, and MRM was the sole independent prognostic factor linked with LRR (HR= 2.5), indicating that MRM alone would not be adequate local therapy in these patients. 681 females with stage I-II TNBC following MRM were randomized to chemotherapy with or without RT in a prospective trial conducted in Shanghai (Wang et al., 2011). Despite the fact that RFS and OS were not their primary goals; the researchers discovered a statistically substantial difference favoring the group that got both adjuvant chemotherapy and post-MRM RT. Following the addition of RT, 5-year RFS enhanced from 75% to 88%, while 5-year OS enhanced from 79% to 90%. Although retrospective and hence prone to unintended bias, they are intriguing yet hypothesis-generating findings that warrant further investigation in a thorough randomized controlled study, but not a change in therapeutic practice (Mir, 2021e).

Most TNBC patients are given neoadjuvant chemotherapy in the expectation to become BCT candidates or to evaluate in vivo responsiveness to systemic treatment. A rising debate has erupted over whether or not to omit post-MRM RT in patients who have seen considerable down-staging as a result of chemotherapy or even to change the RT field layout depending on chemotherapy response.

Targeted therapeutics for TNBC

Because of the significant heterogeneity of TNBC, finding novel therapeutic targets and performing targeted therapy is particularly difficult. There are presently a great number of ongoing clinical trials based on immunohistochemistry staining data that are addressing particular receptors or targeted therapeutics for TNBC (Mir, 2021f).

Antiandrogen treatment

AR is expressed in both healthy and cancerous breast tissues; however, the levels are dramatically varied in distinct cancerous breast tissues. In around 10–15 percent of TNBC patients, AR expression is positive (Barton et al., 2015). AR positivity is characterized as the LAR-subtype TNBC (Farmer et al., 2005; Lehmann et al., 2011). Doane and coworkers analyzed 99 breast tumor patient specimens and eight distinct breast tumor cell lines and found a cell line (MDA-MB-453) that shares features with the LAR subtype, despite the fact that there is little research on the significance of AR in breast carcinoma. They conducted preclinical research on MDA-MB-453 and discovered that it grew in an androgen-dependent manner. AR antagonistic (flutamide) can stop MDA-MB-453 from multiplying. As a result, they recommended a tailored AR-blocking therapeutic regimen for LAR-subtype TNBC patients (Doane et al., 2006). Antiandrogen treatment was used on LAR-subtype TNBC patients by Gucalp and coworkers, who discovered that this patient population may profit from it (Gucalp et al., 2013). Bicalutamide, a selective AR inhibitor, was found to have a 19% clinical benefit rate (CBR) in phase II clinical research for the therapy of women with breast carcinoma with positive AR but negative PR and ER expression (Gucalp et al., 2013). By treating AR-positive TNBC individuals with enzalutamide, an AR inhibitor, Traina and coworkers were able to achieve a 25% CBR (Traina et al., 2018). Aside from AR expression, the LAR-subtype cell lines contain a high proportion of PIK3CA activating alterations and are very sensitive to PI3K inhibitors (Lehmann et al., 2011). The coevolution of PIK3CA alterations with AR reliance is comparable to the higher prevalence of PIK3CA mutations in ER-positive breast tumors (Stemke-Hale et al., 2008; Gonzalez-Angulo et al., 2009). In LAR cell lines, preclinical findings demonstrate that combining bicalutamide with a PI3K inhibitor has an additive/synergistic impact. As a result, this novel therapeutic AR regimen is predicted to be further improved, although further experimental support is required, and the function of AR in TNBC carcinogenesis must be investigated further.

Histone deacetylase inhibitors and heat shock protein 90

Histone deacetylase (HDAC) regulates the rate of transcription and protein levels of numerous DNA-damage response pathway components (Bakkenist and Kastan, 2003; Munshi et al., 2005; Adimoolam et al., 2007; Brazelle et al., 2010; Botrugno et al., 2012). HSP90 chaperones "client" proteins into their original conformations, thereby regulate numerous aspects of protein activity. HSP90 is a client of several components of the homologous recombination and non-homologous end-joining DNA repair mechanism (e.g., CHK1, BRCA1, BRCA2, CHK1, FANCA, RAD51) (Pratt and Toft, 2003; Pearl et al., 2008; Stecklein et al., 2012). HDAC inhibitors cause HSP90 to become hyperacetylated, separating client proteins like BRCA1 from the chaperone. HSP90 inhibitor AUY922 and HDAC inhibitor vorinostat were similarly found to be towards the top of the list for generating HRD-like gene expression patterns in TNBC cell lines in vitro experiments. HDAC inhibitors can thereby improve the treatment effectiveness of DNA-damaging drugs like platinum compounds in TNBC. Furthermore, in vitro investigations reveal that cotreatment with a pan-HDAC inhibitor plus cisplatin causes apoptosis both in BRCA1-mutant and BRCA1-proficient cell lines, and also that HDAC inhibitor therapy promotes synergistic lethality in triple-negative breast carcinoma cell lines (Weberpals et al., 2011; Bhalla et al., 2012; Ha et al., 2014).

HSP90 and HDAC inhibitors are currently in the early stages of clinical trials. In metastatic TNBC, a phase I trial is evaluating the safety and dosage of an HSP90 inhibitor (AT13387) with paclitaxel (NCT02474173). The combination of ganetespib (an HSP90 inhibitor) and paclitaxel is being studied in preoperative research (NCT02637375). A forthcoming phase I research will evaluate the conjunction of PARPi (BMN 673) and HSP90 inhibitor (AT13387)

TABLE 3.1 Trials evaluating the use of histone deacetylase inhibitors and heat shock protein 90.

S. No	Trail id	Intervention/treatment	Cancer type	Phase
1	NCT02474173	Onalespib + paclitaxel	Advanced TNBC	I
2	NCT02637375	Ganetespib + paclitaxel	TNBC	I
3	NCT02627430	Talazoparib + AT13387	Advanced TNBC	I
4	NCT01349959	Azacitidine + entinostat	TNBC	II

in metastatic solid cancers, notably TNBC, based on the preclinical synergy of HSP90 and PARPi (NCT 02627430). In addition, a phase II trial is underway in individuals with chemotherapy-resistant advanced TNBC who are being treated with entinostat (an HDAC inhibitor) in combination with the DNA methyltransferase inhibitor azacitidine (NCT01349959). The combination of cisplatin and romidepsin (a class I HDAC inhibitor) is being tested in metastatic TNCB or BRCA mutation-associated HER2-negative advanced breast carcinoma in a phase I/II trial (NCT02393794) Table 3.1.

Antiangiogenesis therapy

TNBC is linked to greater levels of vascular endothelial growth factor (VEGF)-A expression as well as more frequent amplification (Andre et al., 2009; Linderholm et al., 2009). As a result, it's been suggested that anti-angiogenic medications have a stronger activity in TNBC. Many anti-angiogenic drugs are being developed right now. The sole medicine licensed for the treatment of MBC by the EMEA, however not by the FDA, is bevacizumab, a monoclonal anti-VEGF-A antibody. In the main open-label randomized phase III study E2100, adding bevacizumab to weekly paclitaxel improved median PFS from 5.9 to 11.8 months (hazard ratio 0.6, p 0.001) and increased the response rate by twofold (49.2% vs. 25.2%, p 0.001). OS, on the other hand, did not show any substantial improvement (Miller et al., 2007; Gray et al., 2009).

Two more first-line phase III trials (AVADO and RIBBON-1) supported the advantage of adding bevacizumab to first-line chemotherapy, though to a lesser degree (Miles et al., 2010; Pivot et al., 2011; Robert et al., 2011). Identical outcomes for the combination in the TURANDOT trial (PFS 11 months, ORR 44%) and the CALGB 40502 trial (PFS 10.6 months) may additionally validate the effectiveness of bevacizumab in conjunction with weekly paclitaxel (Rugo et al., 2012; Lang et al., 2013).

Nevertheless, neither one of these individual randomized studies, nor a combined analysis, were able to show a substantial improvement in OS in unselected individuals. Only an exploratory subgroup evaluation of the randomized phase III second-line study, RIBBON-2 (Brufsky et al., 2012), revealed a trend towards increased OS for the TNBC group (17.9 vs. 12.6 months, $p = 0.0534$), as well as a substantial PFS advantage (6.0 vs. 2.7 months; HR 0.45; $p = 0.0006$). Due to the limited number of participants ($n = 159$) and statistical concerns, this may only be considered a hypothesis-generating retrospective study. Second-line therapy with bevacizumab is not permitted.

In a combined subgroup evaluation of all TNBCs in the three randomized phases III first-line studies, the essential question of whether bevacizumab has a particular advantage in

TNBCs was examined. The enhanced ORR (42% vs. 23%) and PFS (8.1 vs. 5.4 months; hazard ratio 0.63; p 0.0001) from these trials were validated in this study, while there was no tendency for a better OS (18.9 vs. 17.5 months; HR 0.96; ns) (Miles et al., 2013). Only triple-negative participants who had already received a taxane-containing adjuvant treatment were shown to have a markedly better OS in the combined study (25.6 vs. 15.0 months; hazard ratio 0.61, 95% confidence interval 0.4-0.94). Nevertheless, this was simply a subgroup study of a subgroup analysis that was exploratory.

Results from the BEATRICE trial (n = 2,591), a major adjuvant phase III study conducted only in TNBC, did not show an increase in DFS or OS for the introduction of bevacizumab to adjuvant chemotherapy following by bevacizumab maintenance treatment (Cameron et al., 2013).

In conclusion, bevacizumab has the same effect on TNBCs as it does in unselected patients. Bevacizumab, on the other hand, may have a unique role in the treatment of metastatic TNBC due to restricted therapeutic alternatives and frequent aggressive disease characteristics. Better response rates and prolonged PFS could more successfully counteract and treat existing or threatening symptoms without using hazardous polychemotherapy.

A variety of tyrosine kinase inhibitors (TKIs) targeting pro-angiogenic kinases including VEGF and PDGF receptors, such as sunitinib, pazopanib, and sorafenib, have been developed besides monoclonal antibodies. Combining these TKIs with chemotherapy drugs has proven challenging due to their enhanced off-target consequences. Their monotherapy effectiveness in MBC is limited, with ORRs varying from 0% to 11% (Cobleigh et al.; Bianchi et al., 2009; Moreno-Aspitia et al., 2009; Taylor et al., 2010).

Estrogen receptor ER-α36

TNBC cells are thought to lack intracellular estrogen signaling pathways because they lack PR, ER, and HER2 expression. They are hormonal treatment insensitive and have no identified therapeutic options. Wang and coworkers were the first to discover, clone, and identify ER-36, a novel estrogen receptor with a molecular weight of 36 kDa. This novel ER is not the same as the ER-α66, which has been investigated extensively. ER-α36 lacks the transcriptional activation domains AF-1 and AF-2 when compared to ER-66, but maintains the DNA-binding regions and certain dimeric ligand domains (Wang et al., 2005). Both ER-positive and ER-negative breast tumor cells express ER-α36, which is mostly found in the cytosol and cell membrane. As a result, ER-α36 is a membrane-expressed estrogen receptor that can swiftly mediate estrogen and antiestrogen signaling in both ER-positive and ER-negative breast tumor cells. Zhang et al. investigated the signaling pathways of ER-α36 in the TNBC cell lines MDA-MB-436 and MDA-MB-231 and discovered a favorable feedback loop between EGFR and ER-α36 in TNBC, suggesting that ER-α36 could be a target for TNBC therapy. Clinical trials are currently lacking in support, and potential treatment regimens have yet to be investigated.

MEK inhibitors

In vitro, a high range of TNBC and BLBC cell lines are responsive to MEK inhibition; BLBC cell lines are more sensitive to MEK inhibitors as compared to PI3K inhibitors (Hoeflich KP, et al., 2009). Certain TNBC cell lines responsive to MEK inhibitors have Ras/MAPK pathway alterations, such as activating mutations in HRAS, BRAF, or KRAS, which are very rare in individuals with TNBC (Barretina et al., 2012). Despite this, many TNBC cell lines demonstrate Ras/MAPK pathway overexpression with no oncogenic mutations in Ras/MAPK pathway components (Giltnane and Balko, 2014). In those circumstances, abnormal Ras/MAPK pathway activation can be thought to be due to stimulation or increased expression of growth factor receptors (like EGFR, FGFR1, IGF1R, or VEGFR, among others), or even to gene copy-number mutations (amplifications and gains) in important Ras/MAPK components including BRAF and KRAS, which were identified at moderate frequencies in BLBC (30 and 33%, respectively) and result in enhanced gene expression (Cerami et al., 2012; Craig et al., 2013). The genetic and/or epigenetic deletion of DUSP4, a negative modulator of ERK1/2 and JNK1/2, which has been linked to BLBC Ras–ERK activation (Balko et al., 2012, Balko et al., 2013), is an additional potential mechanism for Ras/MAPK pathway activation in TNBC. In preclinical investigations, DUSP4 deletion or reduced expression increases chemotherapy resistance in TNBC and leads to the maintenance of the tumor-initiating cancer cell population, which could be targeted, using Ras/MAPK pathway inhibitors and potentially the JNK/AP1 pathway inhibitors (Foulkes et al., 2003; Carey et al., 2010).

MEK activation can aid in the stability of c-Myc, a key oncogene product increased in 30% of TNBC or BLBC cases (Cerami et al., 2012; Horiuchi et al., 2012). While single-agent MEK inhibition can promote c-Myc degradation in TNBC, Duncan et al. (Duncan et al., 2012) found that this impact also causes the expression and stimulation of receptor tyrosine kinases, which can overcome MEK inhibition and create therapeutic resistance (Duncan et al., 2012) (. These findings imply that MEK inhibitors combined with small compounds or monoclonal antibodies addressing receptor-tyrosine kinases could be efficient treatments, but their efficacy has yet to be clinically validated (Duncan et al., 2012). MEK inhibitors are now being studied in conjunction with chemotherapy or other targeted medicines to treat TNBC and BLBC; nevertheless, relevant biomarkers that could allow for optimum patient choice have yet to be identified. There is little evidence on the effectiveness of MEK inhibitors in TNBC, although the only complete response to treatment occurred in a patient having mTNBC in a phase Ib study of patients having solid tumors (n= 31) administered with gemcitabine plus trametinib (an orally accessible strong inhibitor of MEK1/2) (Infante et al., 2013).

Cancer stem-cell population inhibitors

Breast cancer stem cells also called tumor-initiating cells, are a dynamic subset of tumor cells that have the features of breast stem cells, including the potential to recolonize a heterogeneous tumor (including both luminal and basal cytokeratin compartments) from a single cell (Charafe-Jauffret et al., 2009; Qayoom et al., 2021). When compared to non-cancer cells, breast CSCs have slower growth rates and greater levels of chemotherapy resistance (Creighton et al., 2009), and they frequently exhibit phenotypic changes comparable to those seen in epithelial-to-mesenchymal transition cells (Mani et al., 2008; Creighton et al., 2010). In breast malignancies, markers-based approaches such as measuring aldehyde dehydrogenase activity (ALDEFLUOR assay) (Charafe-Jauffret et al., 2009; Huang et al., 2009; Charafe-Jauffret et al., 2010), analyzing the expression of integrin receptors, and the capability to exclude ABC transporter substrates (Pontier and Muller, 2009; Britton et al., 2012) can be used to identify or enrich for the stem cell population. Early findings revealed that this population of cells could represent a subpopulation of tumor cells that contribute to resistance and accelerated recurrence following standard therapies (Creighton et al., 2010; Mir, 2021g), and so would be a suitable target for innovative treatment development in conjunction with chemotherapeutics. Cancer stem cells are abundant in TNBC, BLBCs, and several subtypes described by independent groups (Neve et al., 2006; Herschkowitz et al., 2007). While there is a considerable body of preclinical and clinical data supporting the presence of phenotypic breast cancer stem cells, the routes that lead to the maintenance of this population of cells are not well understood. Independent investigations have found that, based on the model or cell line utilized, the Ras/MAPK (Balko et al., 2013), Wnt (DiMeo et al., 2009), JAK/STAT (Marotta et al., 2011), TGF-β (Bhola et al., 2013), Notch (Harrison et al., 2010), and Hedgehog (Liu et al., 2006) pathways all contribute to the maintenance of breast cancer stem cells.

Antibody-drug conjugates (ADCs)

An ADC is intended to be plasma stable, to target tumor cell surface antigens with great specificity and affinity, and be internalized, cleaved, and to deliver a payload medication that causes anticancer action via direct cytotoxic cell death and causes immunogenic cell death (Mir, 2021a, Fig. 3.5).

Sacituzumab govitecan-hziy (SG) inhibits the expression of a glycoprotein called human trophoblast cell-surface antigen 2 (TROP-2) in greater than 90% of TNBCs. Its carrier is an active metabolite of irinotecan (SN-38) coupled to an anti-TROP-2 antibody via a cleavable linker. There were 108 patients with TNBC in this phase I/II single group research (NCT01631552), and 80% of them developed visceral metastases. Prior treatments comprised chemotherapies and checkpoint inhibitors, with the median number being three (range being two to ten). 57 patients showed medium (2+) to high (3+) TROP-2 expression by IHC, while 5 patients exhibited low or nonexistent TROP-2 expression by IHC, as per data available, despite the lack of biomarker selection. The median duration of response (DOR) was

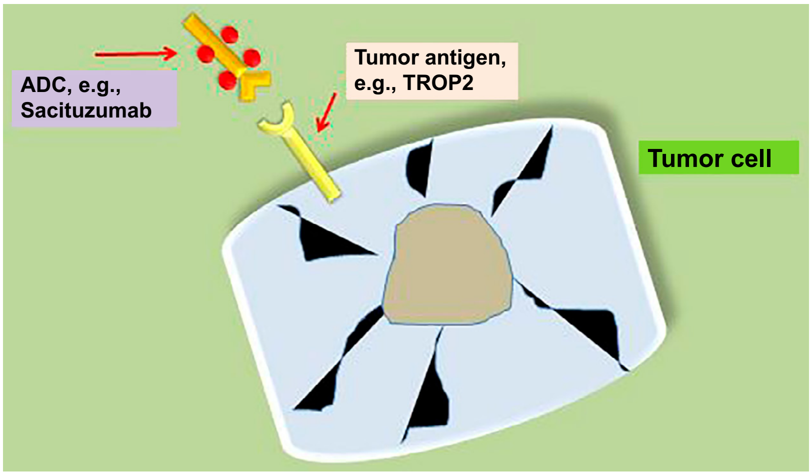

FIG. 3.5 Antibody-drug conjugates target cell-surface molecules and are combined with a powerful cytotoxic chemical to kill cancer cells.

7.7 months, and the ORR was 33%. The median PFS was 5.5 months, while the average OS was 13.0 months. The most common adverse responses were myelotoxic consequences, and grade 3 or 4 AEs included neutropenia and anemia (Bardia et al., 2019). The confirmative ASCENT Phase III research (NCT02574455) of SG in patients with mTNBC compared to therapies of physician's selection was halted due to compelling proof of effectiveness all over multiple endpoints, and the FDA granted SG expedited permission depending on the outcomes of the IMMU-132-01 Phase II clinical trial for the therapy of adult patients with mTNBC who've already received two prior treatments for metastatic cancer. It is also the first FDA-approved anti-TROP-2 ADC for patients with recurrent or refractory metastatic TNBC.

LIV-1, which is expressed in >90% of breast cancers but not in normal tissues, is targeted with ladiratuzumab vedotin (LV). LIV-1 is a transmembrane protein that functions as a zinc transporter as well as a metalloproteinase. LV's cargo is monomethyl auristatin E, a microtubule disruptive chemical (MMAE). Patients with extensively pretreated metastatic TNBC had a 25% ORR and a median PFS of 11 weeks in a phase I trial (NCT01969643). The treatment was usually well tolerated, with anemia, neutropenia, and neuropathy as side effects (Modi S et al., 2018). LV was studied further in combination trials and also in earlier therapy lines. The goal of the SGNLVA-002 Phase Ib/II trial (NCT03310957) was to see if combining LV and pembrolizumab leads to synergistic action via LV-induced ICD, which provides a milieu conducive to increased anti-PD-L1 activity. It was used as first-line therapy for individuals with locally progressed or metastatic TNBC who were unable to be resected. ORR of 35% was found in early dose-finding experiments, with responses independent of PD-L1 status and controllable tolerability (Boni et al., 2019).

Role of immunotherapy in the treatment of TNBC

The immune checkpoint system allows tumor cells to elude detection and destruction by the host immune systems; hence, inhibiting the immune checkpoint system is a viable therapy method for developing efficient antitumor immunity. PD-L1 (programmed cell death-ligand 1) is a transmembrane protein with a size of 40 kDa (Ishida et al., 1992; Mir, 2021b). When foreign antigens amass in the spleen or lymph nodes, the immune system responds by promoting antigen-specific T cell proliferation under normal conditions. When programmed cell death protein 1 (PD-1) binds to PD-L1, it can send signals to T cells that restrict growth and enhance T cell depletion. Tumor cells send inhibitory signals to T cells by bin ding PD-L1 to PD-1 on the surface of T cells (Pardoll 2012; Qayoom et al., 2021). According to one study, 59% of TNBC individuals exhibited high PD-L1 expression, 70% had high PD-1 expression, and 45% had both PD-1 and PD-L1 expression. Furthermore, the degree of tumor lymphocyte infiltration and tumor histological grading is linked to the expression of PD-1 and PD-L1 (Gatalica et al., 2014; Khosravi-Shahi et al., 2018).

Sun and coworkers performed PD-L1 immunohistochemical analysis on 218 TNBC specimens and discovered that TNBC cells exhibited PD-L1, implying that PD-L1 could be a promising TNBC immunotherapeutic focus (Sun et al., 2016). In a 2016 clinical trial with pembrolizumab, a monoclonal antibody against PD-1, for the treatment of TNBC, the ORR was 18.5% (95% CI, 6.3–38.1) in the 27 individuals whose anticancer activity could be assessed. Complete response was reported in one instance (3.7%); the partial response was reported in four cases (14.8%); stable disease was reported in seven cases (25.9%); and advancing disease was reported in 13 cases (48.1%) (Nanda et al., 2016). Likewise, in a 2017 phase I clinical trial for the treatment of TNBC with the monoclonal antibody atezolizumab against PD-L1, roughly 10% of TNBC patients showed a long impact from therapy. Despite the low clinical benefit rate of immune checkpoint drugs that target PD-L1/PD-1, several patients had a favorable prognosis and considerably improved OS rates. As a result, the current key problem is determining ways to increase TNBC patients' responsiveness to anti-PD-1/PD-L1 therapy and turn non-responders into responders. Patients experiencing advanced/metastatic TNBC will benefit from this better treatment, which will assist to lower the number of deaths and give them great hope (Tolba and Omar, 2018). Furthermore, in TNBC, there is a link between the immune response as well as the Ras/MAPK pathway. One study found that the Ras/MAPK system suppresses antitumor immunity by influencing antigen presentation, such as MHC-II, MHC-I, and PD-1, so a combination of MEK inhibition with PD-1/PD-L1 antibodies improved the therapeutic outcome in a mouse syngeneic tumor model (Mir, Giltnane and Balko, 2014).

CTLA-4 prevents T cells from becoming activated by attaching to costimulatory molecules like CD80 and CD86 (Mir and Agrewala, 2007; Mir and Agrewala, 2008; Mao et al., 2010, Mir 2015). The US Food and Drug Administration (FDA) have approved Ipilimumab, an anti-CTLA-4 antibody, for the therapy of metastatic cancer. The ORR for carcinoma patients treated with the monoclonal antibody ipilimumab was 11% (Intlekofer and Thompson, 2013). The conjunction of ipilimumab with nivolumab (PD-1 antibody) as first-line therapy for metastatic melanoma increased the ORR to 61% in a phase I clinical research (NCT01927419) (Postow et al., 2015). Further research (NCT01927419) found that in comparison to monotherapy, combined treatment substantially increased ORR and 2-year OS rates in individuals with metastatic melanoma (63.8% for ipilimumab plus nivolumab combined treatment vs. 53.6% for ipilimumab alone). Nevertheless, the combined treatment group had a considerably higher rate of grade 3–4 adverse events than the monoclonal antibody alone group (59% vs. 20%). Colitis and diarrhea were the most common grade 3–4 side effects (Hodi et al., 2016). Liu and coworkers employed a MUC1 mRNA nanovaccine in conjunction with an anti-CTLA-4 monoclonal antibody to address TNBC and reported a substantial cell-killing impact in TNBC 4 T1 cells as well as a tumor growth inhibitory activity in mice (Liu et al., 2018). Bernier and coworkers (Bernier et al., 2018) used a combination of DZ-2384, a new microtubule-targeting small-molecule drug, and a CTLA-4 inhibitor to considerably prolong the survival duration of mice in a TNBC metastatic mouse model. As a result, improving the combined regimen for TNBC targeted CTLA-4 immunotherapy could be the key.

Specific chimeric antigen receptor T cell (CAR-T) treatment is another immunotherapeutic option. CAR-engineered T lymphocytes targeting the folate receptor (FR) demonstrated extremely effective, selective killing and inhibitory effects on FR-expressing TNBC cells in vitro, according to Song et al. They also discovered that infusing human CAR-T cells that target FRα into immunodeficient mice with MDA-MB-231 tumor xenografts dramatically suppressed tumor growth (Song et al., 2016). Mesothelin is a membrane-bound glycoprotein. It is only expressed in mesothelial cells in healthy human tissues; however, it is substantially expressed in solid tumor tissues like TNBC. As a result, mesothelin could be a potential target for TNBC CAR-T therapy (Pastan and Hassan, 2014). AXL is a receptor tyrosine kinase that was found in individuals with chronic myeloid leukemia alongside two additional kinases, Tyros and MER. TAM (Tyros, AXL, and MER) is a family of proteins that includes AXL. AXL is abundantly expressed on the MDA-MB-231 cell surface in TNBC, according to studies. AXL-CAR-T cells were created for in vitro cell-killing experiments, and the findings revealed that AXL-CAR-T cells killed MDA-MB-231 cells significantly (Wei et al., 2018).

Immunotherapy for TNBC with adenosine pathway blockade

Tumor cells frequently overproduce and release adenosine, which is catabolized from ATP. A cluster of differentiation 73 (CD73), a plasma membrane protein that is increased in several cancer types, converts extracellular nucleotides into it (Allard et al., 2017; Ghalamfarsa et al., 2019). The adenosine 2A receptors (A2aR) and 2B receptors (A2bR) (Duhant et al., 2002; Allard et al., 2016), which are widely expressed on the cell surfaces of myeloid and lymphocyte cells, respectively, are activated by abundant adenosine in the tumor milieu, resulting in immunosuppressive consequences (Fig. 3.6). By removing the inhibitory impact on the immune system and increasing the cytotoxic T lymphocyte (CTL)-mediated immunological response, addressing these receptors and enzymes may contribute to the reactivation of anticancer immunity (Ohta 2016; Buisseret et al., 2018).

Clinical experiments have looked into combining adenosine pathway inhibitors with immune checkpoint inhibitors. NZV930 (SRF373) is an anti-CD73 monoclonal antibody that attaches to CD73 on tumor cells, causing CD73 to internalize and block the conversion of extracellular AMP to adenosine by CD73. In patients with advanced cancers, including TNBC, a Phase I/Ib study (NCT03549000) is being conducted to test NZV930 individually and in conjunction with PD-1 inhibitor PDR001 and/or A2aR antagonist NIR178. NIR178 is an A2aR antagonist that prevents T cells from being inhibited by adenosine/A2aR. NIR178 is being tested in conjunction with the

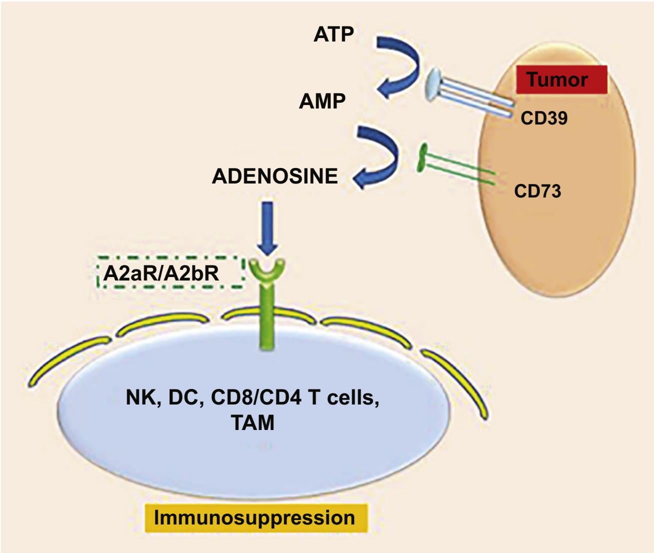

FIG. 3.6 ATP-adenosine pathway: CD39 and CD73 synthesize adenosine from ATP. T cell priming, expansion, and activation are all blocked when it binds to A2 receptors on immune cells. Immunosuppression is caused by NK cell degranulation, DC maturation and activation, and tumor-associated macrophage (TAM) M1 polarization.

PD-1 antibody spartalizumab in several solid tumors including diffuse large B-cell lymphoma (DLBCL) in a Phase II trial (NCT03207867) to see if the adenosine antagonist increases the effectiveness of PD-1 inhibition. In a Phase, I trial (NCT03629756), AB928, a dual adenosine A2aR/A2bR receptor antagonist, is being tested in patients with advanced cancers in conjunction with the PD-1 inhibitor AB122. Early data reveal that AB928 combined treatment has a positive safety profile as well as a predictable PK/PD relationship (Powderly et al., 2019).

Advanced treatment options for TNBC

Chemoresistance is a major issue in the treatment of metastatic cancer (Toh et al., 2014; Mehraj et al., 2021). Despite the fact that chemotherapy has progressed to a new level in treatment techniques (Bagnyukova et al., 2010), there is still a need to lessen the negative consequences of all treatment methods (Ramirez et al., 2009). Furthermore, non-steroidal anti-cancer medications have several adverse effects and are highly hazardous to normal cells in addition to cancer cells (Thun et al., 2002). When it comes to targeting treatments to tumor areas, there are two primary approaches:

(i) A passive transport method known as "enhanced permeability and retention" (EPR) wherein leaky vasculature in peripheral blood arteries to the tumor enhances nanoparticle permeability. Nevertheless, EPR has the disadvantage that not all tumors have leaky vasculature. As a result, a thorough examination of TNBC tumor biomarkers is needed before loading nanoparticles with a ligand specialized in the search for highly expressed receptors such as CXCR4 (folic acid receptor).

(ii) Active transport, which is governed by biomarkers like miRNA, antibodies, proteins, and therapeutic molecules like siRNA and aptamers is another strategy employed by researchers.

MiRNA and lnRNAs

RNA-seq was used to sequence all of the RNA species in a cell and discovered multiple RNA species, including mRNA. MiRNA and Long non-coding RNA are the two main types of non-coding RNA explored in TNBC development and therapy.

MicroRNA (miRNA/miR) is a short non-coding RNA that regulates gene expression and is usually 20–22 nucleotides long. The 3′untranslated region of mRNA is where miRNA is known to bind. The binding either destroys mRNA or prevents it from being translated (Lin and Gregory, 2015). In TNBC, miRNA has a crucial role in carcinogenesis, stemness, and treatment resistance (Ding et al., 2019; Si et al., 2019; Qattan, 2020). Because of their possibility as diagnostic biomarkers, the role of microRNA (miRNA/miR) in cancer therapy has lately increased (Rastogi et al., 2008). MiRNA558 is the overexpressed miRNA in TNBC (Zhu et al., 2017). In addition, a meta-analysis discovered numerous TNBC miRNAs (Lü et al., 2017). MiRNA detection is expected to be part of the armory of oncological research available in hospitals, allowing for more accurate prediction and diagnosis as potent biomarkers. The microRNA profiling investigation, which was the first of its type and focused on primary TNBC and also normal tissues, revealed about 116 microRNAs which had been deregulated. The overexpressed ones included miR-106b, the miR-200 family (miR-200a, miR-200b, and miR-200c), the cluster miR-17/92, miR-155, and miR-21 (Cascione et al., 2013). In addition, miR-424, miR-579, miR-627, miR-101, iR-125a-5P, and let-7g were shown to be highly expressed in lymph nodal tissues in a subsequent module of mRNA profiling of TNBC associated with lymph node metastases (Cascione et al., 2013).

Tumor suppressor miRNAs such as miR-126-5p, miR-136-5p, miR-135b-5p, miR-190a, and miR-182-5p, which are implicated in tumor development, are downregulated in TNBC (Lyng et al., 2012). MiR-22 is related to migration and metastasis and is downregulated in TNBC. The action of miR-22 is mediated by the activation of eukaryotic elongation factor 2 kinase (eEF2K), which stimulates the PI3K signaling pathway (Gorur et al., 2021). MiR-200b, an oncosuppressor, also activates target genes including SRY-box transcription factor 2 (SOX2), zinc finger E-box binding homeobox 1 (ZEB1), and CD133, promoting invasion and migration as well as stemness (Pang et al., 2018; Ding et al., 2019). Radiotherapy resistance arises when sphingosine-1-phosphate signaling is targeted, as evidenced by elevated expression of miR-95 in TNBC. TNBC (Naorem et al., 2019; Tormo et al., 2019) has doxorubicin resistance due to downregulated miR-449, which upregulates CDK2, CCNE2 (Table 3.2). miRNAs are also expressed at distinct stages of TNBC (Liu et al., 2015; Kahraman et al., 2018; Malla et al., 2019), according to various studies. These findings suggest that miRNA-based treatments, such as miRNA mimics or inhibitory oligonucleotides, could be used to treat TNBC (Mei et al., 2020). Shu et al (Shu et al., 2015) employed miR-21 in combination with an aptamer targeting EGFR to inhibit tumor growth in mouse models. Yin et al (Yin et al., 2019) created a CD133-binding RNA aptamer with a sequence complementary to miR-21 carried by a three-way junction motif scaffolding that inhibited TNBC cell migration (Yin et al., 2019).

Long non-coding RNA (lncRNA) affects gene expression at the epigenetic, transcriptional, post-transcriptional, and post-translational stages, with a length of 200 nucleotides. To enhance glycolysis and carcinogenesis in TNBC, the long intergenic non-coding RNA for kinase activation phosphorylates HIF-1 by leucine-rich repeat kinase 2. POU domain class 3 transcription factor 3 (POU3F3) was found to be involved in suppressing apoptosis and

TABLE 3.2 The function of miRNA and lncRNA expressed in TNBC.

	miRNA		
S. No	**miRNA**	**Role in TNBC**	**Reference**
1	miR-126-5p, miR-136-5p, miR-190a, miR-135b-5p, miR-182-5p	Tumorigenesis	(Lyng et al., 2012)
2	miR15a/16, miR-95, and miR-449	Drug resistance	(Huang et al., 2013; Tormo et al., 2019)
3	miR-22 and miR-200 family	EMT	(Pang et al., 2018; Gorur et al., 2021)
	LncRNA		
S. No	**LnRNA**	**Role in TNBC**	**Reference**
1	LINKA	Tumorigenesis and glycolysis	(Lin et al., 2016)
2	DANCR	Inhibits apoptosis	(Jin et al., 2019)
3	NEAT1	Apoptosis, migration, and invasion	(Ke et al., 2016; Jiang et al., 2018)
4	POU3F3	Inhibits apoptosis	(Yang et al., 2019)

increasing proliferation in TNBC by Yang et al (Yang et al., 2019). TNBC metastasis is aided by nuclear paraspeckle assembly transcript 1 (NEAT1) (Ke et al., 2016; Jiang et al., 2018; Wang et al., 2018). Several lncRNAs are reported to co-express with transcription factors implicated in EMT and proliferation such as HOTAIR, LncRNA-ATB, and LincRNA-ROR (Xu et al., 2016). These researches have given information on the possible use of antisense oligonucleotides targeting oncogenic lncRNA as a treatment for TNBC. Vaidya et al (Vaidya et al., 2019) showed in a mouse xenograft model of TNBC that nanoparticle-mediated transport of RNA interference agents directing differentiation antagonizing non-protein coding RNA, a lncRNA that is abundant in TNBC, exhibited modest effectiveness.

siRNA

Following the discovery of the Caenorhabditis elegans plant's qualities, siRNA has ushered in a new era in disease therapy, with siRNA being utilized to turn off or modify tumor genes that cause drug resistance, hence increasing therapeutic effectiveness (Bertrand et al., 2014, Dana et al., 2017). In TNBC cell lines, siRNA screens were done for a variety of genes, and RSK2 was discovered. NCAPD2 (non-SMC condensin I complex subunit D2) (Zhang Y. et al., 2020), Gpx1 (Glutathione peroxidase-1) (Lee et al., 2020) are also prospective therapeutic options for TNBC treatment. The siRNAs which have previously been employed in animal models to combat TNBC could be loaded in non-viral nanoparticles, supramolecular complexes, and viral capsids, enabling gene silencing for proteins that reflect bad prognosis in oncological clinical treatment viability (Guo et al., 2014).

Exosomes are also significant in the delivery of siRNA for the inhibition of TNBC metastases following surgery. Cationic BSA in combination with siS100A4 and exosome membrane-covered NPs aids in the administration of siRNA to prevent aggressive TNBC metastasis (Zhao et al., 2020).

Summary

TNBC is extremely aggressive and has a high rate of early recurrence when compared to other breast carcinoma subtypes. TNBC is resistant to endocrine therapy and targeted therapies due to the negative expression of ER, PR, and HER2. TNBC has a small number of therapeutic options, all of which have low efficacy. Chemotherapy remains the backbone of therapy for patients with early disease due to the absence of approved targeted therapies. Current technological platforms have greatly aided our present grasp of this subtype's molecular diversity. These molecular breakthroughs have allowed us to begin identifying potential treatment targets in TNBC. Several experimental techniques are being pursued, and several promising medication classes, including as immune checkpoint inhibitors, PARPi, platinum agents, and PI3K inhibitors, are being studied in human trials. Traditional medications are being optimized by administering them to patients and tumors that will profit the most, while novel treatments are being studied in biologically chosen patient subgroups. New-generation TNBC studies are beginning to incorporate the concept of heterogeneity, and smaller molecularly defined TNBC subgroups are being investigated. TNBC is a difficult disease to treat, and it's likely that multiple distinct targeted therapies will be required to make significant progress.

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CHAPTER

4

Conventional adjuvant chemotherapy in combination with surgery, radiotherapy, and other specific targets

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Introduction

It is a well-known fact that BC is the frequently diagnosed cancer around the whole globe and is the foremost cause of death occurring due to cancer (Bray et al., 2018; Mehraj et al., 2021). One of the molecular subtypes of BC is Triple-negative breast cancer (TNBC). The characteristic feature of TNBC individuals is that they lack all three receptors, which are ER, PR, and HER2 receptors (Irvin Jr and Carey, 2008). This characteristic feature of TNBC becomes one of the hurdles to several possible therapies that are present for BC treatment (Table 4.1). Because of the molecular heterogeneity, TNBC has become one of the most vulnerable types of BC, accounting for a total of 10–20% of BC cases (Mir et al., 2020). As compared to HR+ BC, TNBC is associated with an augmented rate of proliferation and is badly differentiated (Dent et al., 2007). The worse OS increased rate of recurrence and increased occurrence of distant metastases are all characteristics associated with TNBC (Dent et al., 2007). Since TNBC is associated with worse outcomes and thus doesn't get benefit from hormonal therapy or therapies targeted to HER2. Thus, this specific type of BC must achieve some specific treatment options. Furthermore, the protein EGFR in TNBC, due to its overexpression

TABLE 4.1 Systemic adjuvant therapy options for operable BC.

TNBC subtype	Adjuvant systemic therapy			
Hormone	Over expression of HER2 Receptors	Anti-HER2 therapy	Endocrine therapy	Chemotherapy
_	_	No	No	Yes

Combinational Therapy in Triple Negative Breast Cancer
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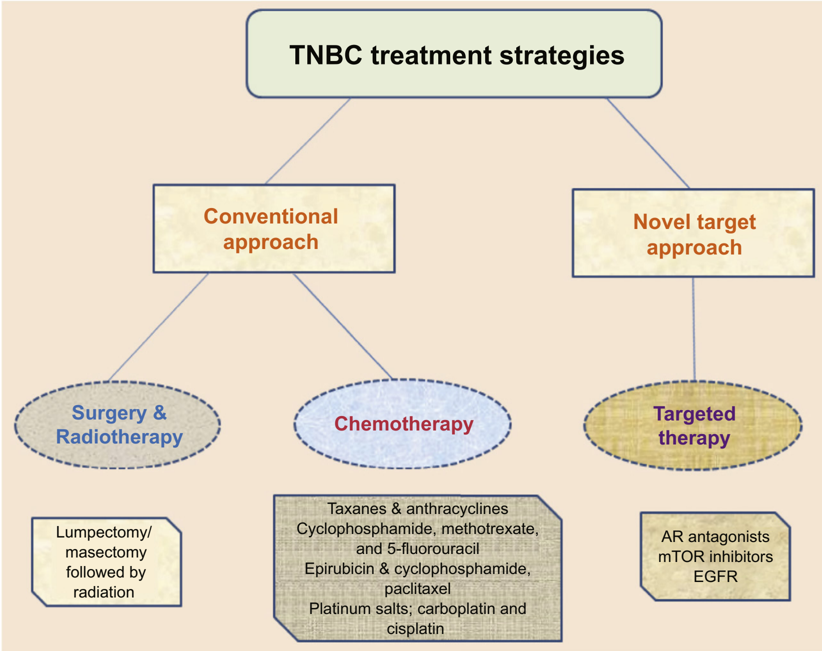

FIG. 4.1 Treatment options for TNBC include radiotherapy, chemotherapy, surgery and targeted therapy. Among them, the most significant one is chemotherapy.

has the ability to increase the resistance of TNBC against the treatment therapies (Nielsen et al., 2004). Thus, the treatment options can take the advantage of this protein by suppressing it and thereby enhancing the effectiveness of the therapies used in TNBC (Mir, 2021).

The treatment options for BC may include radiotherapy, chemotherapy, immunotherapy, and targeted therapy (Qayoom et al., 2021, Fig. 4.1). But at present, chemotherapy is the only treatment that has been approved for TNBC (Lebert et al., 2018). Although TNBC represents the most aggressive type of BC, 20% of TNBC patients show a pathologic complete response (pCR) after being exposed to neoadjuvant chemotherapy (Liedtke et al., 2008; Qayoom et al., 2021). Despite having better pCR, TNBC is associated with poor OS in comparison to non-TNBC patients. This phenomenon is known as the “triple-negative paradox” (Carey et al., 2007). After being administered with neoadjuvant chemotherapy, the clinical outcomes show the difference and thereby suggesting that some TNBC patients are sensitive to chemotherapy in comparison to the patients that form bulk show resistance towards the treatment or are not susceptible (Mir et al., 2021).

An overview of chemotherapy

Chemotherapy is one of the treatments used for treating BC, where cancer cells are destroyed with the help of certain drugs or medicines, thus getting rid of cancer. Depending upon the situation of the BC patients, chemotherapy may be given prior to surgery or it may be given after the surgical process is completed. Chemotherapy treatment options are frequently given in cycles: a treatment for a specified period of time, accompanied by a recovery phase, and then another treatment. It is usually given after surgery and can be administered in a dose-dense manner after every 3 or every 2 weeks.

TNBC is the most threatened form of BC. In spite of the fact that chemotherapy is the better treatment option in TNBC as compared to the other forms of BC, it still shows a worse prognosis (Ismail-Khan and Bui, 2010; Mehraj et al., 2021). The main reason for this is that the disease-free period between neoadjuvant and adjuvant therapy is less and a much-threatened course in the metastatic setting (Mir et al.2021).

The chemotherapy involves strategies that target the cell proliferation process, DNA repair mechanism, P53, and much more (Berrada et al., 2010; Mir, 2021). Various studies involving neoadjuvant chemotherapy demonstrated the advantage of combining novel chemotherapeutics with standard chemotherapy, such as taxanes, anthracyclines, antimetabolites, platinum-based substances, and novel microtubule-stabilizing factors (Amos et al., 2012). Presently, the most significant option available for TNBC involves 3rd generation CT regimens involving dose-dense or metronomic polychemotherapy (Cardoso et al., 2012). Based on various studies, platinum agents causing damage to DNA have shown an association with BRCA1 mutants and DNA repair dysfunctioning, thus developing again an interest in DNA-damaging agents like platinum agents. Also, an association has been shown by ds DNA breaks caused by bleomycin and etoposide (Gluz et al., 2008).

The studies have demonstrated the advantage of anthracyclines based CT in HER-2 positive patients, but in TNBC the efficacy of anthracyclines based CT chemotherapy remains at issue (Slamon et al., 2007; Gennari et al., 2008). Talking about taxanes, they form an important agent in TNBC chemotherapy, but their beneficial role in non- TNBC is not clear (Quinn et al., 2003; Cleator et al., 2007). The chemo sensitivity of tumors with p53 mutations, which is a feature of TNBC, is debatable, as anthracycline resistance in p53 mutated BC has been reported (Geisler et al., 2001). Patients with TNBC having a high rate of visceral metastases have a lower median lifespan of 7-13 months and less duration of response to subsequent lines of CT in the metastatic scenario. It is critical to choose the agents that are most likely to provide a significant benefit (Lin et al., 2008; Kassam et al., 2009).

TNBC is having heterogeneous nature and due to which this subgroup becomes a challenge for us in terms of its treatment (Qayoom et al., 2021). Therefore, predictive biomarkers need to be recognized that will determine response to specific CT, in order to have more advancement in the TNBC treatment field along with the present options of CT and also future combinations (Verma et al., 2011).

Neoadjuvant chemotherapy

This type of CT is given in such a way that some of its cycles are administered before surgery while others are administered after the surgery. To make locally advanced, untreatable BC resectable, neoadjuvant chemotherapy was initially employed. But now it has recently been used to downstage illness in the breast and axilla in curable malignancies, with the goal of preserving breast tissue and decreasing axillary lymph node dissection in some instances (Mieog et al., 2007). Individuals with unicentric malignancies that are greater relative to their breast size and those with HER2+ or TNBC probably get an advantage from NAC. The administration of NAC effectively retards the risk of axillary metastases in clinically node-negative females. More significant systemic therapies following NAC have led to increased response rates in the breast and axilla. Three potential randomized clinical studies looked at the efficacy of sentinel node biopsy following NAC in individuals with nodal metastases. When employing dual-tracer mapping and finding three or more negative sentinel nodes, false-negative rates are less than 10%, according to the ACOSOG Z1071 and SENTINA trials, which is equal to what is acceptable for sentinel nodal biopsy in the basic surgical context (Boughey et al., 2013). In a randomized trial done by Memorial Sloan Kettering Cancer Center, 48 percent of 288 patients with nodal metastases who became clinically node-negative after NAC had a nodal pCR and three or more recognized sentinel nodes, avoiding axillary dissection (Mamtani et al., 2016).

Neoadjuvant CT containing anthracyclines and taxanes

Clinical and pathological RRs in the case of NAC containing AC have been reported by Dees and co-workers (Carey et al., 2007) and it was demonstrated that clinical and pathological RRs are more in ER- and HER-2-negative individuals in comparison to other BC subtypes.

It was reported by Le Tourneau and co-workers (Wahba and El-Hadaad, 2015) that the RRs towards anthracyclines intensified by expanding either dose intensity/density of the administered CT, a rise in pCR rate from 13% - 47% by augmenting conventional neoadjuvant FEC100 CT to E70C 700 mg/m^2 (d1+8) along with standard 5-FU (d1-5).

The effect of Neoadjuvant CT containing Anthracycline and Taxanes was studied in both TNBC as well as non- TNBC patients. It was demonstrated that TNBC patients showed 14% pCR rates as compared to non-TNBC (38% *vs*. 12%). Furthermore, it was seen that those patients who have attained a pCR had an extended DFS and out of the patients who fail to attain a pCR, the TNBC patients revealed significantly poor prognosis (Wang et al., 2009).

Rouzier and co-workers have assessed the impact of CT before surgery in 22 basal-like BC individuals who are given paclitaxel weekly for 12 weeks and later with four cycles of FAC, the outcomes showed a 45% pCR rate (Rouzier et al., 2005).

Neoadjuvant platinum agents in TNBC and BRCA mutation

Various clinical studies have revealed that BC cell lines being BRCA-1 deficient show their sensitivity towards the DNA damaging platinum agents, such as mitomycin and cisplatin, and it was demonstrated that this sensitivity could be reversed with the fixing of normal BRCA1 function or up- regulation of BRCA-1 (Tassone et al., 2003).

Cisplatin has been again reconsidered for treating TNBC because of the better advancements in controlling its poor effects and also because of the various clinical data that revealed the significance of platinum agents in TNBC due to the similarity of TNBC with the BRCA-1 mutation containing BC (Foulkes et al., 2010). Here it should be noted that almost all BRCA-1 mutant tumors are basal-like, but the vice versa is not true, that is all basal-like tumors do not possess BRCA1 mutations (Bhattacharyya et al., 2000). Neo-adjuvant platinum-based CT in TNBC has shown significant promising results. The studies have also revealed the significance of platinum agents in combination with other substances in NAC (Ezzat et al., 2004).

Adjuvant chemotherapy

According to Medical pharmacology and therapeutics, adjuvant chemotherapy is the treatment option that is given to the patients after having the primary treatment, especially surgery, in order to lessen the occurrence of relapse from occult disease.

An abbreviated history of adjuvant systemic therapy

The (NSABP) B-01 was the first clinical trial that was started in 1958 for assessing adjuvant chemotherapy in BC, which revealed in 1968 that an adjuvant alkylating agent (thiotepa) administered after radical mastectomy effectively reduces the rate of relapse in pre-menopausal women having 4 or more positive axillary lymph nodes (Fisher et al., 1968). In 1975, another clinical trial revealed the advantage of one more alkylating agent - L-phenylalanine mustard (Fisher et al., 1975). It was also demonstrated by the Istituto Nazionale Tumori in Milan, Italy, that CMF being one of the CT regimens containing alkylating agent notably decreases the threat of relapse (Bonadonna et al., 1976), thus leading towards the modern era of adjuvant polychemotherapy regimens, frequently utilized in clinical studies. These clinical trials were specifically assessing the benefits of adjuvant CT in premenopausal women having ANP disease at greater threat for relapse (Bonadonna et al., 1976). Later these trials were also conducted for assessing the role of adjuvant CT in under risk post-menopausal women (Albain et al., 2009) and women with ANN disease (Mansour et al., 1989; Fisher et al., 1997; Mansour et al., 1998). It was concluded by the National Institute of Health consensus panel in the US in 2001 that "*Because adjuvant polychemotherapy improves survival, it should be recommended to the majority of women with localized breast cancer regardless of lymph node, menopausal, or hormone receptor status*." (Abrams, 2001). In spite of the fact that the acquisition of a wide range of CT options has resulted in retarding the death rates of BC in the US and also worldwide, (Jemal et al., 2010), still it has led to the overtreatment of so many BC with CT that need not have CT for curing their disease.

Adjuvant chemotherapy: First, second, and third generation regimens

Adjuvant! is a web-based resolving aid generally assisting in clinical practice helping clinicians to recognize the significant advantages of adjuvant therapy, particularly chemotherapy (Loprinzi and Ravdin, 2003). Adjuvant! designates the regimes of adjuvant CT as 1st, 2nd, and 3rd generation (Table 4.2). Among the various combination used, the two remarkable groups used for early as well as advanced stage BC are anthracyclines (Epirubicin, doxorubicin,) and/or Taxanes (docetaxel, paclitaxel) (Fig. 4.2).

TABLE 4.2 Classification of adjuvant chemotherapy regimens.

S. No	Regimen	Advantage
1	1st generation	35% decrease in BC death rate in comparison with no adjuvant chemotherapy
2	2nd generation	20% decrease in BC death rate in comparison with the first-generation regimen
3	3rd generation	BC mortality rate decreases by 20% as compared with a second-generation regimen

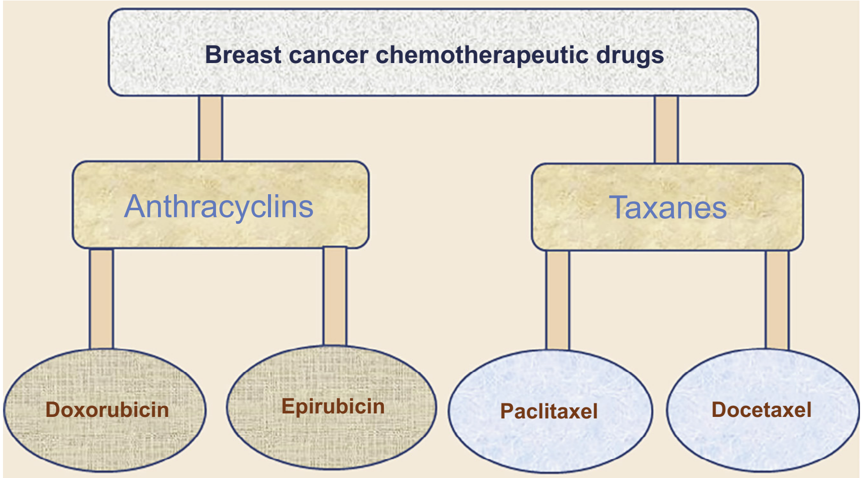

FIG. 4.2 **Some of the chemotherapeutic drugs used in BC chemotherapy.**

Anthracyclines

This CT drug is derived from antibiotic rhodomycin B. Initially, they were extracted they were initially extracted in the 1950s from Streptomyces (gram-positive). One of the most vital single agents in MBC was found to be Doxorubicin, which was extracted from *Streptomyces peucetius* (Shockman and Waksman, 1951), a mutant of the original Streptomyces strain present around the Adriatic Sea, and was thus known as Adriamycin (Tan et al., 1973; Bonadonna et al., 1976). In spite of the fact that Doxorubicin is associated with some kind of toxicity like congestive cardiomyopathy, but the toxicity was controlled by restricting the cumulative lifetime dose (Sparano). Doxorubicin was then replaced by its epimer - Epirubicin, which differs from doxorubicin in the orientation of the C4 OH group on the sugar and shows less or reduced toxicity than doxorubicin (Torti et al., 1986; Ambrosini et al., 1988).

Taxanes

This group of CT drugs belongs to diterpenes. The most broadly used chemotherapy agents of this class include paclitaxel and docetaxel (Mir et al., 2021).

Paclitaxel was discovered from the bark of *Taxus brevifolia*, and its anticancer efficacy was first reported in 1971 (Wani, 1972). Paclitaxel has the ability to bind with microtubules and inhibits their depolymerization, resulting in mitotic arrest (Schiff et al., 1979; Horwitz 2004) and missegregation of chromosomes on aberrant multipolar spindles (Weaver, 2014; Zasadil et al., 2014). Despite its specific method of action, the development of paclitaxel was delayed at first because of its shortage and less solubility. A preparation of paclitaxel solubilized in Cremophor EL was finally produced; however, it was linked to hypersensitivity reactions to the Cremophor EL vehicle (Rowinsky and Donehower, 1995), necessitating pre-medication with histamine and corticosteroids blockers, which almost delays the clinical development of paclitaxel. The US FDA approved Cremophor-EL-paclitaxel in 1994 for treating MBC in individuals who had succeeded after anthracycline-based CT combinations or who relapsed fewer than six months after AT (Rowinsky and Donehower, 1995). The scarcity of paclitaxel was addressed by another chemotherapeutic drug namely docetaxel, an alternative of paclitaxel was generated from the European yew tree *Taxus baccata* (Ringel and Horwitz, 1991). Docetaxel is an inhibitor of microtubule and shows the mode of action identical to paclitaxel, (Ringel and Horwitz, 1991), however, it is much effective in vitro. Docetaxel dissolves in polysorbate-80 and is somewhat more soluble in water than paclitaxel. Premedication is also essential to minimize the threat of acute hypersensitivity reactions and cumulative fluid retention that is related to the infusions of docetaxel (Schrijvers et al., 1993). In a direct comparison between docetaxel vs. paclitaxel among MBC patients, docetaxel exhibited more effectiveness but with greater toxicity (Jones et al., 2005), whereas a direct comparison of paclitaxel vs. doxorubicin as first-line treatment therapy exhibited comparable efficacy (Sledge et al., 2003). Both of these agents have been considerably tested in adjuvant trials depending upon the essential single-agent role for each agent in MBC (Bachegowda et al., 2014).

The case for chemotherapy in TNBC

Various studies have demonstrated the efficacy of cytotoxic chemotherapy in treating TNBC under adjuvant, neo-adjuvant, and metastatic settings. Because much of the earlier investigations were done before the advent of HER2, their applicability to TNBC is restricted. Nonetheless, in retrospect, the first findings showing estrogen receptor levels affecting CT response provided a solid platform on which modern trials could be built. A retrospective analysis of seventy individuals with MBC was one of the first to demonstrate that CT benefits differed depending upon ER status (Lippman et al., 1978). The presence of ER among 25 patients was associated with just a response rate of 12%, compared to 75% in 45 patients who did not have ER expression. However, a contradicting study in the same year claimed that ER- rich group had a greater response rate to CT than the ER-poor group under metastatic scenarios (Kiang et al., 1978).

In a 2005 overview meta-analysis, the Early Breast Cancer Trialist's Collaborative Group (EBCTCG) demonstrated the effectiveness of polychemotherapy in ER-poor BC (Clarke et al., 2008). A significant decrease in relapse and mortality from BC was reported in younger (10 year HR 0.73 and 0.73, respectively) and older females with ER poor disease participated in 46 polychemotherapy trials that began before 2000 (but did not include taxanes) (10 year HR 0.82 and 0.86, respectively). The paucity of data on HER2 status in these trials also limits this study, but it is consistent with the idea that CT has a significant benefit for TNBC. According to a retrospective study of 3 large CALGB trials including 6,444 patients, ER-negative cancers benefit significantly more from contemporary intensified CT (Berry et al., 2006). Furthermore, a comparison was done between the low dose CAF regimen in CALGB8541 with high dose regimen of doxorubicin, cyclophosphamide followed by paclitaxel (AC-T) in CALGB9741 (Citron et al., 2003), it was revealed that decrease in relapse rate was 26% for ER + and 55% for ER - tumors. The complete advancement in risk of relapse at 5 years was 7% for ER-positive patients and 22.8% for ER-negative tumors just treated with tamoxifen. The idea of dose-intensive regimens revealing the highest improvement in results in TNBC is supported by a retrospective investigation assessing 236 high-risk patients in the

WSG AM-01 trial who were administered with a dose-dense regimen of 4 cycles of epirubicin and cyclophosphamide and later were administered with 3 cycles of CMF compared to high dose CT with peripheral stem cell support (Gluz et al., 2008). Despite the fact that high dose CT usually reveals no improvement in OS, at a median follow-up of 62 months, TNBC individuals who are administered with high dose CT show an improved OS of 76% in comparison to 61% in the dose-dense arm. Summing up the results of these two studies, it could be concluded that CT, especially in a dose-dense and dose-intensive setup has got a significant benefits for TNBC.

Principles for adjuvant and neoadjuvant CT

TNBC and other subtypes of BC share the same factors that control the decision to use NAC vs. adjuvant CT. These ideas are largely affected by; the ability to resect the primary tumor and lymph nodes to achieve negative margins and the capability to cytoreduce a BC to enhance breast conservation rather than mastectomy (Palma et al., 2015, Fig. 4.3).

Standard chemotherapy regimens in the neoadjuvant and adjuvant settings

The main aim of NAC is to increase the chances of breast-conserving surgery and to monitor response towards systemic therapy (Kaufmann et al., 2006). Many females with TNBC can be considered for CT at some time in their care due to the paucity of targeted therapy alternatives in the adjuvant situation. Females displaying no histological evidence of residual invasive cancer in the breast or ALN (pCR) show much better long-term results than females who display residual illness (RD) (Cortazar et al., 2014). However, advancements in pCR were not related to identical advancements in OS across BC in a meta-analysis done by Cortazar and co-workers, leading to the fact the NAC results are not an exact substitute for everlasting results for all BC subtypes (Cortazar et al., 2014).

Subsequent anthracycline- taxanes-based CT is a widely utilized standard of care in NAC. According to the NSABP-30 research, subsequent therapy demonstrated a slight but

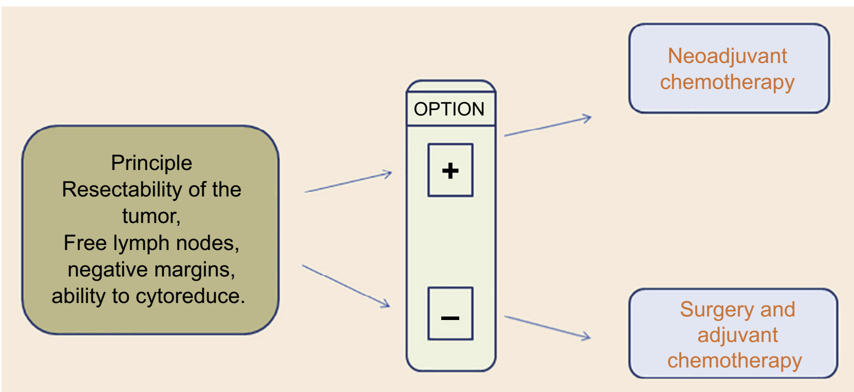

FIG. 4.3 Principle of neoadjuvant chemotherapy strategy.

substantial improvement in DFS when compared to contemporaneous regimens in the adjuvant situation (Swain et al., 2010). There is a lot of curiosity in whether producing new regimens or adding agents to the already existing regimens can have any advancement in pCR rates and long-term results.

Although the subsequent, dose-dense anthracycline-taxane-based CT is the frequently used regimen for moderate to high-risk TNBC patients, there are several alternative regimens for conventional CT for TNBC in the adjuvant situation (Swain et al., 2010). For individuals having average-to-high-risk TNBC illness, an epirubicin-based regimen- FEC followed by docetaxel or paclitaxel is also an option (Martín et al., 2008).

In the US, docetaxel with cyclophosphamide is utilized and seems to be at minimum as successful as AC (Adriamycin plus cyclophosphamide) for various patients; however, this study only involved a small group of hormone receptor-negative individuals (Jones et al., 2009). CMF (cyclophosphamide, methotrexate, and fluorouracil) combination can be used as a substitute that has reduced long and short-term toxicity but with a longer therapy period (Colleoni et al., 2010; Cheang et al., 2012).

Platinum-based CT in TNBC

Data demonstrating a greater incidence of DNA repair errors in TNBC, which may make TNBCs specifically sensitive to cross-linking agents and also high response rates in the metastatic scenario, sparked interest in platinum agents (Isakoff, 2010; Silver, 2010; Isakoff et al., 2015). Individuals having metastatic or recurrent, locally advanced TNBC were randomly administered with docetaxel or carboplatin as 1st line therapy in the TNT study (Tutt et al., 2015). ORRs for a period of 18 months was identical for docetaxel and carboplatin, with 35.6% for docetaxel and 31.4% for carboplatin, revealing the significance of platinum as a feasible 1st line option but not above to taxanes. Additionally, it was demonstrated in a nonrandomized phase II clinical study involving single agent platinum in MTNBC that individuals having 0-1 lines of CT for their MBC had a somewhat low RR of 25.6% (Isakoff et al., 2015).

In the GeparSixto and CALGB/Alliance 40603 studies, the inclusion of platinum to NAC regimens among TNBC patients was investigated prospectively. TNBC patients were randomly assigned to take liposomal doxorubicin, paclitaxel, and bevacizumab along with or without carboplatin in the GeparSixto study (Von Minckwitz et al., 2014). Also in CALGB/Alliance 40603, TNBC individuals were randomized to get concomitant carboplatin four cycles every 3 weeks and/or bevacizumab 9 cycles for every 2 weeks after receiving paclitaxel 12 weeks on weekly basis followed by doxorubicin+ cyclophosphamide 4 cycles for every 2 weeks (Sikov et al., 2015). The platinum dosage and the timing difference between the trials; in 40603, carboplatin with an AUC of 6 was administered every three weeks along with paclitaxel for 12 weeks every week, whereas, in GeparSixto, carboplatin with an AUC= 1.5 was administered weekly along with liposomal paclitaxel and doxorubicin for 18 weeks. The inclusion of carboplatin in both trials resulted in higher rates pCR. The insertion of carboplatin to GeparSixto increased pCR rates (breast/axilla) from 36.9% to 53.2% with BRCA carriers showing a 25% rise in pCR (P= .005) (Von Minckwitz et al., 2014; Von Minckwitz et al., 2014). With the administration of carboplatin for breast/axilla, CALGB/Alliance 40603 showed a rise in pCR (54% vs. 41%; P=.0029) (Sikov et al., 2015). However, endless outcome data introduced at San Antonio Breast Cancer Symposium showed that adding carboplatin in GeparSixto revealed improved DFS (median follow-up 35 months; HR, 0.56; 95% CI.0.33-0.96), whereas adding carboplatin to CALGB/Alliance 40603 did not improve event-free survival (median follow-up 39 months; HR, 0.84; 95% CL, 0.58-1.22) (Von Minckwitz et al., 2014; Sikov et al., 2015).

Although the findings for adding platinum to standard CT in neoadjuvant setup is promising, both the studies were deficient for endless outcome end points, thus making it difficult to draw definitive conclusions. The two trials differ with respect to the addition of alkylating agent; patients in CALGB/Alliance trial were administered with an alkylating agent (cyclophosphamide) besides anthracycline and taxane (with or without carboplatin), whereas in the GeparSixto trial there was no addition of alkylating agent. Differences in platinum dose (every 3 weeks in 40603 vs. weekly in GeparSixto) or time period (12 vs. 18 weeks, respectively) may have influenced the results. Furthermore, in the CALGB/Alliance study, advancements in pCR were linked to higher toxicity like grade 3-4 neutropenia and thrombocytopenia, and also paclitaxel dose modifications (Sikov et al., 2015). It is not clear how platinum will be included, and it is also uncertain whether platinum will be used to replace anthracyclines, taxanes, or alkylators rather than being added to present regimes.

Various ongoing phase III research on platinum could provide more information. The ADAPT study will test nab-ptx along with gemcitabine or carboplatin in TNBC individuals before surgery. In the NRG BR003 trial, adjuvant doxorubicin+ cyclophosphamide was followed by paclitaxel weekly with or without carboplatin for greater risk TNBC or node (+), and the results may provide more information on long term outcomes as well as variations between adjuvant and neoadjuvant setup. For TNBC with the persistent disease following NAC, EA1131 is a randomized trial comparing four rounds of platinum treatment to observation. Platinum is not yet ready to be incorporated in present standard NAC or adjuvant CT regimes for all TNBC patients due to the intricate concerns of toxicity and dose, as well as unknown everlasting effect.

Poly ADP ribose polymerase (PARP) inhibitors in TNBC

In the presence of mutated BRCA1/BRCA2, inhibitors of PARP 1, a base excision repair enzyme, causes synthetic lethality. In TNBC, which frequently has BRCA mutations or impairments in other DNA repair components, PARP1 inhibitors have been investigated (Lord and Ashworth, 2013; Mir and Mehraj, 2019). In a trial, namely I-SPY 2 trial, TNBC and hormone receptor-positive patients were given carboplatin and veliparib along with paclitaxel as a part of NAC. With the addition of platinum/veliparib, the pCR rate for TNBC patients in the PARPi plus carboplatin arm accounts for 52%, compared to 26% for TNBC patients given therapy without platinum or PARP, respectively (Lee et al., 2020). Another phase III trial (NCT02032277), has administered TNBC patients with carboplatin/paclitaxel/veliparib, paclitaxel/carboplatin, or paclitaxel alone, later all are followed by cyclophosphamide in the neoadjuvant setup.

Another study involved individuals with TNBC or BRCA mutations having residual disease and were randomized to cisplatin plus rucaparib or single-agent cisplatin after NAC. The inclusion of the PARP1 inhibitor did not affect the CT toxicity, although it did not increase 1-year DFS appreciably (Dwadasi et al., 2014). Despite the fact that no definitive trial has shown that PARP inhibitors increase OS and/or DFS, upcoming investigations in the

TABLE 4.3 Inhibitors of PARP in neoadjuvant TNBC studies.

S. No	Trials	Treatment	No. of TNBC patients	pCR Rate	p-value
1	I-SPY 2	P and Cb + veliparib followed by AC vs. P followed by AC	39 vs 21	51% vs 26%	Not reported (95% PI, 33–66% vs 9–43%)
2	BrighTNess	Arm 1: P and Cb + veliparib	316 vs 169 vs 58	53% vs 58% vs 31%	Arm 1 vs 2: 0.357
		Arm 2: P and Cb			Arm 1 vs 3: <0.0001
		Arm 3: P			
		All arms followed by AC			
3	GeparOLA	P+ olaparib vs P + Cb, followed by EC	50 vs 27	56.0% vs 59.3%	Not reported
4	NCT02401347	Phase II of talazoparib	Recruiting with an accrual goal of 40	N/A	N/A

AC, doxorubicin and cyclophosphamide; Cb, carboplatin; EC, epirubicin and cyclophosphamide; N/A, not applicable; P, paclitaxel. *pCR in the both breast and axilla (ypT0/is ypN0).
Note:- The table data has been adapted from (Lee et al., 2020).

adjuvant and neo-adjuvant settings may provide more insight into the impact of PARP inhibitors (Table 4.3).

Vascular endothelial growth factor (VEGF) inhibitors in TNBC

Because TNBCs have greater levels of VEGF in their tumors, researchers are looking at using bevacizumab, a VEGF-directed mab, to treat them (Foekens et al., 2001). In a trial namely NSABP B-40, the addition of chemotherapeutic drugs (capecitabine or gemcitabine) to neoadjuvant taxane/anthracycline regimens, as well as the function of neoadjuvant bevacizumab in HER2- breast tumors was assessed (Bear et al., 2015). The administration of either capecitabine or gemcitabine did not result in better results (Bear et al., 2015). With considerably more common grade 3-4 neutropenia, hypertension and hand-foot syndrome, adding bevacizumab was related with enhanced OS (HR, 0.65; 95%, CI, 0.49-0.88; P=0.004)but not disease-free survival(HR, 0.8; 95% CI, 0.63-1.01; P=.06) (Bear HD et al., 2015). In a trial namely GeparQuinto, adding bevacizumab to neoadjuvant cyclophosphamide/epirubicin and then later involving docetaxel resulted in a higher pCR rate for TNBCs (39.3% vs. 27.9%), but no meaningful improvement in OS or DFS (Von Minckwitz et al., 2014).

TNBC patients have also given bevacizumab as adjuvant treatment. TNBC patients were randomized to undergo four cycles of conventional CT along with or without bevacizumab in the BEATRICE experiment, which was an open-label, multicenter, phase III trial. With the addition of bevacizumab, the DFS (82.7% versus 83.7%) OS (HR, 0.84; 95% CI, 0.64-1.12; P=0.23) were not substantially different. Patients who used anthracyclines and bevacizumab at the same time had a modest expansion in cardiac events (Cameron et al., 2013). Due to the increased toxicity and lack of efficacy of bevacizumab in adjuvant setup, bevacizumab is contrary to play role in treating TNBC (BEATRICE and ECOG 5103).

The case of BRCA-mutant TNBCs: PARP and platinum

BRCA- mutant patients may have unique biology and benefit disproportionately from platinum in both adjuvant and neoadjuvant situations, according to emerging evidence. On intrinsic expression profiling, 70% of individuals with a BRCA mutation who develop BC are classed as basal-like and triple-negative (Fig. 4.4). A study of 107 women with BC having BRCA1 mutation who were administered with four cycles of cisplatin had a 61% pCR in neoadjuvant context (Byrski et al., 2014).

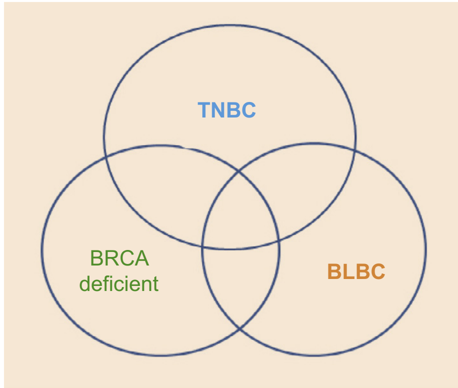

FIG. 4.4 Representation of significant overlap between TNBC, BLBC, and BRCA mutant BC patients.

A nonrandomized phase II study namely TBCR009, employing single-agent platinum in MTNBC showed a RR of 54.2% in NRCA-mutated individuals vs. 19.7% in wild-type BRCA patients (Isakoff, 2010). Low BRCA1 expression, high BRCA1 methylation, and BRCA1 mutation were found to be the biomarkers for platinum response in a trial of neoadjuvant in TNBC (Silver, 2010). Subgroup analysis of patients receiving carboplatin having BRCA1/2 mutations under metastatic setting in TNT trial showed a significant improvement in progression-free survival (Tutt, Ellis et al.).

According to these findings, platinum is likely to be effective in patients having BRCA-mutant BC. In a trial namely INFORM trial (TBCRC 031), BRCA carriers are randomized to either 4 cycles of cisplatin or 4 cycles of AC followed by final surgery of the breast. The trial's final results look at the pCR, comparative toxicities of two regimens, and long-term clinical response rate, and offer more knowledge into how platinum should be used in this unique cohort.

While PARP medicines have failed to demonstrate a clear effect in unselected TNBCs, promising findings in the metastatic context have piqued interest in BRCA-mutant patients (Tutt, Ellis et al., Silver, 2010). Women with advanced, recurrent BRCA-mutated cancer were enrolled in phase II clinical study and were randomly randomized to get either a lower dose or continuous maximum dosage of olaparib. Having acceptable toxicity, those on maximal dose had a greater ORR (41% vs. 22%) (Tutt et al., 2009). The ongoing OlympiA research, which analyses 12 months of PARP inhibitor therapy in BRCA-mutant BC patients, could provide more information about PARP inhibitor's involvement in this unique patient group.

Surgery in TNBC

The surgery is undertaken depending upon the nature and stage of the tumor. The operation may involve lumpectomy; which is the removal of a lump only or it may involve mastectomy; which is the removal of the entire breast. The surgeon must follow a prescribed practice to establish that the cutout tissue in the surgery consists of clear margins of cancer, indicating that cancer has been eliminated. This treatment may involve a pectoralis major muscle; the main muscle of the anterior chest wall and whose part is removed during the surgery (Mir et al., 2021). One of the recent techniques sentinel lymph node (SLN) dissection is a very popular method that involves taking off far fewer lymph nodes, thus possessing few side effects (Ellis et al., 2017; Houssami et al., 2017; Pearson et al., 2017).

Surgery, as with other kinds of BC is the best technique for controlling TNBC locally. On MRI, TNBC is frequently a unifocal mass lesion with a smooth margin, making it a favorable option for BCT with negative resection margins (Uematsu et al., 2009). Several investigations have examined the hazards of regional and local recurrence (Haffty et al., 2006; Nguyen et al., 2008; Freedman et al., 2009; Millar et al., 2009). Some studies have demonstrated that the rate of recurrence in TNBC is not greater than that of non-basal subtypes or non-TNBC (Solin et al., 2009; Voduc et al., 2010) whereas Nguyen and co-workers demonstrated that the local rate of relapse in TNBC is greater than that of HR+ and HER2- subgroups (Nguyen et al., 2008). Although the effect of regional or local recurrence results is unknown, it has a significant effect on patient's QOL. Therefore, on sentinel node biopsy, every effort should be done to keep away from false-negative diagnosis and residual disease on axillar dissection.

The impact of TN status on surgical decision making

Many pieces of research have been conducted to see if individuals with TNBC are more likely to go for mastectomy rather than a lumpectomy. The conclusion was that TN status being related to high-grade tumors, younger age; it has no bearing on surgical treatment options (Mir MA et al., 20210. Though TNBC is regarded as most aggressive form of BC, surgical decisions are likely to be based on more traditional clinicopathological criteria and patient choice (Crutcher et al.). According to Freedman and co-workers, TNBC shows low RR after BCS than other subtypes of BC, so they are still good candidates for BCS.

Chemotherapy before surgery vs after surgery

Both lines of treatment in chemotherapy- preoperative or postoperative are employed in BC treatment. Some of the leading benefits of preoperative CT include higher rates of BCT without local recurrence hazards and close monitoring of tumor response in vivo to the CT. If the aim is to shrink the tumor, neo adjuvant therapy can be employed (Giordano 2003; Chen et al., 2017).

Radiotherapy in TNBC

This approach targets the tumor or post-surgery tumor regions through the use of greater-energy X-rays or gamma rays. It can reduce the need for mastectomies. In the early stages of BC, a combination of RT and lumpectomy is significantly being used instead of mastectomy (Hall and Brenner, 2008). A study was done in India, which enrolled a total of 135 females, the majority of whom had undergone mastectomy. It was demonstrated that there was no local recurrence after hypofractionated RT and among 135 patients only 4 had metastatic disease (Nandi et al., 2014). It was demonstrated by Zhou and co-workers that RT is helpful in early BC patients (Zhou et al., 2012). The studies done by Zhou and co-workers looked at 143 females having BCS and have been given either intraoperative or normal RT.

There was a considerable local control of the tumor after 54 months of follow-up. RT uses high-energy beams to attack tumor cells. RT shows a notable role in the destruction and killing of BC cells that usually remain in the tumor site even after the surgery or appear again where the tumor was excised. An extension of RT brachytherapy involves the use of radioactive catheters. However, this addition has now been replaced by the use of electron beam RT subjected to breast scar. The dosage of RT should be sufficient to kill the cancer cells. This treatment usually occurs for 5-6 weeks, involving 5 days a week. The time duration for each treatment is about 15 minutes (Sharma et al., 2010).

Radiotherapy for TNBC after BCS

Early BCS combined with whole breast radiation has lowered the local rate of recurrence from 10% to roughly 2% over the last 4 decades. This advancement is not just due to the advances in RT, but also to earlier diagnosis, better systemic therapy, and effective pathological evaluation. Individual biological information may help identify individuals with greater recurrence risk using standard prognostic indicators in order to provide intensity-appropriate treatment at the time of molecular typing. The regional recurrence of TNBC and HER2+ is thought to be identical, although Trastuzumab decreases the local rate of recurrence by 50% in patients having overexpression of HER2, which is helpful to repair. Unfortunately, breast surgeons and radiotherapists must decide whether or not to accept BCS and how to lower the local rate of recurrence following surgery for TNBC patients. Abdulkarim's cohort study (Abdulkarim et al., 2011) on how to choose a local treatment approach for 768 early-stage TNBC individuals during 7.2 year follow-up period revealed that the survival rate without relapse of BCS combined with modified radical mastectomy with adjuvant RT, RT, and modified radical mastectomy with adjuvant RT was relatively 85%, 94%, and 87%. The potential of RT in TNBC local management is shown by the fact that postoperative RT can reduce the incidence of local recurrence of TNBC. TNBC patients who have persistent cancer cells under the microscope following BCS may benefit from a dosage increase in RT, according to clinical trials. According to Jone's research, even though TNBC has a greater local recurrence rate, it can be decreased to a significant level by boost; therefore BCS is not inadvisable in patients with TNBC. According to the research of TNBC at the T1-2N0 stage, the complete advantage of BCT in reducing regional recurrence was 6%, suggesting that BCT may be a much better option for TNBC than mastectomy. The European Organization for Research and Treatment of Cancer (EORTC) conducted 22,881 studies on 5000 patients who had a boost in tumor bed after BCS, and the outcomes showed that patients with high-grade tumors and ages under 50, as well as TNBC patients, benefited from local dose escalation. Bartelink et al. (Bartelink et al., 2007) used a 10-year follow-up to establish that the local rate of recurrence of entire breast irradiation with a boost group was 6.2%, in comparison to the whole breast irradiation group where the local rate of recurrence was 10.2% ($P< 0.0001$). Researches (Chen et al., 2017) have also revealed that the prophesy (DFS, OS, and LRFS) among the individuals of TNBC not having lymph node metastasis taking BCS along with post-surgery RT is identical with that of those TNBC patients that are having a mastectomy, simultaneously, the outcomes have revealed that post-surgery RT is helpful for T1/T2 TNBC individuals having >4 positive ALN. As a result, post-surgery RT after BCS among TNBC can decrease the local rate of recurrence, especially among individuals having ≥ positive ALN and a boost is helpful among TNBC individuals.

Radiotherapy after mastectomy in TNBC individuals

The majority of radiation indications for BC depend upon TNM staging. The American Society of Clinical Oncology (ASCO) advises adjuvant radiation following modified radical mastectomy (MRM) for individuals with ≥ 4 positive LN or at stage III, although it states that there is insufficient confirmation to change the guideline at this time. Postoperative radiation is not recommended for individuals with tiny lesions or negative LN, according to the NCNN recommendation. TNBC has a larger local decrease rate than other molecular types, according to clinical studies, and its prognosis is worse. The local recurrence rate is still greater even when the modified radical mastectomy is done for the early stage (T1 2N0-1). Furthermore, whether early patients need RT after radical mastectomy is a debatable issue. The study has shown 10 year CIR LRR of BC at T1-2N0 after radical mastectomy was 5.2%, on multivariate analysis, tumor margin, size, systemic therapy, age, and lymphovascular invasion (LVI) were notably related with LRR. And LRR CIR of patients having no risk factors or > 3 risk factors was 2% and 19.7% respectively (Abi-Raad et al., 2011). The local recurrence rate in the group that received post-surgery RT for 5 years was 11.7%, which was notably lower than the rate in the group that did not receive RT (25.4%) (Abdulkarim et al., 2011). Women with T1-2NO TNBC who were treated with MRM without RT had a considerably higher risk of LRR than those who were having BCT. Marianne Kyndi et al. (Kyndi et al., 2008) selected 1000 individuals with high-risk BC from a total of 3000 patients to study T1 3 (T1-2 84%, T3 15%) and positive lymph nodes (94%, about % > 3 positive lymph nodes), and all of the patients agreed to modified radical mastectomy and were randomly classified into two groups. There were 152 TNBC cases (15%), 7 (9.4%) cases of LRR in the RT group, and 20 (25.6%) cases in the non-radiotherapy group. TNBC was notably associated to an increase in overall death rate ($p = 0.02$), local recurrence rate ($p = 0.01$) and distant metastasis rate ($p = 0.02$), according to a single-factor analysis. The outcome was that early TNBC having the greater risk that refused RT after modified radical mastectomy had a greater local recurrence rate. In a phase III trial, TNBC patients in stages I and II were administered with CT after mastectomy and subsequently were randomly assigned to receive post-mastectomy radiation (PMRT) or not. According to data from a median of more than 7 years of follow-up, lymph nodes of over 80% of patients were negative, tumor diameters of over 70% of patients were less than 2 cm, and 5-year survival with no relapse was considerably enhanced in the PMRT group. Wang and co-workers conducted prospective randomized trials to compare the distinction between TNBC at stage T1-2NOMO with and without RT after modified radical mastectomy and found that the 5-year local rate of recurrence of the RT group and non-radiotherapy group was respectively 11.7% and 25.4% ($p = 0.02$), while the 5-year OS rate of the RT group was also 11.7% and 25.4% ($p = 0.02$). Simultaneously, the 5-year OSR of the RT group was 11.6% greater than that of the non-radiotherapy group ($P= 0.03$) (Wang et al., 2011). In spite of the fact that RT after mastectomy can enhance TNBC outcomes, still more study is needed to back up the findings. Therefore, clinicians must assess the patient's circumstances to determine if patients with TNBC at the N0 stage should have PMRT or not, to select treatment options for each individual.

Radiosensitization

Although it is unclear if TNBC is susceptible to radiotherapy, the ease with which it recurs suggests that treatment resistance exists. After accepting MRM, Kyndi et al. (Kyndi et al., 2008) meta-analyzed prophesy of individuals with BC in different molecular phenotypes and found that the local rate of recurrence of TNBC after RT did not decrease as much as the Luminal type, suggesting that TNBC may be radio resistant. BC with ER+ and HER2 has successful targeted therapy, but TNBC has none. As a result, we can develop new radio sensitizing agents to control TNBC's innate and radiation-induced radiation resistance and improve radiotherapy's local and systemic impacts. Some researchers believe that TNBC's radiation resistance is linked to the overexpression or deletion of multiple gene targets and that the radio sensitivity of cancer cells can be improved by inhibiting the conduction pathway associated with these receptors or molecules. According to a study, deleting and overexpressing various gene targets in TNBC activates the PI3K/Akt pathway, which then regulates radiation resistance through this mechanism (Koboldt et al., 2012). In TNBC individuals with a highly mutated BRCA, adenosine diphosphate (ADP) ribose polymerase (PARP) inhibitors (PARPi) can significantly impede the restoration of damaged DNA, strengthening the cytotoxicity of radiation, and establish a synthetic fatal impact, which has an apparent curing impact. Because overexpression of the EGFR gene and its amplification is found in 45% to 70 5 of TNBC cases, anti-EGFR therapy is coherently beneficial for TNBC patients. ZR-BA1 is a bifunctional targeting molecule inhibitor that can cause DNA alkylating lesions as well as inhibit the TK domain of EGFR. In the TNBC cell lines MDA-MB-468 and 4T1 of mouse BC, a combination of ZR-BA1 and ionizing radiation can cause various ds breaks and repair delay, as well as inhibit the EGFR pathway's conduction (Heravi et al., 2015). There are other radio resistance mechanisms that are mentioned in the (Fig. 4.5).

Chemotherapy in combination with radiotherapy (chemoradiotherapy) in TNBC

As TNBC represents a most threatened type of BC that does not respond to hormone treatment, CT is the main treatment option, despite the fact that even it has a poor prognosis. Adjuvant RT, which reduces BC mortality rate, has not been studied extensively among poor

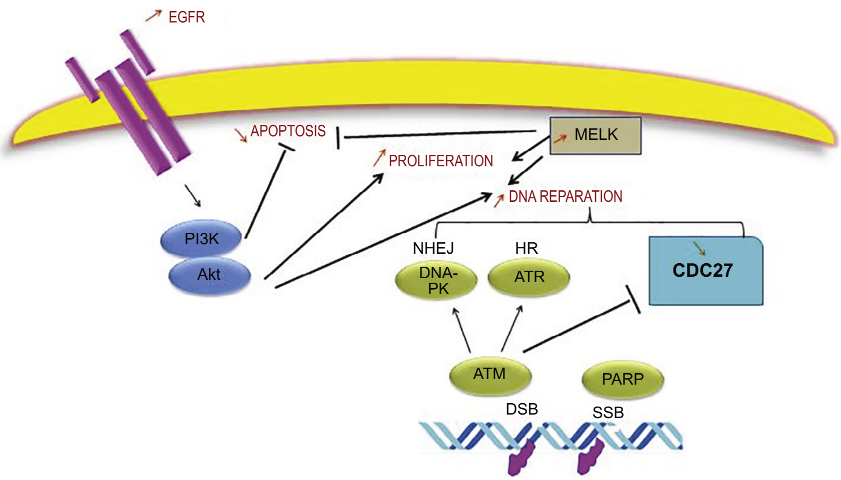

FIG. 4.5 TNBC cells over express genes that are responsible for radio sensitivity. The over expression of these genes like EGFR increases proliferation and DNA damage response and reduces apoptosis. This results in enhanced radio resistance.

TNRC females. The study done by Jianhua Wang and co-workers (Wang et al., 2011) examined the function of chemo radiotherapy on survival outcomes in TNBC females after mastectomy. This prospective randomized controlled multi-center trial including 681 TNBC females having stage 1-11 received mastectomy, among them, 315 cases were administered with systemic CT alone, 366 females were given RT after the course of CT, OS, and RFS were calculated. Systemic and local toxicity were both seen at the same time. The results revealed that after 86.5 months follow-up, 5-year RFS rates were 74.6% and 88.3% for ACT alone and ACT plus RT, respectively, with a notable comparison between the two cohorts (HR 0.77 (95% CI 0.72, 0.58): P=0.02), 5-year OS notably improved in ACT plus RT group as compared with CT alone (90.4% and 78.7%) (HR 0.79 (95% CI 0.74, 0.97): P-0.03). Furthermore, there were no reports of severe toxicity. The conclusion of this study states that females having early TNBC following mastectomy, routine ACT and RT was more beneficial than chemotherapy alone.

The trial (NCT01289353) was done, to assess the effectiveness of chemo radiotherapy in BC patients after breast surgery. This study makes use of carboplatin in combination with RT among TNBC patients. This trial concluded that carboplatin in combination with RT is a well-standard and favorable treatment approach for early-stage TNBC.

Chemotherapy combining specific target molecules in TNBC

Because of the significant heterogeneity of TNBC, finding new therapeutic targets and performing targeted therapy is particularly difficult. There are presently various ongoing clinical trials based on immunohistochemistry staining data that are targeting specific receptors or targeted therapeutics for TNBC (Fig. 4.6) (Table 4.4).

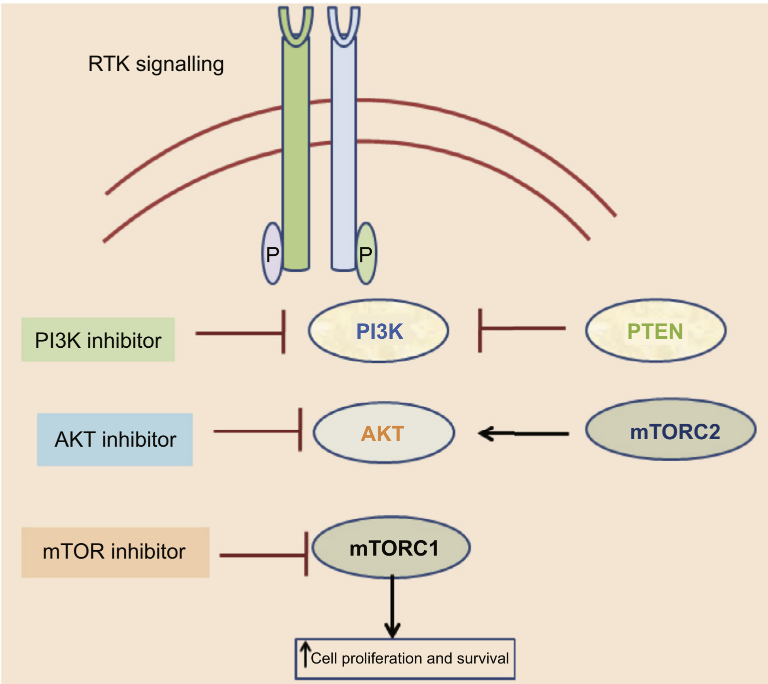

FIG. 4.6 Mechanisms of AKT/PI3K/mTOR pathway activation and targeted therapies.

TABLE 4.4 Possible therapeutic approaches and drug examples for TNBC.

S. No	Possible therapeutic approaches	Drug examples
1	PARP inhibition	Olaparib; ABT-S888
2	Androgen receptor inhibition	Bicalutamide
3	EGFR pathway inhibition	Cetuximab
4	PI3K pathway inhibition	NVP/BEZ235; Everolimus

EGFR

Nielsen and co-workers used DNA microarray investigation on various BLBC samples and discovered that almost 60% of the samples displayed high levels of EGFR (Nielsen et al., 2004). Livasy and coworkers' statistical findings further revealed that around 70–78% of BLTNBC samples showed high levels of EGFR. As a result, it is possible that EGFR could be used as a therapeutic target in TNBC (Livasy et al., 2006). Livasy and co-workers' statistical findings further revealed that around 70–78% of basal-like TNBC patients showed high levels of EGFR. As a result, it is possible that EGFR could be used as a therapeutic target in TNBC (Livasy et al., 2006). However, a randomized phase II clinical study (NCT00232505) of 120 patients of TNBC indicated that cetuximab treatment alone had a RR of < 6%, while cetuximab in combination with carboplatin had an RR of just 17% (Carey et al., 2012). As a result, while the preclinical evidence strongly supported the use of EGFR as a possible target for TNBC targeted therapy, the clinical trial data revealed that EGFR-targeted TNBC treatment did not reach the predicted results.

Androgen receptor (AR)

AR is expressed in both normal and cancerous breast tissues; however, the levels are dramatically varied in distinct cancerous breast tissues (Mir et al., 2021). In around 10–15% of TNBC patients, AR expression is positive (Barton et al., 2015). AR positivity is characterized as the LAR-subtype TNBC (Farmer et al., 2005; Lehmann et al., 2011). Doane and co-workers analyzed 99 BC patient samples and 8 different BC cell lines and uncovered a cell line (MDA-MB-453) that shares features with the LAR subtype, despite the fact that there is little research on the significance of AR in breast cancer. They conducted preclinical experiments on MDA-MB-453 and discovered that it grew in an androgen-dependent manner. AR antagonism (flutamide) can stop MDA-MB-453 from proliferating. As a result, they advocated inhibiting AR as a targeted therapy for LAR-subtype TNBC patients (Doane et al., 2006). Ant androgen therapy was used on LAR-subtype TNBC patients by Gucalp and co-workers, who discovered that this group of TNBC could benefit from it (Gucalp et al., 2013). A 19% of CBR was found in phase II clinical study employing bicalutamide, a targeted AR inhibitor, for treating BC individuals with AR+ but negative ER and PR expression (Gucalp et al., 2013). By treating AR + TNBC patients with enzalutamide, an AR inhibitor, Traina and co-workers were able to achieve a 25% CBR (Traina et al., 2018). LAR-subtype cell lines have a greater PIK3CA activating mutation rate and are sensitive to PI3K inhibitors, in addition to AR expression (Lehmann et al., 2011). The coevolution of PIK3CA mutations with AR dependence is analogous to the high frequency of PIK3CA mutations found in ER+ BC (Stemke-Hale et al., 2008; Gonzalez-Angulo et al., 2009). Bicalutamide in combination with a PI3K inhibitor has an additive effect in LAR cell lines, according to preclinical research. As a result, this new targeted AR regimen is predicted to be further improved, although further experimental support is required, and the function of AR in TNBC tumorigenesis should be investigated further.

Estrogen receptor ER-α36

TNBC cells are thought to lack intracellular estrogen signal transduction because they lack expression of PR, ER, and HER2 expression. They are endocrine therapy insensitive and have no identified therapeutic targets. Wang et al. were the first to discover, clone, and identify ER-36, a novel estrogen receptor with a molecular weight of 36 kDa. This newly developed ER is not the same as the ER-66 that has been researched extensively. ER-36 doesn't possess the transcriptional activator domains AF-1 and AF-2 but possesses the DNA-binding domains and several dimeric ligand domains compared to ER-66. Both ER-positive and ER-negative BC cells contain ER-36, which is mostly expressed in the cytoplasm and cell membrane. As a result, ER-36 is a membrane-expressed ER that can swiftly regulate estrogen and antiestrogen signaling transduction in both ER+ and ER- BC cells. Zhang et al. investigated the signaling pathways of ER-36 in the MDA-MB-231 and MDA-MB-436 TNBC cell lines and discovered a positive feedback loop of EGFR and ER-36 in TNBC, suggesting that ER-36 could be a strong target for TNBC treatment (Zhang et al., 2011). Clinical trials are currently lacking in support, and various treatment programs are still being investigated.

Mammalian target of rapamycin (mTOR)

Mammalian target of rapamycin (mTOR) is an intracellular kinase that is usually associated with cell growth, proliferation, etc. mTOR inhibitors have been proven to assist patients with a variety of cancers, including kidney cancer. PTEN (phosphatase and tensin homolog) loss and mTOR activation are both common in TNBC. There is therefore a case to be made for developing mTOR inhibitors in patients with TNBC who have lost PTEN (Lehmann et al., 2011; Mir, 2015). Interestingly, some findings claim that mTOR activity can contribute to cisplatin resistance, which can be reversed using the mTOR inhibitor everolimus (Beuvink et al., 2005). Beuvink and co-workers (Beuvink et al., 2005) found that combining everolimus with cisplatin increased the loss of viability in vitro by fivefold. These findings show that combining cisplatin and mTOR inhibitors in TNBC patients makes sense.

Summary

Due to its poorer prognosis and fewer treatment options, as well as a lack of targeted use of medicines, TNBC is a challenge for patients and physicians, as evidenced by its high mortality when compared to other BC subtypes. Because there are no known particular therapeutic targets for TNBC, cytotoxic chemotherapy remains the cornerstone of current treatment options. Chemotherapy's benefits for TNBC have now been proven in a number of studies at both the early and advanced stages. TNBC has consistently shown higher RRs to NAC than non-TNBC, and pCR predicts better long-term outcomes for TNBC. However, the possible selective benefit of particular chemotherapeutic medicines over others necessitates careful consideration in order to select the therapy that is most likely to help a given patient while limiting unnecessary damage. The significance of platinum-based CT is indicated by the two most important trials namely CALGB40603 and TNT. PARPi have also revealed their significance, specifically in BRCA-mutant TNBC and various ongoing trials will highlight their significant role in TNBC. With regards to surgery, the decision relies on various pathological variables and patient preferences. The significance of preoperative CT includes higher rates of BCT without local recurrence hazards and close monitoring of tumor response in vivo to the CT. Early BCS combined with whole breast radiation has lowered the local rate of recurrence. The studies have also highlighted the role of chemo radiotherapy, as it was seen that for females having early TNBC following mastectomy, routine ACT and RT was more beneficial than chemotherapy alone. There are presently various ongoing clinical trials based on immunohistochemistry staining data that are targeting specific receptors or targeted therapeutics for TNBC like EGFR, mTOR, etc. Summing up the contention, various ongoing studies are likely to improve and make advancements in the treatment of TNBC patients.

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CHAPTER

5

Role of immune system in TNBC

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Introduction

Breast cancer being a challenging disease has got a significant death rate (Mehraj et al., 2021). It is currently the most frequent type of tumor across the globe and thus is a public health dilemma across the globe. BC has been divided mainly into two categories: invasive and non-invasive. Clinically, there are three categories of BC: BC with positive hormone receptor status, HER2 positive breast cancer, and Triple-negative BC (Mir et al., 2021). TNBC, the most vulnerable type of BC is characterized by the absence of all of the three receptors i.e., the status for Triple-negative BC becomes ER-, PR- and HER2- (Kalimutho et al., 2015). TNBC is further divided into six subcategories based on molecular heterogeneity. Due to this heterogeneity, TNBC has become one of the most vulnerable types of breast cancer, accounting for a total of 10–20% of BC cases. One of the hallmarks of any type of cancer including BC is the stimulation of the immune system. The immune responses shown by the immune system are a result of the coordination between innate and acquired immunity. The immune system is able to perform its function mainly because of the immune cells, that may belong either to the innate (monocytes, APCs, macrophages, and neutrophils) or acquired (B and T cells). The cells of the acquired immune system mainly rely on the innate immune system to recognize the antigens and thus lead to the destruction of these antigens. During the progression of cancer, there occurs a disbalance in the functioning of both the innate and acquired immune systems, thus favoring the cancer growth rather than retarding it (Mir et al., 2021). In this aspect, immune cells had played a significant role in regulating the protumorigenic or anti-tumorigenic functions of the immune system. In other words, we can say that the fate of the cancer cells at different levels of the cancer is greatly influenced by the immune cells (Denkert et al., 2010).

At present, we are equipped with a variety of treatment options for breast cancer; still, there are wide ranges of BC patients who are not responsive to these treatment options. One of the groups of BC patients that are becoming a challenge for us is the group of TNBC

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individuals. In this regard, the role of the immune system is considered to have a significant impact on this particular subtype. This chapter will emphasize the function of the immune system in cancer including the TNBC and the various immune checkpoints in TNBC that could be used in the treatment of these diseases.

Cancer-immunity cycle

The relation between the BC and the immune system has been under study for many years (Berg, 1959). The immune system has the potential to evade and destruct the cancer cells by entering the various stages in a very sequential manner. This step-wise cancer cell destruction is known as the cancer-immunity cycle (Chen and Mellman, 2013) (Fig. 5.1). The cancer immunity cycle, except in the malignant stages, performs a balanced role between the stimulation of a significant immune response and the inhibition of the same to stop immoderate immune response, which would otherwise lead to so-called autoimmunity. During the process of immunoediting, the immune system eliminates the tumor cells and edits the genome of tumor cells, thus acting as dual-faced soldiers during the immunoediting process (Efremova et al., 2018). Taking this aspect into consideration, immune checkpoint inhibitors enhance the process of immunoediting (Mir et al., 2021).

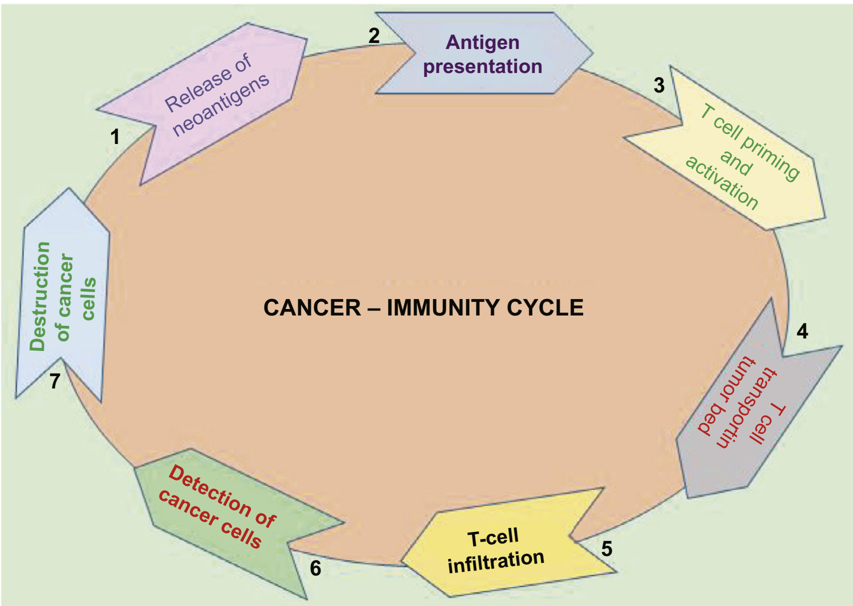

FIG. 5.1 The cancer immunity cycle consists of various steps: 1) In the first step, the tumor cells release neoantigens. 2) The neoantigens are identified by DCs and presented to the T cells; 3) which leads to T cell activation and produces immune response against the tumor antigens. 4) The T cells are migrated into the TME after their activation. 5) There occurs infiltration of T cells in the TME. 6) In the TME T cells identify and 7) destruct tumor cells with the help of their TCRs.

Immune system in cancer –a friend or FOE

In case of the malignant tumors, various intrinsic inflammations are responsible for the development and progression of cancer. Because of the formation of various forms of inflammation, a wide range of innate and adaptive immune cells occur in the BTM (Grivennikov et al., 2010). In addition, these inflammations affect the immune response of an individual toward the tumor cells and thus can be utilized in chemotherapy and immunotherapy (Grivennikov et al., 2010). The tumors are usually defended by the adaptive immune system, mainly involving the immunity that is T cell-dependent (Hwang and Nguyen, 2015). For instance, CD8+ T cells have the ability to secrete granzymes, perforin, and IFN-γ and thus help in the destruction of tumor cells (Töpfer et al., 2011). It has been seen that immune cells may possess either anti-tumorigenic or pro-tumorigenic effects or both. For example, CD8+ and CD4+ T cells, DCs, etc. possess both the effects, whereas, CD4+ T2 cells possess only pro-tumorigenic effects but the pro-tumorigenic effect is not possessed by NK cells (Grivennikov et al., 2010). In addition, the anti- tumor response is significantly triggered by DCs within the TME by antigen presentation to CD8+ and CD4+ T cells (da Cunha et al., 2014). Besides possessing the ability to suppress self-reactive T cells in autoimmune diseases, Tregs cells do possess the ability to inhibit the response against the tumor by inhibiting the various immune cells, such as NK cells, DCs, and CD8+ T cells (Tan et al., 2011). Thus, it is clear that the potential immunotherapy may take the advantage of this, by enhancing the anti-tumorigenic property of DCs, CD8+ T cells, and NK cells and retarding the pro-tumorigenic property of Tregs and thus may prove beneficial for BC patients.

Role of immune cells in cancer progression

The studies suggest that the presence of inflammatory cells within the tumors is an important aspect that needs to have attention to. Since these were the cells that were first to be detected in human cancerous cells their role needs to get a better understanding of cancer biology. Presently, it is clear that the cancer progression is mainly due to the aberration in the immune system of an individual, which results in the cancer cell proliferation and immunosuppression and of course the metastasis (Palucka and Coussens, 2016, Fig. 5.2).

At first, various immune cells assist in the recognition and elimination of the tumor cells, but some of the tumor cells are resistant or non-immunogenic and hide from the elimination phase. These resistant cells divide and divide, although the immune system is accomplished to manage the tumor growth (Teng et al., 2015). As the tumor cells evade the immune response they acquire advanced stages of their progression and there are various inflammatory cells that affect the fate of the tumor. For example, the increased presence of infiltrating macrophages is associated with a bad prognosis (Zhang et al., 2011; Mantovani et al., 2017; Gonzalez et al., 2018). Similarly, the increased presence of tumor infiltrated T cells is associated with a better prognosis. The role of various immune cells in tumor progression or regression is given below:

Macrophages

Macrophages belong to the innate immune system and are very flexible in the sense that they perform their role in tissue hemostasis, a defense mechanism against pathogens, and

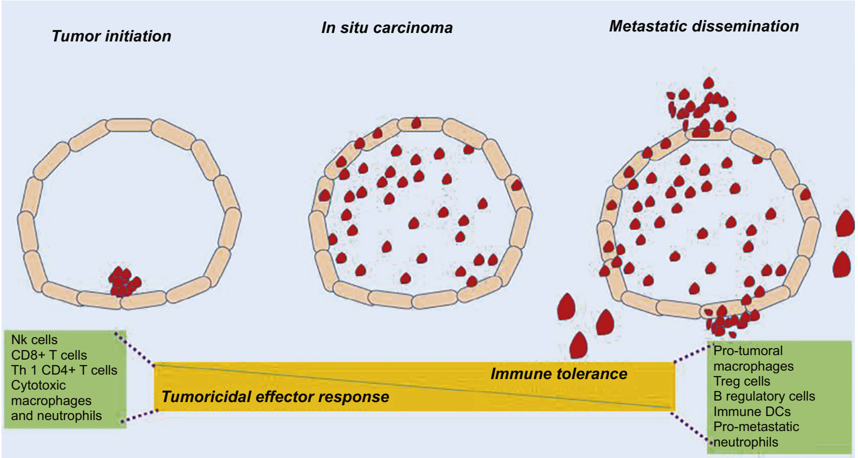

FIG. 5.2 **There occurs a balance between tolerogenic and effector immune response.** During the earlier stages of cancer, cancer cells are eliminated by effector T cells, but in the later stages of disease progression there occur various processes of peripheral immune tolerance and the association of immunosuppressive immune cells that can assist the tumor cells.

assist wound healing (Lavin et al., 2015; Mehraj et al., 2021). Since they are important participants in the tumor-associated inflammation, their association in the cancer progression is seen at each and every step, may it be in the therapy resistance or metastatic progression (Noy and Pollard, 2014; Kitamura et al., 2015; Gonzalez et al., 2018, Fig. 5.3).

Macrophages that inhabit the tumor micro-environment are known as tumor-associated macrophages (TAMs). Studies suggest that high-grade tumor-associated macrophages (TAMs) are associated with worse prognosis and decreased OS (Noy and Pollard, 2014). The

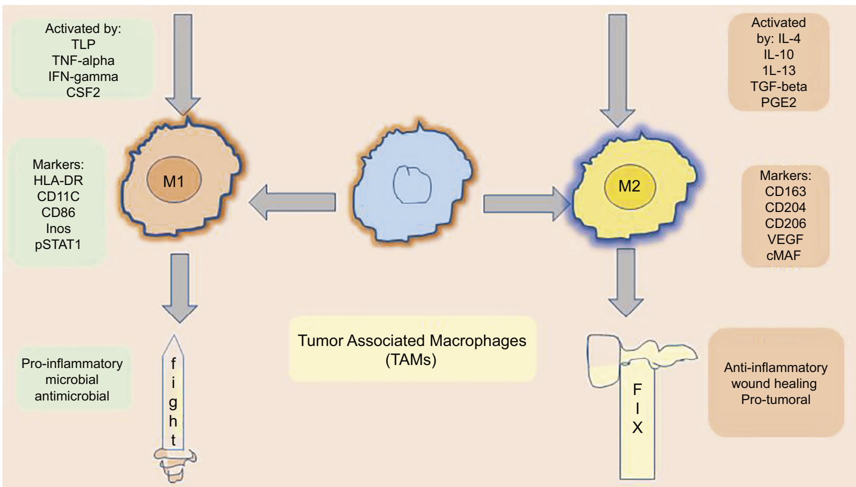

FIG. 5.3 **The distinction between the two phenotypes of macrophages in terms of their IHC markers, their activation mechanism and functions.**

TABLE 5.1 Distinction between M1 and M2 Macrophages.

S. No	Property	M1	M2
1	Phenotype	Proinflammatory	Anti-inflammatory
2	Markers	TNFα, IL-1β, IL-12, 1L-23, CXCL10, Pstat1, MMP9	IL-10, TGFβ, CCL17, CCL22, CD163, CD206, Pstst3/6
3	Phagocytic activity	High	Low
4	Antigen presentation	High	Low

macrophages are divided into two groups based on their phenotype: M1 (activated macrophages) and M2 macrophages (Mantovani et al., 2002). The two phenotypes exhibit opposite functions. M1 macrophages possess antitumor properties and thus assist in the destruction of tumor cells, whereas M2 macrophages favor tumor progression (Table 5.1). During the process of cancer progression, the TME exhibits the M2 phenotype and thus enhances the process (Mantovani et al., 2017). The process of tumor progression by TAMs can be obtained through various ways such as inducing lymph angiogenesis and angiogenesis, triggering the proliferation of cancer cells, stimulating the epithelial-mesenchymal transition, resisting the therapies, ECM remodulation, stimulating metastasis, and stimulating immunosuppression against the anti-cancer immune response (DeNardo et al., 2011; Qian et al., 2015; Mantovani et al., 2017). Usually, TAMs show pro-tumorigenic functions, they may also exhibit antitumor activities (Hanna et al., 2015). There are a wide range of chemokines that are involved in the recruitment of TAMs to the tumor site such as VEGF (Qian et al., 2011), CCL2 (Nakatsumi et al., 2017), CSF1 (Abraham et al., 2010), and CCL5 (Halama et al., 2016). The studies have suggested that VEGF has got the pro-tumor and pro-angiogenic properties. For instance, deletion of VEGF in monocytes retards the metastatic burden in experimental BC metastasis (Qian et al., 2011).

From the above, it is clear that TAMs play their role in cancer progression by switching to a more aggressive phenotype and thus enhancing the tumor progression.

Neutrophils

Neutrophils account for a total of about 50-70% of WBCs, mainly acting as key players of innate immunity. They are hallmarks of acute inflammation. The studies suggest that they are important participants in chronic inflammatory diseases including cancer (Mir et al., 2021). Among the various immune cells, they are the first to reach the injured tissue and start their process of destructing pathogens and regulate the inflammatory processes through various mechanisms including antibacterial protein secretion, phagocytosis, and much more (Kolaczkowska and Kubes, 2013). The studies have revealed that increased levels of tumor-associated neutrophils (TANs) among cancer patients correlate with worse prognosis among different cancers (Donskov et al., 2012). Neutrophils like that of macrophages possess phenotypic plasticity and are categorized into N1 (anti-tumor) and N2 (pro-tumor) phenotypes (Fridlender et al., 2009). The main chemokines are associated with the positioning of TANs to the tumor site are usually CXCR2 ligands such as CXCL5, CXCL2, and CXCL1 (Jamieson et al., 2012; Katoh et al., 2013) and are being displayed by stromal and cancer cells. Not only the CXCR2 ligands are associated with the recruitment of TANs to the tumor site, but TGF-β also plays its role in recruiting and reprogramming the TANs (Fridlender et al., 2009). Neutrophils secrete various factors which are responsible for modulating ECM and thus leading to cancer progression.

Recently, it has been demonstrated that neutrophils secrete a network of structures made up of proteins, chromatin, and the intracellular proteins that possess the ability to destruct and eliminate the cancer cells and were named neutrophil extracellular traps (Cools-Lartigue et al., 2013). The occurrence of NETs near the tumor site is associated with tumor progression in cancer patients and animal models (Papayannopoulos, 2018).

Various studies have concluded that neutrophils play an important role at each and every step of tumor progression; still, a better understanding of the phenotypic plasticity of TANs is required for better outcomes.

NK cells

These are cells of the innate immune system and possess the ability to show the effective cytolytic response toward the infected cells (Cerwenka and Lanier, 2016). These cells are characterized by the presence of various receptors that may inhibit or stimulate a particular signal during immune surveillance (Mir et al., 2021). The inhibitory receptors have the ability to destruct the cancer cells in which no MHC-I is present (Marcus et al., 2014). However, the presence of MHC-1 on the normal healthy cells makes them able to inhibit the NK cell function by binding to their receptors on NK cells (Mir, Lanier 2005). These cells possess a well-defined anti-cancer activity (Marcus et al., 2014; Marcus et al., 2014). Based on the expression pattern of CD16 and CD56, two types of blood NK types have been recognized (Cooper et al., 2001; Vivier et al., 2008). The first one is named CD56 dimCD16+ NK cells, which account for a total of 90-95% circulating NK cells and are characterized by the presence of perforin and granzymes secretion resulting in the cytotoxic activity and also possess ADCC. The second subset is named as CD56 bright CD16- NK cells, which accounts for a total of 5-10% of circulating NK cells and are characterized by the ability to secrete Th1 cytokines, such as IFN-γ and TNF-α (Cooper et al., 2001; Vivier et al., 2008). Additionally, one more subset of NK cells that occurs in the developing decidua is there and are named as decidual NK cells (dNK) (Hanna et al., 2006; Blois et al., 2011). The studies have revealed that in various types of cancers including BC, there occurs some alteration in the phenotype of intratumor NK cells as compared to the peripheral natural killer cells (Mamessier et al., 2011; Bruno et al., 2013; Bruno et al., 2018). Thus, it is clear that the NK cells display aberrant alterations in their phenotype and their function and localization are affected by the neoplastic transformation.

Dendritic cells (DCs)

These are the APCs that play their role both in the innate as well as in the acquired immunity and represent the antigens to T cells with reference to MHC molecules. These types of APCs are present in every tissue, except brain parenchyma (Mildner and Jung, 2014). There are various types of cancers that are associated with Tumor-infiltrating DCs (Janco et al., 2015) and they are important for the T cells to perform their role properly during tumor progression. Inspite of the fact that DCs are present in the TME, immune surveillance still fails during cancer progression. Thus, we can say that DCs also function as one of the contributors of immune non-responsiveness during cancer progression. The studies have revealed that DCs in the tumor have altered differentiation and activation and are not good immune response evoking stimulators (Ruffell et al., 2014). One of the factors that are responsible for the alteration in the function of DCs in the TME is the PDL-1 expression by the tumor cells (Salmon et al., 2016). Another factor responsible for their dysfunctioning is the unusual collection of lipids in them (Herber et al., 2010). Emerging studies revealed that the DCs in the TME is characterized by an altered antigen cross-presentation (Zong et al., 2016). This directly influences the activation and continuation of the antitumor immunity, thereby resulting in cancer progression. More recently, it was also discovered that the tumor-infiltrating DCs can get converted into immunosuppressive regulatory cells by the tumor cells (Tassone et al., 2003). The studies done on various cancer mouse models including the BC mouse model revealed that tumor cells secrete prostaglandin E2 that leads to the impairment in the positioning of DCs to the TME, resulting in the dysfunctioning of tumor-associated NK cells and thus affecting the recruitment of the dendritic cells that is dependent on NK cells (Böttcher et al., 2018).

T cells

T cells are the special type of immune cells that belongs to the acquired immune system and possess the ability to directly destruct the infected cell, trigger the other immune cells to get activated, secrete various cytokines and thus modulate the immune response (Speiser et al., 2016). As the tumor cells are occupied with various types of immune cells, the most frequently occurring among them are the TAMS followed by T cells and thus the T cells are paying strong attention to various types of cancers. The T cells within the TME comprise memory, naïve, effector, and regulatory T cells (Hashimoto et al., 2018). The T cell mechanism of action involves the activation of T cell receptors (TCRs) by the antigens, followed by activation of a cell-intrinsic program that assists the differentiation of T cells into cytotoxic effector cells that will lead to the clearance of antigens. After the destruction of the antigens and a huge increase in the effector cells, most of these cells die, except a few memory T cells that are there for providing long-term immunity against the antigens (Chang et al., 2014). However, the studies have revealed that there occurs a dysfunction and exhaustion of T cells in cancer patients and this dysfunctioning is one of the important features of many cancers (Jiang et al., 2015). The T cell function gets impaired because of the presence of various immunosuppressive cells and chemokines present in the TME leading to tumor progression. For instance, augmented levels of expression of various inhibitory receptors are present on the CD8+ T cells in the TME including TIM-3 and PD-1 receptors that are directly involved in T cell dysfunctioning. Furthermore, this impairment in the T cell functioning leads to the alterations in the secretion of various cytokines including IFN-γ, IL-2, and TNF- α (Chauvin et al., 2015; Wang et al., 2017). Other factors responsible for the T cell dysfunctioning involve checkpoint point up-regulation, alterations in metabolic and transcriptional factors (Le Bourgeois et al., 2018; Liu et al., 2019) (Table 5.2). Among the various checkpoints, the two most significant ones are PD-1 and CTLA-4 that possess the ability to negatively regulate the function of T cells and thus assist in cancer progression by immune evasion (Pardoll 2012).

TABLE 5.2 Examples of factors responsible for T cell dysfunctioning.

S. No.	Transcription factors	Description of dysfunction
1	NR4A	It is a transcriptional factor that is upregulated in T cells, which can alter the anti-tumor activity of T cells and induce PD-1 and TIM-3 expression.
2	mTOR	A metabolic checkpoint that regulates glycolysis through various transcriptional factors, thereby increasing the expression of inhibitory receptors in T cells
3	Eomes	The transcriptional factor that is associated with T cell exhaustion by inducing co-inhibitory molecule B7 superfamily member 1 (B7S1) pathway VC

The role of CTLA-4 in T cell dysfunctioning is highly significant in many cancers including BC (Erfani et al., 2006). PD-1 deregulates the T cell function by binding to PDL-1 that is displayed by various immune cells as well as cancer cells, leading to the inhibition of anti-tumor activities of T cells (Topalian et al., 2015). Thus, these checkpoints have been in use in immunotherapy and had significant success in various types of tumors (Hamid et al., 2013; Gotwals et al., 2017).

Myeloid-derived suppressor cells (MDSCs)

MDSCs are one of the main subsets of inhibitory immune cells displaying phenotypic plasticity and are being mostly observed in human and mouse tumors (Bronte et al., 2016; Tcyganov et al., 2018). The studies have revealed that granulocytic-MDSCs are able to inhibit EMT which is one of the processes in cancer progression. While as, monocytic-MDSCs favor EMT and thus enhance cancer evasion-related processes (Nagaraj et al., 2013). Furthermore, it has been observed in BC murine models, that alterations in the ECM occur due to the occurrence of MDSCs in TME, greatly expressing the cysteine and acidic rich secreted proteins (Sangaletti et al., 2016). Their clinical significance relies on the fact that they play a significant role in developing resistance toward ICB among cancer patients (Gebhardt et al., 2015). The infiltration of this particular subset of immune cells is regulated by CSF-1 and the combination between CSF-1/CSF-1R signaling inhibition and CTLA-4 is also presently being used (Holmgaard et al., 2016).

Cancer-associated fibroblasts (CAFs)

Cancer-associated fibroblasts are a heterogeneous group of cells with multiple origins; most of them have their source of origin as resident fibroblasts (Mir et al., 2021). The other sources of origins include; pericytes, endothelial cells, mesenchymal stem cells, adipocytes, and epithelial cells (Fig. 5.4).

Depending upon the morphological characteristics and also the specific markers, CAFS can get differentiated accordingly in the TME (Liu et al., 2006). During the process of metastasis, there occurs the recruitment of normal fibroblasts to the tumor site and due to the production of various cytokines by the tumor cells, the fibroblasts get activated (Räsänen and Vaheri, 2010). Furthermore, studies have revealed that there is a significant role of miRNAs in the conversion of normal fibroblasts into CAFs (Yu et al., 2010; Enkelmann et al., 2011; Zhao et al., 2012). The secretion of various cytokines by these CAFs enhances tumor

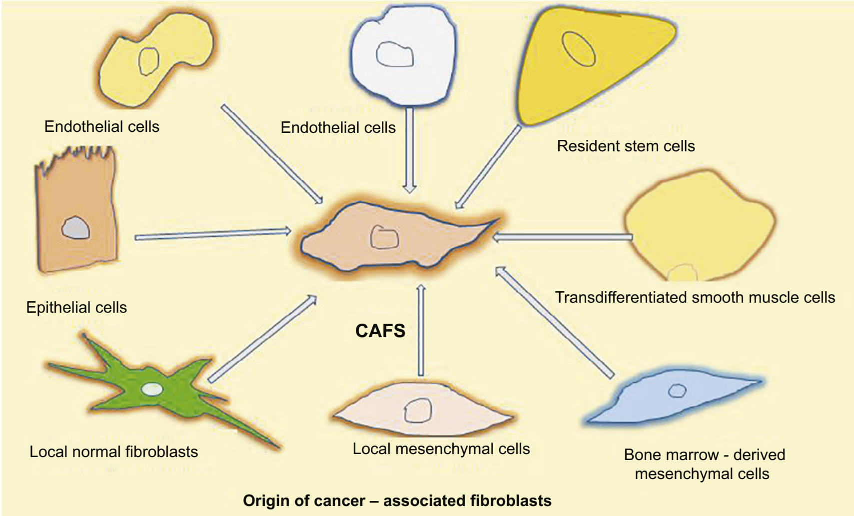

FIG. 5.4 CAFS can have different origins; they mostly develop from local fibroblasts, and other cell types like endothelial cells, BMDMCs, etc.

growth (Östman and Augsten, 2009). Studies have also revealed the role of CAFs in tumor metastasis (Karagiannis et al., 2012; Pavlides et al., 2012). Also, the presence of CAFs in the stroma of TNBC patients has been seen to play role in bone metastasis (Zhang et al., 2013). Thus, CAFs can be considered as an important driver in tumor progression. They acquire a positive impact on tumor development.

B cells

B cells are important components of humoral immunity, as they are able to produce immunoglobulins (antibodies). Their mechanism of action against the antigen involves the recognition of antigen by BCR, followed by the conversion of naïve B cells into their activated forms that possess the ability to get differentiated into PCs leading to the production of Abs (Packard and Cambier, 2013). Upon maturation, B cells get divided into B1, B2, and marginal zone (MZ) B cells and these subsets differ with respect to their location and T cell-dependent or independent activation pathways (Allman and Pillai, 2008). Since TME is home to various types of immune cells, there occur different subsets of B cells in the TME and these subsets may show anti or pro-immune response, leading to cancer progression or tumor evasion (Nielsen et al., 2004; Rubtsova et al., 2015; Mir and Mehraj, 2019).

Immune system and TNBC

Due to the molecular heterogeneity of TNBC, it is associated with worse outcomes (Kast et al., 2015). Since we are having very limited options of treatment for TNBC, it becomes a necessity to develop such kind treatment options that would truly revolutionize the field of

BC (Mir et al., 2021). In this regard, one such strategy is to target the immune system of BC patients which would bring control over the BC tumors (Qayoom et al., 2021).

Immunological portrait of TNBC

TNBC cell

TNBC, unlike the other types of BC, is associated with much more mutations in context with BRCA1/2 and TP53 mutations. The percentage count of these particular mutations in TNBC with respect to other BC types is BRCA1/2 (~30% vs. ~5%) and TP53 (~80% vs. ~33%), leading to the alteration in the normal DNA damage repair mechanism (Nolan et al., 2017; Crosby et al., 2018; Crosby EJ et al., 2018; Nolan E et al., 2017). The other distinction between the TNBC and other BC types is that TNBC is associated with more deficiency in the normal mismatch repair (MMR) as compared with other types of BC (~4.7-6.9% vs ~2%) (Staaf et al., 2019).Because of the presence of such kinds of mutations in TNBC patients, they are having increased TMB, neoantigen levels, and genomic instability. The immunogenicity of TNBC as compared with other BC types is more because of the mutant peptides and DNA, which becomes a significant target for immunotherapy among TNBC patients. Moreover, TNBC is also characterized by the ability to suppress the immune response, to ignore their destruction by the innate immune system. Also, the datasets METABRIC and TCGA-BRCA reveal that there occurs a significant up-regulation of certain genes in TNBC as compared to the other BC types. The examples include LAG3, CTLA4, IDO1/2, PD-L1/2, TIGIT, and PD-1 (Liu et al., 2018). Due to the expression of such types of biomarkers, the cancer cells in TNBC evade immunosurveillance. The studies have revealed the effector T cells secrete IFN γ that leads to the activation of the IFNGR, expressed by the tumor cells and thus results in the PD-L1 expression via through the JAK/STAT1/IRF1 signal transcription machinery (Singh et al., 2020). Another study has demonstrated that the transcription of PD-L1 can also be enhanced by the amplification of MUC1-C that is associated with ~90% of TNBCs. Similarly, JAK/STAT/IFNGR/IRF1 can be activated by MUC1-C leading to the IDO1 expression (Yamashita et al., 2021). TNBC cells are also characterized by up-regulation of CD73, which possess the ability to form adenosine from extracellular adenosine monophosphate (AMP) through adenosine A2A receptor (A2AR) expressed by NK and T cells and thus leads to immunosuppression. An augmented expression of CD73 among TNBC patients has resulted in the retardation of anti-cancer immune response and results in a worse prognosis among TNBC patients (Buisseret et al., 2018). Thus, the response of immune checkpoint inhibition can be enhanced by targeting this adenosinergic pathway.

Tumor microenvironment

TNBC is associated with greater TMB, resulting in the attraction of a greater number of TILs and thus making the TME of TNBC distinct from the TME of other types of BC (Mir MA et al., 2021). These immune cells possess the ability to destruct and kill the cells of TNBC but ultimately are modulated by the tumor cells. The immune cells in the TME of TNBC perform an important function in immunosuppression (Mehraj et al., 2021). This immune suppression is largely regulated by a wide variety of chemokines, such as CCLs, TGFs, CSFs, ILs, and MDSCs (Fig. 5.5).

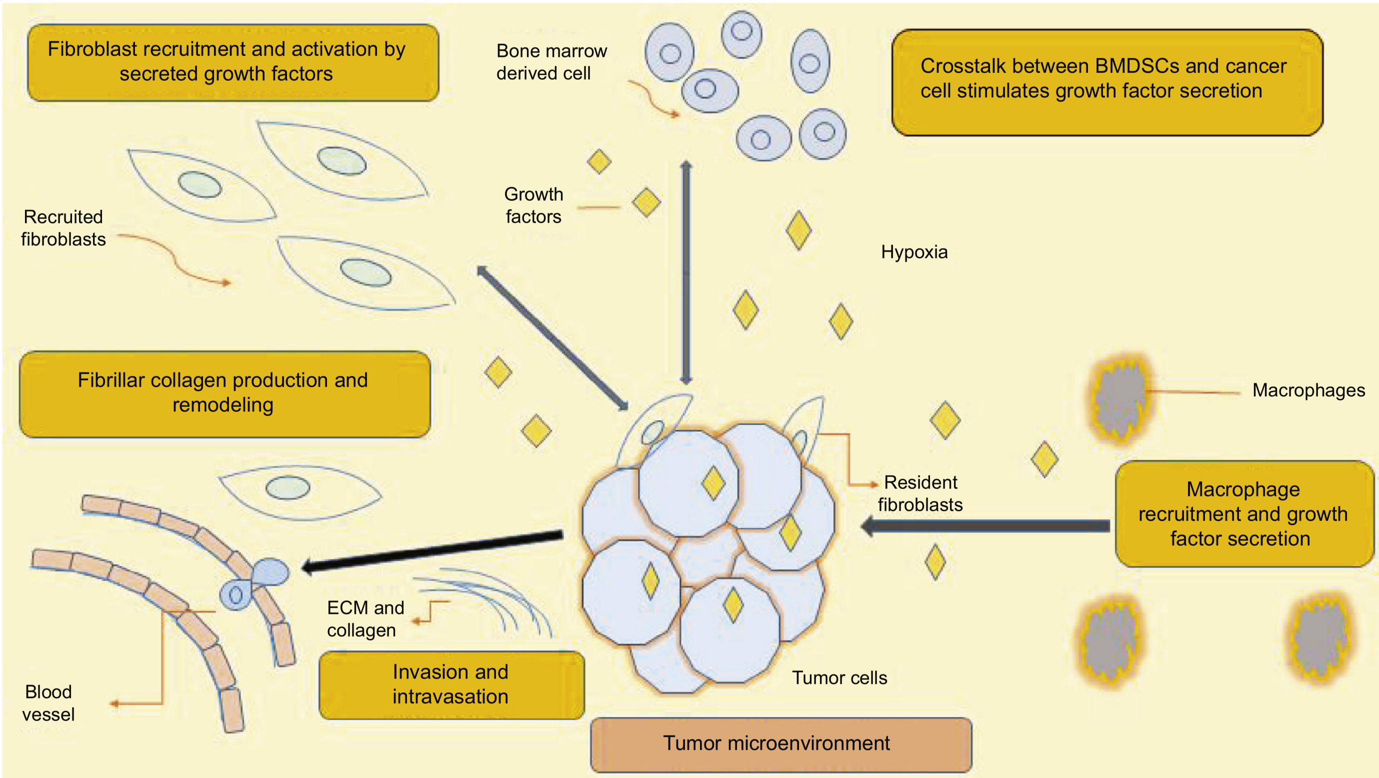

FIG. 5.5 **The tumor microenvironment of TNBC is associated with greater TMB, resulting in the attraction of greater number of TILs and thus making the TME of TNBC distinct from the TME of other types of BC.** These immune cells possess the ability to destruct and kill the cells of TNBC, but ultimately are modulated by the cancer cells. The immune cells in the TME of TNBC perform an important function in the immunosuppression. This immune suppression is largely regulated by wide variety of chemokines, such as CCLs, TGFs, CSFs, ILs, and MDSCs.

Also, in presence of CCL 12 in the TME, the Tregs CD4+CD25+ become active and possess greater proliferative ability because of a group of activated CAFs, which alter the normal function and role of cytotoxic T cells (Costa et al., 2018). Furthermore, in presence of increased CSFs and TGF-β levels, the TAMs in TNBC microenvironment change their phenotype into pro-inflammatory CD163+ M2, which the acts as a store house for chemokines, like IL-10, that retards the function of infiltrating effector T cells (Ruffell et al., 2014; Sami et al., 2020). The CD8+ T cell suppression in TNBC can also be stimulated by the expression of B7-H4 and PDL-1 on M2-like TAMs (Ceeraz et al., 2013; Roux et al., 2019). Therefore, there is developing a significant interest in M2-like TAMs, in order to enhance the effectiveness of immunotherapy (DeNardo and Ruffell, 2019). Studies have revealed that inhibition of CCL2 secretion leads to the augmented anti-tumor immunity among TNBC patients by decreasing the population of M2-like TAMs and MDSC (Liu et al., 2021). Although these studies have revealed the significance of cytokines in the phenotypic switching of immune cells within the TME, their occurrence and the pattern differ considerably with respect to the individual and the microenvironment. Because of the fact that TNBC has got a heterogeneous nature, it becomes necessary to understand its cytokine profile and its pattern in different subtypes, leading to their differential role in the alteration of anti-tumor immunity (Mir et al., 2020).

The intra-tumor immune cells are heterogeneous with respect to their composition. That is, intra-tumoral immune cells acquire phenotypic plasticity and domination within the different local microenvironments, leading to the fact that the immune cells building the heterogeneity of the TME are not that much easy as recruiting, activation and differentiation. Recent studies have also highlighted this characteristic. Azizi and co-workers (Azizi et al., 2018) did a single-cell RNA-seq and observed that BC has got a significant expansion in terms of the phenotypes of myeloid cell lineages and lymphoid cell lineages, revealing 17 distinctive T cell groups and 14 distinctive myeloid cell groups with respect to the normal breast tissue. The occurrence of this diversity among the immune cells within the local microenvironment is because of various factors including various cytokines, hypoxia, and inflammation. One of the examples of this diversity is the occurrence of different subsets of T cells that exists because of the specificity in the expression of TCRs. Similarly, the study done by Wagner and co-workers (Wagner et al., 2019) highlighted the same results in their single-cell mass cytometry technique, where they studied the human BC ecosystem and revealed the phenotypic plasticity among the immune cells within the TME. This feature of the intratumoral immune cell has influenced the effectiveness and the prognostic features of immunotherapy.

Immune checkpoints in TNBC

TNBC like other subtypes of BC is associated with various immune checkpoints; some of which are given below (Table 5.3).

Tumor in filtering lymphocytes (TILs) in TNBC

The BTM is home to a wide variety of immune cells that plays role in tumor progression (Mir et al., 2013; Mehraj et al., 2021). Among the various immune cells of BTM, TILs are one of the significant immune cells that play their role in tumor progression (Dieci et al., 2014).

TABLE 5.3 overview of immune checkpoints in triple negative breast cancer.

S. No.	Immune checkpoints	Overview
1	TILs	These are the immune cells that have migrated from the blood into the tumor. They can identify and destruct the tumor cells.
2	PD-L1 (CD274	One of the immune inhibitory receptor ligands displayed by hematopoietic, non-hematopoietic cells such as B- cells T-cells, and different types of tumor cells
3	PD-1 (CD279)	One of the coinhibitory membrane receptors of which its expression can be induced in active T cells upon T-cell receptor complex stimulation or exposition to different cytokines (Seidel et al., 2018). PD-1 inhibition enhances T cell-mediated immune responses
4	CTLA4 (CD152)	One of the co-inhibitory proteins constitutively expressed by the Treg cells and mostly up-regulated in other types of T cells, like CD4+ T, exhausted T cells, and cells upon activation (Seidel et al., 2018). CTLA-4 inhibition prevents interaction with CD80/86 leading to up-regulation of T-cell activity

The tumors possessing more than 50% lymphocyte to infiltrate are known as lymphocyte-predominant BC (PLBC) and are associated with a better prognosis. TNBC is specifically thought to have LPBC (Loi et al., 2013; Ibrahim et al., 2014). The question here arises, that why is TNBC associated with more lymphocyte infiltrate than other BC subtypes. One of the reasons behind this may be the greater mutations and genomic instability of TNBC (Smid et al., 2011). TILs possess a greater clinical significance in the BC research field, especially TNBC. Augmented levels of immune infiltrate in TNBC can be used as a predictive marker in CT (Ono et al., 2012; Dieci et al., 2014).

Subpopulations of TILs in TNBC

CD8+

One of the most important T-lymphocyte that is important players of acquired immunity against tumor cells are CD8+ T cells. Once they identify foreign Ag, they change into cytotoxic T lymphocytes (CTLs). CD8+ T lymphocytes in BC individuals including TNBC individuals correlate with improved prognosis (Mahmoud et al., 2011; Ibrahim et al., 2014; Ibrahim EM et al., 2014; Mahmoud SMA et al., 2011). 60% of TNBC cases possess an accumulation of CD8+ infiltrates. Some studies reveal that the impact of CD8+ T cells is more effective in BC individuals with negative hormone receptor status. Baker and co-workers (Baker et al., 2011) analyzed a study involving 1854 samples of BC and observed that CD8+ T cells have got prognostic importance only in ER- BC and there was no prognostic importance found in ER+ BC.

CD4+

These are the T cells that upon activation can get differentiated into various cells, and play their function in mediating the activity of the immune system via the regulation of CD8+ T cells, B cells, and macrophages (Ahn et al., 2015). Some of the significant subtypes of CD4+ T helper cells include follicular T helper cells, TH-1 cells, and regular T cells.

Clinical significance of TILs in TNBC

The clinical significance of TILs in TNBC can be described in three different aspects, as given below:

Role of TILs in prognosis

Studies have revealed a great significance of TILs in the survival of individuals with BC (Cimino-Mathews et al., 2015; Mehraj et al., 2021). The occurrence of TILS among TNBC patients receiving no treatment correlates with improved OS, less distant recurrence, and increased metastasis-free survival (Kreike et al., 2007; Adams et al., 2014; Loi et al., 2014). Various studies have revealed the prognostic role of TILs in TNBC. One such study includes a BIG 02-98 trial that involves 256 TNBC patients from 2,009 lymph node + BC individuals administered with adjuvant chemotherapy along with anthracycline. The results of the trial revealed that the stromal TILs (STILs) were having an association with the outcome (Loi et al., 2013). The conclusion of various studies demonstrated that there occurs a 15-20% decrease in the death rate and recurrence for every 10% raise in rich TILs (Adams et al., 2014).

Role of TILs as a predictive factor

TILs in TNBC can act as a predictive factor for better pCR during NACT (Denkert et al., 2010; Ono et al., 2012). After the administration of NACT involving taxane and anthracycline among the TNBC patients, an increase in PCR rates has been observed with increased levels of TILs in them (Loi et al., 2013; Loi S et al., 2013). Additionally, a recent study has revealed that greater TIL levels prior to biopsy were associated with improved pCR toward NACT, and LSO greater pCR rates were correlated with higher TILs in TN (Mao et al., 2014). These studies reveal that TILs could be used as a predictive factor in chemotherapy.

Role of TILs as a biomarker of residual disease

The significant increase in the number of TILs in the residual tumor after having a NACT can be used as a prognostic factor for improved OS and metastases-free survival (Dieci et al., 2014). These TNBC individuals possessing a greater number of TILs show better outcomes, even if pCR is not achieved by them.

Summing up the contention, it is clear that there is a robust augmentation in the levels of TILs among many of the TNBC individuals and this increased level can be associated with improved outcomes.

PD-L1 – expression in TNBC

PD-L1 or B7-H1 or CD274 is an immune-related checkpoint that shows its binding with PD-1 and alters the effector T-cell functioning negatively (Mir, Vranic, Cyprian et al.) (Fig. 5.6). On the basis of the RNA sequencing data from TCGA, Mittendorf and coworkers demonstrated that TNBC is characterized by greater expression of PD-L1 in comparison to other subtypes of BC (Mittendorf et al., 2014). Six studies were included in a meta-analysis

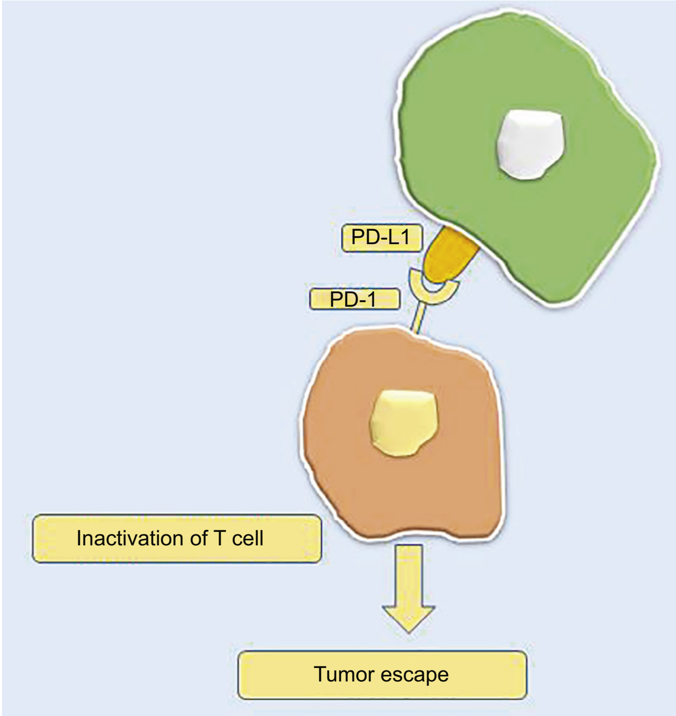

FIG. 5.6 **PDL-1 on tumor cells reacts with PD-1 expressed by the T cells to escape from immune system and thus help in BC progression.**

involving 7877 cases and it was observed that the patients anti-tumor immune response can be reduced by increased PD-L1 expression levels via the activation of the PD-1/PD-L1 pathway, thereby enhancing the potential of tumor cells (Kim et al., 2017) including apoptosis of immune cells, retarding T-cell proliferation and might assist the cancer cells to escape anti-tumor immune responses.

Depending upon the position, there could be different outcomes of the PD-L1 expression. In other words, we can say that their occurrence in different locations may show different results. For instance, PD-L1 expression in cancer cells specifies that the effectiveness and malignancy of the cancer cells are more, high chances of metastasis and bad prognosis. A study in 2014 revealed that augmented PD-L1 expression on cancer cells specifies the immune microenvironment that reflects the resistance against the acquired immune system (Taube et al., 2014). Unlike the expression of PD-L1 on cancer cells, their expression on TILs displays a significant survival time and decrease in clinic-pathological parameters in BC (Huang et al., 2019). Furthermore, a study involving 47 paired metastatic axillary lymph node and breast tumor samples done by Yuan and co-workers revealed that primary tumors are associated with less PD-L1 expression as compared to the metastatic lymph nodes (Yuan et al., 2019).

The percentage account of PD-L1 expression among TNBC patients on immune cells ranges from 40-65% (Beckers et al., 2016). Also, in the IMpassion130 trial, more than 1%

TABLE 5.4 Prognostic significance of TILs and PD-L1.

S. No.	Immune checkpoints	Prognostic significance
1	TILs	High TILs correlated with better survival outcomes and act as a predictive biomarker for increased response to neoadjuvant settings.
2	PD-L1 (CD274)	The high expression relates to increased survival rates in trials with immune check inhibitors.

expression of PD-L1 in immune cells indicates the PD-L1+ group. Surprisingly, most of the TNBC individuals being positive for PD-L1 in immune cells were having the positive status of PD-L1 in tumor cells also (Emens et al., 2019). An increase in the expression levels of PD-L1 leads to an increase in pCR and an increase in survival time (Table 5.4).

CTLA-4- expression in TNBC

CTLA-4/CD152 is an immune checkpoint that is present in Treg cells and T anergic cells. This immune checkpoint consists of a transmembrane protein structure, whose extracellular surface has an identical structure to that of CD28, and its cytoplasmic region consist of two tyrosine-based motifs assisting in the regulation of signal transduction. This immune checkpoint shows its significance in mediating T cell functioning and retards the excessive destruction done by the immune cells. Prior to TCR activation, CTLA-4 occurs in micro vesicles within the cytoplasm, but after TCR activation it gets displayed on the surface of a cell with the help of a T-cell interacting molecule (TRIM) (Fig. 5.7, Rudd et al., 2009).

Phosphorylation of CTLA-4 at Y201VKM results in the CTLA-4 binding to the cell membrane (Rudd et al., 2009). This is followed by a competition between CTLA-4 and its homologue CD28 for the ligands CD80 (B7-1) and CD86 (B7-2). Due to this competitive binding, the function of CD28 as a co-stimulatory molecule of T cell activation is inhibited

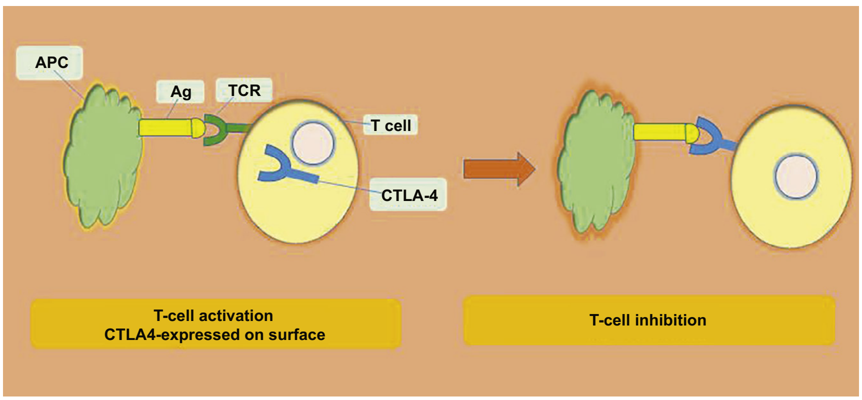

FIG. 5.7 **The CTLA-4 lies inside the cell, but after the T cell activation, CTLA-4 gets expressed on the surface of T cell and inhibits its interaction with B7 co receptors present on APCs, thus results in inhibition of T cell response**

(Egen and Allison, 2002). Therefore, the relative proportion of CTLA-4: B7 Vs. CD28: B7 will decide if T cell undergoes inactivation or activation (Krummel and Allison, 1995). Furthermore, CTLA-4 is also associated with other features of the immune system. CTLA-4 displayed constitutively by Treg play a significant role in the suppressive functions (Takahashi 2000). For instance, in one model, deficiency of genetic CTLA-4 retards the suppressive functions of Treg cells (Wing et al., 2008). Treg cells regulate the effector T cell functioning and play important role in maintaining peripheral tolerance. One of the mechanisms through which Treg cells regulate functions of effector T cells is due to the down regulation of B7 ligands on APCs resulting in the retardation of the co-stimulation of CD28 (Wing et al., 2008; Qureshi et al., 2011).

The TCGA data used to get the expression level of CTLA-4 in different subgroups of BC revealed that CTLA-4 expression is greater in TNBC as compared to other subtypes. Furthermore, expression levels of CTLA-4 were clinically greater in individuals with PR- profiles than the individuals with positive PR status. Also, an increase in CTLA-4 levels in TNBC individuals correlates with improved survival (Peng et al., 2020). Therefore, it is clear that the expression level of CTLA-4 is greater in TNBC than other subtypes of BC.

PD-1

PD-1/CD279 belong to a group of co-stimulatory receptors and is present on various immune cells like myeloid cells, B cells, T cells, and NK cells. PD-1 possesses the ability to mediate the activation of T cells by communicating with its ligands-PD-L1 and PD-L2 (Keir et al., 2008). PD-1 like CTLA-4 retards the proliferation of T cells and IFN-γ, IL-2 production, IFN α and decreases the survival of T cells (Keir et al., 2008). Simultaneous binding of PD-1 and TCR leads to the inhibition of TCR signaling and thus retards the T cell activation (Bennett et al., 2003; Parry et al., 2005). Continuous immune activity results in prolonged PD-1 activation that will directly lead to T cell exhaustion (Barber et al., 2006). This phase of T cell exhaustion takes place during cancer and chronic diseases and leads to dysfunctioning of T cells, thereby altering the normal control over cancer and infection (Mir et al., 2020).

Both PD-1 and CTLA-4 have got some similarities as well as dissimilarities in terms of their distribution, binding effect on T cells, and much more. These differences and similarities and the impact of their association are detailed in Table 5.5.

TABLE 5.5 Similarities and differences between CTLA-4 and PD-1.

S. No.	Similarities	Differences
1	B7 receptor family members	CTLA-4 inhibits T cell response early in an immune response while as, PD-1 inhibits T-cell response later in an immune response
2	Expressed by activated T cells	CTLA-4 is expressed by only T cells while as, PD-1 is expressed by various immune cells including T cells also.
3	Level of expression affected by strength and time of TCR signaling	CTLA-4 affects Treg functioning; while the role of PD-1 on Treg is unclear.
4	Reduce T cell proliferation	PD-1 engagement interferes with more T cell signaling pathways than does CTLA-4 engagement

Newly emerging immune checkpoints

LAG-3 (lymphocyte activation gene-3)

LAG-3 is an immune-related gene that is displayed by various immune cells like activated Th cells and CTL (Workman et al., 2002), some NK cells (Everett et al., 2019), B cells (Kisielow, Kisielow et al., 2005) and nerve cells (Mao et al., 2016). Apart from their expression on the cell surface, they occur in the lysosomes also, which enhances their expression on the cell membrane after the activation of T cells (Bae et al., 2014). Mainly, LAG-3 shows its association with the ligand- MHC-II that appears on the APCs and cancer cells (Salgado et al., 2014). Expression of LAG-3 is also shown by the exhausted CD8+ and CD4+ TITCs that have altered production of cytokines (Gandhi et al., 2006; Yang et al., 2017). LAG-3 is also expressed by Treg cells (Huang et al., 2004). Because of the presence of LAG-3 on the Treg cells, they secrete augmented levels of TGF-β and IL-10 (immunoregulatory cytokines) and thus inhibit the function of T cells that are specific to the tumor. The studies have revealed that an increase in the expression level of LAG-3 and their accumulation in the cancer cells is correlated with worse prognosis, tumor progression, and unfavorable results in many types of cancers including the BC also (Chen and Chen, 2014; Burugu et al., 2017). Thus, it is clear that LAG-3 like that of PD-1 assists in the tumor escape response. LAG-3 can also be released in its soluble form- sLAG-3. The main function of sLAG-3 is to assist the differentiation of monocytes into DCs or macrophages forming APCs that possess less immunostimulatory abilities (Lienhardt et al., 2002). The gene expression of LAG-3 correlates with improved clinical results in almost 80% of TNBC cases and mostly appears with other immune-related biomarkers like TIM3 (Solinas et al., 2019)

B and T lymphocyte attenuator (BTLA)

BTLA is a glycoprotein that possesses an Ig domain and occurs on the surface of resting B cells, dendritic cells, macrophages, and NK cells. The presence e of BTLA on the surface of T cells results in the dysfunctioning of T cells, as is evident from the fact that anti-BTLA approaches lead to the proliferation of T cells (Tao et al., 2005). Since BTLA is a member of IG superfamilies like that of CTLA and PD-1, it possesses the ability to bind with B7 members. In humans, the ligand for BTLA is herpesvirus entry mediator (HVEM). T cell dysfunctioning by the BTLA can be induced by the expression of HVEM on APCs (Murphy et al., 2006). In bioinformatics analyses done by Zhixian Liu and coworkers, it was revealed that there are various immune genes that are associated with TNBC, BTLA was one of them. The expression level of BTLA is specifically higher in TNBC patients as compared to the normal or non- TNBC patients (Liu et al., 2018).

PD-1 H

Programmed death 1 homolog is an emerging checkpoint that has been observed in many human cancers including BC (Cao et al., 2020). This immune-related cell surface molecule has got its homology with PD-1 in terms of its Ig variable region (Flies et al., 2011). It is also named VISTA (v-domain Ig suppressor of T cell activation) because of its function to deaccelerate the activation of T cells (Wang et al., 2011). Despite the homology of PD-1H with B7 family members like PD-L1, PDL2, and PD-1, it possesses specific distinctions in terms of its expression patterns. The expression of PD-H1 is shown by CD4+ T cells and APCs (Wang et al., 2011; Flies et al., 2014). The presence of PD-H1 on APCs retards the functions of both CD8+ and CD4+ T cells and its inhibition results in the autoimmunity that is progressed by T cells (Wang et al., 2011). This specific role of PD-H1 is not dependent upon the presence of PD-1 on T cells, thus revealing that PD-1H on APCs acts as a co-inhibitory ligand and binds to a receptor on T cells that is not yet known. This specific molecule shows its constitutive presence on naïve CD4+ T cells also, where it functions as a co-inhibitory molecule. The studies have revealed that knockout of PD-1H in mice results in the development of autoimmune disease in the mice (Liu et al., 2015). The studies also suggested that PD-1H can function both as an inhibitor ligand as well as an inhibitor receptor (Flies et al., 2014).

The study done by Cao X and co-workers revealed that PD-1H occurs in both tumor and immune cells. There was a greater expression of PD-1H in immune cells than in tumor cells. The presence of greater expression of PD-1H in immune cells correlates with better survival outcomes in TNBC patients (Cao et al., 2020).

TIM/CEACAM1-L

TIM (T-CELL Ig and mucin domain-containing molecule 3) is an immune checkpoint receptor identified in 2002 and is present on various immune cells such as macrophages, DCs, and T cells (Yan et al., 2015; de Mingo Pulido et al., 2018). This particular immune checkpoint is able to perform its suppressive functions on immune cells through its various ligands such as CEACAM-1, phosphatidylserine, and galectin-9 (Zhu et al., 2005; Sabatos-Peyton et al., 2018). The presence of this molecule on activated T cells and its interaction with TC cells results in the formation of an exhausted form of T cells, thereby inhibiting proliferation, reducing effector cytokine secretion, and apoptosis of effector T cells (Das et al., 2017). Furthermore, the TILs possessing TIM can also have PD-1 expression. The inhibition of both the receptors simultaneously leads to a more significant decrease in tumor than either alone (Fourcade et al., 2010).

CEACAM1-L (carcinoembryonic antigen cell adhesion molecule 1) is another immune checkpoint molecule that is present on activated T cells and interacts with TIM-3 so that the function of T cell can be inhibited (Huang et al., 2015). Besides T cells, it is also expressed by NK cells and various tumor cells (Dankner et al., 2017). Depending upon the isoform of CEACAM1-L, it can show both costimulatory and co-inhibitory functions. For instance, the co-inhibitory isoform is expressed by T cells and is the dominant form; while as less common isoform-CEACAM-S is the costimulatory form (Dankner et al., 2017).

Poliovirus receptor (PVR) - like proteins

These are the emerging immune-related checkpoints that are receptors of T cells having an immunomodulatory role. According to the study done by Stamm and co-workers; there is an association of PVR and TNBC subtype and worse survival in BC individuals (Stamm et al., 2019). The two main PVR-like proteins that are involved in immunomodulation are TIGIT (Tyrosine-based inhibitory motif domain) and CD226; these immune-related molecules bind with the ligands CD112 and CD155 and function as immune modulators. The binding of TIGIT with these two ligands causes T-cell inhibition; while as binding of CD226 with both these ligands causes co-stimulation of T cells. The association between TIGIT and CD112 is not so strong leading to the fact that CD155 functions as dominant ligand in this receptor/ligand association (Dougall et al., 2017).The studies have revealed increased TIGIT expression in association with PD-1 expression in tumor infiltrating lymphocytes of many types of cancers (Johnston et al., 2014). Another PVR-like receptor that occurs on NK and T cells is CD96 (earlier known as Tactile). It possesses the ability to bind with CD111 and CD155 but is not able to bind with CD112. There occurs a competition between CD96 and CD226 for CD155 and regulates the functions of NK cells (Chan et al., 2014).

IDO-1 (indole amine 2, 3- dioxygenase 1)

IDO1 is an important cytosolic enzyme encoded by *IDO1* gene and functions as an important enzyme in the L-tryptophan (Trp) catabolism (Lemos et al., 2019). Expression of 1DO in TME induces various phenotypes that are immune tolerogenic such as, retardation in T cell activation, dysfunctioning of B cells, increased infiltration of MDSCs, and enhanced tumor angiogenesis (Fallarino et al., 2006; Yu et al., 2013). In presence of infiltration of T cells and inflammation, IDO expression occurs in TNBC cells also (Godin-Ethier et al., 2009). The clinical investigations are done by Peng Li and co-workers also revealed that IDO1 expression occurs in TNBC cells (Li et al., 2021). They also showed that TNBC samples were having a correlation of PD-L1 and IDO1. It was further assessed by Yu CP and co-workers that increased expression of IDO1 is associated with shorter OS (Yu et al., 2018).

TNBC metastasis and recurrence

One of the main causes of increased mortality among the individuals of BC is metastasis, in which the tumor cells metastasize from the primary site to the other distant site via the blood and results in the formation of secondary tumors. Individuals with TNBC are associated with a greater threat of death or recurrence with respect to the individuals having other types of BC. One study has revealed that because of the 1st site of recurrence among the TNBC individuals, there is a greater threat of the tumor metastasizing to the brain and lungs (Lin et al., 2009). Another study revealed that TNBC individuals possess greater chances of CNS metastasis (Heitz et al., 2008; Saip et al., 2009). TNBC individuals characterized by lack of pathological complete remission rates possess greater chances of recurrences (Liedtke et al., 2008). Mostly, TNBC is characterized by an augmented expression of VEGF, EGFR, and Ki67 and are associated with worse prognosis and decreased survival. Also, low AR expression, P53, and E- cadherin in TNBC, associated with greater histological grade results in metastasis and recurrence (Siziopikou and Cobleigh, 2007; Linderholm et al., 2009).

Summary

BC has got various subtypes, among which TNBC is the most aggressive type due to its heterogeneous nature. Among the various treatment approaches used in treating BC is the treatment approach that modulates the immune system. In this aspect, the role of the immune system has got a great clinical significance in the progression or regression of BC. There occurs a disbalance in the functioning of both the innate and acquired immune systems during the progression of cancer, thereby favoring the tumor growth rather than retarding it. During the process of immunoediting, the immune system eliminates the tumor cells and edits the genome of tumor cells, thus acting as a dual-faced soldier during the immunoediting process Also, immune cells had played a significant role in regulating the pro-tumorigenic or anti-tumorigenic functions of the immune system. Thus, it is the form of immune cells that will decide the profile of the tumor, either it should progress or retard its growth. Various immune cells and immune checkpoints are associated with the BC including TNBC also. Their occurrence in BC will impact the survival outcomes in BC individuals. The expression of immune-related checkpoints in TNBC decides the survival outcomes in TNBC individuals. For instance, the expression of TILs and PD-L1 in TNBC can help in the prognosis of the disease and thereby can give improved survival outcomes. These immune checkpoints and other emerging immune-related molecules could be used in immunotherapy and thus the progression of the disease could be retarded in a much better way.

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CHAPTER

6

The interplay of immunotherapy, chemotherapy, and targeted therapy in tripple negative breast cancer (TNBC)

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Introduction

Clinically, there are three categories of BC: BC with positive hormone receptor status, HER2 positive breast cancer, and Triple-negative BC. TNBC, the most vulnerable type of BC is characterized by the absence of all of the three receptors i.e., the status for Triple-negative BC becomes ER-, PR- and HER2 (Mir et al., 2021). When compared to HR+ and HER2+ BCs, TNBC accounts for 15–20% of all BC cases and is linked with an earlier age of initiation, violent clinical course, and a worse prophesy (Garrido-Castro et al., 2019). Given the scarcity of significant treatment approaches for this subtype of BC, various initiatives turned out to be made in recent years to expand TNBC patients' therapeutic options (Mir et al., 2021).

The immune system's key function in settling the disease course of TNBC has been well-documented during the last ten years. In both neoadjuvant and adjuvant situations of TNBC, the existence of TILs determined by immuno-histochemical labeling is generally acknowledged as a predictor of excellent prognosis (Loi et al., 2013; Criscitiello et al., 2016; Hafeez et al., 2016). Furthermore, a more detailed analysis of immune infiltrates like the existence of various cytotoxic (CD8+) TILs or an increased CD8+/FOXP3+ ratio, can be used to identify TNBC individuals with an improved prognosis after NAC (Miyashita et al., 2015). TILs have been found to alter the prognosis of TNBC, but so has the expression of immune evasion molecules in the TME, such as PD-L1 (Mittendorf et al., 2014; Beckers et al., 2016). These findings, along with the advancement of new immunotherapeutic drugs targeting immune checkpoints, like anti-PD-L1 and anti-PD-1 mabs, support the evaluation of immunotherapeutic techniques among TNBC patients.

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From the results of the IMpassion130 trial ushering BC into the immunotherapy era, new and significant confirmation on the deployment of immune checkpoint-based treatment approaches in TNBC has been developed during the last few years (Mir MA et al., 2021). With the insertion of the anti-PD-L1 drug atezolizumab to first-line treatment along with nab-paclitaxel, Schmid and co-workers (Schmid et al., 2018) established a significant OS value in patients with PD-L1+ metastatic or inoperable locally advanced TNBC. Almost 60% of the registered patients (451 for each treatment group) had a recurrence after previous neoadjuvant /adjuvant treatment, while 37% had stage IV illness for the first time. In addition, roughly 41% of patients in the intent-to-treat (ITT) group had PD-L1 positive illness. The median PFS in the ITT population was notably enhanced following the administration of atezolizumab in contrast to CT alone (7.2 vs. 5.5 months) at a median follow-up of 12.9 months; further, the respective PFS benefit was more noticeable among the PD-L1 positive population (7.5 vs. 5.0 months). In the intent-to-treat (ITT) population, an interim OS analysis revealed that the difference in OS was not statistically significant (21.3 months, median OS (CT + atezolizumab) vs. 17.6 months (CT alone). In the PD-L1 positive population, however, the inclusion of atezolizumab resulted in a statistically significant 9.5-month increase in median OS (25.0 vs. 15.5 months). Furthermore, in the ITT and PD-L1+ populations, the objective response rate (ORR) was numerically greater following the addition of atezolizumab (56% vs. 46% in ITT and 59% vs. 43% in PDL-1+), and more complete responses were seen with atezolizumab than without (PD-L1+ population, 10% vs. 1%: ITT, 7% vs. 2%).

As a result, the above findings raise the question of whether immunotherapy can change mTNBC. Despite the positive results, the IMpassion130 trial has raised a number of questions, like how to exactly evaluate the tumors for the expression of PD-L1 given the advantages of atezolizumab addition in this patient group, which companion diagnostic is best, whether tumor cells or immune cells should be tested for PD-L1, and whether nab-ptx is the best CT helper for ICIs, whether an atezolizumab monotherapy arm was missing that could have been a suitable option for a certain subset of patients, or evaluating what can be learned from the neoadjuvant context.

An overview of immunotherapy in TNBC

Current advancements in the tumor microenvironment's immune landscape have revealed new targeted approaches for TNBC (Mehraj et al., 2021). TNBC's immunologic profile reveals a distinct microenvironment with increased expression of PD-L1 and increased lymphocyte infiltration levels than other subtypes of BC (Mittendorf, Philips et al. 2014). Because of genomic instability, TNBC has a higher frequency of somatic mutations, resulting in the frequent occurrence of neoantigen (Luen et al., 2016). TNBC, based on these data, is more likely to react to immunotherapy. Immunotherapy inhibitors like pembrolizumab and atezolizumab, which target immunological checkpoint proteins, were the first to be successful in treating TNBC (Mir, et al., 2021). The IMpassion130 study found that atezolizumab with nab-PTX enhanced PFS (7.5 vs. 5.0 months) and 3-year OS (36.0 vs. 22.0 months) in PD-L1+ TNBC patients compared to nab-PTX monotherapy (Schmid et al., 2018; Emens et al., 2020). The experiment also demonstrated their efficacy in the Asian subpopulation, marking an effective step forward in addressing TNBC heterogeneity. Overall, we saw considerable development in immunotherapy in TNBC clinical studies (Mir, 2015; Bai et al., 2021). Immunotherapy's arrival will undoubtedly alter the future treatment scenario for TNBC.

Immunotherapy's potential in TNBC: Variables to be considered

Cancer immunotherapy is the new support of cancer treatment, focusing on the tumor microenvironment rather than the tumor itself, and was given the Nobel Prize in Physiology or Medicine in 2018. Various immunotherapy techniques have shown to be helpful in generating long-term clinical responses, with ICI treatment providing the most success stories to it (Cheng et al., 2018; Garon et al., 2018). Tumors use a variety of strategies to avoid identification and elimination by the immune system, including activating inhibitory passages controlled by immunological checkpoints (Mir). Various clinical trials and studies using mabs against CTLA-4, PD-L1, and PD-1 have shown that ICIs frees the immune system from these inhibitory indications and reshape the anti-cancer immune response (Rosenberg and Restifo, 2015; Heimes and Schmidt, 2019). Treatment with ICIs has been shown to enhance clinical outcomes in BC, particularly TNBC (Heimes and Schmidt, 2019). ICI is generally well standard and related to a low toxicity status. However, immune-associated side effects such as colitis, skin rash, pneumonitis, thyroid dysfunction, hypophysitis, and inflammatory arthritis can occur and must be constantly watched (Naidoo et al., 2017).

Immunotherapy success is largely determined by the tumor's immunogenicity, as seen by increased response rates in non-small cell lung cancer and malignant melanoma (Qayoom and Bhat 2020; Davies, 2014). BC has long been thought to be immune-silent cancer that does not respond well to immunotherapy. However, mounting data suggest that BC is a diverse spectrum of tumors with varying intensity of immunogenicity, with TNBC being the most immunogenic subtype (Ali et al., 2016). Furthermore, multiple agents emanating from tumor cells or from within the tumor micro- or macroenvironment, such as neoantigen load and TMB, immune infiltrate diversity, and the microbiome, all influence the immune terrain of a tumor and thus its reaction to immunotherapy (Mir et al., 2013).

Choosing the proper immunotherapy and chemotherapy combination

Despite analyses of the best chemotherapeutic partner for ICIs in combination therapy, a number of questions are there. Nab-PTX was at first chosen in the IMpassion130 trial because it allows for less corticosteroid administration (Aigner et al., 2013). However, better agents, such as platinum salts, anthracyclines, and other taxanes, may be available to improve immunogenicity in BC (Kroemer et al., 2015). Chemotherapy can cause a variety of immunomodulatory alterations in the tumor microenvironment, such as enhanced antigen discharge by tumor cells, overexpression of PD-L1, and enhanced expression of immunogenic cell surface markers (such as, MHC class I). These changes, taken together, may have a favorable impact on immunotherapy efficacy (Pol et al., 2015; Heinhuis et al., 2019). Specifically, different chemotherapy medications often used to treat TNBC can have different impacts on the immune system, as detailed below (Mir et al., 2021).

Anthracyclines

Anthracyclines can cause immunogenic cell death (ICD), a type of apoptosis that might trigger an anticancer immune response by activating dendritic cells and triggering a particular T cell response (Galluzzi et al., 2017). Additionally, anthracyclines can boost CD8+ T cell growth.

Taxanes

The recruitment of TIL can be increased by taxanes in primary BC. Furthermore, taxanes have been demonstrated to reduce Treg cells and MDSCs in the tumor microenvironment, somewhat alleviating immunosuppression (Kodumudi et al., 2010; Roselli et al., 2013).

Cyclophosphamide

Cyclophosphamide can repress Treg cells and boost the multiplication capability of NK cells and CD8+ T cells in combination with its familiar propensity to set on ICD (Ghiringhelli et al., 2007; Kwa and Adams, 2018; Mir et al., 2020).

Gemcitabine

Gemcitabine has been shown to decrease MDSC numbers while increasing CD8+ T cell anti-cancer activity (Vincent et al., 2010; Homma et al., 2014).

Platinum salts

Platinum salts were demonstrated to bring ICD and MHC class I complex on cancer cells (Nio et al., 2000; Jackaman et al., 2012), as well as promote T cell activation and inhibit functions of MDSC.

Immune checkpoint blockades in TNBC

As we are aware of the fact that cancer cells are associated with various types of immune checkpoints, these checkpoints play an important role in decelerating the T cell function (Mir MA et al., 2021, Fig. 6.1). In this regard, the ICI treatment option has proven to be the most competent immune-based treatment approach for generating long-term responses in a range of malignancies. Mabs attacking CTLA-4 and PD-1/PD-L1 have emerged as effective methods for releasing inhibitory T cell activation control (Pardoll 2012; Bansal et al., 2016) (Table 6.1). The US FDA has approved a number of blocking mabs, including the anti-PD1 Abs pembrolizumab, cemiplimab and nivolumab, anti-CTLA-4 Ab ipilimumab, and anti-PD-L1 Abs avelumab atezolizumab, and durvalumab (Ribas and Wolchok, 2018; Voorwerk et al., 2019). Only a small percentage of patients benefit from ICI treatment, with only a minute percentage of patients reporting improved survival rates (García-Aranda and Redondo, 2019). As a result, there is a growing demand for ICI response prediction biomarkers.

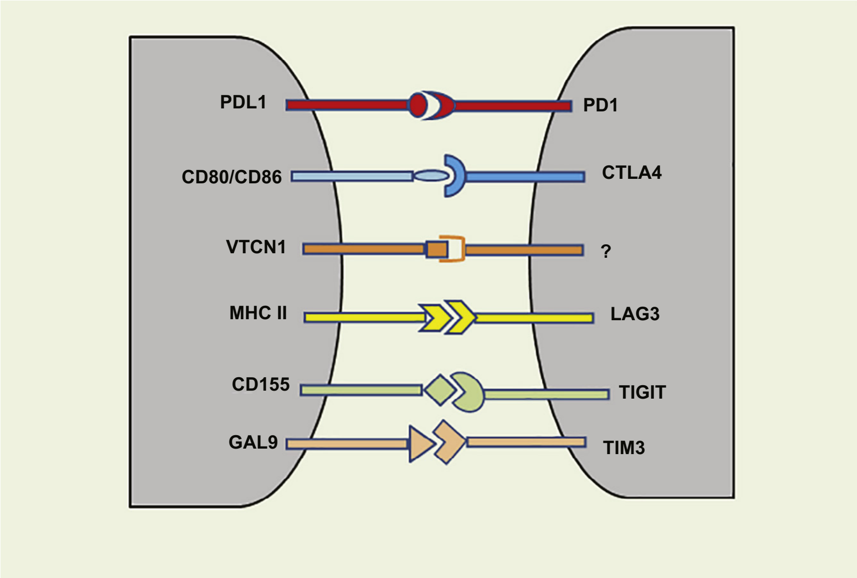

FIG. 6.1 Immune checkpoints associated with T cell inactivation.

Furthermore, only a few preclinical studies have looked into the advantages of targeting various immune checkpoints, such as PD-1, Tim3, Lag3, and CTLA-4 (Cogdill et al., 2017). The majority of BC research currently focuses on inhibiting the pathway PD1/PD-L1. A single-arm pilot research found that combining PD1/PD-L1 blockage with CTLA-4 inhibition resulted in a 43% objective response rate (ORR) in individuals with mTNBC, but no responses in HR+ BC individuals (Santa-Maria et al., 2018; Qayoom et al., 2021).

Inhibiting the PD1/PDL1 pathway in TNBC

The inhibitory receptor PD1 (CD279), which is found CTL surface, is arising as a viable target for ICI. PD1's main function is to suppress T cell activation in peripheral tissues during

TABLE 6.1 Immune checkpoint targeting antibodies.

Target	Antibody
PD-1	Pembrolizumab Nivolumab
PDL1	Atezolizumab Avelumab Durvalumab
CTLA-4	Ipilimumab Tremelimumab

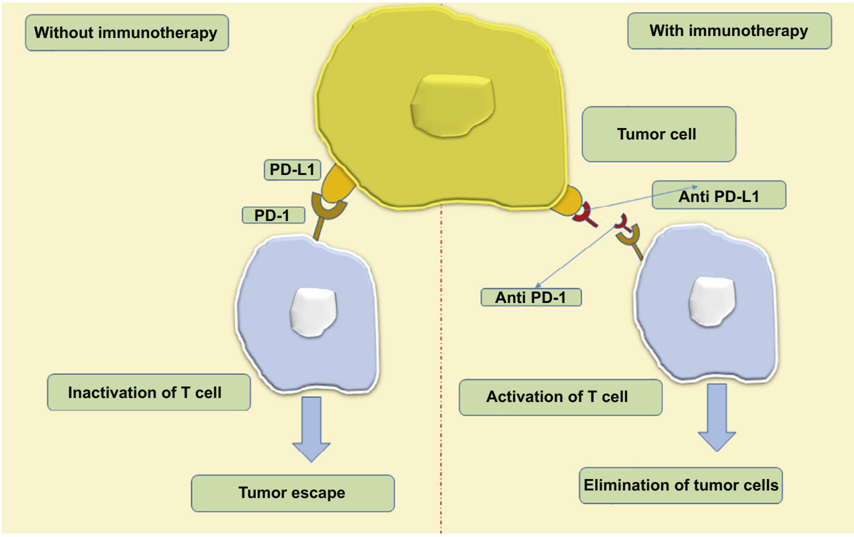

FIG. 6.2 In absence of immunotherapy, the interaction between PD-1 and PDL-1 leads to tumor evasion and thus tumor progression. Whereas, in presence of immunotherapy agents, like anti-PDL-1 and anti- PD-1 tumor cells get eliminated.

an inflammatory response to infection, hence minimizing autoimmunity (Sharpe and Pauken, 2018). The interaction of PD1 with PDL1 (B7-H1 or CD274) on T cells reduces the signals that follow TCR activation (Emens, 2018) (Fig. 6.2). PDL1 expression has been observed in 40–60% of all BCs and has been linked to greater tumor sizes, higher histologic grades, and triple-negative status, all of which are autonomous predictors of worse prophesy in BC (Katz, 2017).

ICI taking Abs that inhibit PDL1/PD1 pathway has enlightened the TNBC treatment. The clinical significance of TNBC has been achieved mostly from the use of immunotherapy in conjunction with radiation or CT (Pan et al., 2018). These combinations should theoretically and practically, increase TMB and optimize the tumor microenvironment, preparing the tumor for immunotherapy and enhancing patients' PFS. Indeed, these combinations have greatly improved the cure rate for TNBC patients.

PD1/PD-L1 antibody monotherapy

In patients with advanced, mTNBC, PD1/PD-L1 monotherapy has shown to have good long-term results. In the KEYNOTE-012 (NCT01848834) clinical trial, pembrolizumab's (an anti-PD1 inhibitor) clinical activity and safety status were initially investigated in extensively pretreated patients having advanced, PD-L1+ BC, urothelial cancer, head, and neck cancer, or gastric cancer. In mTNBC patients, an interim analysis found an ORR of 18.5%, with median response durations varying from 15.0 - 47.3 weeks (Nanda et al., 2016). In a following phase II clinical study, KEYNOTE-086 (NCT02447003), PD-L1 + mTNBC individuals who had not previously taken inherent treatment for metastatic disease had the greatest ORR of 21.4%, median duration of response of 10.4 months at data cut-off, and OS and PFS and OS of 18.0 and 2.1 months, respectively (Adams et al., 2019). PD-L1+ mTNBC individuals that were significantly pretreated had an ORR of 5.7%, having an OS and median PFS of 9.0 and

TABLE 6.2 Main monotherapy clinical trials of immune checkpoint inhibitors in mTNBC.

Drug	Trial Id	BC type	Phase	Recruitment Status
Atezolizumab	NCT01375842	mTNBC	I	Completed
Pembrolizumab	NCT01848834	mTNBC	Ib	Completed
Pembrolizumab	NCT02447003	mTNBC	II	Completed
Avelumab	NCT01772004	mTNBC	Ib	Completed
Tremelimumab	NCT02527434	mTNBC	II	Active, not recruiting

2.0 respectively. Both studies showed that single-agent pembrolizumab in PD-L1+ mTNBC, particularly in the first-line scenario, has feasible safety status and long-term clinical activity. The (NCT02555657) randomized phase 3 KEYNOTE-119 trial then looked at the potency of pembrolizumab monotherapy against CT (gemcitabine, capecitabine, vinorelbine, eribulin,) in PD-L1+ mTNBC that had already been treated. Initial findings showed that individuals administered with pembrolizumab had no meaningful improvement in OS (HR = 0.86) or PFS (HR = 1.35) despite a trend toward improved survival with increased PD-L1 score (Cortes J et al., 2019). The median follow-up time for the CT and pembrolizumab cohorts was 10.9 and 9.9 months respectively, as of the data cut-off date (11th April 2019). As the study progresses, differences in outcomes of survival may get enhanced. These data, on the other hand, could indicate that pembrolizumab monotherapy is more significant as a 1st-line treatment for mTNBC. Abs that attack PD-L1, besides blocking PD-1, have been produced, disrupting PD-L1/CD80 binding as well as PD-L1/PD1, leading to an enhanced anti-cancer immune response by both T cells and APCs (Butte et al., 2007), and Avelumab and atezolizumab, two anti-PD-L1 Abs, have been studied for their safety and potency in BC. In an NCT01375842 trial- a multiphase I trial involving individuals with hematologic malignancies, locally advanced or metastatic solid malignancies, and the clinical activity of single-agent atezolizumab treatment was examined. In mTNBC patients, the ORR with 1st-line atezolizumab administration was 24%, having 17.6 months median OS, compared to 6% in patients who had been previously treated (Emens, 2018). The presence of PD-L1 in at least 1% of TIICs was linked to a greater ORR (12%) and a better overall survival rate (OS) (10.1 vs. 6.0 months). Furthermore, although not substantially, greater levels of PD-L1 positivity (> 10%) were related to improved OS and ORR. The ORR for avelumab in the JAVELIN study (NCT01772004) phase 1b was 3.0% in mBC and 5.2% in mTNBC. Using a PD-L1 cutoff of 10%, larger RRs were reported in PD-L1+ than negative patients (16.7 vs. 1.6%), in particular among patients of TNBC, in line with earlier results (22.2 vs. 2.6%). To summarize, whereas single-agent ICI RRs in mTNBC are limited, the long-term responses of a fraction of PD-L1 + patients show that ICIs combined with other therapy modalities may yield a favorable outcome (Table 6.2).

PD1/PD-L1 Ab chemotherapy combination treatment

CT has been demonstrated to enhance the immune response by increasing tumor cell antigen discharge, inducing the production of class I MHC molecules, PD-L1, and neoantigens, and promoting activation of dendritic cells (Zitvogel et al., 2008; Schmid et al., 2018). Combination regimens of PD1/PD-L1 inhibitors and CT have demonstrated favorable effects in locally progressed, metastatic, and early-stage TNBC (Fig. 6.3).

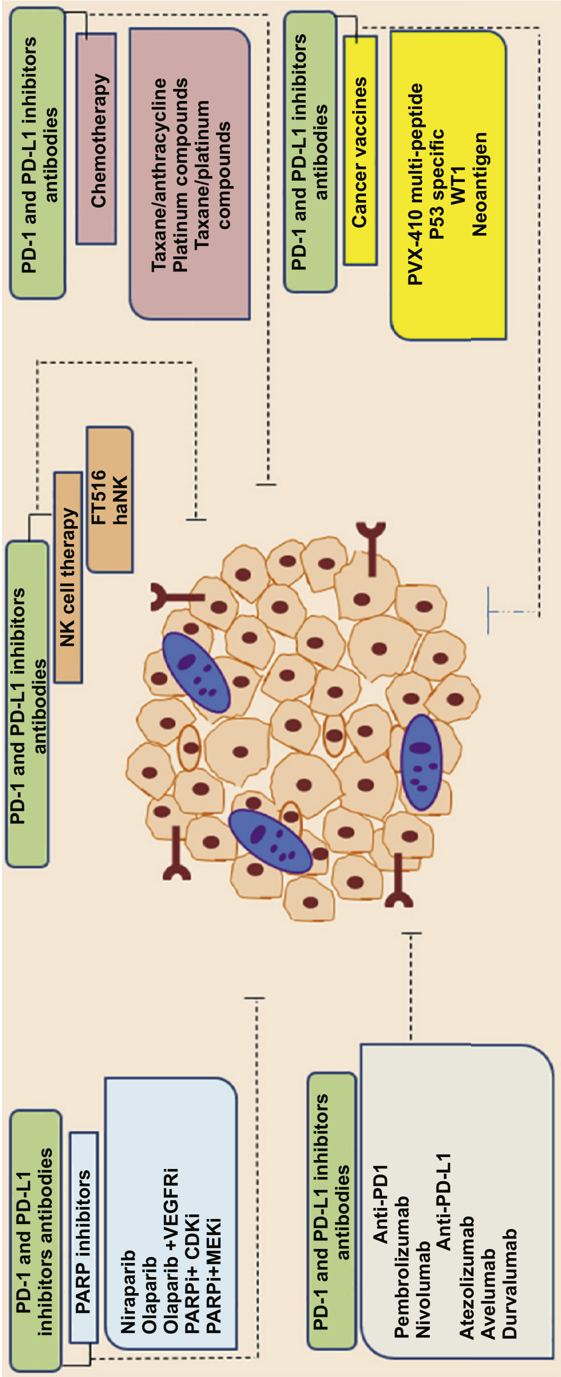

FIG. 6.3 Current approaches for PD-1 and PD-L1 immune checkpoint inhibitions in TNBC.

Pembrolizumab's safety profile and clinical activity have been explored in many trials on PD1 inhibition among TNBC individuals. In strong PD-L1 +, mTNBC patients who have not been previously treated are subjected to receive pembrolizumab in combination with chemotherapy (gemcitabine/carboplatin, paclitaxel, nab-paclitaxel) in the interim evaluation of the phase III KEYNOTE-355 (NCT02819518) and the study indicates a notable advancement in PFS (5.6 vs 9.7 months) (Cortés et al., 2019). Pembrolizumab in conjunction with gemcitabine/ carboplatin in mTNBC is being studied in the phase 2 BR-076 (NCT02755272) clinical trial. Pembrolizumab plus eribulin mesylate the microtubule inhibitor in the KEYNOTE 150/ ENHANCE 1(NCT02513472) trial showed an ORR of 25.6% with a 4.1 month median PFS (Tolaney et al., 2018). The phase II TONIC trial (NCT02499367) looked at the potency of PD1 blockage with nivolumab among mTNBC patients who had already been treated (cisplatin, cyclophosphamide, doxorubicin). The ORR for nivolumab treatment preceded by DXR was 35% in comparison to 17 percent for individuals who did not receive prior CT and 23% for cisplatin, implying that CT can produce an inflammatory tumor microenvironment (Voorwerk et al., 2019). As compared to metastatic TNBC, there has been a lot more research on locally progressed or early-stage TNBC. The addition of pembrolizumab to anthracycline and taxane-based NAC quadrupled the pCR rates of early-stage Her2- BC patients, including TNBC, in the phase 2 I-SPY 2 (NCT01042379) research (Nanda et al., 2020). The phase 1 KEYNOTE-173 (NCT02622074) trial was designed to look at the harmless and anti-cancerous activity of adding pembrolizumab to 6 routinely used NAC regimens in patients with untreated, locally advanced TNBC. The toxicity status of the combined therapy was comparable to that of the separate treatments, indicating a reasonable safety profile. Additionally, with pCR rates of 60% across all treatment groups, combination therapy indicated potential clinical activity (Schmid et al., 2020). In line with previous research, increased pre-treatment PD-L1 expression was linked to improved outcomes. Likewise, an interim evaluation of phase III KEYNOTE-522 trial (NCT03036488) found that adding pembrolizumab to PTX-carboplatin CT in the neoadjuvant setting, followed by giving pembrolizumab enhanced the pCR rates in untreated, locally advanced TNBC individuals from 51.2 to 64.8% (Schmid et al., 2020). It is worth noting that the trial's design prevents a comparison of adjuvant pembrolizumab vs. placebo therapy after NAC alone. Several clinical studies, in addition to PD1 blocking, are examining the safety and potency of PD-L1 inhibition along with CT, particularly in mTNBC patients. For locally advanced or mTNBC individuals treated with atezolizumab + nab-paclitaxel, the phase 1b clinical research NCT01633970 revealed 39.4% ORR and 5.5 months median PFS (Adams et al., 2019). Regardless of treatment history, PD-L1 + mTNBC patients had a non-significant greater ORR (41.4% vs 33.3%), PFS (6.9 vs 5.4 months), and OS (21.9 vs 11.4 months). Regardless of treatment history, PD-L1 + mTNBC patients had a non-significant higher ORR (41.4 vs 33.3%), PFS (6.9 vs 5.4 months), and OS (21.9 vs 11.4 months). Also, although not statistically appreciable, patients who were administered with the treatment regimen in the first-line setting had a greater ORR (53.8 vs 30.0%), increased PFS (8.6 vs 5.1 months), and increased OS (24.2 vs 12.4 months, indicating better results than patients who received atezolizumab monotherapy, which had 24% ORR and 1.6 months median PFS (Adams et al., 2019; Emens et al., 2019). The trial IMpassion130 study (NCT02425891) phase III backs up these findings, showing a clinically substantial increase in 7 months OS of (25.0 vs. 18.0 months) for PD-L1 + mTNBC patients treated with atezolizumab with nab-paclitaxel as 1st line therapy (Schmid et al., 2020).The inclusion of pembrolizumab boosted the ORR from 33 to 53%, according to preliminary findings (Schmid et al., 2018). The FDA and EMA approved atezolizumab + nab-paclitaxel as a 1st line therapy for PD-L1 +, ineradicable, locally progressed, or mTNBC in 2019. The IMpassion131 study (NCT03125902) assesses the safety and potency of atezolizumab with PTX as a 1st -line treatment in patients with TNBC that is either locally progressed or metastatic. Following this, another trial the IMpassion132 trial (NCT03371017) assesses if atezolizumab combined with chemotherapy (capecitabine, gemcitabine/carboplatin,) can help patients with pretreated, untreated, locally progressed, or mTNBC who were not suitable for the IMpassion130 trial. There is currently limited data on the effectiveness of PD-L1 inhibition in conjugation with CT for early-stage TNBC. According to the results of the randomized phase III GeparNuevo trial (NCT02685059), adding durvalumab with taxane-anthracycline-based NAC improves the pCR from 44 – 53% in early TNBC (Loibl et al., 2019). The phase III NeoTRIPaPDL1 (NCT02620280) study, which intends to examine the anti-cancer effect of neoadjuvant atezolizumab + carboplatin and nab-paclitaxel accompanied by adjuvant CT in early-stage high risk or locally progressed TNBC, has no interim data as of July 2020. The preliminary data were shown at the San Antonio BC Symposium 2019 and demonstrated that adding pembrolizumab to the treatment resulted in slightly greater pCR rates (Gianni et al., 2020). The phase III NSABP B-59 (NCT03281954) study combines NAC (PTX with carboplatin) along with atezolizumab, accompanied by adjuvant atezolizumab and CT. The Impassion031 (NCT03197935) study, which combines neoadjuvant atezolizumab with subsequent anthracycline-based and nab-paclitaxel CT in early-stage TNBC, has revealed intermediate data. Patients who were given atezolizumab in combination with CT had a pCR rate of 57.6%, compared to 41.1% in individuals receiving CT plus placebo (Mittendorf et al., 2020). Patients who were administered with atezolizumab in combination with CT had 69% pCR, while those who were administered with CT plus placebo had a 49% pCR. Two current trials in locally advanced TNBC are evaluating the efficacy of CT combined with PD-L1 inhibition in the adjuvant context. The Impassion30 (NCT03498716) study will look at atezolizumab's efficacy in conjunction with adjuvant CT, while the A-Brave (NCT02926196) trial will look at avelumab.

Inhibiting the CTLA4 molecule in TNBC

CTLA4 (CD152) is a co-inhibitory molecule that is displayed exclusively by T cells and is the 1st immune checkpoint receptor to be medically targeted (Lo and Abdel-Motal, 2017). It modulates the amplitude of early-stage T cell activation. CTLA4's ligands, CD80 (B7.1) and CD86 (B7.2) are likewise shared by CTLA4's co-stimulatory receptor CD28 (Krummel and Allison, 1995). CTLA4 has a considerably greater overall affinity for both CD86 and CD80 than CD28. As a result, CTLA4 expression on T cell surfaces suppresses T cell activation by competing with CD28's positive co-stimulatory signal (Mir MA et al., 2021). Because of the prevalence of negative signals from the CTLA4-CD80/CD86 interaction, proliferation of T cells and secretion of IL-2 are reduced (Rudd et al., 2009). The systemic immunological fatal hyper activation phenotype of CTLA4-knockout mice demonstrates CTLA4's important function in suppressing T cell activity (Waterhouse et al., 1995). CTLA4 inhibition, as a cancer immunotherapy method, leads to a wide augmentation of immune responses that rely on helper T cells (Mir, Qayoom, Mehraj et al., 2021). Because of the extremely deadly autoimmune and hyper immune phenotype of CTLA4-knockout animals and the lack of tumor sensitivity to the expression of CTLA4 ligands, the method of inhibiting CTLA4 has been questioned. The blockage of this receptor was initially thought to cause a lot of

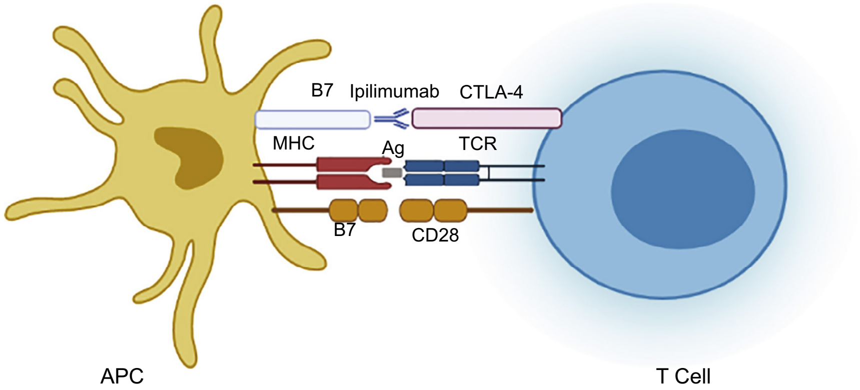

FIG. 6.4 The mechanism of action of anti-CTLA-4 mab (ipilimumab).

immunological damage. Allison and co-workers, on the other hand, employed preclinical models to show that when CTLA4 was partially inhibited by anti-CTLA4 Abs, a therapeutic window was opened (Leach et al., 1996). Further studies have revealed a strong anti-cancer response without apparent toxicities when animals with partially immunogenic tumors were administered with CTLA4 Abs. When anti-CTLA4 Ab was paired with a GM-CSF transduced cellular vaccination, tumors that were poorly immunogenic responded (Chen, Chen et al., 2018). Ab-mediated CTLA4 blockade has the potential to be used in the treatment of immune-related malignancies, according to these preclinical studies.

Humanized Abs against CTLA4

The testing and development of two completely humanized CTLA4Abs was prompted by the preceding preclinical findings. The 1st ICI authorized by the FDA for clinical usage is ipilimumab (brand name Yervoy), a mab that efficiently blocks the binding of CTLA4 to its ligand (Ito et al., 2015) Fig. 6.4. Another anti-CTLA4 mab is tremelimumab. Initial testing was done as a single agent in individuals with advanced melanoma and ovarian tumors who were not giving response to conventional therapy, as with practically other anti-tumor drugs (Hodi et al., 2003). Both Abs exhibited objective clinical responses in about 10% of melanoma patients, although immune-related effects affecting multiple tissue sites were seen in 25–30% of patients, with colitis being the most common problem. Tremelimumab was the subject of the first randomized phase III clinical trial in individuals with advanced melanoma. Tremelimumab (15 mg/kg) was given as a single agent every 3 months in this study, and it was compared to dacarbazine, a conventional melanoma chemotherapy treatment. In comparison to dacarbazine, the trial found no survival benefit with this dose and schedule (Ribas, n.d.). Anti-CTLA4 immunotherapy is now being studied as a monotherapy or in combination with other therapeutic drugs in non-small cell lung cancer and melanoma, with attention on brain metastases (Blank and Enk, 2015; Venur and Ahluwalia, 2017). Anti-CTLA4 antibody clinical trials in TNBC are still ongoing, with no definitive results yet available.

Combination treatments involving PD1/PD-L1

Dual application of ICIs (anti-CTLA-4 and anti-PD-1) in TNBC

Although PD-1 inhibitors have shown to be effective in treating TNBC in various studies, only a minute percentage of patients respond to this treatment. People have progressively understood that inhibiting both pathways concurrently may cause a synergistic impact on anti-cancer immunity, and the combination of both blockages has more than twice the potency of either alone in melanoma and lung cancer (Tanvetyanon et al., 2017). Furthermore, blocking both CTLA-4 and PD-1 improves T lymphocyte refusing activity in cancers, especially when paired with GVAX immunization (irradiated tumors expressing GM-CSF) (Duraiswamy et al., 2013). However, just a few BC-related investigations have been conducted. A combo of these two Abs reduces cancer immunosuppression and significantly treats TNBC, with 80% of tumors regressing. This allows inactivated tumor-specific T lymphocytes to abide to enhance and achieve an effector role, shifting the TME from suppressive to inflammatory (Curran et al., 2010; Mir et al., 2013). As a result, a better knowledge of the pharmacodynamics impact of these 2 Abs in patients will undoubtedly lead to the judicious development of immune-based TNBC combos. Furthermore, using dual anti-CTLA-4 and anti-PD-1 Abs in combination with Cisplatin therapy not only elicited a cytotoxic, rather than suppressive, immune response, as evidenced by increased DC activation, reduced FOXP3+ Treg, and concurrently enhanced activation of CD8+CD4+ T cells (p 0.05), but also more effectively inhibited BRCA-1 deficient growth of tumor ($p = 0.008$) (Nolan et al., 2017).

PD1/PD-L1 Ab – targeted treatment combinations

Triple-negative cancers have a greater TMB, as well as severe genomic instability and DNA damage response deficiencies (Couch et al., 2015). As a result, immunotherapy in conjunction with combination therapy options targeting different oncogenic pathways could be a promising option for TNBC treatment (Mir et al., 2021). Table 6.3 summarises the clinical trials investigating such combination treatments. For example, PARPi, which attacks the homologous recombination repair pathway and causes synthetic lethality in BRCA1/2 mutation carriers, have been licensed for treating TNBC patients having germ line mutations in BRCA1/2 (McCann and Hurvitz, 2018). Because of the stimulation of infiltrating T cells after

TABLE 6.3 Clinical studies involving combinations of PD1/PD-L1 antibody-targeted therapy in TNBC.

Trail	Combination	Phase	Recruiting profile
NCT03167619	Durvalumab + olaparib	II	Active, not recruiting
NCT02657889	Pembrolizumab + niraparib	II	Active, not recruiting
NCT02849496	Atezolizumab + olaparib	II	Recruiting
NCT03801369	Durvalumab + olaparib	II	Recruiting
NCT02079636	Pembrolizumab+ abemaciclib	I	Completed
NCT02484404	Durvalumab + olaparib + VEGFRi	I/II	Recruiting
NCT02322814	Atezolizumab + taxanes + MEKi II	II	Active, not recruiting

the discharge of tumor antigens by PARPi-induced cell death, the addition of PARPi along with ICI in this subset of TNBC patients has the ability to stimulate a greater anti- cancer immune response. PARPi has also been demonstrated to enhance the expression of PD-L1 in cell lines and animal models, adding to the case for using PD1/PD-L1 inhibitors together (Jiao et al., 2017). The mTNBC patients treated with a combination of pembrolizumab and the PARPi niraparib were reported to have an ORR of 29% in the KEYNOTE-162/TOPACIO (NCT02657889) trial. The occurrence of BRCA mutations was linked to a 67% greater ORR (Vinayak et al., 2019). The ORR was greater than what has been observed in the similar patient group for anti-PD1 monotherapy (Adams et al., 2019; Emens et al., 2019). In addition, several clinical studies have been plotted to assess the combination of PD-L1 inhibition with PARPi in mTNBC, including two-phase II trials combining durvalumab with the PARPi olaparib (DORA/NCT03167619 and NCT03801369), as well as a phase II trial combining atezolizumab with olaparib (DORA/NCT03167619 and NCT03801369) (NCT02849496). In addition, triplet combinations of PD-L1 inhibition with VEGF and PARP inhibitors are in development. A phase 1/2 research (NCT02484404) in advanced or recurrent solid cancer is looking at the doublet or triplet combination of durvalumab with the VEGFR inhibitor cediranib and olaparib. According to preliminary findings, the indicated dose was tolerated and resulted in a 67% clinical benefit rate in 9 females with recurrent solid tumors, one of which was TNBC (Zimmer et al., 2019). Another trial namely The MEDIOLA (NCT02734004) clinical trial has the goal to examine the safety and potency of durvalumab along with the PARP i olaparib or along with olaparib plus the VEGF inhibitor bevacizumab in individuals with advanced solid tumors, including BRCA1/2-deficient BC. Furthermore, a study evaluating the therapeutic benefit of conjugating PARPi, PD1/PD-L1 inhibition, and CDK inhibitors might be interesting. Inhibitors of CDK have been shown to sensitize BC cells to PARPi, which may further enhance the treatment response to ICIs. CDKs are eminent regulators of cell cycle progression and DNA repair pathways, and CDKi's have been revealed to sensitize BC cells to PARPi, which may further enhance the treatment response to ICIs (Johnson et al., 2011). CDK4/6 inhibitors have also been observed to boost anti-cancer immunity by stimulating effector T cell function, inducing fibroblast-derived pro-inflammatory cytokines, inhibiting immunosuppressive Treg cell proliferation, and increasing cell surface antigen presentation (Goel et al., 2017). The phase II COLET (NCT02322814) trial looked at the increased advantage of using the MEK1/2 inhibitor cobimetinib in combination with atezolizumab and PTX/nab-ptx as 1st-line therapy in patients with locally progressed or mTNBC. According to preliminary findings, paclitaxel in conjunction with nab-ptx has a 34% ORR, whereas nab-ptx has a 29% ORR (Brufsky et al., 2019).

PD1/PD-L1 Ab – vaccine treatment combinations

Low response rates have hampered the utilization of peptide vaccines for metastatic cancer patients; however, utilizing a multi-peptide vaccine method, RRs have climbed to 9.9% in various cancer types (Sasada et al., 2012). Furthermore, combining cancer vaccines with ICIs may improve the vaccine's anti-cancer immune response. Table 6.4 summarizes the present clinical trials making use of PD/PD-L1 antibody-vaccine combo therapies. In advanced TNBC, a few active trials are looking into the potency of combining cancer vaccines with pembrolizumab, employing either the specific vaccines targeting p53 (NCT02432963)

TABLE 6.4 Current clinical trials for cancer vaccine and immunotherapy.

Trail	Combination	Phase	Recruiting profile
NCT03289962	Atezolizumab + neoantigen vaccine	I	Recruiting
NCT03761914	Pembrolizumab + WT1-specific vaccine	I/II	Recruiting
NCT03199040 I	Durvalumab + neoantigen DNA vaccine	I	Recruiting
NCT03362060	Pembrolizumab + PVX-410	I	Active, not recruiting
NCT03606967	Durvalumab + nab-paclitaxel + neoantigen vaccine	II	Recruiting
NCT02432963	Pembrolizumab + p53-specific vaccine	I	Active, not recruiting

or multi-peptide vaccine PVX-410 (NCT03362060) or WT1 (NCT03761914). There are also few clinical studies investigating the efficiency of combining durvalumab with the neoantigen vaccine (NCT03199040, NCT03606967) or multi-peptide vaccine PVX-410 (NCT02826434) or a as well as atezolizumab with a neoantigen vaccination (NCT03199040, NCT03606967) (NCT03289962).

PD/PD-L1 Ab-NK cell combination treatment

NK cells serve as the body's first line of defense toward aberrant cells and infections from a variety of diseases. Cancer cells, on the other hand, have discovered strategies to evade NK cell-mediated immunosurveillance, like the shedding of stress-inducible ligands MHC class I polypeptide–related sequence A (MICA) and MICB, which only occur in stressed or altered cells (Chan et al., 2020). As a result of the lower cell surface density of the ligands, the activating Natural killer group2 member D (NKG2D) receptor is down-regulated, and susceptibility to NK cytotoxicity is reduced. Ex vivo expanded autologous NK cells, techniques to increase NK cell activity or target inhibitory NK receptors, and the generation of genetically altered NK cells to escape the immunosuppressive environment are all being investigated in NK-based immunotherapy studies (Shenouda et al., 2017; Lorenzo-Herrero et al., 2019). Only two clinical trials in TNBC have been conducted using NK-based immunotherapy in combination with PD-1/PD-L1 immune checkpoint inhibition, as indicated in Table 6.5. Multiple advanced solid tumors, including TNBC, are being studied with the combination of avelumab and iPSC-derived NK cells (FT-516) showing a high-affinity, noncleavable version of the NK activating receptor CD16 (hnCD16) (NCT04551885). Furthermore, the QUILT-3.067 (NCT03387085) trial is evaluating the security and efficiency of NK cell combination immunotherapy in patients with resistant, metastatic, or unresectable TNBC malignancies. In order to boost both the innate and acquired immune systems, the trial

TABLE 6.5 Clinical studies involving PD1/PD-L1 antibody-NK cell combination in advanced or metastatic TNBC.

Trial ID	Combination
NCT04551885	Avelumab + FT-516
NCT03387085	Avelumab + haNK + IL-15 + vaccine + chemo radiation

combines ICI (avelumab) with greater-affinity NK (haNK) cell therapy, IL-15 cytokine injection, metronomic chemo radiation and cancer vaccines. 9 patients had 67% ORR, with a 78% disease control RR and 22% as full RR, according to preliminary statistics (Kistler et al., 2020). In particular, the therapeutic response length is particularly promising, with a median PFS of 13.7 months compared to the typical PFS of 3 months.

Anti-PD-1/PD-L1 in combination with anti-MMP-14 antibodies for potential use

The MMP family enhances cancer spread by mediating ECM breakdown (Shay et al., 2015). MMPs are found in invadopodia, which are F-actin-rich cellular protrusions that breakdown ECM, and MMP-14, a collagen-degrading cell surface receptor, is necessary for the formation of invadopodia, which triggers released MMPs to enhance cancer spread (Devy et al., 2009; Beaty and Condeelis, 2014). Targeting early stages of metastasis, such as ECM breakdown and cancer cell invasion, may enhance TNBC outcomes (Venning et al., 2015). MMP-14 overexpression is linked to increased metastasis in cancer models and a poor outcome in human BC patients (Têtu et al., 2006). VariousMMP-14 Abs with high selectivity has been used. DX-2400, a strong and greatly specific Hum Ab inhibitor of MMP-14 action, reduces MMP-14 action, suppresses TGF-, switches macrophages to an anti-cancer phenotype, and elevates iNOS, resulting in reduced expansion of primary tumor and better radiation therapy response (Ager et al., 2015; Mehraj et al., 2021). Furthermore, several specific scFv Abs bind outside MMP-14's catalytic domain and block its proteolytic capabilities at the cell surface (Botkjaer et al., 2016). Another inhibitory Fab is Fab R2C7, having a significant specificity for MMP-14 (Lopez et al., 2017). ECM breakdown and MDA-MB-231 cell invasion are both inhibited by Fab 3369. The MMP-14i Ab 3369 was observed to decrease MDA-MB-231 tumor xenograft development and metastasis in a study using lung tissue fragment from mice utilizing a human TNBC xenograft model randomized between control IgG and IgG 3369 treatment cohorts (Ling et al., 2017). Binbing Ling also revealed the ability of MMP-14 blockage to break the immunosuppressive TME in MBC, as well as the alteration of various immune regulatory genes (Ling et al., 2017). However, no papers or clinical studies assessing MMP-14 in combination with anti-PD-1/PD-L1 treatment have been published to date; new prospective medicines can be used in individuals with TNBC in the future.

Adoptive cell therapy

TILs, CTLs, NK cells, Th and DC cells, as well as broad ex vivo multiplication and lymphocyte activation for autologous therapy, are all promising and presumably powerful approaches to induce anti-tumor immune responses (Fig. 6.5). CTLs express a specialized TCR that bestows explicitness for the target antigen as the eventual effector cells. The secretion of inflammatory cytokines such as TNF-α, IFN-γ, FASL, TRAIL, and cytotoxic degranulation activates the CTL's effector activities and triggers the killing of the target cell through the discharge of TCR/MHC/antigen complexes on the target-cell surface (Dudley and Rosenberg, 2003; June 2007). In cancer immunosurveillance, natural killer cells (NK cells) play a crucial role. Advances in NK cell expansion techniques (Fujisaki et al., 2009;

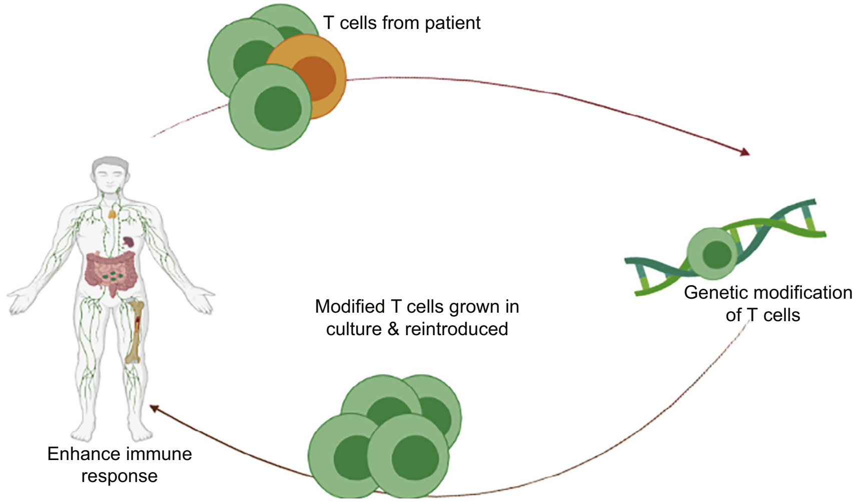

FIG. 6.5 Adoptive cell therapy.

Denman et al., 2012) have shown tremendous promise in NK cell therapies, which are categorized as follows: (i) employing NK stimulants or targeting drugs to harness endogenous responses, and (ii) introducing exogenous NK cells through hematopoietic stem cells transplant or ACT (Ames and Murphy, 2014). NK cells from BC individuals can be increased and have a strong cytotoxic potential to destruct BC cells, according to clinical investigations (Shenouda et al., 2017). ACT has been used strongly in treating metastatic melanoma, neuroblastoma, and leukemia patients (Chodon et al., 2014). As a result, additional new adoptive cell therapies can be used in TNBC individuals in the coming time with host immune environment modification, such as pre-administration host immunosuppression and simultaneous cytokine insertion with the transplanted cells.

Chimeric antigen receptors T-cell containing therapy

Despite nearly 30 years of development since the 1st generation of CARs was begins in 1989 by Gross G (Gross, Gorochov et al.), this option is still in its early stages of exploitation and progress, with significant challenges, including the lack of ability to balance the rate of cytokine secretion and cancer destruction. Rosenberg issued CAR therapy in 2010—a tailored treatment in which a patient's T cells are genetically modified to help them target cancer cells (Rosenberg and Restifo, 2015). Multiple groups are currently working on CARs against a wide range of targets, including mesothelin and EGFRvIII, as well as CD19, CD30, CD20, CD33, and CD138 (Yan et al., 2015). ACT treatment, particularly CAR-T cell therapy, which implants a random specificity onto an immune effector T cell, has derived a lot of interest in the last many years. CARs are fusion receptors made up of an Ab-derived single-chain variable fragment (scFv) linked to the signaling of T cell and co-stimulatory domain via a hinge and Trans membrane regions. IL-7 and CCL19 were recently developed into the novel CAR-T (Adachi et al., 2018).

For TNBC treatment, a few drugs have been produced and put into CAR-T cells. ROR1+ CAR-T cells, NKG2D CAR-T cells, and anti-MUC1 CAR-T cells were among the CAR-T-based ACTs employed in recent clinical trials. There are more candidates in the line. TAB 004 is a proprietary Ab that identifies a tumor-associated version of MUC1 (tMUC1) in > 90% of human TNBC, and the antigenic isoform recognized by TAB 004 is buried in normal epithelia, making it exceptionally safe for CAR-T cell production (Mukherjee et al., 2017). Mesothelin has been examined in 99 primary breast tumors (it was highly expressed in 67% TNBC but only 5% ER (+) or Her2-neu+ type, and not detected in non-neoplastic mammary epithelium) and may hold potential as a distinct TAA for TNBC (Hassan et al., 2016). TEM8 CAR-T cells have recently been proposed as a favorable CAR-T-cell-based treatment, in which the TEM8 CAR T cells trigger the regression of both established, localized patient-derived xenograft cancers (PDX) and lung metastatic TNBC cell line-derived xenograft tumors by destructing the TEM8+ TNBC cancer cells and attacking the tumor endothelium to inhibit tumor neovascularization (Byrd et al., 2018). Furthermore, various potential targets, like FRα and brachyury, have been discovered (Hamilton et al., 2016; Song et al., 2016), which could have consequences for clinical tumor antigen screening for CAR-T cell-based therapy.

T cell receptors (TCRs) - engineered T cells

TCR-engineered T cells are CD8+ T cells that have been effectively engineered to produce TCRs that recognize intracellular antigens processed by MHC proteins, allowing them to target and destroy tumor cells expressing suitable antigens (Ping et al., 2018). TCR-engineered T cells have been studied for over two decades, with a lot of preclinical trials demonstrating their capacity to drive tumor destruction and removal. Due to the increased awareness, this ACT is being developed more aggressively, with promising results in studies of TCR-engineered T cells directed against MAGE, NY-ESO-1, and GP100, as well as potential clinical progress in patients having colorectal carcinoma, metastatic melanoma, synovial sarcoma, and multiple myeloma (Rapoport et al., 2015). PLAC1-specific HLA-A0201-restricted TCR-engineered CD8+ T cells have recently been created to destroy BC cells by generating IFN-γ and TNF-α (Li et al., 2018). However, more extensive use of TCR-engineered T cells in solid tumors like TNBC necessitates improvements in the cells' long-term survival and activity, as well as closed culture techniques capable of multiplying T cells to significant numbers for therapeutic use. Semi-automated devices and modular systems, fortunately, have been produced and deployed in extensive production (Jin et al., 2018). Furthermore, by guiding primary T cells with a pan-cancer reactive TCR in combination with endogenous TCR-knockout, CRISPR/Cas9 technology can advance the role and sensitivity of TCR-engineered T cells to antigens (Legut et al., 2018). TCR-engineered T cells that express an increased level of PD-1 may have reduced functional activity; however, when these cells are employed in combination with anti-PD-1 mAbs, their efficacy may be increased (Perez et al., 2015).

Cancer vaccines

Cancer vaccines are unique cancer immunotherapy method. By delivering BC peptides to T cells, these vaccines boost T cell priming and trigger and strengthen immunological identification of tumor cells. Cancer vaccines are divided into two types: monovalent vaccinations, which give a single tumor-associated antigen (TAA) target for the immune system, and polyvalent peptide vaccines, which provide several TAA targets. Sipuleucel-T is a personalized treatment for prostate cancer that works by programming each patient's immune system. It was recommended by the FDA in April 2010 and has shown to make better OS in patients having castrate-resistant prostate cancer. The MAGE-3 protein-based vaccination is also being tested in melanoma patients and NSCLC in phase III clinical trials. To date, a variety of cancer vaccines are paving the way for TNBC treatment, ranging from cytokine vaccines like combination GM-CSF to Lymphocyte vaccines like a DC-related vaccine and from peptide vaccines like PPV to DNA vaccines like the hDR5 DNA vaccine.

CTA - vaccine target

Cancer-testis antigens (CTAAs) are a diverse set of TAAs that have all the makings of prospective immunotherapeutic targets (Mirandola et al., 2017). SP17, NY-ESO-1, and the MAGE group are among the CTAs that are uniquely expressed in TNBC (Curigliano et al., 2011). SP17 was first discovered in the flagellum of rabbit spermatozoa (Richardson et al., 1994) and has since been found in the human fibrous sheath (FS) of the sperm flagellum during various stages of spermatozoa maturation. Normal donors were successfully used to create SP17-specific cytotoxic T cells (Chiriva-Internati et al., 2009). SP17 is abnormally expressed in esophageal cancers (Gupta et al., 2007), ovarian cancers (Chiriva-Internati et al., 2008), nervous system tumors (Grizzi et al., 2006), NSCLC (Mirandola et al., 2015), myeloma (Chiriva-Internati et al., 2002) and endometrial and cervical cancers (Li et al., 2010) and is related with tumor cell migratory and motility capacity, specifying an association between the gene expression patterns in germinal and cancer cells of diverse histological origins (Arnaboldi et al., 2014), and is considered as a promising immunotherapy target. BC cell lines and actual breast tumors, as well as the TNBC subtype, express SP17. Furthermore, the anti-SP17 Abs found in patient sera were utilized for creating SP17-specific, HLA class I-restricted, CTLs potent for killing BC cells effectively (Mirandola, Pedretti et al. 2017). Early clinical data and tests support the justification for continued research into SP17 for tumor vaccines in several ways. NY-ESO-1 expression is a strong predictive factor in TNBC ($p = 0.046$) and is related to a significant humoural immune response and increased TILs (Ademuyiwa et al., 2012; Lee et al., 2015). As a result, detecting the expression of NY-ESO-1 in TNBC could help doctors identify patients who will benefit from cancer vaccine therapy.

Personalized peptide vaccination (PPV)

In a phase II experiment, Itoh K's unique tailored peptide vaccination combinations were applied, and choose vaccine antigens from a pool of 31 peptides demonstrated increased immune activation and a notable clinical response (Takahashi et al., 2014). As a novel promising vaccine target, intramuscular vaccination with DR5 DNA or TRAIL R2 not only triggers proapoptotic Abs and IFN-γ releasing T cells (p 0.001) but also retards TNBC SUM159 growth by hDR5 immune serum ($p = 0.02$) (Piechocki et al., 2012). In spontaneous BC TA2 mice, GM-CSF, in combination with BC stem cell-associated Ags and cytosine-phosphorothioate-guanine oligodeoxynucleotides (CpG-ODNs), is effective not only in inhibiting tumor growth (p = 0.035), but also stimulating and building up CD3+CD8+ T cells to destruct cancer cells (p = 0.001) (P 0.05) (Liu et al., 2013).

APC and DC-based tumor vaccination

Tumor vaccination using APCs and DCs has been extensively studied and found to be effective in a variety of cancers, including TNBC. During preop treatment, O'Shaughnessy effectively administered autologous monocyte-derived DC vaccines subcutaneously and intratumorally to ten TNBC individuals, which were found to be safe (O'Shaughnessy et al., 2016). Day-3 DCs coupled with entire apoptotic MDA-MB-231 BC cells elicited significant particular anti-cancer T cell responses and could be used as a potential vaccine for BC immunotherapy (Zhang et al., 2014). DCs from healthy donors co-cultured with T cells and transduced with Runx2 generate CTL and destroy TNBC cells (Huang et al., 2016).

Resistance to ICI

To date, there have been few instances of acquired resistance toward ICI among TNBC. However, based on the use of ICI in lung cancer and melanoma, resistance to ICI in TNBC is expected, and an identical situation may be occurring in TNBC. For example, in severely pre-treated TNBC (Polk et al., 2018), the ORR of ICI is only 5-30%, indicating that treatment alters the immune profile of the tumor and hence affects ICI. The mutational landscape of periodic mTNBC revealed a switch in molecular subtypes from immunomodulatory to basal-like and mesenchymal-like phenotypes, as well as reduced immunological activity (Hutchinson et al., 2020; Mehraj et al., 2021). As a result, immunotherapy resistance is a remunerating immune escape mechanism. The expression of the immunological checkpoint is required for ICI to be effective. The JAK/STAT1/IFNGR /IRF1 pathway is activated by the production of IFN by T cells in response to neoantigen, causing cellular PD-L1 and IDO1 expression. This pathway is further boosted in TNBC by deletion of ELF5-FBXW7 or amplification of MUC-C, and it plays a crucial role in TNBC immune escape (Singh et al., 2020). IFN, STAT1, and JAK1 are over-expressed in tumors treated with ICI, and disruption of IFN/JAK signaling caused by loss-of-function mutations in JAK and APLNR (Patel et al., 2017) allelic loss of IRF1 (JL Schwartz et al., 2011), and activation of the PBAF complex (Pan, Kobayashi et al. 2018) may enhance tumor sensitivity to T cell-mediated killing but repudiate the impact of ICI by decreasing PD-L1 expression. Although this link has been seen in patients with melanoma who are resistant to PD-1 blocking treatment, there has been no evidence in TNBC individuals. Sceneay and co-workers (Sceneay et al., 2019) observed a decline in IFN signaling with age, which reduces ICI efficacy in old mice and humans with TNBC (>65 years). In addition, the IFN inducer works in tandem with anti-PD-1 to produce a long-lasting immune response against TNBC in vivo. These findings point to a significant role for IFN system loss-of-function mutations in TNBC immunotherapeutic resistance. Down regulation of proteins in the class I MHC antigen-presenting pathway, such as CALR, TAP1/2, HLA-A, TAPBP, and ERAP1 is a critical strategy for TNBC to elude immune surveillance, especially in repeating tumors. Although IFN signaling promotes class I MHC surface expression, the genetic changes acquired such as the amplification of MEX3B (Huang et al., 2018) and deletion of B2M can still result in MHC class I deficiency, resulting in the loss of antigen-processing machinery and resistance to immunotherapy. Understanding the variations in these mutations, which may impact the response to immunotherapy, not only aids individualized immunotherapy but also assists forecast illness outcomes more precisely.

Moving immunotherapy to early TNBC

Early TNBC appears to have a less immunosuppressive character than metastatic TNBC, according to previous research (Del Alcazar et al., 2017). As a result, investigating immunotherapeutic methods in both adjuvant and neoadjuvant situations are becoming more popular. In stage III melanoma and non-small cell lung cancer, data on the potency of ICIs in early settings are available (Eggermont et al., 2016; Weber et al., 2017; Antonia et al., 2018). Various neoadjuvant investigations in TNBC are presently underway. Neoadjuvant trials provide a good in vivo laboratory for testing immunotherapeutic medicines and their potential interactions with other treatments, including CT, targeted therapies, and other immunomodulatory drugs. The ability to gather baseline biopsies and reassess tumor response and modify in the tumor microenvironment at predetermined time intervals may result in the development of novel biomarkers for patient classification. Findings from the neoadjuvant setting could subsequently be applied to the adjuvant and metastatic contexts. However, in the neoadjuvant situation, another key aspect to consider is whether OS and event-free survival should be prioritized over pathologic full response when selecting objectives for immunotherapy research. Because the true benefit of ICI treatment in other solid tumors is indicated by an improvement in OS, pCR may not be the best surrogate goal for approving these medicines in the neoadjuvant scenario. The use of appropriate endpoints in future clinical trials investigating immunotherapy in TNBC is urgently urged.

TNBC patients at high threat of recurrence and who are unlikely to be cured by the present standard of care may get the most advantage from the inclusion of ICIs in the adjuvant setting. TNBC patients who do not achieve pCR following neoadjuvant CT, for example, have a worse prognosis, and capecitabine therapy in the post-neoadjuvant scenario is the only option for these patients (Masuda et al., 2017). In this situation, the inclusion of ICIs could boost cure rates; various trials are looking at this possibility.

Efficacy of immunotherapy and future perspectives in TNBC

Synergistic impact of immunotherapy and CT

Various pieces of evidence suggest that chemotherapeutic drugs including Cisplatin, anthracyclines, and Carboplatin work against tumors not only by directing cytotoxic impacts but also by altering TIL distribution. In mice, anthracycline-based CT also necessitates stimulation of IFN-producing CD8+ T cells (Ghiringhelli et al., 2009). The efficiency of CT requires immune cells, such as CD8+ cells, and cytokines, like IFN-γ genes, like IL-17, IFN-γ, CD8α/β, IL-1β, and the IL-1β/IL-1R signaling pathway (Mattarollo et al., 2011). There are various studies involving combinations of ICIs, ACT, anti-EGFR antibody, Cisplatin, Cyclophosphamide, Carboplatin, Doxisome, and this combo operates for a variety of mechanistic reasons.

To begin, CT modifies immune gene signatures in TNBC, as well as up-regulating numerous metabolic pathways in reaction to cytotoxic therapy (Gonzalez-Angulo et al., 2012).

Second, both CT and checkpoint Abs improve TNBC patients' outcomes by causing beneficial alterations in the TME. Increased levels of IFN-, TIL numbers and the resulting improved immune response have been linked to superior chemotherapeutic responses and a higher proportion of pCR. Despite the fact that various chemotherapies impair lymphocytes including CD4+, CD68+, and CD20+, cells, they reduce immunosuppressive Foxp3+ Tregs, retain or even enhance CD8+ effectors, and upturn the CD4/CD8 ratio (Ladoire et al., 2008; García-Martínez et al., 2014). Finally, CT-induced cells produce ATP and IL-1 to induce the NLRP3 inflammasome in DCs (Ghiringhelli et al., 2009). As a result, these changing gene profiles in TNBC, as well as cytokines in TME and immune cells, may explain why CT helps immunotherapy, despite the risk of harming lymphocytes.

TNBC responses toward anti-PD-1 or anti-PD-L1 are, on the other hand, modest (less than 20%), and greater expression of PD-L1 is linked with an increased response, demonstrating that ICIs in the neoadjuvant setup accelerates the impacts of conventional NAC alone (Pelekanou et al., 2017). Anti-PD-1 therapy during DC maturation improves DC survival (Park et al., 2014). Doxisome's (liposomal encapsulated formulation of Doxorubicin) synergistic therapeutic effectiveness with anti-PD1 is attributed to enhanced DC infiltration in the TME, which incorporates tumor antigens, stimulates T cell anti-cancer immune responses, and improves therapy response among TNBC patients (Yuan et al., 2016). Chemotherapies incorporating anti-EGFR/VEGF mAbs have also been shown to be effective (Bear et al., 2015; Crozier et al., 2016; Ferrero et al., 2016). Compared to earlier used taxane and anthracyclines based adjuvant chemotherapy, cyclophosphamide, thiotepa, and carboplatin as 1st-line regimens, combined with DC-CIK immunotherapy and then followed by oral low dosage cyclophosphamide as maintenance therapy were effective and safe for mTNBC exposure (Wang et al., 2016). All of these investigations showed that the immune response and TNBC clinical results are likely related to the function of immune cells in cytotoxic CT delivery.

Antibody-drug conjugates (ADC)

Mabs that identify TAAs/TSAs and preferentially internalize when associated with cancer cells to deliver very effective cytotoxic drugs are used in antibody-drug conjugates, a developing novel therapy paradigm (Panowski et al., 2021). ADCs are being tested in at least 100 clinical studies for diseases like melanoma, pancreatic cancer, gastrointestinal cancer, colorectal cancer, cervical cancer, ovarian cancer, and endometrial cancer. Among these clinical trials, Sacituzumab Govitecan (MMU-132, hRS7-SN-38), SGN-LIV1A, and glembatumumab vedotin (CDX-011, CR011-vcMMAE) are used to treat TNBC.

The zinc transporter LIV-1 (SLC39A6) is up-regulated in TNBC and is maintained in primary and metastatic locations despite hormone treatment. SGN-LIV1A is an anti-LIV-1 Ab that is coupled to the micro tube destruct drug monomethyl auristatin E (MMAE) via a cleavable dipeptide linker and exhibits selective cytotoxicity against LIV-1-expressing tumor cells in vivo and in vitro by manifesting and trafficking to the lysosome (Sussman et al., 2014). IMMU-132 is a humanized anti-Trop-2 (expressed in TNBC) mAb (hRS7) conjugated with SN-38 (the active metabolite of irinotecan) that is well adjustable and activates early and potent responses in heavily pretreated patients with mTNBC. It regulates early pro-apoptosis signaling processes (p53 and p21 WAF1/Cip1) and leads to the breakdown of PARP (Bardia et al., 2017). In mice with BRCA1/2-mutated TNBC, a combination of IMMU-132 and PARP inhibitors like talazoparib or olaparib generates much better anti-cancer impacts and slows tumor growth compared to monotherapy (Cardillo et al., 2017; Qayoom and Bhat, 2020). Glycoprotein NMB (gpNMB) is a novel type I Trans membrane protein that fosters metastasis by mediating tissue repair, regulating intercellular adhesion, promoting cell differentiation and growth, and down-modulating anti-cancer T-cell responses. It is up-regulated in most BC. CDX-011 is made up of an anti-gpNMB mAb and MMAE, and in its 1st trial in BC, it showed a clinically acceptable safety profile, with 60% of TNBC patients receiving CDX-011 seeing a 12-week PFS (Bendell et al., 2014).

Summary

To summarize, immune checkpoint blockade clinical studies in TNBC have shown promising results, particularly in the metastatic situation. The immune checkpoint blockades play their role in TNBC by being used with chemotherapy for advanced/metastatic TNBC or chemotherapy or RT as neoadjuvant/adjuvant therapy for early TNBC and also with other targeted drugs. The FDA has approved atezolizumab in combination with nab-paclitaxel for the treatment of mTNBC and is the first BC immunotherapy regimen to be approved by the FDA. ICI in combination with chemotherapy, cancer vaccines, PARP inhibitors, or NK cell treatment has a lot of promise for improving clinical outcomes in TNBC.

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CHAPTER

7

Targeting biologically specific molecules in triple negative breast cancer (TNBC)

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Introduction

TNBC is a unique subtype of BC that lacks immuno-histochemical expression of ER, PR, and HER2 (Mir, 2021a). It accounts to a total of 15–20% of BC cases (Curigliano and Goldhirsch, 2011; Penault-Llorca and Viale, 2012). TNBC has been developed to be more common among American, African, and Hispanic females, with younger females being the most vulnerable (Ismail-Khan and Bui, 2010). Scientific advances in the BC research field have surely given rise to a significant enhancement in the survival rate of BC individuals (Mir et al., 2019). However, this is only true if the cancer is detected early and without metastases (Hafeez et al., 2016). TNBC is the most difficult BC subtype to treat since it is itself divided into various subtypes (Mir and Agrewala, 2008; Mir 2015). Researchers are working hard to categorize these subgroups and find novel treatments for them (Mehraj et al., 2021). Individuals with stage IV TNBC have few therapeutic options, and they are frequently unsuccessful. TNBC is a frequent aggressive BC that grows quicker than other breast cancers and has fewer chances to get detected on annual mammography (Mir, 2021b). It has a high chance to metastasize to other body parts at an early stage in comparison to other BCs (Mir, 2015). TNBC has a worse prognosis than other invasive BCs because of the lack of discovery of driver changes that may be targeted, such as for standard anti-Her2 treatment and endocrine therapy treatment (Lehmann et al., 2015). TNBC treatment possibilities are at the front edge of the BC research field. TNBC individuals presently get a combination of treatments that include radiation, CT, surgery, a recently developed immunotherapy, and targeted therapy (Mir). TNBC is treated locally with lumpectomy, BCS, complete mastectomy, and whole-breast radiation therapy with or without a boost (Mir, 2021c). While some researchers believe that TNBC necessitates a more aggressive locoregional surgical approach that involves removing all of the breast tissue, new research suggests that conservation therapy may

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TABLE 7.1 Different pathways used as targets in TNBC treatment.

S. No.	Pathways	Examples	Trial phase
1	Notch signaling	RO-4929097	I/II
2	Hedgehog signaling	Cyclopamine	II
3	Wnt/β-catenin	Salinomycin	I/II
4	TGF-β signaling	LY2157299	I

enhance locoregional outcomes (Mir et al., 2013; Zumsteg et al., 2013). The cornerstone of TNBC treatment is adjuvant/neoadjuvant CT, which includes taxanes, anthracyclines, and/or platinum drugs, as well as dose-dense TC (docetaxel/cyclophosphamide) and AC (doxorubicin/cyclophosphamide) (Mir, 2021d). Despite the fact that TNBC is susceptible to CT, current treatment choices are insufficient. The insertion of platinum to standard CT, on the other hand, has the potential to raise the fraction of patients who achieve a pCR (Masuda et al., 2013; Petrelli et al., 2014). When specified, CT should be followed by radiotherapy (Mir, 2021e), which includes chest wall radiation, total breast radiation, regional nodal radiation, and enhanced partial breast irradiation (Mir et al., 2020). TNBC was not traditionally thought to be a disease susceptible to immunotherapy until recent research revealed a number of promising immunotherapeutic drugs as well as the immunological signature (Amara et al., 2017; Bottai et al., 2017).

MTNBC is a debilitating illness marked by a greater proliferation index, which leads to visceral and metastasis to CNS (Otvos and Surmacz, 2011), as well as worse prognosis despite the treatment. The average survival time for advanced TNBC is 1 year, which is much less than the average survival time for other advanced types of BC. As a result, identifying particular targets and developing more efficient, strong and hopeful therapy for TNBC patients continues to be a significant clinical challenge. A great focus has been grown in the recently developed targets for TNBC, for instance, the signaling pathways (Table 7.1), like Hedgehog (Hh) pathway, Notch signaling pathway, Wnt/-catenin pathway; the target molecules like (mTOR) inhibitors, EGFR inhibitors, PARP1 inhibitors, angiogenesis inhibitors, chondroitin sulphate proteoglycan 4 (CSPG4) protein targeted monoclonal antibody and TGF-inhibitors (Fig. 7.1).

An overview of targeted therapy

Targeted therapies correspond to that treatment that targets the unique characteristics of tumor cells, like certain proteins, biomarkers, or any signaling pathway (Mir, 2021f). This treatment approach is less likely to damage healthy cells. The various agents to be targeted in this particular approach are mentioned below:

Signaling pathways to be targeted in treating TNBC

Notch signaling pathway

The Notch signaling system regulates essential cellular functions and is an extremely conserved signaling mechanism for cell-to-cell interaction (Al-Hussaini et al., 2011). This

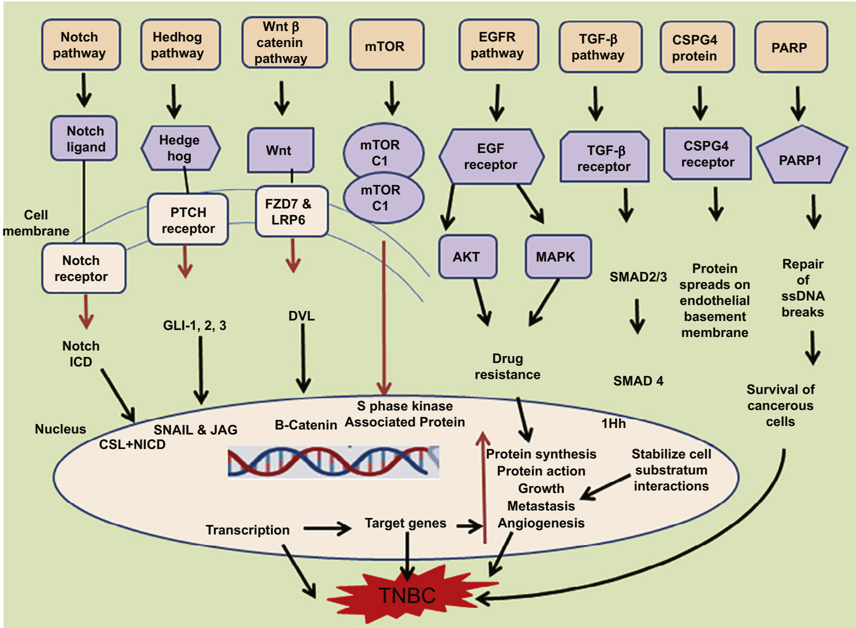

FIG. 7.1 Various pathways involved in the development of TNBC.

pathway plays a role in the onset and progression of BC. TNBC is associated with the regulation of Notch 1 and Notch 4 receptors in vascular endothelial cells and cancer cells with an intracellular position different from hormone-positive BC (Reedijk et al., 2005; Speiser et al., 2012). The association of a Notch ligand to a Notch receptor on a neighboring cell is required for the Notch signaling pathway to be activated. Notch ligands are naturally occurring single Tran's membrane proteins with an extracellular DSL domain that helps in receptor binding and several EGF-like repetitions. When a Notch ligand binds to its Notch receptor, a complex-Notch ligand-receptor complex is formed, which passes through various fundamental cellular processes, including splitting by proteolytic enzymes, which is triggered by ADAM/TACE proteases at an extracellular region and leads to the development of Notch extracellular truncation (NEXT). Eventually, a very distinct enzyme known as γ-secretase is required to get the Notch intracellular domain (ICD) shifted from the cytoplasm toward the nucleus, where it shows its association with the DNA-binding protein CSL and leads to the CSL complex activation, which finally modulates it from a transcriptional repressor to an activator (Shih and Wang, 2007).

The entrance of Notch ICD into the nucleus can be accomplished by utilizing secretase inhibitors, such as RO-4929097or aspartyl protease inhibitors, which is in phase 2 clinical study for recurrent TNBC and is one of the emerging ways for inhibiting this specific pathway of Notch signaling. Furthermore, a phase I clinical study for stage I and II TNBC involving the combination of RO-4929097, carboplatin, and paclitaxel (Olsauskas-Kuprys et al., 2013). The binding association between the Notch ligand and the Notch receptor leads to the activation of a Notch signaling pathway that results in a Notch ligand-receptor complex

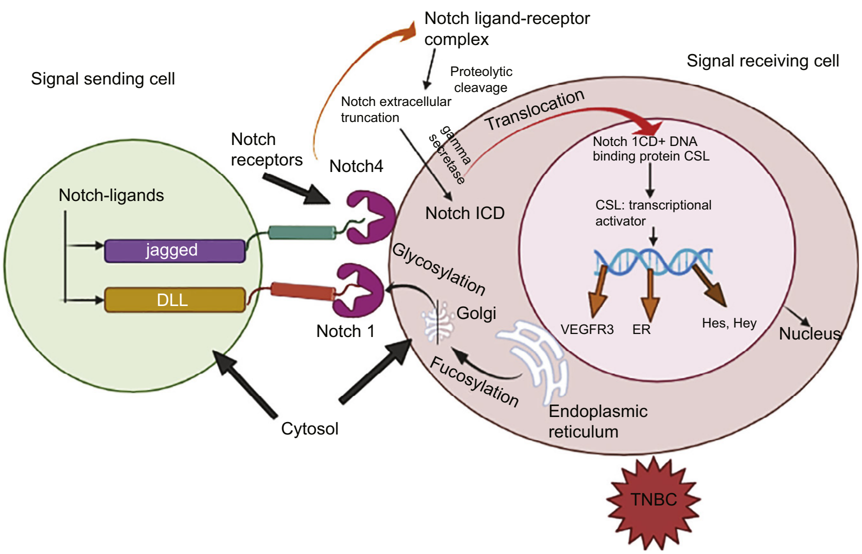

FIG. 7.2 Activation of Notch signaling pathway.

that is transformed into NEXT and subsequently into Notch ICD via γ-secretase. Notch ICD is cleaved into 2 proteins, nicastrin and presenilin by γ-secretase. Presenilin is a catalytic protein, whereas nicastrin promotes gene maturation. Eventually, the Notch ICD is transferred into the nucleus, where it shows its binding association with the transcriptional activator CSL, leading downstream targets like ER, VEGFR3, Hes, and Hey to be transcribed. Transcription factors (NF-B2 and c-Myc), growth factor receptors (HER2), cell-cycle regulators (CD1 and p21), and angiogenesis regulators, and apoptosis regulators are among the transcriptional targets. As a result, disrupting the Notch signaling pathway can have significant consequences for differentiation, apoptosis, angiogenesis, and cell proliferation (Fig. 7.2). As a result, inhibitors of γ-secretase that target the Notch pathway should be further investigated in order to improve TNBC treatment choices.

Hg signaling pathway

This is a signaling pathway that is and functions as a critical signaling pathway system in an embryo's proper development. This signaling system has been linked to a variety of cancers' development, angiogenesis, progression, and angiogenesis. The self-regeneration of stem cells in the skin of the embryo and nervous system is known to be regulated by Hh signaling (Palma and Altaba, 2004). This route has 3 gene homologues: Desert Hh, Sonic Hh (Shh), and Indian Hh, though the Shh pathway is the most targeted gene homologous (Wismar et al., 2000). The Hh pathway is a well-defined and well-coordinated cascade that begins with blocking the 12 Trans membrane protein namely Pathed1 by attaching to Hh ligand and then activates Smoothened (SMO) protein, which is a seven Trans membrane protein (Kasper et al., 2009). On activation, SMO liberates the 5-zinc finger TF Gli from a massive protein complex and is associated with the translocation of Gli into the nucleus as well as transcription of target genes (Jiang and Hui, 2008). As per various studies, Glia is one of the indications of Hh signaling pathway activation (Cayuso et al., 2006). The activation of

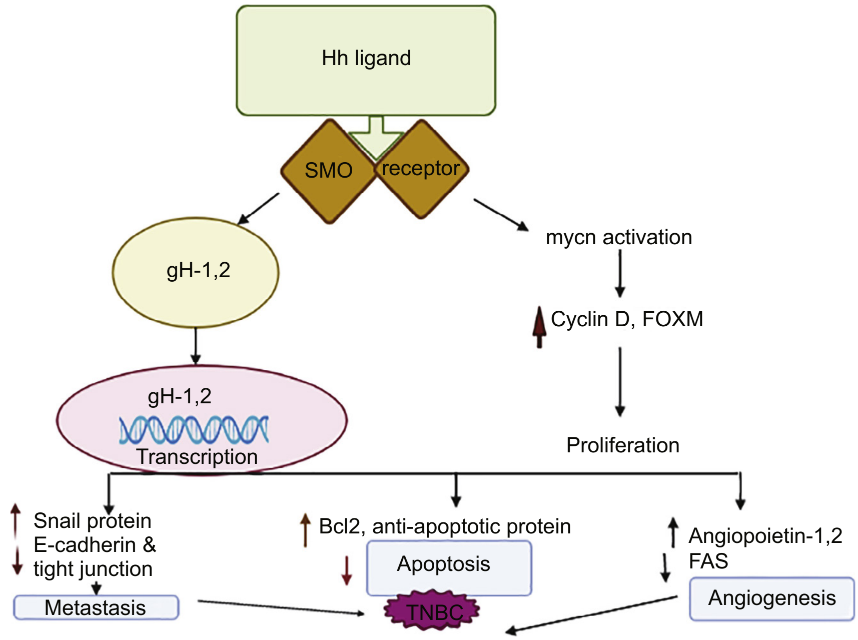

FIG. 7.3 Development of TNBC through HedgehSog signaling.

Glia represents the most important step during the Hh signaling pathway (Fig. 7.3), which is promoted by zinc finger transcription factors such as, Gli1, Gli2, and Gli3, where Gli3 is a pathway inhibitor TF and Gli1and Gli2 are pathway activator TF gene (Kasper et al., 2009), but the exact mechanism of signaling from SMO to Gli protein is not so far fully understood.

However, evidence is rapidly mounting that the primary cilium serves as a better platform for deploying the signals from the membrane to the nucleus (Oro, 2007). The processing sites for Gli TFs are thought to be the main cilia. By altering the balance between TFs and proteins, the activated Glia targeted genes change in the nucleus and involve in transcription that follows apoptosis, angiogenesis, and metastasis, resulting in the formation of TNBC. Angiopoietin-1, 2, and SNAIL proteins are also elevated by this transcription, which are accountable for angiogenesis and metastasis, respectively (Merchant and Matsui, 2010). SMO, on the other hand, plays a direct role in MYCN activation, which results in proliferation by increasing Cyclin D and FOXM 1 expression, which is a TF linked to TNBC growth and progression (Polkinghorn and Tarbell, 2007). FOXM1 also controls the cell cycle-related gene expression that is required for mitosis and DNA synthesis (Teh et al., 2002).

The discovery of cyclopamine which is a steroidal alkaloid derived from Veratrum californicum and an antagonistic to SMO having an oral bioavailability of 33% and a t1/2 of 4 h present in dogs, rodents, and cynomolgus, revealed that the Hh pathway may be clinically blocked. In phase II study for TNBC, formulations involving cyclopamine and derivatives with bioavailability, enhanced specificity, and pharmacokinetics are being tested (Merchant and Matsui, 2010).

Wnt/β-catenin pathway

This signaling system plays a key role in embryonic growth and can lead to tumor formation in an abnormal activation state. Many studies have found that this pathway is aberrantly up-regulated in the tumorigenesis of various malignancies, involving TNBC also (Barker and Clevers, 2006; Bayet-Robert et al., 2010). FZD7 and LRP6 have recently been discovered to be up-regulated among TNBC patients. Furthermore, transcriptional suppression of LRP6 or FZD7 in TNBC tumors has been revealed to inhibit the growth of tumors in vivo (King et al., 2012). The stabilization of cytosolic -catenin, which travels toward the nucleus to start the Wnt-targeted gene activation by binding transcription factors from the T cell factor/lymphoid enhancing factor (TCF/LEF) family (Lu et al., 2011; King et al., 2012), is a hallmark of Wnt/b-catenin signaling. If Wnt ligands are absent, a supramolecular complex combining adenomatous polyposis coli (APC), GSK3, and axin successfully synchronizes -catenin levels. The amino-terminal region of β-catenin is phosphorylated progressively by GSK3 and CK1. The 26S proteasome breaks down phosphorylated -catenin, which becomes multi-ubiquitinated (Ub). Additionally, the association of Wnt with its receptors on the cell surface inhibits the complex's function (Lu et al., 2011; King et al., 2012). The discovery of various Wnt/-catenin target genes, including those that promote apoptosis and cell multiplication had played a role in the initiation and development of tumors (Barker and Clevers, 2006; Bayet-Robert et al., 2010). Furthermore, the Wnt/-catenin pathway can be retarded by nigericin and salinomycin which are selective BCSC assassins that disrupt the Wnt/-catenin signaling system by promoting LRP6 degradation (Lu et al., 2011). Salinomycin is a familiar anti-coccidial antibiotic whose antitumor pharmacokinetic qualities are being studied in a phase I/II trial for TNBC (Naujokat and Steinhart, 2012). As a result, the Wnt/-catenin signaling system, particularly the cell surface Wnt receptors, represents a viable therapeutic target for treating TNBC.

TGF-β signaling pathway

The TGF-β signaling system is associated with embryonic cell proliferation, apoptosis, differentiation, cellular homeostasis, and several cellular roles in adult organs. TGF-β1 is a cytokine that belongs to the TGF superfamily and is encoded by a gene namely the TGFβ-1 gene (Ghadami et al., 2000). It was first seen in human platelets as a 25 KDa protein that plays a major role in wound healing (Assoian et al., 1983). TGFβ-1 is also connected with immune system regulation (Letterio and Roberts, 1998). It was found that TGFβ-1 inhibits the release as well as the activity of several cytokines like IL-2, IFN-γ, and TNF-α. TGFβ-1, on the other hand, has the completely opposite effect on myeloid cells, increasing the release and the production of monocytic cytokines like IL-1α, TNF-α, and IL-1β (Wahl et al., 2006). TGFβ-1 may show a potential role in BCSCs, according to new research, which found that these cells show overexpression of TGFβ-1 and the TGF-β receptor 1 (TGFBR1) (Bhola et al., 2013).

Bhola and co-workers have discovered for the 1st time that TGF inhibitors can stop CT-resistant tumor-initiating cells (TIC) from spreading in vivo (Bhola et al., 2013). This could be the basis for future clinical trials, and their role in associating CT for TNBC individuals should be assessed. TGF- may also cause an EMT in mammary cells, which results in the acquisition of tumor-like features (Mani et al., 2008). In fact, by employing TGFBR1/2 inhibitors and encouraging MET differentiation inside epithelial cells, EMT can be reversed (Bhola et al., 2013). It was also found that TGF-ligands are commonly increased in the TNBC BTM, which can be produced by cancer cells or tumor-associated immune and stromal cells. TGF- also creates SMAD4 and SMAD2/3 which have similar impacts to earlier pathways in terms of proliferation, protein synthesis, angiogenesis, growth, and metastasis. As a result, it is feasible that the TGF-pathway plays a role in the growth of BC. TGF inhibitors are presently being researched as anti-metastatic treatments for cancer patients.

Inhibiting JAK2/STAT3 pathway

JAK and STAT proteins are important components of numerous cytokine receptor systems that control the growth and survival of cells (Aittomäki and Pesu, 2014). When the cytokine binds to the receptor, it causes dimerization, which activates the JAKs involved. JAKs also phosphorylate STATs, causing them to dimerize, translocate to the nucleus and determine the transcription of genes that control differentiation, death dividing ability of a cell (Fig. 7.4).

Disrupting JAK2/STAT3 signaling could be a successful clinical strategy for treating TNBC, according to growing preclinical findings. Genes associated with immune cell signaling and cytokine signaling are also abundant in the IM subtype (Lehmann et al., 2011). The JAK/STAT3 system was found to be particularly active in basal-like BC cells in a preclinical investigation, and inhibiting JAK2 led to xenograft development being inhibited

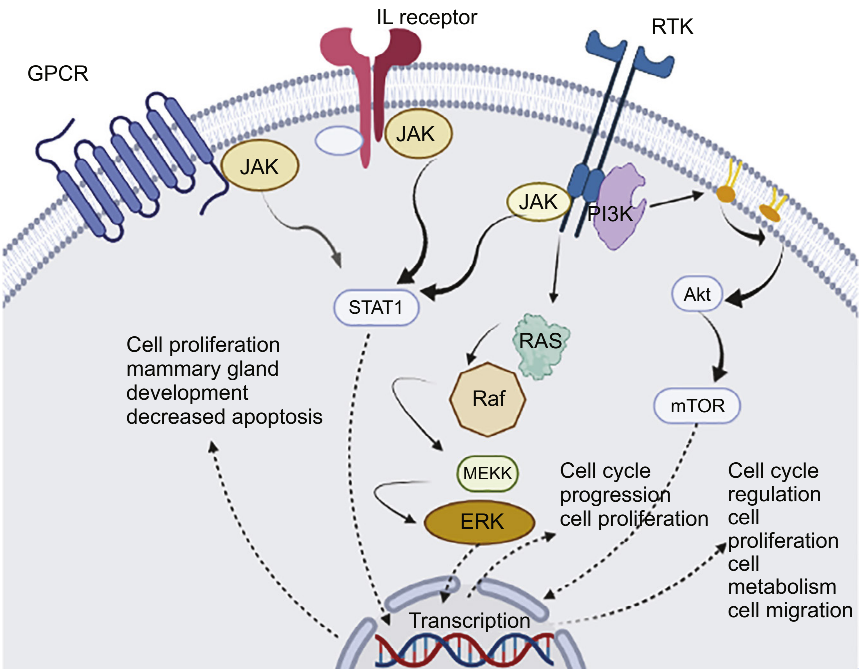

FIG. 7.4 An overview of JAK/STAT pathway. When the cytokine binds to the receptor, it causes dimerization, which activates the JAKs involved. JAKs also phosphorylate STATs, causing them to dimerize, translocate to the nucleus and determine the transcription of genes that control differentiation, death dividing ability of a cell.

(Marotta et al., 2011). The mutations of JAK and STAT, unlike those in myeloproliferative neoplasms, have not been thoroughly studied. JAK2 amplifications were observed to be more common in TNBC administered with neoadjuvant CT in the TCGA than in primary untreated BLBC (Balko et al., 2014; Mir, 2021i). This finding could be used to justify testing JAK inhibitors in patients with JAK2-amplified residual illness. Ruxolitinib, a JAK1 and JAK2 inhibitor that is recommended for the treatment of intermediate and high-risk myelofibrosis, is now being tested in BC. In patients with MBC, a phase I trial (NCT02041429) assess the combination of ruxolitinib that is given 2 times a day with weekly paclitaxel 80mg/m2 for 3 weeks out of four weeks. Patients with inflammatory TNBC will be treated with ruxolitinib orally 2 times a day for 21 days in a 28-day cycle and weekly PTX for 12 weeks, followed by dose-dense AC for 4 cycles, after a recommended phase II dose is found. The trial's primary endpoint is biologic, and looks at pSTAT3 expression in inflammatory TNBC before and after treatment, with a reduction in pSTAT3 expression expected after treatment

Pi3K/AKT/mTOR pathway

Moore and co-workers (1996) identified mTOR also named FRAP1 (FK506 binding protein 12-rapamycin associated protein 1) as a serine/threonine-protein kinase encoded by the gene FRAP1 in humans. MTOR is a member of the PI3K-related protein family, which regulates cell proliferation, motility, survival, growth, transcription, and translation (Hay and Sonenberg, 2004). MTOR is a catalytic subunit comprised of two different complexes – mTORC1 and mTORC2 (Wullschleger et al., 2006) that causes the synthesis of proteins, growth, metastasis, proliferation, and angiogenesis by inducing S-phase kinase association protein (Fig. 7.5).

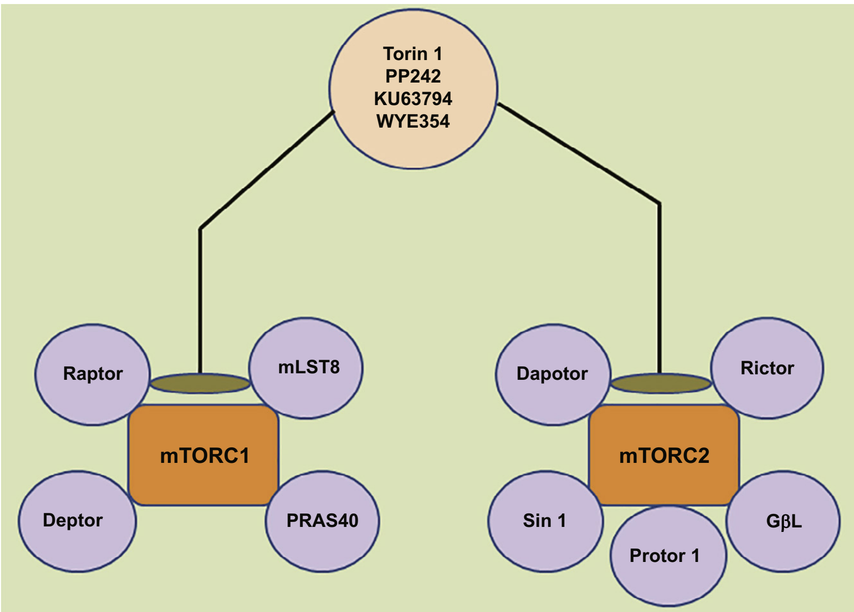

FIG. 7.5 Components of mTOR complex, which can be targeted in treating TNBC.

TABLE 7.2 Main AKT inhibitors in triple negative breast cancer (TNBC).

S. No.	Drug	Trial	Phase	Population
1	Ipatasertib	LOTUS	II	Advanced TNBC
2	Capivasertib	PAKT	II	Advanced TNBC

Multiple biological activities are mediated by the PI3K/AKT/mTOR system, including survival of the cell, dividing ability, invasion, motility, angiogenesis, etc. (Datta et al., 1999). In TNBC, hyper activation of the PI3K/AKT signaling pathway is a common oncogenic change, occurring in about 10% of patients. In TNBC, activating PIK3CA alterations are very common (Marty et al., 2008). The tumour inhibiting phosphatases inositol polyphosphate 4-phosphatase type II (INPP4B) and phosphatase and tensin homolog (PTH) are also lost, resulting in PI3K pathway activation (PTEN) (Koboldt et al., 2012). Furthermore, a tiny percentage of TNBC has an amplification of AKT and translocation of AKT3 (Banerji et al., 2012; Mir, 2021i). PIK3CA activating aberrations appear to be more common in LAR and mesenchymal subtypes (Lehmann et al., 2011). Thus, targeting the PI3K/AKT signaling pathway system is a compelling and sensible possible therapy option.

An inhibitor of all 3 isoforms of the serine/threonine kinase AKT namely Ipatasertib (GDC-0068) is a small molecule inhibitor that is new, selective, and ATP-competitive (Lin et al., 2013). The general side effects identified in a phase Ib study of ipatasertib plus paclitaxel in MBC individuals were nausea, diarrhea, nausea, tiredness, vomiting, rashes, and anorexia (Isakoff et al., 2021). LOTUS (NCT02162719) is a randomized, double-blind, placebo-controlled multinational phase II research in about 120 individuals with locally advanced or MBC and not being treated previously to distinguish the efficacy of ipatasertib paired with paclitaxel to placebo with paclitaxel (Lehmann et al., 2015) (Table 7.2). FAIRLANE (NCT02301988) is another trial that is a multicenter, randomized, double-blind, placebo-controlled, preoperative phase II research comparing the efficacy of ipatasertib combined with paclitaxel to placebo combined with paclitaxel in women with stage IA-IIIA TNBC (primary tumors 1.5 cm).

Overexpressed growth factors in TNBC

Various growth factor receptors, such as EGFR, FGFR, and VEGFR are overexpressed in TNBC. Inhibition of these factors can have a potential effect on TNBC.

Inhibition of fibroblast growth factor receptor (FGFR)

The growth of cells, their migration, survival, and differentiation are all aided by FGFR signaling (Turner et al., 2010). Overexpression of the FGFR1 gene is found in around 9% of TNBC, whereas amplification of the FGFR2 gene is seen in about 4% of TNBC (Koboldt et al., 2012). FGFR mutations are less prevalent in TNBC (less than 1%) (Cerami et al., 2012). In cell line models, cell lines having an amplification of FGFR1 or mutation of FGFR2 or FGFR4 were responsive to an FGFR inhibitor (Turner and Grose, 2010). In addition, inhibiting FGFR along with the amplification of FGFR2 resulted in lower proliferation in basal-like TNBC cell lines (Sharpe et al., 2011; Mir, 2021i). These findings support the clinical testing of FGFR inhibitors in TNBC; however, they may only benefit a limited subset of patients. To date, numerous multitargeted kinase inhibitors with relatively high efficacies against FGFRs are in clinical development. A phase II trial (NCT02202746) in MBC assesses an oral lucitanib in tumors having an amplification of FGFR1 or 11q, and patients with TNBC are eligible. JNJ-42756493, an oral pan-FGFR inhibitor has been tested in a phase I trial in patients with solid tumors, and one group comprises patients with any subtype of BC as long as the tumors have an FGFR activating mutation or translocation (NCT01703481). These trials, which have inclusion criteria that are more precise to specific FGFR mutations, may be more beneficial than prior trials that treated patients with BC who were not selected.

Epidermal growth factor receptor (EGFR) targeted therapy

Nielsen and co-workers used DNA microarray investigation on several BLBC samples and discovered that almost 60% of the samples displayed high levels of EGFR (Nielsen et al., 2004). Livasy and coworkers' statistical findings further revealed that around 70–78% of basal-like TNBC samples showed high levels of EGFR. As a result, it is possible that EGFR could be used as a potential target in TNBC (Livasy et al., 2006). However, a randomized phase II clinical study (NCT00232505) of 120 individuals of TNBC indicated that cetuximab treatment alone had a RR of < 6%, while cetuximab in combination with carboplatin had an RR of just 17% (Carey et al., 2012). As a result, while the preclinical evidence strongly supported the use of EGFR as a possible target for TNBC targeted therapy, the clinical trial data revealed that EGFR-targeted TNBC treatment did not reach the predicted results. Cho and co-workers (Cho, 2019) recently revealed through RNA-seq the ERBB pathway-activated triple-negative cell population. The bulk RNA-seq data indicated no change in the differential expression of 3 subtyping marker genes (ESR1, ERBB2, and PGR), while single-cell transcriptomic revealed intratumor heterogeneity. This finding suggests that ERBB signaling is triggered in an indirect manner and that the molecular subtype of ERBB is altered at the single-cell level. The outcomes of the EGFR signaling pathway study in TNBC individuals revealed that most patients' EGFR downstream signaling pathways remained activated following EGFR-targeted treatment, implying that alternative pathways may be implicated in bypass activation. As the result, EGFR-targeted therapy cannot accomplish potent efficacy on its own. Based on the foregoing findings and Lehmann and co-workers gene expression profile investigation, we believe that using growth factor inhibitors in the MSL, BL-2, and M subtypes in combination with additional downstream signal transduction inhibitors (MAPK, PI3K, and Scr inhibitors) may yield superior outcomes.

Vascular endothelial growth factor (VEGF) inhibitors in TNBC

Because TNBCs have high levels of VEGF in their tumors, researchers are looking at using bevacizumab, a VEGF-directed mab, to treat them (Foekens et al., 2001). In a trial namely, NSABP B-40, the addition of chemotherapeutic drugs (capecitabine or gemcitabine) to neoadjuvant taxane/anthracycline regimens, as well as the role of neoadjuvant bevacizumab in HER2- breast tumors was assessed (Bear et al., 2015). The administration of either capecitabine or gemcitabine did not result in better results (Bear et al., 2015).With considerably more common grade 3-4 neutropenia, hypertension and hand-foot syndrome, adding bevacizumab was related with enhanced OS (HR, 0.65; 95%, CI, 0.49 − 0.88; P = 0.004) but not disease free survival (HR, 0.8; 95% CI, 0.63 − 1.01; P = .06) (Bear et al., 2015). In a trial namely GeparQuinto, adding bevacizumab to neoadjuvant cyclophosphamide/epirubicin and then later involving docetaxel resulted in a higher pCR rate for TNBCs (39.3% vs. 27.9%), but no meaningful improvement in OS or DFS (Von Minckwitz et al., 2014).

TNBC patients have also been given bevacizumab as adjuvant treatment. TNBC patients were randomized to undergo four cycles of conventional CT along with or without bevacizumab in the BEATRICE experiment, which was an open-label, multicenter, phase III trial (Cameron et al., 2013). With the addition of bevacizumab, the DFS (82.7% versus 83.7%) OS (HR, 0.84; 95% CI, 0.64 − 1.12; P = 0.23) were not substantially different. Patients who used anthracyclines and bevacizumab at the same time had a modest expansion in cardiac events (Cameron et al., 2013). Due to the increased toxicity and lack of efficacy of bevacizumab in adjuvant setup, bevacizumab is contrary to play role in treating TNBC (BEATRICE and ECOG 5103).

Targeting the specific agents in the treatment of TNBC

Targeting Trop-2

Several epithelial malignancies overexpress a cell surface protein that is not seen in matching normal tissues (Stepan et al., 2011). Trop-2 a transmembrane calcium signal transducer is associated with cell-cell adhesion control. Trop-2 coupled with the membrane has been linked to a poor outcome in BC (Ambrogi et al., 2014). In TNBC, there is an increasing interest in focusing on Trop-2. The antibody-drug combination IMMU-132 (isactuzumab govitecan) contains the humanized mab hRS7 against Trop-2, which is coupled to the active metabolite of irinotecan, 7-ethyl-10-hydroxycamptothecin (SN-38). The antibody moiety of IMMU-132 binds to Trop-2 alone. The antibody moiety of IMMU-132 binds to Trop-2 alone. SN-38 is administered selectively to tumor cells after internalization and proteolytic cleavage. In MDA-MD-468 TNBC xenograft models, IMMU-132 caused more tumor regression than irinotecan or the antibody-drug conjugate control, according to preclinical findings. In January 2015, the FDA granted the IMMU-132 Fast Track designation for treating TNBC individuals who have progressed on prior therapy for metastatic illness. IMMU-132 was tested in advanced epithelial malignancies, including TNBC, in a phase I/II trial. There was no expression of Trop-2 prescreening. On days 1 and 8 of a 21-day cycle, IMMU-132 was given at a dose of 10 mg/kg intravenously. The main side effects were neutropenia and mild diarrhea. An extended cohort included 23 patients with pretreated metastatic TNBC (median number of prior regimens was 4) who had a 30% response rate (7 partial responses) and a 40% CBR (partial response + stable illness > 6 months) (Bardia et al., 2017). Trop-2 score immunohistochemistry data is being collected. 80 patients having mTNBC who have undergone 2 or more prior regimens with IMMU-132 alone or along with carboplatin were treated in a phase II trial (NCT02161679). To assess the approach of employing antitrop-2 treatments for breast cancer and the association between Trop-2 expression and response, more study is needed.

PARP inhibitors targeting PARP

BRCA genes belong to tumor suppressor genes that use homologous recombination repair to repair broken dsDNA. Mutated BRCA1/2 in the chromosome induces abnormalities in DNA and homologous recombination repair, resulting in genomic instability (Evers et al., 2010). Tumorigenesis is based on this mechanism. BC individuals having BRCA1/2 mutations represent 5–10% of all BC cases. Patients possessing a family history of ovarian or breast cancer (especially first-degree relatives), younger patients (under 45 years), and TNBC patients are more likely to have such mutations (Couch et al., 2015). BRCA1/2 mutations are present in roughly 40% of TNBC individuals, and TNBC is present in approximately 60% of BRCA1-mutated cancers (Atchley et al., 2008), demonstrating that BRCA1/2 and TNBC mutations are associated but not wholly coincidental. The PARP family comprises ribozymes capable of catalyzing the ADP ribosylation, including almost 17 proteins. These members of the family are involved in base repair and resection, which helps to replace ssDNA damage (Anwar et al., 2015; Mir, 2021i). These two repair processes, ssDNA and dsDNA repair, are hindered when PARPi are used in BRCA1/2-mutated cells. Furthermore, a study demonstrated that BRCA2-deficient cells are vulnerable to PARP1i (Bryant et al., 2014). Theoretically, these two assertions support the use of PARPi in the treatment of BRCA1/2-mutated breast malignancies. The relationship between PARP and BRCA closely resembles the notion of "synthetic lethality," which states that a deficit in one of the two genes has no effect, but two deficient genes together cause' cell or organism death (Ashworth and Lord, 2018). Synthetic lethality is a deficiency in tumor suppressor genes that kills tumor cells but has no effect on healthy cells (Lord et al., 2015). This method is ideal for destroying tumor cells accurately. Olaparib, an oral PARPi, has the ability to reduce PARP1, PARP2, and PARP3 all at the same time. This treatment for patients with BRCA1 or BRCA2 mutations in BC is useful. In HER2-MBC patients with hereditary BRCA mutation, the OlympiAD study compared olaparib to therapy of physician's choice (TPC). In this experiment, olaparib significantly increased median PFS as compared to TPC (p0.001; 7.0 months vs. 4.2 months; p0.001). The majority of treatment-related adverse events in the olaparib cohort were mild, including nausea, anemia, vomiting, tiredness, and neutropenia. In addition, the olaparib cohort has a lower rate of grade 3 TRAEs than the TPC group (Robson et al., 2017). Following that, an OS examination revealed no statistically significant comparison between the two groups. The analysis of the subgroup, however, revealed that individuals who got olaparib as 1st-line therapy were more likely to get an advantage from OS. In the olaparib cohort, there was no cumulative impact of long-term toxicity (Robson et al., 2017). Talazoparib is a PARP1/2i that can be used orally. In MBC and genetic BRCA mutated patients, the EMBRACA study compared talazoparib to TPC (eribulin, capecitabine, gemcitabine, or vinorelbine). When compared to the control arm, the talazoparib arm had effectively longer median PFS (8.6 months vs. 5.6 months; p0.001).The talazoparib group had a greater objective response rate (ORR) than the TPC cohort (p0.001; 62.6% vs. 27.2%). Hematologic toxicities were the most prevalent TRAEs in the talazoparib cohort (primarily anemia). The talazoparib cohort had a higher rate of grade 3 or 4 hematologic TRAEs than the TPC cohort (55% and 38%, respectively) (Litton et al., 2018). Studies have revealed that patients who were administered with talazoparib had advancement in QOL and a significant delay in the time to definitive clinically meaningful deterioration using the European Organization for Research and Treatment of Cancer Quality of Life Questionnaire Core 30 and its breast cancer module, QLQ-BR23 (Ettl et al., 2018).

TABLE 7.3 Main PARP inhibitors in MBC/TNBC.

S. No	Drug	Trial	Phase	Population
1	Olaparib	OlympiAD	III	gBRCA-mutated MBC
2	Talazoparib	EMBRACA	III	gBRCA-mutated MBC
3	Veliparib	BROCADE	II	gBRCA-mutated MBC

Furthermore, talazoparib medication helped both germline BRCA mutated individuals who were administered with or were not administered with platinum therapy (Table 7.3) (Turner et al., 2010). Veliparib is another PARP1/2i that is taken orally. The dose schedules for veliparib in different combination regimens are currently varying. In advanced TNBC and/or BRCA mutation-associated BC, a Phase 1 study looked at adding veliparib to vinorelbine and cisplatin. This study revealed that BRCA mutated Patients were more presumably to benefit from study therapy, irrespective of the veliparib dose, and 300 mg veliparib twice a day (BID) schedule was well standed (Rodler et al., 2016). In patients with BRCA-mutated MBC, the BROCADE Phase II trial looked at adding veliparib to temozolomide (VT) or paclitaxel/carboplatin (VCP). VCP (120 mg veliparib BID, days 1–7, 3-week cycle), VT, or placebo plus paclitaxel/carboplatin were given to eligible patients in a 1:1:1 ratio (PCP). VCP significantly enhanced ORR (77.8% vs 61.3%; p = 0.027) but did not affect PFS or OS when compared to PCP. VT had no effect on ORR, PFS, or OS when compared to PCP (Han HS et al., 2018). The equivalent Phase III trial (BROCADE3, NCT02163694), which compares VCP to PCP in HER2- BRCA-associated MBC, has perfect recruitment. So far, no results have been released. When coupled paclitaxel and with carboplatin, an exposure-response examination showed that veliparib dosage from the BROCADE trial provided extra benefit without compromising safety. In the above-mentioned patients, a higher dose of veliparib did not appear to provide a significant advantage in this combination (Nuthalapati et al., 2019). In MBC, a phase I study looked at the effects of veliparib and carboplatin given on an intermittent or continuous basis. The combination of veliparib (2 times a day, 250 mg, d1-d21) and carboplatin (area under the curve 5, 3-week cycle) showed anticancer effectiveness as well as good tolerability (Table 7.3) (Wesolowski et al., 2020). Niraparib, olaparib, rucaparib, and talazoparib are the four inhibitors of PARP presently approved by the FDA. For genetic BRCA mutated patients and HER2- MBC, only talazoparib, and olaparib have been recommended. The other two inhibitors of PARP are only for advanced ovarian cancer patients.

Angiogenesis inhibitors

The majority of TNBC treatment research has highlighted the angiogenesis inhibitors such as VEGF and VEGFR targets. Anti-angiogenesis monotherapy has minimal efficacy in advanced TNBC (Curigliano et al., 2013). However, combining angiogenesis inhibitors with standard CT has demonstrated to be a viable anti-cancer strategy. The ECOG 2100 study found that paclitaxel along with bevacizumab (an anti-VEGF mab) effectively improved median PFS in metastatic HER2- BC patients (p0.001; 11.8 months vs. 5.9 months) when compared to PTX alone as 1st-line treatment (11.8 months vs. 5.9 months, p0.001) (Miller et al., 2007). The FDA recommended bevacizumab in 2008 based on the results of this trial.

The conjunction of bevacizumab and taxane-based CT for advanced TNBC patients improved median PFS by 2.7 months (p0.0001) when compared to CT alone, according to a meta-analysis of the AVADO, E2100, and RIBBON studies. The combination group, on the other hand, had no OS advantage. Neutropenia, hypertension, and sensory neuropathy were the general grade 3 TRAEs with bevacizumab-containing regimens (Miles et al., 2013).

In the past few years, some tailored combination regimens—weekly PTX and carboplatin with bevacizumab—have also shown significant results in advanced TNBC (Symonds et al., 2019). Additionally, the 60-amino-acid polypeptide (ASRPS), endogenous hydrogen sulphide, exosomal-annexin A2, and centromere protein U encoded by long ncRNA have been linked to angiogenesis and recommended as possible targets (Li et al., 2020; Wang et al., 2020).

Estrogen receptor ER-α36

TNBC cells are thought to lack intracellular estrogen signal transduction because they lack expression of PR, ER, and HER2 expression. They are endocrine therapy insensitive and have no identified therapeutic targets. Wang et al. were the first to discover, clone, and identify ER-36, a novel estrogen receptor with a molecular weight of 36 kDa. This newly developed ER is not the same as the ER-66 that has been researched extensively. ER-36 doesn't possess the transcriptional activator domains AF-1 and AF-2 but possesses the DNA-binding domains and several dimeric ligand domains compared to ER-66 (Wang et al., 2005). Both ER-positive and ER-negative BC cells contain ER-36, which is mostly expressed in the cytoplasm and cell membrane. As a result, ER-36 is a membrane-expressed ER that can swiftly promote estrogen and antiestrogen signaling transduction in both ER+ and ER- BC cells (Wang et al., 2006). Zhang et al. investigated the signaling pathways of ER-36 in the MDA-MB-231 and MDA-MB-436 TNBC cell lines and discovered a positive feedback loop of EGFR and ER-36 in TNBC, suggesting that ER-36 could be a potential target for TNBC treatment (Zhang et al., 2011). Clinical trials are currently lacking in support, and various treatment programs are still being investigated.

AR inhibitors

AR belongs to the nuclear steroid hormone receptor family. In around 10–15% of TNBC patients, AR expression is positive (Barton et al., 2015). AR positivity is characterized as the LAR-subtype TNBC (Lehmann et al., 2011). AR, which is a part of the nuclear steroid hormone receptor family, was discovered in 60–70% of all breast tumors and 20–40% of TNBCs (Rahim and O'Regan, 2017). AR mRNA was shown to be nine times more abundant in the LAR subtype than in the other subtypes, according to Lehmann et al. The AR signaling pathway is thought to be responsible for the LAR subtype's growth. 2 The use of AR treatment to target TNBC is a novel technique. Furthermore, the LAR subtype has a high number of PIK3CA mutations, making it susceptible to PI3K/mTOR inhibitors (Lehmann and Pietenpol, 2014).

Bicalutamide is a nonsteroidal inhibitor of AR that is utilized to treat metastatic prostate cancer along with luteinizing hormone-releasing hormone analogues. Bicalutamide has recently been shown to have an anticancer effect in ER/PR-, AR + (>10 percent IHC staining) MBC, with a CBR of 19% and a median PFS of 3. There were no individuals who got

TABLE 7.4 Clinical trials targeting AR in TNBC.

S. No	Trials	Phase	Regimen
1	NCT00468715	II	Bicalutamide 150 mg daily
2	NCT01842321	II	AA 1000 mg daily + prednisone 5 mg twice daily
3	NCT01889238	II	Enzalutamide 160 mg daily
4	NCT02605486	I/II	Palbociclib 100 mg + bicalutamide 100 mg daily, 3 weeks on 1 week off

bicalutamide and experienced TRAEs of grade 4/5 (Gucalp et al., 2013). 17-α-hydroxylase/17, 20-lyase (CYP17) is inhibited by abiraterone acetate (AA), resulting in lower serum testosterone levels (Taplin et al., 2014). In castration-resistant prostate cancer, AA is frequently used (Attard et al., 2008). In AR+ TNBC, Bonnefoi and co-workers found that AA with prednisone resulted in a CBR of 20% CBR and a median PFS of 2.8 months. Hypertension, fatigue, hypokalemia, and nausea with mild grade were general TRAEs in this study (Bonnefoi et al., 2016). Enzalutamide is a more potent AR inhibitor that operates on many levels in the AR signaling pathway when taken orally. In patients with metastatic AR+ malignancies, enzalutamide showed anticancer efficacy and acceptable tolerability. According to Traina and co-workers, 160 mg of enzalutamide on daily basis showed a CBR of 33% at 16 weeks and a CBR of 28% at 24 weeks in AR+ advanced TNBC. In the evaluable subset, the OS and median PFS were 17.6 months and 3.3, respectively. Furthermore, 3% of patients who got enzalutamide exhibited TRAEs of grade 3 and generally experienced fatigue (Table 7.4) (Traina et al., 2018).

Experimental targets for TNBC under study

AMPK (AMP-activated protein kinase)

In cancerous breast tissue, the phosphorylated AMPK (pAMPK, Thr172) level is substantially less than the benign and normal breast tissue. Reduced p-AMPK is linked to axillary node metastases and histological grade. AMPK modulates the cytoskeletal forces of circulating tumor cells (CTCs) in MBC cells, according to immunohistochemistry. AMPK inhibition stimulates the production of micro tentacles, commonly known as microtubule-based protrusions, by increasing microtubule stability and activating cofilin, an actin-severing protein. By enabling CTC aggregation and re-attachment, micro tentacle development improves the metastatic efficacy of circulating breast tumor cells (Chakrabarti et al., 2015). Furthermore, the anti-proliferation ability of tumor cell-mediated activation of AMPK has been linked to the down regulation of TSC2-mTOR and p53-p21 up-regulation (Motoshima et al., 2006). A synthetic allosteric activator of AMPK namely OSU-53 has the ability to augment the levels of p-AMPK (Thr 172) (Lee et al., 2011), which in turn inhibits both p-MDM2 and p-Akt, the E3 ligase that has the ability to ubiquitinate and destruct Foxo3a, resulting in augmentation of Foxo3a nuclear localization and assemblage. The buildup of nuclear Foxo3a caused by an activation of AMPK up-regulated E-cadherin and raised the mesenchymal marker expressions like those of vimentin, YB-1, and snail (Chou et al., 2014). Metformin (Met), a common anti-diabetic biguanide medication activates AMPK. Individuals with diabetes who took metformin had reduced cancer and mortality rates than those who took other diabetic drugs

(Elamin Abdelgadir et al., 2017). Metformin (Met) and its derivative phenformin (Phe) inhibited angiogenic protein synthesis in cultured BC cells and white adipose tissue. The studies showed that Met and Phe both retarded the growth of tumors in HER2-overexpressing and TNBC BCs and also reatarded their metastasis to the lungs (Orecchioni et al., 2015). 5-aminoimidazole-4-carboxamide ribonucleotide (AICAR) has also been studied for its anti-cancer benefits in TNBC; AICAR was the first molecule to be discovered as a direct AMPK activator. AICAR is transformed into AIC. After being taken into cells by the adenosine transporter, AICAR is transformed to AICAR monophosphate (ZMP). Adenosine kinase phosphorylates it, and it then acts as an AMP analogue by attaching to AMPK's AMP binding site (Kim et al., 2016). AICAR reduces the growth of TNBC cell lines, like BT-549 and MDA-MB-231 by reducing c-Myc expression, which leads to down regulation of MTDH expression. The activation of SIRT1 and decrease in p-GSK3 (Ser 9) leads to AICAR-induced MTDH down regulation (Gollavilli et al., 2015). Although none have been examined in relation to TNBC except as indicated above, and AMPK-activating medications have not still entered the clinical study, a recent study extensively reviewed direct and indirect AMPK activators and their method of action (Kim et al., 2016).

Mouse double minute-2 homolog (MDM2)

MDM2, also named as E3 ubiquitin-protein ligase MDM2, binds directly to the tumor suppressor p53 and reduces its transcriptional activity while also promoting its destruction, thus negatively regulating p53. Its N-terminus contains a p53 binding domain; the C-terminus contains a RING domain (Yuan et al., 2011). Soft tissue cancers have the highest rate of MDM2 amplification (20% in 28 human malignancies) while osteosarcomas have the second-highest rate (16%) (Momand et al., 1998). The percentage count of MDM2/4 amplification was found to be 7% of 102 TNBC individuals, according to the Cancer Genome Atlas (TCGA) (Lehmann and Pietenpol, 2014). Small molecule inhibitors have been tried to inhibit MDM2-p53 connection as a therapeutic technique to restore back p53 activity, based on the structural basis of four well-defined hydrophobic residues in p53 - Phe19, Leu22, Trp23, and Leu26 - that are important for the MDM2-p53 relationship (Shangary et al., 2008). The inhibitors of MDM2 inhibitors namely Nutlin-3 and MI-219 bind directly to MDM2 and prevent the MDM2-p53 association. As a result of p53 pathway activation, without producing p53 phosphorylation or DNA damage, tumor cells are arrested and die, making these chemicals far less genotoxic than other anti-tumor drugs (Shangary et al., 2008). PTX and Nutlin-3 were combined in preclinical tests and exhibited synergism with respect to their apoptotic and anti-proliferative effects in TNBC (Wali et al., 2017). RG-7112 has been developed as the 1st clinical inhibitor of MDM2 inhibitor that possesses the ability to show its association with the p53 containing the domain of MDM2 and is more potent than nutlin-3(Vu et al., 2013). Further clinical inhibitors of MDM2, like RG-7388, AMG232 and MI-77301 have been produced and their clinical efficiency examined, although there are currently no clinical trial outcomes for inhibitors of MDM2 in TNBC (Andreeff et al., 2016; Jung et al., 2016). The MDM2-binding protein, MTBP, is well-known as a Myc transcriptional target. This protein has the ability to bind with Myc and interacts with it at Myc-targeted promoters, enhancing Myc's oncogenic role leading to carcinogenesis (Grieb, 2014). According to TCGA data analysis, MTBP is increased in a variety of malignancies. TNBC had the highest levels of MTBP mRNA and protein expression among BC types. BC individuals having greater levels of both MTBP and Myc mRNA had a lower 10-year survival rate than those who had high Myc but low MTBP mRNA levels (Grieb, 2014). TNBC tumor development was lowered and MTBP protein levels in tumors were reduced when MTBP was knocked down utilizing doxycycline-inducible MTBP shRNA (Grieb, 2014).

Metadherin (MTDH)

The astrocyte elevated gene 1 (AEG-1) gene, also named MTDH, is substantially up regulated in breast tumor tissues and is involved in BC angiogenesis, proliferation, metastasis, invasion, and treatment resistance (Li et al., 2008). Increased levels of MTDH are a predictive marker of distant metastasis and lymph node metastases in ovarian, breast, and cervical malignancies, according to a meta-analysis of literature published between 2008 and 2016 (Hou et al., 2016). In TNBC individuals, SU6668 and AICAR reduced MTDH expression, which inhibited tumor cell invasion and proliferation (Gollavilli et al., 2015). TNBC may have a therapeutic target in the form of MTDH.

Heat shock protein

The aggressive nature of TNBC is owing to its heterogeneous and intricate molecular processes; consequently, defining a target that can span many pathways at once is of interest. HSP90 is a well-known molecular chaperone that promotes post-translational modification and stabilization of HIF-1, EGFR, AKT, IGF-1R, and RAF-1, as well as certain important components of DNA repair pathways (e.g., RAD51, BRCA1) (Stecklein et al., 2012). As a result, blocking HSP90 is likely to have a broad impact on a number of key signaling pathways implicated in tumor growth. HSP90 levels that are up-regulated are linked to a higher recurrence rate of TNBC, and multiple studies have shown that TNBC is susceptible to various HSP90 inhibitors (Cheng et al., 2012; Mir, 2021i). By producing a complete response in TNBC xenograft models with considerable tumor shrinkage, PU-H71, a powerful HSP90 selective inhibitor, has shown promise anti-tumor actions without toxicity (Caldas-Lopes et al., 2009). PF-4942847, an orally accessible HSP90 inhibitor, demonstrated a strong anticancer effect in TNBC models in vitro and in vivo by increasing apoptosis and reducing cell proliferation by AKT degradation (Mehta et al., 2011). Some inhibitors of HSP90 inhibitors are now being evaluated for clinical use based on these2 studies: Ganetespib (STA-9090), a second-generation inhibitor of HSP90 with triazolone moiety that is fundamentally different from the 1st generation ansamycin family (e.g., geldanamycin, tanespimycin (17-AAG), and alvespimycin (17-DMAG), is currently in phase II trial (ENCHANT-1 trial; NCT01677455) as monotherapy for HER2-negative breast cancers. It has been shown to potentially slow tumor growth in TNBC xenograft models, either as a monotherapy or along with other drugs, due to its superior safety and efficacy characteristics (Proia et al., 2014). Its anti-tumor properties are due in part to the inhibition of HIF-1 activity, which results in the down-regulation of critical proteins associated with invasion, angiogenesis, and metastasis (Xiang et al., 2014). It also makes TNBC cells more sensitive to PTX in vitro and in vivo by causing the glucocorticoid receptor, another well-known HSP90 client protein, to degrade (Agyeman et al., 2016). ENCHANT-1′s interim results showed that 5 out of 10 evaluable TNBC patients (50 percent) had disease control status (Coburn, 2014). Another effective second-generation HSP90 inhibitor is Luminespib (NVP-AUY922). There are two further phases I trials exploring the combined therapy of onalespib (AT13387), an orally bioavailable inhibitor of HSP90, with paclitaxel (NCT02474173) or olaparib (NCT02898207). In addition to the medications described, several experimental efforts are underway to recognize novel small compounds that target HSP90 in order to establish an effective therapy alternative for TNBC (Oh et al., 2018).

Cell cycle regulating targets: Aurora kinase, CHK1, ATR, WEE1, and CDC25

At numerous levels, the DNA damage response (DDR) and the pathways involved in the cell cycle are intertwined and play a key role in the preservation of TNBC features. G2/M DNA damage checkpoints are triggered when HR is normally active, promoting the repair system and cell cycle advancement. The main regulators in this process are CDC25, WEE1, and their upstream checkpoint kinases CHK1/2, or phosphorylated Polo-Like Kinase 1(PLK1) phosphorylated by ATR (PLK1). Aurora-A kinase (AurA) is a direct upstream activator of PLK1 that prevents RAD51 from being recruited to the destructive site (Cazales et al., 2005). DNA repair pathways have been found to be one of the most deregulated pathways in TNBC (Albiges et al., 2014). As a result, all of the proteins indicated are now being studied in pre-clinical studies as possible therapeutic target agents in TNBC. The various AurA inhibitors are listed in Table 7.5. Two among them are also in clinical trials. Due to the poor clinical effects of aurora kinase inhibitors thus far, more research on combination regimens is likely to be advised.

TNBC is also being studied with a number of WEE1 and ATR inhibitors. A new strategy has evolved that combines a WEE1 inhibitor (AZD1775) with an ATR inhibitor (AZD6738) to improve therapeutic advantages by enhancing overall cytotoxicity. This regimen also showed that RAD51-mediated HR inhibition can make TNBC cells more sensitive to cisplatin and PARPi (Jin et al., 2018). TNBCs can be sensitized to cisplatin without AZD6738, enhancing their cytotoxic response, implying that WEE1 inhibitors have therapeutic potential that should be investigated further in clinical practice (Chen et al., 2018). The degree of Cyclin E expression among TNBC patients could be a decisive factor in its response to AZD1775.

The drug's anti-tumor effectiveness is stronger in Cyclin E-high TNBCs; consequently, previous usage of CDK2 inhibitors for transitory Cyclin E induction to sensitize patients

TABLE 7.5 Aur A targeting by Inhibitors of Aur A.

S. No.	Name	NCT number	Clinical trial identifier
1	Alisertib (MLN8237)	—	—
2	KW-2450	—	—
3	AS703569	—	—
4	Midostaurin	—	—
5	ENMD-2076	NCT01639248	Phase II for advanced or mTNBC who underwent previous treatment
6	AMG900	NCT00858377	Phase I trial of AMG 900 on taxane resistant TNBC patients was conducted but failed to prove the efficacy

to AZD1775 is indicated for Cyclin E-low counterparts (Chen et al., 2018). The rhodium (III) complex has recently been found as a novel WEE1 inhibitor that causes cell death in TNBC cells with p53 mutations (Yang et al., 2018). CHK1 is another crucial target molecule in the DNA repair process that has been found to be overexpressed in the TNBC group. SB218078, V158411 (CHK1 IC50 = 3.5 nM), PF-477736 (CHK1 IC50 = 4.9 nM), and AZD7762 (CHK1 IC50 = 5 nM), all of which inhibit CHK1, have dramatically promoted cell death in TNBC lines by DNA damage and apoptosis enhancement (Albiges et al., 2014; Rundle et al., 2017).

Although CHK1 inhibitors have proven excellent anti-cancer activity when used alone, combination regimens have been revealed to produce greater results. In vitro and in vivo, co-administration of UCN-01 (non-selective CHK1 inhibitor) or AZD7762 with gemcitabine resulted in significantly increased efficacy by producing considerable DNA damage in the tumor, thus inhibiting the growth of cells. CDC25, in addition to ATR and CHK1, has lately come out as a potential therapeutic target for TNBC. The proliferation of RB1-deficient TNBCs was dramatically disrupted when CDC25 was inhibited. The anti-cancer impact of the CDC25 phosphatase inhibitor NSC663284 and the WEE1 inhibitor MK-1775 appeared to be synergistic. Because the PI3K pathway is elevated when CDC25 is inhibited for lengthy periods of time, co-treating TNBCs with PI3K inhibitors and CDC25 modulators may be an appealing option (Liu et al., 2018; Zacksenhaus et al., 2018; Liu et al., 2018).

Summary

TNBC represents the most aggressive type of BC because of its heterogeneous nature. The clinical features associated with this particular type of BC include high metastatic potential, worse prognosis, greater invasiveness, and of course high recurrence rate. Because of the absence of all the three receptors, i.e. ER, PR, and HER2, TNBC shows no response toward endocrine therapy. The treatment options for BC may include radiotherapy, chemotherapy, immunotherapy, and targeted therapy (Mir). But at present, chemotherapy is the only treatment that has been approved for TNBC (Mir, 2021g). In spite of the fact that chemotherapy is the better treatment option in TNBC as compared to the other forms of BC, it still shows a worse prognosis. The main reason for this is that the disease-free period between neoadjuvant and adjuvant therapy is less and a much-threatened course in the metastatic setting. CT in combination with other treatment options may prove beneficial for TNBC patients (Mir, 2021h). With the advancements in the treatment field of TNBC, Various biological agents have been evaluated in this aspect. In view of this, targeted therapy has evaluated various biological molecules and signaling pathways have been targeted for having effective progress in the treatment of TNBC. Additionally, various other targets are in clinical studies for assessing their role in TNBC.

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CHAPTER

8

Different drug delivery approaches in combinational therapy in TNBC

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Introduction

TNBC is a very diverse and invasive type of cancer, making treatments difficult and leading to a greater mortality rate compared to other types of cancer (Brewster et al., 2014; Qayoom et al., 2021). The disease's poor prognosis is due to increased relapse and metastases in the first five years after detection, as well as a lack of tailored drug delivery methods (Fan et al., 2017). TNBC tumors are typically high-grade, large-sized, and infiltrated with lymphocytes. TNBC cells can potentially spread to the brain and lungs (Yao et al., 2017). During identification, TNBC individuals usually reveal early visceral metastases and lymph node linkage (O'Reilly et al., 2015). Patients with advanced TNBC have a median survival duration of 12 months, which is much lower as compared to most advanced breast carcinomas (Meena et al., 2017). TNBC sufferers have a 62% 5-year surviving rate, while non-TNBC sufferers have a 75% 5-year rate of survival (Shan et al., 2017; Mir, 2021). Because of the disease's highly invasive nature, which prohibits identification by conventional diagnostics like mammograms, magnetic resonance imaging (MRI), and ultrasonography, TNBC is usually diagnosed in the advanced stages of the disease when malignant tumor masses are greater than 2.5 cm in diameter (Miller-Kleinhenz et al., 2015; Mir, 2015). The development of new anticancer drugs is a time-consuming and expensive procedure. As a result, the present research is focusing on developing new drug delivery mechanisms to improve the antitumor efficacy of current medicines (Bernabeu et al., 2016). Anticancer drugs are being used to treat TNBC, either alone or in conjunction with surgery or radiotherapy (Yao et al., 2017). Taxanes, anthracyclines, and platinum chemicals are among the medicines used in TNBC chemotherapy (Kalimutho et al., 2015). Patients with TNBC who have BRCA1 abnormalities are more sensitive to platinum treatments and have poorer taxanes responsiveness (Miller-Kleinhenz et al., 2015). Chemotherapy regimens for TNBC individuals are chosen based on tumor size, tumor

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stage, lymph node involvement, and the presence of clinical co-morbidities (O'Reilly et al., 2015). If a tumor is lymph node-negative, it could be excised surgically without chemotherapeutics if it is less than 0.5 cm in diameter; if the size is between 0.6–1 cm in diameter, chemotherapy could be offered post-surgery; but if this is greater than 1 cm in diameter, adjuvant chemotherapy is generally offered post-surgery because there is a greater risk of distant metastasis due to the big size of the tumor (Mir et al., 2021). Resection following by adjuvant chemotherapy using taxanes or anthracyclines is used to treat TNBC tumors with lymph node involvement (Anders et al., 2013). Neoadjuvant chemotherapy, which involves chemotherapy delivered before surgery, helps to decrease the main tumor while also determining the tumor's responsiveness to cytotoxic medicines, which could also benefit with subsequent therapy of recurring TNBC (Miller-Kleinhenz et al., 2015). In TNBC individuals, neoadjuvant CT aids breast preservation (Munzone and Colleoni, 2017).

TNBC cells develop resistance to chemotherapy as a result of the very heterogeneous character of tumors. Drug efflux pump and Anti-apoptotic genes are overexpressed in TNBC cells, allowing them to evade chemotherapy (Darvishi et al., 2017; Mir, 2021). TNBC exhibits intra- and inter-tumor diversity. Various parts of a single tumor can react to treatment differentially (Jhan and Andrechek, 2017). It's critical to figure out how a person reacts to specific chemotherapeutic drugs so that the best effective treatment could be provided instead of subjecting the person to the negative side effects of a medication that doesn't work (Miller-Kleinhenz et al., 2015). Chemoresistance in TNBC is linked to a large number of dormant and pluripotent stem-like cells, that undergo epithelial to mesenchymal transition (EMT) and hence demonstrate resistance to medicines that target proliferating cells (Saraiva et al., 2017; Mehraj et al., 2021). ABC transporters, changes in DNA repair enzymes, amplification of the -tubulin III subunit, alteration of apoptosis-inducing genes, and deactivation of the chemotherapy drug are all key chemoresistance processes in TNBC. Because there are considerable discrepancies among cell lines and actual TNBC tumors, the use of cell lines to assess the effectiveness of newly discovered TNBC medicines is questioned. To imitate the heterogeneous character of clinical TNBC, the effectiveness of newly proposed TNBC medicines must be investigated in a breast tumor model that has been freshly separated from a person and retains all of the elements of the tumor milieu (Sulaiman and Wang, 2017). Due to their cellular, molecular, genomic, as well as epigenetic similarities to clinical tumors, patient-derived xenograft models are much more ideal for assessing recently discovered medicines. The chapter's main goal is to show how nanocarriers could be used to combat TNBC in a variety of methods. The nanocarriers are termed "nanosoldiers," as they may be outfitted with a variety of armaments to fight the difficult struggle against TNBC (Mir, MA et al., 2021).

Nanocarriers as targeted drug delivery systems

Nano-technological developments in medical research for cancer treatment using contrasting compounds and drug delivery vehicles over the last two decades are now leading to more accurate and focused co-delivery of both diagnostic and treatment agents. Lipids, polymers, nucleic acid, carbon, proteins, and metals were used to create a wide range of nano-carriers

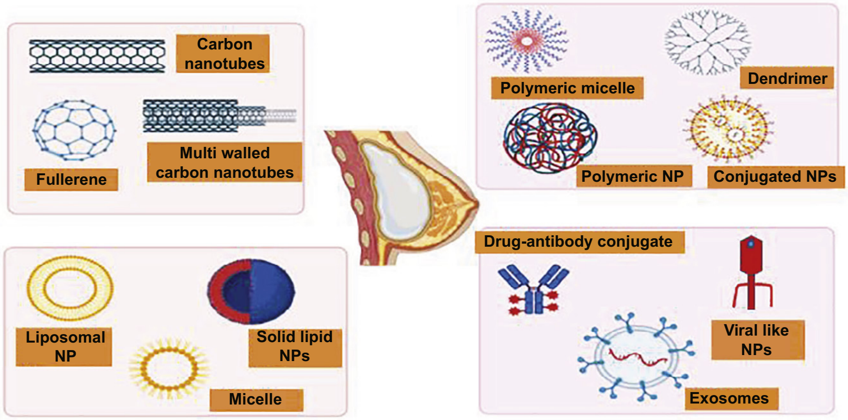

FIG. 8.1 Different types of nanoparticles used in the treatment of TNBC.

such as dendrimers, micelles, liposomes, nanotubes, and DNA tetrahedral/pyramids Fig. 8.1 (Kutty and Feng, 2013; Khodabandehloo et al., 2016; Kumari et al., 2016; Setyawati et al., 2016).

Such smart nanoparticles encapsulate anti-tumor drugs (arsenal) and are surface encased with a particular ligand (key) which ultimately binds with the receptor (lock) displaying on the BC location (target) and destroys the cells, besides the molecular image analysis (tracer agents), enabling us to concurrently make a diagnosis and cure the disease, i.e. Thenanostic strategy for enhancing current tumor diagnosis and therapy regime. Designing a suitable nanoparticle for biomedical use necessitates a variety of biological, physiochemical, and functional features in nanoscience. The main essential factor is size; because desirable nanoparticle size (1-200 nm) plus shape determines the particle's path dynamics, which is critical for nanomedicine development. Furthermore, the nanoparticle's surface charges and encapsulating ability are critical for accurate directed drug administration utilizing particular conjugated ligands against the tumor cell's specific receptor. Other qualities important for nanoparticles to be used as therapeutic applications in cancer diagnosis and therapy include elevated drug loading efficiency, lengthy half-life in the bloodstream with minimal systemic toxic effects, specific localization, elevated adhesion at the tumor environment, improved internalization into the tumor through endocytosis, the prolonged and regulated release of imaging agents as well as cytotoxic drugs over the correct period and time, as well as secure bio-elimination from the body (Setyawati et al., 2014; Mir et al., 2020). For localized drug delivery, most of the nano-delivery systems depend on the increased permeability and retention (EPR) phenomenon. The effectiveness of the study on tumor nanomedicine is also determined by technical practicality (rapid recoveries with regulated drug loading and discharging) and economic security for huge production. Table 8.1 outlines the numerous nanoparticles that may be designed utilizing various materials to transport various therapeutic agents and their important applications.

TABLE 8.1 A list of several nanoparticles that can be used in TNB anticancer therapy.

S. No.	Nanoparticles	Therapeutic agents	Uses
1	Liposomal NPs	DOX, PTX, Rapamycin, Sorafenib	The safety profile is favorable, lengthy circulation half-life, surface functionalization capability
2	Micelles	Docetaxel, RL71	Capacity to solubilize very hydrophobic medicines, increased absorption due to tiny size, biodegradability
3	Dendrimers	AODNs (Antisense-oligo Deoxynucleotides)	Excellent payload ability, active targeting via surface functionalization
4	Polymeric NPs	PTX	Biodegradability, better drug loading, biocompatibility, good drug release
5	DNA nanostructures	DOX	Increased drug loading
6	Metallic NPs	PTX, Cisplatin	High surface-to-volume ratio, surface functionalization potential, Biocompatibility
7	Carbon nanotubes	DOX	The high photoacoustic imaging contrast capabilities of single-walled carbon nanotubes enable improved spatial resolution and deep tissue scanning.

A multifunctional spherical nanocarrier: Liposomal nanoparticles

Liposomes are 400-nanometer round vesicles having a central aqueous core covered with lipid bilayers. Liposomes are the most adaptable carrier systems with improved drug dispersion because they can enclose drugs in lipid membranes or within the aqueous core (Mir et al., 2021, Fig. 8.2).

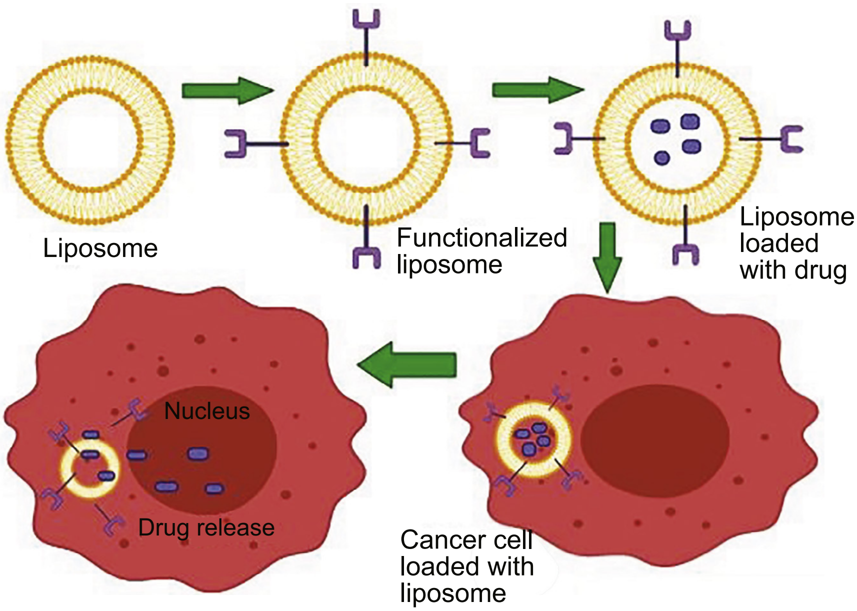

FIG. 8.2 Mechanism of action of liposomal nanoparticles.

Extrusion (the technique of manufacturing NPs with a defined cross-sectional area), solvent injections (the technique of lipid precipitating from a dissolving lipid in solutions), and reversed-phase evaporation are some of the ways used to create liposomal NPs. Dai and coworkers (Dai et al., 2014) used cyclic octapeptide LXY (Cys-Asp-Gly-Phe (3,5-DiF)-Gly-Hyp-Asn-Cys) linked liposomes delivering dual medication rapamycin and DOX to target highly expressed integrin-3 in TNBC models. When contrasted to free drugs, this double-drug-focused method resulted in enhanced effectiveness. Likewise, sorafenib and DOX-loaded liposomes showed improved anticancer efficacy in TNBC mice xenograft (Lee et al., 2010). Nevertheless, because current DOX liposomal preparations are linked to cardiotoxicity, a new micelle-encapsulated DOX formulation (NK911) having greater tumor penetration and lower in vivo cytotoxic effects is now being tested (Matsumura Y et al., 2004). To slow 4T1 cancer development and diminish pulmonary metastasis of breast carcinoma, a liposomal drug delivery method for the simultaneous administration of PTX (anti-cancer) and antagomir-10b (anti-metastasis) was created (Sun et al., 2013). Employing PEG encapsulated PTX nanoparticles targeting naked mice (MDA-MB-231/luc) as well as a lung tumor metastatic model, a substantial inhibition and decrease of 82% in tumorigenesis was reported (Thakur and Kutty, 2019). Estrogenic lipid-conjugated (bioactive; 47.03%) NPs in conjunction with cisplatin were found to decrease breast cancer growth by 87% in xenograft mice (MDA-MB-231 cells) (Andey et al., 2015). EndoTAG-1 and MM-398, which contain PTX and irinotecan, have already been tested in patients with TNBC (Awada et al., 2014).

A cancer therapy miraculous ball: micelles

Micelles are colloidal carriers (5-100 nm) having a hydrophobic interior made up of Van-der Waals interactions and a hydrophilic coating to stabilize them (Sharma et al., 2013; Mir, 2021). Micelle can transport either hydrophobic or water-soluble medicines for cancer treatment due to its amphiphilic properties. Taurin and coworkers (Taurin et al., 2013) created a micellar system utilizing styrene-co-maleic acid (SMA) to administer a hydrophobic curcumin compound, RL71, for TNBC therapy, and found that endocytosis induced increased cellular uptake, as well as a gradual release pattern, resulted in enhanced toxicity to cancerous cells. Even though the aforementioned technique improved drug uptake, it lacked specificity, which remains a significant problem in the therapy of patients with aggressive TNBC. Kutty and Feng and coworkers (Kutty and Feng, 2013) designed cetuximab-conjugated micelles of vitamin E D-alpha-tocopheryl PEG succinate for the tailored delivery of DTX drug, based on the idea of particular ligand-receptor interplay as well as the fact that cetuximab (human chimeric mAB) targets the highly expressed EGFR in TNBCs. In vitro tests with distinct formulations of micelles in a strong EGFR-expressing TNBC cell line (MDA MB 468) revealed an IC50 of 0.1715 g/ml for TPGS micelle containing cetuximab, compared to 1.12 and 35.26 g/ml for TPGS micelle with no cetuximab and free medication, respectively. These findings show promise in TNBC therapy and can be investigated as theranostics if more clinical trials are conducted. Muthu and colleagues (Muthu et al., 2015) created TPGS micelles coupled with transferrin ligand that facilitated co-delivery of therapeutic DXT (drug) and diagnostic nanoclusterAuNc (imaging) for concurrent identification as well as therapy in transferrin receptors expressing MDA-MB-231-Luc breast carcinoma in vitro models. The aforesaid delivery technique was used to picture real-time imaging and tumor inhibition in a mouse xenograft. Sun and colleagues (Sun et al., 2013) created poly (acrylic acid)-g-PEG copolymeric micelles containing DOX (50 wt/wt%) for the effective decrease of pulmonary metastases and 4T1 murine breast cancer growth. Nevertheless, NK012 micelle i.e., SN-38 (irinotecan) containing poly(ethylene-glycol)-poly(glutamic-acid) PEG-PGlu (Matsumura, 2011), is the sole miraculous micelle that has reached phase-II clinical studies in TNBC patients, and it has to be verified in future phases of clinical research.

The siRNA delivering nanoparticles: Dendrimers

Dendrimers are artificial macromolecules (10- 100 nm) made from branching monomers synthesized either divergently or convergently. It has a cavity-rich round structure with a hydrophobic interior and hydrophilic exterior, similar to liposomes, making it a distinctive carrier for siRNA delivery (Bawarski et al., 2008). Utilizing antisense oligo (AODNs) linked poly(amidoamine) dendrimers, Wang and coworkers (Wang et al., 2010) exhibited a decrease in tumor vascularization in a xenograft mice model with TNBC. The greater production of VEGF as AODN receptors is used in this tailored therapeutic strategy. Finlay and colleagues (Finlay et al., 2015) demonstrated the down-regulation of a potential TNBC target, TWISTI-transcriptor gene, using a siRNA coupled poly(amidoamine) dendrimer as a targeted treatment. Zhang and coworkers used a TNBC tumor mouse model to test dendrimer as a tailored detection module. As a dual approach for medication delivery and imaging, a new dendrimer G4PAMAM coupled with GdDOTA (MRI contrast) plus DL680 (NIR dye) was produced and administered in mice subcutaneously. The specific diagnostic applicability of this smaller sized (GdDOTA)42-G4PAMAM-DL680 dendrimeric drug was proven using an MRI scanning and near-infrared (NIR) fluorescence scanning in the TNBC tumor, which showed NP homing and increased fluorescent signals, respectively.

Polymeric nanoparticles

Polymeric nanoparticles (50 nm-10 m) can be categorized as misnomer nanoparticles if their size is less than 10 microns. These NPs can be made from natural or artificial polymers and have the added benefit of enclosing medicines and proteins without the need for chemical modifications. Biodegradable polymeric molecules, such as poly(lactic) and copolymers like poly(lactide-co-glycolide), are been used for nanoparticle manufacturing because of their biocompatibility and low toxicity (Elsabahy and Wooley, 2012; Mir, 2021). The methods of electrospray, nanoprecipitation, and emulsification can effectively enclose drug molecules; nevertheless, Xu and coworkers (Xu et al., 2013) established a revolutionary methodology called PRINT for the manufacture of uniformly sized polymeric NPs. PRINT, or particle replication in nonwetting templates, allows for the tailoring of characteristics for successful cancer treatment. In an in-vivo TNBC mouse model (nude mice containing MDA MB 468 TNBC cells), non-targeted loading of Pt (IV) mitaplatin medication utilizing PLGA-PEG (poly-D, L-lactic-co-glycolic-acid – block-poly-ethylene-glycol nanoparticles) exhibited a better level of tumor suppression (Johnstone et al., 2013). Passarella and coworkers (Passarella et al., 2010) discovered a new peptide (Gle-Ile-Arg-Leu-Arg-Gly) that targets the glucose-regulated protein (GRP78). This group specifically demonstrated apoptosis at the tumor location by targeting specific GIALAG-conjugated PTX enclosed polyester NPs utilizing irradiation TNBC xenograft murine model harboring GRP78 receptor. In a new clinical study, 90% of already treated advanced TNBC individuals displaying elevated protein Trop-2 responded to the IMMU-1322 medication (anti-Trop-2-SN-38 antibodies) had a 33% response rate (Goldenberg et al., 2015). Succinobucol in conjunction with P188 (poloxamer) is proving to be an effective oral therapy for breast carcinoma. Succinobucol NPs have a 13-fold higher bioavailability, which improves the suppression of VCAM-1 invasion and tumor cell motility (Cao et al., 2015). Polymeric NPs have also been found to deliver siRNA and miRNA, as well as therapeutic drugs, to diminish tumor growth and size. Antisense-miR-10b and antisense-miR-21 were co-delivered by PLGA-b-PEG polymeric NPs with a 0.15 mg/kg treatment dosage, while siRNA (multi-drug resistance protein) and DOX co-loaded NPs resulted in an overall decrease in tumor development and size (8-fold drop) (Deng et al., 2013; Devulapally et al., 2015).

In TNBC tumor models, a potential ligand called Arg-Gly-Asp (RGD) either enhances localized delivery of drugs or suppresses tumor invasion in distinct ways. For example, cyclic RGD-functionalized solid lipid NP (RGD-SLN) was found to suppress alphavbeta 3 (v-3) integrin receptors overexpression in aggressive TNBC tumors (Shan et al., 2015). In breast carcinoma cells, this is an excellent instance of targeting ligand and causing inhibition at the same time. Likewise, Zhang and colleagues (Zhang et al., 2017) created RGD-DMPLN, which are hybrid shealth polymer-lipid NPs (PLN) coupled to the peptide ligand RGD as well as co-loaded with mitomycin C (MMC) and DOX. RGD-targeted DMPLN's therapeutic efficacy was tested in an mTNBC mice model created with the MDA-MB-231-luc-D3H2LN cell lines. The DOX-MMC synergistic effect, which is additionally increased by targeting RGD-DMPLN, resulted in increased cytotoxicity in both those aforesaid models.

DNA nanostructures in treatment of cancer

DNA nanostructures make use of DNA's basic essential feature, Watson-Crick complementary base pairing of nucleic acids, to create various structures with specific sizes, shapes, and configurations, such as bipyramids, tetrahedrals, cubes, and cages (Fig. 8.3). For

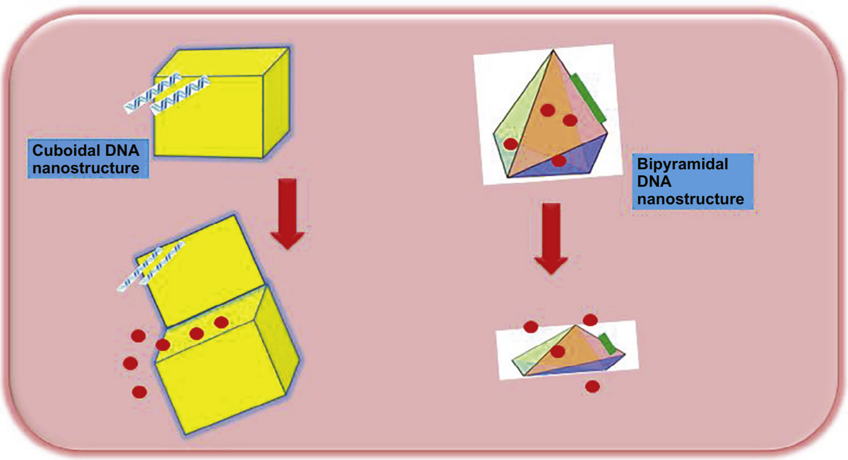

FIG. 8.3 DNA nanostructure-based drug delivery system.

location-specific adhesion and/or bio-imaging, such DNA nanostructures can incorporate ligands or tiny functional molecules. Kutty and colleagues (Setyawati et al., 2014) created a self-assembled DNA nanopyramid containing red-emissive glutathione-protected gold nanoclusters (GSH-Au NCs) near its base plus actinomycin (AMD) in the minor groove of DNA. This therapeutic and diagnostic DPAu/AMD has been created for both detecting and killing of E.coli, but it should be evaluated and modified for additional diseases/cancers. One of the primary hurdles in using these structures is avoiding endosome breakdown of DNA nanostructures in human TNBC. The same research, though, produced new microstructures, DNA tetrahedral (TH), enabling bio-sensing and antibody-mediated targeted delivery of drugs. Because cetuximab is reported to target EGFR overexpressed cancerous cells, cetuximab coupled TH (THC3) plus intercalated DOX medication i.e. THDC3 exhibited favored death of MDA-MB-468 cancerous cells. The lower IC50 value (0.91 μM) of THDC3, in contrast to free DOX, 3.06 μM, indicates that THDC3 has a strong and selective killing efficacy (Setyawati et al., 2016). Due to improved absorption of Cy3-THC3 into MDA-MB-68 cells, additional improved formulation, Cy3-THC3, including one Cy3 probe plus three cetuximab, demonstrates strong signaling intensity. These two minor changes of TH (THDC3 and Cy3-THC3) exhibit improved cancerous cell killing and targeting, making them a good choice for cancer nanomedicine, particularly for TNBC.

Metal nanoparticles

In treating cancer, metallic NPs like silver (Ag), gold (Au), platinum (Pt), titanium dioxide (TiO2), and zinc (ZnO) , and others are utilized. Because of their electrical, magnetic, thermal, and optical capabilities, these NPs may have a broad range of applications in diagnostic and therapeutic assays. Surface alteration of metallic Nps by combining various groups increases their value for treatment outcomes. Various metallic NPs use a variety of chemical mechanisms, including the generation of intracellular ROS, increased oxidative stress, and tumor cell killing (Su et al., 2014; Mir, 2021). Hyperthermia (non-invasive technique) is induced by NPs of a transition metals family, which heats the cells and kills tumor cells by turning electromagnetic energy to heat. Due to their distinct physiochemical features, only a few metallic NPs possess intrinsic anti-cancerous action. The most widely examined and potential metallic NP is known to transport PTX, a well-known anti-cancer medication is gold nanoparticles (AuNPs).

Gold nanoparticles (AuNPs) are the most thoroughly researched and promising metal nanoparticles (NPs) for delivering paclitaxel, a well-known anti-cancer medication. Au nanoparticles (AuNPs) developed and manufactured in various configurations and forms like Au-nanorods (AuNR), Au-nanoshells (AuNS), and Au-nanocages (AuNC) are developing as a diverse nanovehicle for cancer treatment. In a mouse model of breast carcinoma, PEG-coated Au NP, in combination with ionizing radiations, resulted in better survival rates (Kong et al., 2008). Serum-coated AuNR has acquired the capacity to suppress the expression of energy-related genes. Cancerous cells invasion and movement are suppressed in vitro and in vivo due to lower energy. Andey and coworkers (Andey et al., 2015) used a combination of cisplatin-loaded AuNR with NIR laser to inhibit/suppress TNBC tumor growth and migration. On cancerous cells, Ag NPs have antiangiogenic, proapoptotic, and antiproliferative properties. AuNPs, as a radiosensitizing agent, interacts with the acidic medium in cancerous cells, increasing oxidative stress through the generation of ROS, which finally induces destruction and apoptosis. On gliomas, Liu and coworkers (Liu et al., 2013) found good results with AgNPs therapy followed by radiation. These nanoparticles were also found to suppress VEGF on cancerous cells, hence reducing metastasis. For cancer therapy, zinc oxide nanoparticles work similarly to genotoxic medicines. ZnO NPs generate micronuclei within tumor cells, increasing cell apoptosis during mitosis and interphase (Wahab et al., 2014). Because asparaginase is a very well anticancer enzyme that is also utilized as a chemotherapy drug in other cancer therapies, ZnO NPs containing asparaginase improve the stability and selectivity of PTX and daunorubicin when administered together (Baskar et al., 2015). In breast carcinoma cells, ZnO NPs in conjunction with the medicines PTX and cisplatin minimize toxicity while increasing efficacy (Hackenberg et al., 2012).

Copper (CuO NP), iron-oxide (Fe2O3), cerium oxide, silica, and titanium oxide are among the metallic Nps being investigated and employed in breast carcinoma detection and therapy. Because they were manufactured using Acalypha indica and Ficus religioss, copper oxide NPs (CuO NPs) are referred to as green NPs. CuO NPs are used to cure aggressive lung cancers in mice (B16-F10 cells) via ROS production and apoptosis (Wang et al., 2013). In a subcutaneous breast tumor model BT474, double modal treatment using photothermal and radiation using Cu-64 labeled copper sulphide NP (CuS NP) suppressed cancer growth and extended the longevity of mice carrying orthotopic 4T1 breast cancers (Pawar and Prabhu, 2019).

A foldable graphene for cancer treatment: Carbon nanotubes (CNTs)

CNTs are single and/or many-walled cylindrical nanostructures made from benzene ring knitted flattened sheets. Multiple functionalities are conferred by a minor chemical alteration, with enormous potential in cancer treatment (Fig. 8.4). Single-walled NTs with a diameter of 1 nm-2 nm that can penetrate cells have a lengthy dispersion and localized action. By decreasing macrophages and vascular densities in the tumor, oxidized many-walled carbon nanotubes (o-MWNTs) provide a potential method in cancer treatment (Yang et al., 2012; Sharma et al., 2013). Burke and coworkers (Burke et al., 2012) propose that NT enhances cellular

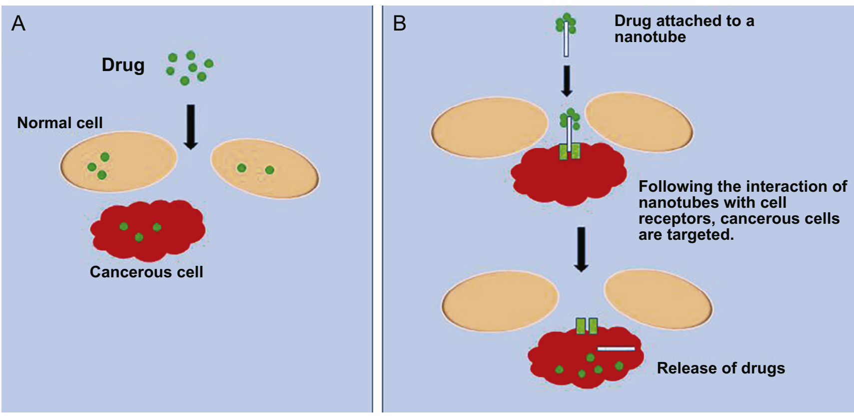

FIG. 8.4 A comparison of untargeted and targeted drug delivery with carbon nanoparticles.

membranes permeabilization, which leads to tumor mass destruction, through hyperthermia. As a result, he offered photothermal-induced excision for TNBC treatment employing many-walled NT. A nanodiamond and DOX combination has been shown to suppress cancer in mice by overcome drug outflow and enhancing apoptosis (Chow et al., 2011) and breast tumor lung metastases (Liang et al., 2016).

Ligands for targeted TNBC treatment

Ligands are short stretches of peptides, nucleotides, or tiny compounds that interact with their receptors. Antibodies, aptamers, peptides, and other tiny molecules such as quantum and carbon dots are common ligands utilized in cancer nanomedicine for directed or probe-based diagnostics.

Nucleic acid-based ligands: Aptamers

Aptamers comprise single-stranded RNA/DNA oligonucleotides with small lengths. The aptamers precisely binds its targeted molecule with great strength and affinity because of its distinctive 3D conformation. Its only drawback is that nucleases degrade it; yet, its exceptional stability has attracted interest in the creation of molecular probes. In a preliminary investigation, Li and coworkers (Li et al., 2014) used the cell-SELEX approach to precisely target surface membranes proteins on TNBC tumors utilizing a recently found LXL-1 aptamer. Huang and coworkers (Huang et al., 2009) used a PDGF-aptamer coupled to Au NPs to identify variable overexpression of PDGF receptors in TNBC cells. Mammaglobin A2 and B1 are reported to be overexpressed in MDA-MB-415 and MCF7 breast carcinoma cells. Hassann and coworkers (Hassan et al., 2019) used very sensitive terahertz (THz) chemical microscopy (TCM) with THz radiations to identify aggressive breast carcinoma utilizing AMB1 and MAMA2 aptamers. In certain breast carcinoma cells, an additional 26-mer G-rich DNA aptamer exclusively targets the nucleolin receptors (Tang et al., 2012). Nevertheless, for TNBC theranostic use, such aptamer-based accurate tailored diagnostic still needs to be enhanced and integrated with drug administration.

Dual-functioning Y-shaped key: Antibodies

Antibodies are Y-shaped proteins containing two epitopes that have great receptor specificity and affinity. They are considered to be the most effective in targeting ligands. Antibodies' value in the diagnosis of cancer outweighs their expensive manufacturing costs. Shi and coworkers (Shi et al., 2015; Mir et al., 2020) proposed and affirmed the use of the anti-TF antibody labeled with copper-64 (anti-TF-antibody-64Cu) utilizing PET scanning in an in-vitro TNBC model to conceptualize the variable up-regulated expression of tissue factor (TF) receptors as well as urokinase plasminogen activator receptor (uPAR) in TNBC. Optical and SPECT scanning were used by Le Beau and coworkers (LeBeau et al., 2014) to identify NIR fluorophore as well as Indium-111 (111In) labeled uPAR antibodies. Anti-VEGFR and anti-EGFR antibodies coupled with fluorescent NP or ultrasound contrasting agents are also identified by fluorescence imaging and ultrasound, respectively.

Peptides

Peptides constitute low-molecular-weight ligands that have a high degree of selectivity when it comes to targeting intracellular entities (Reubi and Maecke, 2008). These target interacting peptide sequences can be fused to bacterial envelope proteins and expressed via genetic engineering, after which they are evaluated utilizing the phage display library method (Yu et al., 2012). P-selection, RGD, tumor metastases targeting (TMT), and chlorotoxin are several peptides that have been used to target metastatic breast carcinoma. NIR fluorescence scanning of CK3 peptides (Cys-Leu-Lys-Ala-asp-Lys-Ala-Lys-Cys) attachment to NRP-1 trans-membrane proteins (neuropilin-1) in TNBC murine models was discovered by Feng and coworkers (Feng et al., 2014). When covalently coupled to cyclic-RGD peptide, Activable cell-penetrating peptides (ACPPs) that target the matrix metalloproteinases (MMP)-2 led to improved tumor uptake as well as contrasted imaging in in-vivo models of TNBC (Crisp et al., 2014). The targeting of $\alpha v\beta 3$ integrin receptors with modified Fe2O3 NPs connected to cyclic RGD peptides was better and effective (Peiris et al., 2012). Furthermore, the dual ligand-linked liposomal NPs (P-selectin and RGD-peptide) can target various tumor locations by over-expressing their specific receptors on breast carcinoma cells (Doolittle et al., 2015).

VLPS (virus-like particles) as unique nanocarriers

Virus-like particles are multi-subunit self-assembled nanostructures (0.1-100 nm) created in heterologous settings by the activation of viral structural genes (Mir MA et al., 2021). VLPs are called virus-like since they are devoid of viral genomic material, making them a diverse nanovehicle for the delivery of drugs. VLP could be derived from plant, microbial, or mammalian viruses and may be filamentous or spherical (Zeltins, 2013). By expressing needed heterologous proteins/peptides/gene sequences on the membrane (capsomers), altered VLPs containing foreign ligands are created. Target mediated treatment is also aided by chemical modifications of the active groups found in the structural capsid proteins. The most notable feature of VLPs is their tiny size, which allows them to travel through the bloodstream, as well as active viral proteins on the cell membrane, which aid cell entrance and penetration. VLP's capacity to encapsulate tiny molecules/drugs could be used to fight cancer by targeting and penetrating tumor cells via energy utilizing receptor-mediated endocytosis, then releasing the encapsulated medication within the cancerous cells. The most remarkable ability is to escape endosomes without lysosomal breakdown; this increases drug accessibility and protects the drugs in the plasma. The major drawback to using VLP as a medication delivery strategy is that it generates an innate immunological response owing to viral proteinaceous particles that are easily uptaken by dendritic cells (Grasso and Santi, 2010), but it provided an optimistic outlook for TNBC therapy when conventional chemotherapy failed. Increased medication absorption and biocompatibility can also mitigate the aforementioned drawbacks. Ebola, Influenza, Human papillomavirus (HPV), Polyovirus, Bacteriophage, Hepatitis E virus (HEV) (Guu et al., 2009), and Tobacco mosaic virus all produce VLPs. Some VLPs have a natural affinity for specific organs or tissues, such as HEV VLPs for hepatocytes, while the bulk of VLPs have an affinity for sialic acids and heparin sulphates, which limits their usage as a tailored nanocarrier. Self-assembled Bacteriophage

MS2 VLP, which is altered using SP94 peptides and enclosed with DOX/cisplatin/and 5-fluoro-uracil to specifically transport and destroy human hepatocellular carcinoma (HCC) in the Hep3B cell line (Ashley et al., 2011), is a typical instance of VLPs as targeted therapy carriers. The adaptability, cell-specific targeting, and rapid cell entrance, as well as the absence of endosomal sequestration, biocompatibility, multivalency, massive encapsulation, and secure delivery mechanism, have all contributed to VLPs' popularity.

Role of nanomedicine in breast cancer treatment: A transition from traditional to nanomedicine

Unfortunately, traditional chemotherapeutic drugs have a number of drawbacks like non-specific targets with systemic harmful effects and negative clinical consequences, harmful to quickly dividing healthy cells with persistent toxicity, and highly common manifestations such as alopecia, thrombocytopenia, and mucositis. The anticancer agent's efficacy is further limited by its weak solubility and poor bioavailability, as well as resistance to drugs due to a putative mechanism that involves higher expression of P-glycoprotein or mutant topoisomerase II. Physical obstacles, intercellular connections governing drug permeability, and extracellular matrix components all contribute to low drug penetration in tumor/cancer cells, limiting therapeutic results (Sharma et al., 2018; Mir et al., 2021). Faster drug elimination and restricted targeting are now a challenge in cancer treatment, necessitating the use of nanomedicines. Breast tumor usually spreads to the bones, lymph nodes, and lungs in the local area; nevertheless, malignant breast carcinoma has traveled to distant locations. Treatment of invasive breast cancer is complicated by vigorous proliferation, complexity, and tumor resistance to treatments. Adjuvant treatment, such as chemotherapeutics (eribulin, paclitaxel) and endocrine therapy (tamoxifen, letrozole), has a variety of long-term adverse effects that might negatively impact a patient's quality of life (Twelves et al., 2016). There is no targeted treatment for triple-negative, aggressive, or recurring breast carcinoma in clinical trials at this time. TNBC also lacks PR, ER, and HER2 and is challenging to treat, making it more prone to return and spread. Its therapy is difficult due to its low overall life and increased risk of metastases. Chemotherapy is the sole therapeutic option for TNBC, including taxane and anthracyclin-based chemotherapy as well as neoadjunctive chemotherapies (Burstein et al., 2015; Gradishar et al., 2015). Although thorough and rigorous treatment, 50% recurrence and 37% death require advanced, new, and successful treatment (Denkert et al., 2017). As a result, multipurpose smart nanoparticles complexed with a targeting, therapeutic, or fluorophore can pass distinct biological barriers, penetrating and targeting cancerous cells via a passive process known as enhanced permeability and retention (EPR), and finally releasing drugs in cancerous cells in a regulated manner.

Active targeting includes the functionalization or decorating of nanocarriers with ligands particular for cancerous cells, allowing for targeted delivery of drugs inside the cancer cell, resulting in improved drug consumption and less unwanted adverse effects at non-target areas (Pérez-Herrero and Fernández-Medarde, 2015). Nanocarriers could be surface functionalized using a range of higher affinity ligands enabling selective absorption by cancer cells displaying the specific receptors for such ligands in the event of active targeting. This reduces chemotherapy medication off-target toxicity and improves effectiveness by increasing

TABLE 8.2 The list of potential TNBC therapy receptors.

S. No	Target receptor	Ligands
1	Urokinase plasminogen activator receptor	Urokinase-type plasminogen activator
2	Folate receptor	Folic acid
3	CD44 receptor	Hyaluronic acid
4	CXCR4 receptor	CXCL12
5	Transferrin receptor	Transferrin
6	EGFR	Heparin-binding EGF-like growth factor, TGF-α, and betacellulin

absorption and retention in tumor tissues. The biotin receptors, folate receptors, CD44 receptor, transferrin receptor, integrin receptor, and others have all been investigated for active targeted therapy. The numerous receptors that can be used to develop targeted therapeutics for TNBC are depicted in Table 8.2.

Nanoparticles relying on the EPR effect for passive targeting of TNBC cells

Palma and colleagues created DOX-loaded PEG-poly(epsilon-caprolactone) nanoparticles that used the EPR mechanism to target cancerous tissues. The nanocarriers allowed for up to 30 days of medication release. The nanoparticles were combined with hydroxypropyl-β-cyclodextrin to improve the formulation's dispersibility and make it easier to administer intravenously. In comparison to the usual nanoprecipitation process, a melt sonication method was used, which led to greater PEG penetration on the nanocarrier surfaces. In an experimental model of TNBC, the nanocarriers revealed remarkable antiproliferative action on MDA-MB-231 cells, as well as comparable efficiency and enhanced survival to Taxotere® (Palma et al., 2014; Mir, 2021). For PTX, Zhang and coworkers used micelle-forming dendritic polymer-drug compounds. Micelle-forming polymer-drug compounds have high plasma stability because their tiny size enhances EPR-mediated penetration into tumor tissues (Fig. 8.5).

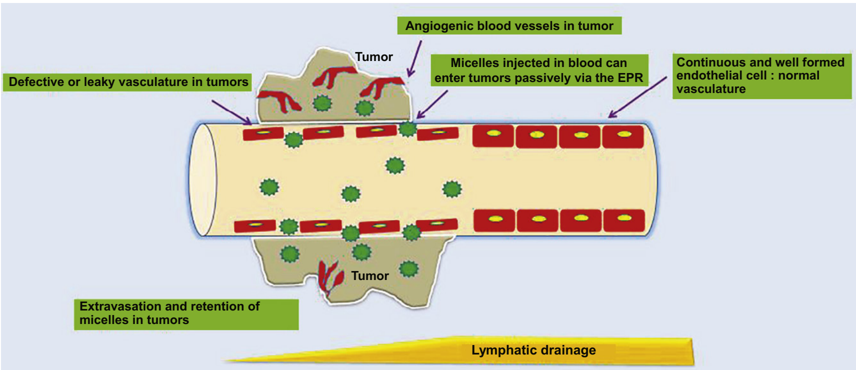

FIG. 8.5 **Enhanced permeability and retention (EPR) and passive targeting: nanoparticles can extravasate into tumors via spaces between endothelial cells and concentrate their due to inadequate lymphatic drainage.**

Large molecular weight straight polymers are used in traditional polymeric drug conjugates, which have a low drug loading capability. Grafted polymeric drug conjugates, on the other hand, can interact with numerous drug compounds via their side chain, although the steric hindrance caused by side chains may make the loading of drugs difficult. The advantages of straight dendritic polymeric-drug conjugates include increased drug loading and high stability profiles. The researchers used 4-nitrophenyl chloroformate as a linker to transform PEG to PEG with eight hydroxy groups that were then connected to PTX. Micelles could form if the conjugate is self-assembled. The micelles are altered with the iNGR peptide that aids in the targeting of TNBC cells through the NRP-1 receptors. Compared to Taxol® and non-targeted micelles, targeted ones showed superior tumor uptake (Zhang et al., 2017).

TNBC treatment using folate receptor-targeted nanoparticles

The notion that folate receptors are only expressed on the apical surface of epithelium cells in healthy tissue is an essential advantage of using them for tailored anticancer medication delivery (Miller-Kleinhenz et al., 2015). Paulmurugan and coworkers created orlistat micelles for targeted administration to TNBC cells via the folate receptors. The active monomers 2-ethylhexyl acrylate and 2-hydroxyethylacrylate were used to create olate targeting diblock polymeric micelles. Orlistat has been shown to inhibit fatty acid synthase's lipogenic action (antigen elevated in breast carcinomas). Nevertheless, the medication has a bioavailability of less than 1%. In SkBR3 and MDA-MB-231 cells, cytotoxicity experiments demonstrated that targeted micelles had a greater impact than the free drug. The targeted micelles induced apoptosis in MDA-MB-231 cell lines at nanomolar levels by activating caspase 3 and inhibiting PARP. When tested in mice with MDA-MB-231 cancer xenografts, the designed micelles showed a significant decrease in tumor size compared to free drug or non-targeted micelles (Paulmurugan et al., 2016).

TNBC therapy using CD44 receptor-targeted nanoparticles

Cerqueira and coworkers developed PTX-loaded PLGA nanoparticles covered with hyaluronic acid (HA) for TNBC intravenous treatment. When compared to uncoated NPs, the coated NPs exhibited better cellular internalization in MDA-MB-231 cells. When compared to uncoated NPs, the nanoparticles had a lower IC50 in case of MDA-MB-231 cells. TNBC overexpresses CD44 receptors, therefore HA is a ligand that binds to them. Coatings with HA aid selective absorption by cancerous cells while also extending circulation duration by avoiding RES-mediated clearance. Because HA can generate a porous layer on the nanocarriers, leading to the hydrolytic breakdown of PLGA, HA-covered NPs released drugs quicker than uncoated NPs. The nanoparticles had a twofold releasing profile, with a burst release in 24 hours and a regulated release lasting up to 5 days. The nanoparticles additionally exhibited non-hemolytic properties, indicating that they may be injected intravenously (Cerqueira et al., 2017). Agrawal and coworkers used a high-pressure homogenized process to create lapatinib nanostructures covered with HA. In mice, intravenous administration resulted in a significant tumor accumulation. In vivo testing in a 4T1 cell lines generated breast cancer model demonstrated that the medication had higher anti-tumor activity than the free drug (Agrawal et al., 2018).

Nanoparticles that target the receptor for advanced glycation end products (RAGE) for the treatment of TNBC

RAGE upregulation had been demonstrated to be associated with tumor proliferation. RAGE overexpression is seen in TNBC cells. Siddhartha and coworkers produced di-allyl-disulfide (DADS) SLNs and coupled them to RAGE antibodies through an interaction involving the RAGE antibody's amine group and the palmitic acid's carboxyl group. The MDA-MB-231 cells demonstrated high absorption of the SLNs with continuous release of the drug. Because of the outflow of the drug through P-gp, plain DADS ladened SLNs had lesser cytotoxic potential as compared to RAGE directed DADS SLNs. Because RAGE-targeted SLNs were absorbed by receptor-mediated endocytosis, they are capable to avoid P-gp caused drug outflow. The Bcl2 protein family (Bcl-xL, Bcl-2, and Mcl-1), as well as survivin, are anti-apoptotic molecules that cause chemoresistance. Caspase-3, Caspase-9, and Bax are three proteins that are pro-apoptotic. Caspase-9 was upregulated in RAGE-targeted SLNs; while survivin and Bcl-2 were both downregulated (Siddhartha et al., 2018).

TNBC therapy using EGFR-targeted nanoparticles

For targeted administration of docetaxel, Kutty and Feng produced cetuximab-altered vitamin E TPGS micelles. Cetuximab, the targeting component, is the first human chimeric mAB to attach specifically to the EGFR's extracellular domain. Micelles size (10–20 nm) was shown to be responsive to enhancing transport to the solid tumor location via the EPR effect (recommended size 10–100 nm). Cellular uptake experiments demonstrated that the micelles were successfully internalized into the cancerous cells, following by cytoplasmic uptake. Furthermore, against MDA-MB-231 and MDA-MB-468 cells, the cetuximab altered micelles demonstrated >200-fold greater cytotoxicity as Taxotere ® (Kutty and Feng, 2013). Nearly 70% of TNBC patients had overexpressed EGFR. Ghosh and coworkers created PLGA NPs loaded with nifetepimine for EGFR-mediated targeting of TNBC cells (Mir et al., 2020). In MDA-MB-468 cell lines, the nanoparticles caused apoptosis. The NPs (30–100 nm) accumulated specifically in breast cancers in mice, resulting in a significant reduction in tumor volume and improved survival. The nanocapsules significantly increased nifetepimine bioavailability, with a 20-fold rise in Cmax and a 12-fold rise in AUC0- ∞ (Ghosh et al., 2016).

The EGFR gene has a key role in tumor growth, infiltration, and metastases (Mir et al., 2020). To target TNBC cells, Jung and coworkers created phospholipid NPs laden with curcumin and coupled with EGF. They produced nanoparticles by combining EGF peptide with N-hydroxysuccinimide-PolyethyleneGlycol-1, 2-Distearoyl-sn-Glycero-3-Phosphoethanolamine (NHS-PEG_{10000}-DSPE) and then hydrating the lipid layer to load curcumin. In MDA-MB-468 cells, the NPs inhibited colony formation and outperformed ordinary curcumin or non-targeted curcumin NPs in terms of cytotoxicity. In mice, the nanoparticles also inhibited tumor development (Jung et al., 2018).

The therapeutic efficacy of aminoflavone (a flavonoid with antitumor action) is threatened by dosage-limiting lung toxicity (Bhat et al., 2021). Brinkman and coworkers created unimolecular micelles containing an aminoflavone linked to a 12-amino-acid peptide called GE11 that targets EGFR. The medication was only delivered at endosomal pH by the targeted micelles, which prevented the release of the drug at basic ph. MDA-MB-468 cells were able to absorb the micelles. A xenograft model of TNBC revealed that the designed micelles inhibited cancer growth better than unbound aminoflavone and non-targeted micelles. Targeted micelles additionally revealed 72-fold and 10-fold greater tumor aminoflavone levels compared to free aminoflavone and non-targeted micelles, correspondingly, owing to improved absorption through the EPR impact and directed absorption into TNBC cells by receptor-mediated endocytosis (Brinkman et al., 2016).

Nanoparticles targeting the transferrin receptors for TNBC therapy

TNBC cells have high levels of transferrin receptor-1. The overexpression of the transferrin receptor-1 gene in both primary and mTNBC cells is due to increased iron absorption required for tumorigenesis. The H-chain of human ferritin binds transferrin receptor-1 in a unique manner. Mazzucchelli and coworkers created a nanoformulated olaparib built on human ferritin H-chain for transferrin receptor-1-targeted TNBC treatment. The nanoformulation showed superior nuclear uptake within tumor cells and a 1000-fold stronger lethal impact than ordinary olaparib (Mazzucchelli et al., 2017).

TNBC therapy using macrophage-targeted nanoparticles

Cancer-associated macrophages are innate immune effectors that are drawn to tumor sites and play a role in tumor development and spread by activating angiogenesis, generating stromal disintegration factors, while inhibiting adaptive immunity. Mannose receptors are overexpressed in these tumor-associated macrophages that can be used to target them. Niu and coworkers created macrophage-targeted nanoparticles for DOX delivery and showed that the method was effective in mice having orthotopic M-Wnt triple-negative breast tumors. To administer DOX, they employed PLGA NPs that were PEGylated using acid-responsive sheddable PEG then surface altered using mannose. When the PEG was stripped inside the acidic tumor milieu, DOX was administered to tumor-associated macrophages (Niu et al., 2016; Mehraj et al., 2021).

TNBC therapy with high-density lipoprotein (HDL) receptor-targeted nanoparticles

The HDL (scavenger receptor class B type 1 [SR-B1]) receptor is reported to be expressed in TNBC cells. The use of reconstructed HDL NPs has a number of advantages, including their tiny size, biocompatibility, increased circulation duration, and capacity to be preferentially absorbed by tumor cells through the SR-B1 receptor. Johnson and coworkers created lapatinib or valrubicin-loaded reconstructed high-density lipoprotein (rHDL) NPs to treat TNBC. MDA-MB-231 cells have been more effectively treated with lapatinib/valrubicin-laden rHDL NPs than with free lapatinib/valrubicin, and they reportedly had a cardioprotective benefit (Johnstone et al., 2013).

TNBC therapy using extracellular matrix-targeted nanoparticles

Lapatinib is a tyrosine kinase inhibitor that has been shown to reduce tumor growth and migration. Nevertheless, because of its limited aqueous solubility and variable oral absorption, it is not suitable for therapeutic use. Furthermore, the high oral dose (Tykerb) generates a variety of adverse effects, including rashes, nausea, and diarrhea. Wan and coworkers created lapatinib with human serum albumin NPs. It has been discovered that 60 kDa glycoprotein receptors on vascular endothelium binds human serum albumin nanovehicles and transports them to tumor cells. In comparison to lapatinib solutions, the nanoparticles induced more rapid apoptosis on 4T1 monolayer cells and had better penetration and inhibition impact in tumor spheroids. In comparison to Tykerb, intravenous administration of NPs led to a 16-times increase in tumor accumulation. At one-tenth the dosage of Tykerb, the NPs inhibited lung metastases. Intimate attachment of human serum albumin NPs to secreted proteins acidic and high in cysteine seen in the ECM of tumor tissues would also have added to nanoparticles effectiveness (Wan et al., 2015; Mir, 2021).

Gene delivery

For cancer treatment, gene delivery is useful for suppressing oncogenes and promoting tumor-suppressing genes (van Elk et al., 2016). The use of small interfering RNA (siRNA) treatment to decrease the expression of specific proteins is incredibly beneficial. Nevertheless, rapid breakdown, limited intracellular absorption, and insufficient endosomal escape pose a challenge to its therapeutic applicability (Yang et al., 2015). Nanocarriers are particularly beneficial for protecting labile genetic material from destruction and allowing it to be taken into cells (Pérez-Herrero and Fernández-Medarde, 2015). For gene delivery, nanoparticles made from a variety of materials like lipids, polymers, and silica have been created. For gene transfection, carbon nanotubes, SLNs, liposomes, dendrimers, and other materials have been employed. Nanoparticles are preferred over viral vectors for gene transfection because of their excellent safety profile (Choi et al., 2014). miR-34a is a potent endogenous tumor suppressor in breast carcinoma. Wang and coworkers created interpolyelectrolyte nanostructures with HA plus protamine sulphate utilizing an electrostatic interaction-based self-assembly approach for miR-34a delivery. In vitro release tests found that miR-34a was released quicker at pH 5.5 than that at pH 7.4, which could be due to the polymers breaking down more quickly at this pH. In MDA-MB-231 cells, miR-34a laden nanoparticles increased miR-34a expression by 20,000–30,000 times over simple nanocomplexes. In xenograft models of breast carcinoma, the nanocomplexes were similarly found to limit tumor development in vivo (Wang et al., 2015).

TNBC is distinguished by elevated expression of CXCR4, a protein that promotes tumor proliferation and chemotaxis. Misra and coworkers used acrylate-functionalized PLGA–acrylate NPs with Plerixafor immobilized as targeting ligands to generate CXCR4 directed endosome detecting nanoparticles. As an endosome-sensitive element, polyethyleneimine (PEI) was incorporated. PEI expands in the acidic endosome environment, breaking the endosome through osmotic expansion and transferring the payloads to the cytoplasm. The tailored nanocarriers bind selectively to CXCR4+ cells and also inhibit CXCR4 signaling, preventing tumor growth and metastases. GFP knockdown was seen when siRNA against the green fluorescent protein (GFP) was incorporated into nanoparticles and incubated with CXCR4-GFP expressing breast carcinoma cells (Misra et al., 2015).

Okamoto and coworkers created siRNA-loaded lipid nanovehicles coupled with Fab antibody against heparin-binding EGF-like growth factors. Because polo-like kinase 1 knockdown is found to trigger apoptosis, siRNA targeting polo-like kinase 1 was used. In MDA-MB-231 tumor-bearing mice, the nanocarriers (160 nm) displayed effective gene transport and suppressed polo-like kinase 1 expression, leading in tumor progression decrease (Okamoto et al., 2018).

Eukaryotic elongation factor 2 kinase has been identified as one of the important pathways for breast tumor development and proliferation. Shahbazi and coworkers created polyethylene-modified Au NPs that were coupled with siRNA (about 60 nm in size) to target eEF2K. When nanoparticles were administered intravenously once a week for 4 weeks in an MDA-MB-231 murine model of TNBC, tumor development was reduced by 90% (Shahbazi et al., 2017).

CPPs (Cell-penetrating peptides) are tiny (near to 30 amino acids) and positive charged peptides. They can move payloads including medicines, proteins, nanocarriers, and genetic materials through biological membranes without the need for receptors. Jing and coworkers created CPP-loaded nanobubbles for TNBC cell delivery of EGFR-targeted siRNA (siEGFR). EGFR mRNA and proteins were found to be downregulated in xenografts produced from TNBC cells in an in vivo investigation (H. Jing et al., 2016), leading in tumor development suppression.

Alshaer et al. created liposomes with a core comprising a siRNA: protamine complex, which were then functionalized by an anti-CD44 aptamer to target CD44 cells. Targeted liposomes had better absorption in MDA-MB-231 cells compared to non-targeted liposomes, and they effectively silenced the luc2 target genes in vivo in a mouse TNBC model (Alshaer et al., 2018).

Photothermal therapy

Photothermal therapy (PTT) is a type of therapy that uses biocompatible vehicles to absorb light energy by converting it to heat that kills tumor cells (Chen and Cai, 2015). Natural molecules found in bodily tissues, including melanin and heamoglobin, can convert photoenergy into thermal energy, leading to harm to health. Due to this, PTT uses near-infrared (NIR) light (700–900 nm wavelength) since bodily constituents absorb very little light in this region. The selection of photothermal carriers necessitates the use of substances with strong NIR photostability, photothermal efficacy, and suitable safety profiles (Wang and Qiu, 2016). Au nanocarriers, polypyrrole, Cu sulphide, carbon nanoparticle, palladium nanosheets, and other probes are often employed for PTT (Li et al., 2017).

Ayala-Orozco and coworkers created 100 nm nano matryoshkas with concentric Au-silica-Au nanocarriers for TNBC PTT. Nanomatryoshkas had a silica-layered Au centre that was then encased in a thin gold coating. Because the nano matryoshkas were shorter (100 nm) compared to silica Au nanoshells (150 nm), they were able to penetrate deeper into tumors, resulting in increased tumor accumulation (4–5 times) in mice. Furthermore, due to larger tumor growth and a greater absorption cross-section, the nano matryoshkas demonstrated improved heat generation in tumors. When contrasted to traditional silica core Au nanoshells in the therapy of big aggressive TNBC tumors, such nano matryoshkas demonstrated a two-fold improvement in survival rates (Ayala-Orozco et al., 2014).

In TNBC cells, the intercellular adhesion molecule-1 (ICAM-1) is overexpressed. Lipocalin 2 (Lcn2) promotes breast tumor proliferation by stimulating angiogenesis and promoting EMT in breast tumor cells. Guo and coworkers created ICAM-1 antibody functionalized liposomes coupled with Lcn2 siRNA to suppress angiogenesis in TNBC specifically. A pH-responsive lipid component, 1,2-dioleoyl-3-dimethylammonium-propane, was incorporated in the lipid nanoparticles to facilitate siRNA escape from endosomes and improve siRNA transfected efficacy. MDA-MB-231 cells were selectively bound by the directed liposomes compared to non-neoplastic cells, and expression of Lcn2 in MDA-MB-231 cells was also reduced. MDA-MB-231 cells administered with the specific formulations also had lower VEGF secretion (Guo et al., 2016).

Photodynamic therapy

Photodynamic therapy (PDT) is the use of light-sensitive compounds known as photosensitizers that when activated by light of a specific wavelength, undergo excitement and create deadly reactive oxygen species (ROS). PDT has several advantages over chemotherapy, including the capacity of photosensitizers to become cytotoxic only after triggered in the tumor location utilizing light, decreasing systemic damage, and lowering the chance of resistance (Jadia et al., 2018). PDT's clinical applicability is hampered by its low tumor selectivity, unfavorable pharmacokinetic properties, and skin photosensitivity to weakly water-soluble photosensitizers (Choi et al., 2015).

The photosensitizer indocyanine green (ICG) has a limited circulatory half-life and low in vivo photostability. Shemesh and coworkers created thermo-sensitive liposomes for encapsulating ICG that contained 1,2-dipalmitoyl-sn-glycero-3-phosphocholine, soy-PC (L-α-phosphatidylcholine), DSPE-PEG 2000 (N-(carbonyl-methoxypolyethyleneglycol 2000)-1,2-distearoyl-sn-glycero-3-phosphoethanolamine), and cholesterol having temp approximately 42°C. Such thermo-responsive liposomes are stable at normal temperatures and have increased lipid layer permeability at temperatures higher than the transition temperatures. In MDA-MB-468 cells, the newly designed method suppressed tumor cell proliferation (Shemesh et al., 2014).

The use of an antibody-photosensitizer combination coupled with exposure to Light sources to destroy tumor cells is known as near-infrared photoimmunotherapy. Nagaya and coworkers created cetuximab-IR700, an antibody-photosensitizer combination for the targeted killing of TNBC cells. The conjugates were found to have anti-tumor effectiveness against both MDA-MB-468 (elevated EGFR expression) as well as MDA-MB-231 (medium EGFR expression) cells, as demonstrated by lowered tumor development as well as prolonged survival in a tumor-bearing mouse model with elevated tumor accumulation and improved treatment effectiveness against MDA-MB-468 cells (Mir, Nagaya et al., 2015).

Nanosoldiers to suppress TNBC metastasis

TNBC metastasis is caused by the EMT. TGF-β is a cytokine that promotes EMT by upregulating the β-3 integrin. Parvani and coworkers used ECO, a cationic lipid

(1-aminoethyl)iminobis[N-oleicylsteinyl-1-aminoethyl)propionamide] that self-assembles with siRNA against β3 to generate a nanoparticle that can then be readily functionalized using the targeted ligand RGD peptide. The targeted nanocarriers were approximately 88 nm in size. The lipid ECO used to construct the system has ph-responsive and amphiphilic properties, which aids in endo-lysosomal escape and prevent lysosomal siRNA destruction. The positive charge of the nanoparticles is increased when the amino groups in ECO are protonated at the acidic pH found in endo-lysosomes, thus enhancing endo-lysosomal membranes fusion. The disulfide bridge formed by autooxidation of the thiols in cysteine residues assists to stabilize the nanoparticles in circulation, while their cytosolic GSH-mediated decrease leads to siRNA releases into the cytosol. In mice, intravenous administration of these nanoparticles reduced the initial tumor burden while also preventing metastases. When mice with orthotopic TGF-stimulated MDA-MB-231 tumors were administered with targeted nanoparticles, there was no evidence of metastases or recurrence following primary tumor excision and up to 4 weeks following treatment release (Parvani et al., 2015; Mir and Mehraj, 2019).

One of the main causes of recurrence and metastases in TNBC is the development of vasculogenic mimicry (VM) channels by remaining TNBC cells. Surgery, radiotherapy, and the usage of cytotoxic medicines are all common treatments for TNBC, although none of these completely eliminate TNBC cells (Qayoom et al., 2021; Mir, 2021). The relapsed TNBC cells are nourished by the growth of the residual TNBC cells caused by the development of linked VM channels. Zeng and coworkers created tailored liposomes containing dasatinib plus vincristine for VM channel destruction. Dasatinib works by blocking VM channels. The upregulated integrin receptor on TNBC cells was targeted with the c(RGD y K), cyclic peptide. Vincristine was present in the inner aqueous cavity of the liposomes, whereas dasatinib was packed within a lipid bilayer. Their particle size ranged from 100 to 107 nanometers. They exhibited prolonged release of drugs, which is advantageous in preventing unwanted drug leaks in the bloodstream and delivering the greatest amount of medicine to the tumor. When comparing directed vincristine + dasatinib liposomes versus non-targeted ones, anticancer activity was assessed in a mice tumor xenografted model with MDA-MB-231 cells. The VM channels number was likewise significantly reduced by the targeted liposomes. In a tumor mice model, the directed liposomes also induced maximum apoptosis. The outstanding action of directed liposomes has been attributed to the respective factors: PEGylation leading to the extended circulation of nanoparticles due to RES evasion, nanoparticle size facilitating entrance and retention into tumor site via EPR effect, directed feature facilitating augmented cell uptake, as well as eventually enhanced apoptosis and eradication of VM channels by combination treatment (Zhang et al., 2015).

Sarkar and coworkers created Au nanomicelles (60–70 nm) using the non-ionic triblock copolymeric PEG-block-polypropylene glycol-block-PEG (PEG-PPG-PEG) for Au NPs stability and reduction. The Au nanomicelles were packed with ZD6474, a dual tyrosine kinase inhibitor that inhibits tumor growth, angiogenesis, and metastases. In addition, it has been shown to cause apoptosis in cancerous cells. Under physiological pH, the nanomicelles released 20% less medication, whereas, at pH 5.2, they released 82%. The Au nanomicelles reduced MDA-MB-231 breast cancerous cells' migration and invasion while simultaneously inducing apoptosis. The nanomicelles were found to have excellent blood compatibility as well as tumor accumulation in mice (Sarkar et al., 2017).

Nanosoldiers to combat brain metastases in TNBC

He and coworkers created docetaxel-containing amphiphilic composite polymer–lipid nanocarriers. DTX acts as a substrate for P-gp efflux across the BBB (Blood Brain Barrier), preventing it from reaching the brain in adequate amounts. DTX's limited aqueous solubility makes it difficult to administer intravenously. The solid lipid in the nanoparticle was ethyl arachidate, while the amphiphilic copolymer was constructed of polysorbate 80, maltodextrin, n-dodecane, and poly(methacrylic acid). The ability of polysorbate 80 covered nanoparticles to penetrate the BBB through LDL receptor induced transcytosis has been demonstrated. Polysorbate 80 was present in the nanoparticles at a significantly lower dosage (10 mg/ml) compared to Taxotere® (80–260 mg/ml), reducing the unwanted hypersensitive reactions found with the latter. The nanoparticles showed a lag period following by a continuous release over a 53-hour period. The lag period is ideal for preventing premature drug release before the NPs approach the tumor's vicinity. The created nanocarriers boosted DTX's circulation duration by 5.5 times, improved Cmax by 3-fold, also improved bioavailability in brain tumors by 5-fold compared to Taxotere.When compared to a similar quantity of Taxotere® in a mice tumor model, the nanoparticles had the ability to delay tumour progression by about 11-fold and prolong median survival rates by 94%. They also had no effect on the histology of mice's vital organs. DTX polymer-lipid nanocarriers outperform Taxotere® due to increased absorption across the BBB through receptor-mediated transcytosis, passively build-up in areas of leaky blood-tumor vasculature, with prolonged release of drugs from the nanoparticles matrix to the tumor (He et al., 2017).

The blood tumor barrier (BTB) is a key impediment to successfully delivering chemotherapy drugs into brain cancer cells for the treatment of TNBC metastatic CNS lesions. Mohammad and coworkers developed irinotecan liposomes (100–110 nm) for the management of TNBC brain metastases. The liposomes increased irinotecan absorption via the BTB, and build-up in brain lesions. Compared to free irinotecan, liposomes had longer average residence duration. In a preclinical TNBC model of brain metastases, the liposomes worked as an irinotecan store inside the brain tumor site, delaying tumor development and resulted in extended longevity (Mohammad et al., 2018).

Nanosoldiers to combat lung metastases in TNBC

Zhang and coworkers created RGD peptide-conjugated composite polymer–lipid nanparticles that were loaded with DOX and mitomycin C. DOX in combination with mitomycin C had remarkable synergistic efficacy against MDA-MB-231 cells. The nanoparticles were between 148 and 165 nm in size. Intravenous administration of the targeted nanoparticles resulted in a 31-fold reduction in the risk of lung metastasis as well as a 57% increase in median survival rates compared to the free drugs. In comparison to free drugs, the nanoparticles showed significantly lower hepatotoxicity and cardiotoxicity. The designed nanoparticles provided both temporal and spatial control of drug release and delivered prolonged doses of DOX and mitomycin C in a synergistic proportion (Zhang et al., 2017).

The CD44 receptor is thought to play a key part in tumor metastases. Combating metastasis, on the other hand, entails addressing both tumor cells and related neovasculature.

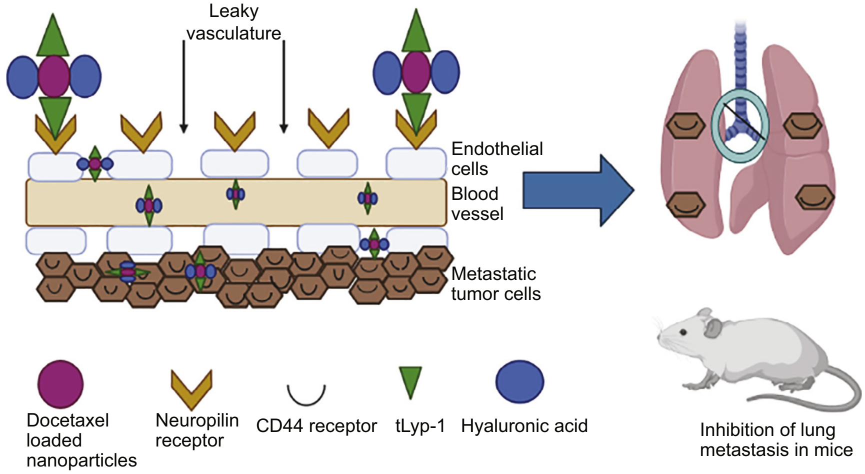

FIG. 8.6 A graphical picture of nanosoldiers coupled with tLyP-1 and hyaluronic acid demonstrating their absorption into the tumor vasculature by the EPR effect and into the tumor tissue via the neuropilin receptor and CD44 receptor.

Neuropilins are VEGF family coreceptors that regulate tumor cell growth and tumor vasculature sprouting. For the treatment of mTNBC, Liang and coworkers created double targeted nanoparticles (CD44 plus neuropilin driven targeting). The neuropilin receptors were targeted with tLyP-1 as a ligand, while CD44 were targeted with HA. tLyP-1 was coupled using tocopheryl succinate (TOS) and PEG, while low molecular weight HA was coupled using d-α-tocopheryl succinate (α-TOS). The NPs were created by molecularly combining the two conjugates. The NPs were about 120 nm in size and can be absorbed through the EPR effect (Fig. 8.6). In vivo effectiveness was proven in a 4T-1 breast orthotopic tumor carrying murine model, which showed a strong anti-tumor impact (79.6% reduction) and totally prevented lung metastases. Metastasis was not cured by Taxotere® or HA NPs. The effectiveness of the formulations was improved by a double targeting through CD44 and neuropilin receptors, as well as the EPR effect (Liang et al., 2017; Mir and Mehraj, 2019).

Stimulus responsive drug delivery

Drug delivery systems that are engineered to deliver their payloads (gene/drug/photosensitizer) at only the target location upon getting exposed to a stimuli or trigger are known as stimulus responsive drug delivery system or smart drug delivery system. The stimuli or trigger can be endogenous (GSH, pH, enzyme) or exogenous (temperature, magnetic field, light, ultrasound). The main benefit of such systems is that they prevent early medication release into systemic circulation that helps to reduce unwanted adverse effects and allows for optimum drug usage. Tumor microenvironments have lower pH, higher GSH levels, and other characteristics that can be used as triggers to elicit payloads discharge from the system by using carriers that deliver the medicine upon contact to these triggers (Yu et al., 2014; Mehraj et al., 2021). Nevertheless, there are some challenges that must be addressed before such smart systems may be used in therapeutic settings. Wavelength and light intensity must be accurately selected in light responsive systems because laser density beyond 1 W/cm2 is harmful to health and UV-visible radiation has low penetration. In vivo, ultrasound responsive systems, like microbubbles, have a short half-life, while magnetic field responsive systems are costly (Yao et al., 2016). However, multiple research teams have developed a variety of nanoparticles (polymer, lipid, and silica based) for delivering payloads within the TNBC cell (Mir et al., 2021).

TNBC therapy with pH-responsive nanosoldiers

Lee and coworkers created doxorubicin-loaded lipidic pH responsive polymeric caged nanobins. They made DOX liposomes first, and then coupled cholesterol terminated PAA to those liposomes. In an acidic environment, the polymer component was essential for activating release of the drugs. Doxorubicin's toxicity was lowered by encapsulating it in the system. In a mouse MDA-MB-231 TNBC xenograft model, an in vivo research revealed a 75% decrease in breast tumor growth (Lee et al., 2010).

TNBC therapy using magnetic hyperthermia responsive nanosoldiers

For the treatment of TNBC, Xie and colleagues developed an injectable self-healing magnetized chitosan hydrogel cross-linked with telechelic difunctional PEG filled with DOX and DTX. An in vitro cytotoxic investigation utilizing MDA-MB-231 cell lines and an in vivo efficiency analysis in MDA-MB-231 tumor-bearing nude mice revealed that the system outperformed DTX-loaded PLGA NPs hydrogel and DOX hydrogel in anti-tumor activity (Xie et al., 2017).

Summary

TNBC remains a very heterogeneous and deadly breast carcinoma with extremely poor survival rates. The very heterogeneous character of TNBC, as well as the associated difficulties of metastases and resistance, present oncologists with difficult tasks. To combat the aggressive TNBC tumor, currently, present medicines are insufficient and must be augmented with innovative targeted therapeutics. With the advancement of nanotechnologies, nanomedicine is also developing in respect of precise and speedy diagnosis, as well as target-directed treatment in malignancies (Mir et al., 2021). Due to their target-specific multipurpose capabilities, nanoparticles are a crucial actor in most tumor research. These nanostructures are well-armed to carry out their mission of eliminating the majority of cancerous cells. The ability to load or enclose drugs not only protects them but also increases their biological half-life, resulting in a reduced overall dosage of administering drugs. Due to increased permeability and retention (EPR), such encapsulating aids the gradual and focused release of medication at the cancerous site, decreasing adverse effects to non-cancerous normal tissues. The effectiveness of treatment is improved when drugs are delivered in a targeted manner. These nanocarriers were approved for diagnostic procedures and therapies due to their flexibility in terms of the size, materials utilized, and production procedures, as well as biocompatibility and biodegradability. Theranostics had been established with a highly potential future in cancer after successfully constructing dual-functionality NPs for concurrently detecting and treating cancer. By combining ligands to NPs, significant multiplexibility is achieved, allowing for combination targeted drug administration at an exact spot to selectively eliminate tumor cells. Despite the wide range of uses, there seem to be a couple of problems that must be solved. The most of nanocarriers used in TNBC research are intended for focused diagnostics or treatment. In-vitro TNBC cell lines as well as in xenograft mice models have been used to demonstrate the efficacy of such nanocarriers in a few investigations. Knowing the cellular and molecular interactions, as well as expertise in combining numerous modalities, in a single system, remains a challenge that requires a potential solution. To summarize, nanosoldiers laden with medications, genes, and immunological components may soon become an important part of the TNBC therapeutic options, assisting in the fight against the disease and contributing to improved survival of patients.

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Glossary

TNBC TNBC is that type of breast cancer that is defined by the absence of ER, PR, and HER2 receptor expression.

Luminal A Luminal A breast cancer is hormone-receptor positive (estrogen-receptor and/or progesterone-receptor positive), HER2 negative, and has low levels of the protein Ki-67, which helps control how fast cancer cells grow.

Luminal B Luminal B breast cancer is hormone-receptor positive (estrogen-receptor and/or progesterone-receptor positive), and either HER2 positive or HER2 negative with high levels of Ki-67.

Mammography Mammography is specialized medical imaging that uses a low-dose x-ray system to see inside the breasts. A mammography exam, called a mammogram, aids in the early detection and diagnosis of breast diseases in women.

Exosomes Exosomes are membrane-bound, extracellular vesicles released by numerous cells in both abnormal and normal situations. Exosomes are largely responsible for carrying biomolecules such as RNA, DNA, lipids, and proteins to recipient cells.

Nanobiosensor A nanobiosensor is a biosensor that combines nanoparticles with transducers to increase biological signaling and transduction processes.

LncRNA LncRNAs are a class of epigenetic regulators that play important roles in epigenetic regulation. LncRNAs regulate epigenetic modification primarily in the nucleus, regulating gene transcription at the transcriptional level by modulating histone or DNA modification, primarily methylation and acetylation.

MiRNA miRNAs are a class of small noncoding RNAs of ~22nt in length which are involved in the regulation of gene expression at the posttranscriptional level by degrading their target mRNAs and/or inhibiting their translation.

Epigenetics Epigenetics is the study of how cells control gene activity without changing the DNA sequence.

DNA methylation DNA methylation is an epigenetic mechanism involving the transfer of a methyl group onto the C5 position of the cytosine to form 5-methylcytosine. DNA methylation regulates gene expression by recruiting proteins involved in gene repression or by inhibiting the binding of transcription factor(s) to DNA.

Biomarker Any form, substance, or factor that is measurable and can influence or anticipate the consequences of a disease.

RNA sequencing RNA sequencing (RNA seq) is a technique that can examine the quantity and sequences of RNA in a sample using next-generation sequencing (NGS). It analyzes the transcriptome, indicating which of the genes encoded in our DNA are turned on or off and to what extent.

Malignancy It refers to the presence of cancerous cells that have the ability to spread to other sites in the body (metastasize) or to invade nearby (locally) and destroy tissues.

Mis-sense mutation It is a type of substitution in which the nucleotide change results in the replacement of one protein building block (amino acid) with another in the protein made from the gene.

Mortality The number of deaths in a certain group of people in a certain period of time.

Relapse The return of a disease or the signs and symptoms of a disease after a period of improvement.

Chemoresistance The ability of cancer cells to evade or to cope with the presence of the chemotherapy.

Tumorigenesis It is the gain of malignant properties in normal cells, including primarily dedifferentiation, fast proliferation, metastasis, evasion of apoptosis and immunosurveillance, dysregulated metabolism, epigenetics, etc.

Xenograft The transplant of an organ, tissue, or cells to an individual of another species.

Lymphoma Lymphoma is a broad term for cancer that begins in cells of the lymph system**.**

Invasive breast carcinoma Cancer that has spread from where it began in the breast to surrounding normal tissue.

Apoptosis A type of cell death in which a series of molecular steps in a cell lead to its death. This is one method the body uses to get rid of unneeded or abnormal cells. The process of apoptosis may be blocked in cancer cells**.**

DNA repair genes DNA repair genes code for proteins whose normal function is to correct errors that arise when cells duplicate their DNA prior to cell division.

Anthracycline A type of antibiotic that comes from certain types of *Streptomyces* bacteria and are used to treat many types of cancer. Anthracyclines damage the DNA in cancer cells, causing them to die.

Neo adjuvant chemotherapy Neoadjuvant chemotherapy is chemotherapy that a person with cancer receives before their primary course of treatment. The aim is to shrink a cancerous tumor using drugs before moving onto other treatments, such as surgery.

Overall survival The length of time from either the date of diagnosis or the start of treatment for a disease, such as cancer, that patients diagnosed with the disease is still alive

Monoclonal antibody A monoclonal antibody is an antibody made by cloning a unique white blood cell.

Transcription factors Transcription factors (TFs) are key proteins that decode the information in our genome to express a precise and unique set of proteins and RNA molecules in each cell type in our body.

Angiogenesis It is the formation of new blood vessels. This process involves the migration, growth, and differentiation of endothelial cells, which line the inside wall of blood vessels.

Masectomy A mastectomy is surgery to remove all breast tissue from a breast as a way to treat or prevent breast cancer.

Lumpectomy Lumpectomy is defined as an excision of a breast lump with a surrounding rim of normal breast tissue, as shown in the image below.

Costimulatory molecules Costimulatory molecules are a heterogeneous group of cell surface molecules that act to amplify or counteract the initial activating signals provided to T cells from the T cell receptor (TCR) following its interaction with an antigen/major histocompatibility complex (MHC), thereby influencing T cell differentiation and fate.

Adjuvant chemotherapy Adjuvant chemotherapy refers specifically to treatment following a surgical procedure that appears to have removed all tumor with the intention of preventing relapse from occult disease.

Neoadjuvant chemotherapy Neoadjuvant chemotherapy is chemotherapy that a person with cancer receives before their primary course of treatment. The aim is to shrink a cancerous tumor using drugs before moving onto other treatments, such as surgery.

Base excision repair Base excision repair (BER) is a cellular mechanism, studied in the fields of biochemistry and genetics, that repairs damaged DNA throughout the cell cycle. It is responsible primarily for removing small, non-helix-distorting base lesions from the genome.

Residual disease It is the occurrence of cancer cells that remain after attempts to remove the cancer have been made.

OS The length of time from either the date of diagnosis or the start of treatment for a disease, such as cancer, that patients diagnosed with the disease is still alive.

Disease free survival It is the period after a successful treatment during which there are no signs and symptoms of the disease that was treated.

Masectomy A mastectomy is surgery to remove all breast tissue from a breast as a way to treat or prevent breast cancer.

Radiosensitization It is a physical, chemical, or pharmacological intervention that increases the lethal effects of radiation when administered in conjunction with it.

Prognosis The likely outcome or course of a disease; the chance of recovery or recurrence.

DNA microarray A DNA microarray is a collection of microscopic DNA spots attached to a solid surface. It is a tool used to determine whether the DNA from a particular individual contains a mutation in genes like *BRCA1* and *BRCA2*.

Immunoediting Immunoediting is a theory that describes the transformation of normal cells to clinically-detectable cancer. The theory implies that while the human immune system protects from cancer, it also drives the development of tumors that will undergo immunogenic "sculpting" and may survive immune cell attacks

Immunosuppression It is the suppression of the body's innate ability to ward off disease and infection.

Innate immunity The nonspecific defense mechanisms that come into play immediately or within hours of an antigen's appearance in the body.

Angiogenesis It is the formation of new blood vessels. This process involves the migration, growth, and differentiation of endothelial cells, which line the inside wall of blood vessels.

Granzymes These are serine proteases that enter the target cell through the perforin-induced channels and activate intracellular enzymes, called caspases that play a pivotal role in the induction of programmed cell death (apoptosis).

Perforin It is a glycoprotein responsible for pore formation in cell membranes of target cells. Perforin is able to polymerize and form a channel in target cell membrane.

Humoral immunity Humoral immunity is the process of adaptive immunity manifested by the production of antibodies by B lymphocytes.

Tumor microenvironment The tumor microenvironment is the environment around a tumor, including the surrounding blood vessels, immune cells, fibroblasts, signaling molecules, and the extracellular matrix.

Immune checkpoints Immune checkpoints are an important part of the immune system that plays a role in the regulation of the immune system. These molecules usually reside on the immune cells and need to get activated for initiating an immune response**.**

Homolog A gene similar in structure and evolutionary origin to a gene in another species

Somatic mutations Somatic mutations are mutations that arise after fertilization, as the cells are replicating, dividing, and differentiating into their individual cell types.

Monotherapy Somatic mutations are mutations that arise after fertilization, as the cells are replicating, dividing, and differentiating into their individual cell types.

Hypophysitis It is the acute or chronic inflammation of the pituitary gland.

Immunogenicity It is the ability of cells/tissues to provoke an immune response and is generally considered to be an undesirable physiological response.

Neo antigens They are mainly tumor-specific antigens generated by mutations in tumor cells, which are only expressed in tumor cells.

Class I MHC Major histocompatibility complex (MHC) class I molecules represent a basic molecular framework that mediates the activation and function of cytotoxic effector cells of the adaptive and innate branches of the immune system, such as $CD8^+$ T cells and natural killer (NK) cells.

Humanized antibodies These are the antibodies from non-human species whose protein sequences have been modified to increase their similarity to antibody variants produced naturally in humans.

Chemoradiation Treatment that combines chemotherapy with radiation therapy.

Cancer vaccines They are a form of immunotherapy that can help educate the immune system about what cancer cells "look like" so that it can recognize and eliminate them.

Targeted therapy It is a type of cancer treatment that uses drugs or other substances to precisely identify and attack certain types of cancer cells.

Translocation A genetic change in which a piece of one chromosome breaks off and attaches to another chromosome.

Steroid A steroid is a biologically active organic compound with four rings arranged in a specific molecular configuration.

Homeostasis It refers to the physiological tendency toward the maintenance of a stable environment controlled internally and coordinately to buffer external changes.

Neoplasm An abnormal mass of tissue that forms when cells grow and divide more than they should or do not die when they should.

Transcription Transcription is the process by which the information in a strand of DNA is copied into a new molecule of messenger RNA (mRNA).

Translation Translation is the process by which a protein is synthesized from the information contained in a molecule of messenger RNA (mRNA).

Isoform A protein that has the same functions as another protein but which is encoded by a different gene and may have small differences in its sequence**.**

Malignancy It refers to the presence of cancerous cells that have the ability to spread to other sites in the body (metastasize) or to invade nearby (locally) and destroy tissues.

Neutropenia It is a condition that in which there occurs lower-than-normal levels of neutrophils in your blood.

Gene amplification Gene amplification is an increase in the number of copies of a gene without a proportional increase in other genes.

Nanocarriers Nanocarriers are nanoparticles that carry medicinal drugs to targeted sites in the body, whilst minimizing damage to the surrounding tissue.

Dendrimers They are nanosized, radially symmetric molecules with well-defined, homogeneous, and monodisperse structure that has a typically symmetric core, an inner shell and an outer shell.

Liposomes Liposomes are small artificial vesicles of spherical shape that can be created from cholesterol and natural nontoxic phospholipids.

Abbreviations

TNBC	Triple negative breast cancer
BC	Breast cancer
BRAC	Breast cancer gene
CNS	Central nervous system
ER	Estrogen receptor
PR	Progesterone receptor
HER2	Human epidermal growth factor 2
MRI	Magnetic resonance imaging
EGFR	Epidermal growth factor receptor
PIK3CA	Phosphatidylinositol-4,5-bisphosphate 3- kinase catalytic subunit alpha
BL1	Basal like-1
BL2	Basal like-2
MSL	Mesenchymal stem like
M	Mesenchymal
IM	Immunomodulatory
LAR	Luminal androgen receptor
ECM	Extracellular matrix
DC	Dendritic cells
AR	Androgen receptor
PAM50	Prediction analysis of microarray 50
PDGFR	Platelet-derived growth factor receptor
DSB	Double stranded break
SSB	Single stranded break
TN	Triple negative
CTCs	Circulatory tumor cells
ctDNA	Circulatory tumor DNA
miRNA	Micro RNA
siRNA	Small interfering RNA
ddPCR	Droplet digital polymerase chain reaction
PET	Positron emission tomography
mAbs	Monoclonal antibodies
dPCR	Digital polymerase chain reaction
qPCR	Quantitative polymerase chain reaction
PTEN	Phosphate and tensin homolog deleted on chromosome 10
JAK2	Janus kinase 2
OS	Overall survival
RFS	Relapse free survival
HRD	Homologous recombination deficiency

HRR	Homologous recombination repair
DNMTs	DNA methyltransferases
DMRs	Differentially methylated regions
LnRNA	Long noncoding RNAs
EMT	Epithelial mesenchymal transition
BET	Bromodomain and extraterminal protein
TP53	Tumor protein 53
BCL2	B-cell lymphoma 2
mTOR	Mammalian target of rapamycin
CDK	Cyclin dependent kinase
pCR	Pathological complete response
HSP	Heat Shock proteins
MEK	Mitogen activated protein kinase
CSCs	Cancer stem cells
PARP	Polyadenosine diphosphate ribose polymerase
CT	Chemotherapy
RT	Radiotherapy
SOC	Standard of care
5-FU	5-Flourouracil
PFS	Progression free survival
VEGF	Vascular endothelial growth factor
FDA	Food and drug administration
SHH	Sonic hedgehog
IHH	Indian hedgehog
DHH	Desert hedgehog
CD	Cluster of differentiation
FZD	Frizzled
WNT	Wingless/integrated
SSBR	Single strand break repair
TGF-β	Transforming growth factor beta
TICs	Tumor-infiltrating immune cells
CSPG4	Chondratin sulfate proteoglycan 4
PTX	Paclitaxel
DOX	Doxorubicin
DTX	Docetaxel
MRM	Modified radial masectomy
BCT	Breast conserving therapy
HDAC	Histone deacetylases
DFS	Disease free survival
DDFS	Distant disease free survival
TK	Tyrosine kinase
mBC	Metastatic breast cancer
mTNBC	Metastatic triple negative breast cancer
AF	Activation factor
BLBC	Basal-like breast cancer

IGFR	Insulin-like growth factor receptor
FGFR	Fibroblast growth factor receptor
ADC	Antibody drug conjugate
DOR	Duration of response
PD-1	Programmed cell death protein 1
PD-L1	Programmed death ligand 1
CTLA-4	Cytotoxic T lymphocyte associated antigen 4
ORR	Overall response rate
CAR-T	Chimeric antigen receptor T
EPR	Enhanced patient response
eEF2K	Eukaryotic elongation factor- 2 kinase
NAC	Neoadjuvant chemotherapy
RRs	Response rates
US	United States
AT	Adjuvant therapy
EBCTCG	Early Breast Cancer Trialist's Collaborative Group
TNT	Triple negative trial
DFS	Disease-free survival
QOL	Quality of life
BCS	Breast-conserving surgery
TAMs	Tumor-associated macrophages
G-CSF	Granulocyte-colony stimulating factor
TANs	Tumor associated neutrophils
NETs	Neutrophil extracellular traps
NK	Natural killer
MHC	Major histocompatibility complex
ADCC	Antibody-dependent cellular cytoxicity
TME	Tumor immune microenvironment
ILs	Interleukin's
IFNs	Interferon's
MDSCs	Myleoid-derived suppressor cells
CAFs	Cancer-associated fibroblasts
MMR	Mismatch repair
TMB	Tumor mutational burden
CSFs	Colony-stimulating factors
TGFs	Transforming growth factor's
LAG-3	Lymphocyte activation gene-3
CEACAM1-L	Carcinoembryonic antigen cell adhesion molecule 1
PVR	Poliovirus receptor
IDO1	Indole amine 2, 3- dioxygenase 1
ICD	Immunogenic cell death
EMA	Epithelial membrane antigen
GM-CSF	Granulocyte-macrophage colony-stimulating factor
TCRs	T-cell receptors
TAAs	Tumor associated antigen

PPV	Personalized peptide vaccination
NEXT	Notch extracellular truncation
ICD	Intracellular domain
NPs	Nanoparticles
DDS	Drug delivery system
AODNs	Antisense-oligo deoxynucleotides
NIR	Near infrared
AuNPs	Gold nanoparticles
CNTs	Carbon nanotubes
VLPs	Virus like particles
HPV	Human papilomavirus
HA	Hyaluronic acid
RAGE	Receptor for advanced glycation end products
HDL	High-density lipoprotein
CPPs	Cell-penetrating peptides
PTT	Photothermal therapy
PDT	Photodynamic therapy

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