RECENT UPDATE ON TRIPLE NEGATIVE BREAST CANCER- REVIEW

Vijayan Venugopal*, P.Vasanth Raj, S.Muralidharan

Faculty of Pharmacy, AIMST University, Semeling, Bedong, Malaysia-08100

ABSTRACT

Triple Negative Breast Cancer (TNBC) is a heterogeneous disease that based on immunohistochemistry (IHC) is estrogen receptor (ER) negative, progesterone receptor (PR) negative and human epidermal growth factor receptor 2 (HER2) negative. TNBC is characterized by a distinct molecular profile, aggressive nature and lack of targeted therapies. The purpose of this article is to review the current and future novel signalling pathways as therapeutic approaches to TNBC. Recent Identification of a new BRCA1 trafficking pathway holds promise in the future for the development of targeted therapies for TNBC.

Keywords: Triple negative breast cancer, HER2, Estrogen receptor

INTRODUCTION

Breast cancer is often referred to as a single disease. But, it is not just a single disease. There are many types of breast cancer [1]. It can even be called a family of diseases. All breast cancers start in the breast. So, they are the same in some ways, but differ in others. The type of breast cancer affects prognosis (outcome) and treatment options. All breast cancer tumors are tested for certain receptors (proteins). These tests look for estrogen, progesterone and HER2/neu receptors. Test results are noted on a pathology report. If the tumors are "positive," there are many receptors. If the tumors are "negative," there are few or none. There are many treatment options for tumors that test "positive," but fewer options for those that don't [2].

Cancer is increasingly a global problem and breast cancer is not only the most common incident form of cancer in women worldwide, but is the first or second most common in all regions of the world, and responsible for 1.4 million new cases annually [3,4]. The incidence of breast cancer is increasing almost everywhere throughout the world, although the mortality rate from breast cancer is declining in many high-income countries.

Triple negative breast cancer (TNBC) gets its name because the cells test negative for three receptors.

Address for correspondence:
Dr.Vijayan Venugopal,
Unit of pharmaceutical technology,
AIMST University,
Bedong, Malaysia-08100.
Mobile: +60103708790.
Email:vijayanv2@gmail.com.

So, TNBC is Estrogen receptor-negative (ER-), Progesterone receptor-negative (PR-) and HER2/neu-negative (HER2-). TNBC does not have receptors that are targets for treatment we have today. About 15-20 percent of all breast cancers in the U.S. are TNBC. Anyone can get this type of breast cancer. But, research shows that it occurs more often in: Younger women, African American women, Women who have BRCA1 gene mutations [5]. TNBC is less likely to be found on a mammogram than some other types of breast cancer. It can also be aggressive. Compared to other types, it tends to grow faster. It can be treated, but it may recur (come back) early and spread to other parts of the body. Part of the reason is due to the lack of targeted treatments. TNBC has a poorer outcome for at least the first five years after diagnosis than estrogen receptor-positive (ER+) tumors. Still, if breast cancer hasn't come back within five years, the chance of survival is higher than for ER+ tumors. This is good news for five-year survivors [6].

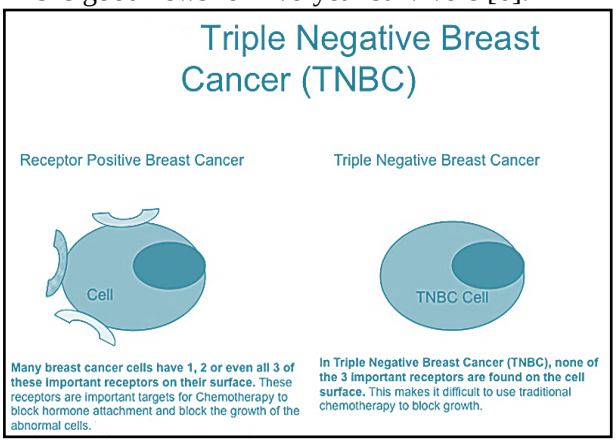

Figure-1: Triple negative breast cancer (TNBC)

Classifying breast cancer based on gene expression: Research on patterns of gene expression has also suggested some newer ways to classify breast cancers. The current types of breast cancer are based largely on how tumors look under a microscope. A newer classification, based on molecular features, divides breast cancers into 4 groups. This testing, called the PAM50, is currently available but it isn't clear that it is any more helpful in guiding treatment than tests of hormone receptors and HER2 [7]:

Luminal A and luminal B types: The luminal types are estrogen receptor (ER)–positive. The gene expression patterns of these cancers are similar to normal cells that line the breast ducts and glands (the inside of a duct or gland is called its lumen). Luminal A cancers are low grade, tend to grow fairly slowly, and have the best prognosis. Luminal B cancers generally grow somewhat faster than luminal A cancers and their outlook is not as good.

HER2 type: These cancers have extra copies of the HER2 gene and sometimes some others. They usually have a high-grade appearance under the microscope. These cancers tend to grow more quickly and have a worse prognosis, although they often can be treated successfully with targeted therapies aimed at HER2 which are often given along with chemotherapy.

Basal type: Most of these cancers are of the so-called triple-negative type, that is, they lack estrogen or progesterone receptors and have normal amounts of HER2. The gene expression patterns of these cancers are similar to cells in the deeper basal layers of breast ducts and glands. This type is more common among women withBRCA1 gene mutations. For reasons that are not well understood, this cancer is also more common among younger and African-American women.

These are high-grade cancers that tend to grow quickly and have a poor outlook. Hormone therapy and anti-HER2 therapies like trastuzumab and lapatinib are not effective against these cancers, although chemotherapy can be helpful. A great deal of research is being done to find better ways to treat these cancers [8].

Treatment options TNBC is treated with a combination of surgery, radiation and chemotherapy. Because it tests negative for the three receptors mentioned above, it isn't treated with hormone or targeted therapy. Yet, chemotherapy works well in TNBC [9]. It may even respond better than other types of breast cancer. Most of the time, chemotherapy is given after surgery. This is called adjuvant chemotherapy. Sometimes chemotherapy is given before surgery. This is called neo-adjuvant chemotherapy. This may shrink a tumor enough so that a woman can have a lumpectomy. How the tumor responds to this treatment may also give information on prognosis. If TNBC responds well, the chance of survival is higher.

DIAGNOSIS OF TNBC

Estrogen receptor (ER) and progesterone receptor (PR):

Receptors are proteins in or on certain cells that can attach to certain substances, such as hormones, that circulate in the blood. Normal breast cells and some breast cancer cells contain receptors that attach to estrogen and progesterone. These 2 hormones often fuel the growth of breast cancer cells.

An important step in evaluating a breast cancer is to test a portion of the cancer removed during the biopsy (or surgery) to see if they have estrogen and progesterone receptors. Cancer cells may contain neither, one, or both of these receptors. Breast cancers that have estrogen receptors are often referred to as ER-positive (or ER+) cancers, while those containing progesterone receptors are called PR-positive (or PR+) cancers [10].

All invasive breast cancers should be tested for both of these hormone receptors either on the biopsy sample or when they are removed with surgery. About 2 of 3 breast cancers have at least one of these receptors. This percentage is higher in older women than in younger women. DCIS should be checked for estrogen receptors, as well.

HER2/neu testing:

About 1 of 5 breast cancers have too much of a growth-promoting protein called HER2/neu (often just shortened to HER2). The HER2/neu gene instructs the cells to make this protein. Tumors with increased levels of HER2/neu are referred to as HER2-positive.

Cancers that are HER2-positive have too many copies of the HER2/neu gene, resulting in greater than normal amounts of the HER2/neu protein. These cancers tend to grow and spread more aggressively than other breast cancers.

All newly diagnosed invasive breast cancers should be tested for HER2/neu because HER2-positive cancers are much more likely to benefit from treatment with drugs that target the HER2/neu protein, such as trastuzumab (Herceptin) and lapatinib (Tykerb). DCIS is not tested for HER2 because is not treated with these drugs [11].

A biopsy or surgery sample is usually tested in 1 of 2 ways:

- Immunohistochemistry (IHC): In this test, special antibodies that identify the HER2/neu protein are applied to the sample, which cause cells to change color if many copies are

present. This color change can be seen under a microscope. The test results are reported as 0, 1+, 2+, or 3+.

- Fluorescent in situ hybridization (FISH): This test uses fluorescent pieces of DNA that specifically stick to copies of the HER2/neu gene in cells, which can then be counted under a special microscope.

Many breast cancer specialists feel the FISH test is more accurate than IHC. However, it is more expensive and takes longer to get the results. Often the IHC test is used first. If the results are 1+ (or 0), the cancer is considered HER2-negative. People with HER2-negative tumors are not treated with drugs (like trastuzumab) that target HER2. If the test comes back 3+, the cancer is HER2-positive. Patients with HER2-positive tumors may be treated with drugs like trastuzumab. When the result is 2+, the HER2 status of the tumor is not clear. This usually leads to testing the tumor with FISH. Some institutions also use FISH to confirm HER2 status that is 3+ by IHC and some perform only FISH.

A newer type of test, known as chromogenic in situ hybridization (CISH), works similarly to FISH, by using small DNA probes to count the number of HER2 genes in breast cancer cells. But this test looks for color changes (not fluorescence) and doesn't require a special microscope, which could make it less expensive. Right now, it is not being used as much as IHC or FISH.

HER2 Division of TNBC: Doctors often divide invasive breast cancers into groups based on the presence of hormone receptors (ER and PR) and whether or not the cancer has too much HER2.

Hormone receptor-positive: If the breast cancer cells contain either estrogen or progesterone receptors, they can be called hormone receptor-positive (or just hormone-positive). Breast cancers that are hormone receptor-positive can be treated with hormone therapy drugs that lower estrogen levels or block estrogen receptors. This includes cancers that are ER-negative but PR-positive. Hormone receptor-positive cancers tend to grow more slowly than those that are hormone receptor-negative (and don't have either estrogen or progesterone receptors). Women with these cancers tend to have a better outlook in the short-term, but cancers that are hormone receptor-positive can sometimes come back many years after treatment. Hormone receptor-positive cancers are more common in women after menopause [12].

Hormone receptor-negative: If the breast cancer cells don't have either estrogen or progesterone receptors, they are said to be hormone receptor-negative (or just hormone-negative). Treatment with hormone therapy drugs is not helpful for these cancers. These cancers tend to grow more quickly than hormone receptor-positive cancers. If they return after treatment, it is more often in the first few years. Hormone receptor- negative cancers are more common in women who have not yet gone through menopause.

HER2 positive: Cancers that have too much HER2 protein or extra copies of the HER2 gene are called HER2 positive. These cancers can be treated with drugs that target HER2.

HER2 negative: Cancers that don't have excess HER2 are called HER2 negative. These cancers do not respond to treatment with drugs that target HER2.

Triple-negative: If the breast cancer cells don't have estrogen or progesterone receptors and don't have too much HER2, they are called triple-negative. These cancers tend to occur more often in younger women and in women who are African-American or Hispanic/Latina. Triple-negative breast cancers tend to grow and spread more quickly than most other types of breast cancer. Because the tumor cells don't have hormone receptors, hormone therapy is not helpful in treating these cancers. Because they don't have too much HER2, drugs that target HER2 aren't helpful, either. Chemotherapy can still be useful, though [13].

Triple-positive: This term is used to describe cancers that are ER-positive, PR-positive, and have too much HER2. These cancers can be treated with hormone drugs as well as drugs that target HER2.

OTHER TESTS OF BREAST CANCERS

Tests of ploidy and cell proliferation rate:

The ploidy of cancer cells refers to the amount of DNA they contain. If there's a normal amount of DNA in the cells, they are said to be diploid. If the amount is abnormal, then the cells are described as aneuploid. Tests of ploidy may help determine prognosis, but they rarely change treatment and are considered optional. They are not usually recommended as part of a routine breast cancer work-up.

The S-phase fraction is the percentage of cells in a sample that are replicating (copying) their DNA. DNA replication means that the cell is getting ready to divide into 2 new cells. The rate of cancer cell division can also be estimated by a Ki-67 test. If the S-phase fraction or Ki-67 labeling index is high, it means that the cancer cells are dividing more rapidly, which indicates a more aggressive cancer [14].

Tests of gene patterns:

Researchers have found that looking at the patterns of a number of different genes at the same time (sometimes referred to as gene expression profiling) can help predict whether or not an early-stage breast cancer is likely to come back after initial treatment. Two such tests, which look at different sets of genes, are now available: the Oncotype DX [15] and the Mamma Print

Oncotype DX: The Oncotype DX test can be helpful when deciding whether additional (adjuvant) treatment with chemotherapy (after surgery) might be useful in women with early-stage breast cancers that are hormone receptor-positive. This test is most often used for tumors that are small (1 cm or less) and have not spread to lymph nodes, but it can be used for more advanced tumors [16]. The test looks at a set of 21 genes in cells from tumor samples to determine a "recurrence score," which is a number between 0 and 100:

- Cancers with a recurrence score of 17 or below have a low risk of recurrence (cancer coming back after treatment) if they are treated with hormone therapy. Women with these cancers would probably not benefit from chemotherapy.
- Cancers with a score of 18 to 30 are at intermediate risk of recurrence. Some women with these cancer might benefit from chemotherapy.
- Cancers with a score of 31 or more are at high risk of recurrence. Women with these cancers are likely to benefit from chemotherapy in addition to hormone therapy.

The test estimates risk and helps predict who would be likely to benefit from chemotherapy. Still, it cannot tell for certain if any particular woman will have a recurrence with or without chemotherapy. It is a tool that can be used, along with other factors, to help guide women and their doctors when deciding whether more treatment might be useful.

Mamma Print: This test can be used to help determine how likely breast cancers are to recur in a distant part of the body after initial treatment [17,18,19].

The test looks at the activity of 70 different genes to determine if the cancer is low risk or high risk. So far though, it hasn't been studied to see if the results are useful in guiding treatment [20,21].

Usefulness of these tests: Many doctors use these tests (along with other information) to help make decisions about offering chemotherapy, but they aren't needed in all cases. These tests are now being looked at further in large clinical trials. In the meantime, women might want to ask their doctors if these tests might be useful for them [22].

THERAPEUTIC STRATEGIES

Although triple negative breast cancers are associated with a generally poor breast cancer specific outcome, most are not resistant to chemotherapy. These patients have an extremely poor prognosis and relapse and die quickly. Several therapies are being developed that target specific biomarkers of TNBC or basal-like subtype [23]. These strategies include EGFR-targeted agents, androgen receptor targeted agents, anti-antigenic agents, and PARP inhibitors are offering an option in triple negative disease; however, their uses as of date are limited to clinical trials and more work is needed to identify targets that yield high therapeutic ratios [23]. TNBC with BRCA1 gene mutations may be more sensitive to agents that cause DNA damage, such as Cisplatin [24]. Other, more recent promising therapeutic targets for TNBC include the NOTCH, Hedgehog and Wnt/b-Catenin signalling pathways [21]. Studies have shown that these therapies alter the apoptotic pathway, inhibiting tumour progression [24].

CHEMOTHERAPY

Adjuvant chemotherapy has been shown to not only prolong disease-free survival in patients but overall survival as a whole; however, TNBC lacks the typical targeted receptors found in luminal or HER-2 disease and therefore cannot be treated with hormonal agents, such as SERMS, aromatise inhibitors or HER2 antagonists [25]. To combat this issue several neoadjuvant studies have been done which accentuated the relationship between chemo sensitivity and outcome, revealing proportionally higher sensitivity to anthracycline or anthracycline/taxane–based chemotherapy such as Doxorubicin and Cyclophosphamide (standard chemotherapeutic agents), for basal like/ER negative breast cancers as compared to the luminal subtype [26]. Highest response rates were noticed among those classified as basal like (85%) and HER2 positive (70%), as compared to luminal (47%, $p<0.0001$). Despite initial chemo sensitivity, disease free survival ($p=0.04$) and overall survival ($p=0.02$) remained poorest among those with basal like and HER2 positive tumours, compared to the luminal subtype [26]. Although triple negative disease is highly responsive to Anthracycline/Taxane chemotherapy treatment, a high risk of relapse still remains if the tumour is not completely eradicated.

Additionally, pre-clinical and clinical studies indicate that tumours with BRCA1 dysfunction are sensitive to platinum agents such as Cisplatin, and Carboplatin which function by causing DNA damage and promote tumour cell apoptosis [25,27]. Studies have shown that p63, a family member of p53, is responsible for controlling a survival pathway that directly mediates Cisplatin sensitivity in TNBC; it therefore, can possibly be used as a biomarker to predict response to platinum therapy in triple negative breast cancer. However, this finding warrants further investigation and currently platinum's are not recommended for the adjuvant treatment of TNBC [25].

EGFR Inhibitors:

EGFR expression is approximately seen in 60% of triple negative breast tumors, thus providing a reasonable targeted treatment approach [6]. A phase II trial evaluating the combination of Cetuximab (a chimeric monoclonal antibody targeting EGFR) and Carboplatin, weekly for 3 to 4 weeks reported a response rate of 18% and overall clinical benefit of 27% among 102 patients with advanced TNBC [28]. Another study evaluating the combination of Irinotecan and Carboplatin with or without Cetuximab reported response rates of 49% and 30% respectively, among 72 patients with pre-treated TNBC. EGFR inhibitor Panitumumab, when used in combination with standard chemotherapeutic agents for neoadjuvant therapy for inoperable TNBC was recently reported to have shown a pathological complete response rate of 65% [25]. Other studies have recently demonstrated that EGFR inhibitors when used in combination with Texans or platinum's may increase the efficacy of the other agents [25]. To date, the majority of data with EGFR inhibitors has generally been interpreted as negative.

PARP Inhibitors:

PARP1, a gene that encodes an enzyme involved in the molecular events leading to cell recovery from DNA damage, when inhibited, leads to the accumulation of double-stranded DNA breaks. Cells deficient in BRCA1 and BRCA2 (required for normal homologous recombination), are exquisitely sensitive to PARP1 inhibition. Several PARP inhibitors Olaparib, Velaparib and PF-01367338 are currently in clinical trials and hold a promising future [25]. The study demonstrated a statistically significant 50% reduction in the risk of death; however, phase III trials failed to show statistically significant benefit for this combination, hence, the drug has been discontinued but some biomarker analysis is still underway to determine if a specific subset of patient may benefit from the drug [16,28,29]. This therefore highlights the need for continued research and clinical trials.

Antiangiogenic Agents:

The antiangiogenic agent Bevacizumab, a monoclonal antibody that targets all forms of VEGF, has been evaluated in a number of large phase III trials as treatment of metastatic breast cancer [25]. The landmark study E2100 illustrated improvement in progression free survival (11.8 vs. 5.9 months, HR=0.60, $p<0.001$) when adding Bevacizumab to Paclitaxel chemotherapy compared with single-agent Paclitaxel alone in first-line treatment of metastatic disease [25]. This subsequently led to subgroup analysis that demonstrated similar progression free survival benefits in both patients with TNBC and non-TNBC with the use of Bevacizumab plus a taxane [25]. Currently, the BEATRICE trial is prospectively investigating this combination as adjuvant therapy in TNBC [25]. Additionally, several small-molecule inhibitors of the VEGF pathway appear to have activity in the subset of pre-treated triple-negative breast cancer and definitive studies showing overall survival benefit will be needed prior to re approval [26-31].

CONCLUSION

Triple-negative breast cancer is clearly a distinct subtype, from the perspective of PR, ER and HER-2, and there may yet be further distinct sub-classifications. This disease presentation clearly represents an important clinical challenge. Triple negative breast cancer is also a surrogate of basal-like breast cancer. Therefore, trials designed to accrue patients with basal-

like breast cancer using ER/PR and HER-2 negativity provide an approximation of the triple-negative population, there is some discordance, including some HER-2 positives and some ER positives among the basals. At present, there is not a clear, proven effective single agent that targets a driving vulnerability in triple-negative breast cancer. However, there are a number of potential therapies currently under investigation that may eventually improve outcomes in these patients.

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