

Current Advances in Breast Cancer Therapeutics: Implication of Gamma Delta T Cells

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ABSTRACT

Breast cancer is one of the leading causes of death in women worldwide. However, it can metastasize to adjacent tissues and distant organs. Breast cancers are classified to different subtypes with various clinical implications, based on various morphological, anatomical, molecular and immunological properties. Depending on these subtypes and different clinical features, therapeutic regime of a breast cancer patient is offered, such as chemotherapy, radiotherapy or immunotherapy. Recent advances in identification of immune-related molecules involved in breast cancer progression opened up new avenue for breast cancer therapy. Current investigations focused on developing methods to reduce side effects of existing chemotherapeutic drugs, and generation of new immunotherapeutic modules like cancer vaccine are in progress. Targeted therapies and combination therapies against breast cancer are getting much attention in recent days. Hence, this chapter explores and summarizes the cause of breast cancer occurrence, intrinsic classification of breast cancer and implication of immunotherapeutic approach using gamma delta T cells (a subset of human cytotoxic T cells), that recognizes only specific molecule expressed on cancer cells for cytotoxic lysis, sparing healthy cells, for potential consideration of clinical application.

INTRODUCTION

Breast cancer is the most common type of invasive cancer among women worldwide. It is the second leading cause of cancer-related death among women, and every year it accounts for 12% of all cancers diagnosed globally [1]. Currently, more than 2.8 million women with a history of breast cancer that includes women currently under treatment and women who have been treated and finished the treatment [1]. Approximately 1 in 8 U.S. women will develop invasive breast cancer at some point in their lives; for men, the lifetime risk is about 1 in 1,000. Usually the breast cancer is detected either during a screening examination, before symptoms have developed, or after a patient notices a lump. In most of the cases the cell masses detected on a mammogram in early screening are benign; do not grow uncontrollably or spread to distant organs, and are not life-threatening. Microscopic analysis of breast tissue is needed for a definitive diagnosis and to determine the extent of its metastatic properties and invasiveness, which characterize the severity of the tumor with cancerous growth. Human breast is composed of two main types of tissues i.e., stromal tissues and glandular tissues. Stromal tissues include fatty and fibrous connective tissues of the breast while glandular tissues house the milk-producing glands (lobules) and the ducts (the milk passages) [2,3]. Lymphatic tissue and immune system are also present in the breast that helps removing cellular fluids and waste generated within the tissue [4]. Because of the presence of various tissues within the breast, many types of tumors may develop within the different areas of the breast according to their cellular constituents. Common form of benign breast tumor is the fibrocystic change, a non-cancerous condition in which women develop cysts (accumulated packets of fluid), lump, fibrosis (formation of scar-like connective tissue), areas of thickening, tenderness, and/or breast pain. However, in case of cancerous growth, most of the abnormal cell proliferations begin in the cells that line the ducts (ductal cancers). In some cases, cancerous growths begin in the cells that line the lobules (lobular cancers), while a small number of cancerous growth start in the other tissues of breast [5]. Various evidences have suggested breast cancers originated from different or same breast tissues having different histo-pathological and biological characteristics, and show distinct features [6]. As a result, different therapeutic strategies are considered for the treatment of cancer. Therefore, accurate detection and grouping of breast cancers are critically important to determine clinically relevant subtypes, which may further help clinicians for making decision for effective therapeutic regime. To simplify this process presence of some classical immune-histo chemistry (**IHC**) markers such as ER, PR and HER2, along with other traditional clinic-pathological variables including, e.g., tumor size, tumor grade and nodal involvement, are conventionally taken in account for patient prognosis and management [7,8]. It is reported that tumor cell response to therapy is not determined by any of the anatomical prognostic factors [9]. Intrinsic molecular characteristics play important roles in breast cancer initiation and progression, which can be probed using advent molecular methods like high-throughput platforms for gene expression analysis such as microarrays [10]. Thus, the change in concept has led to a new paradigm on how cancerous growth of breast tissues are stratified and treated.

This method not only provides an incremental advances on the reproducibility and accuracy of the disease prognosis and therapy, but also integrating information in dissecting this problem in molecular level. Even though the growing number of clinically relevant molecular subtypes is identified, current breast cancer patient management still highly depends on the conventional pathology assessment, which includes biomarker testing using validated commercial assays like MammaPrint, MapQuant Dx and its simplified version, Oncotype DX, and Theros. Though, it is important to standardize the methodology used for molecular sub-typing and precision targeted therapy, but the reproducibility of their efficacy needs to be extensively tested. Immunotherapy is one of the important alternative treatment modalities are being considered in addition to the traditional therapeutics, such as surgery, chemotherapy, radiotherapy, and hormonal treatment. With the expectations for improvement in reduction of toxicity of the current chemotherapies, generation of new non-toxic chemotherapeutic procedures, combination therapies and immunotherapies holds substantial potential as a sustainable therapeutic option for developing personalized medicine, which can target patients' specific tumor characteristics. As a result, most academic interest over the past decade has been dedicated to targeted therapies. The existing chemotherapeutic drugs available for treatment of breast cancer can be divided into several groups like plant alkaloids (paclitaxel, vinorelbine, and vindesine), alkylating agents (cisplatin, carboplatin, and cyclophosphamide), anthracyclines (doxorubicin, epirubicin, and idarubicin), and topoisomerase inhibitors (irinotecan, etoposide, and teniposide) [11]. Another major problem in breast cancer treatment is the drug resistant nature of the tumors, which may occur despite of significant advances in early detection and steady progress in the treatment with systemic agents [12]. Therefore, personalized targeted therapy is much needed as they can target the pathways that promote or sustain growth and invasion of breast carcinoma cells and in turn may cause reduction in cancerous growth. In the past two decades, several small-molecule inhibitors and monoclonal antibodies have been developed and tested in clinical trials that have the capability of targeting cancer progression, such as, cell growth, survival, angiogenesis, and metastasis. Though, out of these targeted agents some causes significant improvement of the breast cancer patient survival and outcome, production of novel molecules with high effectiveness is much needed. With the incremental knowledge on the complex tumorigenesis process of aggressive breast cancer, these novel molecules are gaining their importance, which may contribute to deciphering therapeutic advancement towards treatment of heterogenic breast cancers.

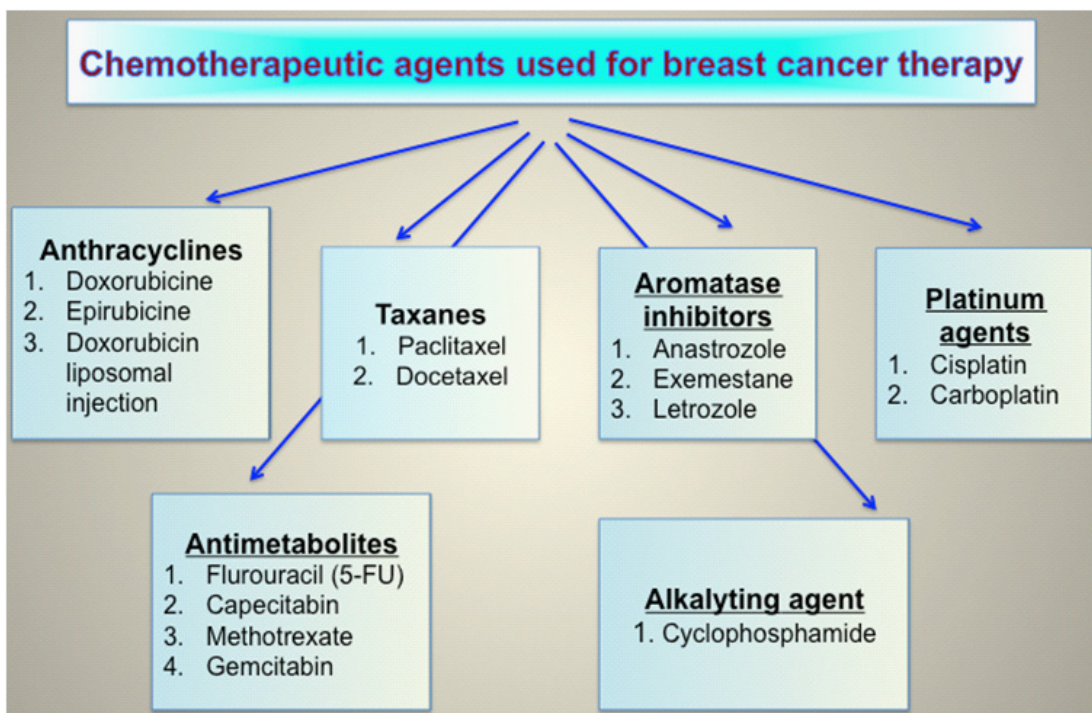


Figure 1: Common chemotherapeutic agents currently used for the breast cancer therapy.

RISK FACTORS FOR BREAST CANCER DEVELOPMENT

Age

Age is one of the predominant factors of breast cancer development. Due to the hormonal changes occur in older women, have the higher risk of developing breast cancer. Age-specific influence of risk factors increase the chance of postmenopausal breast cancer formation in women aged 50-75 or more years [13,14]. In addition, longer we live, there are more chances for genetic damages (mutations) in the body. As we age, our bodies loose regenerative capabilities for repairing genetic damages happening constantly due to the environmental effects. According to the American Cancer Society, about 1 out of 8 invasive breast cancers develop in women younger than 45. However, about 2 out of 3 invasive breast cancers are found in women at the age of 55 or older.

Gene

About 5 to 10% of breast cancers are thought to be hereditary, caused by abnormal genes passed from parent to child. Best-known genes linked to the breast cancer are *BRCA1* and *BRCA2* (breast cancer genes 1 and 2), which is present in all human genome. Normal function of the *BRCA* genes is to repair cell damage and keep breast, ovary, and other cells growing in healthy state.

But when these genes possess mutations impaired proteins are synthesized, abnormal function of the respective breast, ovarian, and other cells occur; as a result the risk of cancer increases substantially. Abnormal *BRCA1* and *BRCA2* genes may account for up to 10% of all breast cancers. Breast cancers associated with an abnormal *BRCA1* or *BRCA2* gene tend to develop in younger women and occur more often in both breasts, than that of cancers in women without these abnormal genes. Women with an abnormal *BRCA1* or *BRCA2* gene also have an increased risk of developing ovarian, colon, and pancreatic cancers, as well as melanoma. Men who have an abnormal *BRCA2* gene have a higher risk of breast cancer than men who don't, and about 8% by the time they're 80 years old [15].

Other genes

Changes in other genes are also associated with breast cancer. These abnormal genes are much less common and don't seem to increase risk as much as abnormal *BRCA1* and *BRCA2* genes, which are considered rare. Because of these genetic mutations are rare, they haven't been studied as much as the *BRCA* genes.

ATM

The ATM gene helps repair damaged DNA. DNA carries genetic information in cells. Inheriting two abnormal copies of this gene causes the disease ataxia-telangiectasia, a rare disease that affects brain development. Inheriting one abnormal ATM gene has been linked to an increased rate of breast cancer and pancreatic cancer in some families because the abnormal gene stops cells from repairing damaged DNA [16].

BRIP1

The BRIP1 gene also involved in repairing damaged DNA. Inheriting one abnormal BRIP1 gene is associated with higher risk of both breast and ovarian cancer [16].

CDH1

Synthesized protein from CDH1 gene helps cells adhere and bind together to form tissue. An abnormal CDH1 gene increases the risk of a rare type of stomach cancer at an early age, where lifetime risk is increased up to 83% . Women with an abnormal CDH1 gene also have a 39% to 52% lifetime risk of developing invasive lobular breast cancer [16].

CHEK2

The CHEK2 gene produces a protein, which is associated with preventing tumor growth. An abnormal CHEK2 gene can at least double the risk of breast and colon cancer, and significantly increase the risk of prostate cancer [16].

MRE11A

The MRE11A gene forms the MRN complex along with the RAD50 and NBN genes, which helps in repairing damaged DNA in cells. An abnormal MRE11A gene is linked to ataxia-telangiectasia-like disorder, a rare disease that affects brain development. The disease also weakens the immune system and increases cancer risk [16].

NBN

Along with the MRE11A and RAD50 genes, the NBN gene forms the MRN complex, which helps repair DNA damage in cells. An abnormal NBN gene causes Nijmegen breakage syndrome, a condition that slows growth in infancy and early childhood. People with nijmegen breakage syndrome are shorter than average; have a higher risk of several types of cancer, including breast cancer and many other health problems. Of the three genes in the MRN complex, researchers think that an abnormal NBN gene has the strongest link to breast cancer [16].

PALB2

The PALB2 gene is called partner and localizer of BRCA2 gene. It provides instructions to make a protein that co-operates with the BRCA2 protein to repair damaged DNA and to stop tumor growth. It was shown that an abnormal PALB2 gene increases breast cancer risk 5 to 9 times higher than average, almost as high as an abnormal BRCA1 or BRCA2 gene. Women with an abnormal PALB2 gene have a 33% to 58% lifetime risk of developing breast cancer. Whereas, women with an abnormal BRCA1 gene have a 50% to 70% risk of developing breast cancer by the age of 70 and little lower risk (40% to 60%) in women with an abnormal BRCA2 gene have a 40% to 60% risk of in developing breast cancer at the same age [16].

PTEN

The PTEN gene helps regulate cell proliferation and growth. An abnormal PTEN gene causes Cowden syndrome, a rare disorder in which people have higher risks of both benign and cancerous breast tumors, as well as growths in the digestive tract, thyroid, uterus, and ovaries. The lifetime breast cancer risk for women with a PTEN mutation is up to 85% [16].

RAD50

Along with the MRE11A and NBN genes, the RAD50 gene forms the MRN complex, which helps repair DNA damage in cells. An abnormal RAD50 gene has been linked to a higher risk of breast cancer in some families because the abnormal gene stops the cells from repairing damaged DNA [16].

RAD51C

The RAD51C gene also associated with repairing damaged DNA. People who have inherited one abnormal copy have higher risk of breast and ovarian cancer [16].

STK11

The STK11 gene helps in regulating cell growth. An abnormal STK11 gene causes Peutz Jegher syndrome, a rare disorder in which people tend to develop a type of polyp, called hamartomatous polyp, mostly in the small intestine but also in the stomach and colon. People with Peutz Jegher syndrome are at higher risk not only of gastrointestinal cancers, but also breast, lung and ovarian cancers. People may also develop freckling around the eyes, nose, and mouth, as well as inside the mouth [16].

TP53

The TP53 gene provides instructions to the body for making a protein that stops tumor growth. Inheriting an abnormal TP53 gene causes Li-Fraumeni syndrome, a disorder that causes people to develop soft tissue cancers at a young age. People with this rare syndrome have a higher-than-average-risk of developing breast cancer and several other cancers, including leukemia, brain tumors, and sarcomas (cancer of the bones or connective tissue). The cancer risk in women with a TP53 mutation is up to nearly 100%, and in men, it is up to 73% [16].

HORMONES

Alteration in hormonal level may initiate development of breast cancer. It could be resulted from hormonal replacement therapy, starting and stopping menstrual cycle, use of oral pills, and pregnancy in early age etc. There are two main types of hormone replacement therapy (**HRT**) is associated with breast cancer.

- (i) Combination HRT, which contains estrogen and progesterone hormones.
- (ii) Estrogen-only HRT, which contains only estrogen.

Combination HRT increases breast cancer risk by about 75%, even when used for short time only. Combination HRT also increases risk of developing breast cancer at more advanced stage, as well as increasing the risk of death from the disease for the diagnosed women with breast cancer. It was found that breast cancer risk increases mostly during the first 2 to 3 years of taking combination HRT. Dose-dependent effect also play critical role in increasing risk for breast cancer development. For instance, higher-dose of combination HRT increases breast cancer risk more than that of lower-dose combination HRT. On the other hand, risk of developing breast cancer decreases to average within two years after stopping intake of combination HRT [16]. Estrogen-only HRT increases the risk of breast cancer, but only when used for more than 10 years.

Cell Density within The Breast Tissue

Breast density assessed by mammography reflects the breast tissue composition. The breast density is a risk factor for developing breast cancer. The histological and other associated factors within breast makes higher density, enhances the risk of developing breast cancer. Women with extensive mammographic density have a greater chance of developing breast cancer [17].

Diet and Life Style

Post-menopausal and obese women may have a higher risk of developing breast cancer. Apart from this, sedentary life style, alcohol consumption may also enhance risk of developing breast cancer. It is known that women who consume alcoholic beverages per day have a greater risk of breast cancer occurrence than that of non-drinkers [18]. Alcohol can increase levels of estrogen and other hormones associated with hormone-receptor-positive breast cancers. Alcohol also may increase breast cancer risk by damaging DNA within cells.

Smoking causes a number of diseases, and is linked to a higher risk of breast cancer in younger, and premenopausal women. Smoking also can increase complications from breast cancer treatment by damaging lungs from radiation therapy, difficulty in healing after surgery and breast reconstruction, higher risk of blood clots when taking hormonal therapy medicines.

Apart from smoking and drinking, lack of exercise increase the chance of breast cancer. Exercise regularly at a moderate or intense level for 4 to 7 hours per week can help in lowering risk of breast cancer. Exercise consumes and controls level of blood sugar and limits levels of insulin growth factor in blood, a hormone that can affect breast cell growth and behavior.

In addition, fat cells also make estrogen. When breast cells are exposed to extra estrogen over time, the risk of developing breast cancer increases.

Environment

Breast cancer is a complex disease, and environment has an impact on initiation and developing disease. We are exposed to multiple chemicals and radiations during the course of our daily lives that potentially enhances risks for breast cancer development. Non-industrialized countries have lower breast cancer rates than industrialized countries. People who move to industrialized countries from countries with low rates of breast cancer develop the same breast cancer rates of the industrialized country. In addition, women who are working with X-rays and CT scans may have slightly higher risk of developing breast cancer. Those who work with low doses of radiation over a long period of time, for example, X-ray technicians are reported to be more susceptible to breast cancer [19].

TYPES OF BREAST CANCER ACCORDING TO THE LOCALIZATION

There are various types of breast cancers according to the cellular localization. By determining the type and its molecular background, clinicians choose for best treatment option for the patient.

Non-invasive

In non-invasive type of cancerous growth, cancer remains within tissues of its origin, and do not migrate to the adjacent tissues, or other part of the body. In this type of cancer, cells are mostly confined to the ducts, and do not invade surrounding fatty and connective tissues of the breast. Ductal carcinoma in situ (**DCIS**) is the most common form of non-invasive breast cancer,

whereas, lobular carcinoma in situ (**LCIS**) is less common and considered a marker for increased breast cancer risk [20,21]. Ductal carcinoma in situ (**DCIS**) is the earliest form of breast cancer, which is determined by the presence of the cancer cells within the ducts of the breast organ. These cancerous cells are restricted (*in situ*) in their movement, and do not spread into normal breast tissues [22]. Whereas, lobular carcinoma in situ (**LCIS**) is the type of breast cancer that causes a sharp increase in the number of cells within the milk glands (lobules) of the breast [23].

Invasive

In this type of cancer, cancerous cells invade through the duct and lobular wall, and spread to the surrounding connective and fatty tissues of the breast. Most of the cases, spreading of the cancerous cells occurs through the bloodstream and lymph nodes. On the other hand, breast cancer also can be invasive without being metastatic to the lymph nodes or other organs [24]. Two most common type of invasive breast cancers are infiltrating lobular carcinoma (**ILC**) and infiltrating ductal carcinoma (**IDC**).

Infiltrating lobular carcinoma (**ILC**) or invasive lobular carcinoma refers to cancer that has begun in the milk glands of the breast and invaded through the wall of the lobule and begun to spread into the tissues of the breast. Over time, invasive lobular carcinoma can spread to the lymph nodes and possibly to the other organs of the body. It is the second most common type of breast cancer.

Infiltrating ductal carcinoma (**IDC**) or invasive ductal carcinoma occurs when the abnormal cancer cells that began forming in the milk ducts spread beyond the ducts, invading the fatty tissue of the breast and possibly other regions of the body. It is the most common type of breast cancer, making up to nearly 80% of all diagnosed breast cancers [25].

BREAST CANCER TYPES ACCORDING TO CELLULAR AND MOLECULAR FACTORS

According to the clinical-pathological paradigm estimation, probability of breast cancer occurrence and recurrence is generally depends upon some biological and physical characteristics of the cancer tissues, such as histological grade, tumor size, and number of metastatic axillary lymph nodes. Expression levels of estrogen and progesterone receptors (**ER/PR**) play major roles in tumor occurrence, and the level of them are mostly determined by immune-histochemistry. Other than hormone receptor expressions, human epidermal growth factor receptor 2 (**HER2**) is an important marker of breast cancer, expression level of which can be determined by immune-histochemistry or *in situ* hybridization. Depending on the expression levels of each of the above-mentioned markers clinicians identify subgroups of breast cancer patients, who are likely to be benefited from anti-estrogen or anti-HER2 specific therapies. Considering these molecular factors, three major subtypes of breast tumors with different biologic behaviors were identified using the traditional immune-histochemical techniques: hormone-receptor positive,

triple negative, and human epidermal receptor (**HER**) 2/*neu* positive breast cancers. All of these subtypes require distinct management approaches as they have different natural histories [26-28]. Recent advancement in genome-wide expression profiling and hierarchical clustering led to identify new additional subtypes of breast cancers. It comprises at least 7 different biologic subtypes [26]. Along with three old subtypes, new subtypes like luminal A, luminal B, luminal C, HER2-enriched, basal-like, claudin-low, and normal breast-like cancers are also considered [29]. Literature studies reveal the distinct features and natural histories of each type of the breast cancer entities [29].

Luminal-like Breast Cancer

This subtype of breast cancer obtains its name from luminal breast epithelium as its expression profile is similar to that of the normal luminal breast epithelium. It can be classified as luminal A, luminal B and luminal C. Luminal A breast cancer is hormone-receptor positive [estrogen-receptor (**ER**) and/or progesterone-receptor (**PR**) positive], HER2 negative, and has low levels of the protein Ki-67 [30]. These parameters control the growth of cancer cells. These types of tumor are considered as low-grade tumors which tend to grow slowly and have the best prognosis. They are sensitive to endocrine manipulation on the other hand, they show less sensitivity to cyto-toxic agents in both neo-adjuvant and metastatic settings. About 60% of all breast cancers are classified as luminal A [31]. Luminal B breast cancer is hormone-receptor positive (ER and/or PR positive), and either HER2 positive or HER2 negative with high levels of Ki-67. Luminal B cancers represent about 20% of breast cancers, generally grow faster than luminal A cancers, and their prognosis is slightly worse than the luminal A [31]. Most of the luminal B tumors harbor mutations in *TP53*, and also have been shown to have genomic instability. The luminal B subtype is less common than the luminal A subtype, it carries a poorer prognosis, and relatively higher risk of relapse [26]. Similar to luminal A, luminal B tumors are also less sensitive towards cytotoxic chemotherapy. This is evident from the low pathological complete response rates observed after neo adjuvant chemotherapy [32-34]. Clinicians and researchers can distinguish the luminal C intrinsic subtype from luminal A and B subtypes by high expression of several genes, function of which is not yet known. The genes which are most commonly identified in luminal-C include transferrin receptor (**CD71**), MYB, nuclear protein p40, SQLE, and GGH [28]. These set of genes are also found to be over-expressed in HER2-enriched and basal-like subtypes.

Her2 Enriched Breast Cancer

This type of breast cancer is hormone-receptor (ER and PR) negative and HER2 positive. Mainly characterized by high expression of HER2/*neu* proliferation genes and low expression of luminal clusters which include luminal cyto-keratins (**CKs**) CK7, CK8, CK18, and CK19, and other luminal-associated markers such as human endogenous retrovirus envelope PL1, X-box-binding protein 1, hepatocyte nuclear factor 3, GATA-binding protein 3, Annexin XXXI, and estrogen receptor 1, among others [35-37]. This subtype of cancers has faster growth rate than

luminal cancers and may have worse prognosis [28]. However, they can be treated successfully with targeted therapies designed against HER2 protein. The occurrence of HER2 enriched breast cancer is approximately 20% to 30% of all breast tumors [38].

Basal-like Breast Cancer

The name of the basal-like intrinsic breast cancer subtype is derived from shared gene expression patterns with normal basal epithelial cells. The set of genes which are common to normal basal epithelial cells and basal-like breast cancer cells includes: keratin 5,6, and 17, integrin- β 4, laminin, and fatty-acid binding protein 7 [26,37]. In most of the cases these tumors are ER-negative, PR-negative, HER2-negative, CK5/6-positive, and/or EGFR (HER1) - positive [39]. Though they are considered ER/PR and HER2/*neu* negative (“triple negative”) due to low expression of the luminal and HER2 gene clusters, they are not same as triple negative (**TN**) breast cancers, as TN breast cancers represent a more heterogeneous group of tumors [40]. Other than this, relatively high occurrence of *BRCA1* (breast cancer type 1 susceptibility gene) mutations, increased genomic instability, high histologic grade, and high expression of the proliferation cluster of genes are the characteristics of this subtype of tumors [41]. Almost 15% of invasive ductal breast cancers represent this subtype of tumors [42].

Claudin-low Breast Cancer

This is a relatively new group of breast cancer, which is characterized by over-expression of genes associated with epithelial to mesenchymal (**EMT**) transition. Several genes are involved in this group of breast cancer subtype function and differentiation, such as, in cell communication (e.g., chemokine ligand 12), extracellular matrix formation (e.g., vimentin and fibroblast growth factor 7), cell differentiation (e.g., Krüppel-like factor 2), cell migration genes (e.g., integrin α 5 and moesin), angiogenesis (e.g., vascular endothelial growth factor C, matrix metalloproteinase 9 or MMP-9), immune-related genes (e.g., CD79b, CD14, and vav1), and stem-cell like genes (e.g., CD44⁺/CD24⁻ and high ALDH1A1). This subtype has shown no positive staining for luminal differentiation markers, HER2, and hormone-receptors, whereas it exhibits meta plastic and medullary differentiation [43].

Normal Breast-like Cancer

This subtype is almost similar to luminal A subtype, where cancer cells are positive for hormone-receptors (ER/PR) and negative for HER2 expression [31]. This group represents low levels of the protein Ki-67 that helps in controlling fast growth of cancer cells [31]. Though prognosis of this subtype of breast cancer is slightly worse than luminal A, it represents better prognosis than the other groups.

OTHER BREAST CANCER TYPES

There are several breast cancer subtypes which are not very common.

Medullary Carcinoma

This subtype is a unique and less-aggressive type of infiltrating ductal carcinoma. It forms a distinct boundary between tumor tissue and normal tissue. Medullary carcinoma is not a very common type of breast cancers, as it represents about 5% of all breast cancers [44].

Tubular Carcinoma

It is a subtype of invasive ductal carcinoma. Tubular carcinomas are usually made up of tube-shaped structures called “tubules” and the cells of these carcinomas look somewhat similar to normal, healthy cells and tend to grow slowly. It has been observed that the women with tubular carcinoma generally have a better prognosis than women with more common types of invasive carcinomas. This subtype of carcinoma accounts for around 2% of breast cancer diagnoses [44].

Mucinous Carcinoma

This subtype of breast cancer is also called as colloid carcinoma. It is a rare form of invasive ductal carcinoma in which the tumor is made up of mucus-producing cancer cells, generally has a better prognosis than other more common types of invasive carcinomas [45].

Inflammatory Breast Cancer

This is a rare and very aggressive form of breast cancer in which cancer cells block lymph vessels in the skin of the breast. Because of the appearance of swollen and red, or inflamed breast this type of breast cancer is called “inflammatory” breast cancer. It is extremely fast-growing form of breast cancer [46].

Phyllodes Tumor

This subtype of tumor is also known as phylloides tumor or cystosarcoma phyllodes. It is a very rare type of breast tumor. Although most phyllodes tumors are benign, some of them are cancerous [47]. All these kinds of phyllodes tumors tend to grow very quickly, and they require surgery to reduce the risk of recurrence.

Paget’s Disease of Breast

Paget disease of the breast is also known as Paget disease of the nipple and mammary paget disease. This is a rare subtype of breast cancers, which involves the skin of the nipple and the darker circle of skin around it, which is called the areola. It has been observed in most cases that patients with paget’s disease of the breast have one or more tumors inside the same breast. In this type of breast tumors the nature of the tumors are fallen under either ductal carcinoma *in situ* or invasive breast cancers [48,49].

INVOLVEMENT OF IMMUNE SYSTEM IN BREAST CANCER PATHOGENESIS

Nk Cells

Natural killer (NK) cells have prominent role in preventing both early non-metastatic and metastatic breast cancers [50]. Lower levels of NK cell activity are noticed in women with lymph node positive breast cancer compared to lymph node negative breast cancer, 18% versus 31% lytic activity, respectively [51]. Additionally, cytotoxic activity of NK cell was significantly lower in the group of people with a high familial incidence of cancer compared to the people with low cancer incidences [52]. The initial stages of human tumourigenesis may involve with the defects in NK cell-mediated cytotoxicity. Decreased NK cell activity may cause an enhancing effect of stress on tumor growth [53]. It was shown that various aspects of host immunity are altered along with the decrease in the number and function of NK cells, which in turn, causes tumor progression in breast cancer patients [54,55]. It has been observed that different immune system parameters are significantly changed from healthy controls to the advanced breast cancer tissues. These changes include decrease in NK and lymphokine activated killer cell toxicity, reduced expressions of interleukin (IL)-2, GM-CSF, interferon- (IFN) γ , and increased expressions of tumor necrosis factor (TNF)- α and IL-6 [56]. Activity of NK cell was observed as 175% lower in the breast cancer patients prior to treatment compared to healthy controls. However, the role of NK cells in breast cancer progression and control is still controversial. Study reported that it is the lymphokine-activated killer (LAK) cells which are positively correlated with the number of axillary nodes development, not the NK cells [57]. Patients with estrogen receptor positive (ER⁺) breast cancer are reported to have higher LAK cell activity. However, benign breast tumor showed NK cell activity that was not much different from NK cell activity in patients with malignant tumors [58]. Despite of the controversy, it is well accepted that NK cell activity may be associated with factors related to cancer initiation and survival. It has been shown that cytotoxic *in vitro* NK cell activity is correlated with remission in pediatric leukemia patients [59]. Though some exciting data have emerged from leukemia biology, more investigations are needed to address whether enhancement of NK cell activity is directly responsible for the greater outcome in remission of breast cancer patients.

T Cells

T lymphocytes produce cytokines which are essential for the effective immune system response. Growth and progression of solid tumors are associated with the release pattern of T-helper type 2 (Th2) cytokine, whereas T-helper 1 (Th1)-induced inflammation inhibits tumor growth [60]. More complicated scenario immerse from the clinical data. Researchers took the blood of 85 women with breast cancer prior to breast cancer surgery and adjuvant therapy. After measurement of intracellular cytokine profiles of T cells in the peripheral blood and by correlating the cytokine levels with the occurrence of micro metastases in lymph nodes and bone

marrow, they found that the percentage of CD4⁺ and CD8⁺ T lymphocytes producing type 1 (IL-2, IFN- γ , or TNF- α) and type 2 (IL-4) cytokines was significantly lower in patients with breast cancer compared with healthy controls [61]. Therefore, it is evident that the shift in the balance of type 1 and type 2 cells causes immune system dysfunction which in turn regulates the severity of the disease. Patients with larger breast tumors had a more reduced cytokine response, whereas advanced breast cancers with micro metastatic properties were also correlated with reduced cytokine levels [62]. It is still debatable that whether these altered cytokine patterns are the cause or the result of breast tumor occurrence. However, this immune system dysfunction is not only limited to advanced breast cancers, but it can be observed in early-stage breast cancer patients also. However, no positive correlation was noticed between cytokine expression in breast cancer and patient's age, cancer stage, or nodal status.

T Regulatory Cells

T regulatory cells or T reg cells are a subpopulation of T cells which can inhibit both cell-mediated (**Th1**) and humoral (**Th2**) responses. These T reg cells are characterized by CD4⁺ and CD25^{hi} and express the transcription factor Forkhead box P3 (**Foxp3**) [63]. These FoxP3-expressing T reg cells are the key mediators of self-tolerance and suppress undesirable immune responses. In scurfy mice, mutations of Foxp3 lead to reduction in T reg populations, which in turn increase the autoimmune disease progression. Many studies have indicated that T reg cells may express different surface molecules, reside at various locations [64]. As the host immune system plays an essential role in the immune surveillance and destruction of cancer cells, there is evidence that induction of T reg cells may occur by tumor formation, and down-regulation of the immune response to tumor antigens [65,66]. T reg cells may be dependent on constant antigenic stimulation to survive, sensitive to apoptosis and it can be induced in the periphery [67]. Induction of T reg cells may occur by tumors, which may inhibit the normal immune system-mediated clearance of the tumor cells. In case of breast cancer, determination of intratumoral T reg cells accumulation and activation helps in the progression of cancer. Intratumoral expression of Foxp3 in invasive breast carcinoma was analyzed and compared with its level in ductal carcinoma in situ and adjacent normal tissue [68]. It was observed that a linear association is present between intratumoral FoxP3 expression and invasion, size, and vascularity of the tumor. This finding further suggested a use for FoxP3, an indicator of T reg cell activity, as a marker of tumor progression and metastasis in breast cancer. In mice, researcher showed that Foxp3 expressing T reg cells can suppress the activity of T cells of a growing tumor, transforming growth factor β (**TGF- β**), and IL-10 secretions [69]. These findings suggest that tumor growth helps to increase T reg cells, and the T reg cells can reduce or shut down the normal immune system response to the tumor. Many other reports also showed that T reg cells are increased in the peripheral blood of breast cancer patient, and greatly increased in the breast tumor microenvironment, whereas depletion of the T reg cells markedly inhibited breast tumor growth and maintained a strong and persistent antitumor immune response [70,71].

CURRENT THERAPIES FOR BREAST CANCER

Chemotherapy

The cytotoxic chemotherapy is commonly used against both advanced and early stage breast cancers. This procedure has made significant progress in last 10 years and achieved several landmark studies representing clear survival benefits as effective therapy. Clinicians may recommend chemotherapy before breast cancer surgery (neo-adjuvant) or after surgery (adjuvant). Neo-adjuvant (or primary systemic) breast cancer chemotherapy is used before surgery not only to decrease the size of large breast tumors, but also to destroy cancer cells. It helps clinicians to determine the effectiveness of a particular regimen on the breast tumor. On the other hand, adjuvant breast cancer chemotherapy is used after surgery or radiation therapy to remove the remaining cancer cells, which may not have been eliminated during breast cancer surgery and/or radiation therapy. This procedure may also help to prevent breast cancer from spreading to other parts of the body. Though, improvements can be noticed in the use of adjuvant therapies for early stage breast cancer, the treatment of metastatic disease remains a major challenge. The main cause of failure in metastatic breast cancer treatment is the chemo-sensitivity of the tumor cells. Chemotherapeutic drugs that are commonly used by clinicians to treat primary and metastatic breast cancer include anastrozole, bevacizumab, capecitabine, cisplatin, cyclophosphamide, doxorubicin, doxorubicin liposomal injection, exemestane, fluorouracil (**5-FU**), gemcitabine, ixabepilone, letrozole, paclitaxel and trastuzumab [72]. Out of these drugs, use of anthracyclines and taxanes in the adjuvant therapy of primary and metastatic breast cancer has led to an increasing number of breast cancer patients with reduced or cured breast tumors. However, in most metastatic breast cancer cases, patients treated with anthracyclines during the adjuvant therapy are less responsive. To overcome this drawback clinicians use taxane based treatment, which is currently the standard of care. This is mainly based on a comparing trial of single agent docetaxel with mitomycin plus vinblastine [73]. However, anthracyclines represent considerable activity in chemo-naive patients or those who received them during the adjuvant therapy more than 12 months. In this case, Response rate towards anthracycline treatment is 30-40% in patients with metastatic breast cancer [74,75]. Though they are effective in adjuvant therapy, due to the high level of toxicity of these anthracycline drugs use are limited to metastatic breast cancer patients. Breast cancer is a very useful model to study the chemotherapeutic effects of new compounds, which have been tested in advanced metastatic settings and having shown efficacy with tolerable toxicity during the adjuvant therapy. Therefore, the ongoing research in understanding of the molecular and biological basis of breast cancer offers the option of generating more novel therapies against potential targets. It is evident that presence and absence of hormonal receptors (like ER,PR) and receptors like HER2 help to determine the effect of many chemotherapeutic agents like taxanes. For example, researchers showed that examination of breast cancer markers expression represented that the addition of docetaxel alone or in combination with other drugs like cyclophosphamide, capecitabine or carboplatin was

highly beneficial in the basal-like subgroup of breast cancers, marked by the absence of ER, PR and HER2 expression [76]. However, data from the clinical trials failed to exhibit the benefit of taxane administration, which was restricted to patients with HER2-positive breast cancer [77,78]. Therefore, development of more specific drug, which can target the markers of breast cancer like HER2 is much needed. To achieve this goal, researchers developed trastuzumab, a humanised monoclonal antibody against HER-2/*neu*. It provides the first example of a rationally designed targeted biological therapy for breast cancer, which was successfully tested in randomized clinical trials [79]. Though trastuzumab is now widely accepted as standard therapy, recent advancement in HER2-directed therapies have been noticed for HER2-positive breast cancer. Pertuzumab is a humanized monoclonal antibody which binds to the extracellular portion of the HER2 receptor. The binding domain of the receptor is very distinct from the binding site of trastuzumab [80]. In patients with HER2-positive metastatic breast cancer, the addition of pertuzumab to trastuzumab results in synergistic tumor cell inhibition that causes significant improve clinical outcomes [80]. Furthermore, trastuzumab is modified by linking cytotoxic maytansinoid, which resulted in a novel antibody-drug conjugate called as ado-trastuzumab emtansine (**T-DM1**). This regimen also emerged as an effective treatment option for HER2-positive aggressive breast cancers. Along with these, conventional combination therapies like trastuzumab and lapatinib; trastuzumab and Paclitaxel /Doxorubicin exert prominent tumor growth inhibitory effect against primary breast cancers and drug resistant cancers [81,82]. Another example of monoclonal antibody that acts against circulating vascular endothelial growth factor (**VEGF**) is Bevacizumab. Clinical trials revealed that Bevacizumab can control metastatic breast cancer progression [83]. It has been noticed that treatment of Bevacizumab in combination with Paclitaxel or Carboplatin significantly prolonged progression-free survival of aggressive metastatic breast cancer patients [84]. For the hormonal receptor positive breast cancer treatment with targeted therapeutic molecules along with hormonal therapies is very common. Drugs like Palbociclib (blocks proteins like cyclin-dependent kinase 4 or CDK4 and CDK6) and Everolimus (blocks mTOR) are used widely for hormone positive breast cancer treatment [85,86]. These drugs are used in combination with hormone inhibiting chemotherapeutic drugs like letrozole, fulvestrant and anastrozole. However, different side effects like mouth sores, diarrhea, nausea, fatigue, feeling weak or tired, low blood counts, shortness of breath, cough, increased blood lipids (cholesterol and triglycerides) and blood sugars are very common during treatment by most of the aforementioned chemotherapeutic drugs. Therefore, invention of side effect/ toxicity free, target specific and highly potent chemotherapeutic agents is highly needed.

Radiotherapy

Radiotherapy is a specialized cancer treatment procedure where measured and controlled high energy x-ray is carefully used. These high energy radioactive rays cause destruction of residual cancer cells that may be left behind in the breast area after surgery of the primary breast cancer. Radiotherapy has very prominent effect on cancer cells, but also causes side effects by

affecting healthy tissue in the area of treatment. However, patients generally recover from these side effects, this procedure is currently in practice for breast cancer treatment. There are two main types of radiation therapy which are presently used by clinicians: (a) external beam breast cancer radiation (traditional cancer-killing rays delivered by a large machine), and (b) internal breast cancer radiation (newer treatment procedure that inject radioactive cancer-killing particles specifically to the affected area) [87].

External Beam Breast Cancer Radiation

This is also known as traditional or whole breast radiation therapy. In this procedure, external beam radiation is given by a highly focused, regular x-ray like beam which targets the cancerous area for few minutes. Though it was the most common type of treatment used for breast cancer treatment, in recent years, internal radiation therapy is getting much attention as more patients are choosing this method for their cancer therapy when it is in the early stage of development. External radiation normally causes fewer noticeable side effects. To reduce the side effects another way of giving external beam radiotherapy is discovered which is called intensity modulated radiotherapy (**IMRT**) [88,89]. In case of IMRT, the intensity of the radiation beam can be modulated. This method allows clinicians to prescribe various amount of radiation to different treatment areas or different volume of tumors, which further enables to spare healthy cells for exposure to radiation.

Internal Breast Cancer Radiation

Internal breast cancer radiation method is applied to the selectively effected breast cancer tissues minimizing radiation to the un effected normal cells. During the treatment, the medico or surgeon inserts radioactive drugs utilizing needles, wires, or a catheter in order to target the area at the close proximity of the cancer cells that pristinely commenced to grow. Advances in internal radiotherapy treatment for breast cancer are being made with the help of brachy therapy [90]. In this particular type of radiation therapy clinicians use to place the radiation source inside the body in the area of tumor growth. Brachy therapy is considered as part of the clinical trial, yet to develop as a standard method of practice. The process of brachy therapy includes insertion of narrow, hollow tubes or a small balloon in the patient body after the removal of major part of cancerous breast tissue. Afterwards, the radioactive wires are inserted through the tubes or into the balloon. The residual cancer cells are exposed to the radioactive drug either continuously for few days or for a short time of each day. Another possible way of giving internal radiotherapy is exposing the tissues during surgical removal of tumor. This is called intra-operative radiotherapy [91]. This is not yet very popular, and not used widely. Instead of the high energy x-rays, in this type of treatment low energy x-rays are used during breast-conserving surgery. Therefore, in this process, after the removal of the cancer tissue mass, direct exposure to radiation occurs to the internal area of cancerous growth.

Because radiotherapy affects healthy tissues, it exerts several side effects like skin reaction, swelling of breast, pain in breast, hair loss, tiredness and fatigue, sore throat, lymphoedema etc. Therefore, usage of this type of therapy is highly depends on the state of breast cancer progression, as it is believed that the benefits that the breast cancer patients will receive from this kind of radiotherapy will outweigh the possible side effects.

IMMUNOTHERAPY

Immunotherapy emerged as an important treatment modality along with the growing interest in the field of targeted therapy for breast cancers. Personalized immunotherapy is getting much attention, as it is non-toxic, sustainable therapeutic option with substantial potentials. In case of personalized breast cancer medication, more immunogenic tumors represent better prognosis and effect than the non-immunogenic one [92,93]. Breast cancer profiling helps to determine the molecular levels of immune-regulatory factors. HER2 is an attractive target for breast cancer therapy as it found to be expressed in 70-80% of breast cancers, and over-expressed in 20-30% of breast cancers [94]. It has been proved that anti-HER2-specific immunity is related with high levels of both cellular and humoral immunity [95,96]. HER-2 is a major player in the field of passive immunotherapy where exogenous antibodies are administered to the patient. As mentioned earlier, the current mainstay of passive immunotherapy includes monoclonal antibodies like trastuzumab, pertuzumab, and T-DM1. Combining chemotherapy with these monoclonal antibody based passive immunotherapy causes positive synergistic effect on breast cancer treatment [97,98]. Despite the success of these monoclonal antibody based passive immunotherapy, innate and acquired tumor resistance remains a major hindrance in breast cancer treatment [99]. To overcome these hurdles another immunotherapeutic procedure is adapted by the checkpoint blockade agents which is called active immunotherapy. The strategy of these agents is to block the activity of inhibitory receptors of molecules such as cytotoxic T lymphocyte-associated protein 4 (**CTLA-4**) and programmed cell death (**PD-1**). These cytotoxic T lymphocytes (**CTLs**) are known for recognizing and destroying tumor cells, Therefore, modulation of their regulators such as regulatory T cells (**Tregs**) and immune checkpoint pathways are novel methods of active immunotherapy which exerts great therapeutic potential. The inhibitory functions T regs are enhanced by CTLA-4 activity that causes inhibition of CD8⁺ T cells. So, designing mAbs to block CTLA-4, present on both CD8⁺ T cells and T regs is a promising approach in target specific immunotherapy [100]. Ipilimumab is the monoclonal antibody that inhibits binding of CTLA-4 to its natural ligand CD80/86, which in turn causes blockage of negative activity of CTLA4 [100,101]. However, Ipilimumab shows high risk of life-threatening autoimmunity and causes autoimmune hypophysitis, hepatitis, and thyroiditis. PD-1 is another CTLA-4 blocker, which initially tested in metastatic melanoma, colorectal cancer, castrate-resistant prostate cancer, non-small-cell lung cancer, and renal cell carcinoma. It exhibited clinical efficacy and acceptably lower side effects in these cancer patients [102]. These results were further validated in breast cancer where lower toxicity is counted for PD1 than Ipilimumab [102]. For more specific active immunotherapy,

usage of cancer vaccine is getting much popularity. These cancer vaccines exhibit promising effect in patients at high risk for cancer recurrences [103]. The mode of action of these vaccines is perpetuated by the secondary release of antigens and cytokines following tumor lysis. These vaccines represent tumor specificity by generating immune response against antigens specifically expressed on tumor cells. HER2 is a major target for vaccine generation and various peptide, protein, plasma DNA, and dendritic cell- based vaccines are in the phase of clinical evaluation, which has the capability to target specifically. It is an immunogenic peptide from the HER2 protein that designed for the prevention of high risk clinical recurrences, and to achieve disease-free condition for breast cancer patients [104]. Combination of active HER2-vaccination and adoptive trastuzumab antibody immunotherapy increases the effectiveness of each approach alone [105]. HER2 DNA-based vaccines are also currently entered into various phases of clinical trials. These vaccines alone or with cyclophosphamide and doxorubicin was administered to breast cancer patients and found to be safe. They induced HER2-specific immunity in patients with metastatic breast cancers [105]. However, challenges of vaccine based immunotherapy include the complex immune escape mechanisms and antigen variability of the cancer cells. Therefore, new approach of multi-epitope vaccine preparation is getting popularity in researchers. Taken together, the field of breast immunotherapy is a dynamic approach, which has more opportunity to grow with the better understanding of the complexity of breast tumor progression.

Gamma Delta T Cells for Immunotherapy

Pre-clinical application of $\gamma\delta$ T cells

Immunotherapy is one of the important areas in developing novel anti-tumor therapeutics apart from surgery, radiotherapy, hormone therapy, and chemotherapy. The adoptive immunotherapy is accomplished by expanding immune effectors cells *in vitro* and transferring the activated immune cells into the hosts, that target against tumor cells or stimulate immune response to eliminate tumor cells [106]. Most of the human peripheral blood T cells express T cell receptor (**TCR**) $\alpha\beta$ but only 1-5% express TCR $\gamma\delta$ receptors. These $\gamma\delta$ T cells can contribute to the immune response against all subtype of breast cancer including triple negative breast cancer directly through their cytotoxic activity and indirectly by stimulating or regulating the biological functions of other cell types required for the initiation and establishment of the anti-tumor immune response, such as dendritic cells and cytotoxic CD8⁺ T cells. They differ from conventional $\alpha\beta$ T cells, since most of $\gamma\delta$ T cells do not express the CD4 and CD8 co-receptors and, as a consequence, antigen recognition by $\gamma\delta$ TCR is not controlled to major histo-compatibility complex (**MHC**) molecules [107]. $\alpha\beta$ TCR interact with peptides bound to MHC class I or class II molecules but $\gamma\delta$ TCR recognize a diverse array of self and non self antigens, such as small peptides, soluble or membrane proteins, phospholipids, prenyl pyrophosphates, and sulfatides. Different mechanism is responsible for TCR-dependent $\gamma\delta$ T cell responses due to various antigenic diversity [108]. Moreover, as $\gamma\delta$ T cell activation does not require antigen processing and presentation by antigen-presenting cells (**APC**), $\gamma\delta$ T cells can be rapidly activated and act during the early phase of the immune response.

Like NK cells, $\gamma\delta$ T cells also respond to stimulation by stress- and/or infection-induced ligands, such as the MHC class I-related molecules H60, RAE1, and MULT-1 in mice [109], or MICA/B and ULBP in humans [110,111].

Most of the breast cancer cells generally expressed MHC class I chain-related molecule **(MICA/B)** on their surface [112,113]. This MICA/B molecule is recognized by $\gamma\delta$ T cells through their natural killer group 2, member D protein **(NKG2D)** receptors. The engagement between NKG2D and its ligands enhances cell-mediated cytotoxicity and cytokine production against breast cancer cells. The ligand binding to NKG2D can affect the release of TNF α , interleukin **(IL)-2**, and increase cytolytic potential of $\gamma\delta$ T cells. The treatment with adoptive transfer of $\gamma\delta$ T cells in combination with IL-2 showed delayed cancer growth. This $\gamma\delta$ T cells can also kill tumor cells through antibody dependent cellular cytotoxicity **(ADCC)**. $\gamma\delta$ T cells expressing CD16 molecule interacts with tumor associated antigens **(TAA)** present in cancer cells via specific monoclonal antibodies, and mediate ADCC. CD16 represent activation/memory status of $\gamma\delta$ T cells, and these CD16 cells have specific phenotypic features that distinguish these from the non-CD16 subset. These cells constitutively express several natural killer receptors (NKG2A/CD94) and high amounts of perforin, but express low levels of chemokine receptors and IFN- γ . CD16/Fc γ RIII receptor binds to Fc portion of immunoglobulin G **(IgG)** and engagement of CD16 by $\gamma\delta$ T cells leads to antibody-dependent cellular cytotoxicity **(ADCC)** [114]. ADCC is a process in which CD16⁺ effector cells actively lyse tumor cells that have been found by specific antibodies. Several studies proven that *in vitro* $\gamma\delta$ T cells become activated via CD16 and mediate ADCC against tumor with presence of monoclonal antibody trastuzumab, atumumab, and lemtuzumab. The $\gamma\delta$ T cells increase the efficacy of trastuzumab *in vivo* Her 2⁺ breast cancer patients. These $\gamma\delta$ T cells favorably kill all types of cancer cells including breast cancer cells and show low reactivity towards non-transformed cells, which make these cells very good candidate for cancer immunotherapy.

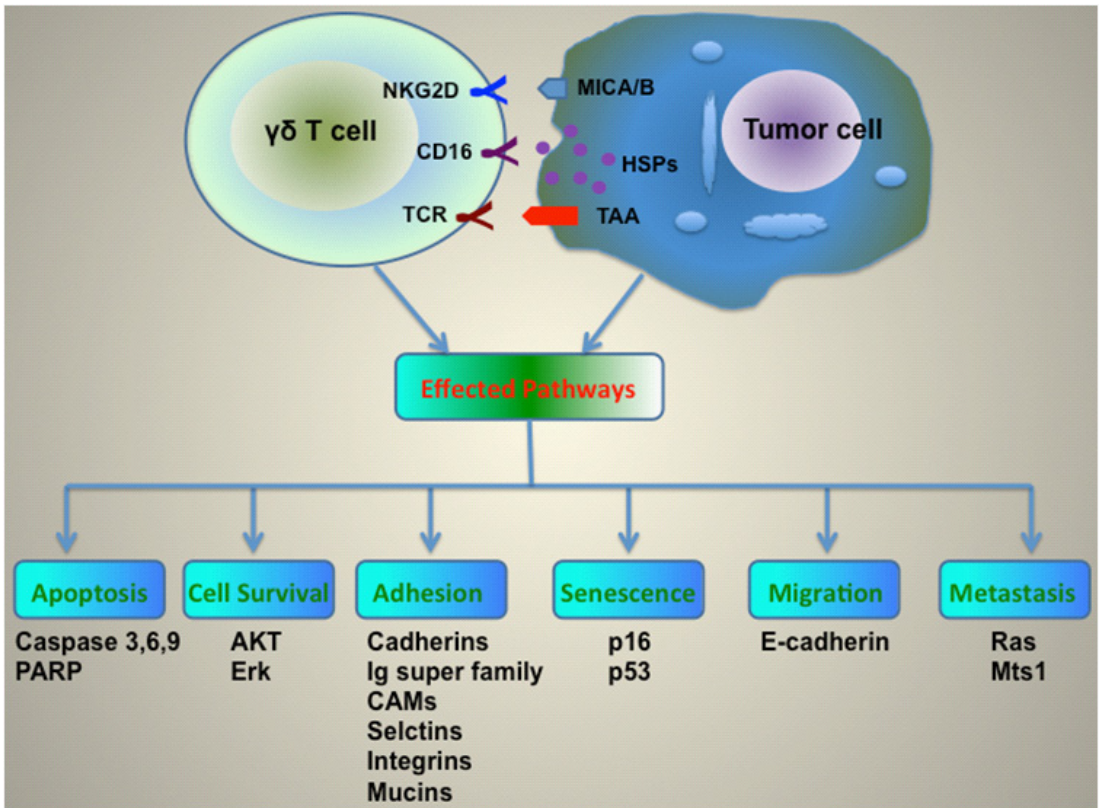


Figure 2: Gamma delta T cell interaction with tumor cell, effected pathways and relevant signaling molecules.

Clinical application of $\gamma\delta$ T Cells

$\gamma\delta$ T cells can be utilized for antitumor therapies in an unconventional manner [115], because they exhibit a potent MHC-unrestricted lytic activity against a wide variety of tumor cells. Given the potent anti-tumour effect or function of $\gamma\delta$ T cells and broad reactivity to many different types of tumours has raised a great interest to explore their clinical trial. In clinical studies, $\gamma\delta$ T cells have been shown to infiltrate into various kinds of tumors, including lung carcinomas [116], renal cell carcinomas [117], and breast carcinomas [118]. Also, an important feature of $\gamma\delta$ T cells is that these favourably kill cancer cells and show low (if any) reactivity towards non-transformed cells which makes these very attractive candidates for cancer immunotherapy [119]. Therefore, the safety and efficacy of $\gamma\delta$ T cell-based immunotherapy have been evaluated in several clinical trials [120]. Presently, two strategies were applied for $\gamma\delta$ T cell-based cancer immunotherapy. One is the adoptive cell transfer of $V\gamma9V\delta2$ T cells, and second one is the administration of pAg or aminobisphosphonates along with low-dose recombinant IL-2 to stimulate $V\gamma9V\delta2$ T cells. To examine the anti-tumor activity of $\gamma\delta$ T cells in breast cancer, a phase I trial was conducted, in which zoledronate and low-dose IL-2 were administered to ten advanced metastatic breast

cancer patients who were therapeutically terminal [121]. The treatment was well tolerated and promoted the effect or maturation of V γ 9V δ 2 T cells in all patients. It is noteworthy that there was a statistically significant linkage between clinical outcome and the number of peripheral V γ 9V δ 2 T cells, as seven patients who failed to sustain V γ 9V δ 2 T cells showed progressive clinical deterioration, while three patients who sustained robust peripheral V γ 9V δ 2 cell populations displayed one instance of partial remission and two of stable disease, respectively. In addition, the treatment of patients with V γ 9V δ 2 T cells plus zoledronate could efficiently enhance the lysis of breast tumor cells and prostate carcinoma cells [118].

MICA AS A TARGET MOLECULE

The MICA/B genes encode for non conventional class I HLA molecules which have no role in antigen presentation. But MICA/B is up-regulated by many stress conditions such as heat-shock, oxidative stress, neoplastic transformation, and viral infection. A stress-inducible MICA, which is identified as a human ligand of NKG2D [122] expressed on the membrane of viral-infected cells and many carcinoma cells such as in lung, breast, ovary, prostate, colon cancer, but is usually absent from normal tissue [123,124]. MICA expression was also increased in epithelial cell lines following infection by adherent Escherichia coli, in dendritic and epithelial cells after mycobacterium tuberculosis infection [111], and in cytomegalovirus-infected fibroblasts and endothelial cells [125]. Human macrophages infected with influenza virus or sendai virus exhibited augmented levels of MICB [126].

Heat shock treatment also up-regulates MICA on epithelial cells. Indeed, the MICA and MICB promoters contain heat shock transcriptional elements similar to those found in the promoters of heat shock proteins, such as heat shock protein 70 (**HSP70**). Recently, a wide variety of stimuli causing genotoxic stress or resulting in DNA replication arrest, including treatments with ionizing radiation, cisplatin, aphidicolin, mitomycin C or hydroxyurea, were shown to significantly up-regulate NKG2D ligands in transformed murine ovarian epithelial cells and normal adult fibroblasts, and in human secondary foreskin fibroblasts [127]. MICA/B is the ligand for the NKG2D expressed on the NK cells and V γ 2V δ 2 T cells. Many cancer cell which express stress-related molecule MICA/B on their surface that is recognized by V γ 2V δ 2 T cells through their NKG2D receptor. The expression and modulation of MICA on tumor cells have effects on cell survival. It was showed that the tumor cell survival from the recognition of $\gamma\delta$ T cells is correlated with the down regulation of the MICA/B molecule novel mechanisms for tumor evasion against innate host immune response [113,128,129].

MOLECULAR MECHANISMS

Relatively little is known about the molecular mechanisms that trigger NKG2D ligand gene expression. Several pathways such as DNA damage-induced ataxia-telangiectasia-mutated (**ATM**), Rad3-related protein kinases (ATM-ATR) signaling and oncogene-induced PI3K-AKT signalling pathway is responsible for activation of NKG2D ligands.

The DNA damage response pathway, initiated by the ataxia telangiectasia mutated (**ATM**) or ataxia telangiectasia and Rad3 related (**ATR**) kinases, is implicated in the regulation of NKG2D ligands. Inhibition of ATM, ATR and of a downstream mediator of these pathways, Checkpoint kinase 1 (**Chk1**), suppressed the observed up-regulation of the ligands for NKG2D in response to the different treatments, demonstrating that these pathways play an important role in ligand regulation [127,129]. Furthermore, down-regulation of ATM pathway resulted down-regulation of MICA, and reduced V γ 2V δ 2 T cell-mediated cytotoxicity. p53, which is also a downstream mediator of the ATM/ATR pathways, [130,131] is not necessary for the induction of ligand expression in response to the various treatments [127]. But treatments that modify chromatin, resulting in a more open structure, have also been shown to up-regulate NKG2D ligand expression [127,132].

Apart from ATM, ATM-ATR or PI3K-AKT signaling pathway, mevalonate pathway is responsible to activate TCR- $\gamma\delta$ cells. Human cells utilize the mevalonate pathway for isoprenoid and IPP biosynthesis [133,134], which also activates V γ 9V δ 2 T cells *in vitro* by professional antigen-presenting cells or tumor cells [135] but only at concentrations not achieved physiologically in non-transformed cells. However, certain tumors yield higher concentrations of IPP, which can be sensed by V γ 9V δ 2 T cells.

CONCLUSIONS AND FUTURE DIRECTIONS

Along with the increased complexity of various types of breast cancers, application of different mode of therapeutic procedures is needed. However, the best solution of the problem could be achieved by avoiding the causes of breast cancer occurrence and taking extra precautions for the early prognosis of the disease. But, in case of clinically advanced, highly metastatic life threatening breast cancers, usage of novel approaches like neo-adjuvant, adjuvant chemotherapy, radiotherapy with minimum side effects and passive/active/vaccine based immune therapies may provide better therapeutic outcome to the patients. Recent findings have allowed researchers to refine breast cancers not only based on their popular prognostic markers, but also by the other molecules involved in cancer immunology and pathogenesis. Recently, the $\gamma\delta$ T cell-mediated therapy is showing a great promise. Clinical trials of immune therapies using $\gamma\delta$ T cells conducted in the past few years. Multiple trials proved that immune therapies using $\gamma\delta$ T cells were safe and well tolerated. Combinations of newly emerging therapy with established treatments could minimize the potential side effects of immune reconstitution in the future. But there are some aspects that could be improved in future clinical trials. Immunotherapy may induce significant adverse reaction. Activated $\gamma\delta$ T cells can produce proinflammatory cytokines that may elicit severe adverse reactions. The clinical efficacy of $\gamma\delta$ T cell immunotherapy should be further assessed.

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