Review on breast cancer immunotherapy

Selamawit FentahunAli

School of Veterinary Medicine, Wollo University

ABSTRACT

Cancer immunotherapy consists of approaches that modify the host immune system, and/or the utilization of components of the immune system for treatment and prevention of cancer. Eventhough breast cancer has historically not been considered a favorable target for immunotherapies as compared to those solid tumors such as melanoma and renal cell carcinoma, several preclinical and clinical studies have provided important insights and clinical data that support its potential to improve clinical outcomes for patients with breast cancer. In addition, the recent definition of tumor-specific immunity in breast cancer patients and the identification of several breast cancer antigens has generated enthusiasm for the application of immune based therapies to the treatment of breast malignancies. In general, immunotherapies can be considered either non-specific, such as cytokine, or tumor specific for example a vaccine that targets breast cancer tumor antigens. Current immunotherapeutic approaches either through immunization of the patient (e.g., by administering a cancer vaccine), in which case the patient's own immune system is trained to recognize tumor cells as targets to be destroyed, or through the administration of therapeutic antibodies as drugs, in which case the patient's immune system is recruited to destroy tumor cells by the therapeutic antibodies, Cell based immunotherapy is another major entity of cancer immunotherapy. The review concluded that immunotherapeutic approaches combined with novel sequences of chemotherapies, radiation, and immunomodulating agents hold promise for enhancing the treatment of breast cancer.

Keywords: immunotherapy, monoclonal antibodies, cytokine, breast cancer, immune system and cancer vaccines.
INTRODUCTION
The word “cancer” is an umbrella term that refers to about 200 diseases that share two common characteristics: first, an uncontrolled, abnormal growth of cells and second, the ability to invade and damage normal tissues either locally or at distant sites in the body. Some human cancers arise in the epithelium that is the layers of cells covering the surface of the body and the lining of internal organs and various glands; these cancers are called carcinomas. Cancers of the supporting tissues of the body, such as bone, muscle and blood vessels are sarcomas. Cancers of the blood and the lymph glands are called leukemias and lymphomas, respectively. Gliomas are cancers of the nerve tissue. Melanomas arise from darkly pigmented cells, usually in the skin [1].

Breast cancer is the most common type of cancer among women worldwide other than skin cancer. Ductal carcinoma and lobular carcinoma are two common types of breast cancer that begins in cells that line a breast duct and in a lobule of the breast respectively. It is a heterogeneous disease embracing a range of clinical patterns, biologic behavior, prognostic characteristics, and response to different types of treatment [2].

Increased risk for breast cancer is associated with a personal or family history of the disease and inherited genetic mutations in the breast cancer susceptibility genes BRCA1 and BRCA2. These mutations account for approximately 5%-10% of all breast cancer cases, but are rare in the general population (less than 1%). Women with BRCA1 and BRCA2 mutations have an estimated 40% to 85% lifetime risk of developing breast cancer. Other known risk factors including obesity, using a hormone therapy that combines progestin and estrogen, high breast tissue density, alcohol consumption, and physical inactivity. Each year, breast cancer represents 11 percent of all cancers diagnosed globally, and it is the second leading cause of cancer-related death among women. In 2008, there were 1.38 million new diagnoses worldwide and 458,000 deaths. Approximately 1 in 8 U.S. women will develop invasive breast cancer at some point in their lives. Therefore it is crucial to understand and control cancer because to date, apart from heart disease, more people die from cancer than any other disease [2].

International efforts have been made to improve survival rate by early diagnosis and multiple therapies. Despite the clinical success of conventional anti-cancer therapies such as Surgery, radiation therapy, chemotherapy, and hormone therapy which have many limitations. Among the limitations of these therapies are low overall response rate, a very narrow therapeutic index (NTI), rapid and severe systemic toxicity, and frequent resistance. However, limitations of current therapeutic modalities have led to increasing enthusiasm for developing highly targeted therapies to improve the efficacy and safety of cancer treatments [3].

Immunotherapy is one of the current therapeutic modality and it has been well documented since 1890 when Coley demonstrated that bacterial products known as Coley’s toxins and recognition that antibodies might be “magic bullets” for cancer therapy and the subsequent application of Bacillus Calmette-Guerin (BCG) and other crude immunostimulants showed benefits that led to regulatory approval of their use in some solid tumors such as bladder cancer [4].

Cancer biotherapy, or immunotherapy, is the manipulation of the immune system, or utilization of components of the immune system for treatment and prevention of cancer. The immune system is perfectly structured to distinguish self from non-self, as demonstrated by anti-microbial immune responses. Moreover the immune system has the potential to recognize self from “altered-self”, which is the case for cancer. The immuno-editing theory suggests that the immune system is capable of recognize and eradicate subclinical tumors, but at some point equilibrium is reached and further many tumors escape from this equilibrium state, and cancer becomes clinically apparent. In this
case the objective of the cancer immunotherapist is to understand the mechanisms by which cancer is able to escape the immune system and to therapeutically intervene at critical points to promote anti-tumor immune responses [5].

Historically breast cancer has not been perceived as an immunogenic tumor when compared with diseases such as melanoma and renal cell carcinoma, which have long used immunotherapy such as interleukin 2 (IL-2) with some success, several preclinical and clinical studies have provided important insights and clinical data that support its potential to improve clinical outcomes for patients with breast cancer. Cells with a greater influx of tumor-infiltrating lymphocytes tend to respond better to neoadjuvant chemotherapy compared with less immunogenic tumors [6].

The antigens which are found on breast cancer should be primarily identified and then understanding of how antigens interact with T cells and antibodies at the molecular level, has given rise to the engineering of specific immunotherapies that target breast tumors. Many of these immune based treatment strategies are currently being tested in human clinical trials.

Recent advances in cellular and molecular immunology have greatly improved our understanding of how T cells recognize cancer-related proteins. CD8+ cytotoxic T cells (CTL) can kill cells expressing antigen. CD4+ T helper cells have the potential to enhance the antigen-specific immune response via cytokine secretion. Subsets of CD4+ T cells are Th1 and Th2. Th1 secrete cytokines such as (interleukin-2 [IL-2]), interferon [IFN]- γ, tumor necrosis factor [TNF]-α (IL-2) and that stimulate the proliferation and activity of cytotoxic T cells where as Th2, secrete cytokines such as IL-4, 5, 6, and 10 that result in more effective antibody formation [4]. The interplay and inter-action between the various arms of the immune system give emphasis to the complexity of those interactions as well as the potential for utilizing multiple immunotherapeutic modalities in the treatment of breast cancer [4].

Patients on experimental procedures together with informative studies of murine tumor models have clearly demonstrated that their immune system plays a major role in tumor outcome [7]. These findings have led to the development of therapeutic strategies that aim to enhance immune-mediated tumor destruction and to counteract tumor-induced immune suppression. This review describes the major immunotherapeutic approaches that have the potential to enhance or generate an antibrust cancer immune response: (i) cytokine therapy; (ii) cancer vaccines; (iii) monoclonal antibody therapy, and (IV) Adoptive cellular therapy and explores how each approach has been applied to the treatment of breast cancer.

**SIGNS AND SYMPTOMS OF BREAST CANCER**

Most commonly, the first sign of breast cancer is a new lump in the breast. The lump is usually painless.

Other signs of breast cancer include
- A new area of thickened tissue in the breast.
- Nipple discharge or a change in the nipple.
- Dimpling or puckering of the skin of the breast.
- A change in breast size or shape.

While these symptoms may not be related to breast cancer, it is important to see a doctor promptly for assessment and accurate diagnosis if any of these symptoms are present. Early detection is vital in the successful treatment of breast cancer [8].

**DIAGNOSIS**

In order for an accurate diagnosis to be made the three step approach of clinical examination, imaging, and surgery will be required.

**Clinical examination**

Check the abnormal lump's size, location and other characteristics such as whether it is mobile, hard or soft, regular or irregular. The
doctor will ask about the history of the lump such as how long it had been there, has it grown, and is it painful. Risk factors such as family history or previous breast lumps will be asked about.

**Imaging tests**

**Diagnostic mammography**- Diagnostic mammography is similar to screening mammography except that more views (pictures) of the breast are taken, and it is often used when a woman is experiencing signs, such as nipple discharge or a new lump. Diagnostic mammography may also be used if something suspicious is found on a screening mammogram.

**Ultrasound**- An ultrasound uses high-frequency sound waves to create an image of the breast tissue. An ultrasound can distinguish between a solid mass, which may be cancer, and a fluid-filled cyst, which is usually not cancer.

**MRI**- An MRI uses magnetic fields, not x-rays, to produce detailed images of the body. A contrast medium (a special dye) is injected into a patient’s vein to create a clearer picture of the breast. A breast MRI may be used once a woman has been diagnosed with cancer to check the other breast for cancer or to find out how much the disease has grown throughout the breast.

**Surgical tests**

**Biopsy**- A biopsy is the removal of a small amount of tissue for examination under a microscope. Other tests can suggest that cancer is present, but only a biopsy can make a definite diagnosis. The sample removed during the biopsy is analyzed by a pathologist. There are different types of biopsies, classified by the technique and/or size of needle used to collect the tissue sample.

- A fine needle aspiration biopsy uses a thin needle to remove a small sample of cells.
- A core needle biopsy uses a thicker needle to remove a larger sample of tissue. This is usually the preferred biopsy technique for finding out whether an abnormality on a physical examination or an imaging test is cancer.

- Image-guided biopsy is used when a distinct lump can't be felt, but an abnormality is seen with an imaging test, such as a mammogram. During this procedure, a needle is guided to the location with the help of an imaging technique, such as mammography, ultrasound, or MRI. A stereotactic biopsy is done using mammography to help guide the needle.

- A surgical biopsy removes the largest amount of tissue. This biopsy may be incisional (removal of part of the lump) or excisional (removal of the entire lump). Because definitive surgery is best done after a cancer diagnosis has been made, a surgical biopsy is usually not the recommended way to diagnose breast cancer. Most often, non-surgical core biopsies are recommended to diagnose breast cancer [8].

**ADVANTAGES OF IMMUNOTHERAPY FOR BREAST CANCER**

Immunotherapy holds several key advantages over conventional chemotherapeutic treatments, as well as over new targeted therapies. The most common type of pharmacological anticancer treatment has been conventional chemotherapy. This type of treatment does not discriminate between rapidly dividing normal cells and tumor cells, thus leading to severe systemic side effects, where as targeted therapies depend on the cell’s oncogenic dependency on specific pathways. However, it is known that many cells can activate compensatory signaling to make them resistant to these therapies. For instance several strategies for targeting tyrosine kinase have been developed, the most successful being monoclonal antibodies and small molecule tyrosine kinase inhibitors. However, increasing evidence of acquired resistance to these drugs has been documented. New clinical and laboratory studies have suggested that multiple receptor tyrosine kinase inhibitors against
neoplastic cells could help to increase patient survival and possibly reduce the emergence of cells resistant to single-target inhibitors. This increased activity will have to be balanced by the expected increased toxicity due to the association of the drugs that may limit their feasibility. Moreover, combination of mAbs and multi-target small molecules could be also a very promising therapeutic approach. In contrast, the ability of immunotherapy to target and eradicate micro metastatic disease is dependent on tumor cells’ protein expression [9].

Another advantage with some immunotherapies such as vaccines is that they can be given over much longer periods in the adjuvant setting. An activated immune response maintained through booster vaccines can patrol the body for residual disease over an extended period without significant toxicity to the patient or a lengthy time commitment. The initial results of the HER2 E75 peptide vaccine studies demonstrated initial protection over the first few years, but this protection waned as the immune response declined. This finding led the investigators to incorporate a booster schedule in the ongoing phase III E75 adjuvant trial [6].

**IMMUNE SYSTEM FIGHT AGAINST CANCER**

The reason why immunotherapy is so attractive is the specificity; an immune response can be directed against tumor-associated antigens (TAA) or tumor specific antigen (TSA) through two mechanisms these are:- the humoral response and the cellular response. The humoral response is triggered by the interaction between the variable regions of an antibody with specific epitopes on cell-surface molecules. The cellular response involves recognition of antigens by T-cell receptors (TCRs) when they are presented by the cell in conjunction with the major histocompatibility complex (MHC) molecules. Antibodies are not capable of detecting the small processed peptides on MHC molecules on the cell surface, so the nature of the antigens that are recognized by the humoral and cellular arm is different [10].

The initiation of an antitumor immune response rests with antigen presenting cells (APCs) to process and present tumor-related antigens. Proteins are phagocytosed by APCs, partially digested into smaller polypeptides, and bound to MHC class II molecules. After the antigen-MHC complexes are transported to the cell surface they can be recognized by naïve T lymphocytes through the TCR. When a naïve helper (CD4⁺) T cell recognizes the antigen, as well as costimulatory molecules that are present on the APC, it becomes activated. Activation results in proliferation and differentiation and the activated helper T cell can then help to promote a cellular response (Th1) or a humoral response (Th2). Th2 response ultimately leads to the stimulation of B cells to proliferate and differentiate into plasma cells through the secretion of B-cell stimulatory cytokines (interleukin [IL]-4, IL-5, IL-6, and IL-10). Antibodies that are produced by the plasma cells can recognize breast cancer cell surface antigens and kill tumor cells by a variety of methods. One important method is antibody dependent cell-mediated cytotoxicity (ADCC), which involves the attachment of tumor-specific antibodies to tumor cells and the subsequent destruction of the tumor cell by immunocompetent cells, most commonly the natural killer (NK) cell. Another way in which antibodies lead to tumor death is through complement-dependent cell-mediated cytotoxicity (CDCC), where the recognition and attachment of complement-fixing antibodies to tumor specific surface antigens is followed by complement activation and cell death [6].

A cellular response occurs when a naïve cytotoxic (CD8⁺) T cell recognizes antigen that is being presented on the surface of an APC. The TCRs on cytolytic T cells recognize antigen that is presented on MHC class I molecules. In the presence of costimulatory molecules on the APC and cytokines that are released from the Th1 helper T cell (IL-2, interferon [IFN]- γ, tumor necrosis factor [TNF]- α) the cytolytic T cell is activated. Once activated, cytolytic T cells destroy tumor cells by way of TCR recognition of...
tumor-specific antigen that is presented on MHC class I molecules at the tumor cell surface. They bind to the MHC class I receptor–tumor antigen complex and destroy the tumor cell [10].

**BREAST CANCER–ANTIGENS**

The success of immunotherapy rests upon the presence of breast cancer antigens to which either a humoral or cellular response can be initiated. Tumor antigens have been classified into two broad categories: tumor-specific shared antigens and tumor-specific unique antigens. Shared antigens or tumor-associated antigens (TAAs) are expressed by more than one type of tumor cells. A number of TAAs are also expressed on cancer cells and normal cells; typically, they are over expressed in cancerous tissue such as human epidermal growth factor receptor–2 (HER-2/neu), mucin (MUC-1) cancer-testis antigens, and carcinoembryogenic antigen (CEA) [11].

In breast cancer, the most important of these antigens is the human epidermal growth factor receptor–2 (HER-2/neu). This transmembrane growth factor receptor protein is expressed at low levels on normal tissue but is over expressed in many types of malignancies, including 30% of breast cancers. This over expression makes HER-2/neu as an appealing molecular target for drug therapy. Another breast cancer antigen that is of interest is mucin (MUC-1), a membrane-bound glycoprotein that consists of a polypeptide core and numerous carbohydrate side chains. The version of MUC-1 that is expressed on breast cancers differs from that in normal tissues in that the carbohydrate side chains are shorter and the peptide backbone is more exposed. As a cell surface glycoprotein, it is most efficient in inducing a humoral response; the presence of a MUC-1 specific IgG and IgM antibody response may be associated with a longer disease-free survival [10].

Cancer-testis antigens in breast cancer include MAGE-3 and SSX-expressed proteins are antigens that present on germ line cells but not on somatic cells. Changes in transcriptional regulation in cancer cells can lead to expression of these antigens. Because germ line cells do not express MHC molecules, these antigens normally are silent; however, when expressed on cancer cells, they are capable of eliciting an immune response. Whereas unique tumor antigens result from mutations induced through physical or chemical carcinogens; they are therefore expressed only by individual tumors. Tumor-specific unique antigens encompass melanocyte/melanoma differentiation antigens, such as tyrosinase, MART1 and gp100, prostate-specific antigen (PSA) and Idiotype (Id) antibodies [11].

1. **SPECIFIC IMMUNOTHERAPY FOR THE TREATMENT OF BREAST CANCER**

1.1 **Monoclonal Antibody**

Immunotherapy is based on the production of humanized monoclonal antibodies (mAbs) that bind with high specificity to secreted proteins or to the extracellular domain of membrane-bound proteins. Many copies of a specific antibody can be made in the laboratory. These are known as monoclonal antibodies (mAbs or moAbs). The use of mAbs relies on the principle that most of the targeted molecules are expressed at higher levels on neoplastic cells, when compared to normal cells, where they play an important role in sustaining cancer progression. Monoclonal antibodies are now used to treat many diseases, including some types of cancer. A major advantage of these drugs is that because they are so specific, they may have only mild side effects, unlike some other cancer treatments. But researchers first have to identify the right antigen to attack. Two types of monoclonal antibodies are used in cancer treatments such as; Conjugated mAbs and naked mAbs [9].

1.1.1 **Conjugated monoclonal antibodies**

Monoclonal antibodies attached to a radioactive substance, drug, or toxin are called conjugated mAbs. This mAb is used as a homing device to take one of these substances directly to the cancer cells. It circulates in the body until it can find and catch the target antigen. The mAb then delivers the toxic substance where it is needed.
most to diminish the damage to normal cells in other parts of the body.

Conjugated mAbs are also sometimes referred to as tagged, labeled, or loaded antibodies. They can be divided into groups depending on what they are linked to mAbs with radioactive particles attached are referred to as radiolabeled, and treatment with this type of antibody is known as radioimmunotherapy (RIT), mAbs with chemotherapy drugs attached are referred to as chemolabeled, mAbs attached to cell toxins are called immunotoxins [9].

1.1.2 Naked monoclonal antibodies

These are antibodies that work by themselves with no drug or radioactive material attached to them. Most naked mAbs attach to antigens on cancer cells, but some work by binding to antigens on other, non-cancerous cells, or to even free-floating proteins. Naked mAbs can work in different ways. Some may boost a person's immune response against cancer cells. Others work by blocking specific proteins that help cancer cells grow and some may do both. Some naked MAbs attach to cancer cells to act as a marker for the body's immune system to destroy them. An example of this is alemtuzumab (Campath®), which is used to treat some patients with chronic lymphocytic leukemia. Alemtuzumab is an antibody that binds to the CD52 antigen, which is found on immune cells called B cells and T cells. Once attached, the antibody triggers the destruction of the cell by the immune system. Some naked mAbs work mainly by attaching to and blocking specific antigens that are important signals for cancer cells (or other cells that help cancer cells grow or spread). For example, trastuzumab (Herceptin®) is an antibody against the HER2/neu protein. A large amount of this protein is present on the cells in some types of cancer. When HER2/neu is activated, it helps these cells grow. Trastuzumab stops these proteins from becoming active. It is used to treat breast and stomach cancers that have large amounts of this protein [12].

Trastuzumab (Herceptin®), which is FDA-approved for the treatment of HER-2/neu expressing breast cancer, facilitates down regulation of the HER2 receptor (overexpressed in about 30 percent of breast cancer cases). It was the first monoclonal antibody to be approved for treating solid tumors. The therapeutic activity of trastuzumab has been evaluated in women with metastatic breast cancer as a single agent given before or after traditional chemotherapy, and in combination with a variety of chemotherapy agents. The first phase II trials demonstrated objective response rates from 12 to 15 %. These studies established that trastuzumab therapy can be effective in breast cancer patients. On the basis of the convincing preclinical studies, clinical trials were conducted and demonstrated the benefits of combining chemotherapy with trastuzumab therapy; one such study enrolled women who had not received previous adjuvant therapy to examine the combination of trastuzumab therapy and chemotherapy. The addition of trastuzumab therapy to chemotherapy was associated with a longer time to disease progression, a higher objective response rate, and longer survival. Based on these results, the FDA approved trastuzumab, given either alone or in combination with chemotherapy, for treating patients with metastatic breast cancer over expressing HER2/neu [12].

Randomized trials have confirmed that disease free interval was significantly improved with 52 weeks of trastuzumab added to adjuvant chemotherapy. Based on risk benefit ratio the researcher recommends that trastuzumab be incorporated into a concurrent regimen with taxane chemotherapy as an important standard-of-care treatment alternative to a sequential regimen [13].

The mechanisms of action of unconjugated monoclonal antibodies such as Trastuzumab (Herceptin®) include blocking a pro-survival signal that inhibits the capacity to promote tumor growth, as well as facilitating tumor cell destruction by the binding of the Fc portion of the
antibody to Fc Receptors on natural killer (NK) cells—promoting the ability of NK cells to lyse their targets through a process known as antigen-dependent cytotoxicity (ADCC). Monoclonal antibodies can also mediate cytotoxicity by binding to complement receptors on effector cells, a process known as complement-dependent cytotoxicity (CDCC). The Fc portion of a monoclonal antibody plays a major role in determining the immune mechanisms induced, with monoclonal antibodies of the human IgG4 isotype primarily functioning as "blockers". One interesting aspect involved in the development of monoclonal antibodies for the clinic involves their affinity, while higher antibody affinity results in increased target engagement and ADCC, higher affinities can also result in decreased tumor penetration and compromised efficacy [5].

The most significant toxicity associated with trastuzumab use is cardiac dysfunction, or congestive heart failure, that typically is mild and reversible, but may leave behind permanent subclinical damage. In the presence of clinical heart failure, or documentation of left-ventricular dysfunction, trastuzumab should be discontinued until cardiac function has improved. As a single-agent, trastuzumab was associated with a 4%-5% rate of heart failure. Clinical trials have shown that cardiotoxicity is markedly increased when trastuzumab is administered with or follows cardiotoxic agents such as anthracyclines. Therefore combination therapy of trastuzumab with such agents should be avoided [4].

The targeting of other breast cancer antigens, including MUC-1 and epithelial cell adhesion molecule (EpCAM), with monoclonal antibody therapy is under clinical and laboratory investigation. MUC-1 is expressed in 80% of breast malignancies, so passive immunotherapy against MUC-1 would be applicable to a much larger fraction of women who has breast cancer. The low level of glycosylation on the aberrant MUC-1 that is present on breast cancer cells unmask epitopes that are not exposed on normal mucin and creates targets for tumor-specific antibodies. Preclinical research identified several anti–MUC-1 mAbs that are effective in killing breast cancer cells. DF3 is mAb that is directed against MUC-1; it increased antigen-directed phagocytosis and cytolysis of MUC-1–expressing human breast cancer cells in vitro [10].

Other monoclonal antibody targeting a protein known as SFRP2 has been shown by researchers at the University of North Carolina to inhibit tumor growth in pre-clinical models of breast cancer and angiosarcoma. As Dr. DeMore said “We previously micro dissected blood vessels from malignant human breast cancers and compared gene expression to blood vessels micro dissected from normal tissue. We found a number of genes that were highly over-expressed in the malignant blood vessels compared to normal. One of those genes was SFRP2,” [14].

Researchers confirmed the over-expression of Nectin-2 protein in breast and ovarian cancer tissues by immunohistochemistry (IHC) and found that Nectin-2 protein was abundantly present in these cancer tissues while it was undetectable in normal breast and ovary tissues. A more extensive IHC study indicated that Nectin-2 protein was over-expressed in more than 80% of breast cancer tissue samples and approximately 50% of ovarian cancer tissues samples. This study demonstrated that over-expression of Nectin-2 in breast and ovarian cancer tissues and showed that the anti-Nectin-2 human mAb Y-443 exerts an in vivo anti-tumor effect on OV-90 and MDA-MB-231 cells via ADCC as the main mechanism of action. Thus, Nectin-2 was shown to be a promising target for antibody-based breast and ovarian cancer therapies [15].

1.2 Breast Cancer Vaccines

The term of cancer vaccine refers to a vaccine that prevents either infections with cancer-causing viruses or the development of cancer in certain high risk individuals known as prophylactic cancer vaccine and eliminate the
cause of a given disease, e.g., to eliminate cancer cells or virally infected cells and to treat the disease. Their activity is mostly dependent on antigen-specific CD8+ T cells that generate cytotoxic T lymphocytes (CTLs) to reject cancer or infected cells known as therapeutic cancer vaccine [16]. Prevention or treatment with a cancer vaccine is a very attractive therapeutic option because the mechanism of action is eventually an enhanced endogenous immune response against the host’s malignancy. The approaches of vaccine are by utilization of tumor antigens and antigen-presenting cells to enhance a preexisting antitumor immune response or perhaps in some cases, to induce an antitumor immune response that did not previously exist [4].

Different studies have identified a large number of cancer-associated antigens, which are the bases for cancer treatment vaccines both in basic research and clinical trials [16]. Both tumor-specific shared antigen such as the cancer-testis antigens, human epidermal growth factor receptor 2 (HER2/neu protein) and carcinoembryonic antigen (CEA) and tumor-specific unique include melanocyte/melanoma differentiation antigens, such as tyrosinase, MART1 and gp100, prostate-specific antigen (PSA) and Idiotype (Id) antibodies are applied as a basis for the new cancer vaccines production. To achieve positive outcome cancer vaccines should combine the best tumor antigens with the most effective immunotherapy agent’s delivery strategies [11].

Many vaccines were initially tested in patients with metastatic disease who had undergone multiple previous therapies. Clinical studies have now shown that patients will generally respond better to vaccines when they have been treated with fewer previous chemotherapeutic regimens and when a longer time has elapsed since their last chemotherapy. In many other forms of cancer therapy that kill tumor cells when ample doses of the therapeutic agent are delivered, the limiting factor is usually host toxicity. By contrast, cancer vaccines have demonstrated minimal toxicity, but their success is limited by the number of host-induced effector cells induced by the vaccine vs immunosuppressive entities. Consequently, patients with large tumor burdens would be even less likely to respond to vaccines than to chemotherapy. The reason for this is the typical numbers of antigen specific cytotoxic T cells that are activated as a result of a vaccination schedule represent a small subset of the total cell population [17].

Generally, several vaccination types are available against cancer, including protein and peptide-based, viral-based, dendritic cell, and plasmid DNA vaccines [16].

### 1.2.1 MUC-1/CEA/TRICOM viral vector vaccine

PANVAC is a recombinant poxviral vaccine that expresses carcinoembryonic antigen (CEA) and mucin-1 (MUC-1), in addition to three costimulatory molecules named TRICOM. TRICOM consists of three T-cell costimulatory molecules, including B7-1, intercellular adhesion molecule 1(ICAM)-1, and lymphocyte function-associated antigen 3(LFA)-3. When expressed in APCs, TRICOM has been shown to elicit a stronger immune response, along with a greater number of T cells specific for the TAA [18].

CEA is glycoprotein overexpressed on the majority of adenocarcinomas. Its selective overexpression also makes it a potential target for immunotherapy [20]. (MUC-1) is breast cancer antigen which is membrane-bound glycoprotein that consists of a polypeptide core containing 20-amino acid sequence and numerous carbohydrate side chains. As a cell surface glycoprotein, it is most efficient in inducing a humoral response; the presence of a MUC-1 specific IgG and IgM antibody response may be associated with a longer disease-free survival. There also seems to be evidence for a natural immunization against MUC-1 during pregnancy, which has been proposed as one explanation for why breast cancer is less common in multiparous women [10].
MUC-1 is commonly found on a variety of normal epithelial cells, including lung, breast, pancreas, stomach, colon, salivary gland, kidney, endometrium, and prostate, as well as malignant cells of breast, ovary, pancreas, endometrium, colon, lung, and prostate origin. On normal cells, mucins are located on the apical surface and are extensively glycosylated where as in tumor cell, the usual structure of the tissue is disrupted so that mucin may be found on multiple cell surfaces and presence of abnormal glycosylation in the cancer results in lesser complexes and fewer carbohydrate side chains. Therefore, in tumors, aberrant glycosylation and cellular architecture may allow for greater exposure of mucin epitopes to antibodies and T cell as compared to normal cells. The study demonstrates that vaccination of breast cancer patients with this vaccine results in significant production of both IgM and IgG antibodies against synthetic MUC1, but no evidence of T lymphocyte activation. For most patients, the IgM and IgG antibody levels decreased with time. IgG titers remain elevated for a minimum of 106–137 weeks after the first vaccination. Therefore, the patients may require subsequent “booster” vaccinations [20].

In a study of PANVAC vaccination with in twelve metastatic breast cancer patients had a median time to progression of 2.5 months following vaccination. Five of the twelve breast patients had stable disease or tumor regression for 4 months or more. These five responders had a mean of 1.8 chemotherapies prior to vaccination and lower tumor-marker levels. On the other hand, non responders had a mean of 4 previous chemotherapy treatments and much higher mean serum CEA levels. This study showed that patients with a smaller tumor burden and a lower number of previous chemotherapy treatments responded better to vaccine therapy. Therefore In the metastatic setting, the objective activity of vaccine treatments is low due to the sheer tumor burden and underlying immunosuppression caused by the tumors [18].

Cancer vaccine monotherapy in patients with heavy tumor burdens or much prior chemotherapy is not likely to produce significant clinical benefit. However, vaccines may result in slower progression of disease or enhanced sensitivity to subsequent lines of salvage chemotherapy in this setting. If patients respond and do well with the treatment, they can receive booster shots during scheduled interruptions in therapy used in a “stop-and-go” fashion [6].

1.2.2 E75 peptide vaccine
Peptide-based vaccines target tumor-associated antigens, which are differentially over expressed in tumor cells. Human epidermal growth factor receptor (HER2) is a commonly over expressed antigen in breast cancer tissue and can be targeted by peptide vaccine. The E75 vaccine is comprised of a nine–amino acid peptide (HER2 369–377) that binds to the type I major histocompatibility complex in order to elicit a cytotoxic T-cell response against the HER2 protein. Because peptides are not immunogenic by themselves, the E75 HER2/neu vaccine is administered with granulocyte/macrophage colony–stimulating (GM-CSF) factor as an adjuvant to stimulate antigen-presenting cells (APCs). (GM-CSF) is a cytokine that is used as an adjuvant in vaccines due to its capacity to cause proliferation of dendritic cells and macrophages. The major attraction of this approach is the potential to generate immunological memory, which may protect against relapsing tumors [18].

Preclinical breast cancer vaccine strategies targeting Her2/neu and other breast cancer antigens established the proof of principle of the approach. Encouragingly, early-phase clinical studies using peptides derived from the Her2/neu protein (E75) have shown evidence of immunological responses and a more recent study suggests a potential clinical benefit in subsets of patients treated with the E75 peptide vaccine [21]. A study by Mittendorf etal, also suggests the vaccine was demonstrated to be safe and effective in stimulating HER2-specific immunity. Those early results suggested that the
vaccine may have clinical efficacy in preventing or delaying disease recurrence in patients at high risk for relapse. However, large phase III clinical trials have largely failed to deliver major clinical benefits, despite the induction of humoral and cellular immune responses, indicating that improvements in vaccine design are clearly required [22].

Vaccine had clinical efficacy, with the vaccinated group having a breast cancer recurrence rate of only 5.6% compared with those of (control) group that is 14.2%. After 5 years the trial was observed that late recurrences in the vaccinated group corresponded to weakening immunity, as demonstrated by decreased levels of E75-specific cytotoxic T lymphocytes (CTLs). This finding suggested that a booster inoculation may be necessary to maintain significant immunity. After 24-month analysis further evidence suggesting that patients whose tumors have low HER2 expression benefit from vaccination, showing a statistically significant improvement in disease free survival (DFS) rates compared with controls. These studies suggest that E75 vaccine could represent targeted therapy for patients whose tumors have some degree of HER2 expression but who do not meet current clinical criteria to receive trastuzumab and also for patients with HER2-overexpressing tumors benefit from vaccination in addition to trastuzumab [21].

A disease recurrence was not observed in 11 patients who received both E75 vaccine and trastuzumab compared with recurrences in almost 20% of patients who received E75 vaccine in the absence of trastuzumab. These findings demonstrating that treatment with trastuzumab enhanced the sensitivity of HER2-expressing tumor targets to HER2-specific CTLS and also patients who received the vaccine after trastuzumab were more likely to maintain long-term specific immunity [21].

1.2.3 Dendritic cell vaccine

Dendritic cells (DCs) are a discrete leukocyte population in the monocyte/macrophage family, that display antigen as short peptides on their extracellular surface in conjunction with major histocompatibility complex (MHC) class I and II molecules to T-lymphocytes. DCs are believed to be the most important class of antigen-presenting cells (APCs) and are sometimes referred to as professional APCs. DCs are able to take in, process, and present antigen; are able to migrate through tissues; and are able to stimulate antigen-specific T-cell responses. DCs drive both CD4+ and CD8+ antigenic responses. Helper T cells (CD4+) recognize their cognate antigens (MHC class II molecules) on DCs, where CD8+ cytotoxic T lymphocytes (CTLs) recognize foreign or cancer cells that display the complementary peptide-MHC class I molecule on their cell surface. Immature DCs are phagocytic and take up and process antigen, but as they mature their phagocytic capacity diminishes in association with antigen presentation. For this reason, most of the interest in DCs has been to present antigen for immunization or vaccination purposes for a primary response or to boost weak existing responses [23].

Various cytokines optimize the production of DCs, especially the combination of IL-4 and GM-CSF. DCs express a variety of cluster designation (CD) markers. CD11c, CD80 (B7.1), CD86 (B7.2), and CD83 are commonly used to define and characterize DCs and to differentiate between immature and mature DCs. Therefore, antigen-loaded DCs are capable of producing either an antiantigen immune response or tolerance [4].

DCs can conduct all of the elements of the immune orchestra and they are therefore a fundamental target and tool for vaccines. Leukapheresed APCs can be activated with TAAs ex vivo and reintroduced into patients to present these antigens to effector T cells, thereby activating the immune system. On this basis, vaccination strategies employing DCs have been regarded as a promising therapeutic approach, even for advanced cancer. In contrast to preventive vaccines, therapeutic vaccines
attempt to influence the patient’s immune system to respond to an existing cancer [23].

Autologous tumor vaccines are the most promising vaccine in which antigen-presenting cells (APCs) are isolated from a patient and then cultured in the presence of a cancer antigen before being infused back into the patient. Autologous tumor cell vaccines have a great advantage because they present the unique set of TAAs, such as particular point mutations or fusion gene products, from a given patient's own tumor. DCs and autologous tumor cells are fused together before immunization of the patient. DC–tumor cell fusions combine the unique properties of whole tumor–cell vaccines with the enhanced antigen-presenting power of DCs. At this point, the APCs can present the antigen to cytotoxic T cells and activate them to attack the tumor. The first such vaccine to come to market is Sipuleucel-T (Provenge®), which targets advanced, metastatic prostate cancer and was approved by the FDA in April 2010 [17].

Sipuleucel-T is created by exposing a patient's dendritic cells to a fusion protein that is composed of prostatic acid phosphatase (a PAP, a prostate antigen) and granulocyte-macrophage colony-stimulating factor (GM-CSF) before reinfusion. These cells are then reinfused to the patient for the purpose of conferring immunity; this process is repeated three times at biweekly intervals. Treatment with Sipuleucel-T resulted in a 4-month-prolonged median survival in subjects with prostate cancer [24, 25].

DCs have been administered by various routes. From a product-classification standpoint, pure DC population that has not been antigen-loaded would be considered as an adoptive immunotherapy. DCs that are antigen-loaded and administered by subcutaneous (s.c.), intradermal (i.d.), intranodal, or lymphatic routes of administration, are considered to be vaccines [4].

1.2.4 DNA vaccine

DNA vaccines have emerged as an attractive approach for antigen-specific T cell-mediated immunotherapy to combat cancers. T cell-mediated immunity is critical for cancer immunotherapy and vaccine development. Tumor antigens that are recognized by T cells are likely to be the major inducer of tumor immunity and most promising candidates for tumor vaccines. Clearly, the current approach to immunotherapy mainly relies on the role of CD8+ cytotoxic T lymphocytes (CTL). Generally, various strategies have been developed to enhance the potency of DNA vaccines such as increasing the number of antigen-expressing dendritic cells (DCs) or antigen-loaded DCs, improving antigen expression, processing and presentation in DCs and enhancing DC and T cell interaction. Various DNA delivery systems as a powerful research tool for elucidating effective anti-tumor immune responses. The method of delivering a DNA vaccine can influence the type of immune response induced by the vaccine. DNA may be administered by different methods such as intradermal (i.d.), intramuscular (i.m.), intranasal (i.n.) and subcutaneous (s.c.). In many cases, cutaneous administration has been associated with immunological benefits, such as the induction of greater immune responses compared with those elicited by other routes of delivery. However, there is still a need to improve the delivery of DNA vaccines and to increase the immunogenicity of antigens expressed from the plasmids. For example, tumor burden has been decreased by novel DNA vaccine strategies that deliver cytokines as plasmids directly into tumors in both mouse and human models [16].

Plasmid DNA vaccine was designed to stimulate an immune response against CYP1B1 epitopes commonly expressed on the surface of transformed cells. Vaccination of Seventeen patients with advanced stage disease to determine safety and immunogenicity of this approach [18]. The vaccine contains plasmid DNA encoding an inactivated form of CYP1B1. While six of the 17 patients developed CYP1B1-
specific immunity, it is interesting to note that five of the patients that developed immunity exhibited a significant response to their next salvage therapy. Of the eleven that did not develop immunity, three died before receiving salvage therapy, seven progressed after salvage therapy, and one had complete remission. There is increasing evidence that once a vaccine activates the immune system, patients have a better response to subsequent therapies [18].

This finding help to determine reasons why some patients developed immunity and some did not. The numbers of prior treatments that they received bring a difference. It was thought that patients with a greater number of previous treatments would not be as capable of developing an immune response. In addition, the number of vaccinations had no effect on immunity, as all six patients developed immunity between vaccinations two and six, with no increase in immunity during further vaccination. One explanation could be that aggressive disease inhibits the patient’s ability to generate immunity to the TAA. It was also theorized that the tumor burden might be influence the level of immune response generated, due to regulatory T cells and immunosuppressive cytokines [18].

**Influence of the tumor microenvironment and immunosuppressive factors on vaccine**

One of the major reasons for the limited success of therapeutic cancer vaccines to date is likely to be the negative influence of the tumor microenvironment and other immunosuppressive factors. Preclinical studies have shown that the interstitial pressure within a large tumor mass diminishes diffusion of macromolecules, such as antibodies, and effector cells like T cells. Most solid tumors also lack T-cell costimulatory molecules. Due to lack of costimulatory molecules, they are anergized and lose lytic capacity. The tumor microenvironment has been shown to contain a range of immunosuppressive immune cell types including CD8+ T cells, tumor-associated macrophages (TAMs), and regulatory natural killer (NK)/NKT cells. Analysis of peripheral blood mononuclear cells (PBMCs) from patients with several types of cancer has also shown increased levels of MDSCs and Tregs, as well as an increased suppressive function of Tregs on effector T cells. These suppressive cells, tumor cells, and other cells in the tumor microenvironment can also release into the microenvironment a number of soluble immunosuppressive factors, including TGF-β, IL-10, indoleamine-pyrrolole 2, 3 dioxygenase (IDO), and VEGF. Combination therapies are one of the Strategies to combat these immunosuppressive entities [17].

**COMBINATION THERAPY**

One way to elicit a stronger immune response and possibly derive clinical benefit would be to combine vaccines with other cancer treatments, such as chemotherapy, monoclonal antibodies, or radiation.

One study showed patients with improved clinical outcomes when receiving the PANVAC vaccine plus the chemotherapy agent docetaxel, as compared to PANVAC or docetaxel alone [18].

Preclinical studies have now demonstrated that certain small-molecule targeted therapeutics have the ability to enhance vaccine-mediated T-cell lysis of tumors. Both a BCL-2 inhibitor and the tyrosine kinase inhibitor have been shown to enhance the ratio of TAA-specific T cells to regulatory cells, resulting in enhanced vaccine efficacy [17].

In a recent study, Vaccines are also being used with monoclonal antibodies such as the HER2/neu inhibitor trastuzumab, before administering the E75 HER2/neu peptide vaccine resulting in enhanced HER2/neu-specific immune responses in breast cancer patients. An increased CTL response against the E75 peptide was observed, possibly due to the increased processing of the HER2/neu antigen. This would provide a greater number of peptides
available to be presented by the APC, and potentially a greater number of tumor cells that are recognized and eliminated. In all of the above vaccine combination therapies, the dose and scheduling of the combining agent, the appropriate patient population, and the clinical endpoint are very important points should be considered [18].

2. NON SPECIFIC IMMUNOTHERAPY AS A TREATMENT OF BREAST CANCER

2.1. Bacille Calmette Guerin (BCG)

Coley’s toxin represented the earliest form of active immunotherapy that used a nonspecific immunostimulant. A more familiar approach is the use of bacille Calmette-Gue´rin (BCG), an attenuated form of the tubercule bacillus. Although first used as a vaccine against tuberculosis, it ultimately was discovered to be a potent immunostimulant that was capable of preventing tumor growth in mice [8]. BCG has been studied in several tumor types and remains an intralesional therapy of early stage bladder cancer. BCG was studied as an adjuvant to chemotherapy for disseminated breast cancer. Although rates of reduction were similar between chemotherapy and BCG combination compared with chemotherapy alone, reductions seemed to last longer with the addition of BCG [10].

2.2. Cytokines

Cytokines are proteins that are secreted by lymphocyte and macrophage cells and affect the immune response, typically via effects on other cells through receptors. They are crucial in controlling the growth and activity of other immune system cells and blood cells in the body. Anticancer effects of cytokines are generally believed to be mediated by their effects on immune cells. There are numerous cytokines that involves in cancer therapy, including, hematopoietic colony-stimulating factors (CSFs), interferons (IFNs), at least 35 different interleukins (ILs), tumor-necrosis factor (TNF), and a number of other protein ligands [4]. As Campbell and his colleagues measured intracellular cytokine profiles of T cells in the peripheral blood of 85 women with breast cancer and correlated cytokine levels with the presence of micrometastases in lymph nodes and bone marrow. Blood was drawn prior to breast cancer surgery and adjuvant therapy. 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. The presence of micrometastatic cells in bone marrow was correlated with depressed cytokine levels. Altered cytokine patterns may be both the cause and the result of breast tumors [26].

2.2.1 Tumor necrosis factor- α (TNF-α)

Tumor necrosis factor- α (TNF-α) is principally produced by lymphocytes and NK cells. Defects in NK cell-mediated cytotoxicity may play a role in the initial stages of human tumorigenesis. Significant differences were observed in several immune system parameters between healthy controls and women with advanced breast cancer prior to initiating a course of taxane-based chemotherapy, including depressed NK cell and lymphokine activated killer cell (LAK) cytotoxicity, decreased levels of interleukin (IL)-2, GM-CSF, interferon-γ (IFN)γ, and increased levels of tumor necrosis factor (TNF)-α and IL-6. NK cell activity was 175% lower and TNF activity was 100% higher in the breast cancer patients prior to treatment compared with healthy controls. Nevertheless, the role of NK cell activity in controlling breast cancer disease is still controversial. One study reported that women patients with benign breast cancer showed NK cell activity that was not significantly different from NK cell activity in patients with malignancies in each of four different NK cell activity assays. In addition the study found that in breast cancer patients LAK cell activity was reduced but not the activity of NK [26].

In particular, interleukin-6 (IL-6) and tumor necrosis factor-alpha (TNF-α) have been reported to be elevated in the blood serum of patients diagnosed with advanced stage breast
tumor and correlate with an increased number and size of metastatic sites. IL-6 and TNF-α have been shown to promote the growth and invasiveness of colon and prostate cancer epithelial cells in vitro and in vivo. The recruitment of tumor-associated immune cells during the inflammation that accompanies tumor progression has been shown to promote tumor growth and contribute to angiogenesis, invasion, and metastasis. Infiltration of these tumor-promoting immune cells as well as various inflammatory cytokines, particularly TNF-α and IL-6 has been found in the breast tumor microenvironment. A clinical study reported elevated levels of proinflammatory cytokines in the serum of breast cancer patients when compared to healthy individuals [27]. The results from these studies have led to think about the possible usefulness of targeting these cytokines in breast cancer patients. As a result, understanding both the mechanisms by which inflammatory mediators promote breast cancer and the effectiveness of anti-inflammatory drugs in treating breast cancer will lead to novel therapeutic regimens to treat this devastating disease [28].

2.2.2 Interferon-Gamma (IFN-γ)

Interferon-γ (IFN-γ) is a pro-inflammatory mediator produced principally by T cells (CD4+ and CD8+) and NK cells. Key functions include activating cells of the monocyte/macrophage lineage, promoting differentiation of naïve CD4+ T cells into Th1-like cells and inhibiting differentiation of CD4+ cells into Th2-like cells. A research demonstrated that IFN-γ is a critical component in regulating an innate phagocytic response against metastatic breast cancer. Furthermore, incubated peripheral blood mononuclear cells (PBMC) from breast cancer patients with IFN-γ and found that this increased the LAK activity. The use of intravesical instillation of recombinant IFN-γ has been demonstrated to be effective against tumour recurrence in patients with early-stage superficial transitional cell bladder cancer. For this to be feasible in breast cancer, new delivery systems allowing targeted cytokine release are required [29].

2.2.3 Granulocyte-Macrophage Colony-Stimulating Factor (GM-CSF)

Granulocyte-macrophage colony-stimulating factor (GM-CSF) is also a cytokine that has profound effects on the differentiation and actions of myeloid cells. It has not been approved as a monotherapy for cancer treatment, but is one of several hematopoietic stimulating factors that have been approved for clinical use because of their positive effects on recovery of hematopoietic cells. Due to a greater effect of GM-CSF’s on monocytes, macrophages, and dendritic cells, it is so important for immunotherapy. The anti-tumor activity of GM-CSF appears to be related to its ability to activate macrophages and dendritic cells that is used as an adjuvant in vaccines [4]. The potential for GM-CSF to stimulate immune responses has been shown in many tumor models, including a murine melanoma in which transgenic expression of GM-CSF provided protection to subsequent tumor challenge in over 90% of the animals, and promising results have been observed in other tumors [30].

2.2.4 Interleukin-2 (IL-2)

IL-2 (Proleukin ) was the first biological approved for the treatment of cancer based on immune modulatory effects where as IFN-α has direct antitumor cytostatic effects in addition to its immune-modulating effects. IL-2 has no direct antitumor effects but induces cytotoxic natural-killer (NK) and T-lymphocytes. Originally, IL-2 was described as the “T-cell growth factor,” it was focused on its role in the ex vivo culture of T-lymphocytes for the differentiation and proliferation of activated T cells, especially for possible clinical application of cytotoxic T-lymphocytes (CTLs) [30]. As such, it seems like an ideal choice for immunotherapy. This is why most early trials focused on IL-2 in combination with adoptive cell-therapy products known as lymphokine-activated killer (LAK) cells, or tumor-infiltrating lymphocytes (TILs) [4].
Systemically delivered interleukin-2 (IL-2), a cytokine that induces T-cell activation and proliferation, has clinical efficacy as a stand-alone therapy for patients with metastatic melanoma and renal cell carcinoma [30]. The most encouraging results were in melanoma and renal cell cancer, which were generally considered to be immunoresponsive tumors, but some activity was seen in virtually every tumor type. Unfortunately, this therapy is so toxic that it requires careful patient selection, and can only be safely infused by physicians and nursing staffs experienced in its administration. Many of the combination regimens appear to have higher RRs, and perhaps longer PFS, but long-term OS benefits have not been documented. Because high-dose IL-2 regimens are only appropriate for younger, healthier patients having normal organ function and good performance status in practice, more melanoma patients are treated with combination regimens that use lower doses of IL-2 [4].

3. ADOPTIVE CELLULAR THERAPY

Adoptive T cell therapy involves the isolation and ex vivo expansion of tumor specific T cells to achieve greater number of T cells than what could be obtained by vaccination alone. The tumor specific T cells are then infused into patients with cancer in an attempt to give their immune system the ability to overwhelm remaining tumor via T cells which can attack and kill cancer. Although it is more complex than delivering monoclonal antibodies, the cellular arm of the immune system also may be used as passive immunotherapy. A cellular immune response to cancer may be more effective than an antibody response; however, attempts to stimulate a cellular response through immunostimulants or vaccines have been difficult. Therefore, the passive administration of cells with antitumor activity to the tumor-bearing host has generated significant interest [10].

This cellular infusion therapy, known as adoptive immunotherapy, involves harvesting cytotoxic T lymphocytes, NK cells, or mononuclear cells from the patient (autologous) or a donor (allogeneic); selecting and expanding them in vitro; and delivering the activated, tumor-specific cellular inoculum to the patient. Early trials of the adoptive transfer of allogeneic lymphocytes demonstrated little or no clinical effect. Slightly more promise has been seen with harvesting autologous lymphocytes, activating them in vitro, and delivering them back to patients. These lymphocytes may be obtained from the peripheral blood or bone marrow of patients who have breast cancer. The primary obstacle has been the generation of sufficient numbers of tumor-specific cells for transfer. Minimal benefit is seen when lymphocytes are activated in a nonspecific manner because there is a scarcity of tumor-specific T cells in the periphery. Stimulating lymphocytes in the presence of autologous tumor cells, genetically-modified tumor cells, or tumor-pulsed dendritic cells can increase the specificity of the lymphocytes, and potentiate the clinical response [10].

3.1 Natural-Killer Cells and Lymphokine-Activated Killer Cells

In addition to T cells, NK cells may have the potential for adoptive immunotherapy of cancer because their natural role in the body is fighting virally-infected cells and cancerous cells. NK cells are inherently cytotoxic against tumor cells in the absence of immunization or activation. NK cells are cytotoxic against K562 cells, virally infected autologous cells, and autologous tumor cells that lack expression of major histocompatibility complex (MHC) class I antigens or B7 molecules. NK cells can also affect ADCC via the CD16 FcgRIII that binds to the Fc portion of antibody coated cells. The majority of NK cells is phenotypically CD2-, CD3-, CD4-, CD8-, CD19-, CD20-, CD16+, CD25+, and CD56+; are directly cytotoxic; and can serve as effector cells in ADCC. Although unstimulated NK cells can recognize and kill tumor cells in vitro, there have been no large-scale clinical trials of unstimulated NK cells. Instead, such cells have typically been stimulated with interleukin-2 (IL-2) and infused into patients because it enhances cytotoxic effects of NK
cells. When PBMC are stimulated in vitro with IL-2 for 4-6 days, the resulting cell population is referred to as lymphokine-activated killer (LAK), and exhibits greater cytolytic Properties than unstimulated NK cells. It was demonstrated that LAK cells in combination with IL-2 were very effective in eradicating tumors in mice with established pulmonary sarcoma metastases [4].

3.2 Tumor-Infiltrating Lymphocytes and Cytotoxic T-Lymphocytes

Recent reports have suggested that patients whose tumors were heavily infiltrated with lymphocytes have better prognoses. Some of the lymphocytes infiltrating tumors have specific antitumor activity. Tumor-infiltrating lymphocytes (TIL) are derived from primary or metastatic tumors, or regionally draining lymph nodes, and expanded in vitro in media containing IL-2. A study demonstrated that TIL could have as much as 10–100 times more cytolytic activity than autologous LAK cells after IL-2 stimulation. Efforts were made to utilize TIL as a cancer therapy in combination with IL-2. The original goal was to isolate and expand cytotoxic CD8+ cells that would be HLA class I restricted and only kill autologous tumor cells, but most trials have utilized whatever final product emerged after culturing in IL-2 for several weeks [4].

CONCLUSION

Critical evaluation of the effects of cytokine infusions, cancer vaccines, monoclonal antibody and adoptive cell therapy for the treatment of breast cancer will give direction to subsequent clinical trial design. Recent promising clinical trials justify hopes that immunotherapy could become a keystone of future breast cancer treatments. Some cytokines will stimulate cancer cell growth and may contribute to metastatic spread, this cytokine may led to think about the possible usefulness of targeting these cytokines in breast cancer patients. Breast cancer vaccines provide a means to elicit an immune response to antigens specifically expressed by tumor cells. There are different ways of introducing these antigens, including peptide-based, viral-based, dendritic cell, and plasmid DNA vaccines. These vaccines, may be more effective in the minimal residual disease state, when used alone than metastatic setting because vaccines may not have the proper amount of time to induce an immune response. Immunotherapeutic approaches combined with novel sequences of chemotherapies, radiation, and immunomodulating agents hold promise for enhancing the treatment of breast cancer.

Abbreviation & acronyms

APC- Antigen presenting cell
CEA - Carcinoembryonic Antigen
BCG - Bacille Calmette-Guerin
CTL - Cytotoxic T lymphocyte
GM- CSF - Granulocyte macrophage colony stimulating factor
HLA- Human leukocyte antigen
IL- Interleukin
TNF- Tumour necrosis factor
DC- Dendritic cells
LAK - Lymphokine activated killer
MHC- Major histocompatibility complex
NKcell - Natural killer cell
LPS- Lipopolysaccharide
PBMC - Peripheral blood mononuclear cell
PMN- Polymorphonuclear cells
TIL - Tumor infiltrating lymphocyte.
ICAM- Intercellular adhesion molecule-1
ADCC - Antibody-dependent cytotoxicity
TCRs - T-cell receptors
FDA- Food and Drug Administration
TAA - T tumor-associated antigens
CEA - Carcinoembryonic antigen
HER-2/neu- Human epidermal growth factor receptor
MUC-1 mucin
mAbs or moAbs - monoclonal antibodies
CDCC - Complement-dependent cytotoxicity
EpCAM- Epithelial cell adhesion molecule
NCI - National Cancer Institute website
RIT - radioimmunotherapy
Tregs - Regulatory T cells
MDSCs- Myeloid derived suppressor cells
IDO- Indoleamine-pyrrole 2,3 dioxygenase
ACT- Adoptive cell therapy
MART1- Melanoma antigen recognized by T cells
PD-1- Programmed death 1
CTLA4- Cytotoxic T Lymphocyte Antigen4
TGF- Transformed growth factor
RR- Response rate
PFS- Progression free survial
OS- Overall survial
BCL2- B-cell lymphoma 2
DFS- Disease free survial
NTI- Narrow therapeutic index
EFRG- Epidermal growth factor receptor

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