Immunotherapy for human breast cancer

A thesis submitted in partial fulfillment of the requirements for the degree of Master of Science.

By

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I HEREBY RECOMMEND THAT THE THESIS PREPARED UNDER MY SUPERVISION BY Nasrah Al Kamal ENTITLED Immunotherapy for human breast cancer BE ACCEPTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF Master of Science

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Abstract
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The study focuses on the methodologies that are used in breast cancer therapy. The review finds major evolutions of these methods and the influence they have made on the increased survival rates in cancer patients. There is also a projection of the processes that are involved in the therapy practices and the way the protocols have been improved over time. The various stages of cancer treatment project the various aspects of better performance articulations in the therapy sector. There is also reference to what is supposed to be done to reduce the limitations in cancer therapy contingencies.
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Dedication

I would like to dedicate my research project to my family for unlimited support, encouragement and love.
Introduction

The Immune system is very critical in suppression of cancer development. However, cancer cells have evolved mechanisms to destruct by these immune cells. Immune cells are actively involved in suppression of cancer cells in early stages of development, but with time the cancer cells alter the immune cells to the extent that they begin promoting tumor progression through the provision of conditions that select tumor cells able to grow in the host and facilitating their growth. In most cases the tumors are contained by the active immune system to allow active involvement of innate and adaptive immune responses involving the macrophage system. However, the cancer cells bypass immune cells by modifying the basic cell systems such as apoptosis and tumor suppressor gene mutations, leading to a shift in the expression of the cancers (Arteaga, 1992).

Cancers develop more easily with advance age in most cases, and breast cancer is no exception. The continued shortening of Telomeres in the chromosomes can lead to deletions in tumor suppressor genes, apoptosis mediating genes and proto-oncogene modification to oncogenes. These factors in an immunocompromised individual usually allow the growth and survival of the cancer cells. Cancers can be metastatic or non-metastatic in an individual. Metastatic cancers are usually disastrous and difficult to treat because they spread to various body tissues and organs and resist chemotherapy and other forms of treatment become challenge (Moen & Stuhr, 2015).

Breast cancer is among the most studied forms of cancer. It is among the cancer treatments that have received immune-based treatment strategies in the biomedical science field. Currently, this form of cancer is the mostly commonly diagnosed cancer among women in the
world, forming 12% of all the cancer diagnoses. (Vetvicka et al., 1999). Therefore, Breast Cancer has been declared by the World Health Organization as the second leading cause of deaths associated with cancer in women, with 520,000 deaths out of the approximately diagnosed 1.8 million women in U.S in 2012. The data is an equivalent of about 1 out of 8 woman at risk as compared with the exposure risk of 1 man per 1000. Higher risks of breast cancer are usually associated with genetic mutations that can be passed to on the hereditary generations. These mutated genes such as BRCA1 and BRCA2 may invariably be expressed with time in an individual to bring about the cancer and they are including the tumor susceptible genes. (Patt et al., 1993)

The 5-10 % risk of developing breast cancer if it inherited predisposition chance which is direct contrast to other kind of cancer form acquired by other means that accounts to less than 1% in the general population. Other factors that culminate the development of breast cancer include but they not limited to hormonal therapies which both estrogen and progestin, heavy alcohol intake, reduced physical activity, high breast tissue density and obesity. In general, mutations in BRCA1 and BRCA2 are the leading cause of breast cancer, accounting for a 55-65% of all breast cancers (Stähli et al., 1985).
Figure 1: Female breast cancer incidence rates, by age group in Ontario between the year 1981 – 2009, (Adapted from qap.sdsu.edu).

This figure shows that the older people were more susceptible to breast cancer than younger people.
Figure 2: Incidence and mortality rates of breast cancer in USA between the years 1971 and 2009, (Adopted from qap.sdsu.edu).

The figure 2 shows the incidence rates of breast cancer in USA between the years 1971 and 2009 was higher than mortality rates.
Incident source: Combined data from the National Program of Cancer Registries as submitted to CDC and from the Surveillance, Epidemiology and End Results Program as Submitted to the National Cancer Institute in November 2014.


Figure 3: Female Breast Cancer Incidence Rates by Race and Ethnicity, U.S., 1999-2012, adapted from (kff.org).

Death rate was also analysed with a report obtained, as from 1999-2012, variations in death rates were evident, depending on race and ethnicity. Black women showed elevated death rate compared to other groups. (Zhou et al., 2007)

Mortality source; U.S. Mortality files, National center for Health Statistics, CDC

Female Breast Cancer

Death Rates by Race and Ethnicity, U.S, 1999-2013
Breast cancer has had misconceptions about race or ethnicity relationships, below is an analysis of cancer victims across all races. It shows the number of women out of 100,000 got infected with breast cancer each year during the years 1999-2012. Figure 8 shows high rate of white women infected by breast cancer as at 2012 (Hilgers et al., 1985).

Figure 4: Death Rates by Race and Ethnicity, U.S, 1999-2013, (adapted from kff.org).
Breast cancer treatment

Just like other cancer therapies, the main aims of treating breast cancer are to completely eliminate the cancer and to prevent recrudescence of the cancer in the individual at any given point in life. In most cases, cancer therapists evaluate the type of treatment before actual administration. The type of breast cancer an individual suffers from, the extent of spread of the tumor and its size, age and presence or absence of receptors for HER2 protein, progesterone as well as estrogen. (‘Breast Cancer Research and Treatment’, 2003).

Treatment options include surgery to remove the affected breast, or lumpectomy which involves removal of the affected tissues only, leaving the breast intact. Radiation therapy, a commonly used form of treatment, uses high frequency waves to kill the cancer cells. Other therapies such as chemotherapy and hormone therapy are currently receiving attention by scientists since they target treatment at genetic levels of the individuals. For instance, hormone therapy involving the targeting of the genes responsible for the production of estrogen that may promote feeder hormone for breast cancer cells. Such drugs include tamoxifen used by premenopausal and postmenopausal women and anastrozole, exemestane and letrozole that are used for postmenopausal women. In some instances, there may be an option of combining the therapies to offer maximum achieved therapy. Targeted hormone and chemotherapy may be supplemented with irradiation of the cancer cells so as to completely kill them (Bunnell, 2000). However, this is require higher levels of expertise to avoid life threatening or metastasis encouragement (Poulin et al., 1988).

Immunotherapy is the most recent therapy that is being to treatment of Breast Cancer and has advantages over the forms of treatment such as chemotherapy and targeted therapies since it
is highly effective and less traumatizing. Immunotherapy conventionally have very few side effects and thus can be administered to an individual for much longer periods of time with little or no toxicity. This kind of therapy has also received minimal resistance by most patients and target specific cancer antigens by using specific immune cells such as macrophages. For instance, the use of immune check point inhibitors such as programmed cell death protein 1 have led to an overall extension of survival for patients with metastatic cancers as well as breast cancer (Rocha et al., 2012).
### Treatment approaches for each therapy in breast cancer.

<table>
<thead>
<tr>
<th>Type of immunotherapy</th>
<th>Drugs/approach used</th>
</tr>
</thead>
<tbody>
<tr>
<td>Monoclonal antibodies</td>
<td>Trastuzumab</td>
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<td></td>
<td>Ado-trastuzumab</td>
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<tr>
<td>Therapeutic vaccines</td>
<td>NeuVax</td>
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<tr>
<td></td>
<td>GVAX</td>
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<tr>
<td>Checkpoint inhibitors</td>
<td>Ipilimumab</td>
</tr>
<tr>
<td></td>
<td>Pembrolizumab</td>
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<tr>
<td>Adoptive T cell therapy</td>
<td>Chimeric Antigen Receptors(CARs)</td>
</tr>
</tbody>
</table>

**Table 1: Treatment approaches for each therapy in breast cancer, (Adapted from National Cancer Institute, 2015).**
**Hypothesis**

This new breast cancers therapy is designed to elicit immune responses that target the cancer associated antigens to destroy the marked cancer cells. This study addresses the role of macrophages in breast cancer development and its sustenance.

**Aims of the study**

- To determine the effectiveness of therapeutic vaccines such as GVAX and NeuVax in breast cancer treatment
- To evaluate the effectiveness of checkpoint inhibitors and their contribution to breast cancer therapy
- To establish the effectiveness of adaptive T-Cell therapy in breast cancer treatment
- To evaluate the role of genetically engineered monoclonal antibodies in targeting breast cancer antigens
Background

Macrophages

Macrophages are a core participant in general cellular immunity of an individual following an infection. They have a very important role in maintaining the host defense system involving both the acquired and innate immunity (Djawari et al., 1978). These cells have their origin in the bone marrow bound from pro-monocytes and monocytes that are then released into the blood stream. Macrophages are characterized for their ability to shift their roles in response to various microenvironments

Development and differentiation of the Macrophage system.

Macrophages also develop in line with monocytes that form the Mononuclear Phagocyte System (MPS). They have a common precursor called the myeloid progenitor that also produces granulocytes. These myeloid progenitor cells give rise to monoblasts that produce promonocytes. These promonocytes develop to form monocytes which further move to tissues and blood in the body. Colony Stimulating factor 1 (CSF-1), FMS-like Tyrosine Kinase 3 Ligand and Granulocyte Macrophage Colony stimulating factor (GM-CSF) stimulate the myeloid precursor cells to initiate the macrophage cell development. CSF-1 is an essential component in the overall production of tissue macrophages and normal cell development. The GM-CSF is actively involved in stimulating the differentiation of the bone marrow as well as the monocyte population in response to Antigen Presenting Cell’s activity. The production of macrophages largely depends on the presence of Interleukin 1 and 3 target with CSF-1, GM-CSF and Interferon gamma (Djawari et al., 1978).
Macrophage polarization

Macrophages can be broadly classified into two basic categories: Type I and Type II macrophages. Type I and Type II cells are defined as M1 and M2, respectively, depending on the receptors, functions, and their production cytokine. Pro-inflammatory macrophages are produced by M0 cells through activating of Interferon gamma and a set of lipopolysaccharides. (Pro-inflammatory macrophages are also termed M1 macrophages). The anti-inflammatory Macrophages (M2) usually develop following an exposure to Interleukin-1, Interleukin-10 and Interleukin-13. Interferon gamma as well as the Lipopolysaccharides activate the anti-inflammatory macrophage phenotype lineage. When the anti-inflammatory macrophages are associated with tumor suppression, they are regarded as Tumor-Associated macrophages (TAMs). To a larger extent, the M1 cells are characterized by their anti-tumor activity with the ability to initiate the destruction of tissues. On the other hand, the M2 line of macrophages are associated with the initiation of repair of the affected tissues, remodeling as well as promoting the tumor cells. However, the production of TAMs lead to the reduction of anti-tumor events as well as a rise in the production of the mediators for angiogenesis (Sapra et al., 2005).

Figure 5 illustrates the activation of macrophages, the (M1) pro-inflammatory and (M2) anti-inflammatory, as well as their effects on the growth of tumors. TAM (M2 macrophage) predominate in tumor responses. Cells with the M1 phenotype contain antitumor activity and initiate the destruction of tissue. The M2 phenotype, on the other hand, develops after IL-4 activation, initiating tissue repair, remodeling and promotion of tumor growth. Most TAMs are M2 cells, which result from multiple signals produced in the tumor microenvironment. The alternate activation of TAMs results in a reduction in the antitumor activities and increased production of angiogenic mediators. Inhibition of the M2 phenotype and the induction of M1
signals can reestablish TAMs as an effective therapy in reducing tumor size (Leyva-Illades et al., 2012).

Figure 5: The Impact of Macrophages on the Growth of Tumor and their Differential Activation. M1 (pro-inflammatory), M2 (anti-inflammatory) (adapted from Leyva-Illades et al., 2012).
Role of Tumor Infiltrating Lymphocytes in Breast Cancer

Tumor Infiltrating Lymphocytes (TILs) has been observed mostly in a number of presented breast cancer cases. The developing forms of the cancers that have a negative status enlargement of the axillary lymph nodes characteristic a relatively small size of tumor and usually low grade ones are studied by use of the Tumor infiltrating lymphocytes. Tumor infiltrating lymphocytes have a negative correlation with the age of a patient and their count too is positively associated with the survival span of the affected patients with the estrogen receptors. TILs are the predictive and potential prognostic markers in breast cancer. When a local relatively developed breast cancer is treated with neoadjuvant chemotherapy, any occurrence of tumor infiltrates of lymphocytes is an evident predictor of the response (Criscitiello et al., 2014).

Macrophages in Cancer Development

Macrophages and their monocytes are converted to tumor associated macrophages (TAMs) when there is a tumor in the body of an individual. These cells are usually attracted towards the tumor cells when there is sufficient signaling as they move past the blood to the tissues. The attraction is facilitated by a cascade of events that are mediated by chemokine production. The most common chemokines include CCL2, and CCL5 (RANTES). Of the two, CCL2 is a chemoattractant that mediates the attraction of monocytes towards the point of tumor location. The Chemokine is produced by malignant breast cells which facilitates the movement of monocytes and macrophages as the immediate responders to kill the mutated cells. The absence of chemokine CCL2 does not regulate the number of macrophages that can be found in a malignant cell. Instead this may directly affect the number of monocytes moving towards this tissue. When the levels of CCL2 are high, the infiltrating macrophage number increasingly
becomes so bulk and this may mediate the destruction of a tumor at its early levels of development. The Macrophages initially show a high potential of clearing the cancerous cells with the production of apoptotic factors and the secretion of extracts of highly reactive oxygen species such as Nitric Oxide (NO). CCL5 is also produced by Breast Tumor cells alongside Naïve T cells. CCLs in turn stimulates the monocyte infiltration and expression of CCL2, CCL3, CCL4 and IL-8. The chemokines further facilitate the movement of myeloid cells and other monocytes and macrophages to the site of the tumor to fight the inflammation (Venglinskaya & Shubich, 1974).

Macrophages and monocytes are also recruited to tumor sites by cytokines. The Tumor cells produce Colony Stimulating Factor 1. Colony Stimulating Factor on its own facilitates the survival, growth, proliferation and differentiation of the tumor cells and macrophages. Monocytes and macrophages are also characterized the production of cytokines. Therefore, this stream of independent production of Colony Stimulating Factor allows for a competing colony of proliferating tumor cells. At some point, the macrophages facilitate the production of cytokines that stimulate of the growth of the tumor colonies as a well as encourage the process of angiogenesis (Venglinskaya & Shubich, 1975).

The Angiogenic Growth Factor, Vascular Endothelial Growth Factor (VEGF), is associated with the presence of the macrophages and monocytes in tumor cells. This factor also plays the role of a chemoattractant by attracting free circulating macrophages that can destroy the tumor cells before macrophages get overwhelmed and mutated. The attraction occurs by the activation of tyrosine kinase receptors in the macrophages. Similarly, Endothelins, ET-1, ET-2 and ET-3 are also associated with macrophages. These endothelins usually bind to peptides that
further bind to G-protein linked transmembrane receptors. In face of tumors, endothelins are produced in presence of macrophages. These endothelins in turn have a duty in stimulating tumor development and facilitating apoptotic inhibition, enhancing metastasis, angiogenesis and mutagenesis. Therefore the macrophage cell-lines may fall as victims of mutation in presence of the endothelins thus allowing for development of the tumor cells (Schumacher et al., 1993).

**Role of tumor Associated Macrophages in Tumor sustenance**

Normally, TAMs are associated with cytotoxic activity on tumor cells and direct recruitment of immune response in tumor clearance. The M2 TAMs may stimulate the growth of tumors, development of new blood vessels (Angiogenesis) and metastasis. M2 TAMs are associated with the stimulation of tumor growth and development. Facilitates of the production of cytokines that stimulate the proliferation of malignant cells through direct recruitment of endothelial cell proliferation as well as angiogenesis. The M2 TAMs also produce Tumor Growth Factor-Beta (TGF-β) which is an immunosuppressive component that inhibits the anti-tumor responses of the Cytotoxic T-Cells. Tumor Associated Macrophages also facilitate the proteolytic remodeling of the extracellular matrix that is essential in various points during the multistage development of the tumors (Schneble et al., 2014)

**Macrophage contribution in Angiogenesis**

Angiogenesis is developing new blood vessel supply from the existing ones. The ability of macrophages to secrete a wide range of chemokines, growth factors, and a varied range of enzymes allows them to facilitate the angiogenesis process which results to the establishment of new and fully independent blood vessels from the wild ones. Three steps are associated with angiogenesis mediated by Macrophages. TAMs produce endothelins, chemokines, Interleukin-
17, Interleukin-23, TGF-β and VEGFs to promote angiogenesis. The initial step is the initiation of endothelial cell growth and further proliferation of breast tissue cells. (Fortier et al., 1994). This is followed by a coordinated production of metalloproteases that degrade basement membranes and the vessels that supply them. This process of degradation involves mostly proteases called Metall. This protease activity allows an easy sprouting and migration of the host endothelial cells into the developing malignant cells of the breast. Finally, there is an unregulated process of new tube formation similar to those of embryonic origin. This tube forms a new blood vessel that is supplied with numerous capillaries and venules. The blood vessel undergoes further stabilization with the attachment of mural cells and to collagen fibers to attain maturity. In turn, the tumor cells become independent and continue multiplying that cannot be recognized as non-self by the immune system (Venglinskaya & Shubich, 1975).
Figure 6: Adaptive T Cell patient monitoring (Adapted from wikipedia, 2015).
Figure 7: Understanding the adaptive T cell (Adapted from Eggermont et al., 2014).


**Literature review**

Nearly all the currently available therapeutic approaches in Breast cancer treatment and management focus on the improvement of the activity of immune cells including macrophages and monocytes. The entire research work that has been done over decades has been supplemented with knowledge on the production of chemokines, cytokines and the activity of the biomolecules that facilitate tumor cell multiplication and angiogenesis. T cells and macrophages have been studied with their potential targets understood, a factor that offers information on the establishment of therapeutic measures (Segal et al., 2013).

Adenocarcinoma, a cancerous tumor that can affect various body organs including human breast, has clinically shown great host resistance factors. Human breast cancer develops from breast tissues. Its signs can vary depending on the victim, but the common ones include; a lamp in the beast, a unique fluid discharge from the nipple, for instance a milky discharge when a woman is not breastfeeding, enlargement of pores around the breast skin, change in breast or nipple appearance, for instance, dimpling on the breast, shrinkage of the breast, and the nipple might become slightly inverted (Murray et al., 1994).

Monoclonal antibodies (MAbs) can be used for cancer treatment. It is possible to produce these antibodies in the laboratory, by injecting an animal, for example mice or horse with an antigen from an affected human cell. The antibody producing cells are collected from the animal and fused with a cancerous B cell, this process will lead to production of hybridoma cells. Genetic engineering process can then be used to humanize the hybridoma cells. Cancer vaccines are not designed to prevent malignancy but rather to treat cancer that has already developed (Liu, 2014).
Breast cancer, just like other forms of cancer, can be diagnosed at its very early stages of development and the correct therapy initiated to destroy the cancerous cells. The basic form of offering therapy begins with carrying out surgery, belong by other forms of treatment such as hormonal therapy, drug chemotherapy and irradiation of the cells. Breast cancer immunotherapy has previously been focused with the potential of fast improvement of the treatment plans in the host individuals. Immunotherapy in breast cancer treatment has been associated with the low or no side effects that are experienced, less chances of developing immune resistance and the ability of the therapy to be offered to patients for quite longer periods of time in association with other forms of drugs (Davies, 2009).

A number of immunotherapy approaches have been developed to counter the severity of progression and development of breast cancers. Such therapies include: use of therapeutic vaccines such as Nelipepimut-S (Neuvax/ E75), GVAX and trastuzumab conjugated to an adjuvant, use of checkpoint inhibitors, adoptive T cell Therapy and genetically engineered monoclonal antibodies designed to target antigens produced by breast tumor cells (Schneble et al., 2014). The stage of the cancer, type and the age of the patient form the basic factors to consider when carrying out a therapy. Any case diagnosed early enough is an advantage to the patient (King, 2004).

Advances are being made in research to offer the best in breast cancer therapy. For instance, Ming Li (A grantee at Memorial Sloan Kettering Cancer Center) has over time studied the activated pathways in cancer associated cells that mark beta-blockers. Beta blockers are prescribed to high blood pressure patients and this has to facilitate longer survival to the breast cancer patients. In his study, T cells are targeted if they can respond to beta-blockers thus to a
greater extent opening up more targets for the anti-cancer drugs. Other studies are based on inhibition of the signaling pathways that aid in the maintenance of tumor survival that will lead to the production of specific antibodies that can aid in breast cancer therapy (Davies, 2009).

**Therapeutic vaccines in the treatment of cancer**

Immuoediting has proved to suppress growth of tumors through attacking and subsequent destroying of tumor cells. These modalities in immunotherapy have made a new perception of therapeutic clinical trials that potentially improve and encourage survival to some level (Patients with hormone-refractory, metastatic prostate cancer treated with GVAX cancer vaccine have demonstrated encouraging survival outcomes, 2007).

**Cancer Immunoediting**

The immune system changes the conformation of a tumor in a process called editing in a very selective manner. Immunoediting involves pure selection against the tumor variants which do not express or poorly express the specific antigens that are recognized by the immune system. This is done through a cascade of events that eliminate those epitopes, which in some way do not express the antigenic epitope thus all those epitopes that are negative do not become visible to the Cytotoxic T-Lymphocytes. However, over time there has been a problem that is attributed to lack of complete clearance of the tumor cells by the Cytotoxic T-Lymphocytes. This has been attributed to the fact that a number of antigens expressed by tumor cells are directly subjected to simple by the immune systems and thus the Cytotoxic T-Lymphocytes associated are destroyed by the thymus gland in a process referred to as central tolerance ('Cancer vaccine-GVAX: potential in NSCLC', 2004).
**Patterns of Cancer Vaccine Development**

The application and the use of therapeutic vaccines has been complemented by an understanding of the antigens being produced by the breast tumor cell that are specific to infiltrating T cells of the patient’s immune systems. Previously, patients have been treated with vaccines that contained about 8 to 10 amino acid long Cytotoxic T lymphocyte peptides that were obtained from the understood tumor antigen sequences. This was attributed to the fact that CD8+ T cells are involved in the active process of attacking and lysing the invading cells including tumor. However, this trial never demonstrate the clearance of tumor cells as the specific anti-tumor cells in the CD8+ colony proved to be sub-optimal and had limited life spans. The patients in the clinical trials were those with heavy tumors with high levels of mutant antigen production and high suppression levels (‘Cancer vaccine-GVAX/leuporelin/nadroparin calcium', 2009).

A vaccine called GVAX was among the early anti-tumor vaccines developed in breast cancer treatment. This vaccine used irradiations to the breast cells that were producing GM-CSF that did not show any success in meeting the target. Current studies are focusing on priming the naïve T cells to play the role of effectors in clearance of cancers. The US FDA has approved the use of a vaccine that has been designed to stimulate of immune responses to breast cancer cells in a more personalized form (Singh & Gulley, 2014).

On the other hand, recombinant pox viruses have been used in the delivery of vaccines. These viruses are used as vectors to transmit the desired therapeutic agent or cause recombinant mutations in the tumor cells to cause normal sequence alignments of the genes that code for the tumor cells. The use of recombinant viruses that encode for PANVAC and PROSTVAC have
been an active therapy in patients with different forms of cancer including prostate and breast cancer. The use of viruses in drug delivery includes both uses of in vitro methods to integrate the active molecules of the therapeutic agents into the virus’ genome (Sousa-Canavez et al., 2008).

The sequence of the virus must be understood to correlate with that of the gene responsible for a given tumor and due to this, the cell is targeted to modify the mutated sequence in an effort to change the expression of the contributing molecules for cancer development. Results from studies involving the use of pox viruses as chemotherapeutic agents have proved that the slow and progressive activation of the immune system on infusion of a vaccine has benefited most patients with breast cancer. This kind of therapy is highly effective to patients with metastatic forms of the cancer (Stein et al., 2004).

**Current trends in cancer vaccine therapy**

More advances are being made towards establishing fully active chemotherapeutic agents that can aid in clearing and preventing growth of tumors even in predisposition to the causative agents. Chemotherapeutic programs aim at the generation of specific responses that mediate cellular immune responses to kill any abnormal cell or tissue. This aims at making a change in reversal of the immunosuppressive systems and in a way through the stimulation of the immune system and its effectors. To some other level, it is to be understood that the chemotherapeutic agents that are being administered usually after vaccines have showed to take advantage of any hyper-response by the immune system and lead to more desirable outcomes in the treatment strategy (Limacher et al., 2013).

The use of vaccination and subsequent chemotherapy follow ups confirm a better response in terms of low tumor recurrence as compared to the previous patterns of reappearance
of the tumors. The survival rate is also increased in such patients as compared to those that receive chemotherapy alone. Another form of current technology in vaccines is limited to the use of monoclonal antibodies that are modified to specifically act as antagonist’s bodies that act to inhibit receptors of the antigens or as agonists that target the potential immune enhancing receptors thus the overall antitumor immunity is achieved. Vaccines are currently being combined with monoclonal antibodies especially the immunostimulatory antibodies that are based on the initiation of immune response to the tumors as well as the active inducing of anticancer responses with specific cancer responses since the antigens that are produced are easily marked by the modified immune systems with active maintenance of the system by use of memory cells (Madan et al., 2011).
The current survival rate is 90% up from 63% as per 1960s.

When Cancers rapid a protein receptor is called HER2. This kind of cancer can be treated with the use of targeted therapies. For example, pertuzumab and trastuzumab and in case of advanced cancer lapatinid (tykerb) or ado-trastuzumab emtansine (Kadcyla). The newest treatment choices are pertuzumab as well as ado-trastuzumab emtansine. The Pertuzumab was the first-line treatment of HER2+ metastatic breast cancer in conjunction with trastuzumab or (Herceptin) and the chemotherapy docetaxel (Taxotere). Ado-trastuzumab emtansine or (kadcyla) which is a HER2 targeted antibody with chemotherapy attached by linker, and is premeditated to deliver chemotherapy straight to the tumor through HER2-positive ('The GVAX cancer vaccine plus ipilimumab is active in the treatment of patients with advanced prostate cancer;', 2007).

GVAX can be best examined in patients with stage 4 breast cancer that does not express HER2. It is a cancer vaccine that is obtained from cancer cell that have been irradiated and engineered to express the immune molecules GM-CSF, these genetically modified tumor cells can help the immune system to effectively respond to cancer cells. Cyclophosphamide together with and trastuzumabs can elevate the number of immune cells hence, boosting the immune system (Davies, 2009).

Neuvax is made up of peptide, obtained from combination of HER2 protein and GM-CSF. Prescription for its dosage is ones, each month, for a period of six months. Neuvax vaccine is aimed at preventing or controlling the regeneration of cancer cells that are known to course breast cancer (Davies, 2009). Below is a cancer vaccine trial table presented at American society of Clinical Oncology Breast Cancer Symposium in September 2014.
<table>
<thead>
<tr>
<th>Clinical trials. Gov Identifier</th>
<th>Type of vaccine</th>
<th>Patients with;</th>
<th>Time frame</th>
<th>status</th>
</tr>
</thead>
<tbody>
<tr>
<td>NCT00524277</td>
<td>Her2- derived HLA class 1 peptide (GP2) with GM-CSF</td>
<td>Her2+ breast cancer</td>
<td>Five years</td>
<td>Active, preventing</td>
</tr>
<tr>
<td>NCT00854789</td>
<td>Her2- HLA class 1 peptide (E75) with GM-CSF</td>
<td>Her2+ breast Cancer</td>
<td>vaccine to induce an in vivo peptide-specific immune response Period needed to determine maximum tolerated and optimal biologic doses (30 days after each monthly dose.)</td>
<td>Active, preventing</td>
</tr>
</tbody>
</table>

Table 2: Cancer Vaccine Trial table
In table 1, this was a phase ii trial, which showed effective response to these Vaccines. Treatment with trastusumab(Herceprin), either received the Her2-derived HLA Class I peptide called GP2 in combination with GM-CSF, significantly reduced the risk of regeneration and so the patients benefited greatly from this vaccination (Lollini et al., 2015).

**GVAX**

GVAX is an anticancer drug that was developed by Somatix, a gene therapy company in 1993. This vaccine has revolutionized the field of medicine especially in oncology. GVAX is made of genetically modified cancer cells which are manipulated to produce a potential cytokine called Granulocyte Macrophage Colony Stimulating factor, GM-CSF. This vaccine has been previously used as a stimulator of neutrophils and macrophages in recombinant Leucine production with the purpose of reducing the toxicity of intensive chemotherapy. The vaccine has undergone several trials that have however proved to be successful. These trials have evolved through phases. In phase I, there was a low response amongst the patients involved but with much more research, the vaccine has gained success in subsequent phases (Rowe & Beverley, 1984).

GVAX aids in the delivery of Tumor Associated Antigens and the subsequent secretion of Granulocyte Macrophage Colony Stimulating Factor through paracrine models. This secretion of Granulocyte Macrophage CSF leads to a cascade of events that activate the dendritic cells of the bone marrow. The activated dendritic cells then present the Tumor Associated Antigens that are expressed by the tumor. The dendritic cells have a role of preparing CD4+ and CD8+ cells that recruit mechanisms of tumor destruction via lysis (Singletary, 1999). The Major
Histocompatibility Complex of the patient in this case does not need any matching with the vaccine since antigens are involved in priming the T cells in relation to the tumor secretions (Mazanet et al., 2012).

**NeuVax**

Neuvax is mostly used in the treatment of breast cancer patients with elevated HERs expression and is commonly administered after surgery. This is a peptide vaccine that was developed with the aim of reducing the possibility of the reappearance of cancer following surgery or irradiation of tumor cells. The vaccine is comprised of an active form of E75 peptide that is isolated from the cells expressing HER2. This peptide is then combined with GM-CSF obtained from yeast and an adjuvant is then added. The extracellular domain HER2 acts as the Nona peptide source for this drug during development. Through a number of steps, the responsible CD8+ cells are activated after presentation of antigens to the cells. Activated CD8+ (CTLs) are able to identify tumor cells, neutralize them and destroy them via lysis. The cancer cells that are targeted are the HERs protein producing cells and metastatic precursors. The immune response can as well direct cytotoxic T lymphocytes to new immunogenically characterized peptides via inter and intergenic spreading of active epitopes. Basically NeuVax has been used in Prevention of Recurrence in Early Stage Positive Breast Cancers with Low to Intermediate HERs expression (PRESENT) trials in which the Human Leucocyte Antigen 2 and 3 (HLA A2/A3) also called Nelipepimut-s was being evaluated. Since this vaccine is usually used with an adjuvant, it is used in patients who are expressing minimum residual nature of the cancer (Rowe & Beverley, 1984).
Breast tumor antigen is important to determine the efficiency of vaccines used in treatment and prevention breast cancer because breast tumor antigen works as tumor rejection target. In Table 2, Emens, 2012 summarized clinical trial of using vaccines to target HER-2 and MUC1. He examined the vaccines that was taking lone or low doses of cyclophosphamide and doxorubicin in 28 patients who had metastatic breast cancer. It was an initial clinical trial that using vaccines. He found that induction of induction of CD4+ T cell-dependent HER-2-specific immunity can measured by delayed-type hypersensitivity (DTH) and the level of antibodies. In addition, the level of CD4+ T cells-dependent immunity was lower when use vaccine a lone. Chemotherapy work better with vaccine than a lone.
Table 3: Summary of clinical trial of using vaccines to target HER-2 and MUC1 (Adapted from Emens, 2012).

<table>
<thead>
<tr>
<th>Vaccine</th>
<th>Patient population</th>
<th>n</th>
<th>Antigen-specific immune response</th>
<th>Clinical benefit</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>HER-2 peptide, E7S+GM-CSF</td>
<td>Stage IV breast and ovarian cancer</td>
<td>186</td>
<td>New E7S-specific CD8+ T cells and DTH</td>
<td>Recurrence rate in vaccinated vs control patients: 14.2 vs 5.6%</td>
<td>[67–76]</td>
</tr>
<tr>
<td>HER-2 peptide, E7S+GM-CSF</td>
<td>Early stage LNN99 and LNN99 breast cancer patients</td>
<td>18</td>
<td>New E7S-specific CD8+ T cells</td>
<td>NR</td>
<td>[77]</td>
</tr>
<tr>
<td>HER-2 peptide, E7S+GM-CSF</td>
<td>Early stage LNN99 breast cancer patients</td>
<td>15</td>
<td>New AIE7S-specific CD8+ T cells</td>
<td>NR</td>
<td>[78–79]</td>
</tr>
<tr>
<td>HER-2 peptide, E7S+GM-CSF</td>
<td>Early stage LNN99 breast cancer patients</td>
<td>18</td>
<td>New E7S-specific CD8+ T cells</td>
<td>NR</td>
<td>[77]</td>
</tr>
<tr>
<td>HER-2 peptide, E7S+GM-CSF</td>
<td>Stage III IV HER-2+ lung, breast, ovarian</td>
<td>64</td>
<td>New HER-2-specific IgG, T cell and DTH</td>
<td>NR</td>
<td>[80–85]</td>
</tr>
<tr>
<td>HER-2 ICD, protein+GM-CSF</td>
<td>Stage III IV, HER-2+ breast and ovarian</td>
<td>29</td>
<td>New HER-2-specific IgG, T cells</td>
<td>NR</td>
<td>[86]</td>
</tr>
<tr>
<td>HER-2 DC</td>
<td>DCIS</td>
<td>13, 27</td>
<td>New HER-2-specific CD4+ and CD8+ T cells; inversion of CD8+ T cells from CD4+CD8+CD29 to CD8+CD29+HLA-A2</td>
<td>Decreased HER-2 expression and less residual DCIS</td>
<td>[87,88]</td>
</tr>
<tr>
<td>HER-2 DC (Lapalasecl-T)</td>
<td>Stage IV HER-2+ breast cancer</td>
<td>18</td>
<td>New HER-2-specific T-cell proliferation</td>
<td>1 partial response, 3 stable disease &gt;12 months</td>
<td>[117]</td>
</tr>
<tr>
<td>HER-2 DC</td>
<td>Stage II/IIIY NED breast cancer</td>
<td>7</td>
<td>New HER-2-specific T-cell responses</td>
<td>100% DFS at &gt;4 years follow-up</td>
<td>[118]</td>
</tr>
<tr>
<td>Influenza virusomes-HER-2 peptides</td>
<td>Stage IV HER-2+ breast cancer</td>
<td>10</td>
<td>New HER-2 peptide and protein-specific antibody in 80% increased T-cell activity; decreased peripheral Tregs</td>
<td>NR</td>
<td>[119]</td>
</tr>
<tr>
<td>NeurG GM3- VSSP+Montanide™ (SEPPIC, Paris, France)</td>
<td>Stage III IV breast cancer</td>
<td>21</td>
<td>New vaccine-specific IgA, IgG, IgM</td>
<td>10% stable disease (for 18 and 40 months)</td>
<td>[120]</td>
</tr>
<tr>
<td>NeurG GM3- VSSP+Montanide™ vs best supportive care</td>
<td>Stage IV breast cancer</td>
<td>79</td>
<td>New vaccine-specific IgG and IgM</td>
<td>Trend toward survival improvement</td>
<td>[121]</td>
</tr>
<tr>
<td>GL1001-H-KLH+Q8-21</td>
<td>Stage IV breast cancer</td>
<td>27</td>
<td>New IgG, CDC, ADCC, no IgG</td>
<td>56% 2-year DFS</td>
<td>[98–94]</td>
</tr>
<tr>
<td>Phase III THERATOPE: CY+KLH vs CY+Stns-KLH</td>
<td>Stage IV breast cancer</td>
<td>1028</td>
<td>New vaccine-specific IgG</td>
<td>No difference</td>
<td>[96]</td>
</tr>
<tr>
<td>CEA-MUC-1-TRECOM poxvirus</td>
<td>Stage IV breast and ovarian cancer</td>
<td>26</td>
<td>Inconsistent</td>
<td>Possible clinical benefit in patients with minimal disease</td>
<td>[122]</td>
</tr>
<tr>
<td>hTERT peptide+montanide+GM-CSF</td>
<td>Stage IV breast cancer, HLA A2*</td>
<td>19</td>
<td>New TILs post-vaccine: Functional hTERT-specific peripheral CD8+ T cells</td>
<td>Improved survival associated with hTERT immunity</td>
<td>[123]</td>
</tr>
</tbody>
</table>
Adoptive T cell therapy for breast cancer

T cells are essential elements in cell mediated immunity especially for recognition of stray antigens in the host. They have been used in the current environment in fighting against the invading cancer cells through a cascade of modifications. Substantial advances have been made in an effort to modify the T cells by use of genetic engineering methods and the use of antibody recognition in Chimeric Antigen Receptors (CARs). Clinical trials have been done to evaluate the efficacy of genetically modified T cells in the treatment of breast cancer of which have proven to be very successful. The different tumor types in breast cancer have however posed a great challenge in the development of specific T cells for a given kind of tumor thus posing a great setback in this research (Libutti, 2015).

Adoptive T cell therapy is achieved when there is a transfusion of lymphocytes with an aim of treating an individual with cancer, in this case breast cancer. T cell therapy basically has an overall enhancement feature of antitumor immunity, augmentation of efficacy of vaccines and the subsequent limitation of graft-host infections. The ultimate passage of new allogenic T-cells into individuals with cancers has helped in the control of the growth of tumors in human populations especially if diagnosis is made early enough. According to Chester Southam and his Colleagues in 1970, leukocytes with a given ability to inhibit the implantation and growth of tumor cells were found in a number of patients who had advanced cancer to be used as the potential pol for adoptive T cell therapy (Libutti, 2015).

In comparison with the therapeutic cancer vaccines, the use of adoptive T cell therapy has not been previously practiced by most oncologists since there was no a formal approval of their use. Currently, the knowledge on the application of T cell therapy has opened up the dream of
treating breast cancer with ease. This is destined to be much more effective in with the advent of transfusion medicine. Emphasis is being put on Cytotoxic T lymphocytes (CTLs) and Tumor Infiltrating Lymphocytes (TILs) (Murray et al., 1994).

CTLs are obtained from Peripheral blood lymphocytes and are potential targets that have been used for different sets of tumors including melanoma and Breast Cancer. Tumor Infiltrating lymphocytes basically demand that new and fresh isolates of T cells are obtained through biopsy with a subsequent selection of cancer specific cells with the use of very high interleukin 2 in cell culture. If the host is prepared early enough with chemotherapy and then infused with the adoptive T cells, there is an increased response (Libutti, 2015).

Sets of clinical models have been evaluated and they suggest that the associated transfer of concomitant Hematopoietic Stem Cells (HSCs) may show a greater result in promoting anti-tumor effectiveness of the infused modified T cells. The problem scientists are currently facing lies on the efficiency of producing much more satisfactory specimens that can be used in treatment of Breast cancer as only 30-40% of Tumor Infiltrating Lymphocyte populations have been successfully obtained from biopsy specimens. If the current technical problems on tissue culture be overcome, then the whole exercise of using TILs can create a better milestone in the treatment of breast cancer (Murray et al., 1994).

This kind of therapy manipulate host T cell family with an aim of destroying cancer breast cancer cells. The procedure involves an aseptic collection of patients’ immune cells that are further genetically engineered in vitro like CAR so as to identify breast tumor cells in the large pool of body cells. This is done through manipulating the cells to specifically recognize the types of chemoattractants and cytokines that are being produced by the tumor cells. These cells are
then purified and then re-introduced to the patient intravenously (Dudley, 2011). These T cells that have been modified are now capable of identifying the specific antigens that are being produced by the tumor cells thus can be easily destroyed. The process of T cell therapy involves a number of developmental stages. The sample is initially collected from the breast cells expressing the cancerous condition followed by hybridoma based multiplication of the tumor cells in the laboratory. High aseptic techniques need to be employed in the entire exercise. T cells are then obtained from the patients’ bloodstream and then are manipulated genetically in order to identify the cancer cells with specific antigens being expressed. These cells are modified with the incorporation of special receptors that aid in the recognition of the proteins being expressed on the surface of the tumor cells. These receptors are called Chimeric Antigen Receptors (CARs) (‘Adoptive T-cell Therapy Has Clinical Activity in Metastatic Cervical Cancer’, 2015). The adoptive T cells transfer is working by taking from patient and active them in vitro. These cells can activate by DCs, or by artificial T cells or by engineering T cell and then rejection to patient. Trying to treat HER+ breast cancer patients by transfer of autologous HER2 specific T-cell clones and monitoring patients (Bernhard et al., 2008).

Figure 8 shows the structure of CAR. The CAR is engineered receptor consists of two parts: ectodomain and endodomain. Ectodomain has a signal peptide that point protein to endoplasmic reticulum and linked the amino- terminal. In addition, it has the antigen recognition domain that is commonly be a single-chain variable fragments (scFv) derived from monoclonal antibodies. ScFv described from alpha and beta signal chain of naive T cell receptor (TCR) and it can link cytokine that lead to realization to cells which have cytokine receptor. The transmembrane domain that is expansion the membrane is a hydrophobic alpha helix and is
located in endodomain. Connecting between the transmembrane domain and the antigen binding domain by a spacer region in ectodomain. Endodomain is in the last part in the receptor. Signals and receptors transfer to cell after antigen recognition.

Figure 9 is a representation of engineered receptors comprised of pieces from different sources that were focused against both another receptor called tyrosine-protein kinase ErbB-2 and MUC1. In figure 9A, an MUC1-specific scFv cloned from HMFG2 was coupled through an IgD hinge and the IgG1 Fc was connected to a human CD28+ CD3 endodomain that generate IL-2 when co-cultivated with MUC1-expressing target cells. The CD28+ was seen to lack the scFV while HDF28 and HDFTr conflict the intercellular component consisted of the full or truncated CD28 endodomain respectively. Further, in IzI, an ErbB2-specific scFv, which was cloned from ICR 12 was connected through a CD8 alpha hinge and transmembrane domain to CD3ζ endodomain. In 128ζ hinge, endomain of CD28 and transmembrane were followed by CD3ζ endodomain. The arrangement MUCI/ErbB2 construct was indicated. Figure 9B was a structure of the retroviral vector used to express IzI to gether with HDF28 or HDFTr while figure 9C three upper panel shows detection of MUC1-specific CARs when transduced in T-cells. They found the expression of ErbB2 IgG FC was not detectable in both ITH and ITHTr. Finally, figure 9D was a Western blotting showing how IZI expresses itself in dual targeting constructs. The rsusult was the mass of IZI was coordinated with efficient proteinmeadieted by retrovial vector. In conclusion, the modified T Cells can be used as therapy for hematologic malignancies like breast tumors. As a result, the co-express of IZI with MUC1- spesfic HDF28 CAR can destroy various breast cancer monolayers even of the expression IZI was reduced (Wilkie et al., 2012).
Figure 10 is a graphical representation of interleukin-2 production by T-cells engineered to express the dual target constructs. The human T-cells that express the indicated CAR molecules were cultivated a night earlier with an equal number of Jurkat E6 cells or MDA-MB-435 cells in (Figure 10A). They found the level of IL-2 decrease when ErbB2 co-express with MUC1. Figure 10B expresses MUC-1, ErbB2 or both antigens. The human T-cells in 10C express the indicated CAR molecules that were cultivated earlier with a confluent monolayer of the indicated mammary carcinoma cell line. In all the cases, two separate transduction with ITH were also tested. The supernatants were harvested and analyzed IL-2 content in triplicate. In this diagram, it describes a dual targeting of the ErbB2 and MUC1 in breast cancer. they demonstrate that T-cell activation and IL-2 production was depended on balance between CAR expression on the T-cell and intensity of antigen on target cell (Wilkie et al., 2012).

In figure 11 Bernhard et al., 2008, were monitoring the effect of transfer T cell to the patients by monitoring the frequency of HER2 specific T cells in both peripheral blood and bone marrow before and after transfer T cells. Figure 11A is in peripheral blood after 1 hours, measured that the frequency of HER2 specific T cells rise of CD8 T cells. After 1 day, CD8 T cell decreased which means is there are no reveal of the frequency of HER2 specific T cells. Figure 11B is in BM, also they are found rise of the frequency after 24 hours in patient with fifth transfer using A2/HER2. Therefore, the HER2- specific T cell can residence in bone marrow.

In summary, T cells can destroy the single neoplasm cells in case of there no surrounding tumor cells. This sequence of events described by Bernhard et al., 2008, supports my hypothesis.
Figure 8: The structure of chimeric antigen receptor (CAR) (Adapted from wikipedia, 2015).
Figure 9: Engineered receptors against both ErbB-2 and MUCI (Adapted from Wilkie et al., 2012).
Figure 10: Interleukin-2 production by T-cells engineered to express the dual target constructs (Adapted from Wilkie et al., 2012).
Figure 11: Monitoring the frequency of HER2-specific T cells in peripheral blood and bone marrow in patients before and after transfer adaptive T cells. A- Peripheral blood. B- Bone marrow (Adapted from Bernhard et al., 2008).
Monoclonal antibodies in targeting Breast cancer antigens

Monoclonal antibodies mediate cell killing by use of Antigen Dependent Cell Cytotoxicity (ADCC). There are approaches that have been embraced in the successful use of antibodies in the elimination of tumor cells including immune modification of T cell functions by use of ipilimumab which is monoclonal antibody can activate immune system, induction of ADCC by use of rituximab (chimeric monoclonal antibody) and nullification of cancer cell signaling by use of drugs such as centuximab (which is an epidermal growth factor receptor (EGFR) inhibitor used for the treatment of metastatic cancer) and trastuzumab (is a monoclonal antibody that interferes with the HER2/neu receptor) (Annibali et al., 1994).

Monoclonal antibodies have been used in treatment of breast cancer especially when the nature of the antigens is well known. The secreted antigens easily bind to the monoclonal antibodies and they prevent other antibodies from binding to the tumor in the developing cells. Rapid internalization of the antigen occurs in order to maximize the availability of the antigen binding site to the immune cells as well as the complementary proteins (Berinstein, 2009).

The current technologies in treatment of breast cancer largely depend on monoclonal antibodies targeting the specific molecules being produced by the tumor cells. HER2/neu is produced by tumor cells and expressed on their surface. Too much of this antigen on the surface of the cells facilitates rapid growth of the cells with minimum achievements of treatment. There are a number of drugs otherwise termed as monoclonal antibody extracts that target the antigen produced by the tumor cells. These include but not limited to, Trastuzumab (Herceptin), Pertuzumab (Perjeta), Ado-trastuzumab emtansine (Kadcyla) and Lapatinib (Tykerb) (Murray et al., 1994).
Trastuzumab and pertuzumab are basically in vitro monoclonal antibodies that are administered intravenously with high immune protein specificity. Ado-trastuzumab emtansine is usually attached to a chemotherapeutic drug that is also administered intravenously. Of these drugs, only lapatinib is given as a pill.

**Role of genetically engineered monoclonal antibodies in targeting Breast cancer antigens.**

Rowe & Beverley, (1984) flag the cells, cancer cells, and make them identifiable for destruction. The monoclonal antibodies are specialized to identify specific receptors on the surface of the tumors and subsequently bind them at the Fc region. The flagging makes the cancer cells easily recognized by the other immune cells that migrate to the sites of cancer to kill the cells by opsonization, lysis and by use of Antigen Dependent Cell Cytotoxicity (ADCC).

Monoclonal antibodies also act by blocking the basic signal producing processes as well as the receptor sites for the signals and antibodies. In the process of mAb development, scientists can opt to limit tumor cell growth by blocking the mechanisms that facilitate the process of proliferation. The genetically engineered antibodies block the major pathways such as signal transduction pathways, pathways that lead to initiation angiogenesis and those that allow for cell maturation. Since there has to be a reliable mechanism of transporting nutrients, cancer cells initiate the process of angiogenesis. Monoclonal antibodies that limit the process of angiogenesis therefore deprive the tumor cells of basic nutrients for their survival leading to cell death or slowed proliferation (Rowe & Beverley, 1984).

Monoclonal antibodies are used to directly deliver drugs to the cancer cells. This process involves prior modification of cancer cells to carry therapeutic agents that they deliver directly to the cancer cells. This lies on the fact that the antibodies are capable of attaching specifically to
the designated breast cancer cells. Therefore, therapeutic agent is bound to the monoclonal antibody in vitro and the antibody marked to target specific lines of cells. The antibody is then introduced into the bloodstream via intravenous infusion. This antibody travels to the breast cancer cells where it binds with the tumor cells with subsequent delivery of the drugs. This happens by engulfment of the monoclonal antibodies by the cancer cells which begin to. The process of internalization breaks off the bound drug to release it to the environment thus causing cell death. The process is termed as self-suicide since the tumor cell initiates the process of its death. (Rodriguez-Bigas, 1992)

Radiation particles alongside chemotherapeutic agents can also be combined with monoclonal antibodies and then allowed to deliver in small doses. The radiations cause death of the cancer cells selectively allowing the survival of adjacent cells. The monoclonal antibodies tagged with radiation particles are also used in the diagnosis and monitoring of the cancers using radioimaging techniques. Cytokines are the latest form of combination that is conjugated to the monoclonal antibodies. This complex is called an immunocytokine. Immunocytokines are used to directly deliver cytokines to the tumor cells with an aim of initiating an immune response. Trastuzumab, also called Herceptin, and Kadcyla (ado-trastuzumab) are the currently applied monoclonal antibodies (Rowe & Beverley, 1984).

Shi et al., (2015) emphasized that neutral killer cells (NK) have significant function in ADCC. Also, these cells can rise infiltration of NK cells in breast cancer that bind to activity trastuzumab. By peripheral blood mononuclear cell (PBMC) and macrophages while Trastuzumab can intermediate ADCP to against HER2-expressing tumor cells. They used old mice that vaccinated with high HER2-expression BT474 breast cancer cells to show the
antitumor effectiveness of trastuzumab in infiltration of macrophages in neoplasm site. Treated mice with Trastuzumab and isotype IgG. Macrophages in spleen in Mice were treated with trastuzumab was greater than mice were treated with control isotype IgG (figure 12A). Using clodrosome nanoparticles to deplete macrophage in vivo. Prevent tumor development via trastuzumab with clodrosome (figure 12B). In (figure12 C) mice treated with trastuzumab had higher to kill cancer cells. This experiment demonstrated that trastuzumab had higher power to inhibit BT474 breast cancer cells. Also, Xenograft tumor growth and infiltration of macrophages in tumor site had a great significant for efficiency of trastuzumab.

Shi et al., (2015) also studied the function of FcyRIV that expressed in macrophages in reach of trastuzumab-intermediate ADCC efficiency. They were using mice macrophage cell lines. These cells can be upregulated via combine with INF-gamma or knock down by shRNA lentivector system. They found that the expression of FCYRIV was higher with WT plus INF-gamma as showed in (figure13 A). In (figure 13B) showed the percentage of killing tumor cell also was elevation with WT + INF- gamma compared with WT. In addition, the activity of phagocytes was rising in WT plus INF- gamma in the existence of trastuzumab (figure 13C). The INF-gamma catalyze BBM within existence trastuzumab had a power to kill HER2 cancer cells and increase the percentage of phagocytes (figure13 D-F).

Shi et al., (2015) found FCYRIV had an important function in macrophages intermediated ADCP and trastuzumab to destroy cancer cells.
Figure 12: The antitumor efficacy of trastuzumab depends on macrophage recruitment in the tumor tissues. A) Macrophages (CD11b+) in the splenocytes and tumor-infiltrating cells of mice treated with trastuzumab or isotype control IgG with or without clodosome treatment. (B) Xenograft tumor sizes of the different treatment groups. (C) The splenocytes (Adapted from Shi et al., 2015).
Figure 11: The function of FCYRs that are expressed in macrophages in trastuzumab-mediated ADCP activity (Adapted from Shi et al., 2015).
Checkpoint inhibitors and their contribution in Breast Cancer Therapy.

There are a number of checkpoint inhibitors that have proven to be very effective in the treatment of a wide range of cancers such as lung cancer, lymphomas and breast cancers. However, breast cancers have numerous features that have a different regimen of treatment. This breast cancer cells may be affected by female hormones that stimulate abnormal cell growth (Estrogen and progesterone). (Marques et al., 2015) found that these forms of Breast Cancer that have been studied involve those that are associated with HER2+. These cancers come about due to the limitless expression of the epidermal growth factor on receptor 2 which is a cascade member in the growth of normal body cells. The latter kind of cancer is forms the most rampant kind of cancer with 2 out of 10 women with breast cancer.

Other breast cancers may not be caused by the above hormones and are not HER2 negative. These are cancers collectively termed as triple negative breast cancers. These kinds of cancers are really active and very aggressive in terms of progression with high chances to metastasize to peripheral tissues. Therefore an early diagnosis of these cancers reduces the risk of early death due to cancer. In a study to test on the activity of the above stated checkpoint inhibitors, there was a recruitment of 32 cancerous individuals who had the triple negative breast cancer and Keytruda administered for a period of 14 days in a regular case until all the patients showed a response as either effective in lowering the progression or maintaining the rate of progression or no effect in controlling the progression. Six patients (18.5 percentage) showed a complete response while twenty one showed a stable condition of the disease with the rest having a substantial increase in the levels of progression (Libutti, 2015).
Patients with HER2 cancer have also been treated with a varied number of therapies including the use of endocrine / HER2 targeted therapy in single forms or in a coupled combination in the specific individuals affected. This therapy has been known as a single or a lone therapy that is preferred when the patients have an asymptomatic condition of breast cancer. Pertuzamab is the currently preferred first line of treatment for the onset of the cancer with a second line of treatment being on trastuzumab. On a contrary base, as there are advances in the non-targeted therapy in the triple negative therapy, there are further scientific research processes in progress that target to investigate the novel type of surface receptors through use of checkpoint inhibitors with the identification of the specified targets to benefit from therapies based on platinum and adenosine diphosphate ribose inhibitors (Libutti, 2015).

On the aspect of targeting the inhibition of angiogenesis, the Eastern Cooperative Oncology group (ECOG) has found that if bevacicumab conjugated with paclitaxel is a random set of breast cancer patients, the nature of metastatic cancer is in a great fold improved. The process of angiogenesis uses a given set of factors known as The Fibroblast growth factor receptor gene (FGFR) that is a common contributor to resistance of the anti-fibroblast growth factor component as well offering many variations of this gene in breast cancer. Therefore in 1 out of 10 patients with breast cancer, FGFR variations affect subsequent prognosis in the oncology centers. This has caused a great shift of research work on the possible means to develop target therapy for this growth factor. If successful, all the processes of angiogenesis by tumor cells are expected to be altered to in order to treat breast cancer completely (Marques et al., 2015).
Checkpoint inhibitors are not target specific and hence can work in many different tumor types. Check point inhibitors have a unique mechanism of action, where they target molecules that act as checks and balances in the regulation of immune response. Therefore, enhancing the cancer immune response (Hilgers et al., 1985). Mononuclear antibodies (mAbs) are known to be effective in blocking one or many immune checkpoints, hence, boosting the power of the antitumor cells. In clinical trials, anti-cytotoxic T-lymphocytes antigens 4 (CTLA -4) Mabs, has been tested.

Figure 15 shows that combining the anti-CTLA-4 mAb 9H10 with local radiation therapy caused an extension of the survival of mice with non-immunogenic 4T1 carcinoma. Mab 9H10 is monoclonal antibody that can block CTLA-4. 4T1 is BALB/C mouse – derived mammary carcinoma cell line. They using IgG antibody as a control. Figure 15 (A) shows the growth of tumor volume (in mm3) over a period of 40 days after tumor inoculation under different treatment conditions (IgG only, 9H10 only, RT+IgG, and RT+9H10). The graph shows that growth of the tumor was highest in the first two treatment (IgG only and 9H10 only). However, tumor volume was much lower for RT + 9H10 and RT + IgG treatments. Figure 15 (B) shows the survival rates of mice subjected to the four treatment options after tumor inoculation. The highest survival rate was shown to be for mice subjected to the RT + 9H10 treatment. Survival was lowest for mice treated with IgG and 9H10 only. The two figures show that the combination of radiation therapy with 9H10 monoclonal antibodies against CTLA-4 was most effective in treatment of tumor. However there us limitation in this study which is the survival for five days more (Demaria et al., 2005).
Figure 16, Demaria et al., 2005 want to make sure of the effect of the combination of RT with 9H10 on initial tumor grow and survive of mice. In part A, they found that the neoplasm degradation in two of seven mice treated with RT+ IgG. In mice treated with RT+9H10 found that tumor degradation in four of seven mice as shows in part B. In figure 16C there was not degradation of tumor in mice treated with IgG alone. In part D, they found benefit of treating mice with RT+9H10 in increase the degradation of tumor with long survival.

Figure 17 shows the level of lung metastases in the lungs of mice treated with four treatment options: IgG only, 9H10 only, RT+IgG, and RT+9H10. The mice were sacrificed and the number of metastases counted. Figure 17 (A) shows that the number of metastases was lowest for the last treatment group RT + 9H10 and highest in the control group, which was treated in IgG only. This implies that the combination of radiation therapy and 9H10 monoclonal antibodies against CTLA-4 has high therapeutic value in lung metastases in breast cancer. Figure 167(B) shows that CD8+ T cells were depleted in the reduction of lung metastases in the RT+9H10 treatment while CD4+ cells were depleted in RT+IgG treatment. The figure shows that CD8+ T cells are required in the inhibition of lung metastases by radiation therapy and CTLA-4 blockade (Demaria et al., 2005).
Figure 14: Therapeutic inhibitors using checkpoint process in cancer therapy treatment, (Adapted from Khalil et al., 2012).
Figure 15: Combining the anti-CTLA-4 mAb 9H10 with local radiation therapy caused an extension of the survival of mice with non-immunogenic 4T1 carcinoma (Adapted from Demaria et al., 2005).
Figure 16: Effects of two fractions of local RT in combination with CTLA-4 blockade on the primary tumor growth and survival of mice bearing the nonimmunogenic 4T1 carcinoma (Adapted from Demaria et al., 2005).
Figure 17: Level of lung metastases in the lungs of mice treated with four treatment options (Adapted from Demaria et al., 2005).
Conclusion

Development of highly potent immunotherapy against breast cancer has been a successful in controlling the risks of metastasis. However, clinical evidence confirms that if the cancer is diagnosed early enough before advancement to complex levels, either of the discussed therapies can offer higher percentages of treatment. The development of monoclonal antibodies and T cell therapy is expected to help in complete clearance of breast cancers especially with the ability of monoclonal antibodies to elicit a number of mechanisms in killing cancer cells (Zhou et al., 2007).

Considering the current statistics regarding breast cancer immunotherapy, there is great correspondence to the statistical representation in the above tables. Cancer deaths are so many and eminent but are decreasing because of the available aspects that are regarded to induce objective inclination towards survival rates. In the past five years, there has been an increase in this survival rate to a consistent of 90% of the cases prospected. This is described to be a dramatic improvement considering that the rates of cancer infections have been so much (Zhou et al., 2007). This is quite different as compared to the earlier years where, the infections were much few as compared to present times. If this consideration is expressed in terms of the stage of the disease, the survival rates are quite variant and can be explained using the table that is shown below.
<table>
<thead>
<tr>
<th>Number</th>
<th>Stage</th>
<th>Rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1</td>
<td>95 %</td>
</tr>
<tr>
<td>2</td>
<td>2</td>
<td>84 %</td>
</tr>
<tr>
<td>3</td>
<td>3</td>
<td>60 %</td>
</tr>
<tr>
<td>4</td>
<td>4</td>
<td>24 %</td>
</tr>
</tbody>
</table>

Table 4: Cancer survival rates against infection stages, (Adapted from immcellther.com).

The above table shows particular aspects that will always recommend that cancer treatment at the early stages is so crucial and will decisively be articulated with its infection levels. In the past 3 decades, the level 1 survival rates were obligated at 63 % which also shows great improvement in the treatment of breast cancer. This is where immune therapy is so crucial in contribution to the current survival rates. This is why the medical positions have always advised that the treatment of cancer is started early and is prospected to have every indication intact in the sense that it will be articulated to have better performance in order to improve the rates from the current 24 %. Immunotherapy has had a greater hand in this improvement, however, with the drawbacks like having expensive inputs in the therapy articulations. Every inclusion is therefore important and must be accorded that the basic requirements are meant to reduce the risk of cancer deaths (Winchester, 1991).
Figure 18: Survival rates graph, (Adapted from mmcellther.com).

The discussed aspects that aid in cancer immunotherapy are always induced to better performance hence. These aspects named as the prospective and justified therapy methodologies will include the use of vaccines, the use of check point inhibitors, the use of antibodies and the use of the adoptive T cell therapy. All these aspects are considerably an important aspect and are regarded with the objective of improving the survival rates for cancer and the positions that are meant to design the current statistics to further decrease in cancer cases, cancer deaths and increment of the survival rates of cancer and other prospects. Every consideration is therefore prospective towards some input in the positive influence in cancer treatment aspects. (Yee, 2013)
Limitations to the study and therapy

Breast cancer is not a single disease

Breast cancer has always been considered a multitude of diseases. Hence given the description of not being a single disease. There are particular biological processes that are involved as well as various and distinct pathways that are genetic. These pathways are also associated with the prognosis of the chemotherapy alongside the sensitivity that is involved. Ensuring that individuals who have the disease have the normal way to lead their life is quite a problem and is addressed with the articulated importance of less targeted agents in different types of breast cancer. This implies that there are numerous types of these infections that can be induced and lead to deaths if it is not well managed (Paoletti et al., 1993).

Cancer immunotherapy is always a recurrent process

This is also relented to its classification. This is because it is classified as part of the molecular events. This is also considered a primary challenge, especially when future drug development is a consideration. Every aspect in breast cancer is articulated to give a distinction of genes as well as the pathways that drive cancer proliferation. The identification of these functional pathways which are enriched for the articulated mutated genes will devolve the influence in subpopulations of patients that are prone to sensitivity to the agents that are biologically driven (Kramer et al., 1994).

Immunotherapy is a complex process

There is also one consideration that cancer treatment which involves immunotherapy is a complex process. However, we are moving to an era of stratified therapy as well as that which is
personalized. This is based on the precepts that there is a detailed molecular characterization of the patient tumor as well as its micro environment components. This will give rise to the tailored therapies as well as a decreased toxicity in the process. Every aspect must be ignited towards the numerous challenges that are meant to improve the stature of personalized immunotherapy protocols (Paoletti et al., 1993).

**Tumor Heterogeneity**

The processes will also include tumor heterogeneity as well as molecular evolutions as well as the costs. There is also potential morbidity as a factor of the biopsies, technical limitations in the process of therapy and the decreased effective drugs because of the genomic aberrations. There is also a consideration of the reimbursement and the regulatory hurdles. Critically, these aspects are mainly influential and have forced the improvement of the practices against cancer and the recurrent limitations of the therapy processes. This will influence the increased survival rates against the cancer prone cases (Dizdar et al., 2007).
Challenges in future research

Cancer immunologists have presented various efforts towards proving the adaptive and innate immune cells which are meant to recognize tumor cells. This has always influenced injections of tumors as well as injection in the existing models of the treatment. This means that there is a prospective huddle in this therapy and will have the most problematic process that is capable of better performance and other related aspects. There is a rationale of study in the future in this argument in the sense that it is articulated towards various aspects of ever changing stages of the immunotherapy aspects. The strategies are influenced by the most difficult facial situations that are anti-tumor immune responses. We therefore focus on changing aspects in this process of therapy which will always require new studies as well. In addition, using older mice in trail is the best because the old women are more susceptible to breast cancer.
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