JACOBS PUBLISHERS

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Jacobs Journal of Cancer Science and Research

Review article

Emerging Role of Immunotherapy in Breast Cancer

Timothy Allen[1], MD, Ph.D., Ghazaleh Shoja E Razavi*[1] MD

Global Allied Pharmaceutical, Center for Excellence in Research & Development

**Corresponding author: Dr. Ghazaleh Shoja E Razavi MD, Dir. Clinical Development- Oncology and Respiratory, Global Allied Pharmaceutical, Tel: 1- 416-520-8835; Email: ghazaleh.shoja@gapsos.com*

Received: 11-17-2015

Accepted: 01-18-2016

Published: 02-26-2016

Abstract

Breast cancer is the second most common cancer in women, after non-melanoma skin cancer. One out of every 8 woman in the United States will develop breast cancer. It can be caused by genetic mutations, using certain medicines, or even hereditary disorders. Traditionally breast cancer would be treated through surgery, radiation therapy or chemotherapy. However, novel approaches in targeting the tumor cells rather than normal cells are changing both the therapeutic options and the prognosis of breast cancer. Immunotherapy, is one of these targeted therapeutic approaches that has been shown promising results in the treatment of specific subtypes of breast cancer and has been subjected to significant development and clinical studies.. This kind of therapy stimulates one's own immune system and exploits immunologic anti-tumor mechanisms such as antibodies, as well as cell mediated cytotoxicity to fight the malignant tumor. In this paper, we discuss the causes, epidemiology, and potential immunotherapeutic techniques to treat breast cancer.

Keywords: Breast Cancer; Immunotherapy; Estrogen; Monoclonal Antibodies; T-Cell Therapy; Vaccines; Checkpoint Inhibitors

Introduction/Epidemiology

Breast cancer is one of the leading causes of death worldwide. In 2014, it was estimated by the National Cancer Institute that there would be 232,670 new cases of breast cancer in females and 2,360 new cases in males. In the same year, they also estimated that there would be 40,000 cases of deaths in females and 430 cases of deaths in males in the United States [1]. As per the statistical analysis, in the United States, breast cancer is the second most common leading cause of death in women and around 1 in every 8 women will develop invasive breast cancer in their lifetime [2]. The annual age-standardized incidence rates (per 100,000 women) were as follows: in North America, 90; Central America, 42; Western Europe, 78; Northern Europe, 73; Southern Europe, 56; South and Eastern Europe, 49; East Asia, 18; North Africa and Western Asia, 28; South-East Asia, 26; South Central Asia, 22; Oceania, 74; and sub-Saharan Africa, 22. The United States have a higher incidence as compared to rest of the world [3].

Generally, breast cancer initiates in the lining of the milk ducts or lobules that supply them with milk. A breast cancer that initiates in the ducts is called a ductal carcinoma. If it initiates in the lobules, it is called a lobular carcinoma [1]. Race, ethnicity, breast feeding, hormone therapy, oral contraceptives, age at menarche, parity, first live birth, menopausal status, alcohol, diet, anthropometric factors, physical activity, environmental exposures, occupational exposures, and mammographic breast density are the most common risk factors of breast cancer [4].

White women are more prone to breast cancer than African-American women. But African-American women are more likely to develop more advanced-stage breast cancer, be diagnosed at a young age and are more likely to die from this cancer. However, in women under 45 years of age, breast cancer is more common in African-American women. Asian, Hispanic, and Native-American women have a lower risk of developing and dying from breast cancer. Breastfeeding for a year or more slightly reduces a woman's overall risk of breast cancer.

Results from observational studies indicate that hormone replacement therapy after menopause increases the risk of breast cancer. Use of a regimen that includes both progesterone and estrogen has been linked with a higher risk of breast cancer than the use of estrogen alone. Women who do not use hormone therapy may undergo screening mammography less frequently as it plays a role in decreasing the incidence of breast cancer. Various studies have found that women using oral contraceptives (birth control pills) have a slightly greater risk of breast cancer than women who have never used them. Once women stop taking these pills, the risk might reduce over time. Women who stopped using oral contraceptives for more than 10 years do not appear to have any increased breast cancer risk.

The use of alcohol is clearly linked to an increased risk of developing breast cancer. The increase in risk is directly proportional to the amount of alcohol intake. High-fat diets can lead to being overweight or obese, which is a breast cancer risk factor. A diet high in fat has also been shown to influence the risk of developing several other types of cancer. Also, intake of certain types of fat is clearly related to heart disease risk. Women who have had more menstrual cycles because of early menarche (started menstruating before age 12) and/or went through menopause late (after age 55), have a slightly higher risk of breast cancer. The increase in risk may be due to a long exposure to estrogen and progesterone.

Pathophysiology and molecular basis:

Breast cancer includes well-defined molecular subtypes i.e. luminal (luminal A and luminal B) or hormone-sensitive, HER2-positive, molecular apocrine subtype and triple negative tumors [5]. The luminal molecular subtype includes estrogen (ER) and progesterone (PR) receptor [6]. The identification of Luminal B subtype is done by the co-expression of HER2, in addition to ER and PR, HER2-negative luminal-A subtype, or by higher proliferative activity [6,7]. There is a lack of expression of ER and PR in HER2 positive breast cancer, but it is defined by the overexpression of HER2 protein by immunohistochemistry and/or HER2/ neu gene amplification [6]. Breast cancer negative for ER, PR and HER2 protein expression is called triple negative. It partially overlaps with basal-like subtype showing expression of basal cytokeratins that is normally present in the basal cell of mammary ducts.

New molecular subtypes have also been described in breast cancer. The claudin-low subtype includes triple negative breast cancers, which lacks cytokeratin 5/6 and epidermal growth factor receptor in contrast to basal triple negative subtype [6,8]. Molecular apocrine breast cancers are characterized by ER negativity and androgen receptor positivity in addition to apocrine morphology with the presence of intracellular vacuoles [9].

Both HER2-positive and triple negative breast cancers have a higher tendency to develop metastases in visceral locations or immediately in the central nervous system. The molecular type also act as an indication for treatment: luminal type can be targeted by hormone therapy, HER2-positive tumor – by anti-HER2 agents, and triple negative – by chemotherapy. Triple-negative breast cancer cells also are dependent on poly (ADP) Ribose polymerase (PARP) to repair single strand breaks in DNA. Additionally, PARP inhibition also serves as an effective treatment modality [5].

Immunotherapy

A. Monoclonal Antibodies (mAbs):

1. Trastuzumab: [10]

Trastuzumab is approved by FDA for the adjuvant treatment of HER2, over-expressing, node positive breast cancer combined with multi-modality anthracycline based therapy. The first line treatment of this subtype of breast cancer should be with trastuzumab in combination with Paclitaxel. It is also indicated as first line treatment for metastatic, HER 2, over-expressing breast cancer in combination with aromatase inhibitors in hormone receptor positive tumors. Trastuzumab is also used as a single agent for the treatment of HER2, over-expressing, metastatic breast cancer in those patients who have already been receiving one or more lines of chemotherapy.

The mean half-life increases and clearance decreases with increasing dose level. The half-life ranges from 2 to 12 days. The quantity of distribution of trastuzumab is around serum volume (44 mL/kg). The mean peak serum concentration is 377mcg/mL. When administered concurrently with anthracyclins and taxanes, trastuzumab can cause sub-clinical and clinical cardiac failure manifesting as CHF and reduced left ventricular ejection fraction (LVEF). Evaluation of cardiac function prior to and during treatment with trastuzumab is strongly recommended. Besides, treatment with trastuzumab must be discontinued in the presence of cardiomyopathy. Other warnings are pulmonary toxicity and infusion reactions. Acute respiratory distress syndrome and interstitial pneumonitis are other indications for discontinuation of trastuzumab. The most important adverse effects of trastuzumab are rash, cough, insomnia, headache, chills, fever, angioedema, anaphylaxis and CHF.

2. Pertuzumab: [11]

Pertuzumab is an HER2/neu receptor antagonist, which is approved by FDA for neo-adjuvant treatment of locally advanced (more than 2 cm diameter or node positive) or inflammatory breast cancer in combination with docetaxel and trastuzumab. It is also approved for the treatment of HER2 positive, metastatic breast cancer in combination with trastuzumab and docetaxel for patients who have not received prior HER 2

directed treatment The median clearance (CL) rate is 0.24 L/day and the half-life is 18 days. The steady-state concentration of pertuzumab has been achieved after the first maintenance dose. Pertuzumab is contraindicated in patients with known hypersensitivity to this immunotherapeutic agent or to any of its excipients.

Pertuzumab can cause sub-clinical and clinical cardiac failure manifestations, such as CHF, and reduced left ventricular ejection fraction (LVEF). Cardiac function needs to be evaluated prior to and during the treatment. Trastuzumab should be discontinued in cases of decreased left ventricular function. Pertuzumab can cause fetal harm when received by pregnant women. Women should advise not to get pregnant during the course of treatment. The most common adverse effects of pertuzumab with docetaxel and trastuzumab have been reported to be peripheral neuropathy, rash, fatigue, nausea, neutropenia, alopecia, and diarrhea in both neo-adjuvant and metastatic breast cancer. The most common adverse effects noted with pertuzumab alone are cardiotoxicity, allergic reactions, and fetal toxicities.

3- Trastuzumab emtansine (TDM-1): This medication is an antibody-drug conjugate (ADC) consisting of the recombinant anti-epidermal growth factor receptor 2 (HER2) monoclonal antibody trastuzumab conjugated to the maytansinoid DM1 via a nonreducible thioether linkage (MCC) with potential antineoplastic activity. The trastuzumab moiety of this ADC binds to HER2 on tumor cell surface surfaces; upon internalization, the DM1 moiety is released and binds to tubulin, thereby disrupting microtubule assembly/disassembly dynamics and inhibiting cell division and the proliferation of cancer cells that overexpress HER2. Linkage of antibody and drug through a nonreducible linker has been reported to contribute to the improved efficacy and reduced toxicity of this ADC compared to similar ADCs constructed with reducible linkers.

It has been approved for metastatic Her 2 positive breast cancer patients who were previously treated with trastuzumab, another anti-HER2 therapy, and taxanes, a class of chemotherapy drugs commonly used for the treatment of breast cancer. The efficacy of TDM-1 has been evaluated in a clinical study of 991 patients randomly assigned to receive that or lapatinib plus capecitabine, another chemotherapy drug. Patients received treatment until either the cancer progressed or the side effects became intolerable. The end points of the study have been assigned to be progression-free survival (PFS) and overall survival (OS). TDM-1 has shown a median PFS of 9.6 months compared to 6.4 months in patients treated with lapatinib plus capecitabine. The median overall survival was 30.9 months in the TDM-1 group and 25.1 months in the lapatinib plus capecitabine group.

The most common side effects reported in patients treated with this ADC were nausea, fatigue, pain in the muscles or joints, low levels of platelets in the blood (thrombocytopenia), increased levels of liver enzymes, headache, and constipation. Its liver toxicity, heart toxicity and death have gained special boxed warning. The drug can also cause severe life-threatening birth defects, and pregnancy status should be verified prior to starting TDM-1 treatment.

There are many mAbs are under clinical trials in phase I, II, and III as in Table 1 below:

1. Glembatumumab: It is an antibody-drug conjugate consisting of the fully human monoclonal antibody CR011, directed against glycoprotein NMB (GPNMB) and conjugated via a cathepsin B-sensitive valine-citrulline (vc) linkage to the cytotoxic agent, monomethyl auristatin E (MMAE), with potential antineoplastic activity. Upon administration, the monoclonal antibody-CR011 moiety binds to glycoprotein nmb (GPNMB), which is expressed on the surfaces of a variety of cancer cell types. Upon endocytosis, the synthetic dolastin analogue, MMAE, is released via enzymatic cleavage into the tumor cell cytosol. This is where it binds to tubulin and inhibits tubulin polymerization, which may result in G2/M phase arrest and apoptosis. The vc linkage system is highly stable in serum, rendering the cytotoxicity of Glembatumumab vedotin specific for GPNMB-positive cells. GPNMB is a transmembrane protein overexpressed on the surfaces of various cancer cell types, including melanoma, breast, and prostate cancer cells.

2. Bevacizumab: A recombinant humanized monoclonal antibody directed against the vascular endothelial growth factor (VEGF), a pro-angiogenic cytokine. Bevacizumab binds to VEGF and inhibits VEGF receptor binding, thereby preventing the growth and maintenance of tumor blood vessels.

3. Cetuximab: It is a recombinant, chimeric, monoclonal antibody directed against the epidermal growth factor (EGFR) with antineoplastic activity. Cetuximab binds to the extracellular domain of the EGFR, preventing the activation and subsequent dimerization of the receptor; the decrease in receptor activation and dimerization may result in an inhibition in signal transduction and anti-proliferative effects. This agent may inhibit EGFR-dependent primary tumor growth and metastasis. EGFR is overexpressed on the cell surfaces of various solid tumors.

4. IMMU-132: It is an antibody-drug conjugate containing the humanized monoclonal antibody, hRS7, against tumor-associated calcium signal transducer 2 (TACSTD2 or TROP2) and linked to the active metabolite of irinotecan, 7-ethyl-10-hydroxycamptothecin (SN-38), with potential antineoplastic activity. The antibody moiety of IMMU-132 selectively binds to TROP2. After internalization and proteolytic cleavage, SN-38 selectively stabilizes topoisomerase I-DNA covalent complexes, resulting in DNA breaks that inhibit DNA replication and

trigger apoptosis. TROP2, also known as epithelial glycoprotein-1 (EGP-1), is a transmembrane calcium signal transducer that is overexpressed by a variety of human epithelial carcinomas; this antigen is involved in the regulation of cell-cell adhesion and its expression is associated with increased cancer growth, aggressiveness and metastasis.

5. Panitumumab: It is a human, monoclonal antibody produced in transgenic mice that attaches to the transmembrane epidermal growth factor (EGF) receptor. Panitumumab may inhibit autocrine EGF stimulation of tumor cells that express the EGF receptor, thereby inhibiting tumor cell proliferation.

6. Nimotuzumab: It is a humanized, monoclonal antibody directed against the epidermal growth factor receptor (EGFR) with potential antineoplastic activity. Nimotuzumab binds to and inhibits EGFR, resulting in growth inhibition of tumor cells that overexpress EGFR. This agent may act synergistically with radiation therapy.

7. MGAH22: It is a Fc-domain optimized, IgG, monoclonal antibody directed against the human epidermal growth factor receptor 2 (HER2) with potential immunomodulating and antineoplastic activities. After binding to HER2 on the tumor cell surface, anti-HER2 monoclonal antibody MGAH22 may induce an antibody-dependent, cell-mediated cytotoxicity (ADCC) against tumor cells overexpressing HER2. HER2, a tyrosine kinase receptor, is overexpressed by many cancer cell types. Compared to other anti-HER2 monoclonal antibodies, the Fc domain of MGAH22 is optimized with increased binding to the activating Fc gamma receptor IIIA (CD16A), which is expressed on cells like natural killer (NK) cells and macrophages, thereby mediating an enhanced ADCC; the Fc domain also shows decreased binding to the inhibitory Fc gamma receptor IIB (CD32B).

mAbs	Clinical trial identifier number	Phase	Study design	Target
Glembatumumab	NCT01997333	Phase II	Randomized, Open Label Efficacy Study	GPNMB
Bevacizumab	NCT01250379	Phase II	Randomized, Open Label, Safety/Efficacy Study	VEGF
Cetuximab	NCT00353717	Phase I/II	Non-Randomized, Safety/Efficacy study, Open label	EGFR
IMMU-132	NCT01631552	Phase I/II	Safety/Efficacy study, Open label	DNA replication
Panitumumab	NCT01036087	Phase II	Safety/Efficacy study, Open label	EGF
Nimotuzumab	NCT01939054	Phase I/II	Randomized, Efficacy study, Open label	EGFR
MGAH22	NCT01148849	Phase II	Safety study, Open label	HER2
Vantictumab	NCT01973309	Phase I	Non-Randomized, Safety/Efficacy study, Open label	CSC

Table 1. Monoclonal antibody drugs in phase I-III [12-191]

8. Vantictumab: It is a monoclonal antibody directed against the Wnt signaling pathway with potential antineoplastic activity. Vantictumab binds to certain receptors in the Wnt signaling pathway, thereby preventing the activation of the Wnt signaling pathway. This may result in an inhibition of cancer stem cell (CSC) activity and a subsequent inhibition of cancer cell proliferation. The Wnt signaling pathway is dysregulated in many cancer cell types and appears to play a major role in CSC regulation and activity; CSCs are tumor initiating cells that are able to self-renew and are responsible for tumor growth and resistance.

B. Adoptive Cell Therapy:

There is no adoptive T-cell therapy that is currently approved by the FDA for breast cancer. However, many of them are under clinical trials in phases I, II, and III as in Table 2 below.

1. HER2 Bi-armed activated T cells: These are activated T cells (ATC) that have been coated with bispecific antibodies (BiAb), with potential antineoplastic and immunomodulating activities. In vitro, T cells are activated through exposure to the anti-CD3 murine monoclonal antibody, OKT3, and interleukin 2 for 14 days. They are then armed with anti-CD3 × anti-Her2 bispecific antibody (Her2Bi). Upon administration, HER-2Bi-armed activated T cells attach to CD3-expressing T cells and HER2/neu-expressing tumor cells, selectively cross-linking T cells and tumor cells; this may result in the recruitment and activation of cytotoxic T lymphocytes (CTLs), CTL perforin-mediated tumor cell cytolysis, and the secretion of antitumor cytokines and chemokines.

2. cMet RNA CAR T cells: This is a preparation of autologous T-lymphocytes that have been electroporated with mRNA encoding a chimeric antigen receptor (CAR). This receptor consists of an anti-human hepatocyte growth factor receptor, (HGFR or cMet) scFv, (single chain variable fragment) and the zeta chain of the TCR/CD3 complex (CD3-zeta), which is coupled to the co-stimulatory molecule, 4-1BB (CD137), with potential antineoplastic activities. Upon intratumoral administration, cMet CAR-mRNA electroporated autologous T lymphocytes direct T-cells to cMet-expressing tumor cells, which induce a selective toxicity in cMet-expressing tumor cells and causes tumor cell lysis. The 4-1BB co-stimulatory molecule signaling domain enhances activation and signaling after recognition of cMet. The inclusion of the 4-1BB signaling domain may increase the antitumor activity as compared to the inclusion of the CD3-zeta chain alone. The mRNA CAR is expressed for a limited amount of time, which can prevent serious, unforeseen side effects. cMet, a receptor tyrosine kinase overexpressed or mutated in many tumor cell types, plays a key role in cancer cell growth, survival, angiogenesis, invasion, and metastasis.

T-cells	Clinical trial identifier number	Phase	Study design	Target
HER2Bi-armed activated T cells	NCT01022138	Phase II	Interventional, Open Label	Cancer cells
cMet RNA CAR T cells	NCT01837602	Phase I	Interventional, Safety/Efficacy Study	Cancer cells

Table 2. Adoptive T-cell therapy [20,21]

C. Vaccine Based Immunotherapy:

There are no vaccines that are currently approved by the FDA for breast cancer. However, many vaccines are under clinical trial in phases I, II, and III as in Table 3 below:

1. Nelipepimut-S plus GM-CSF vaccine: It is a cancer peptide vaccine comprised of a human leukocyte antigen (HLA), A2/A3 restricted HER2/neu (ERBB2) peptide, from the extracellular domain of the HER2 protein (E75 peptide) and combined with the immunoadjuvant granulocyte-macrophage colony-stimulating factor (GM-CSF), with potential immunomodulating and antineoplastic activity. Upon intradermal injection, nelipepimut-S plus GM-CSF vaccine may induce a specific cytotoxic T-lymphocyte (CTL) response against HER2/neu-expressing tumor cell types. HER2/neu, a tumor-associated antigen and a member of the epidermal growth factor receptor family of tyrosine kinases, is overexpressed in various tumor cell types. GM-CSF potentiates the antitumor immune response.

2. Allogeneic GM-CSF-secreting breast cancer vaccine: It is an allogenic vaccine consisting of irradiated breast cancer cells transfected with the granulocyte macrophage-colony-stimulating factor (GM-CSF) gene. Upon vaccination, the genetically modified cells secrete GM-CSF, thereby potentiating a tumor-specific T cell response against breast cancer cell-associated antigens.

3. HER-2/neu intracellular domain protein: The cytoplasmic domain or intracellular domain (ICD) of the HER2/neu protein that exhibits tyrosine kinase activity. Based on sensitization theory, co-administration of trastuzumab (anti-HER-2/neu monoclonal antibody) and HER-2/neu intracellular domain protein may result in the potentiation of a HER2/neu-specific cytotoxic T lymphocyte (CTL) response against tumor cells overexpressing the HER2/neu protein. HER-2/neu protein, a glycoprotein cell surface receptor that is composed of an extracellular domain (ECD), a transmembrane domain, and an ICD, is overexpressed by many adenocarcinomas including breast adenocarcinoma.

4. E39 peptide: It is a cancer vaccine comprised of human leukocyte antigen (HLA) A2 restricted folate binding protein (FBP) epitope E39 (amino acids 191 to 199), with potential immunostimulatory and antineoplastic activity. Upon intradermal injection, FBP E39 peptide vaccine may induce a specific cytotoxic T-lymphocyte (CTL) response against FBP-expressing tumor cell types. FBP is a membrane-bound, tumor-associated antigen highly overexpressed in various tumor cell types, such as in breast, ovarian and endometrial cancers; E 39 is a strong immunogenic peptide.

5. Mammaglobin-A DNA Vaccine: This is a cancer vaccine containing a plasmid encoding the mammaglobin-A gene with potential immunostimulating and antineoplastic activities. Upon administration, mammaglobin-A DNA vaccine may induce both humoral and cytotoxic T lymphocyte (CTL) immune responses against tumor cells that express mammaglobin-A, which may result in decreased tumor growth. The 10 kilo-Dalton (kD) glycoprotein mammglobin-A is expressed in over 80% of human breast cancers.

Vaccines	Clinical trial identifier number	Phase	Study design	Target
NeuVax™ vaccine	NCT01479244	Phase II	Randomized, Double blind, Safety/Efficacy Study	HER2-positive breast cancer cells
Allogeneic GM-CSF-secreting breast cancer vaccine	NCT00971737	Phase II	Randomized, Open Label	Tumor surface proteins and breast cancer cells
E39 vaccine then J65 vaccine	NCT02019524	Phase I/II	Randomized, Efficacy study, Single blind	HER2-positive breast cancer cells
Mammaglobin-A DNA Vaccine	NCT02204098	Phase I	Randomized, Open Label, Safety Study	CTL
HER-2/neu intracellular domain protein	NCT01922921	Phase I	Randomized, Double blind, Safety Study	HER2-positive breast cancer cells

Table 3. Approved Vaccines [22-26]

D. Checkpoint Inhibitors and other immunostimulating antibodies:

There are no checkpoint inhibitors that are currently approved by the FDA for breast cancer. However, many of them are under clinical trials in phase I, II, and III as in Table 4 below.

1. MEDI4736: This is a monoclonal antibody directed against B7H1 (B7 homolog 1; programmed cell death ligand 1) with potential immunostimulating activity. Upon intravenous administration, MEDI4736 binds to the cell surface antigen

B7H1, thereby blocking B7H1 signaling. This may activate the immune system to exert a cytotoxic T-lymphocyte (CTL) response against B7H1-expressing tumor cells. B7H1, a member of the B7 protein superfamily and a negative regulator of cytokine synthesis, is overexpressed on certain tumor cell types. It is an anti-PDL-1 checkpoint inhibitor, which is made by MedImmune/AstraZeneca. It is used to treat breast cancer.

2. Nivolumab: This is a fully human, monoclonal antibody directed against the negative immunoregulatory human cell surface receptor, PD-1, (programmed death-1 or programmed cell death-1/PCD-1) with immunopotentiation activity. Nivolumab binds to and blocks the activation of PD-1, an Ig, superfamily transmembrane protein, by its ligands PD-L1 and PD-L2, resulting in the activation of T-cells and cell-mediated immune responses against tumor cells or pathogens. Activated PD-1 negatively regulates T-cell activation and effector function through the suppression of P13k/Akt pathway activation.

3. MEDI6469: A monoclonal antibody directed against programmed cell death-1 ligand 1 (PD-L1), with potential immunomodulating and antineoplastic activities. Anti-PD-L1 monoclonal antibody MEDI6469 binds to PD-L1 and blocks PD-L1, binding to and activation of its receptor, programmed death 1 (PD-1), which may both enhance the T-cell-mediated immune response to neoplasms and reverse T-cell inactivation. PD-L1 is overexpressed by many human cancer cell types. PD-L1 binding to PD-1 on T-cells suppresses the immune system and results in immune evasion. PD-1, a transmembrane protein expressed on activated T-cells, is a negative regulator of the immune system that limits both the expansion and survival of CD8+ T-cells.

4. Anti-OX40 monoclonal antibody: An agonistic, monoclonal antibody against receptor OX40 (CD134) with potential immune-stimulatory activity. Mimicking the natural OX4 ligand (OX40L), anti-OX40 monoclonal antibody selectively binds to and activates the OX40 receptor. Receptor activation induces proliferation of memory and effector T lymphocytes. In the presence of tumor associated antigens (TAAs), this may promote an immune response against the TAA-expressing tumor cells. OX40, a cell surface glycoprotein and member of the tumor necrosis factor (TNF) receptor family, is expressed by CD4 T cells and provides a costimulatory signal for T cell activation.

5. AlloStim: A preparation consisting of allogeneic, differentiated Th1-like T cells bound to T cell-stimulating monoclonal antibodies with potential antitumor activity. More specifically, allogeneic CD4+ memory Th1-like T cells/microparticle-bound anti-CD3/anti-CD28 are composed of a proprietary preparation of mismatched, allogeneic differentiated CD4+ memory Th1-like T cells bound to paramagnetic, epoxy-covered 4.5 micron microparticles with covalently bound anti-CD3/anti-CD28 monoclonal antibodies at a 2:1 bead to cell ratio. The CD4+ memory Th1-like T cells are derived from precursors found in the circulation of a normal donor. Stimulated by the microparticle-bound monoclonal antibodies, the infused T cells produce pro-inflammatory, anti-tumor cytokines such as IFN-gamma, TNF-beta, and IL-2, disabling tumor immune avoidance mechanisms and stimulating the host immune system to both reject the infused T cells and kill tumor cells.

Checkpoint inhibitors	Clinical trial identifier number	Phase	Study design	Target
MEDI4736	NCT01693562	Phase I/II	Non-Randomized, Open label, Safety/Efficacy Study	PDL1
Nivolumab	NCT01928394	Phase I/II	Randomized, Open label, Efficacy Study	PD-1
MEDI6469	NCT02205333	Phase I/II	Non-Randomized, Safety study, Open label	OX40
anti-OX40 antibody	NCT01862900	Phase I/II	Non-Randomized, Open Label, Safety/Efficacy study	OX40
AlloStim	NCT01741038	Phase II/II	Randomized, Double Blind, Safety/Efficacy study,	Cancer cells

Table 4. Check point inhibitors [27-31]

E. Cytokine Therapy:

There are no cytokine therapies that are currently approved by the FDA for breast cancer. However, many of them are under clinical trials in phase I, II, and III as in Table 6 below:

1. Aldesleukin: A recombinant analogue of the endogenous cytokine interleukin-2 (IL-2) with immunoregulatory and antineoplastic activities. Aldesleukin binds to and activates the IL-2 receptor, followed by heterodimerization of the cytoplasmic domains of the IL-2R beta and gamma(c) chains; activation of the tyrosine kinase Jak3; and phosphorylation of tyrosine residues on the IL-2R beta chain, resulting in an activated receptor complex. Various cytoplasmic signaling molecules are recruited to the activated receptor complex and become substrates for regulatory enzymes that are associated with the receptor complex. This agent enhances lymphocyte mitogenesis, stimulates long-term growth of human IL-2 dependent cell lines, enhances lymphocyte cytotoxicity, induces lymphokine-activated killer (LAK) cell and natural killer (NK) cell activities, and induces expression of interferon-gamma. Aldesleukin may induce T cell-mediated tumor regression in some tumor types.

2. Anakinra: A recombinant human nonglycosylated interleukin-1 (IL-1) receptor antagonist with potential antineoplastic activity. Anakinra binds to the IL-1 receptor, thereby blocking

the binding of the IL-1 to and activation of its receptor. Blockade of IL-1 activity may inhibit the cascade of downstream pro-angiogenic factors such as vascular endothelial cell growth factor, tumor necrosis factor-alpha, and IL-6, resulting in inhibition of tumor angiogenesis.

Drugs	Clinical trial identifier number	Phase	Study design	Target
Aldesleukin	NCT00673829	Phase I	Randomized, Open Label, Safety/Efficacy Study	Cancer cells
Anakinra	NCT01802970	Phase I	Open Label, Safety/Efficacy Study	IL-1

Table 5. Cytokine therapies [32,33]

Conclusion

Immunotherapy has proven to be effective in the treatment of breast cancer. Trustuzumab is the first monoclonal antibody that has been approved and successfully applied in the treatment of Her 2 positive breast cancer. It has shown to be rather safe and effective in both advanced and metastatic breast cancer cases as well as adjuvant setting and has dramatically changed the course of Her 2 positive subtype of breast cancer that has been identified as a poor prognosis disease. Moreover, this monoclonal antibody has been a pioneer in exploiting immune based therapeutic approach in breast cancer patients. Pertuzumab, another monoclonal antibody targeting Her 2 receptor and TDM-1 an antibody drug conjugate has both developed on the basis of what has been learnt from trustuzumab studies and have shown their efficacy and safety in Her 2 positive breast tumors, respectively. Identification of specific tumor associated antigens on the surface of tumor cells also suggest them as potential targets for cell mediated cytotoxicity activating against tumor cells while sparing the regular cells lacking these tumor specific antigens. Cancer vaccines and adoptive immunotherapeutic approaches exploiting dendritic cells generally follow this concept of reactivating cell mediated cytotoxicity against tumor cells. However, many of these suggested therapeutic approaches such as monoclonal antibodies, adaptive T-cell therapies, vaccines and checkpoint inhibitors, are under clinical trials regarding their safety and efficacy as well as being approved by regulatory authorities as an specific therapeutic approach. Our success in treating breast cancer is increasing and advancing with the knowledge of the function of the immune system. Researchers are still challenged in exploring innate and adaptive immune systems and combining these novel targeted therapies with the available therapeutic modalities for the optimized cancer treatment. There has been a promising development in immunotherapy in the past few years. The recent activities have increased our understanding of the tumor microenvironment, various immunotherapeutic modalities or combination therapy (like chemotherapy with immunotherapy and even monoclonal antibodies in combination with the checkpoint inhibitors or adoptive immunotherapeutic approaches). Additionally, the effects of such modalities in combination with immunotherapy in cancer patients are still in the exploratory phase. The complete perspective of immunotherapy treatment has not been realized and utilized. Proper preclinical and clinical designs are the important pillars in understanding the future of immunotherapy in treating breast cancer patients.

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