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REVIEW ARTICLE **Open Access**

Active Immunotherapy of Breast Cancer Treatment

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ABSTRACT

Background: Immunotherapy as a specific molecular targeted therapy could modulate immune response through cytotoxic CD8+ pathway so that generate anti-tumor activity on breast cancer. HER2/Neu is the one of tumor associated antigen which extensively studied to reduce cancer progression. CTLA-4 has important role in immune checkpoint blockade that it is used for studying breast cancer either in early or advanced level
Content: In this review, we discussed principle of immunotherapy in tumor microenvironment and some kinds of active immunotherapies, which included cancer vaccination and immune checkpoint blockade. Majority of those immunotherapies are ongoing clinical trial.
Conclusion: Active immunotherapy may become a promising new field in breast cancer therapies in a future and will ultimately change the current status of breast cancer therapies.

Keywords: CTLA-4; HER2/Neu; immunotherapy; immune checkpoint; MUC-1; PD-1

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Introduction

Breast cancer is a carcinoma originating from breast tissue that is often found in women, but is sometimes found in men as well. Data from The International Agency for Research on Cancer (IARC) 2020 states that breast cancer, together with lung and colorectal cancer, are the three cancers with the highest incidence and are among the top five causes of death.[1] In Indonesia alone, GLOBOCAN 2020 data shows breast cancer is the first cancer with new case findings (16.6%) in both men and women.[2] In 2018, The Center of Data and Information of Health Ministry of Indonesia (Infodatin) noted that prevalence of cancer among women was 2,85.[3]

The exact cause of breast cancer is not fully known. However, like cancer in general, breast cancer occurs due to various factors that are not fully related, such as genetic factors in the family, endogenous and exogenous hormonal exposure, environment, and sociobiology associated with an unhealthy lifestyle.[4] Breast cancer therapy has continued to evolve in recent decades and involves a variety of strategies including surgery, chemotherapy, ablation, radiotherapy, hormonal therapy, and interventions with molecules, antibodies, or antigens/adjuvants to inhibit cell growth, proliferation, survival and tumor invasiveness.[5] Another therapy, namely neo-adjuvant can also be used to reduce the size of the tumor before surgery.[5,6] Although breast cancer treatment continues to develop and increase cure rates, the problem of multidrug resistance (MDR) and a survival rate of only 2 – 3 years is still a major problem in the management of patients with advanced breast cancer.[6]

For the purposes of treatment options and patient prognostic assessment, tumors were classified according to their intrinsic subtypes obtained on routine histology and immunohistochemistry (IHC) examination as described in Table 1. The subtype classifications are: luminal A, luminal B, human epidermal growth factor receptor 2 (HER2)/ Neu enriched, basal-like and triple negative breast cancer (TNBC).[5,7] The subtype with hormonal receptors, luminal A, has a good prognosis and should be considered for hormonal therapy only. Luminal B with HER2 negative generally received hormonal therapy and chemotherapy, while luminal B with HER2 positive was treated with chemotherapy + anti-HER2 + endocrine therapy. The HER2 subtype has a poor prognosis with a high rate of progression, and is recommended for anti-HER2 therapy and chemotherapy. The last subtype, TNBC, has a different character and shows strong heterogeneity because it does not have expression of ER, PgR, or HER2. There is no standard therapy for this type, but chemotherapy is still recommended for patients with this subtype.[8]

Table 1. Definition of intrinsic subtypes of breast cancer.[7]

Intrinsic Subtype	Definition
Luminal A	'Luminal A-like' ER (+) HER2 (-) Low Ki67 High PgR Low risk of molecular signs (if available)
Luminal B	'Luminal B-like (HER2 negative)' ER (+) HER2 (-) Which include High Ki67, or Low PgR High risk of molecular signs (if available) 'Luminal B-like (HER2 positive)' ER (+) HER2 (+) Present Ki67 Present PgR
HER2	'HER2-positive (non-luminal)' HER2 (+) ER and PgR undetected
Basal-like	'Triple-negative' ER dan PgR undetected HER2 (-)

ER, estrogen receptor; HER2, human epidermal growth factor receptor 2; PgR, progesterone receptor; Ki67, proliferation marker.

Breast cancer is immunogenic and has several tumor antigens (tumor-associated antigens, TAAs) such as Mucin 1 (MUC 1) and HER-2 has been observed on that tumor. These properties make immunotherapy a new and very promising therapy in breast cancer therapy.[5,8].

Immunotherapy Principal

Cancer immunotherapy is the term used by the body's immune system to fight cancer cells. The principle of this therapy is to use tumor-specific immune responses to recognize abnormal proteins expressed by cancer cells, namely tumor antigens.[9] Immunotherapy basically aims to accelerate the loss of tumor cells by increasing the proliferation and activity of T lymphocytes, antigen presentation, and production of inflammatory mediators (cytokines and chemokines). Other specific goals of this therapy are to reduce the potential for metastasis, reduce the incidence of recurrence, and prevent the formation of secondary resistance.[5]

The basic principle of immunotherapy against tumor eradication is the killing of tumor cells mediated by cytotoxic T lymphocytes (CTL). Several studies have shown that breast tumors are infiltrated by immune cells, especially T lymphocytes and both show a positive correlation with a good prognosis.[5,10]

The majority of tumor antigens that can trigger an immune response are cytosolic or nuclear proteins that are endogenously synthesized which are then exposed to the cell surface by major histocompatibility complex (MHC) class I peptides. Many breast cancer tumor antigens have been identified and proven to be recognized by T lymphocytes. The tumor antigens associated with breast cancer are human epidermal growth factor receptor 2 (HER2), mucin 1 (MUC-1), carcinoembryonic antigen (CEA), human telomerase reverse transcriptase (hTERT), sialyl Tn (STn), and Wilms' Tumor. Gene (WT1).[11] T cell receptors (T cell receptors, TCR) on the surface of CD8+ cells will bind to the MHC-antigen complex and then differentiate and proliferate into cytotoxic T cells. This interaction can take place at the site of the tumor, secondary lymphoid organs, or in peripheral tissues.[9,10]

The process of antigen initiation and naive T cells also takes place due to cross-presentation of antigen presenting cells (APCs). APCs eat dead cells and extracellular peptides, and the resulting peptides are expressed on the APC surface with the help of MHC-1. Antigen presentation by APCs also stimulates expression of costimulatory molecules. Proteins B7-1 (CD80) and B7-2 (CD86) are costimulatory molecules that have been extensively studied in T lymphocytes. Some members of this protein are stimulatory and some are inhibitory to the immune response. The binding between B7-1 (CD80) and CD28 that occurs in the initial phase is stimulatory, while the binding between costimulator B7-2 (CD86) and cytotoxic T-lymphocyte antigen-4 (CTLA-4) that occurs after T cell activation is inhibitory. Another costimulator of protein B7, programmed cell death ligand-1 and -2 (PD-L1 and PD-L2), which binds to programmed cell death -1 (PD-1) also inhibits the immune response following T cell activation.[8,10]

CTLA-4 and PD-1 have important roles in suppressive function of T cell regulation. These receptors prevent the immune response against the body's own antigens and genetic deletions that prevent autoimmune disease. CTLA-4 and PD-1 are also involved in the inhibitory response in several types of tumors that cause the immune system to fail to eradicate tumors, causing uncontrolled proliferation.[10]

Breast Cancer Vaccination

A form of stimulating an active immune response against tumors is vaccination.

Several vaccines for various types of tumors currently being developed are made from various immunogenic sources, namely whole tumor cells, tumor antigen peptides, DNA, RNA, and viruses. In addition, these components can be combined with immunoadjuvant ingredients that function to stimulate the activation of the immune system.[5]

Antigen vaccine of HER2/Neu

This vaccine model is the most potent vaccine in various published research results. The HER2 protein is a receptor protein with tyrosine kinase activity and has homology with the epidermal growth factor receptor (epidermal growth factor). HER2 is expressed in many epithelial tumors, but is overexpressed in approximately 25% of cases of primary breast cancer.[9,11] Overexpression of this protein is associated with poor prognosis in these patients. HER2 is a good target antigen because it is involved in the extracellular domain so that it can be reached and targeted by antibodies produced by B lymphocytes.[11]

There are several models of the HER2 vaccine currently under development and in different phases of clinical trials. Nelipipimut-S (NeuVax, Galenda Bipharma) is a model of the HER2 vaccine that has been extensively studied and is currently in clinical phase III in the evaluation of clinical efficacy in early-stage breast cancer (NCT01479244).[9] The tumor antigen in this vaccine, E75, is a peptide of the extracellular domain of HER2. This peptide vaccine displays a peptide bound to HLA-class 1 of the extracellular domain of the HER2/Neu protein. The E75 antigen has been shown to be immunogenic and capable of stimulating a specific CD8+ cytotoxic response to HER2. This vaccine is also currently under evaluation for its combination with GM-CSF adjuvant compounds aimed at treating tumors at various levels of HER2 overexpression. While the increase in patient outcomes (survival and immune response) with this vaccine was observed more in patients with medium or low HER2 expression.[9]

The first study using the HER2 vaccine was published in 2009. A phase I clinical trial of Lapuleucel-T (APC8024) with a combination of GM-CSF adjuvants was conducted on 18 subjects who had undergone surgery and HER2/Neu 1 status in breast cancer, ovarian cancer, endometrial cancer and colorectal. Subjects were divided and received three dose levels of the HER2 ICD protein vaccine. The vaccine is injected intradermally and monitored monthly for a year. The results obtained were good vaccine tolerance, the majority of subjects were able to produce specific T cell immunity against ICD HER2, and the vaccine dose did not predict the high T cell response.[12] Review of others ongoing clinical trials in breast cancer vaccine can be seen in Table 2.

Table 2.Ongoing breast cancer vaccine clinical trials [13]

Register Number (ID)	Vaccine Name	Study Phase	Vaccine substance	Study Participant	Study Period
NCT01479244	NeuVax (nelipepimut-s)	II	E75-GM-CSF conjugated in combination with herceptin	KP HER2 IHC 1+/2 dan nodus+	2012 – currently ongoing
NCT02297696	NeuVax (nelipepimut-s)	II	E75-GM-CSF conjugated in combination with transtuzumab	High risk BC, HER+ or nodes+	2014 - currently ongoing
NCT01390064	P10s-PADRE	I	Pan-T-Cell Epitope and neolactoseries antigen Lewis Y (LeY) and the ganglioside GD2 mimicking carbohydrate peptide (CMP) + MONTANIDE™ ISA 51 VG adjuvant	Stage IV BC	2011 - currently ongoing
NCT022229084	P10s-PADRE (Chemovax)	I/II	P10s-PADRE with chemotherapy, MONTANIDE™ ISA 51 VG adjuvant or chemotherapy and adjuvant	KP stadium I, II, atau III ER+	2014 - currently ongoing
NCT02938442	P10s-PADRE (Chemovax)	II	P10s-PADRE with MONTANIDE™ ISA 51 VG adjuvant	TNBC	2016 - currently ongoing
NCT02826434	PVX-410	Ib	tetra-peptida vaccine XBP1, CD138 and CS1 + durvalumab	Stage II/III TNBC	2016 - currently ongoing
NCT03362060	PVX-410	I	Tetra-peptide vaccine XBP1, CD138 and CS1 + pembrolizumab	TNBC	2017 - currently ongoing
NCT02960594	INO-1400, INO-9012, and INO-1401	I	A plasmid encoding hTERT	Many types of cancer and BC patients who are at high risk of tumor relapse	2014- currently ongoing
NCT02348320	-	I	Personalized polyepitope DNA	TNBC	2015 - currently

			vaccine encoding patient's own immunogenic TAAs which are selected after genome porifling of the patient's BC cells.		ongoing
NCT01730118	Ad/HER2/Neu DC	I	Autologous adenovirus HER2 – transduced DC vaccine	KP metastasis	2012 – currently ongoing
NCT00923143	-	I	HER2/Neu pulsed DC1	DCIS	2009 – currently ongoing

HER2, *human epidermal growth factor receptor* 2; BC, breast cancer; ER, *estrogen receptor*; TNBC, triple negative breast cancer; DC, *dendritic cell*; GM-CSF, *granulocyte macrophage colony stimulating factor*; hTERT, *human telomerase reverse transcriptase*.

Mucin-1 Vaccine

Mucin 1 (MUC-1) is a cell membrane glycoprotein present in various types of ductal epithelium, namely pancreas, breast, lung and gastrointestinal tract. This protein is overexpressed and glycosylated in malignant cells.[8,11] More than 70% of cancers exhibit MUC-1 overexpression which makes this antigen a potential target for immunotherapy. Although this vaccine is able to induce a specific T-cell response, the clinical outcomes achieved in this study are still unsatisfactory.[14] The largest phase III clinical trial using STnkeyhole limpet hemocyanin (KLH) vaccine (Theratope ®; Biomira, Inc., Edmonton, Canada), a conjugated STn synthetic tumor antigen in combination with an adjuvant, in 1028 patients with metastatic breast cancer found that this vaccine was well tolerated with good and able to stimulate the formation of antibodies against MUC-1 antigen, but did not provide benefits in patient outcome and survival.[15]

Preclinical studies using tumor cells with expression of MUC-1 protein or peptide antigen concluded that MUC-1 can induce a humoral immune response without inducing a cellular response. This may be due to the induction of T cell anergy by MUC-1-containing tumors. [11] Preclinical studies using tumor cells with expression of MUC-1 protein or peptide antigen concluded that MUC-1 can induce a humoral immune response without inducing a cellular response. This may be due to the induction of T cell anergy by MUC-1-containing tumors.[16]

Early clinical trials with PANVAC-V, a CEA/MUC-1/TRICOM recombinant vaccine inserted into a poxvirus vector, in patients with breast or ovarian cancer have found that this vaccine is beneficial for patients with less than three history of chemotherapy.[17]

Therapy at Immune Checkpoints

Various studies over the past decade have reported that T cell infiltration in tumors plays a role in tumor immune surveillance in humans. T cell infiltration is used as a measure of the success of therapy and also the patient's prognosis in various subtypes of breast cancer. [18,19] However, tumor cells surrounded by infiltrated T cells were still able to survive and escape T-cell immunity through the mechanisms of antigen expression deletion, T-cell regulator removal, and indoleamine-2,3-dioxygenase (IDO).[20]

Immune checkpoints are surface molecules that have a role in modulating the body's immune response, preventing autoimmunity against self-antigens, and maintaining self-tolerance. The process of T cell recruitment involves a balance between the process of stimulation with the help of co-stimulators and also inhibition in the presence of co-inhibitors. Co-inhibitors normally play an important role in maintaining immune system homeostasis, but in cancer cells these molecules become a way of escaping the body's immune response.[21]

Inhibitory antibodies that act to inhibit immune system checkpoints have shown promising potential in several studies with subjects with melanoma, bladder cancer, non-small cell lung cancer (NSCLC), and breast cancer. For breast cancer, therapies targeting immune system checkpoint co-inhibitors such as CTLA-4 and PD-1 are currently in clinical trials.[8]

Inhibitor CTLA-4

CTLA-4 is a molecule that is expressed on the surface of T cells and functions to suppress T cell activity. Signaling by CTLA-4 on CD8+ specifically will directly inhibit T cell activation.[9,10,21] CTLA-4 is also expressed by CD4+ FOXP3+ regulatory T cells and is an important immunosuppressor in these cells.[21] CTLA-4 is also able to competitively bind to the B7 ligand APCs, thereby interfering with the co-stimulatory signaling mediated by CD28 binding to B7-1.[9] Thus CTLA-4 as a checkpoint for the immune system has a crucial role in the function of CD8+ effector T cells in the early response stage to the formation of memory immune cells.

The study using anti-CLTA-4 monoclonal antibody in melanoma patients gave satisfactory results. Phase III clinical trials with Ipilimumab, a cytotoxic T-cell lymphocyte antigen 4 inhibitor, with or without the glycoprotein 100 peptide (gp100) found that there was an extension of the patient's lifespan, namely 10.0 months on average in patients receiving ipilimumab with gp100 compared to 6.4 months in patients receiving ipilimumab with gp100. the group of patients receiving only gp100 (hazard ratio for death, 0.68; $P < 0.001$). Whereas

in the group receiving only ipilimumab, the mean survival of patients was 10.1 months (hazard ratio for death compared to the gp100 group, 0.66; P = 0.03).[22] In 2011, Ipilimumab was subsequently approved by the United States FDA as the first immune system checkpoint inhibitor as adjuvant therapy for patients with cutaneous melanoma who had undergone total surgery and total lymphadenectomy in tumors with regional metastases.[21]

Currently, Ipilimumab has been widely studied for the treatment of various other cancers such as, lung cancer (non-small cell lung carcinoma, NSCLC), breast cancer, and renal carcinoma (RCC). The clinical phase 1 trial of the combination of Tremelimumab with Exemestab was conducted on 26 breast cancer patients with hormone positive response type. The side effects of this inhibitor therapy are mild to moderate (diarrhea, pruritus, constipation, fatigue) and increase the expression of co-stimulators on CD4+ and CD8+ T cells. [23]

There are two cohort studies of combined CTLA-4 inhibitors that are still ongoing. They are the combination Tremelimumab with anti-B7H1 monoclonal antibody MEDI4736 for the treatment of HER2 negative type breast cancer (NCT02536794) and the combination Ipilimumab with anti-B7H3 monoclonal antibody MGA271 in TNBC patients (NCT02381314). This inhibitor therapy still requires further investment to identify its safety profile and synergies with other therapeutic modalities.[8]

Inhibitor PD-1/ PD-L1

The major ligands for PD-1 are PD-L1 (CD274 or B7-H1) and PD-L2 (CD273 or B7-DC).[8] In contrast to CTLA-4, PD-1 inhibits the activation of T-cell immune responses at the effector stage, at the site of the reaction both in the periphery and in tumor tissue. PD1 is expressed on nearly all surfaces of several immune cells, including CD4+ and CD8+ T cells, B cells, NK cells, and regulatory T cells. Because regulatory T-cell infiltration is found in a wide variety of tumor types, inhibition of the PD1 pathway may help enhance the anti-tumor immune response by reducing the number and/or suppressing the activity of intra-tumor regulatory T cells.[24] Although clinical experience with PD1 antibodies is currently less than with CTLA4 antibodies, preliminary results look very promising. Resume of the current study of CTLA-4 and PD-1 inhibitors is shown in Table 3.

The first phase 1 clinical trial with this inhibitor was a clinical trial using a single anti-PD-1 antibody (MDX-1106) in 39 metastatic cancer patients. The results obtained in the form of good tolerance and showed anti-tumor activity. Some cases even experienced tumor regression.[25] In 2014, for the first time a PD-1 target therapy, Nivolumab (Opdiva®, Bristol-

Myers Squibb) was approved by the United States FDA. This therapy was developed purely from human IgG4κ monoclonal antibody. Nivolumab is approved as first-line therapy for untreated melanoma and has been shown to have no mutations in BRAF. More recently, nivolumab has also been approved as adjuvant therapy in stage III/IV melanoma in patients who have undergone total surgery.[21]

Table 3. Several ongoing clinical trials of CTLA-4 and PD-1 inhibitors.[8]

Register Number (ID)	Formula	Study Phase	Study participant
CTLA-4			
NCT02536794	MEDI4736 + Tremelimumab	II	HER –
NCT02381314	MGA271 + Ipilimumab	I	TNBC
PD-1			
NCT02661100	Pembrolizumab + CDX-1401 + Poly ICLC	I/II	TNBC advanced stage
NCT02453620	Entinostat + Iplimumab + Nivolumab	I	HER –
NCT02129556	Pembrolizumab	I/II	HER2 + (Transtuzumab resistance)
NCT02309177	Nab-Paclitaxel + Nivolumab + Gemcitabine + Carboplatin	I	BC
NCT02404441	PDR001	I/II	TNBC
NCT02555657	Pembrolizumab + Capecitabine + Eribulan + Gemcitaine + Vinorelbine	III	TNBC

HER2, *human epidermal growth factor receptor* 2; BC, Breast Cancer; TNBC, *triple negative breast cancer*

Atezolizumab (Tecentriq®), an IgG1κ monoclonal antibody, is the first anti-PD-L1 monoclonal antibody approved as an immune checkpoint inhibitor therapy. This drug is approved for use in cases of localized or metastatic urothelial cancer and NSCLC.[21]

PD-L1 protein expression was detected in 20-30% of breast cancer patients, especially in the TNBC type. Animal model studies and clinical trials show that PD-1 and PD-L1 inhibitors have potential for breast cancer therapy. In a non-randomized phase 1 trial of 32 recurrent/metastatic PD-1 positive TNBC patients showed that pembrolizumab monotherapy was well tolerated and only 15.6% of patients had single symptomatic side effects. These results are similar to those of a combination therapy study between atezolizumab and nab-paclitaxel in metastatic breast cancer with positive PD-L1, ER, and negative profiles. HER2. These results then became the basis of a phase 3 clinical trial aimed at evaluating the combination therapy of Atezolizumab and Nab-paclitaxel in TNBC metastases that are still ongoing. (NCT02425891).[8]

It is very important to find an effective therapy in TNBC breast cancer with

significant therapeutic activity. This is because this type of breast cancer is easy to metastasize and is recurrent. Until now there is no ideal treatment protocol for TNBC, so many patients receive different types of therapy to treat the nature of this type of breast cancer.

Conclusion

Scientific evidence regarding tumor antigens and co-stimulatory compounds capable of activating anti-tumor immune responses, makes immunotherapy a promising therapy for success in preventing cancer recurrence and metastasis in the future. However, the current application of immunotherapy for breast cancer therapy is still far from satisfactory. Currently, several types of breast cancer vaccines are still in the clinical research stage. The immune system checkpoint inhibitor therapy in combination with other therapies in several studies has shown good results, although it has not been able to completely inhibit tumor regression. The current massive development of molecular biology and genomics is expected to perfect this therapeutic model product so that it can reduce the mortality rate of this disease in the future.

Conflict of Interest

Nothing to declare

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Nothing to declare

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