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The overexpressed antigens in triple negative breast cancer and application in immunotherapy

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ABSTRACT

Breast cancer is the most common cancer in women worldwide. Triple-negative breast cancer (TNBC) is considered a poor-prognosis subtype breast cancer with insensitivity to hormonal and targeted therapies. TNBC has several overexpressed tumor-associated antigens (TAAs) that contribute to cancer progression and prognosis determination of the patients. Interestingly, the overexpressed TAAs can induce the host immune response which may provide an alternative potential treatment option for TNBC patients. In this review, four overexpressed antigens commonly reported in TNBC, their clinical significance, and current ongoing preclinical and clinical studies to target these antigens in immunotherapy including immunotoxin/drug-conjugated antibody, bispecific antibody, cancer vaccine and chimeric antigen receptor-T cells (CAR-T cells) are described. These immunotherapy approaches using the overexpressed proteins as targets are proposed as important alternative treatments for advanced TNBC patients.

Keywords: breast cancer; triple negative breast cancer; tumor-associated antigen; immunotherapy

INTRODUCTION

Breast cancer remains the most common malignancy in women and one of the leading causes of death worldwide (Siegel *et al.*, 2019). Based on the expressions of estrogen receptor (ER), progesterone receptor (PR) and human epidermal growth factor 2 (HER2), breast cancer can be classified into three major subtypes; luminal (ER and/or PR positive), HER2 (HER2 positive) and triple-negative breast cancer (TNBC; ER, PR and HER2 negative) (Watkins, 2019).

TNBC represents 10-20% of all breast cancers and is considered as a poor-prognosis subtype (Agarwal *et al.*, 2016). TNBC is common in patients with young age and high histological grade and visceral organ metastatic setting that implies a much-shortened median survival time from relapse to death (Luo *et al.*, 2017). Lack of ER, PR and HER2 expression renders TNBC insensitive to the hormonal treatment and HER2-targeted therapies. Although TNBC is more sensitive to chemotherapy, high recurrence rate cases with very poor prognosis are also observed (Foulkes *et al.*, 2010). Hence, a novel effective treatment for TNBC is still needed.

Cancer immunotherapy is a treatment that aims to modulate a patient's immune response to eradicate cancer cells. Cancer cells have genetic and epigenetic alterations resulting in aberrant expressions of proteins which can be recognized by cytotoxic T lymphocytes (CTLs) (Zhang *et al.*, 2015). Targeting or redirecting the immune cells to the antigen expressed in the cancer cells using immunotoxin/drug-conjugated a monoclonal antibody, bispecific-antibody (BsAb), cancer vaccines and chimeric antigen receptor-T (CAR-T) cells has been proven as an effective treatment of malignancies by induction of cancer cell death (**Figure 1**). The immunotoxin and drug-conjugated antibody can deliver toxin or anti-tumor drug specifically to the cancer cells by specific binding with the antigen expressed on cancer cell (Birrer *et al.*, 2019); the antibody can be either an intact antibody (both variable or antigen-binding domain and constant domains) or a variable fragment. BsAb is an engineered antibody with two different antigen-binding domains; one is on cancer cells and the other is on immune cells. BsAb can induce the engagement between immune cells (i.e. dendritic cells, DCs) and cancer cells and then promote the anti-tumor immune response by recruiting antigen-specific CTLs to destroy cancer cells (Dahlén *et al.*, 2018). Immunization using cancer-derived antigens to promote anti-tumor immune response is termed cancer vaccine. This approach utilizes a variety of antigens including DNA, RNA, protein, and peptide which are able to be presented to DCs to specifically activate antigen-specific CTLs (Kruger *et al.*, 2019). CAR-T cells are genetically-engineered T cells that express receptors composed of an

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extracellular domain specific to tumor antigens and signaling domains of the immune cells, i.e. immunoreceptor tyrosine-based activation motifs, or cytosolic motifs of CD28, which promote robust T cell activation. Upon binding to the specific antigen on cancer cells, CAR-T cells can easily secret multiple cytokines, granzyme and perforin leading to cancer cell apoptosis (Kruger *et al.*, 2019). These immunotherapies are new hope for advanced cancer patients who have no effective conventional treatments available.

In this review, four potential tumor-associated antigens (TAAs) in TNBC including mesothelin (MLSN), folate-receptor alpha 1 (FOLR1), cancer-testis antigens (CTAs) and trophoblast surface antigen 2 (Trop2) are discussed for their clinical relevance and the available approaches to target these antigens in context of immunotherapy in TNBC patients.

Mesothelin (MSLN**)**

Expression and function in cancer

MSLN is a membrane-bounded glycophosphatidylinositol-linked glycoprotein normally slightly expressed in mesothelial cells form a monolayer of specialized pavement-like cells that line the body's serous cavities and internal organs (Hassan *et al.*, 2016). The *MSLN* gene encodes a 72-kDa precursor protein composed of four domains including N-terminal signaling domain, C-terminal domain, mature megakaryocyte promoting factor (MPF) and GPI-anchored domain. This precursor MSLN is cleaved into two fragments after being exported from the endoplasmic reticulum by furin protease creating 32-kDa MPF secreted from the cell and 40-kDa membrane-bounded mature MSLN (Hassan *et al.*, 2016). Expression of MSLN has been reported in many solid tumors with poor clinicopathological features and short patient survival time (Li *et al.*, 2014; Thomas *et al.*, 2015). MSLN expression was found in 34-67% of TNBC (Bayoglu *et al.*, 2015; Li *et al.*, 2014; Parinyanitikul *et al.*, 2013; Tchou *et al.*, 2012; Tozbikian *et al.*, 2014) (**Table 1**). High levels of MSLN were correlated with tumor grade, lymph node metastases and decreased overall survival in TNBC patients (Bayoglu *et al.*, 2015; Li *et al.*, 2014; Tozbikian *et al.*, 2014).

Though the function of MSLN in normal tissue is still unclear in TNBC, MSLN facilitates the anchorage-independent growth and inhibits anoikis by downregulating the pro-apoptosis protein (Uehara *et al.*, 2008). Moreover, overexpression of MSLN upregulates matrix metalloproteinase-9 via ERK signaling in breast cancer metastasis (Servais *et al.*, 2012).

Immunotherapy against MSLN in TNBC

LMB-100 (previously RG7787), an immunotoxin of MSLN-specific humanized antigen binding domain of the antibody fused with toxin derived from Pseudomonas exotoxin A (PE24) can bind to MSLN expressed on the cancer cells and results in internalization of the drug into the cell where the toxin is cleaved and inhibits the protein synthesis (Liu *et al.*, 2012). A preclinical study of LMB-100 demonstrated impressive anti-tumor activity in the MSLN-expressing TNBC cell line (Alewine *et al.*, 2014). Furthermore, in a xenograft model of the TNBC tumor, administration of LMB-100 alone delayed tumor growth, and with co-administration with chemotherapy it synergistically inhibited tumor growth (Alewine *et al.*, 2014).

Utilizing MSLN as a TNBC-associated marker for induction of the anti-tumor immune response was also explored in the BsAb system. Recently, a BsAb against MSLN and CD16 (FcγRIII), a crucial receptor for NK cell-mediated antibody-dependent cell cytotoxicity (ADCC) or MSLN/CD16, BsAb was developed and the *in vitro* data demonstrated that it efficiently induced anti-tumor immune response mediated by NK cells against MSLN expressing TNBC cell lines (Del Bano *et al.*, 2019). Using humanized NOD-SCID gamma mice bearing MSLN-expressing TNBC tumor, the results confirmed the anti-tumor activity of MSLN/CD16 BsAb. Moreover, MSLN/CD40 BsAb (ABBV-428) demonstrated an anti-tumor activity in both syngeneic and xenograft MSLN-expressing tumors (Ye *et al.*, 2019). This anti-tumor effect was dependent on MSLN expression and activation of antigen presenting cells via CD40 signaling which subsequently activated the tumor-specific T cells response (Ye *et al.*, 2019). The phase I clinical trial of ABBV-428 in combination with nivolumab in several solid tumors was recently completed (NCT02955251) (**Table 1**), however, no results have yet been published.

MSLN-specific CAR-T cells specifically and efficiently killed MSLN-expressing TNBC cell lines *in vitro* (Tchou *et al.*, 2012). A phase I clinical trial of MSLN-specific CAR-T cells in TNBC patients is still ongoing and expected to finish in 2021 (NCT02792114) (**Table 1**).

Folate receptor alpha 1 (FOLR1)

Expression and function in cancer

FOLR1 is a member of the folate receptor protein family that comprises alpha, beta, gamma and delta isoforms (Elnakat and Ratnam, 2004). FOLR1

is a membrane-bounded glycophosphatidylinositol-linked glycoprotein that binds to folate with high affinity and facilitates the folate absorption (Elnakat and Ratnam, 2004). It can limitedly be found in luminal epithelial cells such as the gastrointestinal tract and female reproductive tract (Kelemen, 2006). In cancers, FOLR1 has been found overexpressed in ovarian and lung cancers, and TNBC (Necela *et al.*, 2015; O'Shannessy *et al.*, 2015). Overexpression of FOLR1 was associated with high grade tumors and decreased survival time in breast cancer patients (Aboulhagag *et al.*, 2018). FOLR1 overexpression has been revealed in 20 to 87% of TNBC samples (Aboulhagag *et al.*, 2018; Ginter *et al.*, 2017; Necela *et al.*, 2015; Zhang *et al.*, 2014) (**Table 1**).

FOLR1 regulates many cancer cells signaling, proliferation and invasion by acting as a transcriptional factor which regulates several gene expressions involved with carcinogenesis and progression of cancer (Boshnjaku *et al.*, 2012). In TNBC, *FOLR1* knockdown reduced cell proliferation via the Src/ERK signaling pathway (Cheung *et al.*, 2018; Necela *et al.*, 2015). Moreover, FOLR1 also activated the JAK/STAT3 pathway involving cancer cell progression (Hansen et al, 2015).

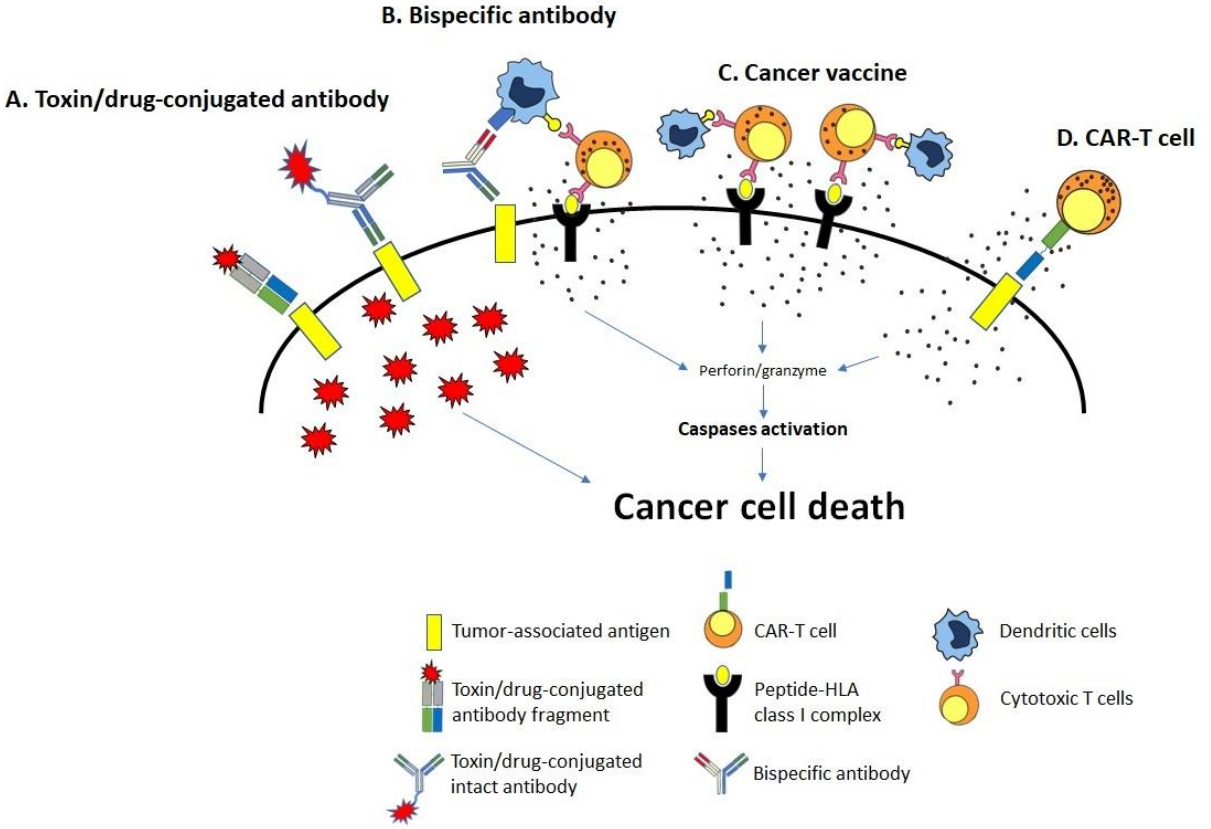

Figure 1 Different immunotherapies targeting the overexpressed TAAs. **A.** Toxin/drug-conjugated antibody can bind to antigen on the cancer cell resulting in endocytosis of toxin/drug that induce cell apoptosis. **B.** Bispecific antibody bridges the immune cell such as dendritic cells (DCs) to cancer cells which then subsequently induces antigen-specific cytotoxic T lymphocytes (CTLs) to kill cancer cells. **C.** Cancer vaccine utilizes a variety of antigens including DNA, RNA, protein, and peptides which are able to be presented onto DCs to specifically activate antigen-specific CTLs. **D.** CAR-T cells can recognize antigens on the cancer cell surface in an MHC independent manner and induce robust cancer cell killing.

Table 1 Summary of overexpressing antigens in TNBC, their clinical significance and potential immunotherapy targeting these antigens.

Antigen in TNBC/Study	% Expression by IHC (positive case/total cases)	Clinicopathological correlation	Prognostic value	Targeted immunotherapy in clinical trial	Roles in breast cancer progression
MSLN					
(Parinyanitikul *et al.*, 2013)	34% (37/109)	Lymphovascular metastasis	No	1. **ABBV-428, anti-MSLN/CD40 BsAb** Phase I (NCT02955251)* 2. **MSLN specific CAR-T cells** Phase I (NCT02792114)	Promote anoikis-induced apoptosis resistance (Uehara *et al.*, 2008) Increase cancer invasion via MMP-9 production (Servais *et al.*, 2012)
(Tozbikian *et al.*, 2014)	36% (82/226)	Age of patient, basal phenotype and distant metastasis	Poor		
(Bayoglu *et al.*, 2015)	42% (30/71)	Tumor grade	No		
(Li *et al.*, 2014)	63% (44/70) cohort A 66% (76/116) cohort B	TNBC status	Poor		
(Tchou *et al.*, 2012)	67% (29/43)	ND	ND		
FOLR1					
(Ginter *et al.*, 2017)	20% (15/76)	No	Poor	1. **IMGN-853, drug-conjugated anti FOLR1 antibody** Phase I (NCT03106077)* Phase II (NCT02996825) 2. **FOLR1 peptide vaccine** Phase I (Kalli *et al.*, 2018) Phase II (NCT03012100)	Induce oncogenic genes expression (Boshnjaku *et al.*, 2012) Promote cell proliferation (Cheung *et al.*, 2018; Necela *et al.*, 2015) Facilitate oncogenic STAT3 activation (Hansen et al, 2015)
(Boogerd *et al.*, 2016)	47% (7/15)	No	No		
(O'Shannessy *et al.*, 2012)	67% (12/18) 50% (19/38) metastasis TNBC	ER, PR expression and tumor grade	ND		
	47% (7/15)	No	No		
(Zhang *et al.*, 2014)	80% (44/55)	Tumor grade and lymph node metastasis	Poor		

Table 1 continued.

Antigen in TNBC/Study	% Expression by IHC (positive case/total cases)	Clinicopathological correlation	Prognostic value	Targeted immunotherapy in clinical trial	Roles in breast cancer progression
CTAs: MAGE-As					
(Chen *et al.*, 2011)	24% (54/225) MAGE-A	Tumor grade and tumor size	ND	1. **MAGE-A peptide vaccine** Phase I (Dillon *et al.*, 2017; Zhang *et al.*, 2017)	Increase chemotherapy resistance (Monte *et al.*, 2006)
(Curigliano *et al.*, 2011)	26% (13/50) MAGE-A	ND	ND		
(Wang *et al.*, 2016)	76% (13/17) MAGE-A	Tumor grade, TNBC status and lymph node metastasis	ND	2. **MAGE peptide vaccine** Phase II (NCT03093350)	Promotes p53 degradation rate, increase apoptosis resistance (Marcar *et al.*, 2015)
(Mrklić *et al.*, 2014)	69% (56/81) MAGE-A1 16% (13/81) MAGE-A10	Clinical stage	ND		Increase cancer invasion and EMT process (Wang *et al.*, 2016)
(Tessari *et al.*, 2018)	86% (18/21) MAGE-A3	No	No		
(Ayyoub *et al.*, 2014)	32% (17/54) MAGE-A3/6	ER, PR expression and tumor grade	Poor		
(Badovinac Crnjevic *et al.*, 2012)	10% (5/50) MAGE-A10	ER and HER2 expression	No		
CTAs: NY-ESO-1					
(Lee *et al.*, 2015)	10% (59/612)	TILs infiltration	Good	1. **NY-ESO-1 peptide vaccine** Phase II (NCT03093350)	Increase cell invasion and correlated with cancer-stem cell markers (Liu *et al.*, 2019)
(Ademuyiwa *et al.*, 2012)	16% (27/168)	TILs infiltration	No		
(Raghavendra *et al.*, 2018)	17% (11/65)	No	No		
(Curigliano *et al.*, 2011)	18% (9/50)	No	No		
(Chen *et al.*, 2011)	19% (43/225)	Tumor grade	ND		
(Mrklić *et al.*, 2014)	27% (22/81)	No	No		
(Tessari *et al.*, 2018)	29% (6/21)	ER and PR expression	No		
(Badovinac Crnjevic *et al.*, 2012)	86% (42/49)	TNBC status	ND		

Table 1 continued.

Antigen in TNBC/Study	% Expression by IHC (positive case/total cases)	Clinicopathological correlation	Prognostic value	Targeted immunotherapy in clinical trial	Roles in breast cancer progression
Trop2					
(Khoury *et al.*, 2019)	49% (17/35) 64% (21/33) metastasis TNBC	*PI3KCA* and *RB1* mutation	ND	1. **IMMU-132, drug-conjugated anti-Trop2 antibody** Phase I (Starodub *et al.*, 2015)	Promote oncogenic NF-kB, MAPK and ERK activation (Guerra *et al.*, 2013) Enhance cancer invasion (Zhao *et al.*, 2018) Promote chemotherapy resistance (Caldon *et al.*, 2012; Scaltriti *et al.*, 2011)
(Zhao *et al.*, 2018)	78% (75/96)	Clinical stage, lymph node metastasis, distant metastasis, TNBC status and E-cadherin expression	Poor	Phase I/II (Bardia *et al.*, 2019) Phase I/II (NCT03424005) Phase I/II (NCT01631552) Phase I/II (NCT04039230) Phase II (Bardia *et al.*, 2017 Phase II (NCT04230109) Phase III (NCT02574455)	

CTAs: cancer-testis antigens, EMT: epithelial to mesenchymal transition, FOLR1: folate receptor alpha-1, MAGE-As: melanoma-associated antigens, NY-ESO-1: New York esophageal squamous cell carcinoma 1, Trop2: trophoblast surface antigen 2, No: no correlation reported, ND: no data available, NCT (number of clinical trial)*: finished clinical trial but no results published, and NCT number: active or ongoing clinical trial

Immunotherapy against FRA1 in TNBC

The internalization of FOLR1 upon binding to folate is a potent target for tumor imaging and a tumor-specific drug delivery approach (Kayani *et al.*, 2018; Khandelwal *et al.*, 2020). In TNBC, folate-drug conjugated, folate-decorated nanoparticles or liposomes carrying anti-tumor drugs have been largely explored in preclinical and some clinical settings. EC1456, a folate conjugated tubulysin, a microtubule destabilizing agent was being tested in a preclinical study of FOLR1 expressing tumors including TNBC (Lu *et al.*, 2017; Reddy *et al.*, 2018). EC1456 inhibited the growth of tumors *in vitro* and in TNBC-patient derived xenograft mice (Lu *et al.*, 2017; Reddy *et al.*, 2018). There is a phase I clinical trial of EC1456 in TNBC (NCT01999738); however, the results from this clinical trial are still not available (at the time of this manuscript preparation). The folate-conjugated beta-lactoglobulin nanoparticle containing doxorubicin delivered the drug at clinically relevant doses to a TNBC cell line, inhibited tumor growth and induced apoptosis (Kayani *et al.*, 2018). Moreover, folate-conjugated polymeric nanoparticles delivered paclitaxel to breast cancer that metastases to lung and bone which improved patient survival rate without overt adverse effects (Gregory *et al.*, 2020). The folate-coated particle targeting TNBC is an active ongoing area of research that will surely be investigated in a clinical trial in the future.

Mov18-IgG1, a humanized FOLR1-specific antibody that has shown an anti-tumor activity in ovarian cancer has been recently investigated in TNBC (Cheung *et al.*, 2018). Mov18-IgG1 conjugated with A-419259, a Src-inhibitor, reduced TNBC growth in both orthotropic and xenograft models with no toxicity observed (Cheung *et al.*, 2018). IMGN853 (mirvetuximab soravtansine), a FOLR1-specific humanized antibody-conjugated with DM4, a microtubule destabilizing toxin that demonstrated significant anti-tumor activity in ovarian cancer, is currently being investigated in phase II and III clinical trials (Altwerger *et al.*, 2018; Moore *et al.*, 2018). A trial on the maximum tolerated and recommended phase II doses of IMGN853 in combination with gemcitabine in TNBC patients is now ongoing (NCT02996825) (**Table 1**). In a phase II clinical trial, the clinical benefit of IMGN853 as a single agent in TNBC patients was recently finished with no results currently published (NCT03106077).

E39 is an antigenic peptide derived from FOLR1 that was presented to monocyte-derived dendritic cells and induced E39-specific CTL in ovarian and breast cancer patients (Kim *et al.*, 1999). The presence of naturally FOLR1-specific T cell responses was detected in ovarian and breast cancer patients, and the immunity was not associated with autoimmunity; furthering the indications of the antigenic potential of FOLR1 for cancer immunotherapy. Phase I clinical trials showed the response of FOLR1 antigenic peptide vaccines in combination with GM-CSF to induce a safe and detectable FOLR1-specific T cell response up to 12 months in ovarian and breast cancer patients (Kalli *et al.*, 2018). The randomized phase II clinical trial to investigate the survival outcome of FOLR1 antigenic peptide vaccine treatments in TNBC patients is now being conducted (NCT03012100) (**Table 1**). The study is expected to be completed in 2024.

Targeting FOLR1 via CAR-T cells was successful to inhibit tumor progression in TNBC in preclinical studies (Song *et al.*, 2016). T cells with CAR constructed from single chain variable fragments (scFV) of MOv19, a FOLR1-specific antibody fused with CD3ζ and CD28 signaling domains inhibited the growth of TNBC *in vitro* and *in vivo* in an antigen-specific manner (Song *et al.*, 2016). Further investigation of FOLR1-specific CAR-T cells in clinical trials is another promising approach to target FOLR1 in TNBC.

Cancer-testis antigens (CTAs)

Expression and function in cancer

More than 50 proteins have been identified as CTAs that included forkhead box protein M1 (FOXM1), ATPase family AAA domain containing 2 (ATAD2), melanoma-associated antigens (MAGE-As), New York esophageal squamous cell carcinoma 1 (NY-ESO-1) and preferentially expressed antigen in melanoma (PRAME) (Mahmoud, 2018). CTAs are ideal TAAs because of its limited expression in specific tissues and high immunogenicity (Fratta *et al.*, 2011). In breast cancer, multiple CTAs were reported to be overexpressed with a preference for different subtypes. FOXM1 and ATAD2 expressions were correlated with ER+ breast cancer (Khongkow *et al.*, 2016) whereas MAGE-As and NY-ESO-1 were predominantly expressed in TNBC (Curigliano *et al.*, 2011). MAGE-As and NY-ESO-1 overexpression were reported in 16-86% and 8-20% of TNBC patients, respectively (Ademuyiwa *et al.*, 2012; Ayyoub *et al.*, 2014; Badovinac Crnjevic *et al.*, 2012; Chen *et al.*, 2011; Mrklić *et al.*, 2014; Raghavendra *et al.*, 2018; Tessari *et al.*, 2018; Wang *et al.*, 2016) (**Table 1**). MAGE-As expression was also associated with poor clinical

features such as clinical stage, tumor grade, and lymph node metastasis (Ayyoub *et al.*, 2014; Mrklić *et al.*, 2014; Wang *et al.*, 2016). MAGE-As has been reported as a poor or good prognostic marker in breast cancer (Ayyoub *et al.*, 2014; Lian *et al.*, 2012; Raghavendra *et al.*, 2018). These conflicted results may come from the differences in MAGE-A isoform being evaluated (Ayyoub *et al.*, 2014). NY-ESO-1 was revealed as a good prognostic marker in TNBC patients (Lee *et al.*, 2015). Expression of MAGE-A3 in breast cancer inhibited p53, a crucial tumor-suppressor protein by reducing the degradation rate of MDM4, a p53 suppressor protein, resulting in apoptosis resistance (Marcar *et al.*, 2015). The p53 transactivation inhibitory effect of MAGE-A has led to resistance to chemotherapy and apoptosis (Monte *et al.*, 2006). Moreover, MAGE-A may contribute to the metastatic property of breast cancer cells by promoting the epithelial-mesenchymal transition (EMT) process. Using siRNA against MAGE-A in the TNBC cell line resulted in decreased migration and invasion capacities (Wang *et al.*, 2016). This effect was found in concordance with lower EMT markers (Wang *et al.*, 2016). The let-7, a miRNA known for tumor-suppressing function, could inhibit MAGE-A1 expression in breast cancer cells and reduced cell proliferation, migration, and invasion (Mi *et al.*, 2019). The possible role of NY-ESO-1 expression in breast cancer stem cells promoted high migration and invasion (Liu *et al.*, 2019). The expression of NY-ESO-1 was found to correlate with CD44 and CD24 expression in metastatic breast cancer tissues (Liu *et al.*, 2019).

Immunotherapy against CTAs in TNBC

Cancer vaccine is the main approach to target CTAs (Wei *et al.*, 2019). Vaccination of NY-ESO-1-derived antigenic peptide together with Montanide ISA-51 and CpG ODN 7909 adjuvant induced NY-ESO-1 specific antibodies and T cell response in breast cancer patients (Valmori *et al.*, 2007). This vaccination caused detectable NY-ESO-1 specific CTL that lysed NY-ESO-1 expressing tumor cells *in vitro* (Karbach *et al.*, 2010). Moreover, the favorable clinical outcome was observed in 6 of 9 patients who developed NY-ESO-1-pecific immune responses after vaccination with 27 to 49 months overall survival (Karbach *et al.*, 2010). Treatment of 5-Aza-2′-deoxycytidine (DAC), a DNA demethylating agent, induced *de novo* NY-ESO-1 expression in breast cancer cells that augmented the recognition of antigen-specific T cells, resulting in apoptosis of cancer cells (Klar *et al.*, 2015). A phase II clinical trial for NY-ESO-1 peptide vaccination in breast cancer is still ongoing (NCT03093350) (**Table 1**). A pilot study evaluating the outcome of peptide vaccination including MAGE-A and NY-ESO-1 together with poly I:C in breast cancer patients demonstrated that 4 of 11 patients developed an antigen-specific immune response with no adverse effects (Dillon *et al.*, 2017). Vaccination of HLA-A2 specific MAGE-A11 peptide elicited antigen-specific CTL response that lysed MAGE-A11 expressing breast cancer cells *in vitro* and *ex vivo* (Zhang *et al.*, 2017). Similarly, immunization of HLA-A2 specific MAGE-A3 peptide generated MAGE-A specific CT that not only recognized MAGE-A3 but also cross-reacted to other MAGE-A antigens across different cancer cells such as melanoma, lung cancer and TNBC (Chinnasamy *et al.*, 2011). Although the immunogenicity of MAGE-A and NY-ESO-1 are well characterized, clinical studies targeting these antigens in breast cancer are still under investigation (Wei *et al.*, 2019) which may be due to their lower expressions compared to other cancers. Nevertheless, targeting CTAs in TNBC is still a potential approach as these antigens have already been proven to improve the clinical outcome in other cancer patients (Wei *et al.*, 2019).

Trophoblast surface antigen 2 (Trop2)

Expression and function in cancer

Trop2 is a 36 kDa glycoprotein firstly discovered in trophoblast cells. In normal tissue, Trop2 can be found in skin, breast, prostate, cervix, and placental tissues (Stepan *et al.*, 2011). Trop2 was reported to be overexpressed in various tumors and its expression was associated with poor clinical features and prognosis (Goldenberg *et al.*, 2018). The overexpression of Trop2 in breast cancer was reported (Ambrogi *et al.*, 2014; Khoury *et al.*, 2019; Zhao *et al.*, 2018). High expression of Trop2 in breast cancer was associated with high tumor grade, clinical stage, lymph node metastasis, and distant metastases with decreased patient survival time (Ambrogi *et al.*, 2014; Zhao *et al.*, 2018). Trop2 overexpression was found in 56 to 78% of TNBC samples (Khoury *et al.*, 2019; Zhao *et al.*, 2018) (**Table 1**).

Trop2 can bind to several ligands, notably insulin-like growth factor 1 and claudin 1/7 which regulate many cell functions (Vidmar *et al.*, 2013). Activation of Trop2 increases endogenous Ca^{2+} which subsequently induces NF-κB and ERK/MAPK pathways, enhances cell proliferation and apoptotic resistance (Guerra *et al.*, 2013). Studies in breast cancer patients revealed the correlation of Trop2

overexpression with high tumor grade and invasive capacity (Guerra *et al.*, 2013; Zhao *et al.*, 2018). Trop2 expression was inversely correlated with levels of E-cadherin expression in breast cancer cell lines indicating a highly invasive property (Zhao *et al.*, 2018). Trop2 played a role in multiple drug resistance in breast cancer including tamoxifen and trastuzumab which were linked with increased cyclin D and E expressions (Caldon *et al.*, 2012; Scaltriti *et al.*, 2011).

Immunotherapy against Trop2 in TNBC

The immunogenicity of Trop2 has long been recognized in breast cancer (Mangino *et al.*, 2002) which indicates the potential of Trop2 in immunotherapy such as Trop2-specific monoclonal antibody and drug-conjugated antibody. The hRS7, a humanized Trop2-specific antibody had shown anti-tumor activity in cervical and ovarian cancers by inducing the ADCC response to the cancer cells (Raji *et al.*, 2011; Varughese *et al.*, 2011). Several drug-conjugated hRS7 antibodies were proposed (Bardia *et al.*, 2017; Chang *et al.*, 2010). 2L-Rap(Q)-hRS7 or Rap-hRS7 is a hRS7 conjugated with ranpirnase, a protein synthesis inhibitor that inhibited the growth of TNBC, cervical, lung, colon, pancreatic, prostate and ovarian cancers, and increased the survival rate of tumor-bearing mice without adverse effects (Chang *et al.*, 2010).

The hRS7 conjugated with SN-38, a topoisomerase inhibitor called sacituzumab govitecan or IMMU-132 (Goldenberg *et al.*, 2015) had a potent anti-tumor activity in murine and monkey models of different solid tumors including TNBC (Cardillo *et al.*, 2011; Goldenberg *et al.*, 2015). The toxicity profile of IMMU-132 was highlighted in a study using monkeys with severe neutropenia and diarrhea in high dose treatment (Cardillo *et al.*, 2011). The efficacy of IMMU-132 was evaluated in a phase I clinical trial for advanced stage metastatic cancers e.g. colon, lung, pancreatic cancer and TNBC (Starodub *et al.*, 2015). No grade 4 adverse effects were observed, while grade 3 adverse effects including diarrhea, fatigue and neutropenia were observed in a small fraction of patients (six of twenty-five) (Starodub *et al.*, 2015). Two patients (TNBC and colon cancer) demonstrated a partial response and sixteen patients had a stable disease (Starodub *et al.*, 2015). A phase II clinical trial of IMMU-132 in 69 TNBC patients was investigated and the complete and partial responses were observed in two and twenty-one patients (Bardia *et al.*, 2017). The median overall survival and progression-free survival were 16.6 and 6 months (Bardia *et al.*, 2017). A recent report of a phase I/II clinical trial of IMMU-132 in 108 TNBC patients demonstrated complete and partial responses in three and thirty-three patients (Bardia *et al.*, 2019). Although 32% of patients showed a serious adverse effect including neutropenia, febrile neutropenia and anemia, only 3% of patients had to withdraw from study because of the toxicity (Bardia *et al.*, 2019). Currently, a multicenter randomized phase III clinical trial of IMMU-132 in metastatic TNBC patients who were refractory or relapsed after at least 2 prior chemotherapies (including a taxane) is expected to be soon finished (NCT02574455). Moreover, four phase I or II clinical trials of IMMU-132 in combination with other agents in TNBC patients are still ongoing, suggesting the potential of IMMU-132 for TNBC treatment (NCT04230109, NCT03424005, NCT01631552 and NCT04039230) (**Table 1**).

CONCLUSION

Among all types of breast cancer, TNBC is considered for alternative immunotherapy because of its limited treatment options (Watkins, 2019). Targeting overexpressed antigens in the cancer cells by immunotherapeutic approaches promotes anti-tumor immune responses and improves the survival time of cancer patients (Bardia *et al.*, 2019). Several proteins have been reported to be aberrantly overexpressed and contributed to the increased aggressive nature of TNBC. Hereafter, it can be summarized that there are four potential targets for a clinical phase of immunotherapies that may benefit patients with TNBC. Preclinical and clinical studies of immunotoxin, monoclonal antibody, drug-conjugated monoclonal antibody, CAR-T cells and cancer vaccines have been evaluated in TNBC with a significant clinical response with low toxicity Therefore, using immunotherapy to target these overexpressing antigens may provide a potential novel approach for TNBC treatment.

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