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Current State of Cell Therapies for Breast Cancer

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Abstract: Metastatic breast cancer (BC) is an aggressive form of cancer and is an absolute challenge to treat. This review discusses the standard treatments available for metastatic BC. It further highlights the rationale for targeting oncodrivers, tumor-associated antigens, and neoantigens in BC. Explaining the significance of immune response in successful immunotherapeutic studies, it draws attention towards how adoptive cell therapy can be a useful immunotherapeutic tool. We focus on adoptive cell therapy in BC covering tumor-infiltrating lymphocyte therapy, engineered T cell receptor therapy, chimeric antigen receptor therapy, dendritic cell therapy and natural killer cell therapy. In this work, we aim to provide an overview of clinical data regarding the use of cellular immunotherapies in BC. Eventually, we conclude by proposing future adoptive cell therapy approaches, which can be used to cure BC.

Key Words: Adoptive cell therapy, oncodrivers, tumor associated antigens, TIL therapy, engineered TCR therapy, CAR therapy, DC therapy, NK therapy

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In the United States, an estimated 6% of women with breast cancer (BC) are metastatic at diagnosis, with 20% to 30% of early-stage BC patients eventually progressing to metastatic disease.[1,2] Metastatic BC (MBC) is considered incurable with goals of treatment aimed at quality of life and extending survival. The 5-year survival for MBC is a mere 27% compared with more than 86% for women with local disease. Overall survival for MBC ranges from 6.3 to 55.8 months with a median of 24 months, depending on subtype.[3] Over the past 30 years, all-stage BC deaths have declined by 40%, which can be partially attributed to a growing list of treatment options.[4]

Metastatic BC is a varied disease that can be characterized based on molecular subtype, which also provides prognostic information. The most common subtype is hormone receptor (HR)–positive (either estrogen receptor [ER]–positive or progesterone receptor [PR]–positive), which accounts for approximately 68% of newly diagnosed BCs. Hormone receptor–positive BC can be further divided into luminal A and luminal B based on Ki-67 expression. Subtypes that are known to be more aggressive and have worse outcomes include those overexpressing human epidermal growth factor receptor 2 (HER2) and triple-negative ($HR^-/HER2^-$).[5] Molecular subtypes have also been found to have an impact on local and regional recurrence. Luminal A subtype has been shown to have the best prognosis with the lowest frequency of metastatic disease, consisting of bone-only disease in 45% of cases. This differs drastically to $HER2^+$ and triple-negative BC (TNBC) subtypes, which present more often with visceral-only metastasis.[6] Patients with brain metastasis compared with bone have a worse overall prognosis partly due to the fact that most systemic therapies fail to cross the blood-brain barrier, which limits treatments.[5]

One of the biggest struggles with treating MBC is the amount of intertumor and intratumor heterogenicity. These differences can be due to natural or treatment selection pressures, which can alter treatment response.[7] Currently, the National Comprehensive Cancer Network recommends assessment of the following biomarkers in MBC: HRs, HER2, programmed death ligand 1 (PD-L1) in triple-negative, and germline BRCA1 and BRCA2 status, with the option to test for PIK3CA as a second line in $ER^+/HER2^-$ cancers and in certain circumstances testing for mismatch repair protein and tumor mutational burden.[8] A perfect example of the importance that subtypes play in MBC is seen in the evolution of $HER2^+$ disease, which used to confer a poor prognosis until anti-HER2 therapies were developed.[9,10]

STANDARD TREATMENT FOR MBC

Although MBC is considered an incurable disease, there have been significant advances in systemic treatment leading to improved progression-free survival (PFS) and even overall survival.[11] Current systemic treatments vary based on subtype of MBC and sites of metastasis.

$HR^+/HER2^-$

Up to 68% of MBCs are $HR^+/HER2^-$ subtype, which have a high incidence of bone metastasis.[12] Endocrine therapy (ET) is the recommended frontline treatment in addition to bone-modifying agents such as bisphosphonates and denosumab if bony metastases are diagnosed. Patients who relapse during the first 2 years of ET or have disease progression within 6 months are considered primary endocrine resistant.[13] Resistance is thought to be caused by a mutation of the ligand-binding domain of estrogen receptor 1 encoding ERα upregulating the HER and PI3K/Akt/mTOR pathways.[14,15] If visceral disease is present, then chemotherapy should be given to minimize organ failure with eventual goal of maintenance ET.[11,15] One major advancement for HR^+ MBC has been treatment with CDK4/6 inhibitors in combination with ET. CDK4/6 inhibitors include palbociclib, ribociclib, and abemaciclib and act by inhibiting the transition from G0/G1 to S phase of the cell cycle.[16] Multiple studies have shown improved overall survival and PFS with CDK4/6 inhibitors combined with ET versus ET alone and less toxicity compared with standard chemotherapy.[17–21] Patients with recurrent BC or those who develop ET resistance, the PALMOMA-3 study showed that fulvestrant combined with palbociclib improved PFS in this patient population.[22]

Patients who progress on combination ET and CDK4/6 inhibitor can be tested for PIK3CA and estrogen receptor 1 mutations as well as germline BRCA1/2 to determine second-line therapy. PIK3CA mutations occur in an estimated 40% of patients with HR^+HER2^- BC. SOLAR-1, a randomized, placebo-controlled trial, showed that treatment with alpelisib (PI3K inhibitor) and fulvestrant prolonged PFS 11.0 versus 5.7 months in patients with mutated PIK3CA. Adverse effects of alpelisib caused disruptions

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in treatment in 70% of patients, most notably hyperglycemia, leading to its recommendations as second-line therapy after CDK4/6 inhibitor with ET.[13,23,24] Another option for patients who progress on ET is addition of everolimus (mTOR inhibitor).[25] Patients with continued progression despite the aforementioned targeted therapies should then be considered for single-agent chemotherapy such as anthracyclines, taxanes, capecitabine, platinums, and other agents.[13]

HER2+

The CLEOPATRA trial established pertuzumab, docetaxel, and trastuzumab as the criterion-standard, first-line treatment of HER2+ MBC regardless of HR status. The addition of pertuzumab, which stimulates antibody-dependent, cell-mediated cytotoxicity, complements trastuzumab for a more thorough blockade of HER2 signaling, improving PFS, 18.5 versus 12.4 months.[26] For patients with HER2+/HR+ MBC ET can be added to trastuzumab-pertuzumab maintenance therapy. Patients who progressed on the above regimen used to receive ado-trastuzumab emtansine (T-DM1) as second-line treatment based on TH3RESA[27] and EMILIA[28] studies. However, in 2019, the Food and Drug Administration approved trastuzumab deruxtecan (T-DXd) in MBC who had been treated with one or more anti-HER2 therapies based on studies showing improved PFS versus T-DM1.[29]

Triple Negative

Treatment of metastatic TNBC has proven difficult over the years given a lack of therapeutic targets. Taxane- and anthracycline-based chemotherapy remains first-line therapy with addition of targeted treatments based on PD-L1 and BRCA status.[13] Patients who harbor a PD-L1 mutation account for 20% to 40% of triple-negative MBC. The KEYNOTE-355[30] and Impassion130[31] supported the addition of immune checkpoint inhibitors to a chemotherapy regimen as first-line treatment. Patients with a BRCA mutation should be offered a poly(ADP-ribose) polymerase inhibitor (olaparib or talazoparib) as an alternative to chemotherapy because of improved PFS.[32,33]

TARGETING ONCODRIVER, TUMOR-ASSOCIATED ANTIGENS, AND NEOANTIGENS IN BC

Tumor-associated antigens (TAAs) are more frequently overexpressed in various BC subtypes. Studies have reported that overexpression of TAAs is associated with poor clinical outcomes in many invasive BC (IBC) and MBC patients.[34] Some of the examples for TAAs in BC subtypes include overexpressed cellular proteins HER2, HER3, EGFR, mucin 1 (MUC1), carcinoembryonic antigen (CEA), Wilms tumor gene (WT1), and mutated tumor suppressor protein p53.[35] The TAAs such as HER2, HER3, EGFR, ER, PR, and MUC1 are expressed on normal cells, but these oncodrivers are overexpressed on tumor cells.[36] Various studies have identified a collection of highly immunogenic peptide epitopes for HER2, HER3, EGFR, and MUC1 oncodrivers, and these peptides are being applied to develop various types of immunotherapies.[37–41] The immunogenic peptides from the TAAs have the ability to elicit CD4+ T cell–, CD8+ T cell–, and B cell–mediated antitumor response in BC.[42] HER2 is overexpressed in 20% to 25% of all primary, IBC, and MBC patients and is also responsible for poor prognosis and recurrence. HER2 oncodriver is an ideal target to develop immunotherapy approach for HER2+ BC patients because it involves extracellular domain (ECD) and intracellular domain, and it can be targeted to trigger both T cell– and B cell–mediated antitumor immunity.[43] Both major histocompatibility complex (MHC) classes I and II recognizing immunogenic tumor antigenic peptides from the ECD and intracellular domain portion of HER2 protein can target T cell component and antibodies produced by B cells. One of the critical advantages of antibody production by using cancer vaccine (e.g., dendritic cell [DC]–based immunotherapy) is that these antibodies can block TAA-specific functional signaling pathway and induce antibody-dependent cell-mediated cytotoxicity in BC.[42] Very recently, DC-based immunotherapy targeting HER3 oncodriver has shown a highly promising outcome and was able to generate CD4+ T cell–specific antitumor immune response in preclinical models for HER2+, TNBC, and melanoma.[41] The immunogenic peptides derived from HER3 oncodriver are being tested in clinical trials for HER2+ TNBC and brain metastasis patients. Various studies have also been focused on targeting neoantigens for potential use of immunotherapy development in BC treatment. Neoantigens are tumor-specific antigens that are overexpressed from the results of nonsynonymous mutations on tumor cells in BC.[44] A recent study has shown a major advantage of utilizing tumor neoantigens for cell-based immunotherapies and generation of neoantigen-reactive tumor-infiltrating lymphocytes (TILs) and enhanced antitumor response in metastatic cancer patients.[45]

SIGNIFICANCE OF IMMUNE RESPONSE IN BC TREATMENT THERAPIES

Breast tissue has a complex immune environment with cytotoxic CD8+ T cells, CD4+ helper T cells, natural killer (NK) cells and B cells infiltrating the normal tissue. Cytotoxic T cells and DCs are uniformly present in breast lobules and are in close association with the breast epithelium creating a defense system.[46] Development, involution, and lactation of the breast tissue are reported to be assisted by immune cells present in breast parenchyma.[47] Furthermore, the immune cells also contribute toward cancer immunosurveillance.[48] Despite this, immunity is breached, leading to development of tumors.

We are using the standard line of treatments for ER+, HER2+ and TNBC tumors as discussed in the previous section. However, over time, the patients become resistant to most of these treatments and fall trap to tumor reoccurrence or progressing into MBCs. It is noteworthy that breast tumors are referred to as immunologically cold tumors. According to the National Cancer Institute, cold tumors are defined as a tumor that is unlikely to trigger a strong immune response.[49] They tend to be surrounded by immune suppressor cells keeping T cells from attacking tumor cells.[49] Along the same lines, it has been reported that HER2+ BC patients show infiltration of immune suppressor cells such as regulatory T cells, M2-polarized macrophages, myeloid-derived suppressor cells in the tumors leading to evasion of T cell, DC, B cell function and inhibition of M1 macrophage polarization and NK cell cytotoxicity.[50,51] Not only this, the expected response to neoadjuvant chemotherapy trastuzumab with lapatinib was prevented by the immune-suppressive tumor microenvironment (TME), resulting in lower TILs in HER2+ BC patients.[52]

Hence, intervention into the breast tumor as an attempt to convert it from a cold tumor into a hot tumor might be helpful. To further emphasize this point, we would like to quote a few compelling studies highlighting the pivotal role of immune responses significantly contributing toward promising antitumor therapies in the BC field. Progressive loss of T_H1 immunity against HER2 oncodriver in correlation with poor prognosis was observed in HER2+ ductal carcinoma in situ (DCIS) and IBC patients.[53,54] Our group reported that improved survival in HER2+ BC patients was due to the restoration of the anti-HER2 T_H1 response.[54,55] We investigated further and found that T_H1 cytokine, interferon γ (IFN-γ), was the key player. Interferon γ led to augmented levels of cullin-5, an E3 ubiquitin ligase, which in turn caused ubiquitination and degradation of surface HER2 receptors leading to diminished

tumor growth and tumor senescence.[56] In addition, an interaction between trastuzumab and IFN-γ and tumor necrosis factor (TNF) has shown to be beneficial, resulting in efficient cytotoxic responses by HER2-specific $CD8^+$ T cells and increased MHC-I expression on tumor cells.[57] To examine the effects of IFN-γ, a clinical trial (NCT03112590) aimed at testing a combination of IFN-γ with paclitaxel, trastuzumab, and pertuzumab in $HER2^+$ BC was initiated.[58,59] Interestingly, stimulation of IFN-γ/STAT1 pathway is identified as a prognostic marker of chemotherapy resistance in patient-derived xenograft ER-BC model.[60] A progressive loss of T_H1 immunity against HER3 in IBC patients has been reported by our group, with most profound effects in TNBC patients compared with the healthy donors.[61] Taken together, it is evident that immune-factor intervention in breast tumors can be a game changer. Therefore, in the following sections, we discuss the clinical studies that have been recruited for adoptively transferring immune cells. These trials could have multitude antitumorigenic effects in BC patients. In this review, we discuss the following therapies as a part of the adoptive cell therapy (ACT) in BC: TIL therapy, engineered T cell receptor (TCR) therapy, chimeric antigen receptor (CAR) T cell therapy, DC therapy, and NK cell therapy. Clinical trials mentioned under each of these categories are adapted from clinicaltrials.gov and listed in Table 1.

TIL THERAPY

Metastatic BC proves to be a moving target for therapies as cancer cells continuously undergo different mechanisms of tumor escape from the innate and adaptive human immune system. HER2-positive BC and TNBC often exhibit brisk TILs, indicating a host antitumor immune response.[62] More than 3700 patients treated with neoadjuvant chemotherapy were analyzed for the presence of stromal TILs showing that increased TIL concentration was associated with an improved response to neoadjuvant chemotherapy across all subtypes of BC. Higher TIL concentration was also found to be associated with longer survival in triple-negative and $HER2^+$ BC.[63]

Adoptive cell therapy using expanded autologous TILs after lymphodepleting chemotherapy has had promising outcomes in patients with metastatic melanoma, albeit a tumor with high mutational burden.[64] Improvements in TIL therapy have been attempted to better outcomes in MBC and other less immunogenic epithelial cancers. Selecting TILs against tumor antigens identified by whole-exome sequencing and RNA sequencing, tumor recognition, and killing potential can be improved.[65] NCT01174121 is a phase II study looking at response rate and safety of TILs plus pembrolizumab in metastatic cancers. Pembrolizumab was given pre-TIL infusion to prevent blockade of activated cells. A case report of a patient with HR^+ MBC treated on this clinical trial who underwent TILs reactive against 4 mutated proteins had a regression of her MBC ongoing for greater than 22 months.[66] T cell somatic mutations have been found in 67% of patients with treatment-refractory MBC.[67]

Three additional clinical trials are listed on clinicaltrials.gov including NCT04111510, a phase I trial evaluating TILs in triple-negative MBC patients who have progressed on 3 prior systemic therapies. Tumor-infiltrating lymphocyte therapy does have limitations including need to obtain sufficient tissue, generating enough T cells reactive against tumor mutated antigens and, clinically, a few weeks' hospital stay for lymphodepletion. With melanoma TIL therapy paving the way, researchers now know that nonselected TILs have little efficacy in MBC, and a more tailored approach to TIL therapy has a promising future for MBC.

ENGINEERED TCR THERAPY

The cellular immune system in particular utilizing T cells' cytotoxic abilities against tumor cells is an appealing approach for longstanding cancer therapy. T cells isolated from cancer patients may have low-affinity TCRs because of the development of tolerance, limiting cytotoxic abilities. This limitation of the immune system has been overcome by using gene transfer to express transgenic TCR α and β chains of high affinity.[68] Engineered TCRs utilize polyclonal T cells with tumor antigens of choice not normally present, which recognize epitopes presented by MHC molecules allowing personalized treatment.[65] Engineered T cells can then be activated in the laboratory while the patient's immune system is being optimized for cell transfer. The greater the extent of lymphodepletion, the more effective the treatment, as host immunosuppression allows elimination of regulatory T cells and cytokines produced by host stromal cells.[69]

The first clinical trial for TCR therapy was in 2004, and trials have increased exponentially, with majority of trials studying melanoma and gastrointestinal cancers and only 4% including BC patients.[70] Currently, there are 7 clinical trials registered that include BC patients looking at TCR therapy. A possible risk of TCR immunotherapy is toxicity to normal tissues as some tumor antigens are expressed on normal host cells. T cell receptors directed against tumor antigen of epithelial cancers can have serious adverse effects such as uveitis, vitiligo, and even death of melanoma patients targeting MART-1 and gp100,[71] which is why routine testing of cross-reactivity of novel TCRs is done during preclinical trials. Li et al.[72] studied TCRs engineered against placenta-specific 1 (PLAC1), a novel antigen found in multiple tumor types including 82% of primary BCs with limited expression in normal tissue. They found that PLAC1 TCR-transduced $CD8^+$ T cells significantly suspended tumor progression in mice displaying BC, providing a promising target for future clinical trials.[72] NCT01147016 is a phase II clinical trial studying women with stage II–III HER2-negative BC who received neoadjuvant chemotherapy followed by HER2Bi-activated T cells, which is awaiting results. NCT03093350 is one of the only BC–specific clinical trials looking at TAA-specific cytotoxic T lymphocyte treatment efficacy and safety in 12 patients. Antigens that investigators looked at included NY-ESO-1, MAGEA4, PRAME, survivin, and SSX2. With TCR therapy being a fairly new and personalized treatment modality for BC, results of clinical trials, mostly phases I and II, are still pending.

CAR T CELL THERAPY

Chimeric antigen receptor T cells are a class of engineered T cells designed to express an artificial receptor consisting of 4 domains: an extracellular antibody-derived recognition motif that binds the target antigen such as single-chain variable fragment, a transmembrane domain that anchors the CAR to the T cell membrane, a extracellular hinge region that provides flexibility required by the antigen-specific domain to bind to the targeted epitope, and 1 or more intracellular signaling domains.[73–78] The first-generation CAR T cells contained CD3ζ or FcRγ signaling domain.[79] However, second- and third-generation CAR T cells contained CD3ζ signaling domain along with 1 or 2 extra costimulatory domains, respectively.[80–82] Recognition of glycolipid and carbohydrate antigens and MHC-independent binding are critical advantages offered by CAR T cell therapy.[76,83,84]

Chimeric antigen receptor T cell therapy has achieved immense success in the field of hematological cancers.[76,77,85,86] However, it has been quite a challenge to design efficient CAR T cells for solid cancers. Selection of a suitable tumor antigen is a major challenge as it should be highly expressed on tumor cells

TABLE 1. List of Clinical Trials Registered on ClinicalTrials.gov Under Different ACT Categories for BC

ACT	Identifier (Clinical Trials.gov)	Phase	Status	Target Molecule
TIL therapy	NCT01174121	II	Recruiting	
	NCT04111510	I	Recruiting	
	NCT00301730	I	Completed	
	NCT01462903	I	Unknown	
TCR therapy	NCT01147016	II	Unknown	HER2Bi-armed activated T cells
	NCT03093350	II	Active, not recruiting	TAA-specific cytotoxic T cells: NY-ESO-1, MAGEA4, PRAME, survivin, and SSX2
	NCT04102436	II	Recruiting	TCRs reactive against mutated neoantigens in patients with metastatic cancer
	NCT03970382	I	Active, not recruiting	NeoTCR-P1 ACT
	NCT03412877	II	Recruiting	TCRs reactive against neoantigens in patients with metastatic cancer
	NCT03159585	I	Completed, no results	NY-ESO-1–specific TCR
	NCT02457650	I	Unknown	TCR targeting NY-ESO-1
	NCT02111850	I/II	Completed, results	Anti–MAGE-A3-DP4 TCR peripheral blood lymphocytes (PBLs)
	NCT01967823	II	Completed, results	Anti–NY-ESO-1 mTCR PBL
CAR therapy	NCT02547961	I/II	Withdrawn	HER-2–targeting CAR T cell infusion
	NCT03696030	I	Recruiting	HER2-CAR
	NCT03740256	I	Recruiting	HER2-CAR
	NCT04430595	I/II	Recruiting	CAR T cells targeting HER2, GD2, and CD44v6 surface antigen in BC
	NCT04511871	I	Recruiting	T cell modified CAR (CCT303-406)
	NCT04025216	I	Recruiting	CART-TnMUC1 cells (glycosylated MUC1 form)
	NCT04020575	I	Recruiting	huMNC2-CAR44 CAR T cells (truncated version of MUC1)
	NCT01837602	I	Completed, no results	cMet RNA CAR T cells
	NCT02414269	I/II	Active, not recruiting	iCasp9M28z T cell infusions (CAR targeting mesothelin)
	NCT02792114	I	Active, not recruiting	MSLN-CAR
	NCT04348643	I/II	Recruiting	CEA-targeted CAR-T
	NCT04107142	I	Unknown	NKG2DL-targeting chimeric antigen
	NCT02915445	I	Recruiting	CAR T cells recognizing EpCAM
	NCT04427449	I/II	Recruiting	CD44v6-specific CAR gene-engineered T cells
	NCT02830724	I/II	Recruiting	Anti-hCD70 CAR-transduced PBL
	NCT02706392	I	Terminated	ROR1 CAR-specific Autologous T lymphocytes
	NCT02541370	I/II	Completed, no results	Anti-CD133-CAR vector–transduced T cells
DC therapy	NCT00082641	I/II	Completed, has results	P53
	NCT03630809	II	Recruiting	HER2
	NCT03387553	I	Active, not recruiting	HER2
	NCT03384914	II	Recruiting	HER2
	NCT02061423	I	Active, not recruiting	HER2
	NCT02063724	I	Active, not recruiting, has results	HER2
	NCT00879489	I/II	Unknown	Oncofetal antigen/iLRP
	NCT00499083	II	Completed, has results	
	NCT00197522	I	Completed, no results	HER2
	NCT00162929	I	Completed, no results	HER2
	NCT00107211	I	Completed, no results	HER2
	NCT04348747	II	Recruiting	HER2 and HER3
	NCT04105582	I	Active, not recruiting	Neoantigen
	NCT03450044	I/II	Completed, no results	
NK therapy	NCT04319757	I	Recruiting	ACE1702 (anti-HER2 oNK cells) is an off-the-shelf NK cell product that targets human HER2-expressing solid tumors

Continued next page

TABLE 1. *(Continued)*

ACT	Identifier (Clinical Trials.gov)	Phase	Status	Target Molecule
	NCT03841110	I	Recruiting	
	NCT03634501	I/II	Recruiting	
	NCT03319459	I	Completed, no results	
	NCT02839954	I/II	Unknown	Anti-MUC1 CAR-pNK cells
	NCT02030561	I/II	Unknown	
	NCT01105650	II	Completed, with results	

and be absent or be negligibly expressed on normal cells. Chimeric antigen receptor T cells are highly sensitive and therefore are activated by even low levels of antigen expression. Hence, they exhibit off-target effects and cellular toxicity.[86–88] Another challenge is the vast heterogenicity in terms of antigen expression within the tumors, which may lead to tumor escape.[77,88] Moreover, immunosuppressive tumor environment of solid tumors poses yet another challenge for efficient CAR T cell trafficking and infiltration.[89]

Despite these hurdles, various *in vitro* and *in vivo* CAR T cells, preclinical studies have been conducted targeting different tumor antigens and oncodrivers. Human epidermal growth factor receptor 2/receptor tyrosine-protein kinase erbB-2 (HER2/Erbb2) is the most prominent oncodriver investigated in BC. It belongs to the ErbB family comprising the transmembrane receptors. HER2 overexpression is associated with poor prognosis and is reported in 15% to 20% of tumors.[90–92] In 2015, a phase I/II clinical trial (NCT02547961) was initiated evaluating the safety and short- and long-term efficacy of HER2–CAR T cell infusion for the first time in HER2-positive recurrent and MBC patients. Another phase I clinical trial (NCT03696030) was aimed at studying the adverse effects and optimum dose of HER2–CAR T cells in treating patients wherein cancer had metastasized to the brain and was recurrent. $HER2^+$ BC patients were also a part of this study. Another human phase I clinical trial (NCT03740256) pertaining to $HER2^+$ cancer was initiated; this trial investigated the efficacy and safety of HER2-specific autologous CAR T cells in combination with intratumor injection of an oncolytic adenovirus, CAdVEC, which was hypothesized to boost the immune system and enhance the capacity of HER2–CAR T cells to kill tumor cells. In 2020, a phase I/II clinical trial (NCT04430595) was initiated to assess the efficacy, feasibility, and safety of CAR T cells targeting HER2, GD2 and CD44v6 surface antigens in BC. This study also looked at the activity and persistence of the multi-CAR T cells in the patients. Another phase I clinical trial (NCT04511871) was initiated in 2020 that assessed the antitumor activity, safety, and tolerability autologous T cells with modified CAR (CCT303-406) in patients with relapsed or refractory $HER2^+$ solid cancers.

A considerable number of clinical trials are testing CAR constructs against multiple oncodrivers and tumor antigens in TNBC. MUC1 is an oncodriver that is overexpressed in TNBC. In addition to this, it shows a modified glycosylation profile in a tumor setting, making it an ideal target for CAR therapy. To evaluate the preliminary efficacy, tolerability, feasibility, and safety of autologous CART-TnMUC1 cells, directed against the glycosylated MUC1 form, a tumor antigen, and activate T cells, a phase I first-in-human clinical trial (NCT04025216) was launched.[93] Recently, another phase I CAR T cell clinical trial (NCT04020575) targeting truncated version of MUC1 ECD, referred to as MUC1*, exclusively expressed on tumor cells was commenced. cMET is a tyrosine receptor kinase expressed in BC inclusive of TNBC.[94] A phase I clinical trial (NCT01837602) began investigating feasibility and safety of the intratumoral administration of autologous cMET-directed T cells (cMet RNA CAR T cells) in patients with TNBC and MBC. Significant proportion of TNBC expresses mesothelin (MSLN), whereas normal cells express mesothelium. MSLN promotes local invasion, metastases and proliferation leading to malignant transformation.[95,96] A phase I/II clinical trial (NCT02414269) is being conducted to figure out the safe dose of autologous CAR T cells targeting MSLN in malignant pleural disease patients; BC patients are also recruited as a part of this study. In addition, 1 more phase I clinical trial (NCT02792114) was held recruiting patients with MBC to evaluate the tolerability and safety of MSLN-CAR T cells. Moreover, other CAR T cell clinical trials targeting CEA, natural killer group 2D (NKG2D) ligands, epithelial cellular adhesion molecule (EpCAM), CD44 isoform, Cd44v6, CD70, receptor tyrosine kinase–like orphan receptor 1 (ROR1) and CD133 have also been initiated as listed in Table 1. Most of these clinical trials discussed in this section are not yet completed or are awaiting results. In conclusion, all these studies highlight the importance of comprehending the expression pattern of varied molecules expressed on tumors, exploiting the advantage to design CAR T cells to target these antigens.

DC THERAPY

Dendritic cells are the primary antigen-presenting cells and play a master regulatory role in inducing protective immunity against infectious pathogens and various cancers.[97] Dendritic cells can be utilized to trigger antitumor immunity via loading various tumor antigens or highly immunogenic tumor antigen peptides leading to presentation and recognition by $CD4^+$ and cytotoxic $CD8^+$ T cells, activation, and their infiltration into tumor sites.[98] Since, DC-based immunotherapy approach can activate tumor antigen–specific effector immune cells to eliminate tumor cells, this approach has been applied to treat various cancers. Various clinical trials are underway that utilize DC-based immunotherapy for the treatment of BC subtypes including $HER2^+$, ER^+ and TNBC subtypes.[36] Tumor suppressor gene TP53 is the most frequently mutated gene in approximately 30% of all BC patients.[99] Dendritic cell vaccination approach targeting p53 protein can be an effective therapeutic strategy to trigger immune response against p53 (p53 mutant) overexpressing BC.[100] A phase I/II clinical trial has been recently completed in testing the efficacy and adverse effects of adenovirus p53–infected DC vaccine in stage III BC patients receiving neoadjuvant or adjuvant chemotherapy and adjuvant radiation therapy (NCT00082641). A combination treatment approach of adenovirus p53–transduced DC vaccine with 1-methyl-D-tryptophan was investigated in MBC patients and observed enhanced antitumor response (NCT01042535).

The oncodriver HER2 overexpression accounts for approximately 20% to 25% of BC patients, and this tumor antigen is of particular interest and effectively being targeted to develop DC

immunotherapy.[43] A phase II clinical trial is currently investigating the antitumor immune response and potential booster HER2-DC1 vaccine treatment response in HER2$^+$ BC patients, in those who previously received HER2-DC1 vaccine (NCT03630809). In addition, early-phase I trial to test HER2-DC1 vaccine during neoadjuvant therapy in HER2$^+$ IBC patients is ongoing (NCT03387553). A multicenter phase II study also currently evaluates the safety of combination vaccine therapy WOKVAC with HER2-DC1 and their effect on disease-free survival in HER2$^+$ BC patients (NCT03384914). Next, HER2-DC1 vaccine is being applied in phase I clinical trial to test the safety and antitumor immune activity in post–neoadjuvant chemotherapy setting for high-risk HER2$^+$ BC patients (NCT02061423). Dendritic cell–based immunotherapy in combination with chemotherapy may enhance the antitumor response and TIL infiltration into tumors and increase complete response in BC patients.[101] The HER2-DC1 vaccine combined with chemotherapy with or without trastuzumab is being tested in a phase I study in high-risk HER2$^+$ IBC patients (NCT02063724).

Dendritic cell immunotherapy targeting other potential tumor antigens is also under investigation for BC patients with locally advanced or metastatic disease. A phase I/II clinical trial currently investigated the efficacy of autologous DC-loaded oncofetal antigen/iLRP in MBC patients. Previously, a phase II study examined the efficacy of chemotherapy followed by combination of autologous DC intratumoral delivery with or without radiotherapy in patients with HER2$^-$ BC and reported enhanced treatment benefits (NCT00499083). CD34$^+$-derived DCs transduced with an adenovirus-expressing HER2/neu vaccine approach is being tested in patients with metastatic BC and locally recurrent BC (NCT00197522, NCT00162929). Next, a phase II clinical trial is studying the effect of DC vaccine targeting 2 oncodrivers HER2 and HER3 in combination with anti-PD1 immune checkpoint inhibitor pembrolizumab in TNBC or HER2$^+$ BC patients with asymptomatic brain metastasis (NCT04348747). A phase I/II study showed better treatment efficacy of cyclin B1/WT-1/CEF tumor antigen–loaded DC vaccine in combination with preoperative chemotherapy for ER$^+$/HER2$^-$ BC patients (NCT02018458). Previously, DC vaccine transfected with various tumor antigens such as survivin, hTERT, and p53 mRNA has been applied in a phase I study to treat patients with metastatic BC or malignant melanoma (NCT00978913). Another phase I trial also utilized autologous DC pulsed with CEA RNA vaccine to examine the safety and efficacy in various metastatic cancers expressing CEA including MBC (NCT00004604).

NK CELL THERAPY

Natural killer cell therapy is different from the aforementioned treatments as it utilizes the innate immune system to destroy cancer cells. Natural killer cells are terminally differentiated cells that can spontaneously kill virally infected, stressed, and cancerous cells in an antigen-independent manner. In addition to their cytotoxic ability, NK cells also secrete a large amount of proinflammatory cytokines preventing tumor angiogenesis.[102] Unlike the above T cell therapies that can be evaded by loss of MHC molecules, NK cells activity is stimulated and independent of the same. Natural killer cells can be manufactured in large quantities from primary NK cells, stem cells, and clonal cell lines, of which the Food and Drug Administration approved NK-92 for use in clinical trials.[65]

Recent advancement in NK cell therapy has focused on cytokine supplement, monoclonal antibody, modification of internal

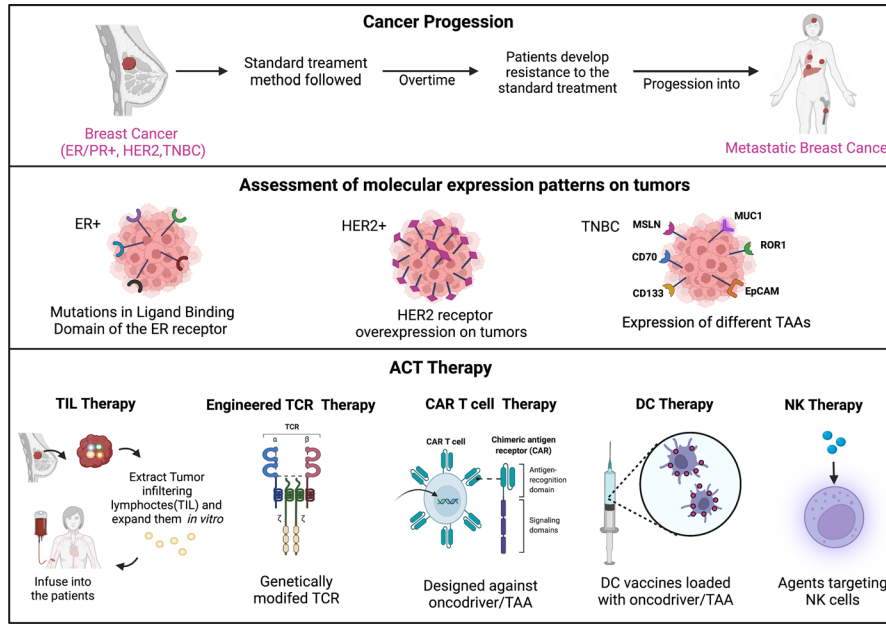

FIGURE 1. Overview of BC progression, molecular characterization of subtypes and ACTs used for treatment. This figure highlights 3 different aspects of BC. (1) Cancer progression. It is the process wherein the patients receiving standard treatment depending on a specific cancer subtype (ER/PR$^+$, HER2, TNBC) develop resistance to the treatment regimen, progressing into MBC. Metastatic BC is likely to spread to the bone, lungs, brain, or liver as depicted here. (2) Assessment of molecular expression patterns on tumors. This is a crucial aspect that forms the rationale for devising the treatment strategy. As shown here, ER$^+$ tumors can develop somatic mutations in the ligand-binding domain of the ER receptor, leaving them to signal constitutively and develop aggressive cancers. HER2 tumors show an abnormally increased expression of HER2 receptors as compared with the normal BC tissue. Triple-negative BC expresses different TAAs as shown here, therefore, attracting varied therapies specifically targeting cancer cells. (3) Adoptive cell therapy. This aspect depicts the various ACTs discussed in the review used in treating BC.

signal pathway, adoptive transfer, and genetic engineering of NK cells.[103] The addition of cytokines such as interleukins 2 and 15 has been shown to intensify NK cells cultured from peripheral blood without affecting their cytotoxicity.[104] As discussed previously, HER2-positive BC can be treated with monoclonal antibody–targeted therapy called herceptin with the severe adverse effect of cardiotoxicity. Tian et al.[105] stimulated NK cells with cytokines and engaged them with herceptin to treat HER2-positive BC cells. A single patient was treated and after 4 cycles saw a 33% size decrease in a lung metastasis with no cardiac adverse effects. Similar to CAR T cell therapy, CAR NK cell therapy has been studied to minimize cytokine release and tumor-lysis syndrome. In TNBC, tissue factor has been discovered as a selective molecule for CAR NK cell therapy in mouse models with need for further investigation.[106]

CONCLUDING REMARKS

Metastatic BC is a devastating cancer state wherein cancer spreads or metastasizes to other parts of the body, predominantly in the bone, lungs, brain, or liver. Treatment for MBC is based on the BC subtype (ER/PR$^+$, HER2$^+$, or TNBC) aiming to only prolong patient's life expectancy. It is noteworthy that the most likely cause of progression of cancer into the metastatic stage is the resistance that patients develop over time, towards the treatment they are initially subjected to.

Despite this daunting scenario, immunotherapy, which focuses on enhancing the vigor of immune cells, has transformed the field of cancer immunology. ACT is an important branch of immunotherapy which that stirred hopes for treating BC. Adoptive cell therapy is inclusive of TIL, engineered TCR, CAR, DC, and NK therapies, as discussed in this review. Various clinical trials discussed here (Table 1) are pointing toward remarkable advances in ACT, surpassing immune escape mechanism and antigenic heterogenicity prevalent in advanced BC. However, several questions are still needed to be catered as this ACT approach is still in its infancy. Intriguingly, there is yet another challenge for TIL isolation in BC. In this solid tumor, tissue is not always accessible to harvest TIL; hence, alternative sources such as blood or lymph nodes could be used for adoptive transfer of T cells.[107]

We now propose a few future approaches to enhance ACT. To begin with, a rigorous screen for neoantigenic discovery in different MBC subtypes must be practiced as neoantigen targets are substantially more immunogenic in nature as well as exhibit lesser off-target effects. Another critical question to be answered is whether it is possible to design broad-spectrum ACT by virtue of which we can target shared neoantigens in different BC subtypes. Another future approach could be targeting tumors using a combination of immune cells for ACT. For instance, a therapy can be designed such that DC vaccines can be combined with the delivery of CD4$^+$ T cells to target a particular TAA. This could be similar to providing an immune inoculum so that antigen-specific DC and CD4$^+$ T cells can initially keep tumors in check, and later on, DCs can also stimulate CD4$^+$ T cells that could perhaps potentiate antitumor effects by further recruiting CD8$^+$ T cells and stimulating humoral responses via B cells. Eventually, maybe this immune loop could continue to bring about tumor regression. Hence, it is integral to comprehend the functioning and properties of immune cells in monotherapeutic clinical trials to exploit them at the optimum in combinatorial therapies. Interestingly, a multitude of studies have reflected that the metabolic state of an immune cell dictates its ability to function in each condition. For instance, a T cell has different metabolic needs dictating its function during its development in thymus and in the naive versus activated states in the periphery.[108] Studies are also focusing on relating the mitochondrial morphology to functioning of various T cell states such as naive, activated, or memory.[109,110] To this end, another immunotherapeutic strategy, combining any metabolic agent targeting to enhance the functioning of a specific immune cell (without affecting the tumor) along with ACT of that same immune subset, could serve as a major boost to regulate antitumor responses. We have summarized this review covering all the essential aspects in Figure 1. Taken together, it is certain that ACT can be certainly explored and tweaked in multiple ways to devise strategies to cure BC.

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