

Emerging CAR-T Cell Therapy for the Treatment of Triple-Negative Breast Cancer

Sundee Dees, Rajkumar Ganesan, Sanjaya Singh, and Iqbal S. Grewal



ABSTRACT

Triple-negative breast cancer (TNBC), a highly aggressive breast cancer subtype that lacks estrogen receptor, progesterone receptor, and HER2 expression, does not respond to traditional endocrine and anti-HER2-targeted therapies. Current treatment options for patients with TNBC include a combination of surgery, radiotherapy, and/or systemic chemotherapy. FDA-approved therapies that target DNA damage repair mechanisms in TNBC, such as PARP inhibitors, only provide marginal clinical benefit. The immunogenic nature of TNBC has prompted researchers to harness the body's natural immune system to treat this aggressive breast cancer. Clinical precedent has been recently established with the FDA approval of two TNBC immunotherapies, including an antibody-drug conjugate and

an anti-programmed death-ligand 1 monoclonal antibody. Chimeric antigen receptor (CAR)-T cell therapy, a type of adoptive cell therapy that combines the antigen specificity of an antibody with the effector functions of a T cell, has emerged as a promising immunotherapeutic strategy to improve the survival rates of patients with TNBC. Unlike the remarkable clinical success of CAR-T cell therapies in hematologic cancers with Kymriah and Yescarta, the development of CAR-T cell therapies for solid tumors has been much slower and is associated with unique challenges, including a hostile tumor microenvironment. The aim of the present review is to discuss novel approaches and inherent challenges pertaining to CAR-T cell therapy for the treatment of TNBC.

Introduction

In 2020, breast cancer is projected to be the most diagnosed cancer among American women and the second leading cause of death after lung cancer (1, 2). The pathogenesis of breast cancer begins with the active proliferation of breast epithelial cells, leading to the development of malignant cancer in either the ductal or lobular compartment of the breast (2–4). *In situ* cancers constitute 20% to 25% of breast cancers and remain confined within the site of origin, whereas invasive cancers make up the remaining 75% to 80% of all breast cancer diagnoses and traverse the myoepithelial cell layer and basement membrane to invade locally into the surrounding breast stroma (5–7). Breast cancers are further classified according to histologic grade and stage to evaluate the aggressive potential and advancement of a breast tumor, respectively (4, 8–11). In addition, breast cancers are categorized into four molecular subtypes based on IHC expression of classic hormone and growth factor receptors including the estrogen receptor (ER), progesterone receptor (PR), and HER2, as well as their proliferative index in terms of Ki-67 expression (2, 12–14). Patients diagnosed with Luminal A (ER⁺ and/or PR⁺, HER2⁻, Ki-67 < 14%), Luminal B (ER⁺ and/or PR⁺, HER2⁺ or HER2⁻, Ki-67 > 14%), or HER2 amplified (ER⁻, PR⁻, HER2⁺) breast cancer subtypes (13) are candidates for receiving HER2-targeting monoclonal antibodies, antibody-drug conjugates, or tyrosine kinase inhibitors as well as endocrine therapies including selective estrogen receptor modulators, aromatase inhibitors, or ER degraders (15–19). While these three

subtypes are associated with favorable clinical outcomes due to their responsiveness to targeted therapies, poor prognosis is readily observed within a major subdivision of the fourth breast cancer subtype referred to as triple-negative breast cancer (TNBC) that tests negative for ER, PR, and HER2 overexpression due to lack of targeted treatment options for this patient population (16, 20).

TNBCs are a heterogeneous group of basal-like tumors that account for 15% to 20% of all breast cancer diagnoses (21, 22). TNBC is most often diagnosed in premenopausal African American women (23, 24). A high mutational burden is a characteristic feature of TNBC. In fact, there is an elevated percentage of mutations in TNBCs such as tumor protein 53 (TP53; 74.51%–82.80%), breast cancer type I susceptibility gene (BRCA-1; 1.96%–21.55%), and phosphatidylinositol 3-kinase catalytic alpha polypeptide (PIK3CA; 8.60%–23.28%) that contribute to its aggressive phenotype (22, 25–27). Patients diagnosed with TNBC experience heightened metastatic potential within the first 3 years of initial diagnosis (28, 29). These metastases are primarily identified in the lung, brain, liver, and bones, and fewer than 30% of patients diagnosed with metastatic TNBC will survive beyond 5 years (27, 28). As such, the aggressive clinical course and poor survival rates in TNBC warrant a focus on developing effective treatment options for this patient population. Because TNBCs are unresponsive to endocrine and anti-HER2 therapies (21), surgical excision in the form of a mastectomy or breast-conserving surgery, radiotherapy, and chemotherapy remain the current treatment modalities for patients with TNBC (30–32). However, chemotherapeutic drugs including taxanes, anthracyclines, and alkylating agents are nonspecific in nature and can result in systemic toxicities (31). TNBCs often carry mutations in BRCA1 (1.96%–21.55%) and/or BRCA2 (1.63%–18.10%), and PARP inhibitors have been approved to treat these advanced-stage TNBCs, which show some clinical efficacy (2, 25, 32). In addition, a few other molecular targets for TNBC including proteins in the Janus kinase 2/Signal transducer and activator of transcription 3 (Jak2/Stat3) pathway have been discovered, and several new therapies including PI3K inhibitors and angiogenesis inhibitors are being investigated in the clinic in combination with chemotherapeutic agents (2). Although

Janssen Biotherapeutics, Janssen Pharmaceutical Companies of Johnson & Johnson, Spring House, Pennsylvania.

Corresponding Author: Iqbal S. Grewal, Janssen Biotherapeutics, Janssen Pharmaceutical Companies of Johnson & Johnson, 1400 McKean Road, Spring House, PA 19477. Phone: 425-753-8263; E-mail: igrewal@its.jnj.com

Mol Cancer Ther 2020;19:2409–21

doi: 10.1158/1535-7163.MCT-20-0385

©2020 American Association for Cancer Research.

the above-mentioned targeted therapies show some promise in the clinic, additional treatment options tailored toward TNBCs are greatly needed.

Tumor-host immune interactions are considered prominent drivers of cancer progression. Recent evidence of the interplay between the immune system and the disease course of TNBC has led to the identification of TNBC as an immunogenic malignancy (33–37). High tumor mutational burden, mismatch repair deficiency, microsatellite instability, the presence of cytotoxic CD8⁺ and regulatory FOXP3⁺ tumor-infiltrating lymphocytes, and the expression of immune checkpoint molecules such as programmed death-ligand 1 (PD-L1) are prime examples that contribute to the immunogenic nature of TNBC. In fact, some of these immunogenic attributes are predictive of patient response to immunotherapy and positively correlate with increased survival and good prognosis (33, 38–42). Immune checkpoint blockade drugs such as anti-programmed death-1 (PD-1)/PD-L1 antibodies and bispecific antibodies that redirect CD3⁺ T cells to tumors are under investigation in the clinic (33, 43). The discovery of six molecular subtypes of TNBC, one of which being the immunomodulatory subtype, has further accelerated the development of immunotherapy strategies for this disease indication (44). The recent FDA approval of atezolizumab in combination with the chemotherapeutic agent nab-paclitaxel for the treatment of PD-L1-positive unresectable, locally advanced, or metastatic TNBC has fueled an immunotherapy era in TNBC (45). Market approval was based on the Impassion130 clinical trial, which demonstrated that median overall survival (OS) for atezolizumab/nab-paclitaxel-treated patients with PD-L1-positive TNBC was extended nearly 10 months in comparison with patients that were treated with placebo/nab-paclitaxel (NCT02425891). Since this landmark approval, multiple active areas of immunotherapy research for TNBC have emerged which include additional immune checkpoint inhibitors, cytokines, oncolytic viruses, bispecific antibodies, cancer vaccines, and adoptive cell therapy (34, 36). The aim of the present review is to describe how a type of adoptive cell therapy called chimeric antigen receptor (CAR)-T cell therapy is being exploited to eradicate TNBC (34). A comprehensive list of promising targets and ongoing clinical trials for CAR-T cell therapy in TNBC will be presented in the current review as well as inherent challenges associated with CAR-T cell therapy in solid tumors such as TNBC and key strategies to overcome these limitations.

Promise of CAR-T Cell Therapy in TNBC

CAR-T cells: structure, function, and production

A peripheral blood T cell modified to express a CAR is referred to as a CAR-T cell (46–48). A typical ectodomain of a CAR usually comprises of a tumor antigen specific antibody-derived recognition motif such as a single-chain variable fragment (scFv). The scFv, which drives specificity of the CAR, contains a variable heavy (V_H) chain of an antibody covalently linked to a variable light (V_L) chain of an antibody. A flexible spacer serves as a hinge that connects the scFv to a transmembrane domain. The endodomain of a CAR comprises of a CD3ζ chain of the T-cell receptor that facilitates intracellular signaling (49–51). Although first-generation CARs rely on CD3ζ signaling mechanisms alone, second- and third-generation CARs incorporate one or two intracellular costimulatory domains, respectively, to augment T-cell effector function (Fig. 1A). Inclusion of CD27, CD28, 4-1BB, OX40, and/or ICOS costimulatory domains into CAR constructs has been shown to increase T-cell expansion and persistence (50, 52–53). Introduction of CAR constructs into autologous or allogeneic T cells can be achieved using viral vectors

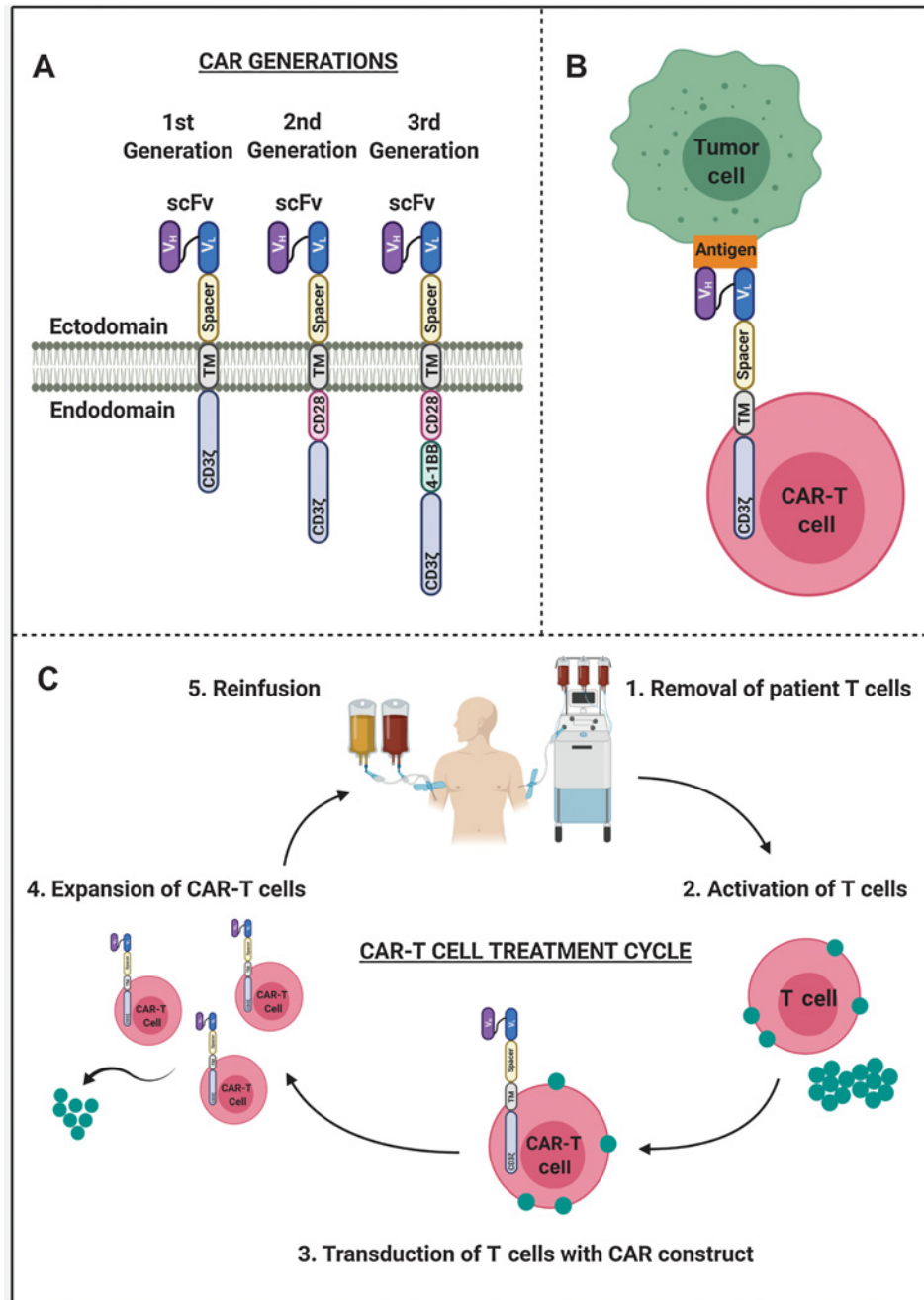
(lentivirus, retrovirus, adenovirus), nonviral vectors (synthetic DNA, mRNA transposons, CRISPR-Cas9), or plasmids (48, 54). CARs function by redirecting T cells toward specific antigens on the surface of tumor cells and providing critical signals to drive T-cell activation and cytolytic activity against tumor cells (Fig. 1B; ref. 55). Recognition of cognate tumor antigen by CARs is independent of MHC, which is particularly advantageous in the context of cancer, where tumor cells frequently downregulate MHC expression as a mechanism of resistance to immunotherapies (47, 49). The production of autologous CAR-T cells for clinical application is a well-established process that adheres to good manufacturing procedure guidelines. In brief, autologous CAR-T cell production involves the removal of patient T cells, activation and introduction of CAR constructs into patient T cells, expansion of CAR-modified patient T cells, and administration of lymphodepletion chemotherapy followed by infusion of CAR-T cells into the patient (Fig. 1C; refs. 48, 50, 56).

CAR-T cell therapy successes and challenges

The most prominent successes of CAR-T cell therapy have been documented in hematologic cancers with the FDA approvals of Kymriah and Yescarta, two second-generation CAR-T cell products targeting the B-cell antigen CD19 (NCT02435849, NCT02445248, and NCT02348216; refs. 46, 48–50). Since these ground-breaking approvals, additional antigenic targets in hematologic malignancies have demonstrated promise in ongoing CAR-T cell therapy clinical trials including B-cell maturation antigen, CD20, CD22, CD30, CD33, CD38, CD123, and CD138 (56–58). However, the remarkable clinical benefit of CAR-T cell therapy derived in hematologic cancers has not been matched in solid tumors (46, 48, 52). The first major obstacle associated with CAR-T cell therapy in solid cancers relates to identifying appropriate tumor target antigens that are absent or expressed at very low levels on normal tissues, particularly in vital organs. This issue has been amplified by the fact that a given CAR-T cell only needs to recognize a few receptors on the target cell to achieve full activation. In order to avoid off-target effects and associated toxicity, an ideal target antigen should be selected (i.e., overexpressed on cancer cells, with minimal to no detectable expression observed on healthy tissue; refs. 48, 59, 60). In the case of hematologic cancers, tumor antigen expression on normal tissues can be tolerated in the context of B-cell-specific antigens. CD19, the antigenic target for both Kymriah and Yescarta, is a prototypical example of an optimal target antigen for CAR-T cells due to its overexpression in greater than 95% of B-cell malignancies (61). Although CD19 is expressed on nonmalignant (normal) B cells as well, immunoglobulin replacement therapy can be administered to mitigate patient complications arising from B-cell aplasia (49, 61). Another issue with CAR-T cell therapy in solid cancers is intratumor heterogeneity in terms of antigen expression, which could potentially lead to tumor escape (50, 60). An additional challenge relates to the immunosuppressive tumor microenvironment (TME; ref. 51). The TME consists of multiple interacting components including cancer cells, immune cells, stromal cells, chemokines, cytokines, and extracellular matrix. In solid cancers, the TME is highly immunosuppressive in nature due to the recruitment of tumor-associated macrophages (TAM), cancer-associated fibroblasts (CAF), myeloid-derived suppressor cells (MDSC), and regulatory T cells (Treg), as well as the production of immunosuppressive cytokines and soluble factors (e.g., IL-10, VEGF, TGFβ, indoleamine 2,3-dioxygenase, and adenosine). Furthermore, expression of immune checkpoint molecules on T lymphocytes also suppresses antitumor immune responses. Remodeling the solid TME from an immunosuppressive state to a proinflammatory state remains an active area of research that can

Figure 1.

A, Schematic displaying the structural backbone and generations of CARs. A prototypical CAR consists of an extracellular antigen binding scFv (V_H and V_L regions of antibody linked by a glycine-serine peptide sequence), a flexible spacer or hinge region, a transmembrane domain, and an intracellular CD3ζ activation domain. First-generation CARs contain only a CD3ζ activation domain. Second-generation CARs contain one costimulatory domain (e.g., CD28), and third-generation CARs contain two costimulatory domains (e.g., CD28 and 4-1BB). **B**, Diagram depicting a first-generation CAR-T cell engaging a cell surface antigen on a tumor cell. **C**, Schematic outlining a CAR-T cell treatment cycle. 1. Removal of T cells from patient with cancer. 2. Activation of T cells with anti-CD3 anti-CD28 activation beads. 3. Transduction of activated T cells with CAR construct. 4. *Ex vivo* expansion of CAR-T cells and removal of activation beads. 5. Administration of lymphodepletion chemotherapy followed by infusion of CAR-T cell product into patient.



lead to enhanced antitumor immunity (54, 55, 62). Additional obstacles associated with CAR-T cell therapy in solid cancers include insufficient trafficking and infiltration of the tumor as well as lack of persistence (50, 63). Taken together, the major challenges with CAR-T cell therapy for the treatment of solid malignancies include lack of target antigen specificity, intrinsic target antigen heterogeneity, an immunosuppressive TME, expression of immune checkpoint molecules, inefficient intratumoral trafficking/infiltration, and poor persistence (Table 1). Despite these obstacles, a number of potential targets for CAR-T cell therapy in TNBC have recently emerged in preclinical studies that have begun to address some of the inherent challenges in solid tumors.

Promising targets for CAR-T cell therapy in TNBC

The quantity and quality of TNBC immunotherapy research being conducted in the CAR-T cell space has exponentially increased over the last 3 years (34). The antitumor activity associated with targeting over a dozen TNBC antigens with CAR-T cells has been demonstrated in preclinical *in vitro* and *in vivo* proof of concept (POC) studies. Tumor antigens are classified as tumor-specific, tumor-associated, or cancer germline antigens. Tumor-specific antigens are exclusively expressed on malignant tissue, whereas tumor-associated antigens are either lineage-restricted in terms of their expression or are expressed on malignant tumors to a greater extent compared with healthy tissue. Cancer germline

Downloaded from <http://aacrjournals.org/mct/article-pdf/19/12/2409/1862874/2409.pdf> by guest on 05 October 2024

Table 1. Key approaches to mitigate challenges associated with CAR-T cell therapy in solid tumors.

Challenges associated with CAR-T cell therapy in solid tumors	Key approaches to overcome the challenges with CAR-T cell therapy in solid tumors	Examples of mitigation strategies in TNBC
Target antigen heterogeneity/lack of target antigen specificity	<ul style="list-style-type: none"> • AND-gate circuits: dual CARs, synthetic notch CARs • NOT-gate circuits: inhibitory CARs • OFF switches: suicide gene safety switches • ON switches: split CARs • Bifunctional switches: bispecific CARs 	<ul style="list-style-type: none"> • Integrin $\alpha_v\beta_3$-CAR-T cells engineered with an EGFRt safety switch (94) • Bispecific CAR-T cells targeting FRα and mesothelin (85)
Immunosuppressive tumor microenvironment	<ul style="list-style-type: none"> • Targeting of antigens on multiple cell types • Dominant negative receptors • Inverted cytokine receptors • TRUCKs 	<ul style="list-style-type: none"> • Target antigen expression on primary TNBC tumor cells and stromal cells (e.g., MDSCs, CAFs, TAMs, Tregs, angiogenic endothelial cells)
Expression of immune checkpoint receptors	<ul style="list-style-type: none"> • Immune checkpoint blocking antibodies • Antibody-secreting CAR-T cells 	<ul style="list-style-type: none"> • PD-1 knockout mesothelin-CAR-T cells (97)
Inefficient intratumoral trafficking and infiltration	<ul style="list-style-type: none"> • Coexpression of chemokine receptors 	<ul style="list-style-type: none"> • Lack of TNBC preclinical studies that address this challenge
Poor persistence	<ul style="list-style-type: none"> • Coexpression of cytokine receptors 	<ul style="list-style-type: none"> • AXL-CAR-T cells with constitutively active IL7 receptor (70)

antigen expression is restricted to adult somatic tissue such as the ovary or testis, thus limiting on-target/off-tumor toxicity (55, 60, 62). Although many CAR-T cell antigenic targets are proteins overexpressed on the surface of tumor cells, target antigens for CAR-T cells can also include posttranslational modifications, such as aberrant glycosylation patterns, or alterations in cell surface proteins (62, 64). A comprehensive summary of promising CAR-T cell targets in TNBC is displayed in **Table 2**. Each of these targets will be discussed in further detail.

Receptor tyrosine kinase AXL

AXL is a receptor tyrosine kinase that mediates the activation of downstream signaling pathways involved in tumor progression including the PI3K, MAPK, and NF- κ B signaling cascades (65). Overexpression of AXL on tumor cells correlates with poor prognosis in several cancers. Moreover, AXL has emerged as a therapeutic drug target for TNBC treatment (65, 66). In fact, AXL-CAR-T cell therapy in TNBC led to significant *in vitro* cytotoxicity and cytokine secretion as well as a reduction in tumor growth in an MDA-MB-231 xenograft mouse

Table 2. Antigenic targets being exploited for CAR-T cell therapy in TNBC.

CAR-T cell target	Class of CAR-T cell target	Key preclinical results of CAR-T cell therapy in TNBC
AXL	Receptor tyrosine kinase	AXL-CAR-T cells show <i>in vitro</i> cytotoxicity in TNBC cells and reduce tumor cell growth in a TNBC xenograft mouse model (67). AXL-CAR-T cells engineered with a constitutive IL7R show enhanced <i>in vitro</i> anti-TNBC activity and increased survival in a TNBC xenograft model (70).
CD32A	Fc gamma receptor II (Fc γ RII) Cell surface glycoprotein	CD32A-CAR-T cells in combination with cetuximab or panitumumab lead to <i>in vitro</i> killing of EGFR-expressing TNBC cells (72).
CSPG4	Cell surface proteoglycan	CSPG4-CAR-T cells elicit cytolytic activity against TNBC cells <i>in vitro</i> (77).
EGFR	Receptor tyrosine kinase	EGFR-CAR-T cells trigger TNBC cell lysis <i>in vitro</i> and inhibit growth of TNBC tumors <i>in vivo</i> (74).
FR α	GPI-linked membrane protein	FR α -CAR-T cells induce <i>in vitro</i> killing of TNBC cells and promote tumor regression in a TNBC xenograft mouse model (84).
GD2	Disialoganglioside	GD2-CAR-T cells drive cytotoxicity of TNBC cells <i>in vitro</i> and prevent lung metastasis in a xenograft mouse model of TNBC (88).
ICAM-1	Cell surface glycoprotein	ICAM-1-CAR-T cells mediate <i>in vitro</i> killing of TNBC cells (92).
Integrin $\alpha_v\beta_3$	Transmembrane cell adhesion receptor	Integrin $\alpha_v\beta_3$ -CAR-T cells engineered with an EGFRt safety switch inhibit TNBC cell growth <i>in vitro</i> (94).
Mesothelin	Cell surface glycoprotein	PD-1 knockout mesothelin-CAR-T cells demonstrate superior <i>in vitro</i> and <i>in vivo</i> anti-tumor activity (97).
c-Met	Receptor tyrosine kinase	mRNA c-Met-CAR-T cells mediate lysis of TNBC cells <i>in vitro</i> and significant tumor shrinkage in a TNBC xenograft mouse model (101).
MUC1	Cell surface glycoprotein	tMUC1-CAR-T cells promote cytotoxicity in TNBC cells <i>in vitro</i> and reduce TNBC tumor growth <i>in vivo</i> (103). TnMUC1-CAR-T cells are also under preclinical investigation for TNBC (104).
NKG2D ligand	Stress ligand	NKG2D-CAR-T cells demonstrate prolonged <i>in vivo</i> persistence and tumor regression in a TNBC mouse model (106).
ROR1	Receptor tyrosine kinase	ROR1-CAR T cells infiltrate <i>in vitro</i> 3D cultures of TNBC cells and elicit antitumor activity (107).
SSEA-4	Glycosphingolipid	SSEA-4-CAR-T cells induce cytotoxicity to TNBC cells <i>in vitro</i> (109).
TEM8	Cell surface glycoprotein	TEM8-CAR-T cells demonstrate antitumor activity in a TNBC xenograft mouse model (111). TEM8-CAR-T cells mediate on-tumor off-target toxicity in an <i>in vivo</i> model of TNBC (112).
TROP2	Cell surface glycoprotein	TROP2-CAR-T cells demonstrate cytotoxicity in TNBC cells <i>in vitro</i> (116).

model (67). Furthermore, there is evidence that AXL-CAR-T cell therapy has the potential to overcome the immunosuppressive TME by inhibiting the release of suppressive chemokines and cytokines from TAMs (67, 68). In addition, because AXL expression is found on MDSCs, it is possible that CAR-T cells targeting AXL may deplete MDSCs from the TME and fundamentally alter the TME to a proinflammatory state (69). More recently, it was demonstrated that AXL-CAR-T cells with constitutive expression of the IL-7 receptor had enhanced *in vitro* antitumor activity and prolonged survival in a TNBC subcutaneous xenograft model (70). Collectively, these data underscore the importance of AXL as a novel CAR-T cell therapy target in TNBC and its potential to polarize the TME to a proinflammatory state conducive for effective antitumor immune responses.

Fc-gamma receptors

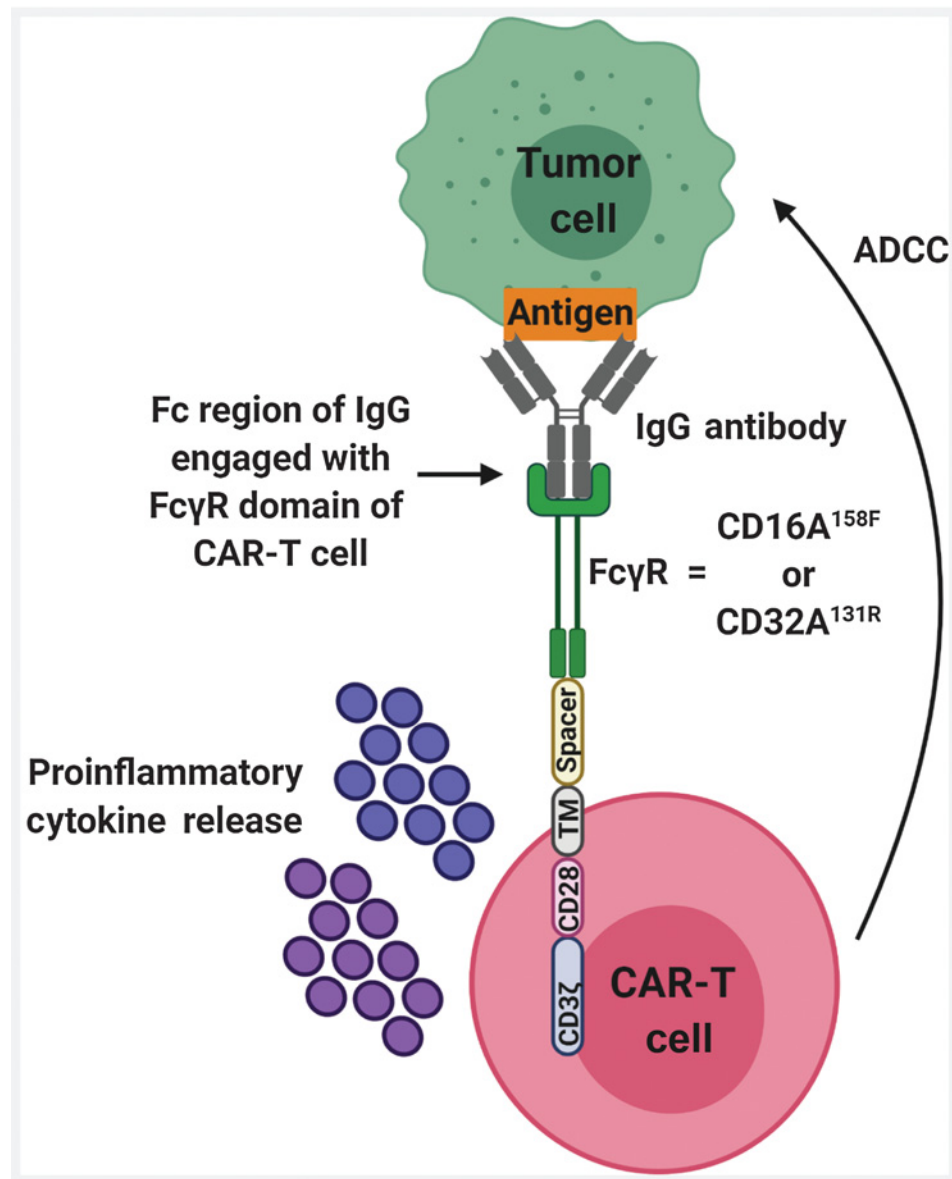
The standard form of CAR-T cell redirection is restricted to a single tumor-associated antigen expressed on the tumor cell surface.

A universal CAR-T cell that expresses a Fc-gamma receptor (FcγR)-CAR can be used to facilitate multiple therapeutic antibodies to redirect T cells to virtually any antigen expressing tumor cell (71). Since therapeutic antibodies that target antigens on solid tumors are available, use of FcγR-CAR-T cells in combination with these antibodies is a viable option to eliminate solid tumors. Understanding the antibody-dependent cellular cytotoxicity (ADCC) mechanism and linking it to antitumor activity of tumor-specific antibodies has stimulated interest in engineering T cells with FcγR-CARs. In these CAR-T cells, the CAR is composed of a FcγR extracellular domain that is fused with signaling and costimulatory domains. FcγR-CAR-T cells bind the Fc portion of the antibody resulting in activation of the CAR-T cell and efficient targeting of tumor cells (Fig. 2; refs. 71, 72).

In humans, the main FcγRs of potential interest include CD16A and CD32A, which have been considered for generating CAR-T cells. CD16A is predominantly expressed on natural killer (NK) cells, whereas CD32A is more widely expressed on monocytes,

Figure 2.

Diagram depicting an autologous T cell endowed with a second-generation CAR engineered with a FcγR extracellular domain (CD16A^{158F} or CD32A^{131R}). Engagement of the FcγR domain on a CAR-T cell with the Fc region of an IgG antibody specific for a tumor antigen results in tumor cell killing through ADCC mechanisms as well as proinflammatory cytokine release.



Downloaded from <http://aacrjournals.org/mct/article-pdf/19/12/2409/1862874/2409.pdf> by guest on 05 October 2024

macrophages, dendritic cells, and NK cells. These receptors function in transmitting activating signals through immunoreceptor tyrosine-based activation motif-containing adaptor molecules (71, 73). One interesting feature of CD16A and CD32A is that they are expressed in polymorphic forms and thus manifest different binding affinities for IgG (Fc binding region). For example, low affinity receptors are characterized by a phenylalanine residue located at position 158 of CD16A (CD16A^{158F}) and an arginine residue located at position 131 of CD32A (CD32A^{131R}), whereas high affinity receptors are characterized by a valine residue located at position 158 of CD16A (CD16A^{158V}) and a histidine residue located at position 131 of CD32A (CD32A^{131H}; ref. 72). Engagement of CD16A or CD32A with the Fc region of therapeutic antibodies results in ADCC and target cell depletion. This ADCC activity is dependent on the subclass of the antibody because different subclasses have different affinities for CD16A and CD32A. Two therapeutic antibodies, cetuximab (IgG1) and panitumumab (IgG2), are approved drugs to treat EGFR-positive tumors. Although cetuximab has been shown to mediate ADCC in EGFR-positive cancer cells through CD16 engagement, panitumumab has demonstrated a lack of ADCC activity due to suboptimal binding affinity of human IgG2 for CD16. Interestingly, both cetuximab (IgG1) and panitumumab (IgG2) have been shown to engage with CD32A. EGFR is also overexpressed in TNBC, thus, these antibodies in conjunction with FcγR-CAR-T cells could be considered as novel modalities to target TNBC (72, 74).

Recent published work used cetuximab and panitumumab as a model system to determine which FcγR variant-based CAR-T cell gives rise to optimal cytotoxicity. Results with CD16A^{158F} engineered CAR-T cells in combination with cetuximab demonstrated significant *in vitro* antitumor activity against TNBC cells overexpressing EGFR. On the other hand, CD32A^{131R} CAR-T cells in combination with cetuximab or panitumumab led to the elimination of EGFR-positive MDA-MB-468 TNBC cells as well as the secretion of proinflammatory cytokines including IFNγ and TNFα (72). These data establish the utility of CD16A^{158F} and CD32A^{131R} CARs in FcγR-CAR-T cell therapy for the treatment of EGFR-overexpressing TNBC. Thus, depending on the subclass of the therapeutic antibody, an appropriate FcγR-CAR-T cell can be designed to achieve optimal tumor cytotoxicity. FcγR-CAR-T cell therapy has been pursued by pharmaceutical companies such as Unum Therapeutics, who have pioneered the Antibody-Coupled T Cell Receptor (ACTR) platform. Recently, Unum Therapeutics initiated a clinical trial for an ACTR T cell product in combination with trastuzumab for the treatment of patients with HER2-positive advanced cancers (NCT03680560). The next-generation ACTR platform is a promising technology that can be applied to other indications such as TNBC.

Chondroitin sulfate proteoglycan 4

Chondroitin sulfate proteoglycan 4 (CSPG4) is an immunogenic cell surface proteoglycan that functions in stabilizing cell-substrate interactions in melanoma. Compared with normal tissue, CSPG4 is highly expressed in hematologic malignancies and solid tumors, including TNBC. Recently, CSPG4 was discovered to be overexpressed in glioblastoma tumors with limited heterogeneity, thus making CSPG4 a good target for CAR-T cell therapy in glioblastoma. Indeed, studies with CSPG4-CAR-T cells have shown control of tumor growth in mice implanted with glioblastoma tumors (75, 76). In TNBC, second-generation CSPG4-CAR-T cells have demonstrated *in vitro* antitumor activity in MDA-MB-231, Hs578T, and MDA-MB-468 TNBC cells, resulting in proinflammatory cytokine release and cyto-

toxicity to tumor cells (77). A major advantage of CSPG4-CAR-T cell therapy is the ability to target both primary TNBC cells and CAFs because CSPG4 is highly expressed on stromal cells in the TNBC TME (76). To limit the potential of on-tumor/off-target toxicity due to low CSPG4 expression on normal tissue, the incorporation of suicide genes into CSPG4-CAR constructs has been proposed (77). Suicide genes such as inducible caspase-9 function as safety switches that trigger cell death upon activation to protect against unwanted toxicity (78).

Receptor tyrosine kinase EGFR

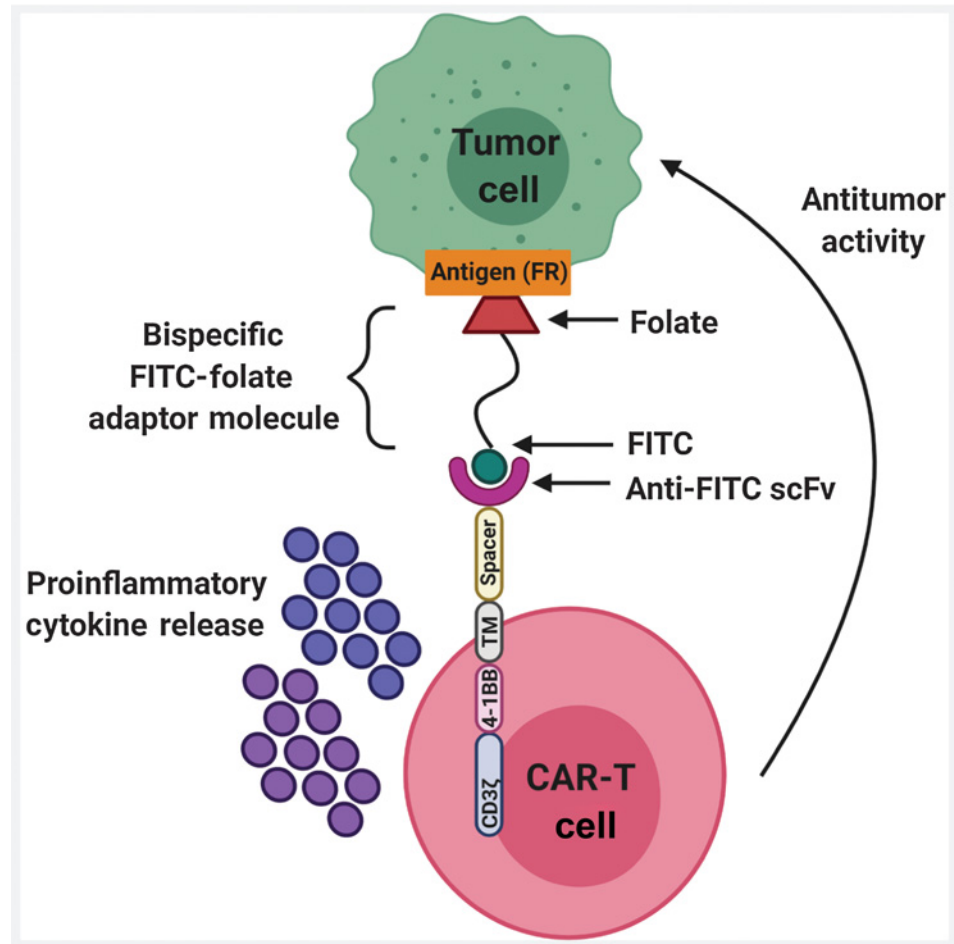
EGFR is a receptor tyrosine kinase that mediates oncogenic signaling cascades which drive the growth, survival, and invasion of cancer cells (79). Approximately 45% to 70% of patients with TNBC overexpress EGFR, and efforts to target this receptor in TNBC are well underway (74). Classic small-molecule EGFR inhibitors such as gefitinib and neratinib result in poor patient response and resistance. More effective therapies targeting EGFR in TNBC are greatly needed. Encouragingly, recent *in vitro* data demonstrated that third-generation CAR-T cells targeting EGFR in Hs578T, MDA-MB-468, and MDA-MB-231 TNBC cells led to enhanced cytokine secretion and cytolytic activity. Furthermore, EGFR-CAR-T cells inhibited TNBC tumor growth in cell line-derived xenograft and patient-derived xenograft mouse models (74). Engineering T cells to incorporate dual or tandem CARs that recognize multiple antigens is a promising strategy for future next-generation CAR-T cell therapies to address the current limitation of tumor-associated antigens, such as EGFR, being expressed on normal tissue (58). EGFR variant III (EGFRvIII) is an attractive target for CAR-T cell therapy because its expression is tumor restricted, thereby reducing the risk of on-target/off-tumor toxicity mediated by the CAR recognizing antigens on normal tissue (80). EGFRvIII-specific CAR-T cells that reduce immune exhaustion and enhance antitumor therapeutic function have been recently reported (81). Novel CAR-T cells that incorporate checkpoint blocking antibodies such as anti-PD-1 and anti-cytotoxic T lymphocyte associated protein 4 (CTLA-4) into EGFR-CAR-T cells are also being studied in the clinic. Because CAR-T cells will secrete these antibodies, this treatment modality will serve as combination therapy and may negate the immunosuppressive TME (82).

Folate receptor alpha

Folate receptor alpha (FRα) is a glycosylphosphatidylinositol (GPI)-linked membrane protein that binds to and mediates the intracellular transport of folate. The water-soluble vitamin, folate, is necessary for the biosynthesis of DNA and facilitates metabolic reactions for proliferating cells. FRα is overexpressed in cancers of epithelial origin compared with normal tissue, including lung, colorectal, ovarian, and breast tumors, and has emerged as an attractive anticancer therapeutic target (83). An inverse correlation between FRα and ER expression has been established. FRα expression in ER-negative, stage IV metastatic TNBC is approximately 70% to 80%, whereas other breast cancer subtypes show only 30% expression. Thus far, FRα-CAR-T cells have demonstrated potent *in vitro* killing of TNBC cells and significant tumor regression in an MDA-MB-231 xenograft mouse model. Moreover, the antitumor activity of FRα-CAR-T cell therapy strongly correlated with FRα surface antigen expression levels on tumor cells, suggesting that FRα could serve as a selection biomarker to improve clinical response rates (84). To help tackle the issue of potential on-tumor/off-target toxicity associated with FRα-CAR-T cell therapy, POC for bispecific CAR-T cells simultaneously cotargeting two different tumor-associated antigens, FRα and mesothelin, has been demonstrated (85). Moreover, a folate-FITC bispecific adaptor

Figure 3.

Diagram representing a universal CAR-T cell. A bispecific FITC-folate adaptor molecule is used to recruit anti-FITC universal CAR-T cells to folate receptor overexpressing tumor cells, resulting in tumor cell killing and proinflammatory cytokine release.



molecule has been shown to redirect universal anti-FITC-CAR-T cells to target tumor cells expressing the folate receptor (Fig. 3; ref. 86).

Disialoganglioside GD2

GD2 is a glycosphingolipid that facilitates the tethering of tumor cells to extracellular matrix proteins. GD2 expression is upregulated in cancerous tissue and highly restricted in normal tissue. The cell surface and tumor-selective expression of GD2 has made this molecule an appealing anticancer target and has led to the development of antibody-based immunotherapies, as evidenced by the FDA approval of the anti-GD2 monoclonal antibody dinutuximab beta for the treatment of neuroblastoma (87, 88). In fact, the efficacy and long-term persistence of Epstein-Barr virus specific T cells (EBV-CTLs) and activated T cells expressing GD2-CARs for the treatment of neuroblastoma are under clinical investigation (NCT00085930; ref. 89). Recently, GD2 was found to be highly expressed on a subpopulation of CD44^{high} CD24^{low} human breast cancer cells that phenotypically resemble cancer stem cells. Since then, third-generation CAR-T cells have been engineered with a scFv derived from dinutuximab beta to target GD2 on cancer stem cells in TNBC to eliminate disseminated tumor cell populations and prevent metastasis formation. GD2-CAR-T cells demonstrated *in vitro* antitumor activity in terms of increased persistence and target cell lysis. Furthermore, an orthotopic xenograft mouse model of human TNBC revealed an effective antitumor immune response that arrested tumor growth and prevented the formation of lung metastases (88). In aggregate,

these data identify GD2 as a relevant antigenic target for CAR-T cell therapy in highly aggressive TNBC.

Intracellular adhesion molecule-1

Intracellular adhesion molecule-1 (ICAM-1) is a cell surface glycoprotein that facilitates endothelial transmigration of leukocytes and functions in stabilizing cell-cell interactions (90). Gene and protein expression data have revealed ICAM-1 to be upregulated in MDA-MB-231 TNBC cells compared with ER-positive breast cancer cells (MCF7) and nontumorigenic mammary epithelial cells (MCF-10A; ref. 91). Recent work demonstrated *in vitro* antitumor activity of CAR-T cells targeting ICAM-1 in TNBC. Approximately 85% of MDA-MB-231 TNBC cells were killed by ICAM-1-CAR-T cells (92). Although these preliminary data are promising, the results from ongoing *in vivo* xenograft mouse models will provide better insight into the feasibility of ICAM-1-CAR-T cell therapy for the treatment of TNBC.

Integrin $\alpha_v\beta_3$

Integrins are receptors expressed on the surface of all nucleated cells that function in cell adhesion. Physiologically, integrins are involved in transmitting signals between cells and their microenvironment, but can also modulate oncogenic processes including proliferation, migration, invasion, and survival (93). Integrin $\alpha_v\beta_3$ is one of the most characterized integrins in oncology. Elevated expression of integrin $\alpha_v\beta_3$ is observed in TNBC as well as components of the TME including

TAMs, CAFs, and angiogenic endothelial cells. Second-generation CAR-T cells have been generated to target integrin $\alpha_v\beta_3$ in TNBC and have demonstrated potent antitumor functions in preclinical *in vitro* models, including tumor growth inhibition and secretion of proinflammatory cytokines such as IL-2 and IFN γ (94). Importantly, these integrin $\alpha_v\beta_3$ -CAR-T cells are equipped with an EGFRt safety switch. Upon administration of the anti-EGFR monoclonal antibody cetuximab, integrin $\alpha_v\beta_3$ -CAR-T cells can be rapidly depleted through endogenous ADCC mechanisms to prevent unwanted toxicity (78, 94). Moreover, integrin $\alpha_v\beta_3$ -CAR-T cells have the potential to shift the TME to a proinflammatory state because the adhesion molecule is highly expressed in the stromal compartment (94). Together, these data identify integrin $\alpha_v\beta_3$ as an important TNBC target for CAR-T cells and its therapeutic potential.

Mesothelin

Mesothelin is a cell adhesion glycoprotein expressed on the surface of mesothelial cells. In the context of cancer, mesothelin promotes oncogenesis through activation of NF- κ B, PI3K, and MAPK signaling pathways (95). Until recently, the role of mesothelin in breast cancer remained unexplored. Interestingly, IHC analysis revealed that approximately 67% of TNBC tissue specimen overexpress mesothelin (96). Because this cancer antigen is highly immunogenic and is expressed on normal tissues at very low levels, mesothelin has emerged as a target for anticancer therapy in TNBC. In fact, mesothelin knockout mice exhibited normal reproduction and development, confirming the nonessential biological function of mesothelin in healthy tissue. Moreover, favorable safety profiles have been observed in mesothelin-targeted immunotherapies thus far (95). CAR-T cells have been successfully engineered to target mesothelin in TNBC and have been shown to induce *in vitro* cytotoxicity and cytokine production, and significant tumor regression. Importantly, in these mesothelin-CAR-T cells, PD-1 expression was knocked down using clustered regularly interspaced short palindromic repeats (CRISPR) gene-editing technology (97). PD-1 is an immune checkpoint receptor on T cells that engages with PD-L1 expressed on tumor cells to suppress the T-cell response and allow tumor cells to escape immune surveillance (40). Accordingly, PD-1 knockout mesothelin-CAR-T cells showed superior effector function in an orthotopic xenograft mouse model of TNBC due to downregulation of this inhibitory receptor (97). This seminal work demonstrates POC for using immune checkpoint blockade in combination with mesothelin-CAR-T cell therapy to treat TNBC. Clinical testing of mesothelin-CAR-T cells is underway in patients with HER2-negative breast cancer, including TNBC (NCT02792114).

Receptor tyrosine kinase c-Met

c-Met is a tyrosine kinase receptor that mediates physiologic processes such as wound healing and organogenesis. Through activation of signaling cascades including PI3K, Stat3, beta-catenin, and RAS pathways, c-Met plays an important role in the progression of many solid cancers by driving their proliferation, angiogenesis, migration, and metastasis (98, 99). c-Met has been recently identified as a therapeutic target in TNBC. Indeed, small interfering RNA-mediated silencing of c-Met in human TNBC cell lines led to a significant reduction in cell proliferation and migration (99). c-Met overexpression occurs in over 50% of patients with TNBC and correlates with poor OS (99, 100). Although low level expression of c-Met is observed in normal epithelial tissues such as hepatocytes, c-Met has emerged as a promising target for CAR-T cell therapy in TNBC. To avoid the potential for on-target/off-tumor toxicity due to c-Met expression on healthy tissue, mRNA electroporation was utilized to induce transient

c-Met-CAR expression in autologous T cells (100). Compared with stably transduced CAR-T cells, mRNA-based CAR-T cell therapy provides a safer approach due to transient expression and limited persistence of cells. For example, c-Met-CAR-T cells were shown to persist for 4 days, which was enough time to induce cytolytic activity in TNBC cells, and then disappeared by day 7 after mRNA electroporation which limited off-target toxicity. The antitumor activity of c-Met-CAR-T cells was further demonstrated by a reduction in tumor growth in a TNBC xenograft mouse model with an intact immune system (100). Most recently, the safety of intratumorally injecting mRNA c-Met-CAR-T cells into patients with TNBC was observed in a clinical setting (NCT01837602).

Mucin1 glycoprotein

MUC1 is transmembrane protein that lines the apical surface of epithelial cells and functions in protecting host cells from infection by serving as a protective mucosal barrier. Serine and threonine residues present in the variable number tandem repeats region of the MUC1 extracellular domain serve as attachment sites for O-glycans. Thus, posttranslational modifications are readily observed in the MUC1 protein (102). Interestingly, TNBC cells overexpress an aberrantly glycosylated tumor form of MUC1 (tMUC1) in greater than 95% of all TNBC, and no significant tMUC1 expression is detected on normal breast tissues. Efforts to target this tumor-specific antigen in TNBC are well underway. Indeed, second-generation tMUC1-CAR-T cells recently demonstrated potent tumor cytolytic activity and Th1 cytokine and chemokine production *in vitro*. Tumor cell growth was significantly inhibited in a TNBC xenograft mouse model while sparing normal breast epithelial cells (103). All in all, tMUC1 represents an important target for CAR-T cell therapy in TNBC given its high tumor antigen specificity. Similarly, another aberrant glycoform of MUC1 (TnMUC1) is also abundantly expressed on TNBC. CAR-T cells recognizing this glycoform are being considered as bonafide CAR-T cell targets for TNBC (103, 104). Taken together, redirecting CAR-T cells to MUC1 glycoforms is a viable avenue to treat TNBC. Clinical trials to evaluate CAR-T cells targeting these glycoforms are ongoing (NCT04020575 and NCT04025216).

Natural killer group 2, member D

Natural killer group 2, member D (NKG2D) is an NK cell activating receptor that functions in recognizing stress ligands on infected or transformed cells and subsequently eliminating these unwanted cells through cytotoxic mechanisms (105). NKG2D ligands, including MHC class I-related chain and UL16-binding protein/retinoic acid early transcript (ULBP/RAET) family members, are highly expressed on TNBC cells with minimal to absent expression observed on healthy tissue. Furthermore, NKG2D ligands are also expressed on immunosuppressive cells of the TME such as MDSCs and Tregs. It was recently demonstrated that TNBC cells expressing NKG2D ligands were sensitive to NKG2D-CAR-T cell therapy, as evidenced by *in vitro* cytolytic activity and proinflammatory cytokine release as well as TNBC growth inhibition in a xenograft mouse model. Moreover, second-generation NKG2D-CAR-T cells engineered with 4-1BB or CD27 costimulatory domains demonstrated superior *in vivo* antitumor activity in terms of increased T-cell persistence and tumor regression compared with first-generation NKG2D-CAR-T cells (106). Collectively, these data identify NKG2D ligand as a promising target for CAR-T cell therapy in TNBC primary tumors and the surrounding TME. Clinical testing of NKG2D-CAR-T cells is scheduled to begin shortly in TNBC (NCT04107142). Indeed, early clinical safety and feasibility have already been demonstrated in leukemia (NCT02203825).

Receptor tyrosine kinase–like orphan receptor 1

Receptor tyrosine kinase–like orphan receptor 1 (ROR1) is a protein that facilitates neuronal growth in the central nervous system. Uniform and high expression of ROR1 is observed in hematologic malignancies and epithelial cancers, including TNBC. Moreover, it is well established that ROR1 expression is limited in healthy adult tissue. In fact, nonhuman primate toxicology studies revealed no toxicity to vital organs in response to ROR1-based immunotherapies, making this tumor antigen an ideal anticancer target (107). The antitumor activity of ROR1-CAR-T cells in TNBC was recently evaluated in a vascularized three-dimensional (3D) complex *in vitro* model. Such microphysiologic systems are predicted to improve preclinical-to-clinical translation, as they more effectively recapitulate the TNBC phenotype and microenvironment (108). Indeed, ROR1-CAR-T cells were shown to infiltrate and migrate through TNBC 3D cultures and elicit potent antitumor immune responses (107). This study provides POC for the development of novel 3D microphysiologic systems to test the antitumor activity of CAR-T cell therapies in TNBC, compared with conventional two-dimensional culture systems and xenograft animal models. Encouragingly, ROR1-CAR-T cell therapy for TNBC is beginning to be tested in the clinic (NCT02706392).

Stage-specific embryonic antigen-4

Stage-specific embryonic antigen-4 (SSEA-4) is a glycosphingolipid used to identify human embryonic stem cells. SSEA-4 promotes invasion and metastasis of tumor cells and is associated with poor prognosis and chemoresistance in solid cancers such as TNBC. Expression of SSEA-4 is limited in normal tissue and upregulated in approximately 30% of TNBC tumor cells (109). The antitumor activity of second-generation SSEA-4-CAR-T cells was investigated in TNBC cells, where *in vitro* T-cell degranulation, cytokine secretion, and tumor cell killing were achieved. Although a significant decrease in TNBC tumor burden was observed in an MDA-MB-231 xenograft mouse model, hematopoietic multipotent progenitor cells in the bone marrow and epithelial pluripotent cells in the lungs were cotargeted by the SSEA-4-CAR-T cells due to low level antigen expression (109). These data emphasize the importance of incorporating safety mechanisms into CAR-T cell constructs to avoid on-tumor/off-target toxicities and potential life-threatening side effects.

Tumor endothelial marker 8

Tumor endothelial marker 8 (TEM8) is a collagen-binding cell surface glycoprotein that promotes the migration of endothelial cells (110). TEM8 is overexpressed on the surface of primary TNBC cells and in the vasculature of solid tumors. Using CAR-T cells to concomitantly target an antigen such as TEM8 on the tumor surface and stroma represents a combinatorial approach to achieve antitumor activity with reversal of the immunosuppressive TME. Initial reports demonstrated both safety and antitumor activity upon injecting TEM8-CAR-T cells into a TNBC mouse model (111). However, other published work showed off-target toxicity in healthy tissues when using TEM8-CAR-T cells generated from the same antibody (112). Due to conflicting data around the safety profile of TEM8-CAR-T cells, more thorough investigation is warranted before progressing to clinical trials.

Trophoblast cell-surface antigen 2

Recently, the FDA granted accelerated approval of sacituzumab govitecan, an antibody–drug conjugate targeting trophoblast cell-surface antigen 2 (TROP2), for the treatment of relapsed or refractory

metastatic TNBC. This ground-breaking approval was based on a multicenter phase II clinical trial which demonstrated a 33.3% objective response rate and a 7.7-month median duration of response in patients with TNBC receiving sacituzumab govitecan who had previously been administered conventional systemic therapies (NCT01631552; ref. 113). The antibody–drug conjugate sacituzumab govitecan consists of the active metabolite of the topoisomerase I inhibitor irinotecan (SN-38) conjugated to a humanized IgG antibody against the cell surface glycoprotein TROP2 (113). TROP2 is overexpressed in approximately 90% of TNBC tumors and correlates with poor prognosis in breast cancer due to the induction of pro-oncogenic signaling. The safety of TROP2 targeting has been demonstrated in the clinic with minimal toxicity observed in normal tissues, and has thus emerged as an attractive therapeutic target in TNBC (113, 114). Recently, TROP2 has been identified as a promising target for CAR-T cell therapy in epithelial cancers. Bispecific CAR-T cells targeting TROP2 and PD-L1 have shown *in vitro* and *in vivo* antitumor activity in gastric cancer (115). Moreover, TROP2-CAR-T cells have been generated against TNBC cells. To address the challenge of tumor antigen heterogeneity in solid tumors, exosomes from TROP2-expressing tumors were transferred to TROP2-negative tumor cells to skew the proportion of targetable tumor cells by TROP2-CAR-T cells (116). *In vitro* evaluation of this novel concept is underway.

Other target antigens

Given the promise of CAR-T cell therapy in preclinical models of TNBC, novel approaches for identifying additional antigenic targets in TNBC are under investigation. Recently, nano-ultra performance liquid chromatography tandem mass spectrometry (nUPLC-MS²) was used to analyze the immunopeptidomic profiles of samples from patients with TNBC and adjacent normal tissue specimens. Five proteins were identified to be preferentially expressed in patients with TNBC, including IL-32, proliferating cell nuclear antigen, syntenin-1, ribophorin-2, and cofilin-1 (117). These data provide candidate proteins for future validation as CAR-T cell targets in TNBC. Such studies are of importance because they promote the repurposing of antigen-directed CAR-T cells for TNBC, that otherwise would not have been pursued.

Clinical Trials for CAR-T Cell Therapy in TNBC

Several antigenic targets evaluated in preclinical *in vitro* and *in vivo* studies for CAR-T cell therapy in TNBC have progressed to first-in-human studies, as depicted in **Table 3**. A phase I clinical trial was initiated to evaluate the safety of mesothelin-CAR-T cell therapy for the treatment of relapsed and/or chemotherapy refractory TNBC, although the current status of this trial remains unknown (NCT02580747). Since then, the Memorial Sloan Kettering Cancer Center has started recruiting individuals for an open-label phase I trial to identify a safe dose for mesothelin-CAR-T cell therapy in patients with metastatic HER2-negative breast cancer (NCT02792114). Recently, a phase I clinical trial was completed at the University of Pennsylvania which investigated the safety of c-Met-CAR-T cells intratumorally injected into patients with TNBC (NCT01837602). The results of this trial demonstrated that c-Met-CAR-T cell therapy was well-tolerated by patients and elicited an inflammatory response within TNBC tumors, with no evidence of drug-related adverse effects greater than grade 1 (101). Thus far, MUC1-CAR-T cell therapy has been the most investigated in clinical trials. The safety and efficacy of autologous MUC1-CAR-T cells in patients with relapsed or refractory

Table 3. CAR-T cell therapies in clinical trials for the treatment of TNBC.

CAR-T cell target	Clinical Trials.gov identifier	Clinical trial phase	Clinical trials for CAR-T cell therapy in TNBC
Mesothelin	NCT02580747	Phase I	Clinical study of chimeric mesothelin antigen receptor–modified T cells in relapsed and/or chemotherapy refractory malignancies.
Mesothelin	NCT02792114	Phase I	A phase I clinical trial to evaluate the safety and tolerability of mesothelin-specific chimeric antigen receptor–positive T cells in patients with metastatic mesothelin-expressing breast cancer.
cMet	NCT01837602	Phase I	Clinical trial of autologous cMet redirected T cells administered intratumorally in patients with breast cancer.
MUC1	NCT02587689	Phase I/II	Phase I/II study of anti-MUC1 CAR T cells for patients with MUC1+ advanced refractory solid tumor.
TnMUC1	NCT04025216	Phase I	A phase I open-label, multicenter first-in-human study of TnMUC1-targeted genetically modified chimeric antigen receptor T cells in patients with advanced TnMUC1-positive solid tumors and multiple myeloma.
MUC1*	NCT04020575	Phase I	Adoptive immunotherapy for advanced MUC1*–positive breast cancer with autologous T cells engineered to express a chimeric antigen receptor, huMNC2-CAR44 specific for a cleaved form of MUC1 (MUC1*).
NKG2D Ligand	NCT04107142	Phase I	A phase I dose-escalation trial to evaluate haploidentical/allogeneic natural killer group 2D ligand (NKG2DL)-targeting chimeric antigen receptor-grafted gamma delta ($\gamma\delta$) T cells (CTM-N2D) in subjects with relapsed or refractory solid tumor.
ROR1	NCT02706392	Phase I	Phase I study of adoptive immunotherapy for advanced ROR1 ⁺ malignancies with defined subsets of autologous T cells engineered to express an ROR1-specific chimeric antigen receptor.

TNBC were proposed to be evaluated in a phase I/II study (NCT02587689). More recently, Minerva Biotechnologies Corporation started recruiting for individuals with metastatic breast cancer to participate in a phase I trial investigating the safety and MTD of autologous CAR-T cells targeting a cleaved form of MUC1 antigen (NCT04020575). Furthermore, a multicenter, open-label phase I trial was initiated by Tmunity Therapeutics to study the safety, tolerability, feasibility, and preliminary efficacy of TnMUC1-CAR-T cells in patients with TNBC (NCT04025216). Additional CAR-T cell targets under examination in the clinic for TNBC include NKG2D ligands and ROR1. The safety and tolerability of allogeneic gamma delta ($\gamma\delta$) T cells transduced with CARs targeting NKG2D ligands on TNBC cells will be investigated in a phase I clinical trial upon recruitment of participants (NCT04107142). Moreover, a phase I dose-escalation study is recruiting patients with TNBC to evaluate the safety of ROR1-CAR-T cell therapy (NCT02706392). Collectively, the number of TNBC CAR-T cell therapies in clinical trials is continuing to grow, thus paving the road to an exciting immunotherapy era in solid cancers.

Future Perspectives and Concluding Remarks

There is an unmet medical need to develop effective treatment modalities for patients with TNBC that experience heightened metastatic potential and poor survival rates (22, 27, 28). Recently, CAR-T cell-based immunotherapeutic strategies have been developed to redirect T cells to kill solid immunogenic tumors such as TNBCs (34). The present review describes novel TNBC CAR-T cell targets including AXL, CD32A, CSPG4, EGFR, FR α , GD2, ICAM-1, integrin $\alpha_v\beta_3$, mesothelin, c-Met, MUC1, NKG2D, ROR1, SSEA-4, TEM8, and TROP2. Although CAR-T cell therapy has been successful in B-cell malignancies, as observed with Kymriah and Yescarta, additional obstacles arise when targeting solid cancers that can limit antitumor activity (46, 49, 50, 54, 59). Key challenges associated with CAR-T cell therapy in solid tumors, potential approaches to overcome these

challenges, and examples of such mitigation strategies in TNBC are depicted in **Table 1**.

For example, expression of antigenic targets on both TNBC primary tumors and cells residing in the TME (e.g., MDSCs, TAMs, CAFs, Tregs) can facilitate CAR-T cell-directed targeting of multiple cell types to overcome the immunosuppressive TME and enhance antitumor activity (69, 76, 94). Additional approaches that can be explored to reprogram the hostile TME in TNBC to a proinflammatory state include arming CAR-T cells with dominant-negative TGF- β receptors or inverted cytokine receptors, as well as engineering TRUCKs (T cells redirected for universal cytokine-mediated killing) that release proinflammatory cytokines upon CAR engagement (50, 118, 119). Combining CAR-T cell therapy in TNBC with immune checkpoint blockade (e.g., anti-PD-1) has also been demonstrated as a promising strategy to combat the immunosuppressive TME and unleash more powerful anticancer activity (97). CAR-T cells that secrete blocking antibodies against immune checkpoints such as anti-CTLA-4 and anti-PD-1 would make for an attractive alternative to combination therapy and is being considered (82). To reduce the potential for on-tumor/off-target toxicity arising from low target antigen expression on healthy tissue, different suicide gene “safety switches” have been engineered into TNBC CAR-T cell constructs (94). In addition to elimination switch-based control mechanisms, “on switches” such as split CARs have been developed to treat solid cancers, in which the signaling domain is dissociated from the antigen-binding domain, to enable precise regulation of CAR-T cell activation (50). Moreover, autologous T cells endowed with bispecific CARs targeting multiple TNBC tumor antigens have demonstrated POC in preclinical studies (84). Dual or synthetic notch CARs that utilize AND-gating logic or inhibitory CARs that utilize NOT-gating logic have shown success in reducing on-tumor/off-target toxicity in other solid tumors (58). Another major challenge associated with CAR-T cell therapy in solid cancers is inefficient trafficking to the tumor site, which has not yet been addressed in TNBC. Engineering TNBC CAR-T cells to express chemokine receptors such as CCR-2 and CCR-4 is one strategy that has the potential to improve tumor trafficking and infiltration because

TNBCs overexpress the associated chemokine ligands (50, 63). In fact, engineered anti-FITC CAR-T cells that coexpress CCL-19 and IL-7 to enhance tumor infiltration are being considered in other malignancies (120). Furthermore, efforts to prolong the persistence of CAR-T cells in solid tumors are underway and include the incorporation of constitutively activated IL-15 or IL-7 receptors into CAR-T cell constructs (50, 58, 70).

The use of combination therapy to simultaneously target different mechanisms of action has proven to be a viable approach to treat cancer. CAR-T cell therapy for TNBC is unlikely to replace chemotherapy in the foreseeable future, but rather be useful in combination therapy. In fact, the FDA-approved anti-PD-L1 immunotherapy atezolizumab is marketed for use in combination with the chemotherapeutic agent nab-paclitaxel for the treatment of metastatic TNBC (45). In addition to evaluating CAR-T cell therapy and chemotherapy combination regimens, the addition of targeted treatment modalities to CAR-T cell therapy also holds promise. Intriguingly, the PARP inhibitor olaparib was recently demonstrated to induce CD8⁺ T-cell infiltration via STING pathway activation in an *in vivo* model of BRCA1-deficient TNBC, thus providing rationale for combining PARP inhibitors with CAR-T cell therapy for the treatment of TNBC (121). Furthermore, PI3K inhibition during *ex vivo* CAR-T cell expansion was shown to induce a memory phenotype, leading to enhanced *in vivo* persistence and antitumor activity in leukemia (122). In addition, the safety and efficacy of an oncolytic adenovirus in combination with CAR-T cells targeting HER2-positive tumors are under evaluation in the clinic (NCT03740256). In aggregate, targeted therapies such as

PARP inhibitors and PI3K inhibitors, as well as oncolytic viruses, should be evaluated in combination with TNBC targeting CAR-T cell therapy. Emerging platforms including $\gamma\delta$ -CAR-T cells and CAR-NK cell immunotherapy are promising next-generation approaches that should also be explored for the treatment of TNBC (123, 124).

Taken together, substantial progress has been made in the CAR-T cell therapy space regarding the development of novel treatments for patients with TNBC, as evidenced by the numerous therapies under evaluation in preclinical studies and clinical trials. There is much to learn from CAR-T cell therapy clinical trials being conducted in liquid tumors and other solid cancers, and these novel concepts can potentially be applied to TNBC. Continued momentum in the field will rely on engineering TNBC CAR-T cells to achieve specificity, safety, and efficacy and selecting the most appropriate combination therapies to improve clinical outcome in patients with TNBC.

Disclosure of Potential Conflicts of Interest

All authors are paid employees of the Janssen Pharmaceutical Companies of Johnson & Johnson and receive salary and other compensation.

Acknowledgments

The authors thank Jennifer Nemeth-Seay for her guidance as JBIO Post-Doctoral Coordinator.

Received May 7, 2020; revised July 6, 2020; accepted October 8, 2020; published first October 21, 2020.

References

- American Cancer Society. Cancer facts & figures 2020. Atlanta: American Cancer Society, Inc.; 2020.
- Harbeck N, Penault-Llorca F, Cortes J, Gnant M, Houssami N, Poortmans P, et al. Breast cancer. Nat Rev Dis Primers 2019;5:66.
- Zucca-Matthes G, Urban C, Vallejo A. Anatomy of the nipple and breast ducts. Gland Surg 2016;5:32–6.
- Feng Y, Spezia M, Huang S, Yuan C, Zeng Z, Zhang L, et al. Breast cancer development and progression: risk factors, cancer stem cells, signaling pathways, genomics, and molecular pathogenesis. Genes Dis 2018;5:77–106.
- American Cancer Society. Breast cancer facts & figures 2019–2020. Atlanta: American Cancer Society, Inc.; 2019.
- Adriance MC, Inman JL, Petersen OW, Bissell MJ. Myoepithelial cells: good fences make good neighbors. Breast Cancer Res 2005;7:190–7.
- Wu X, Chen G, Qiu J, Lu J, Zhu W, Chen J, et al. Visualization of basement membranes in normal breast and breast cancer tissues using multiphoton microscopy. Oncol Lett 2016;11:3785–9.
- Rakha EA, Reis-Filho JS, Baehner F, Dabbs DJ, Decker T, Eusebi V, et al. Breast cancer prognostic classification in the molecular era: the role of histological grade. Breast Cancer Res 2010;12:207.
- Schwartz AM, Henson DE, Chen D, Rajamarthandan S. Histologic grade remains a prognostic factor for breast cancer regardless of the number of positive lymph nodes and tumor size: a study of 161 708 cases of breast cancer from the SEER Program. Arch Pathol Lab Med 2014;138:1048–52.
- Koh J, Kim MJ. Introduction of a new staging system of breast cancer for radiologists: an emphasis on the prognostic stage. Korean J Radiol 2019;20: 69–82.
- Cserni G, Chmielik E, Cserni B, Tot T. The new TNM-based staging of breast cancer. Virchows Arch 2018;472:697–703.
- Russnes HG, Lingjaerde OC, Borresen-Dale AL, Caldas C. Breast cancer molecular stratification: from intrinsic subtypes to integrative clusters. Am J Pathol 2017;187:2152–62.
- Makki J. Diversity of breast carcinoma: histological subtypes and clinical relevance. Clin Med Insights Pathol 2015;8:23–31.
- Malhotra GK, Zhao X, Band H, Band V. Histological, molecular and functional subtypes of breast cancers. Cancer Biol Ther 2010;10:955–60.
- Libson S, Lippman M. A review of clinical aspects of breast cancer. Int Rev Psychiatry 2014;26:4–15.
- Waks AG, Winer EP. Breast cancer treatment: a review. JAMA 2019;321: 288–300.
- Tremont A, Lu J, Cole JT. Endocrine therapy for early breast cancer: updated review. Ochsner J 2017;17:405–11.
- Lumachi F, Santeufemia DA, Basso SMM. Current medical treatment of estrogen receptor-positive breast cancer. World J Biol Chem 2015;6:231–9.
- Pernas S, Tolane SM. HER2-positive breast cancer: new therapeutic frontiers and overcoming resistance. Ther Adv Med Oncol 2019;11:1–16.
- Dai X, Li T, Bai Z, Yang Y, Liu X, Zhan J, et al. Breast cancer intrinsic subtype classification, clinical use and future trends. Am J Cancer Res 2015;5:2929–43.
- Uscanga-Perales GI, Santuario-Facio SK, Ortiz-Lopez R. Triple negative breast cancer: deciphering the biology and heterogeneity. Medicina Universitaria 2016;18:105–14.
- Kumar P, Aggarwal R. An overview of triple-negative breast cancer. Arch Gynecol Obstet 2016;293:247–69.
- Amirikia KC, Mills P, Bush J, Newman LA. Higher population-based incidence rates of triple-negative breast cancer among young African American women: implications for breast cancer screening recommendations. Cancer 2011;117: 2747–53.
- Dietze EC, Sistrunk C, Miranda-Carboni G, O'Reagan R, Seewaldt VL. Triple-negative breast cancer in African-American women: disparities versus biology. Nat Rev Cancer 2015;15:248–54.
- Niyomnaitam S, Parinyanitikul N, Roothumngong E, Jinda W, Samarntai N, Atikankul T, et al. Tumor mutational profile of triple negative breast cancer patients in Thailand revealed distinctive genetic alteration in chromatin remodeling gene. PeerJ 2019;7:e6501.
- Prat A, Pineda E, Adamo B, Galvan P, Fernandez A, Gaba L, et al. Clinical implications of the intrinsic molecular subtypes of breast cancer. Breast 2015; 24:S26–S35.

27. Foulkes WD, Smith IE, Reis-Filho JS. Triple-negative breast cancer. *N Engl J Med* 2010;363:1938–48.
28. Dent R, Trudeau M, Pritchard KI, Hanna WM, Kahn HK, Sawka CA, et al. Triple-negative breast cancer: clinical features and patterns of recurrence. *Clin Cancer Res* 2007;13:4429–34.
29. Pogoda K, Niwinska A, Murawska M, Pienkowski T. Analysis of pattern, time and risk factors influencing recurrence in triple-negative breast cancer patients. *Med Oncol* 2013;30:388.
30. Wahba HA, El-Hadaad HA. Current approaches in treatment of triple-negative breast cancer. *Cancer Biol Med* 2015;12:106–16.
31. Zeichner SB, Terawaki H, Gogineni K. A review of systemic treatment in metastatic triple-negative breast cancer. *Breast Cancer* 2016;10:25–36.
32. Bergin ART, Loi S. Triple-negative breast cancer: recent treatment advances [version 1; peer review: 2 approved]. *F1000Res* 2019;8:F1000 Faculty Rev-1342.
33. Marra A, Viale G, Curigliano G. Recent advances in triple negative breast cancer: the immunotherapy era. *BMC Med* 2019;17:90.
34. Li Z, Qiu Y, Lu W, Jiang Y, Wang J. Immunotherapeutic interventions of triple negative breast cancer. *J Transl Med* 2018;16:147.
35. Katz H, Alsharedi M. Immunotherapy in triple-negative breast cancer. *Med Oncol* 2017;35:13.
36. Jia H, Truica CI, Wang B, Wang Y, Ren X, Harvey HA, et al. Immunotherapy for triple-negative breast cancer: existing challenges and exciting prospects. *Drug Resist Updat* 2017;32:1–15.
37. Garcia-Aranda M, Redondo M. Immunotherapy: a challenge of breast cancer treatment. *Cancers* 2019;11:1822.
38. Miyashita M, Sasano H, Tamaki K, Hirakawa H, Takahashi Y, Nakagawa S, et al. Prognostic significance of tumor-infiltrating CD8+ and FOXP3+ lymphocytes in residual tumors and alterations in these parameters after neoadjuvant chemotherapy in triple-negative breast cancer: a retrospective multicenter study. *Breast Cancer Res* 2015;17:124.
39. Ibrahim EM, Al-Foheidi ME, Al-Mansour MM, Kazkaz GA. The prognostic value of tumor-infiltrating lymphocytes in triple-negative breast cancer: a meta-analysis. *Breast Cancer Res Treat* 2014;148:467–76.
40. Mittendorf EA, Phillips AV, Meric-Bernstam F, Qiao N, Wu Y, Harrington S, et al. PD-L1 expression in triple negative breast cancer. *Cancer Immunol Res* 2014;2:361–70.
41. Wimberly H, Brown JR, Schalper K, Haack H, Silver MR, Nixon C, et al. PD-L1 expression correlates with tumor-infiltrating lymphocytes and response to neoadjuvant chemotherapy in breast cancer. *Cancer Immunol Res* 2015;3:326–32.
42. Thomas A, Routh ED, Pullikuth A, Jin G, Su J, Chou JW, et al. Tumor mutational burden is a determinant of immune-mediated survival in breast cancer. *Oncoimmunology* 2018;7:e1490854.
43. Yu L, Wang J. T cell-redirecting bispecific antibodies in cancer immunotherapy: recent advances. *J Cancer Res Clin Oncol* 2019;145:941–56.
44. Lehmann BD, Bauer JA, Chen X, Sanders ME, Chakravarthy AB, Shyr Y, et al. Identification of human triple-negative breast cancer subtypes and preclinical models for selection of targeted therapies. *J Clin Invest* 2011;121:2750–67.
45. Schmid P, Rugo HS, Adams S, Schneeweiss A, Barrios CH, Iwata H, et al. Atezolizumab plus nab-paclitaxel as first-line treatment for unresectable, locally advanced or metastatic triple-negative breast cancer (Impassion130): updated efficacy results from a randomized, double-blind, placebo-controlled, phase 3 trial. *Lancet Oncol* 2020;21:44–59.
46. June CH, O'Connor RS, Kawalekar OU, Ghassemi S, Milone MC. CAR T cell immunotherapy for human cancer. *Science* 2018;359:1361–5.
47. Rossig C. CAR T cell immunotherapy in hematology and beyond. *Clin Immunol* 2018;186:54–8.
48. Bagley SJ, O'Rourke DM. Clinical investigation of CAR T cells for solid tumors: lessons learned and future directions. *Pharmacol Ther* 2020;205:107419.
49. Yang X, Wang GX, Zhou JF. CAR T cell therapy for hematological malignancies. *Curr Med Sci* 2019;39:874–82.
50. Abreu TR, Fonseca NA, Goncalves N, Moreira JN. Current challenges and emerging opportunities of CAR-T cell therapies. *J Control Release* 2020;319:246–61.
51. Titov A, Valiullina A, Zmievskaia E, Zaikova E, Petukhov A, Miftakhova R, et al. Advancing CAR T-cell therapy for solid tumors: lessons learned from lymphoma treatment. *Cancers* 2020;12:125.
52. Comoli P, Chabannon C, Koehl U, Lanza F, Urbano-Ispizua A, Hudecek M et al.; European Society for Blood and Marrow Transplantation, Cellular Therapy & Immunobiology Working Party-Solid Tumor Sub-Committee. Development of adaptive immune effector therapies in solid tumors. *Ann Oncol* 2019;30:1740–50.
53. D'Aloia MM, Zizzari IG, Sacchetti B, Pierelli L, Alimandi M. CAR-T cells: the long and winding road to solid tumors. *Cell Death Dis* 2018;9:282.
54. Martinez M, Moon EK. CAR T cells for solid tumors: new strategies for finding, infiltrating, and surviving in the tumor microenvironment. *Front Immunol* 2019;10:128.
55. Newick K, O'Brien S, Moon E, Albelda SM. CAR T cell therapy for solid tumors. *Annu Rev Med* 2017;68:139–52.
56. Zhao Z, Chen Y, Francisco NM, Zhang Y, Wu M. The application of CAR-T cell therapy in hematological malignancies: advantages and challenges. *Acta Pharm Sin B* 2018;8:539–51.
57. Ali N, FCPS Haematology. Chimeric antigen T cell receptor treatment in hematological malignancies. *Blood Res* 2019;54:81–3.
58. Charrot S, Hallam S. CAR-T cells: future perspectives. *Hemisphere* 2019;3:e188.
59. Ma S, Li X, Wang X, Cheng L, Li Z, Zhang C, et al. Current progress in CAR-T cell therapy for solid tumors. *Int J Biol Sci* 2019;15:2548–60.
60. Abbott RC, Cross RS, Jenkins MR. Finding the keys to the CAR: identifying novel target antigens for T cell redirection immunotherapies. *Int J Mol Sci* 2020;21:515.
61. Chavez JC, Locke FL. CAR T cell therapy for B-cell lymphomas. *Best Pract Res Clin Haematol* 2018;31:135–46.
62. Liu B, Yan L, Zhou M. Target selection of CART cell therapy in accordance with the TME for solid tumors. *Am J Cancer Res* 2019;9:228–41.
63. Knochelmann HM, Smith AS, Dwyer CJ, Wyatt MM, Mehrotra S, Paulos CM. CAR T cells in solid tumors: blueprints for building effective therapies. *Front Immunol* 2018;9:1740.
64. Steentoft C, Migliorini D, King TR, Mandel U, June CH, Posey AD Jr. Glycan-directed CAR-T cells. *Glycobiology* 2018;28:656–69.
65. Zhu C, Wei Y, Wei X. AXL receptor tyrosine kinase as a promising anti-cancer approach: functions, molecular mechanisms and clinical applications. *Mol Cancer* 2019;18:153.
66. Zhang S, Xu XS, Yang JX, Guo JH, Chao TF, Tong Y. The prognostic role of Gas6/Axl axis in solid malignancies: a meta-analysis and literature review. *Oncotargets Ther* 2018;11:509–19.
67. Wei J, Sun H, Zhang A, Wu X, Li Y, Liu J, et al. A novel AXL chimeric antigen receptor endows T cells with anti-tumor effects against triple negative breast cancers. *Cell Immunol* 2018;331:49–58.
68. Ye X, Li Y, Stawicki S, Couto S, Eastham-Anderson J, Kallop D, et al. An anti-Axl monoclonal antibody attenuates xenograft tumor growth and enhances the effect of multiple anticancer therapies. *Oncogene* 2010;29:5254–64.
69. Brenig R, Pop OT, Triantafyllou E, Geng A, Singanayagam A, Perez-Shibayama C, et al. Expression of AXL receptor tyrosine kinase relates to monocyte dysfunction and severity of cirrhosis. *Life Sci Alliance* 2020;3:201900465.
70. Zhao Z, Li Y, Liu W, Li X. Engineered IL-7 receptor enhances the therapeutic effect of AXL-CAR-T cells on triple-negative breast cancer. *Biomed Res Int* 2020;2020:4795171.
71. Caratelli S, Sconocchia T, Arriga R, Coppola A, Lanzilli G, Lauro D, et al. FCγ chimeric receptor-engineered T cells: methodology, advantages, limitations, and clinical relevance. *Front Immunol* 2017;8:457.
72. Caratelli S, Arriga R, Sconocchia T, Ottaviani A, Lanzilli G, Pastore D, et al. In vitro elimination of epidermal growth factor receptor-overexpressing cancer cells by CD32A-chimeric receptor T cells in combination with cetuximab or panitumumab. *Int J Cancer* 2020;146:236–47.
73. Anania JC, Chenoweth AM, Wines BD, Hogarth PM. The human FcγRII (CD32) family of leukocyte FcR in health and disease. *Front Immunol* 2019;10:464.
74. Liu Y, Zhou Y, Huang KH, Li Y, Fang X, An L, et al. EGFR-specific CAR-T cells trigger cell lysis in EGFR-positive TNBC. *Aging* 2019;11:11054–72.
75. Ilieva KM, Cheung A, Mele S, Chiaruttini G, Crescioli S, Griffin M, et al. Chondroitin sulfate proteoglycan 4 and its potential as an antibody immunotherapy target across different tumor types. *Front Immunol* 2017;8:1911.
76. Harrer DC, Dorrie J, Schaft N. CSPG4 as target for CAR-T-cell therapy of various tumor entities-merits and challenges. *Int J Mol Sci* 2019;20:5942.
77. Beard RE, Zheng Z, Lagisetty KH, Burns WR, Tran E, Hewitt SM, et al. Multiple chimeric antigen receptors successfully target chondroitin sulfate proteoglycan 4 in several different cancer histologies and cancer stem cells. *J Immunother Cancer* 2014;2:25.

78. Yu S, Yi M, Qin S, Wu K. Next generation chimeric antigen receptor T cells: safety strategies to overcome toxicity. *Mol Cancer* 2019;18:125.
79. Nakai K, Hung MC, Yamaguchi H. A perspective on anti-EGFR therapies targeting triple-negative breast cancer. *Am J Cancer Res* 2016;6:1609–23.
80. Heimberger AB, Hlatky R, Suki D, Yang D, Weinberg J, Gilbert M, et al. Prognostic effect of epidermal growth factor receptor and EGFRvIII in glioblastoma multiforme patients. *Clin Cancer Res* 2005;11:1462–6.
81. Caruso HG, Tanaka R, Liang J, Ling X, Sabbagh A, Henry VK, et al. Shortened ex vivo manufacturing time of EGFRvIII-specific chimeric antigen receptor (CAR) T cells reduces immune exhaustion and enhances anti glioma therapeutic function. *J Neurooncol* 2019;145:429–39.
82. Yoon DH, Osborn MJ, Tolar J, Kim CJ. Incorporation of immune checkpoint blockade into chimeric antigen receptor T cells (CAR-Ts): combination or built-in CAR-T. *Int J Mol Sci* 2018;19:340.
83. Cheung A, Bax HJ, Josephs DH, Ilieva KM, Pellizzari G, Opzooomer J, et al. Targeting folate receptor alpha for cancer treatment. *Oncotarget* 2016;7:52553–74.
84. Song DG, Ye Q, Poussin M, Chacon JA, Figini M, Powell DJ Jr. Effective adoptive immunotherapy of triple-negative breast cancer by folate receptor-alpha redirected CAR T cells is influenced by surface antigen expression level. *J Hematol Oncol* 2016;9:56.
85. Lanitis E, Poussin M, Klattenhoff AW, Song D, Sandaltzopoulos R, June CH, et al. Chimeric antigen receptor T cells with dissociated signaling domains exhibit focused antitumor activity with reduced potential for toxicity in vivo. *Cancer Immunol Res* 2013;1:43–53.
86. Kim MS, Ma JS, Yun H, Cao Y, Kim JY, Chi V, et al. Redirection of genetically engineered CAR-T cells using bifunctional small molecules. *J Am Chem Soc* 2015;137:2832–5.
87. Voeller J, Sondel PM. Advances in anti-GD2 immunotherapy for treatment of high-risk neuroblastoma. *J Pediatr Hematol Oncol* 2019;41:163–9.
88. Seitz CM, Schroeder S, Knopf P, Krahl AC, Hau J, Schleicher S, et al. GD2-targeted chimeric antigen receptor T cells prevent metastasis formation by elimination of breast cancer stem-like cells. *Oncoimmunology* 2019;9:1683345.
89. Louis CU, Savoldo B, Dotti G, Pule M, Yvon E, Myers GD, et al. Antitumor activity and long-term fate of chimeric antigen receptor-positive T cells in patients with neuroblastoma. *Blood* 2011;118:6050–6.
90. Benedicto A, Romayor I, Arteta B. Role of liver ICAM-1 in metastasis. *Oncol Lett* 2017;14:3883–92.
91. Guo P, Huang J, Wang L, Jia D, Yang J, Dillon DA, et al. ICAM-1 as a molecular target for triple negative breast cancer. *Proc Natl Acad Sci U S A* 2014;111:14710–5.
92. Yang Y, Vedvyas Y, McCloskey JE, Min IM, Jin MM. ICAM-1 targeting CAR T cell therapy for triple negative breast cancer [abstract]. In: Proceedings of the American Association for Cancer Research Annual Meeting 2019; 2019 Mar 29–Apr 3; Atlanta, GA. Philadelphia (PA): AACR; *Cancer Res* 2019;79 (13 Suppl):Abstract nr 2322.
93. Bianconi D, Unseld M, Prager GW. Integrins in the spotlight of cancer. *Int J Mol Sci* 2016;17:2037.
94. Wallstabe L, Madas A, Frenz S, Einsele H, Rader C, Hudecek M. CAR T cells targeting $\alpha_5\beta_1$ integrin are effective against cancer in preclinical models. *Adv Cell Gene Ther* 2018;1:11.
95. Morello A, Sadelain M, Adusumilli PS. Mesothelin-targeted CARs: driving T cells to solid tumors. *Cancer Discov* 2016;6:133–46.
96. Tchou J, Wang LC, Selven B, Zhang H, Conejo-Garcia J, Borghaei H, et al. Mesothelin, a novel immunotherapy target for triple negative breast cancer. *Breast Cancer Res Treat* 2012;133:799–804.
97. Hu W, Zi Z, Jin Y, Li G, Shao K, Cai Q, et al. CRISPR/Cas9-mediated PD-1 disruption enhances human mesothelin-targeted CAR T cell effector functions. *Cancer Immunol Immunother* 2019;68:365–77.
98. Gaule PB, Crown J, O'Donovan N, Duffy MJ. cMET in triple-negative breast cancer: is it a therapeutic target for this subset of breast cancer patients? *Expert Opin Ther Targets* 2014;18:999–1009.
99. Kim YJ, Choi JS, Seo J, Song JY, Lee SE, Kwon MJ, et al. MET is a potential target for use in combination therapy with EGFR inhibition in triple-negative/basal-like breast cancer. *Int J Cancer* 2014;134:2424–36.
100. Zhao X, Qu J, Hui Y, Zhang H, Sun Y, Liu X, et al. Clinicopathological and prognostic significance of c-Met overexpression in breast cancer. *Oncotarget* 2017;8:56758–67.
101. Tchou J, Zhao Y, Levine BL, Zhang PJ, Davis MM, Melenhorst JJ, et al. Safety and efficacy of intratumoral injections of chimeric antigen receptor (CAR) T cells in metastatic breast cancer. *Cancer Immunol Res* 2017;5:1152–61.
102. Nath S, Mukherjee P. MUC1: a multifaceted oncoprotein with a key role in cancer progression. *Trends Mol Med* 2014;20:332–42.
103. Zhou R, Yazdanifar M, Roy LD, Whilding LM, Gavril A, Maher J, et al. CAR T cells targeting the tumor MUC1 glycoprotein reduce triple-negative breast cancer growth. *Front Immunol* 2019;10:1149.
104. Posey AD Jr, Schwab RD, Boesteanu AC, Steentoft C, Mandel U, Engels B, et al. Engineered CAR T cells targeting the cancer-associated tn-glycoform of the membrane mucin MUC1 control adenocarcinoma. *Immunity* 2016;44:1444–54.
105. Wensveen FM, Jelencic V, Polic B. NKG2D: a master regulatory of immune cell responsiveness. *Front Immunol* 2018;9:441.
106. Han Y, Xie W, Song DG, Powell DJ Jr. Control of triple-negative breast cancer using ex vivo self-enriched, costimulated NKG2D CART cells. *J Hematol Oncol* 2018;11:92.
107. Wallstabe L, Gottlich C, Nelke LC, Kuhnemundt J, Schwartz T, Nerretter T, et al. ROR1-CAR T cells are effective against lung and breast cancer in advanced microphysiological 3D tumor models. *JCI Insight* 2019;4:126345.
108. Wikswo FP. The relevance and potential roles of microphysiological systems in biology and medicine. *Exp Biol Med* 2014;239:1061–72.
109. Pfeifer R. Evaluation of SSEA-4 as a CAR T cell therapeutic target for the treatment of chemoresistant triple negative breast cancers. Dissertation. Eberhard Karls Universitat Tubingen. 2018;1–149.
110. Byrd T, Fousek K, Pignata A, Wakefield A, St Croix B, Fletcher BS, et al. TEM8 specific T cells target the tumor cells and tumor-associated vasculature in triple negative breast cancer. *J Immunother Cancer* 2014;2:P7.
111. Byrd TT, Fousek K, Pignata A, Szot C, Samaha H, Seaman S, et al. TEM8/ANTXR1-specific CAR T cells as a targeted therapy for triple-negative breast cancer. *Cancer Res* 2018;78:489–500.
112. Petrovic K, Robinson J, Whitworth K, Jinks E, Shaaban A, Lee SP. TEM8/ANTXR1-specific CAR T cells mediate toxicity in vivo. *PLoS One* 2019;14:0224015.
113. Bardia A, Mayer IA, Vahdat LT, Tolaney SM, Isakoff SJ, Diamond JR, et al. Sacituzumab govitecan-hziy in refractory metastatic triple-negative breast cancer. *N Engl J Med* 2019;380:741–51.
114. Goldenberg DM, Stein R, Sharkey RM. The emergence of trophoblast cell-surface antigen 2 (TROP-2) as a novel cancer target. *Oncotarget* 2018;9:28989–9006.
115. Zhao W, Jia L, Zhang M, Huang X, Qian P, Tang Q, et al. The killing effect of novel bi-specific Trop2/PD-L1 CAR-T cell targeted gastric cancer. *Am J Cancer Res* 2019;9:1846–56.
116. Bedoya DM, King T, Posey AD. Generation of CART cells targeting oncogenic TROP2 for the elimination of epithelial malignancies. *Cytotherapy* 2019;21:11–2.
117. Ternette N, Olde Nordkamp MJM, Muller J, Anderson AP, Nicastrì A, Hill AVS, et al. Immunopeptidomic profiling of HLA-A2-positive triple negative breast cancer identifies potential immunotherapy target antigens. *Proteomics* 2018;18:1700465.
118. Kloss CC, Lee J, Zhang A, Chen F, Melenhorst JJ, Lacey SF, et al. Dominant-negative TGF- β receptor enhances PSMA-targeted human CAR T cell proliferation and augments prostate cancer eradication. *Mol Ther* 2018;26:1855–66.
119. Wang Y, Jiang H, Luo H, Sun Y, Shi B, Sun R, et al. An IL-4/21 inverted cytokine receptor improving CAR-T cell potency in immunosuppressive solid-tumor microenvironment. *Front Immunol* 2019;10:1691.
120. Adachi K, Kano Y, Nagai T, Okuyama N, Sakoda Y, Tamada K. IL-7 and CCL19 expression in CAR-T cells improves immune cell infiltration and CAR-T cell survival in the tumor. *Nat Biotechnol* 2018;36:346–51.
121. Pantelidou C, Sonzogni O, Taveira MDO, Mehta AK, Kothari A, Wang D, et al. PARP inhibitor efficacy depends on CD8+ T-cell recruitment via intratumoral sting pathway activation in BRCA-deficient models of triple-negative breast cancer. *Cancer Discov* 2019;9:722–37.
122. Zheng W, Jones LL, Geiger TL. Modulation of PI3K signaling to improve CAR T cell function. *Oncotarget* 2018;9:35807–8.
123. Hu Z. Tissue factor as a new target for CAR-NK cell immunotherapy of triple-negative breast cancer. *Sci Rep* 2020;10:2815.
124. Wu Y, Kyle-Cezar F, Woolf RT, Nacuer-Lombardelli C, Owen J, Biswas D, et al. An innate-like $V\delta 1^+ \gamma\delta$ T cell compartment in the human breast is associated with remission in triple negative breast cancer. *Sci Transl Med* 2019;11:9364.