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Adoptive T-Cell Therapy for Solid Tumors

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OVERVIEW

Chimeric antigen receptor (CAR) T-cell therapy is an innovative form of immunotherapy wherein autologous T cells are genetically modified to express chimeric receptors encoding an antigen-specific single-chain variable fragment and various costimulatory molecules. Upon administration, these modified T cells traffic to, and recognize, cancer cells in an HLA-independent manner. CAR T-cell therapy has shown remarkable success in the treatment of CD-19–expressing B-cell acute lymphocytic leukemia. However, clinical gains to the same magnitude have not been reported in solid tumors. Several known obstacles to CAR T-cell therapy for solid tumors include target antigen identification, effective trafficking to the tumor, robust activation, proliferation, and in vivo cytotoxicity. Beyond these T-cell intrinsic properties, a complex and dynamic immunosuppressive tumor microenvironment in solid tumors hinders T-cell efficacy. Notable advancements in CAR design to include multiple costimulatory molecules, ligands, and soluble cytokines have shown promise in preclinical models, and some of these are currently in early-phase clinical trials. In this review, we discuss selected solid tumor malignancies and relevant preclinical data and highlight clinical trial results that are available. Furthermore, we outline some obstacles to CAR T-cell therapy for each tumor and propose strategies to overcome some of these limitations.

CAR T-cell therapy for solid tumor malignancies is an exciting frontier in cancer immunotherapy. The general architecture of a CAR consists of a single-chain variable fragment (scFv) derived against a predetermined tumor-associated antigen (TAA) followed by a CD3ζ domain required for provision of signal 1 and T-cell activation upon antigen recognition.[1] Upon transfection into autologous T cells, first-generation CAR T cells targeting *HER2/Neu*-expressing breast and ovarian cancer cell lines showed increased interleukin-2 (IL-2) production and cytotoxicity.[2] However, it was subsequently realized that sustained activity and proliferation after receptor engagement required a secondary signal, or signal 2.[1] Additional genetic modifications to include costimulatory molecules, such as CD28[3] and 4-1BB,[4] to the CD3ζ signaling domain led to second-generation CARs (28ζ and 4-1BBζ, respectively). Acting in concert, provision of both signal 1 and signal 2 mitigated the anergy and activation-induced cell death observed with first-generation CAR T cells.[5] Direct comparison of first- and second-generation CARs directed against CD19, a TAA expressed on malignant B cells, revealed superior expansion, tumor infiltration, and

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persistence in favor of the second-generation CAR design.[6] Additional genetic modifications have yielded third-generation CARs composed of two distinct costimulatory domains, such as CD28/4-1BB/CD3ζ or CD28/OX-40/CD3ζ, all with varying degrees of efficacy.[7–9] More recently, other approaches to optimize CAR T-cell efficacy via engineered expression of tethered or soluble ligands, cytokines, or scFvs[10,11] also have been reported.

However, despite ongoing success in the management of CD19+ B-cell hematologic malignancies, progress in the solid tumor landscape has been met with many obstacles. One is the identification of suitable neoantigens or TAAs to serve as targets for CAR T-cell therapy. The biologic heterogeneity of solid tumor malignancies does not lend to an approach of one antigen fits all. This difficulty is compounded by the frequent expression of putative target antigens on normal tissues that leads to on-target, off-tumor toxicity.[12] Despite this, acceptable antigens, such as EGFR variant III (EGFRIII),[13] GD2,[14] mucin 1 (MUC-1),[9] mucin 16 (MUC-16),[15] carcinoem-bryonicantigen,[16] mesothelin,[17] CA-IX,[18] and prostate-specific membrane antigen (PSMA)[19] have been characterized and are in various stages of clinical development (Table 1). Besides identification of a suitable TAA, trafficking of administered CAR T cells to the tumor is another challenge to effective therapy. Consequently, experimental models to improve innate CAR T-cell trafficking via coexpression of chemokine receptors[20] and compartmental/intercavitary administration of CAR T cells are being investigated.[21] Perhaps the most notable limitation lies in the dynamic, complex, and often inhibitory tumor microenvironment present in many solid tumor malignancies. For instance, myeloid-derived suppressor cells and tumor-associated macrophages (TAMs) decrease local tryptophan levels in the tumor microenvironment,[22] depriving CAR T cells of an essential amino acid necessary for optimal function. In addition, regulatory T cells, myeloid-derived suppressor cells, and TAMs elaborate inhibitory cytokines such as IL-4, IL-10, leukemia inhibitor factor, and transforming growth factor β—all of which further repress T-cell function.[23–25] Strategies aimed at overcoming these limitations are currently areas of intense investigation.

GLIOBLASTOMA

IL-13 receptor α2 (IL-13Rα2) and EGFRIII are two major targets that have been investigated for CAR T-cell therapy against glioblastoma. IL-13Rα2 is overexpressed in more than 50% of glioblastomas, but limited expression on normal brain tissue is retained.[34] Importantly, IL-13Rα2 expression has been reported on both stem-like and more differentiated malignant cells, making it a favorable target with the potential to eliminate tumor-initiating cells and prevent tumor recurrence. Kahlon et al[35] generated a first-generation IL-13Rα2–specific CAR that redirected human CD8+ cytotoxic T lymphocytes to eradicate established glioblastoma tumor in an orthotopic xenograft model. In a separate study, IL-13Rα2–specific CAR T cells targeted glioma stem–like cancer-initiating cells and abrogated their tumor-initiating activity in mice.[36] A phase I trial was conducted in three patients with recurrent glioblastoma who received repetitive intracranial infusions of first-generation IL-13Rα2–specific CAR T cells without nonmyeloablative preconditioning.[26] Only transient antiglioma responses were observed in two patients. The unsatisfactory response may be explained by poor expansion and persistence of CAR T cells in vivo, because the trial used first-generation CAR T cells. As previously mentioned, first-

generation CAR T cells show diminished expansion upon repeated antigen stimulation.[37] In a recent case report, a patient showed tumor regression after multiple intracranial infusions of second-generation IL-13Rα2–specific CAR T cells.[38] Interestingly, CAR T cells with intracavitary administration prevented only local tumor recurrence but failed to control tumor progression at distant sites. In contrast, intraventricular infusions resulted in tumor regression in all intracranial and spinal tumors. *EGFRIII* is a tumor-specific, mutated form of wild-type *EGFR* and is commonly expressed in glioblastoma. Because of an absence in normal tissues, EGFRIII is ideally suited to minimize on-target, off-tumor toxicity. Multiple preclinical studies demonstrate that EGFRIII-specific CAR T cells recognize and eliminate antigen-positive glioblastoma tumors in vitro and in vivo without cross-reacting with wild-type receptors present on normal tissues.[1339–41]

NEUROBLASTOMA

In contrast to glioblastoma, neuroblastoma originates from immature neurons and mostly occurs in infants and young children. Multiple targets, including GD2 and CD171, have been identified and tested for development of CAR T-cell therapy. GD2 is expressed on tumors of neuroectodermal origin, including neuroblastoma and melanoma.[42] In a preclinical study, GD2-specific CAR T cells exhibited potent cytotoxicity and cytokine production in response to antigen stimulation.[43] A phase I clinical trial by Louis et al[27] reported a complete remission rate of 27% (three of 11 patients) in patients treated with first-generation GD2-specifc CAR T cells without lymphodepletion. Furthermore, CAR T-cell persistence was observed for up to 192 weeks in this study.[27] CD171 is a surface antigen expressed on many types of cancer, including neuroblastoma. Functionally, CD171 has been reported to enhance tumor cell activity.[44] The first CD171-specifc CAR was developed by Gonzalez et al,[45] and the engineered T cells displayed robust antitumor activity in vitro. However, subsequent treatment with first-generation GD2-targeting CD8+ lymphocytes in clinical trials failed to control disease progression, and CAR T-cell persistence was inversely correlated with disease burden.[28] The authors speculated that the minimal antitumor response was due in part to the lack of coadministration of IL-2, which is especially critical to support the function of first-generation CARs. It is also worthwhile to note that absence of a CD4+ subset in transferred T cells may have compromised function and persistence; emerging data indicate that optimal CAR T-cell efficacy requires both CD4+ and CD8+ compartments.[46]

Prospects

Efficient CAR T-cell trafficking and localization to the tumor site are prerequisites for optimal antitumor efficacy. This is especially challenging for neuro-oncological malignancies such as glioblastoma because of limited T-cell infiltration in brain. CAR T cells modified to express chemokine receptors, such as chemokine receptor 2, have shown improved trafficking and tissue homing in a neuroblastoma model.[47] An alternative strategy is to target the tumor vasculature. Local delivery of tumor necrosis factor α (TNF-α) has been reported to upregulate the expression of adhesion molecules, such as vascular cell adhesion protein 1 and intracellular adhesion molecule 2 on endothelial cells, and to enhance T-cell infiltration.[48] Therefore, genetically modifying CAR T cells to secrete TNF-α is one

potential approach to overcome this limitation and improve CAR T-cell efficacy. Combining CAR T cells with lenalidomide has been reported to enhance the formation of immune synapses and improve persistency of CAR T cells in vivo,[49] providing a rationale for combinatorial approaches for CAR T-cell therapy.

HEAD AND NECK CANCER

A target of particular interest is the ErbB receptor family, which contains four members, designated EGFR (or ErbB-1), ErbB-2 (HER2 or neu), ErbB-3, and ErbB-4.[50] ErbB receptors are transmembrane tyrosine kinase proteins that promote cell growth and inhibit apoptosis. Overexpression of these receptors, especially ErbBl and ErbB2, have been observed in many malignancies, such as head and neck, breast, and lung cancers.[51–53] ErbB receptors can exist either in homodimeric or heterodimeric configurations,[54] and it has recently been appreciated that the transforming potential of the heterodimeric configuration is superior.[55] In addition, targeting individual ErbB receptors often results in acquired resistance because of enhanced activity of nontargeted receptors. In light of this, Davies et al[56] developed a second-generation CAR that incorporates a chimeric polypeptide, TIE, designed to achieve broad specificity for the ErbB network. ErbB-specific CAR T cells recognized and lysed several ErbB-positive tumor cell lines in vitro. These cell lines showed expression of a broad range of receptor combinations. In SCID-beige mice, CAR T-cell administration led to the eradication of established xenografts derived from ErbB1/2-overexpressing and ErbB2/3-overexpressing tumors. All four ErbB receptors are widely expressed in normal tissues, albeit at lower levels, which could lead to on-target, off-tumor toxicity. Van der Stegen et al[57] examined treatment toxicity in SCID-beige mice after delivery of the ErbB-specific CAR T cells via different routes. Compared with the intraperitoneal route, intratumoral delivery promoted tumor regression without eliciting any cytokine release syndrome. Consideration of intratumoral delivery has been proposed in clinical trials.[58]

Prospects

Multiple mechanisms have been exploited by cells in head and neck squamous cell carcinoma to escape immune surveillance. Data suggest that 55% to 65% of head and neck squamous cell carcinomas express PD-L1, which binds to its cognate receptor PD-1 on T cells, and suppress immune responses.[59] The presence of infiltrating regulatory T cells also contributes to the immunosuppressive tumor microenvironment via secretion of IL-10 and transforming growth factor β and via direct inhibition of T cells.[60] Therefore, strategies to optimize T-cell efficacy for head and neck squamous cell carcinoma could involve rational combinations of anti-PD-1/PD-L1 antibody with CAR T cells or armored CAR T cells modified to secrete blocking PD-1/PD-L1 scFvs.

BREAST CANCER

HER2 and mesothelin are two TAAs currently under investigation. Amplification of *HER2* oncogene leads to uncontrolled cell proliferation and occurs in approximately 20% of breast cancers.[61] Globerson-Levin et al[62] generated a HER2-specific, second-generation CAR containing CD28 and fragment crystallizable receptor (FcyR) signaling domains and tested

its efficacy in a syngeneic mouse mammary tumor model. Transduced T cells exhibited potent cytotoxic capacity and cytokine secretion upon antigen recognition.[62] In addition, repeated injections of HER2-directed CAR T cells eliminated spontaneous HER2-positive tumors and enhanced survival in transgenic mice. Mesothelin is a glycoprotein expressed on a broad range of solid tumors, with limited expression on normal tissues.[63] Mesothelin expression has been shown to be enriched in triple-negative breast cancer and is associated with poor outcomes.[64] Patients with triple-negative breast cancer are not suitable for targeted therapy or hormone therapy, so adoptive transfer of mesothelin-specific CAR T cells offers an alternative option. Tchou et al[65] engineered mesothelin-specific CAR T cells and reported a cytolytic capacity against primary breast tumor cells in vitro. However, in vivo antitumor activity was not evaluated in this study.

Prospects

A major therapeutic challenge to therapy in breast cancer is acquired resistance that results from antigen escape. For instance, under selective pressure, HER2 can undergo proteolysis to cleave the extracellular domain without compromising kinase activity. One approach to overcome this limitation is to use a dual-targeting CAR system, in which engineered T cells coexpress two CARs that recognize two distinct antigens. Redirected T cells can be activated in the presence of either antigen, in essence creating an or-switch, to mitigate antigen-loss escape.[66] Alternatively, CAR T cells can be modified to secrete inflammatory cytokines, such as IL-12, or costimulatory ligands, such as 4-1BB ligand, to stimulate an endogenous immune response against tumor cells via epitope spreading.[6768]

NON–SMALL CELL LUNG CANCER

Overexpression of *EGFR* is commonly seen in patients with non–small cell lung cancer, and small molecules inhibiting EGFR kinase activity have shown therapeutic benefits. Feng et al[29] reported efficacy of second-generation EGFR-specific CAR T cells that incorporate CD137 and CD3ζ signaling domains. In vitro antitumor efficacy was demonstrated via potent cytotoxicity and by interferon γ (IFN-γ) and IL-2 secretion in response to EGFR-positive lung carcinoma cells. In a phase I clinical study, two of 11 patients with refractory non-small cell lung cancer experienced a partial response after treatment with second-generation EGFR-specific CAR T cells after lymphodepletion. CAR T cells were detected in the peripheral blood of treated patients along with detection of CAR T cells at tumor sites, and eradication of EGFR-positive tumor cells was noted in post-treatment biopsies.[29] Mesothelin and carcinoembryonic antigen are also two attractive targets because of their elevated expressions in non-small cell lung cancer.[69,70] Multiple preclinical studies have reported antitumor efficacy of mesothelin- and carcinoembryonic antigen–specific CAR T cells against antigen-positive tumors, such as ovarian and liver cancers. However, direct evidence of antitumor efficacy against primary tumor samples or lung cancer cell lines has not been evaluated.[71–74]

MESOTHELIOMA

In addition to breast and lung cancer, mesothelin is overexpressed on the majority of mesotheliomas. Carpenito et al[71] engineered several mesothelin-specific CARs that used

different combinations of costimulatory domains and compared their antitumor efficacy. Despite equivalent cytotoxicity in vitro, third-generation CARs, which contained CD137 and CD28 costimulatory domains in tandem, showed marginally superior tumor rejection in a subcutaneous mesothelioma tumor model compared with second-generation CARs that had either costimulatory domain alone. In a separate study, a fully humanized second-generation anti-mesothelin CAR mediated tumor elimination in vitro and in vivo.[72] Importantly, CAR T-cell activation was not subverted by soluble tumor-secreted or recombinant mesothelin. This mitigates the concern that CAR T cells could be blocked or preoccupied by the soluble portion of mesothelin detected in some patients. In addition to CAR development, identifying an optimal route of administration has been explored. Using an orthotopic mesothelioma xenograft model, Adusumilli et al[73] showed that intrapleural delivery of second-generation mesothelin-directed CAR T cells vastly outperformed intravenous delivery, requiring 30-fold fewer CAR T cells to induce tumor eradication. In a phase I clinical trial, four patients with advanced mesothelioma or pancreatic cancer were treated with repetitive intravenous infusions of second-generation mesothelin-specific CART cells. Moderate antitumor responses were observed, and CAR T cell persistence and trafficking to the tumor site were detected. Interestingly, this study also reported induction of an antitumor humoral immune response after CAR T-cell therapy, evidenced by an elevated antibody response to a variety of tumor-associated proteins. This observation highlights the potential of CAR T-cell therapy to elicit a systemic immune response targeted to a broader range of antigens mediated via epitope spreading.[17] One patient experienced anaphylaxis and cardiac arrest after the third infusion on this trial, and this adverse event was believed to be associated with the development of antibodies against the murine-derived scFv.[75]

Prospects

Like many other solid tumors, lung cancer and mesothelioma possess an immunosuppressive microenvironment. Overexpression of inhibitory molecules, such as PD-L1 and indoleamine 2,3-dioxygenase (IDO) by tumor cells and myeloid-derived suppressor cells have been reported in patients with non-small cell lung cancer or mesothelioma.[76–78] Multiple strategies, including additional modification of CAR T cells and combinatorial approaches, can be adopted to overcome these obstacles and enhance CAR T-cell efficacy. For instance, CAR T cells can be engineered to express dominant negative PD-1 receptors[79] or anti-PD-1/PD-L1 agents to promote resistance to such inhibition.[11] In addition, rational combinations with PD-1/PD-L1 blockade antibody or IDO inhibitors may restore CAR T-cell activity.

OVARIAN CANCER

Several antigens have been exploited as targets for CAR T-cell therapy in ovarian cancer. Barber et al[80] engineered a first-generation NKG2D receptor CAR that recognizes the cognate NKG2D ligand expressed on ovarian cancer cell lines and patient-derived primary ovarian cancer samples. In both cell lines and primary samples, these CAR T cells were activated, secreted proinflammatory cytokines, and lysed tumor cells in an NKG2D-dependent fashion. In vitro efficacy and repression of flank-implanted ovarian cancer cells in a xenogeneic model using HER2/neu-directed second-generation CAR T cells also have

been reported.[81] The Lewis-Y (Le^{Y+}) antigen is a carbohydrate molecule that has been shown to be overexpressed on 70% of epithelial-derived tumors.[82–84] Westwood et al[85] designed a CD28ζ second-generation CAR directed against Le^{Y+} tumors, one of which included ovarian cancer in an OVCAR-3 tumor model. These CAR T cells showed significantly enhanced IFN-γ production, proliferation, and cytotoxicity when exposed to Le^{Y+} OVCAR-3 cells.[85] Furthermore, treatment with Le^{Y+}-specific CARs inhibited growth of flank-implanted OVCAR-3 in immunodeficient NOD-SCID mice. Another TAA under development is MUC-16. MUC-16 is a membrane-associated molecule that belongs to the mucin family of glycoproteins.[86] The extracellular domain of MUC-16 is cleaved into a soluble antigen (cancer antigen 125 [CA-125]), leaving a retained portion (MUC-16-CD) that can be targeted by adoptively transferred engineered T cells.[15] Chekmasova et al[15] engineered a second-generation (CD28ζ MUC-16-CD–directed CAR that showed efficacy against OVCAR-3 and patient-derived tumor samples. Armored CAR T-cells which have been engineered to secrete IL-12 directed against MUC-16-CD have been shown to be superior in vitro and in vivo to second-generation MUC-16-CD–directed CARs.[87] Similarly, mesothelin, a glycoprotein molecule expressed on pleural, pericardial, and peritoneal cells[88] has been explored as a TAA in ovarian cancer. Carpenito et al[71] reported notable in vitro cytotoxicity using mesothelin-directed third-generation (CD28/4-1BBζ CAR T cells. Folate receptor α(FRα) is a cell surface–anchored glycosylphosphatidylinositol molecule[89] that is highly expressed on ovarian cancer cells,[90] and it has been shown to be predictive of negative outcomes in patients with ovarian cancer.[91] On the basis of the preclinical efficacy of folate receptor–directed CAR T cells,[92] Kershaw et al[32] conducted a phase I clinical trial using first-generation FR-positive–specific CAR T cells with or without exogenous IL-2 in patients with relapsed/refractory epithelial ovarian cancer. All 14 patients treated in this study had progressive disease. There was no reported decline in CA-125 or antitumor response.[32] In one of the cohorts in this study, the adoptively transferred cells were labeled with indium-111 to facilitate in vivo imaging. After intravenous administration, most of the labeled T cells persisted in the lungs, without any evidence of specific localization to the tumor sites. This finding partially explained the decreased systemic persistence and lack of efficacy in this trial.

Prospects

The inhibitory tumor microenvironment in ovarian cancer, including the highly suppressive ascitic microenvironment,[93] is an important obstacle that needs to be addressed for CAR T cells to be successful in this disease. One approach is to armor the CAR T cells with soluble cytokines, such as IL-12,[21] a proinflammatory cytokine that has been shown to enhance the cytotoxic capability of effector T cells[94] and to reprogram dendritic cells and myeloid-derived suppressor cells.[95] Potential combinations of checkpoint blockade with second-generation or armored CAR T cells also could be explored as a means to augment CAR T-cell efficacy via recruitment of endogenous effector T cells.[96,97]

PROSTATE CANCER

Prostate stem-cell antigen and PSMA are two of the most commonly used target antigens for CAR T-cell therapy for prostate cancer. Predominantly found on prostate tissue, prostate

stem-cell antigen is a glycosylphosphatidylinositol-anchored antigen located on the cell surface.[98] In contrast, PSMA is a type II transmembrane protein that reportedly is present at low levels on the cytosolic/apical surface of normal prostate tissue.[99] However, during malignant transformation to prostate adenocarcinoma, it translocates to the extracellular/luminal side of the epithelium.[100] Zhong et al[8] generated a PSMA-directed third-generation CAR by engineering the 4-1BB receptor costimulatory molecule in tandem with CD28 and CD3ζ (named P28BBζ) and tested its efficacy against a human prostate cancer cell line in an SCID/beige mouse model. These CAR T cells showed robust proliferation and cytotoxicity in vitro. In tumor-bearing mice, treatment with P28BBζ greatly enhanced survival compared with control mice. Mechanistically, these T cells showed increased intracellular signaling and enhanced production of granzyme, IFN-γ, TNF-α, and granulocyte-macrophage colony-stimulating factor. Hillerdal et al[101] also have reported efficacy of a prostate stem-cell antigen–directed third-generation CAR that uses CD28 and OX-40 costimulatory molecules. In addition to robust proliferation, cytokine production, degranulation, and cytotoxicity upon recognition of prostate stem-cell antigen-expressing cells, these CAR T cells also were able to significantly delay subcutaneous tumor growth and prolong survival in nude mice. A phase I clinical trial by Junghans et al[102] reported a response rate, by prostate-specific antigen level, of 40% (two of five patients) with a first-generation PSMA-directed CAR after non-myeloablative preconditioning and concurrent IL-2 administration. In another phase I report, Slovin et al[30] reported tolerability and systemic persistence of up to 2 weeks with second-generation PSMA-directed CART cells.

Prospects

TAMs have been implicated in prostate cancer.[103] Specifically, TAMs are recruited to and infiltrate the tumor stroma in a colony stimulating factor-1 (CSF-1)/CSF-1 receptor (CSF-1R) –dependent fashion,[104] where it has been shown to promote tumor and vascular growth[105] and to mediate resistance to hormonal therapy.[106] In experimental models, clodronate-mediated depletion of TAMs led to notable inhibition of tumor growth.[105] One approach to optimize CAR T-cell therapy for prostate cancer might involve preconditioning therapy with either pharmacologic (AZD6495) or antibody-mediated (anti–CSF-1R) depletion of TAMs before CAR T-cell administration. Alternatively, second-generation CAR T cells can be armored via additional genetic modifications to secrete soluble CSF-1R inhibitors.

RENAL CELL CARCINOMA

Carboxy-anhydrase-IX (CA-IX) expression in metastatic renal cell carcinoma has been exploited as a target for adoptive transfer of engineered T cells.[18] CA-IX is a metalloprotease that reversibly catalyzes the hydration of carbon dioxide.[107] Although it is useful as a TAA in renal cell carcinoma, it also is expressed on several normal tissues, such as the gastric mucosa epithelium, small intestine epithelium, duodenum, and biliary tree.[108] In addition, expression of CA-IX is inducible in many other tissues under hypoxic conditions.[109] In preclinical studies, Weijtens et al[110] showed robust cytokine production and cytotoxic activity of first-generation CA-IX–directed engineered T cells against renal carcinoma cells. Lamers at al[31] initially treated three patients with CA-IX–positive

metastatic clear cell renal cell carcinoma with first-generation CA-IX–specific CAR T cells and exogenous IL-2 administration without nonmyeloablative preconditioning. Two of these patients developed grade 2 to 4 liver enzyme toxicity, and liver biopsies showed cholangitis that involved T-cell infiltration around bile ducts and confirmation of CA-IX expression on the biliary ductal epithelium. Furthermore, all three patients developed antibodies against the murine-derived scFv. To abrogate any more toxicity, the investigators pre-administered unmodified antibody from which the scFv was derived (cG250) to saturate and protect the liver before CAR T cell administration. With this amended approach, Lamers et al[18] successfully eliminated treatment-associated hepatoxicity in all four patients who received antibody pretreatment. Curiously, they were unable to detect any human anti-mouse antibodies against the cellular product in patients who underwent antibody pretreatment, which suggests that perhaps the nonspecific inflammation caused by the cholangitis contributed to the generation of human anti-mouse antibodies. Despite CAR T-cell persistence of 3 to 5 weeks, there were no clinical responses.[18]

Prospects

Myeloid-derived suppressor cells[111,112] have been shown to facilitate T-cell suppression via arginase-mediated down-regulation of the T-cell receptor ζ chain.[113] Increased levels of circulating regulatory T cells also have been reported in patients with renal cell carcinoma[114] and are inversely corelated with survival.[115] Sunitinib is a U.S. Food and Drug Administration–approved multikinase inhibitor for the treatment of metastatic renal cell carcinoma, and it has been shown to decrease myeloid-derived suppressor cells,[116] enhance type-I IFN responses, and decrease regulatory T cells function in patients with renal cell carcinoma.[117] Could sunitinib be used as preconditioning and maintenance therapy after CAR T-cell administration? This hypothesis could readily be subject to testing with a second-generation or armored CARs in a syngeneic model of metastatic renal cell carcinoma.[118]

SARCOMA

Although sarcomas represent a heterogeneous group of mesenchymal-derived neoplasms, there has been some success in identifying TAAs that are expressed across different sarcoma subtypes. Ahmed et al[119] exploited the expression of HER2 on osteosarcomas by engineering a second-generation HER2-directed CAR construct. These HER2-specific T cells showed robust cytokine production, proliferation, and cytotoxicity in vivo. Adoptive transfer of these genetically modified T cells effectively treated both localized and metastatic osteosarcoma in SCID mice. Second-generation (CD28ζ NKG2D ligand-directed CAR T cells also have shown efficacy in preclinical in vitro models of Ewing sarcoma.[120] Another approach reported by Huang et al[121] involved generation of an anti–IL-11 receptor α chain (IL-11Rα) second-generation CAR. IL-11Rα expression has been reported on multiple tumor types, including osteosarcoma,[122] prostate cancer,[123] and breast cancer.[124] Signaling via the IL-11/IL-11Rα pathway has been shown, among many other things, to promote osteoclastogenesis.[125,126] IL-11Rα–specific CAR T cells were effective against both primary tumors and pulmonary metastasis in a nude mouse model of osteosarcoma.[121] In a phase I/II trial by Ahmed et al,[33] 19 patients with HER2-positive sarcoma were treated with

second-generation HER2-specific CAR T cells without nonmyeloablative preconditioning. Adoptively transferred cells were detectable for up to 9 months in a fraction of treated patients. Furthermore, in patients who underwent metastatectomy 9 to 15 weeks after CAR T-cell therapy, HER2-specific CAR T cells were detected in the tumor samples by qualitative polymerase chain reaction.[33] Of the 17 evaluable patients, four had stable disease for as long as 12 weeks to 14 months. Three patients who underwent metastatectomy after CAR T-cell therapy remained in remission for up to 16 months.

Prospects

The importance of angiogenesis and vascular invasion in sarcoma has been well described.[127] In addition, the presence of M2-polarized TAMs has been reported, and these cells also could contribute to pathologic vasculogenesis via VEGF production.[128] Could CAR T cells be additionally modified to secrete soluble VEGF inhibitors? Perhaps they could be used in combination with anti-VEGF antibodies or multikinase inhibitors like pazopanib or sunitinib? Preconditioning or combination with immune-modifying agents, such as trabectedin[129] or mifamuritide, which act against monocytes/macrophages, could be explored as a means to optimize CAR T-cell efficacy for this disease.

CONCLUSION

Despite enthusiasm for adoptive immunotherapy, many obstacles must be addressed before CAR T-cell therapy joins the armamentarium for management of solid tumors. In tumor types that have more than one TAA, there is the question of which is the optimal target to minimize tumor escape via antigen loss/downregulation. When more than one TAA is expressed, could scFvs against both antigens be engineered in an or-activation or and-activation configuration to combat tumor heterogenicity or to improve safety, respectively? The prerequisite for nonmyeloablative preconditioning also must be rigorously assessed in syngeneic solid tumor models and clinical trials. There might be a hypothetical benefit to remodeling the endogenous lymphoid populations in anticipation of activation/recruitment by specifically armored CAR T cells, but this remains to be tested. Appropriate preclinical models and mechanisms of efficacy and resistance to CAR T-cell therapy also should be explored, ideally before clinical development. Driven mostly by the importance of demonstrating antitumor efficacy against human cancer cell lines, the clear majority of preclinical CAR T cell validation experiments have been in the context of SCID/beige or other immunodeficient tumor models. These models potentially could underestimate the immunomodulatory effect of the endogenous immune systems of the hosts and the effects of the immunosuppressive tumor microenvironment on adoptively transferred T cells. Consequently, more effort is being directed at understanding the interaction of the tumor microenvironment and the endogenous immune system in immunocompetent mouse models in addition to the prerequisite xenogeneic research. The route of CAR T-cell administration also could be tailored to each solid tumor malignancy according to what is known about each tumor’s biology. For example, clinical trials of intrapleural and intraperitoneal administration of CAR T cells for mesothelioma and ovarian cancer, respectively, are in progress. Lingering issues with toxicities in the form of cytokine release syndrome, neurotoxicity, and off-tumor cytotoxicity also are being investigated. Ultimately, knowledge

of how best to mitigate these toxicities, coupled with rational combinations of chemotherapy, surgery, radiotherapy, or immunomodulators, will pave the way for the next breakthroughs in CAR T-cell therapy for solid tumor malignancies.

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KEY POINTS

- CAR T-cell therapy has emerged as a promising immunotherapeutic approach for solid tumor malignancies and several promising candidates are in early-phase clinical trials.
- Despite tumor and antigen heterogeneity, several TAAs such as MUC-16, GD2, EGFRIII, mesothelin and PSMA have been identified as targets for CAR T-cell therapy.
- Clinical responses have been reported in a small subset of solid tumor malignancies; however, increased response rates and responses across a broader range of tumor types are required.
- CAR T-cell efficacy is limited by various intrinsic and extrinsic factors, including poor trafficking to tumor site and an immunosuppressive tumor microenvironment.
- Further genetic engineering to optimize CAR design (armored CAR T cells) or combinatorial approaches with cytotoxic, targeted therapy, and immunomodulatory agents are currently under investigation.

TABLE 1

Selected Clinical Trials of CAR T-Cell Therapy for Solid Tumors

Trial	Tumor	Target	CAR Design	Phase	Best Response Data
Brown et al[26]	Glioblastoma	IL-13Rα2	CD3ζ	I	*
Louis et al[27]	Neuroblastoma	GD2	CD3ζ	I	CR, 27%; 19 patients**
Park et al[28]	Neuroblastoma	CD171	CD3ζ	I	PD
Feng et al[29]	Non–smallcell lung cancer	EGFR	4-1BB/CD3ζ	I	PR, 18%; SD, 45%; 11 patients
Beatty et al[17]	Mesothelioma/pancreatic cancer	Mesothelin	4-1BB/CD3ζ	I	PR, 50%; 2 patients
Junghans et al[30]	Prostate cancer	PSA	CD3ζ	I	PR, 40%; 5 patients
Lamers et al[18,31]	Renal cell carcinoma	CAIX	FcRγ	I	PD
Kershaw et al[32]	Ovarian cancer	Folate receptor α	FcRγ	I	PD
Ahmed et al[33]	Sarcoma	*HER2*	CD28/CD3ζ	I/II	SD, 24%; 17 patients
NCT02209376	Glioblastoma	EGFRIII	4-1BB/CD3ζ	I	N/A
NCT01454596	Malignant glioma	EGFRIII	CD28/CD3ζ	I/II	N/A
	Glioblastoma				
	Brain cancer				
NCT02664363	Glioblastoma	EGFRIII	†	I	N/A
NCT02208362	Glioblastoma	IL-13Rα2	4-1BB/CD3ζ	I	N/A
NCT02311621	Neuroblastoma	CD171	4-1BB/CD3ζ	I	N/A
	Ganglioneuroblastoma		CD28/41BB/CD3ζ		
NCT01822652	Neuroblastoma	GD2	CD28/OX40/CD3ζ	I	N/A
NCT01818323	Head and neck cancer	*ErbB*	CD28/CD3ζ	I	N/A
NCT02547961	Breast cancer	*HER2*	CD28/CD3ζ	I/II	N/A
NCT02349724	Lung cancer	CEA	†	I	N/A
	Colorectal cancer				
	Gastric cancer				
	Breast cancer				
	Pancreatic cancer				
NCT02414269	Malignant pleural disease	Mesothelin	CD28/CD3ζ	I	N/A

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Trial	Tumor	Target	CAR Design	Phase	Best Response Data
	Mesothelioma				
	metastases				
	Lung cancer				
	Breast cancer				
NCT02159716	Pancreatic cancer	Mesothelin	4-1BB/CD3ζ	I	N/A
	Ovarian cancer				
	Mesothelioma				
NCT01583686	Cervical cancer	Mesothelin	†	I/II	N/A
	Pancreatic cancer				
	Ovarian cancer				
	Mesothelioma				
	Lung cancer				
NCT01140373	Prostate cancer	PSMA	CD28/CD3ζ	I	N/A
NCT02498912	Ovarian cancer	Muc-16	CD28/CD3ζ	I	N/A
NCT00902044	Sarcoma	*HER2*	CD28/CD3ζ	I	N/A
NCT02107963	Sarcoma	GD2	OX40/CD28/CD3ζ	I	N/A
	Osteosarcoma				
	Neuroblastoma				
	Melanoma				

*Patients underwent craniotomy before CAR therapy.

**Patients with NED before CAR therapy were not included in denominator of responders.

†Not listed on clinicaltrials.gov.

Abbreviations: CAR, chimeric antigen receptor; CEA, carcinoembryonic antigen; CR, complete response; EGFRIII, EGFR variant III; FcR, fragment crystallizable receptor; GD2, disialoganglioside GD2; IL-13Rα2, interleukin-13 receptor α2; MUC-16, mucin 16; N/A, not applicable; NED, no evidence of disease; PD, progressive disease; PR, partial response; PSA, prostate-specific antigen; PSMA, prostate-specific membrane antigen; SD, stable disease.