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Review Article

Chimeric Antigen Receptor T-Cell Therapy for Solid Tumors: The Past and the Future

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ABSTRACT

Chimeric antigen receptor (CAR) T-cell therapy is the new standard treatment for various indications in patients with advanced hematologic malignancies. Despite the several preclinical and early phase clinical trials, the overall clinical experience has been disappointing when applying this innovative therapy in solid tumors. The failure of CAR T-cell therapy and its limited antitumor activity in solid tumors have been attributed to several mechanisms, including tumor antigen heterogeneity, the hostile tumor microenvironment and poor trafficking of CAR T cells into tumor sites, and the unacceptable toxicities in some settings, among others. However, remarkable improvements have been made in understanding many of these failure mechanisms for which several emerging novel approaches are being applied to overcome these challenges. In this review, after a brief historic background for immunotherapy in solid tumors, we highlight the recent developments achieved in CAR T-cell designs, summarize completed clinical trials, and discuss current challenges facing CAR T-cell therapy and the suggested strategies to overcome these barriers.

Keywords: immunotherapy, chimeric antigen receptor, CAR T-cell, solid tumors

INTRODUCTION

Despite the major advances in chimeric antigen receptor (CAR) T-cell therapy for hematologic malignancies and several preclinical data to support its utility in solid tumors, the clinical experience has been disappointing when applying this innovative therapy in solid tumors. Various hypotheses, and perhaps resistance mechanisms, have been suggested to explain the limited antitumor activity and failure of CAR T-cell therapy in solid tumors. However, remarkable advances have recently been made in our understanding of many of these failure mechanisms. Several emerging novel approaches are currently being applied in the clinic to overcome these challenges and improve CAR T-cell outcomes for these high-risk solid tumor patients with an unmet need.

Immunotherapy has emerged over the past decade as one of the most powerful therapeutic modalities for hematologic and solid malignancies, with breakthrough Food and Drug Administration (FDA) approvals in several cancer subtypes. The principle of immunotherapy and its introduction in the clinic to treat cancer patients dates to the late 19th century with what was modified and known later as the Coley toxin.[1] Several immune-based therapies have been tried since, but most of which failed due to excessive toxicity and/or lack of activity. However, several of these failed attempts and with some encouraging results in selected cancer patient populations, provided the "proof of concept" for further developments in the field to harness the immune system in fighting and perhaps curing cancer. One of the most evidenced indications for the ability of immunotherapy to cure cancer comes from a long-term experience with the use of allogeneic stem cell transplantation in advanced hematologic malignancies. The initial concept of allogeneic stem cell transplantation, "the prototype" for immunotherapy in hematologic neoplasms, was based on delivering myeloablative doses of chemotherapy and/or radiotherapy to treat cancer; however, we learned with time that the real benefit leading to cure is mediated by the donor-derived T lymphocytes resulting in a graft-versus-tumor effect.[2] In solid tumors, the success of immunotherapy dates back to the 1980s; it was most evidenced by the efficacy of cytokine therapy (which promotes immune cell activity) in kidney cancer and melanoma. Interferon alpha and high-dose interleukin (IL)-2 were associated with durable remissions and

potential cures in a small subgroup of patients with renal cancer and melanoma.[3–7]

Since these earlier modest achievements, the spectrum of cancer immunology research and immunotherapy has been growing rapidly. There have been remarkable achievements in cancer immunology using the active (treatments that trigger an endogenous immune response) and passive (relies on ex vivo generation of immunotherapeutics) immunotherapy-based approaches. Although the overall experience with cancer vaccines has been disappointing, checkpoint inhibitors (CPIs) emerged as one of the most successful active immunotherapeutics in the past decade. The immune checkpoints are thought to blunt the immune surveillance mechanisms against cancer; therefore, targeting these receptors or their ligands could potentially unleash the immune system through different mechanisms and promote T-cell activity.[8,9] Ipilimumab was the first CPI to obtain FDA approval in 2011 for metastatic melanoma.[10] However, several CPIs have since been granted approvals and are currently incorporated into the standard treatments of various cancer subtypes, mostly in solid tumors.

Monoclonal antibodies and adoptive cellular therapy (ACT) are two passive immunotherapy strategies that have been successfully applied to several cancer subtypes. In contrast to CPIs, which have more indications and clinical activity in solid cancers, most of the FDA-approved monoclonal antibodies are being used in patients with hematologic malignancies, with rituximab (CD20 monoclonal antibody) being the first to get approval in 1997 for patients with B-cell non-Hodgkin lymphomas (NHL). Similarly, the major successes of ACT have been noted in patients with hematologic malignancies. The following three ACT strategies have been extensively studied and are now being explored in clinical trials:

(1) Tumor-infiltrating lymphocytes, which are based on naturally existing lymphocytes within the tumor that are collected and expanded ex vivo before they are reinfused to target cancer cells.
(2) Engineered T-cell receptor (TCR) T-cells, which rely on peripheral blood mononuclear cells collection to obtain nontherapeutic T-cells; they are then genetically engineered to express a tumor-specific TCR capable of targeting a specific peptide–human leukocyte antigen complex on the tumor cell.
(3) CAR T-cells, the focus of our review and which also require peripheral blood mononuclear cells collection. The T cells are genetically engineered similar to TCRs but the CARs are non-major histocompatibility complex (MHC) restricted and rather target a specific tumor surface antigen through a single-chain variable fragment derived from a tumor-specific antibody.

Similar to the paradigm shift of immunotherapy in solid tumors that occurred with immune CPIs, CAR T-cell therapy has caused a major paradigm shift in cancer immunotherapy for hematologic malignancies; it is considered one of the most innovative immunotherapeutic approaches in cancer history. Six CAR T-cell products have been approved since 2017, making CAR T-cell therapy a new standard for several indications in patients with advanced relapsed or refractory hematologic malignancies. In contrast, except for the very recent first breakthrough historic FDA approval in January 2022 of a TCR product in patients with metastatic uveal melanoma,[11] CAR T-cell therapies and other ACTs remain investigational in solid tumors.

In this review, we have provided a brief overview of the recent developments and improvements in CAR T-cell designs, summarized the completed clinical trials using CAR T-cell therapy in solid tumors highlighting the negative and/or positive findings, and finally discussed the current challenges facing CAR T-cell therapy in solid tumors and the suggested strategies to overcome these barriers.

PROGRESS IN CAR T-CELL DESIGNS

The concept of developing CARs capable of triggering T-cell activation and recognizing any potential antigen, independent of the MHC status (unlike the TCRs), dates back to at least 1987, as described by Kuwana et al.[12] Briefly, the CARs are recombinant receptors that consist of an antigen-binding domain derived from a monoclonal antibody (single-chain variable fragment is commonly used for this purpose) and an intracellular signaling or stimulatory domain (CD3ζ molecule is the most commonly used), hence the name *chimeric*. The CAR is then genetically transfected into T cells using different viral or nonviral methods. When infused into subjects with targeted tumors, these "redirected" engineered CAR T cells can bind to tumor antigens, leading to T-cell activation and tumor killing by cytokine release and direct cytotoxicity mechanisms. The initial CAR designs (first-generation CARs) showed encouraging antitumor activity in preclinical studies but, when used in early phase clinical trials, were limited in their clinical activity, likely due to suboptimal T-cell activation and persistence in vivo. One of the earliest first-generation CAR T-cell studies in solid tumors was published in 2006.[13] The CAR construct in this study was designed to target ovarian cancers expressing an associated antigen α-folate receptor. The results showed the feasibility and safety of these first-generation CAR T cells, but no responses were noted, and large numbers of circulating CAR T cells were only identified in the first 2 days after the infusion with rapid clearance afterward.

There has been remarkable progress in developing better CAR constructs over the past two decades, with five generations of CARs tested and/or being tested in several preclinical and clinical studies. Figure 1 illustrates the key differences in the five generations of the CAR constructs. All the currently FDA-approved commercially

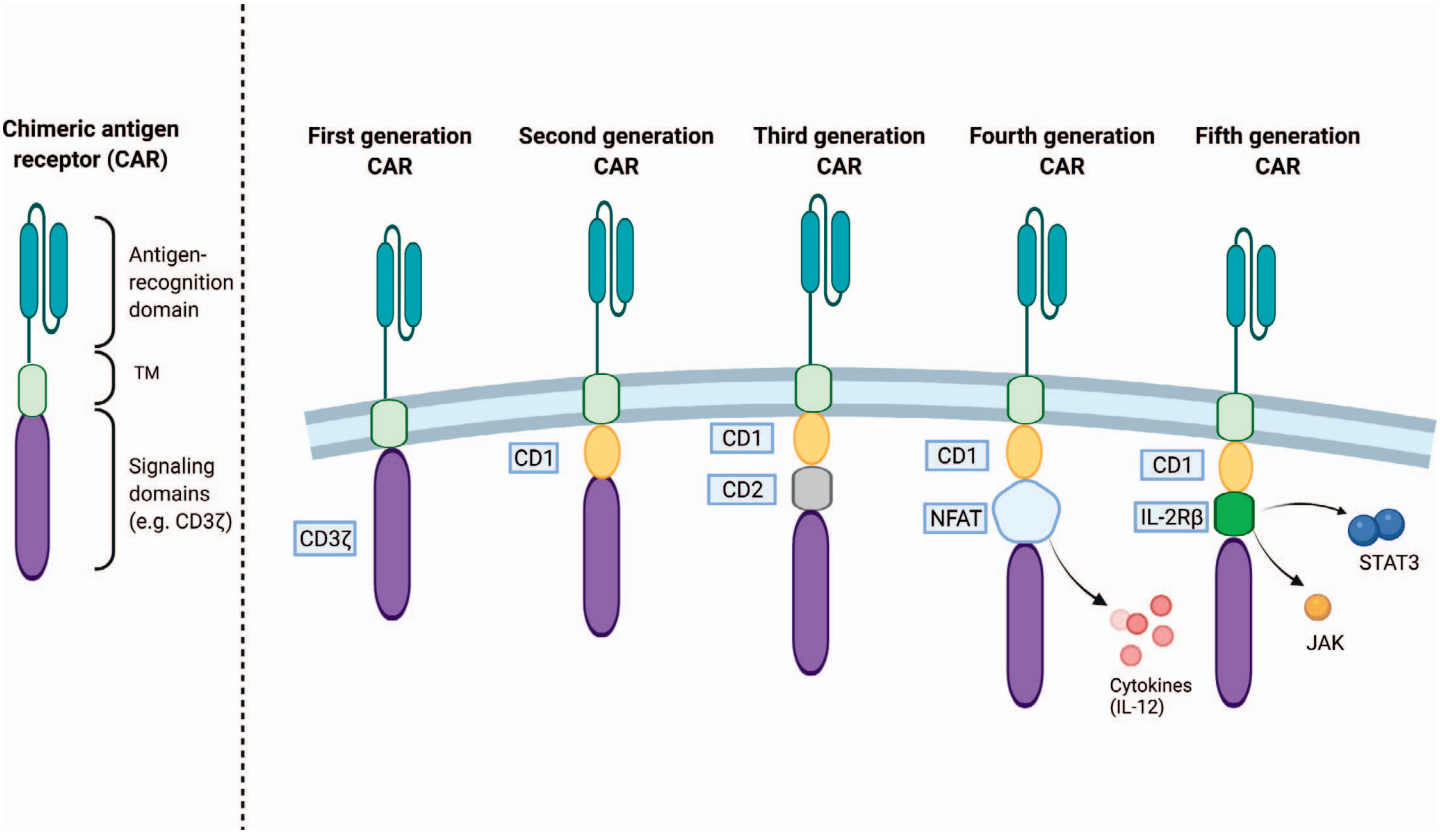

Figure 1. (Left panel) Basic characteristics of the original CAR T-cell design consisting of an extracellular antigen-recognition domain and intracellular signaling domains linked by a TM domain. (Right panel) Evolution of CAR T-cell generations. The first-generation CARs contained only CD3ζ as the signaling domain that activates T cells. CD3ζ usually maintains the cytotoxic effector function of CAR-T cells. The next generations of CARs consist of modifications added to the first and second CAR generations. Second-generation CARs included one CD (CD1) linked to CD3ζ. Third-generation CARs consisted of two CDs (CD1, CD2) linked to CD3ζ to improve CAR T-cell cytotoxicity and persistence; CD28, OX40, and 4-1BB are examples of the CDs. The fourth-generation CARs consisted of second-generation CARs paired with gene cassettes for cytokine (e.g., IL-12) production under the control of an NFAT transcription factor. The fifth-generation CARs are also derived from the second-generation CARs, with the addition of a JAK-STAT activation domain derived from intracellular domains of cytokine receptors (e.g., IL-2Rβ).

CAR: chimeric antigen receptor; CD: costimulatory domain; IL: interleukin; JAK: Janus kinase; NFAT: nuclear factor of activated T-cells; STAT: signal transducer and activator of transcription protein; TM: transmembrane. Created with BioRender.com.

available CAR T-cells belong to the second-generation CAR T cells. The key difference from the first-generation CARs is the incorporation of costimulatory domains (e.g., CD28 and 4-1BB) in the second-generation CARs, which have led to significant improvement in T-cell activation, expansion, and persistence and translated into an excellent antitumor activity in clinical trials in hematologic malignancies. Most early phase clinical trials in solid tumors (as described in the following section) used second-generation CAR T-cell constructs but with limited antitumor activity compared with what has been achieved in hematologic malignancies. Third-generation CARs were designed by encompassing two costimulatory domains to improve the potency against tumor cells.[14] A phase 1 clinical trial by Ramos et al[15] compared third-generation CD19 CAR T cells with second-generation CD19 CAR T cells with encouraging results, showing superior expansion and longer persistence with third-generation CARs. However, clinical data are still limited on whether third-generation CAR T cells would lead to clinically meaningful improvements in patient outcomes without increasing the risk of CAR T-cell–related toxicities. The fourth-generation CARs are being explored to overcome some of the limitations of the second and third generation CAR constructs, particularly in solid tumors where antigen-negative cancer cells are thought to be one of the key reasons for inadequate response and progression. These constructs are like second-generation CARs in design but add an inducible expression cassette encoding a transgenic cytokine (e.g., IL-12), which could lead to cytokine release in the tumor lesions; hence activating and attracting the innate immune cells that can target the antigen-negative cancer cells. Most fourth-generation studies remain in the preclinical phase,[16–18] but some phase 1 clinical trials reported encouraging results in hematologic malignancies.[19,20] Finally, a fifth-generation CAR construct is currently under investigation. Instead of adding an inducible expression cassette as in the fourth-generation CARs, it incorporates intracellular

cytoplasmic domains of cytokine receptors with additional binding capabilities (e.g., IL-2 receptor β-chain domain with a STAT3-binding tyrosine-X-X-glutamine motif).[21,22] This fifth-generation CAR modeling demonstrated in preclinical studies improved expansion and superior effector functions compared with second-generation CARs.

CAR T CELL THERAPY IN CLINICAL TRIALS FOR SOLID TUMORS

The unprecedented success of CAR T-cell therapy in hematologic cancers envisioned a new hope for similar achievements in solid tumors. Over the past few years, an exponential rise in preclinical and clinical studies exploring the role and applicability of CAR T-cell therapy in solid tumors was witnessed. Several preclinical and clinical studies targeting almost all solid tumor subtypes have been published and/or are still ongoing. Although CAR T-cell therapy in solid tumors has not yet proven effective, many tumor-associated antigens and neoantigens have been identified as potential targets. The tumor antigens that are frequently targeted in clinical trials include carcinoembryonic antigen (CEA), human epidermal growth factor receptor 2 (HER2), mesothelin, disialoganglioside 2 (GD2), glypican-3, CD133, epidermal growth factor receptor variant III (EGFRvIII), IL-13 receptor subunit alpha 2 (IL13RA2), among others (Table 1). Table 2 summarizes the published CAR T-cell clinical trial data in solid tumors. We review here the key findings from selected published CAR T-cell clinical trials that targeted various tumor-specific antigens in several solid tumors.

The first published phase 1 clinical experience in solid tumors was in patients with advanced metastatic epithelial ovarian cancer and used first-generation CAR T cells targeting folate receptors.[13] No responses were noted in this study, with notable poor in vivo expansion and persistence of the engineered T lymphocytes. Another disappointing experience with first-generation CARs was in patients with renal cell carcinoma–targeting carbonic anhydrase IX (CAIX): no responses were noted, but significant on-target off-tumor hepatobiliary toxicity was observed.[23,24] Among other urogenital neoplasms, prostate cancer held earlier promises to benefit from immunotherapeutic approaches after the accelerated FDA approval of sipuleucel-T for metastatic castration-resistant prostate cancer in 2010.[25] Despite strong preclinical data, clinical trials have had limited activity in prostate cancer with either CPIs or CAR T-cell therapies. However, two more recent phase 1 prostate-specific membrane antigen (PSMA)-directed CAR T-cell therapy clinical trials hold some promise. The first was a first-generation CAR but used adjunctive IL-2, and the second used a second-generation CAR armored with a dominant-negative transforming growth factor (TGF)-β receptor.[26,27]

Table 1. Summary of the potential targeted antigens in solid tumors

Tumor Antigen	Tumor Type
AFP	Liver
AXL	Kidney
B7-H3 (CD276)	Ovary, pancreas, sarcoma, neuroblastoma, glioblastoma
CAIX	Kidney
CD44v6	Sarcoma, colorectal
CD70	Kidney
CD133	Cholangiocarcinoma, liver
CD147	Liver
CEA	Colorectal, pancreas
CEACAM5	Gastrointestinal
Claudin18.2	Pancreas, gastric
DLL3	Lung
EGFR	Lung, cholangiocarcinoma, sarcoma
EGFRv3	Glioblastoma
EpCAM	Gastrointestinal, prostate
FRα	Ovary
GD2	Melanoma, sarcoma, neuroblastoma
Glypican-3	Liver
HER2	Pancreas, glioblastoma, colorectal, breast, sarcoma
HLA-G	Kidney
IL13-Rα2	Glioblastoma
L1-CAM (CD177)	Neuroblastoma
LMP-1	Nasopharyngeal
MAGE-A4	Melanoma, sarcoma, ovary, gastrointestinal
Mesothelin	Mesothelioma, breast, lung, pancreas, ovary
MUC1	Breast, pancreas, lung
MUC16	Ovary
NKG2D	Colorectal
PD-L1	Lung
PSCA	Prostate, pancreas
PSMA	Prostate cancer
ROR1	Breast
TAG-72	Ovary, colorectal
VEGFR2	Metastatic cancers

AFP: alpha-fetoprotein; AXL: AXL receptor tyrosine kinase; CAM: cell adhesion molecule; CAIX: carbonic anhydrase IX; CD: cluster of differentiation; CEA: carcinoembryonic antigen; DLL3: delta-like 3; EGFR: epidermal growth factor receptor; EGFRv3: epidermal growth factor receptor variant 3; EpCAM: epithelial CAM; FRα: folate receptor alpha; GD2: disialoganglioside 2; HER2: human epidermal growth factor receptor 2; HLA-G: human leukocyte antigen G; IL: interleukin; IL13-Rα2: IL-13 receptor alpha 2; L: ligand; LMP: Epstein-Barr virus latent membrane protein 1; MAGE-A4: melanoma antigen gene protein A4; MUC: mucin; NKG2D: natural killer group 2D; PD-L1: programmed cell death ligand-1; PSCA: prostate stem cell antigen; PSMA: prostate-specific membrane antigen; ROR1: receptor tyrosine kinase-like orphan receptor 1; TAG-72: tumor-associated glycoprotein 72; v: variant; VEGFR2: vascular endothelial growth factor receptor 2.

Cancers known to have poor a prognosis, such as glioblastoma and pancreatic ductal adenocarcinoma, have been of special interest for a potential role of CAR T-cell therapy. Several tumor-specific antigens, such as HER2, IL-13Ralfa2, and EGFRv3, have been tested in brain tumors. HER2-specific CAR T cells were used without dose-limiting toxic effects, but objective responses were noted in one patient only ($n = 24$).[28] Local administration (intracranial into the resection cavity) of

Table 2. Summary of the published clinical trials for CAR T-cell therapy in solid tumors

Year	Tumor Subtypes	Target	Sample Size	Outcome	Reference
2006	Ovary	FRα	14	No response	Kershaw et al[13]
2007	Neuroblastoma	CD171	6	NR	Park et al[82]
2010	CRC	HER2	1	Died	Morgan et al[36]
2011	Neuroblastoma	GD2	19	3 CR of 11 active disease	Louis et al[34]
2013	RCC	CAIX	12	No response	Lamers et al[24]
2015	GBM	IL13Ra2	3	Transient	Brown et al[29]
2015	Ovary	MUC16	6	NR	Koneru et al[83]
2015	GBM	HER2	16	1 PR and 4 SD for up to 24 mo	Ahmed et al[84]
2015	Sarcoma	HER2	19	17 evaluable: 4 with SD for up to 14 mo	Ahmed et al[37]
2016	Prostate	PSMA	5	2 PR	Junghans et al[26]
2017	CRC	CEA	10	2 PR 7 SD up to 30 wk	Zhang et al[48]
2017	Neuroblastoma	GD2	11	5 SD	Heczey et al[35]
2017	CEA-positive tumors	CEA	14	No response	Thistlethwaite et al[49]
2017	GBM	EGFRv3	10	1 SD for 18 wk	O'Rourke et al[3]2
2018	CD133-positive tumors	CD133	23	3 PR, 14 SD	Wang et al[56]
2018	HNSCC	EGFR	13	ORR: 69%	Papa et al[85]
2018	Biliary and pancreatic cancers	HER2	11	1 PR 5 SD	Feng et al[39]
2018	PDAC	Mesothelin	6	3 SD	Beatty et al[40]
2019	Pleural tumors	Mesothelin	20	14 with PD1 therapy: 2 CR, 5 PR, 4 SD	Adusumilli et al[42]
2019	Mesothelin-positive tumors	Mesothelin	15	11 SD	Haas et al[86]
2019	Gastric, pancreas	Claudin18.2	12	1 CR, 3 PR, 5 SD	Zhan et al[51]
2019	MUC1-positive tumors	MUC1	13	9 SD	Li et al[87]
2019	GBM	EGFRv3	18	No response	Goff et al[33]
2019	CEA-positive tumors	CEA	8	2 SD	Katz et al[47]
2019	PSCA-positive tumors	PSCA	15	8 SD	Becerra et al[88]
2019	GD2-positive tumors	GD2	12	1 CR, 2 PR	Yankelevich et al[89]
2019	TNBC	ROR1	4	1 PR 2 SD	Specht et al[90]
2019	CRC	NKG2D	8	NR	Van Cutsem et al[91]
2020	Lung	PD-L1	1	Serious AE	Liu et al[92]
2020	HCC	Glypican-3	13	2 PR and 1 SD for 44 mo	Shi et al[93]

AE: adverse event; CAIX: carbonic anhydrase IX; CEA: carcinoembryonic antigen; CR: complete response; CRC: colorectal carcinoma; EGFR: epidermal growth factor receptor; EGFRv3: epidermal growth factor receptor variant 3; FRα: folate receptor alpha; GBM: glioblastoma multiforme; GD2: disialoganglioside 2; HCC: hepatocellular cancer; HER2: human epidermal growth factor receptor 2; HNSCC: head neck squamous cell carcinoma; IL13-Rα2: interleukin-13 receptor alfa 2; MUC1: mucin1; NR: not reported; ORR: overall response rate; PD: programmed cell death; PDAC: pancreatic ductal carcinoma; PD-L1: programmed cell death ligand 1; PR: partial response; PSCA: prostate stem cell antigen; PSMA: prostate-specific membrane antigen; ROR1: receptor tyrosine kinase-like orphan receptor 1; SD: stable disease; TNBC: triple negative breast cancer.

IL13Rα2-specific CAR T cells is feasible and safe, with encouraging clinical responses reported in a first-in-human pilot study in 2015.[29] Furthermore, the same group reported a case of recurrent multifocal glioblastoma who received IL13Rα2 CAR T cells via multiple intracavitary infusions with an excellent response that lasted 7.5 months.[30] A more recent study by another group reported on the feasibility of repeated locoregional infusions of HER2-specific CAR T cells in the first three treated patients with relapsed or refractory central nervous system tumors.[31] Intravenous administration of EGFRvIII-specific CAR T cells was examined in two studies with high-grade gliomas. The first study included 10 patients, and lymphodepletion was not used. Although no responses (one stable disease) were achieved, infiltration of CAR T cells into tumor was noted in five of seven tested patients,[32] which holds promise for the ability of intravenously infused CAR T cells to traffic into the intracranial tumor sites. The second study allowed lymphodepletion and included 18 patients, but no responses were noticed again.[33] GD2 is another tumor antigen receptor that was targeted in a few CAR T-cell studies for patients with neuroblastoma with promising early results. In one study, which included 11 patients with active disease at the time of infusion, three achieved complete remission, and no dose-limiting toxicities were observed.[34] In a more recent study that included 11 patients and used third-generation GD CAR T cells, lymphodepletion was associated with improved CAR T-cell expansion but adding a programmed cell death-1 (PD-1) inhibitor did not further enhance the expansion or persistence of these cells.[35]

In addition to brain tumors, HER-2 is known to be expressed in several other cancers, including breast, gastrointestinal, pancreatic and hepatobiliary, lung, and sarcomas. Hence, HER2 can be a substantiated target for CAR T-cell therapy. A fatal on-target off-tumor lung toxicity hampered earlier attempts for its successful progress in a young patient with metastatic colon cancer.[36] Since then, a few studies have shown the feasibility of using HER2-targeted CAR T-cell therapy. Two main studies reported encouraging outcomes in sarcoma, an orphan heterogeneous malignancy that can frequently be chemoresistant with poor prognosis. Nineteen patients with HER-2–positive sarcomas were treated in one study; four had stable disease (some of

which lasted up to 14 months).[37] In the second phase 1 study (published in abstract format), 10 patients were treated with HER2 CAR T-cell therapy, two and three patients achieved CR and stable disease, respectively, and five had progressive disease.[38] For patients with pancreatic and biliary carcinomas, 11 patients were enrolled in a phase 1 clinical trial, all of whom received HER2 CAR T-cell infusions.[39] One patient achieved an objective partial response; five had stable disease with a median progression-free survival of 4.8 (range, 1.5–8.3) months.[39]

Mesothelin is a cell-surface antigen expressed in several solid tumors, including lung cancer, mesothelioma, ovarian, and pancreatic carcinomas. Its tumor expression is generally associated with a more aggressive cancer disease and worse prognosis. Several ongoing and published studies are exploring CAR T-cell therapy for mesothelin-expressing tumors. In a small phase 1 study in pancreatic ductal adenocarcinoma ($n = 6$), two patients had stable disease for 3.8 and 5.4 months with messenger RNA CAR T-cell therapy targeting mesothelin with no notable associated toxicities.[40] In another small report (published in abstract format), six patients (four did not receive lymphodepletion) with epithelial ovarian cancer were treated with second-generation intravenous mesothelin-targeting CAR T-cell therapy.[41] Three of four patients with tumor samples had evidence of CAR T-cell infiltration, and all patients reported having stable disease at 1 month, but no information was provided on the duration of stable disease.[41] In a recent phase 1 clinical trial that included 27 patients (25 with malignant pleural mesothelioma (MPM) and 1 each with metastatic lung and breast cancers), regional (rather than intravenous) mesothelin-targeted CAR T-cells were administered with no dose-limiting toxicities.[42] Eighteen patients (all with MPM) in this study also received intravenous anti–PD-1 pembrolizumab, which is hypothesized to decrease T-cell exhaustion and improve antitumor efficacy.[42] Among the 23 MPM patients who received lymphodepletion with or without pembrolizumab, two achieved a partial response, and 11 had stable disease. Of the patients who received pembrolizumab and had measurable disease ($n = 16$), two achieved partial response, and nine had stable disease, eight of which had disease control for over 6 months.[42]

Oncofetal antigens are proteins typically present only during fetal development but are expressed in some cancer subtypes in adult patients. CEA is known for its prognostic value and to be nearly expressed in all colorectal cancers,[43,44] but the antigen is also expressed in various other cancers,[44,45] which makes it an attractive target for CAR T-cell directed therapy. Two phase 1 clinical trials conducted by the same study group reported their experience using hepatic intraarterial CEA CAR T-cell infusions for patients with CEA-positive metastatic carcinomas to the liver with no safety concerns, including no on-target off-tumor toxicity.[46,47] Only one achieved stable disease from the six evaluable patients in the first study.[46] In the more recent study, investigators added adjunct therapy using selective internal radiotherapy, but only two patients achieved stable disease.[47] Two CEA-targeted CAR T-cell clinical trials reported their results using intravenous systemic mode. The first study included 10 patients with metastatic colorectal cancers, the CEA CAR T-cell therapy was tolerated, and seven patients achieved stable disease, of which two remained with disease control for over 30 weeks.[48] The second study included 14 patients with various CEA-positive metastatic carcinomas, including colorectal, gastric, esophageal, gastroesophageal, and pancreatic.[49] No objective responses were noted, but seven had stable disease, with three maintaining response at 12 weeks.[49] However, this study was prematurely terminated, given the safety concerns for on-target off-tumor toxicity (acute respiratory toxicity) and the lack of prolonged CAR T-cell persistence.[49]

Claudin 18.2 is another tumor antigen marker of interest which is expressed in various cancers,[50] including gastric and pancreatic carcinoma, which are generally associated with poor prognosis. One study reported interim results in an abstract format where 12 patients with gastric ($n = 7$) and pancreatic ($n = 5$) adenocarcinomas were treated with claudin 18.2-specific CAR T cells.[51] The preliminary results were very encouraging, where no safety on-target off-tumor toxicities were noted. For the 11 evaluable patients for efficacy, four (33.3%) had an objective response (including one patient with gastric carcinoma who achieved a complete response) with a median progression-free survival of 130 days.[51] Five additional patients had stable disease, and only two had disease progression at first assessment after CAR T-cell infusion. In a more recent phase 1 clinical trial, which included 37 patients (gastric carcinoma, $n = 28$; pancreatic cancer, $n = 5$; other cancers, $n = 4$) treated with claudin18.2-targeted CAR T-cell therapy, the results were very encouraging: an objective response rate of 48.6% (all partial responses) and a 6-month duration of response rate of 44.8%.[52] Of note, the cytokine release syndrome (CRS) rate was 94.6% in this study, all of which were low grades 1 to 2.

This brief overview of most of the published clinical trials exploring the role of CAR T-cell therapy in solid tumors, although disappointing when compared with the achievements made in hematologic malignancies, does highlight several facts and developments in the field that help us better understand the barriers facing CAR T cells in solid tumors. In addition to the safety lessons learned from these studies, we saw some very encouraging results, such as the successful trafficking and infiltration into tumors and the remarkable objective responses noted in selected patients, all of which reaffirm and provide a proof of concept for the utility of CAR T cells in solid tumors. We will summarize in the following section some of the challenges facing CAR T-cell therapy in solid tumors and the suggested strategies for improvement.

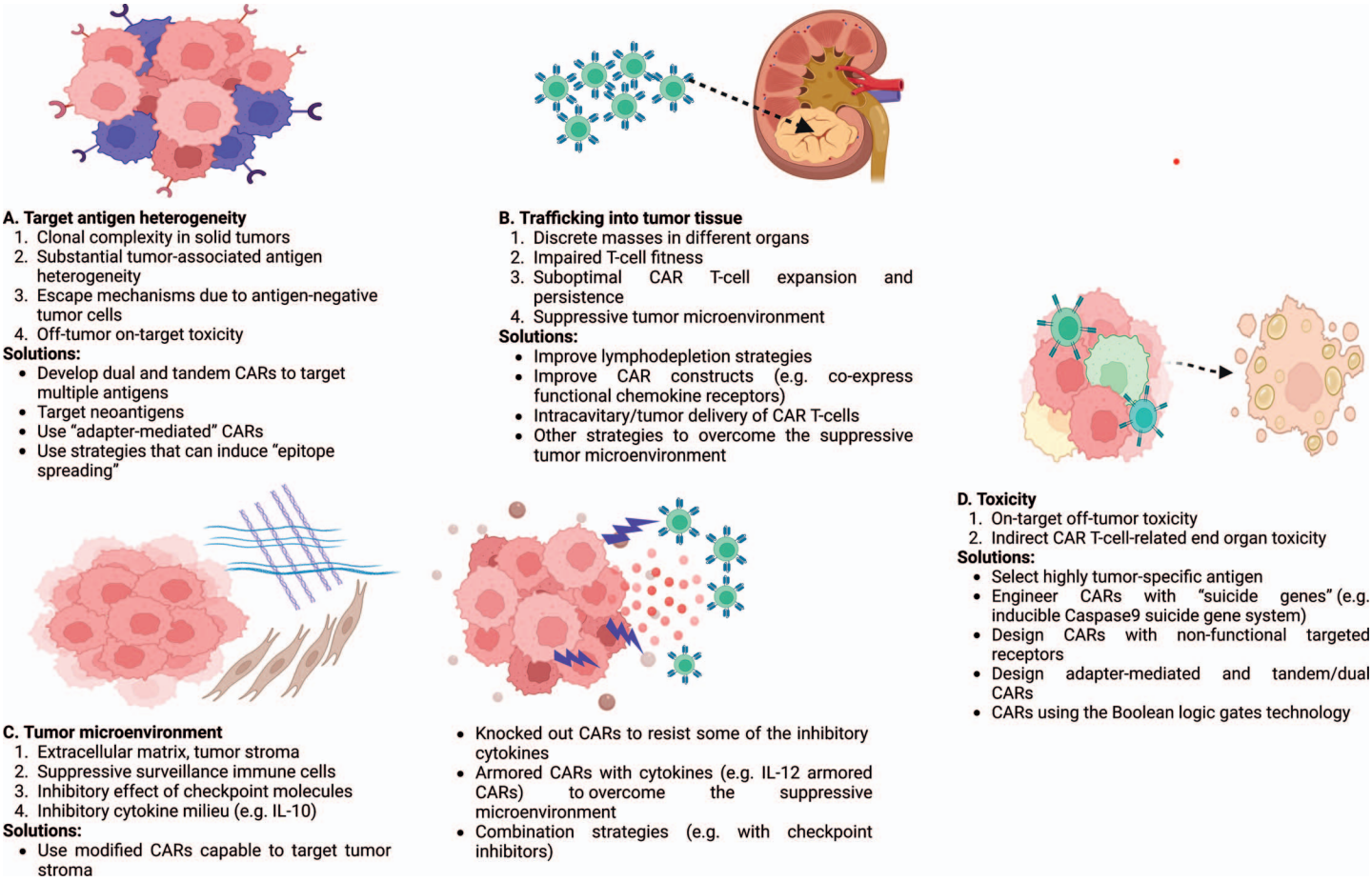

Figure 2. Challenges facing CAR T-cell therapy in solid tumors and the proposed strategies to overcome them. (A) Target antigen heterogeneity. (B) Trafficking into tumor tissue. (C) The suppressive tumor microenvironment. (D) Toxicity related to CAR T-cell therapy.

CAR: chimeric antigen receptor; ECM: extracellular matrix; IL: interleukin; PD: programmed cell death. Created with BioRender.com.

HURDLES FACING CAR T-CELL THERAPY IN SOLID TUMORS AND IMPROVEMENT STRATEGIES

Despite adapting a similar CAR T-cell manufacturing platform used in hematologic neoplasms, most clinical trials in solid tumors failed to show consistent deep and/or durable responses. However, the overall experience and findings from these clinical trials lead researchers to continue their efforts to better understand the reasons behind the failure mechanisms and propose strategies to overcome them. In the following section, we will summarize some of these hurdles and challenges that may have contributed to the failure of CAR T cells in solid tumors and will briefly highlight some of the suggested strategies to overcome these hurdles. Figure 2 provides a visual summary of the failure mechanisms and the proposed strategies for improvement.

Target Antigen and Tumor Heterogeneity

Selection of appropriate tumor-associated antigen with high affinity and specificity and no or minimal expression on healthy tissues is the first critical step for engineering a "directed" CAR T-cell therapy to serve the purpose of targeted tumor cytolytic activity. The safety and efficacy of the designed construct greatly rely on identifying an ideal target that is predominantly and selectively expressed on tumor cells and that would be an essential molecule for sustaining the survival of the tumor cells. The expression of a selected tumor antigen in healthy tissues, even at lower levels, could potentially produce life-threatening adverse effects. In contrast to hematologic malignancies, there is much more clonal complexity in solid tumors and a substantial tumor-associated antigen heterogeneity that makes the selection of a suitable target more complex and challenging. This heterogeneity in tumor antigens contributes partly to the immune escape mechanisms that can lead to a lack of response and/or relapse. For instance, the low-antigen or antigen-negative tumor cells can proliferate and cause disease progression. Hence, a potential solution has been proposed to target multiple antigens simultaneously or sequentially. To that point, significant advances in the potential use of dual and tandem CARs have been achieved. For instance, HER2/mucin-1–specific dual CAR T-cells for breast cancer and PSMA/prostate

stem cell antigen dual CAR T-cell for prostate cancer showed feasibility in preclinical studies.[53,54] Unlike the dual CARs, tandem CAR T cells contain a single, bivalent CAR capable of identifying two different antigens.[55] Fourth-generation CARs, which can activate innate T cells to eliminate antigen-negative tumor cells, are being explored as another reasonable intervention with encouraging preliminary results. Several ongoing studies are trying to overcome this heterogeneity obstacle; one such attempt targets CD133 cancer stem cells.[56]

In contrast to tumor-associated antigens that can also be expressed in healthy tissues, neoantigens are more tumor-specific antigens that are exclusively expressed on tumor cells; hence when applicable, these neoantigens should be selected as alternative ideal targets in solid tumors. One clinical experience targeting neoantigens was with the EGFRvIII CAR T cell in glioblastoma multiforme, which showed encouraging results with no on-target off-tumor toxicity.[57] However, most neoantigens are intracellular and cannot be easily targeted by the CAR T-cell. In contrast to TCR T cells, CAR T cells are generally directed against surface antigens. There have been recent attempts to improve the CAR constructs, allowing them to target intracellular tumor-associated antigens and neoantigens. Another strategy being explored to overcome tumor antigen heterogeneity is through "epitope spreading" process; this phenomenon was observed in a preclinical study combining CAR T-cell with a vaccine therapy (acts via triggering an extensive proliferation of T cells to infiltrate tumors) induced remissions and decreased disease relapse. Furthermore, "adapter-mediated" CARs are being explored for their potential to improve directed CAR T cells against the targeted tumor. In this model, CARs are similar to the conventional second-generation CAR constructs; however, the extracellular domain does not recognize and interact directly with the tumor-associated antigen but to a binder site in the adaptor molecule that directs the CAR T cell to the targeted antigen-expressing tumor cells. These adapted-mediated CARs can potentially have the advantage of precisely controlling CAR T-cell activity by controlling the administration of the adaptor molecule.[58]

Tumor Microenvironment

Solid tumors are surrounded by a complex and hostile tumor microenvironment (TME), preventing CAR T cells from penetrating the tumors and/or inhibiting the activity of the infiltrating intratumoral T cells. Several mechanisms have been described to explain this hostile TME. In addition to several suppressive surveillance immune cells and the inhibitory cytokine milieu (e.g., TGF-β, PGE2, IL-10), physical (tumor stroma), and metabolic (nutrient starvation, hypoxia) factors can contribute to the hostile TME.[59,60] Regulatory T cells, myeloid-derived suppressor cells, and tissue-associated macrophages are examples of suppressive surveillance immune cells. Checkpoint molecules, PD-1, and CTLA-4 are shown to contribute to the inhibitory T-cell functions. Additionally, the extracellular matrix is one of the main barriers preventing T-cell infiltration into the tumor. This matrix contains heparan sulfate proteoglycans, and T cells lack heparanase to degrade it.[61]

Several attempts are being explored to overcome the hostile TME and improve CAR T-cell homing into the tumors. GD-2–targeting CAR-T cells with exogenous coexpression of heparanase was developed in a neuroblastoma xenograft model with encouraging results to eliminate the suppressive effect of the extracellular matrix.[62] TGF-β is one of the inhibitory factors in the TME, markedly elevated in patients with prostate cancer. In a recent phase 1 clinical trial that included 13 patients who received second-generation PSMA CAR T cells with knocked out TGF-β, encouraging antitumor responses were observed along with CAR T-cell expansion in blood and tumor trafficking.[63] To counteract the inhibitory effects of PD-1/CTLA-4, combining CPIs with CAR T cells is a promising strategy.[64] In mice models, PD-1 knockout CAR T cells secrete IL-12 and IL-18 and have a higher percentage of less differentiated T cells.[65] Carbonic anhydrase-targeted second-generation CAR T-cells armored to secrete anti–PD-L1 antibodies showed enhanced antitumor activity in orthotopic mice models of human clear cell renal cell carcinoma.[66] Despite the several encouraging preclinical studies using different approaches to address the inhibitory PD-1/PD-L1 pathway in the TME,[67] there remains no strong clinical data to support its efficacy. In a small report for patients with neuroblastoma using GD2-targeted third-generation CAR T cells, adding pembrolizumab did not improve CAR T-cell expansion and persistence or tumor responses,[33] and further studies are needed to prove the benefits of this approach. Other strategies are being explored, including combining CAR T cells with oncolytic viruses (armed with chemotactic cytokines) and armored CARs.[68,69] In one report, IL-12–armored CARs tested in murine ovarian peritoneal carcinomatosis to overcome the PD-L1–mediated inhibitory TME effect, inhibit tumor-associated macrophages, and alter the ascitic cytokine microenvironment.[70]

Trafficking to Tumor Site

Unlike hematologic tumors, whose cells can be readily available in circulation and an easy target for the CAR T cells, solid tumor cells form discrete masses in different organs where T cells cannot easily traffic through and infiltrate into the tumor cells to produce strong cytolytic activity. In addition to the suppressive TME itself described above, several other factors can affect CAR T-cell trafficking into tumor sites, including impaired T-cell fitness and survival before reaching tumor sites, suboptimal expansion and suboptimal persistence of CAR T cells. In addition to the strategies used to overcome the suppressive TME and the strategies to improve CAR design, several studies explore multimodality approaches to include modifications in the lymphodepletion plat-

form (to further suppress the host immune reactivity against CAR T-cells). The overall intent of these attempts is to improve CAR T-cell expansion and persistence, but with less T-cell exhaustion, and hence the increased chance for tumor infiltration with higher numbers of CAR T-cells, which could potentially lead to better antitumor activity. Chemokines have a critical role in T-cell activation and recruitment into tumor sites, and the expression of chemokine receptors in tumor sites correlates with T-cell infiltration and outcomes. Hence, one strategy that showed promise in several preclinical in vivo models for improved CAR T-cell homing and persistence was by using modified CAR constructs to co-express functional chemokine receptors (such as CCR2 and CXCR).[71–74] Another obvious way to overcome the trafficking barrier is to deliver CAR T cells directly into the tumor sites, which also allows for direct on-target activity with a lower chance for systemic absorption and on-target off-tumor toxicity.[75] This strategy has been tried in preclinical and clinical studies with mixed results.[76,77] One of the disadvantages of this invasive route of administration is its limited utility for most patients who will frequently have either advanced metastatic malignancy with multiple sites of involvement and/or metastatic lesions in sensitive hard-to-deliver areas.

Toxicity

Despite the excellent responses in hematologic malignancies, CAR T-cell therapy is associated with unique toxicities that can be potentially serious. Hence, CAR T-cell therapy requires patients to be treated in highly skilled centers and monitored closely after treatment. CRS and immune effector cell-associated neurotoxicity syndrome (ICANS) are two classic and frequently encountered toxicities in patients with hematologic cancers; however, their incidence and severity are relatively low in solid tumor patients receiving CAR T-cell therapy.[78] Based on the heavy experience in hematologic malignancies, most CRS and ICAN cases can be manageable with current standard therapies, including the use of systemic IL-6 monoclonal antibodies and corticosteroids. However, there remain some other rare but potentially serious and hard-to-treat complications, such as hemophagocytic lymphohistiocytosis, (which is associated with a high mortality rate) and profound, prolonged cytopenias that require, at times, rescue stem cell transplantation. Fortunately, these toxicities are not highly prevalent as reported in solid tumor clinical trials with published data, however, the possibility of on-target off-tumor toxicities remains a more concerning safety issue to watch out in these patients. The tumor antigens often expressed and targeted in solid tumors are mostly of epithelial origin and can be expressed in healthy epithelial cells, which could potentially cause on-target off-tumor toxicity. Despite the extensive preclinical and several clinical studies trying to predict tumor antigen specificity, the safety evidence remains elusive until the CAR construct is tried in first-in-human clinical trials. Even a low level of tumor antigen expression in healthy tissues may have detrimental side effects. This deleterious on-target off-tumor toxicity in solid tumors has been reported in several studies.[79] For instance, one extreme example was the case of acute respiratory distress and sudden death from anti-HER2 CAR-T in a patient with metastatic HER2 colorectal cancer.[36] In another experience using CAIX-targeted CAR T-cells for renal cell carcinoma, the study was terminated early because of severe on-target off-tumor hepatobiliary toxicity.[24]

Selecting a highly tumor-specific and tumor-selective antigen remains a priority for a safer and more effective CAR T-cell therapy. However, several studies have shown the feasibility of adapting different approaches to overcome the antigen specificity and some of these potentially serious toxicities. Using genetically engineered new CAR designs that carry "suicide genes" (e.g., through an inducible Caspase9 suicide gene system) showed promise in preclinical studies, and it had been validated in the allogeneic stem cell transplantation setting.[80] The suicide gene requires the administration of a synthetic dimerizing molecule so it can be activated, leading to rapid death of CAR T-cells. Another strategy, similar in concept to the suicide gene system, but instead designed the CAR constructs by co-expressing nonfunctional receptors that can be targeted by monoclonal antibodies leading to antibody-mediated CAR T-cell killing. One of the disadvantages of these models is the irreversible depletion of CAR T-cells, which could potentially compromise some of the clinical benefits for the responding patients. As described earlier, adapter-mediated CARs and tandem or dual CARs are alternative approaches. In addition to its ability to provide reversible control on the CAR T cells (controlling the adapter administration serves as a molecular safety switch), another advantage of the adapter-mediated CARs is that it is modifiable to target different tumor-associated antigens. Another interesting approach introduced recently is using the Boolean logic gates to generate autonomous CARs. These are self-controlled CARs of which activation and antitumor activity are triggered by more than one targeted surface antigen. This concept has been applied successfully in two preclinical approaches. First, through the "logic AND gates," which resembles dual CAR design but uses one receptor for signaling (SynNotch), which can, in turn, activate a fully functional CAR to target the tumor-associated antigen; hence, a tumor must have both of these antigens to be targeted that allows for high CAR T-cell tumor specificity. In a recent preclinical study targeting neuroblastoma, SynNotch gated CAR-T cell was developed, where GD2 served the signaling gate and B7H3 as the target, leading to high tumor specificity and activity.[81] The second approach is called the "logic NOT gates," where an inhibitory CAR is incorporated into the CAR construct. The inhibitory CAR targets an antigen expressed on

healthy tissues, and as such, the CARs will be deactivated when trafficking into the antigen-expressing healthy tissues.

CONCLUSION

Immunotherapy has revolutionized the landscape of treatment options for solid tumors, but only a small proportion of patients with advanced and/or metastatic cancers achieve durable responses and long-term survival. Hence, the continued unmet need for novel therapeutics to improve the outcomes of these high-risk patients. The success of CAR T-cell therapy in hematologic malignancies and the encouraging proof-of-concept anecdotal successes in some of the clinical trials in solid tumors, indicate a promising future for the use of CAR T-cell in solid tumors. Identifying tumor-specific antigens to serve as suitable targets and refining the CAR construct designs to improve T-cell trafficking and overcome the hostile TME are two main pathways of active current research activity in the hope of developing effective CAR T-cell therapies in solid tumors. Results of several ongoing clinical trials are eagerly awaited but based on the advances made in the field to date, the premise is to see in the coming few years breakthrough approvals for CAR T-cell in at least some of the solid tumors.

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