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REVIEW

Current Clinical Evidence and Potential Solutions to Increase Benefit of CAR T-Cell Therapy for Patients with Solid Tumors

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ABSTRACT

Immunotherapy by chimeric antigen receptor (CAR)-modified T-cells has shown unprecedented clinical efficacy for hematological malignancies. Recently two CAR T-cell based therapeutics, Kymriah (Tisagenlecleucel) and Yescarta (Axicabtagene ciloleucel) were approved by the US Food and Drug Administration and by the European Medicines Agency. Despite the progress in treating hematological malignancies, challenges remain for the use of CAR T-cell therapy in patients with solid tumors. Barriers yet to overcome for achieving effective CAR T-cell therapy include antigenic heterogeneity of solid tumors, an immune-suppressive microenvironment, and organ-specific properties that limit T-cell entry. This review will summarize available clinical data for CAR T-cell therapy in solid tumors, including present obstacles and promising strategies to advancement.

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Introduction

The genetic engineering of T lymphocytes with chimeric antigen receptors (CARs) has rapidly advanced from preclinical tumor models to Food and Drug Administration (FDA) and European Medicines Agency approval (EMA) for hematologic malignancies, and clinical-grade production. To date, however, solid tumors are less susceptible to CAR therapies and instead have been treated more successfully with immune checkpoint inhibitors (ICIs)[1] or tumor-infiltrating lymphocyte (TIL) therapy.[2] The interactions between antigen-presenting cells and T-cells allow high precision host protection against pathogens and cancer cells. T-cells have unparalleled ability to not only recognize these antigens (Ag) but also to establish long-term memory, allowing rapid and robust response upon rechallenge against a given Ag. Tumors express Ags that are recognized by T-cells, whereby mutations of self-Ags or germline cancer Ags differ sufficiently from normal Ags, or those that are less easily detected, such as overexpressed self-Ags or differentiation Ags, expressed by the tumor-originating tissue.[3] Hence, tumors that are more similar to normal cells, and particularly those with highly immune-suppressive features, escape surveillance (i.e.*via* immune editing), which results in their uncontrolled growth. Technological advances have created opportunities to enhance the effector functions of T-cells against cancer through reeducation and intelligent design to overcome the immune evasion mechanisms established by solid tumors. Adoptive cell therapy (ACT) consists in *ex vivo* enrichment of autologous tumor-specific cells and expansion to large numbers, and subsequent reinfusion into the patient to specifically target and kill cancer cells. ACT is conducted *via* two methods: (1) naturally arising TILs can be directly expanded *ex vivo* from a tumor lesion[2] or (2) non-therapeutic host lymphocytes obtained from the peripheral blood can be artificially rendered tumor specific *via* genetic engineering with a T-cell receptor (TCR)[4] or a chimeric Ag receptor (CAR).[5] The CAR is a hybrid antigen receptor, part antibody and part TCR, and is composed of an extracellular Ag-binding domain and intracellular signaling domain(s).[5] Genetic modification of a T-cell with a CAR provides a new Ag-specificity through the single-chain variable fragment (scFv), which is derived from a tumor-specific antibody.[5] The scFv allows the T cell to bind a tumor Ag and the T-cell activation cascade is initiated through the intracellular domains, derived from CD3ζ ITAM domains.[6] To complete the genetic construct for the CAR, a hinge and a transmembrane domain (TM), commonly from CD8α or immunoglobulin, bridges the extracellular scFv and intracellular CD3ζ ITAM domains. Its first use by Kuwana et al. and Gross et al. in the late 1980s revealed that redirection of a T-cell with this receptor could induce Ag recognition through the scFv, as for a native Ig, without classical major histocompatibility complex (MHC) restriction required by a TCR recognizing Ag-derived peptide.[7,8] These first-generation CAR T-cells had very limited persistence and anti-tumor efficacy *in vivo*.[9,10] The modular nature of the CAR technology allows constant optimization, which is how first-generation CARs, containing only the CD3ζ portion of the TCR were replaced with second-generation CARs containing an added costimulatory element such as CD28 or 4–1BB. The specificity of a TCR is for only a short peptide (8–12 amino acids), so there is potential for cross-reactivity to similar sequences of amino acids.[11] TCR ligation of self Ag can lead to T-cell activation, autoimmunity, and even death. To

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minimize this risk, T-cells require at least two signals to fully activate.[12] Second-generation CARs contain the two-signal model of T-cell activation including a CD28 costimulatory domain in tandem with CD3ζ ITAM domain. This supports *in vitro* T-cell activation and killing, but more importantly efficient tumor killing and long-term T-cell persistence*in vivo*.[13] In addition, costimulatory domains other than CD28, such as CD27, 4–1BB, and OX40, provide similar improvement to CAR T-cell function and persistence *in vivo*.[14,15]

CAR-redirected T-cell therapies have been successful in hematologic malignancies but are less effective in treating the majority of patients with solid tumors to date. This review will summarize available data from completed clinical trials of CAR T-cells in solid tumors and discuss present obstacles and promising strategies to advancement.

Overcoming tumor heterogeneity: which target? At what price?

CAR T-cell-based therapy is an innovative anticancer approach based on the specific recognition of a tumor Ag by the patient's own engineered T-cells. However, attempts to recapitulate the success achieved with CAR T-cells in B-cell malignancies for solid tumors have been disappointing. Table 1 summarizes the clinical trials of CAR T-cells that have been completed to date in patients with solid tumors and reports the clinical outcome and the toxicity profile.[16–33] The three main hurdles encountered for the application of CAR T-cell therapies to solid tumors are (1) the presence of tumor-associated Ags, which are generally cell-surface molecules not present on normal tissue, (2) the limited trafficking of adoptively transferred cells to tumor sites and (3) the immunosuppressive effect of tumor microenvironment (Figure 1).

Tumor-specific Ags, like the epidermal growth factor receptor variant III[29] (EGFRvIII), are among the ideal targets in the sense that they are uniquely present on cancer cells. Therefore the CAR T-cell with engineered specificity toward EGFRvIII will attack only tumor cells, and normal tissue cells should theoretically be ignored. Additional attractive targets of tumor cells are represented by proteins resulting from unique post-transcriptional modifications such as alterations of the glycosylation patterns of MUC1, MUC16, TAG72 or B7-H3.[34–36] Targeting tumor-selective Ag is an additional strategy: the Ag has to be expressed by tumor cells but at a much higher level than healthy cells, such as Human Epidermal growth factor Receptor 2 (HER2).[16] Consequently the effect on healthy cells should be negligible compared to that of tumor cells. A third class of Ag, represented by the Prostate Stem Cell Ag (PSCA),[37] is a tissue-specific Ag with very low expression in other tissues like pancreas or bladder. In this case, it is hoped that the side effects on healthy tissues will be minimal, as the elimination of PSCA-positive cells would not cause vital organ failure. Theoretically, even if the perfect Ag for a solid tumor could be identified and targeted, CAR T-cell therapies for solid tumors face further obstacles including poor trafficking to the tumor site,[38] as well as limited proliferation and persistence within the host.[39] Moreover, CAR T-cells can be functionally suppressed within the hostile tumor microenvironment.[40] These collective hurdles set solid tumor CAR-based therapies apart from liquid tumors.

Tumor heterogeneity is often major and makes it a crucial problem for CAR- T-cells.[41,42] A difficulty with the principle of CAR T-cells lies in the fact that cytotoxicity is based on a single Ag, even an improved one. Indeed, the tumor tissue, whatever the primitive, is known to evolve over time but also in its different metastatic locations. One solution is to attack several tumor Ags concomitantly, as this should allow maintenance of cytotoxic activity despite loss of one of the target Ags. Several strategies[43] are possible for targeting two Ags: the co-administration of two CAR T-cells each targeting a different Ag, the use of a bicistronic vector that leads to the expression of two distinct CARs on the same T-cell, the co-transduction of two vectors each encoding for one CAR, or the expression of a bispecific Tandem CAR.[44] Tandem CARs are constructed with two Ag specificities built in series in order to recognize two different tumor targets or to stimulate cytotoxicity with the second CAR recognizing a ubiquitous viral Ag such as CMV. Efficacy for bispecific CD19/CD22 CAR T-cells is under clinical evaluation for relapsed/refractory B-cell acute lymphoblastic leukemia.[45] In addition to increasing the specificity of the CAR to the tumor, this technique potentially minimize the "on-target/off-tumor" toxicity toward healthy cells with low-level single Ag expression.[46]

Tumor heterogeneity over time also includes loss or down-regulation of expression of the Ag of interest, leading to "Ag-negative" relapse, while tumor heterogeneity in space leads to the risk of dissociated response between different metastases. Targeting Ag expressed by the cells of the tumor microenvironment, such as the fibroblast activation protein (FAP), particularly expressed on cancer associated fibroblasts (CAFs), seems an attractive option.[47,48] Tran and colleagues[49] showed in a mouse model that, despite anti-FAP CARs displayed specific degranulation and production of effector cytokines in response to Ag stimulation *in vitro*, they did not mediate an efficient antitumor response *in vivo*, and unexpectedly, anti-FAP CARs caused severe cachexia and lethal bone toxicities. The FAP protein is also expressed by multipotent bone marrow stromal cells (BMSCs), hence the observed toxicity is linked to their expression. Interestingly, Kakarla and colleagues,[48] using an anti-FAP CAR with a different scFv, demonstrated antitumor efficacy without toxicities in a mouse model of lung cancer. The safety concerns generated by the work of Rosenberg et al.[49] are likely related to the specificity and affinity of the scFv, given that the last two studies with CAR T-cells with different scFvs recognizing highly positive FAP cells have a good toxicity profile. Given the potential for multi-modal antitumor effects of FAP targeting, rational combinations for future immunotherapeutic approaches should include stroma-targeting CAR T-cells with either antitumor CAR T-cells or ICIs.

As a living therapy, CAR T-cells bear the potential for rapid and massive activation , which contributes to their therapeutic efficacy but simultaneously underlies their side effects. The most well-
documented toxicity is called cytokine release syndrome (CRS), a systemic inflammatory response characterized by fever,

Table 1. Completed clinical trials of CAR T-cells in patients with solid tumors: target, outcome, and relevant toxicities.

Author	Clinical Trial	Journal	Year of publication	Antigen	Organ	N	Clinical outcome	Type of toxicity
Ahmed, N	NCT00902044	Journal of Clinical Oncology	2015	HER2	HER2 + Sarcoma	19	4 SD 13 PD 2 NE	CRS, off target
Ahmed, N	NCT01109095	JAMA oncology	2017	HER2	Glioma	24	1 PR 7 SD 8 PD	seizures and/or headaches
Beatty, GL	NCT01355965	Cancer Immunology Research	2013	Mesothelin	MPM/PDAC	2	1 PR 1 SD	CRS
Brown, CE	NCT00730613	Clinical Cancer Research	2015	IL13 Ra2	Glioma	3	NE	Headache, fatigue, myalgia, olfactory auras
Brown, CE	NCT02208362	The New England Journal of Medicine	2016	IL13Ra2	Glioma	1	1 PR	Headache, fatigue, myalgia, olfactory auras
Feng, K	NCT01869166	Science China-Life Sciences	2016	EGFR	EGFR+ NSCLC	11	2 PR 5 SD 4 PD	CRS, skin toxicity
Feng, K	NCT01935843	Protein Cell	2018	HER2	biliary tract and pancreatic cancers	11	1 PR 5 SD 5 PD	fever, fatigue, nausea/vomiting, Myalgia/ arthralgia, lymphopenia
Hege, KM	C-9701 and C-9702	Journal for ImmunoTherapy of Cancer	2017	TAG72	Colorectal cancer	23	No objective response	CRS, on target/off tumor
Junghans, RP	NCT00664196	The Prostate	2016	PSMA	Prostate cancer	5	2 PR	No toxicity attributed to PSMA CAR T-cells
Katz, SC	NCT01373047	Clinical Cancer Research	2015	ACE	CEA+ liver metastases	6	1 SD 5 PD	flulike symptoms, elevated liver enzymes, rash
Lamers, CHJ	DDHK97-29/ P00.0040 C	Biochemical Society Transactions	2016	CAIX	CAIX+ RCC	12	No objective response	CRS, on target/off tumor
Louis CU	NCT00085930	Blood	2011	GD2	Neuroblastoma	11	3 CR 1 PR 1 SD	Neurotoxicity
Louis CU	NA	Journal of Immunotherapy	2010	EBV	EBV+ nasopharyngeal carcinoma (8 in remission, 15 with active disease)	23	5/8 remain disease free 7/15 had CR or PR	None
O'Rourke, DM	NCT02209376	Science Translational Medicine	2017	EGFR vIII	Glioma	10	NE	CRS, off target, neurotoxicity
Tchou, J	NCT01837602	Cancer Immunology Research	2017	c-Met	Breast cancer	6	No objective response	CRS
Thistlethwaite FC	NCT01212887	Cancer Immunology, Immunotherapy	2017	CEACAM5	CEACAM5+ malignancy	14	No objective response	CRS, on target/off tumor
You, F	case report	Science China-Life Sciences	2016	MUC1	Seminal vesicle cancer	1	1 PR	None
Zhang, C	NCT02349724	Molecular Therapy	2017	ACE	ACE + colorectal cancer	10	7 SD 3 PD	CRS

Table legend: CRS: cytokine release syndrome; NA: not applicable; NE: not evaluable; PR: partial response; SD: stable disease; PD: progressive disease; CR: complete response; NSCLC: non-small cell lung cancer; RCC: renal cell carcinoma; MPM: malignant pleural mesothelioma; PDAC: pancreatic ductal adenocarcinoma.

The main hurdles avoiding CAR T-cells efficiency in solid tumors are schematized here, in association with promising approaches to overcome them; ICB: immune checkpoint blockade.

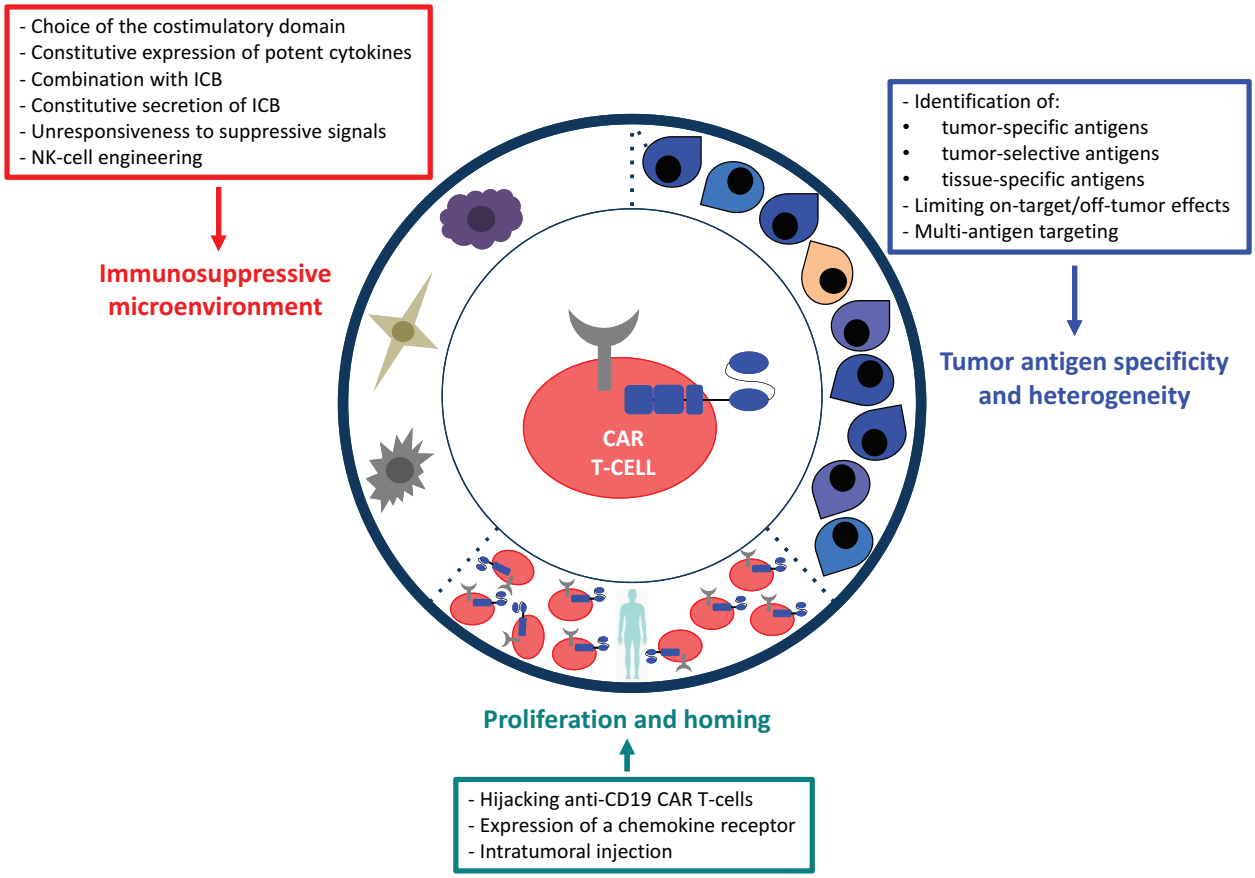

Figure 1. Challenges and solutions of CAR T-cell therapy in solid tumors.

hypotension and hypoxia. CRS is triggered by the activation of CAR T-cells and their subsequent production of pro-inflammatory cytokines including IFNγ, IL-6, and IL-2.[50] This is thought to result in additional activation of bystander immune and nonimmune cells (i.e. macrophages, endothelial and stromal cells) which further produce cytokines, including IL-10, IL-6, and IL-1beta and inflammatory mediators (i.e. ferritin).[51] The severity of CRS ranges from a mild fever to life-threatening, multi-organ failure.[52] Neurologic toxicity is another serious adverse event, which can occur alongside CRS.[53] Tocilizumab, a monoclonal IgG1 directed against the IL-6 receptor, is the current standard treatment for CRS.[54]

It is important to highlight that lack of tumor Ag specificity increases the potential risk of significant on-target/off-tumor toxicity. This was the case for a patient with metastatic colon cancer, who received an infusion of HER2 (ERBB2)-targeting CAR T-cells and died 5 days later.[55] The cause of death was attributed to CAR T cytotoxicity against the pneumocytes, which express low levels of HER2. Another example of on-target, off-tumor toxicity has been described in a preclinical model with a high affinity anti-GD2 CAR for neuroblastoma, in which low levels of GD2 in the brain resulted in fatal encephalitis.[56] Thistlethwaite et al. also described a patient who developed acute respiratory distress due to the on-target/off-tumor effect of CEACAM5-specific CAR T-cells exerting cytotoxicity against pneumocytes and lung-associated macrophages, and the trial was closed due to this severe and unexpected toxicity.[31] These fatal events underscore the importance of choosing a safe tumor-associated Ag, as even low level expression of the target Ag on normal tissues can result in severe toxicity. These acute responses also highlight that the binding affinity of a CAR is tightly linked to both safety and efficacy, and that higher affinity is not necessarily better. As an example, an *in vivo* study found that CAR T-cells targeting ICAM-1, a marker associated with many solid tumors including thyroid cancer (but also expressed on normal tissues as an adhesion molecule), was safer and more effective, when the CAR specificity for the Ag had only micromolar affinity.[57,58]

In order to specifically control CAR T-cell activity toward the Ag, several models of adapter-mediated CARs, also known as universal CARs (UniCAR), have been developed.[59–61] A shared feature is their method of tumor recognition, which is achieved by linking an adaptor, a molecule recognized by the CAR, to an antibody or ligand that specifically recognizes the tumor Ag. While current clinically approved CARs are designed to be constitutively active, adapter-mediated CAR T-cells have the distinct advantage to only recognize and kill the Ag-expressing target cell when the adapter is administered, allowing for titratable and reversible control of the CAR T-cells. As an example, the UniCAR02-T associated with the CD123 Target Module is currently in phase I in patients with hematologic malignancies expressing CD123 (NCT04230265).[62]

Improving expansion and homing

Trafficking to the tumor does not seem to be a major issue for hematologic tumors but is likely to be a challenge for CAR T-cells targeting solid tumors. The majority of solid tumors present with

a fibrotic stroma[63] and may be more difficult for engineered T-cells to infiltrate (Figure 1). Contrary to B-cell malignancies, CAR T-cells targeting solid tumors do not rapidly encounter their target once infused. This necessary time to migrate into the tumor certainly hinders the efficacy of CAR T-cells for solid tumors by limiting their proliferation and persistence. The high objective response rate observed with anti-CD19 CAR T-cells in refractory large B-cell lymphoma was found to be associated with CAR T-cell expansion following infusion.[64] Thus normal CD19 + B-cells act as an immediate and self-renewing source of Ag. A new immuno-oncology company proposed to tweak anti-CD19 CAR T-cells, thus making them able to recognize multiple different targets via the expression of fusion proteins while retaining their proliferation and persistence properties.[65] The fusion protein contains a CD19 extracellular domain and an anti-tumor antigen binding domain, thus it creates a bridge, which helps redirecting anti-CD19 CAR T-cells cytotoxicity against multiple tumor-associated Ags. This strategy seems attractive for the treatment of solid tumors by CAR-engineered T-cells.

Some studies have shown that modifying CAR T-cells to express a chemokine receptor (CCR2,[66] CCR4,[67] CXCR2[68,69]) matching to the chemokine secretion by the target tumor cells leads to improved T-cell homing into the tumor and enhanced antitumor efficacy in vivo. The enforced expression of a chemokine receptor such as CXCR1 or CXCR2 also augments intratumoral CAR T-cells persistence and tumor regression in xenograft mouse models of glioblastoma, ovarian, and pancreatic cancer.[70]

Another way to solve this migration issue could be to inject CAR T-cells directly into the tumor. Several preclinical studies showed higher CAR T-cell activation, efficacy and persistence when a regional delivery is performed as compared to intravenous injection.[71–73] This is particularly relevant for tumors localized within difficult-to-access niches, such as the central nervous system (CNS). Mulazzania et al.[74] used *in vivo* intracranial 2-photon microscopy to demonstrate that intracerebral injection of anti-CD19 CAR T-cells resulted in a deeper infiltration and an enhanced control of the tumor growth, than intravenous infusion in an orthotopic murine model of primary CNS lymphoma. Interestingly, 28 days after intracerebral injection, CAR T-cells were detected in distant non-draining lymph nodes. Anti-CD19 CAR T-cells persisted in the brain and the bloodstream for up to 159 days, even after complete regression of the CNS lymphoma.

Both intracranial[20] and intravenous routes are currently being tested in brain tumor clinical trials, but intracranial injection is a more risky procedure compared with intravenous infusion. Moreover, a recent publication reported on 8 patients with secondary CNS lymphoma treated with commercial tisagenlecleucel (anti-CD19 CAR T-cells containing a 4–1BB costimulatory domain) at a single institution.[75] CAR T-cells were administered as a single intravenous injection and the overall response rate was 50%. There was no increased rate of CRS or neurotoxicity. This retrospective analysis suggests that CAR T-cells can efficiently traffic to the CNS after intravenous injection, but larger studies are needed to clarify the optimal route of delivery. One remaining question could be whether a dose reduction of CAR T-cells is appropriate when injected directly into the tumor region.

Intratumoral injection of CAR T-cells has been tested in 6 patients presenting with a metastatic (accessible cutaneous or lymph node metastases) breast cancer.[30] The investigators used the previously published mRNA-transfected c-Met CAR T-cells,[18] whose transient expression of the c-Met CAR limits its possible on-target/off-tumor effect. The downside of this transient expression system is the rather rapid loss of the transgene, especially in proliferating cells, as the CAR-encoding RNA is not replicated during cell division. Despite an inflammatory response noted within the tumor, no objective clinical response was reported.

In conclusion, various approaches have been tested in preclinical models in order to enhance expansion, homing, and persistence of CAR T-cells in solid tumors. Some strategies have been evaluated in clinical studies, but more trials are needed to better assess their efficacy.

Overcoming the immunosuppressive microenvironment

Several solid tumors produce an immunosuppressive environment impairing the efficacy of ACT.[76] Multiple improvements of CAR T-cells have been proposed to allow their proliferation, persistence and cytotoxicity within an immunosuppressive environment. Regarding second-generation CAR T-cells, the choice of the costimulatory domain is certainly a key point and still a matter of debate. It has been shown, however, that UniCAR T-cells redirected to PSCA and harboring CD28 costimulation resist regulatory T cell (Treg) suppression, both *in vitro* and *in vivo*, via the secretion of Th1-related proinflammatory cytokines, in contrast to 4–1BB-based CARs, which are efficiently suppressed by Tregs.[77] Of note, 4–1BB costimulation is associated with an increased central memory differentiation and a prolonged persistence of the CAR T-cells.[78] Ideally, clinical trials should randomize CAR T-cells directed against the same target but bearing different costimulatory domains, and the optimal approach may be a defined ratio of CAR T-cells with different costimulatory domains.

One potential option for shaping the tumor microenvironment to enhance ACT efficacy is to induce the local release of stimulatory factors that promote antitumor immune responses.

The last generation of “armored” CAR T-cells, so called TRUCKs for T-cells redirected for universal cytokine killing, is particularly promising for the treatment of solid tumors associated with a suppressive microenvironment. These CAR T-cells are genetically modified to constitutively express potent cytokines. In this context IL-12 and IL-18 represent promising candidates to favorably remodel the tumor environment. In particular, IL-12 is a pro-inflammatory cytokine, able to improve T-cell activation and induce a Th_1 CD4 + T-cell response, CD8+ clonal expansion, and effector function. It is also able to recruit NK-cells to the tumor site, reactivate anergic TILs, inhibit Tregs and the secretion of IL-10, IL-4 and transforming growth factor beta (TGFβ) by tumor-associated macrophages. IL-12 TRUCKs have shown efficacy in preclinical models of hematologic[79,80] and solid tumors.[81–83]

Chmielewski and colleagues performed a cytokine screen, which identified IL-18 as inducing a T-Bet^{high} $FoxO1^{low}$ signature in CAR T-cells.[84] The authors engineered an IL-18

TRUCK, which improves the survival of immune-competent mice with advanced pancreatic cancer when compared to CAR T-cells without cytokine secretion. In addition, IL-18 CAR T-cell therapy induces a favorable remodeling of the tumor microenvironment. This model is of particular interest for pancreatic ductal adenocarcinoma, as it is one the most lethal human cancers, and its resistance to immune checkpoint inhibitors could be due to a predominance of immunosuppressive cells in the microenvironment.[85]

Combining CAR T-cells with ICIs (such as programmed cell death protein 1 (PD-1) or its ligand PD-L1) is another obvious way to modify the tumor microenvironment. Preclinical data demonstrated that the administration of an anti-PD-1 antibody enhances the antitumor activity of CAR T-cells against HER2+ sarcoma and breast cancer cell lines.[86] The anti-PD-1 therapy acts on TILs as well as on the CAR T-cells themselves, whose PD-1 expression is often upregulated following Ag stimulation. In order to limit the toxicity related to systemic delivery of ICIs and to increase tumor concentration, Rafiq et al. proposed to "armor" CAR T-cells to secrete a PD-1 blocking scFv only in the local tumor site.[87] Interestingly, in a xenograft model of metastatic ovarian cancer, mice treated with this innovative strategy had improved survival compared with mice receiving the anti-MUC16^{ecto} CAR T-cells plus an anti-PD-1 antibody.[87] This approach is promising to make CAR T-cells efficient in tumors with an immunosuppressive microenvironment.

Regarding PD-L1, CAR T-cells targeting carbonic anhydrase IX (CAIX) and engineered to secrete anti-PD-L1 antibodies have shown better control of the tumor growth than anti-CAIX CAR T-cells alone in a humanized mouse model of clear cell renal cell carcinoma.[88]

To date, the clinical benefit of the combination of CAR T-cells with ICIs is not proven. In a phase 1 clinical trial, the administration of a PD-1 inhibitor together with anti-GD2 CAR T-cells did not improve antitumor responses of patients with neuroblastoma, although the number of treated patients was small.[89]

Other research teams in the field have engineered CAR T-cells that are unresponsive to suppressive signals. For instance, genome editing has been used to remove the PD-1 receptor from CAR T-cells, making them inert to the PD-1/PD-L1 inhibitory pathway.[90–92] One can also express a dominant-negative form of some receptors (like TGFβ[93] or PD-1[94,95]) rendering CAR T-cells unresponsive to inhibitory signals. Nonetheless, such strategies raise the risk of uncontrolled CAR T-cell activation, as the suppressive pathways are essential to modulate T-cell effector functions. Special attention should be paid to the toxicity profile of such approaches, which should be monitored cautiously.

Very recently, Porter et al. published the combination of an oncolytic virus armed with a bispecific tumor-targeted T-cell engager (BiTE) molecule specific for CD44v6 plus IL-12 plus an anti-PD-L1 antibody (so called CAdTrio) with anti-HER2 CAR T-cells.[96] They showed that the association of both the CAdTrio and the CAR T-cells leads to a more sustained control of an orthotopic head and neck squamous cell carcinoma model than any component alone. Albeit a bit futuristic, this strategy that employs both intratumoral and intravenous routes, may be able to counteract both Ag heterogeneous expression and immune suppression by the solid tumor microenvironment.

NK-cells belong to the innate immune system and mediate cytotoxic functions against cancer cells through a complex network of activating and inhibitory receptors.[97] Interestingly, the density, phenotype and functions of tumor-infiltrating NK-cells have been associated with a favorable outcome in various solid tumors[98-101] but the microenvironment can impair their natural properties.[102] NK-cell based immunotherapy encompass multiple promising approaches, including CAR engineering,[103] which is under preclinical[104-106] and clinical[102,107] development for treating solid tumors. NK-cells can also be engineered to overcome the suppressive effect of the tumor microenvironment on their function. Interestingly, Parihar et al. produced modified NK-cells with a chimeric NKG2D receptor comprising the extracellular domain of the native NKG2D fused to the intracellular ζ-chain of the TCR (NKG2D.ζ), instead of the physiological DAP10 that is commonly downregulated by suppressive factors secreted by the microenvironment, such as TGFβ.[108] They showed that NKG2D.ζ NK-cells, but not unmodified NK-cells, killed NKG2D ligand-expressing myeloid-derived suppressor cells (MDSCs) in a xenograft model of MDSCs-containing neuroblastoma and enhanced infiltration and antitumor activity of co-injected anti-GD2 CAR T-cells.[108] Finally we are experiencing a new and exciting era almost resembling a science fiction movie, where the engineering of CAR T-cells seems to have no limit to overcome the evasion mechanisms of solid tumors. It is now time to assess if all these preclinical data will translate into clinical benefit for patients with aggressive solid tumors.

Conclusions

In the past few years, CAR T-cells made a huge breakthrough in the treatment of B-cell malignancies. Second generation CAR T-cells encompass one costimulatory domain (commonly CD28 or 4–1BB) and are now commercialized for the treatment of relapsed/refractory B-cell acute lymphoblastic leukemia and diffuse large B-cell lymphoma. This proof of concept generated great interest for the development of CAR T-cells directed against solid tumors. Unfortunately, clinical trials evaluating second generation CAR T-cells in solid tumors have shown disappointing results. While a few complete responses have been observed, the duration of response is still limited. This anecdotal success is due to several hurdles encountered with solid tumors, including the heterogeneous and nonspecific expression of tumor-associated Ag, the homing capacity, and the immunosuppressive tumor microenvironment. Figure 1 summarizes the solutions that have been proposed to face these challenges. Because no single CAR T-cell modality will likely defeat all evasion mechanisms of solid tumors, including plasticity of tumor Ag expression and active immune suppression by the tumor environment, "armored" CAR T-cells strategy (TRUCKs) is likely to increase the breadth, potency and duration of antitumor activity of second generation CAR T-cells. This last generation of CAR T-cells has demonstrated promising results in preclinical studies.

In addition, as CAR-related toxicities often arise acutely, control mechanisms should ideally allow fast control over CAR

T-cell activity. Permanent elimination of CAR T-cells could abrogate their long-term antitumor effect, and many methods therefore aim at reversible, ligand-enabled control, allowing to swiftly turn off the CAR T-cells when toxicities occur, such as with the design of adapter CAR T-cells. In addition, the use of boolean logic gates and tumor selectivity strategies is under intense investigation to generate autonomous CARs with a higher target specificity and tissue selectivity, capable of better distinguishing tumor from healthy cells.[109,110] In the future, the choice of CAR T-cell should also be tailored to the tumor-type targeted, as tissue-specific vascularization can hinder adequate CAR T-cell biodistribution, concentration, and persistance in the involved organs. Positive results from clinical trials are now awaited to hold the promise of this emerging category of cell-based therapy.

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Declaration of interest statement

The authors have no conflict of interest to disclose.

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