Review Article

The emerging role for CAR T cells in solid tumor oncology

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Abstract: In recent years, treatment with chimeric antigen receptor (CAR) T-cells has revolutionized the outcomes of patients with relapsed or refractory hematological malignancies with long-term remissions in >30% of patients. Similarly, the introduction of immune checkpoint inhibitor therapy changed the therapeutic landscape for several solid malignancies also leading to impressive long-term remission in patients. However, so far CAR T-cell therapy in solid tumors has shown low response rates and especially a lack of long-term remissions. This review focuses on the latest clinical advances and discusses promising results seen with CAR T-cells exploring new target antigens. We then review relevant challenges limiting long-term responses with CAR T-cell therapy in solid tumors like CAR T-cell persistence and target antigen expression. In addition, there is an increasing understanding on T-cell function and dysfunction within the immunosuppressive tumor microenvironment. This comprises of inhibitory cytokines and checkpoint molecules limiting the killing capacity of CAR T-cells. Finally, we will discuss how this deeper knowledge can be used to develop CAR T-cell therapies overcoming these inhibitory factors and results in CAR T-cell products with higher efficacy and safety. These technological developments will hopefully lead to enhanced clinical activity and improved solid tumor patient outcomes in the near future.

Keywords: Chimeric antigen receptor T-cells (CAR T-cells); solid tumors; adoptive T-cell therapy

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Introduction

In the past decade, chimeric antigen receptor (CAR) T-cell therapy emerged as novel and highly innovative therapeutic modality for hematological malignancies, with durable complete response rates of >30% in heavily pre-treated patients (1). CAR T-cell therapy is nowadays considered to be standard of care for patients with refractory or relapsed hematological cancers, including B-cell acute lymphoblastic leukemia (B-ALL), large B-cell lymphoma (LBCL), primary mediastinal B cell lymphoma (PMBCL), follicular lymphoma, mantel cell lymphoma and multiple myeloma (2-4). CAR T-cell therapy aims at the generation of a robust anti-tumor immune response (5). Most commonly, autologous T-cells are isolated and genetically engineered, via retro- or lentiviral transduction or more recently by clustered regularly interspaced short palindromic repeats

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(CRISPR) gene editing, which results in the expression of a synthetic CAR that redirects T-cell specificity and reactivity towards the selected membrane-bound tumor-associated target antigen (6). Hereto, the CAR contains several functional domains including an antigen recognition domain (antibody-derived single-chain variable fragment), a hinge domain, a transmembrane domain, a costimulatory domain and a T-cell activation domain (5). In this way, the CAR integrates primary TCR-driven activation with secondary stimulation provided by co-stimulatory molecules. After *in vitro* expansion, the CAR T-cell product is infused into the patient, which is most frequently preceded by chemotherapy to increase the plasma levels of lymphoproliferative cytokines [e.g., interleukin (IL)-7, IL-15, IL-21] and to limit suppressive effects of immunomodulatory cells, such as regulatory T-cells. CAR T-cells proliferate *in vivo* after administration into the patient and long-term persistence has been described. A single injection of these cells would theoretically be sufficient to induce long-term anti-tumor efficacy in patients with hematological malignancies. The cytokine release syndrome (CRS) and immune effector cell associated neurologic syndrome (ICANS) are well-known toxicities of immune effector cells therapy, as recently reviewed (7). These toxicities are in general well manageable with supportive care and immunosuppressive agents, e.g., the IL-6 receptor antagonist tocilizumab or corticosteroids (7).

In contrast, the results of CAR T-cell therapy in solid oncology have been less encouraging so far. Important factors that hamper the success of this cellular immunotherapy in solid tumors include the selection of an appropriate tumor-associated target antigen, the complex hostile and immunosuppressive tumor microenvironment (TME) and the limited persistence of the CAR T-cells (8). However, the field changes at rapid pace and many innovative strategies are currently being developed to improve CAR T-cell efficacy for solid tumors. This review focusses on the emerging role of CAR T-cell therapy in solid tumor oncology by discussing promising clinical studies as well as the challenges that need to be overcome.

Clinical studies

Numerous clinical trials have evaluated the efficacy of CAR T-cells in solid tumors, including gastro-intestinal cancer, central nervous system malignancies, prostate cancer, melanoma and many other tumor types. The results of the majority of these phase I trials have been modest and often disappointing. However, some studies, which are discussed below, show promising outcomes and provide important leads to further improve the efficacy of CAR T-cell therapies.

Claudin18.2 CAR T-cells for advanced gastro-intestinal cancers

A recent study investigated the therapeutic potential of claudin18.2-directed CAR T-cells in gastro-intestinal cancers (9). Claudin18.2 is an isoform of the tight junction protein claudin18 that is predominantly expressed on gastric mucosal cells (10). Qi *et al.* recently published a phase I trial on the effects of claudin18.2 targeted CAR T-cells in patients with claudin18.2^{+} gastro-intestinal cancer following at least one line of systemic therapy (9). Fifty-nine patients underwent apheresis and 49 patients received the CAR T-cell product following conditioning chemotherapy. The median time from apheresis to administration of the CAR T-cell product was 27 days, consequently, the majority of patients received at least one cycle of bridging therapy. Almost half of the subjects received two or three cycles of preconditioning/claudin18.2 CAR T-cells. Patients with a large tumor (≥4 cm) and subjects with brain metastasis, widespread lung or liver metastasis, and an active gastric ulcer or GI bleeding were not included in the study (9).

The published interim analysis included the first 37 patients who completed at least 12 weeks of follow-up. Claudin18.2 therapy was in general safe and no dose-limiting toxicities were observed within the first 28 days. However, one patient developed grade 4 GI hemorrhage as a result of rapid tumor regression following the second administration of the CAR T-cell product. All patients developed grade ≥3 hematologic toxicity, which was attributed to the conditioning chemotherapy and generally recovered within 4-9 days. Following claudin18.2 CAR T-cell administration, 94.6% of the patients developed grade 1 of 2 CRS with a median onset of 2 days post-infusion and a duration of 6 days. Tocilizumab was administered to 77.1% of the patients and 11.4% also received corticosteroids. Importantly none of the patients developed ICANS or grade ≥3 CRS. Claudin18.2 CAR T-cell therapy induced tumor regression in 83.3% of the patients. The overall response rate (ORR) was 48.6% and the disease control rate (DCR) was 73% with a median progression-free-survival (PFS) of 3.7 months. Circulating CAR T-cell numbers peaked 7 days post-infusion and the median persistence in the circulation was 28 days. Factors

that were associated with a beneficial response included the immunological phenotype of the final CAR T-cell product, as discussed below. Importantly, anti-drug antibodies (ADA) were found in 28 patients, but the presence of ADA was not associated with clinical activity.

The study of Qi demonstrated that claudin18.2 CAR T-cells have clinical activity in the majority of patients with advanced GI cancer, also the subgroup of subjects who failed PD-(L)1 targeting therapies. Prior clinical studies have evaluated the effects of the monoclonal claudin18.2 antibody zolbetuximab, in patients with claudin18.2 positive recurrent or refractory advanced gastric of gastroesophageal junction (GEJ) cancers and reported an ORR of 9%. The combination of zolbetuximab with chemotherapy (epirubicin, oxaliplatin, capecitabine) as first line therapy resulted in an ORR of 39% as compared to an ORR of 25% in the cohort that received chemotherapy alone (10). Moreover, the ORR of claudin18.2 CAR T cells was also higher than response rates of other therapeutic modalities, including PD-(L)1 targeting immune checkpoint inhibitors (11,12). Thus, claudin18.2 CAR T-cell therapy has an acceptable safety profile and shows promising activity in patient with refractory of recurrent advanced GI malignancies.

Claudin6 is another member of this protein family, which is frequently expressed in testicular and ovarian cancer patients, but also at lower frequencies in other tumor types like lung and uterine adenocarcinoma (13). In a first-in-human trial (NCT04503278), 22 metastatic claudin6$^+$ cancer patients were treated with different dose-levels of claudin6 CAR T-cells (14). Beside a manageable safety profile, 7 from 21 evaluable patients showed a partial response 6 weeks after CAR T-cell infusion. These first encouraging clinical data describe claudin6 as a novel interesting target for CAR T-cells in solid tumors.

Armored PSMA CAR T-cells in advanced prostate cancer

Prostate-specific membrane antigen (PSMA) is highly expressed in metastatic castration-resistant prostate cancer (mCRPC) and has been explored as therapeutic target for CAR T-cells (15). A major challenge for adoptive cell therapy in prostate cancer is the immunosuppressive TME. The cytokine transforming growth factor-β (TGF-β) is markedly elevated in the TME of mCRPC and a major suppressor of anti-tumor immune responses (16,17). In preclinical studies, inhibition of TGF-β signaling, via overexpression of a dominant-negative TGF-β receptor II (TGFβRDN) or knock-out of the endogenous TGF-β receptor II (TGFBR2) improved the efficacy CAR T-cells in *in vitro* and *in vivo* models (16,17).

The safety and clinical activity of PSMA targeting TGF-β resistant (PSMA TGFβRDN) CAR T-cells in patients with mCRPC and >10% PSMA$^+$ tumor cells were recently explored in a phase 1 trial (15). All 14 patients received prior treatment with at least one standard 17α-lyase inhibitor or second-generation anti-androgen therapy. Fourteen patients underwent apheresis and the CAR T-cell product was administered after a median period of 35 days in 13 patients (one patient withdrew consent). The final product contained 98% CD45$^+$CD3$^+$ cells with a median CD4/CD8 ratio of 3. Importantly, the frequency of anti-PSMA CAR$^+$ T-cells varied between 56-91%, with a median of 70%, which may have contributed to the heterogeneous clinical activity of the product.

The use of conditioning chemotherapy prior CAR T-cell infusion varied between the cohorts. No treatment related grade ≥3 adverse events were observed in the cohort that received 1×10^7–3×10^7 cells without lymphodepleting chemotherapy. Two patients in the cohort that received 1×10^8–3×10^8 cells without conditioning chemotherapy developed grade 3 CRS within 12 hours after administration and one of these subjects subsequently developed grade 3 ICANS, which was successfully managed with tocilizumab and corticosteroids. A dose-limiting toxicity occurred in one subject who received 1×10^8–3×10^8 cells following cyclophosphamide/fludarabine. This patient developed grade 4 CRS and died 30 days post-infusion due to multiorgan failure in the context of enterococcal septicemia. Following this dose-limiting toxicity, 6 patients were treated with 3×10^7 cells preceded by cyclophosphamide/fludarabine. All subject developed CRS (grade ≤2). Circulating CAR T-cell numbers peaked within 14 days after infusion. Following CAR T-cell administration, a decrease in PSA levels of ≥30% was observed in 4/13 patients, with a higher frequency in patients who received conditioning chemotherapy. One patient achieved a PSA level of <0.1 ng/mL within 2 weeks after infusion of the CAR T-cells and before the occurrence of a grade 5 AE. Radiologically, the best response was stable disease in 5/13 patients three months after treatment. Future clinical studies are required to further determine the efficacy of this treatment modality and its position in the therapeutic landscape of mCRPC.

Additionally, this study also provides important insight in the potential effects of CAR T-cell therapy on the TME. Ten days after PSMA TGFβRDN CAR

T-cell administration, a biopsy was performed and CAR T-cells were detected in 7/9 metastasis. An increased expression of the proliferation marker Ki67, OX40L and granzyme B was observed in the TME, which may reflect increased T-cell activation. The presence of other immune cell subsets in the TME was unaltered following CAR T-treatment. Functionally, the increased expression of indoleamine 2,3-dioxygenase 1 (IDO1) and CD40 may point towards enhanced activation of myeloid cells, whereas the increased expression of the co-inhibitory molecules T-cell immunoglobulin and mucin domain-3 (TIM3), programmed death-ligand2 (PD-L2), V-domain Ig suppressor of T-cell activation (VISTA) and B7-H3/CD276 may reflect a suppressive T-cell phenotype. Together this indicates that PSMA TGFβRDN CAR T-cell therapy has dual effects on the TME in mCRPC. Similar observations were found in a clinical trial for recurrent glioblastoma with an increased expression of inhibitory mediators in the TME after infusion of unarmored EGFRvIII CAR T-cells (18). These findings highlight the need for additional strategies targeted at the modulation of the TME following CAR-T treatment of patients with solid tumors.

CAR T-cells for malignancies of the central nervous system (CNS)

The disialoganglioside GD2 is highly expressed in diffuse intrinsic pontine glioma (DIPG) and other diffuse midline gliomas (DMGs) with histone H3 K27M mutations (19). These malignancies have a poor prognosis with an average life expectancy of 10 months from diagnosis. GD2 CAR T-cell showed efficacy in preclinical glioma models and the safety and therapeutic activity has recently been evaluated in a phase I trial (19,20). In this study, 3 DIPG patients and 1 spinal DMG patient received 1×10^6 cell/kg GD2 CAR T-cells intravenously following conditioning chemotherapy (cyclophosphamide/fludarabine). In case of clinical benefit, patients were eligible for an intracerebroventricularly administration of CAR T-cells. During manufacturing of the CAR T-cell product, rapid tumor progression occurred in the patient with spinal DMG and participation in the study was ended. All three patients developed CRS (grade ≤2), which was well manageable with tocilizumab and corticosteroid. In addition to this well-known toxicity, worsening of neurological symptoms as a results of CAR T-cell-induced inflammation in regions of the CNS occurred in all patients. This toxicity was termed tumor inflammation associated neurotoxicity (TIAN). Patients with bulky disease, thalamic tumors, cerebellar tumors, and patients with DIPG requiring placement of an Ommaya reservoir were expected to be at an increased risk for TIAN and were therefore excluded from this study. Management of TIAN included removal of CSF, hypertonic saline, and anti-inflammatory agents, e.g., tocilizumab, corticosteroids, and anakinra. The three patients treated in this study, experienced worsening of neurological symptoms around the 6/7 days post-infusion, which was attributed to TIAN and well manageable.

Radiological progression was observed in a patient, one months after CAR T-cell administration, which resulted in death 3 months post-infusion. Post-mortem analyses demonstrated lymphocytic infiltration of the tumor, but not in the unaffected brain areas. The GD2-CAR transgene was detected in the tumor, indicating the presence of GD2 CAR T-cells in the tumor. Another patient experienced improvement of neurological symptoms two weeks post-infusion which was accompanied by a 17% reduction in tumor volume 4 weeks after the first treatment. When neurological symptoms worsened, 2–3 months later, the patients received a second dose of CAR T-cells intracerebroventricular. Two weeks later an improvement of neurological symptoms and 27% reduction in tumor volume were observed. After the fifth CAR T-cell treatment, ten months following the first treatment, the patient died as a result of intratumoral hemorrhage, a relatively common complication of DIPG. The third patients died 7 months after the first GD2 CAR T-cell infusion as a result of tumor progression, following initial improvement of neurological symptoms. Interestingly, the spinal DMG patient, who received GD2 CAR T-cells off-study showed a >90% reduction in volume of the spinal tumor. As compared to intravenous administration, intracerebroventricular infusion of CAR T-cells resulted in higher IFNγ, TNF, IL-2 and IL-6 levels, which also correlated with the number of CAR T-cells in the CSF and may reflect an anti-tumor immune response. Interestingly, intravenous administration of CAR T-cells was associated with higher numbers of immunosuppressive regulatory T-cells in the CSF as compared to intracerebroventricular infusion. Together, this study demonstrated some clinical activity of GD2 CAR T-cells in aggressive CNS malignancies and highlights that CAR T-cell therapy for these diseases may be challenged by the occurrence of TIAN due to CAR T-cell-induced CNS inflammation, in addition to the well-known CRS and ICANS. Moreover, the study shows that repeated intracerebroventricular infusions of CAR T-cells may be

used to prolong responses and limit systemic toxicities as compared to intravenous administration. These important insights can be used to improve the efficacy of CAR T-cell therapy for these aggressive malignancies.

Other adoptive T-cell strategies for CNS malignancies include GD2 CAR NKT-cells, Her2 CAR T-cells, and epidermal growth factor receptor variant III (EGFRvIII) CAR T-cells, which are in general safe, but have mixed responses (18,21,22). For example, the clinical activity of repetitive locoregional administration of CAR T-cells targeted at the epidermal growth factor receptor Her2 has been evaluated in children and young adults with recurrent/refractory $Her2^+$ CNS tumors (22). All patients had undergone multiple surgical interventions, radiotherapy and at one line of chemotherapy. Patients received infusions in week 1, 2 and 3 of a 4-week course via CNS catheter into either the tumor cavity or the ventricular system for a maximum of 6 cycles. Conditioning chemotherapy was not applied in this study. One subject received 6 infusions and 3 patients received 2 infusions. Headache, pain and worsening of neurological symptoms were most frequent adverse events. One subject experienced worsening of neurological symptoms within 24 hours of the first dose, which was accompanied by radiological signs of locoregional inflammation, including peritumoral vasogenic edema, local mass effect and intensified contrast enhancement. Additional analyses showed signs of CNS inflammation following CAR T-cell administration, such as elevated CSF levels of CXCL10 and CCL2. Two patient developed fever and CRP levels were elevated in all three patients. This peak overlapped with headache and pain. Her2 CAR T-cell were not detected in the peripheral blood. After six administrations, 2 patients had progressive disease, while the other patients had stable disease. Together these findings indicate that locoregional administration of Her2 CAR T-cells was relatively well tolerated. The transient worsening of neurological symptoms may be related to TIAN, as observed in GD2 CAR T-cells. Effective trafficking of CAR T-cells into CNS tumor following intravenous administration has been demonstrated following treatment with epidermal growth factor receptor variant III (EGFRvIII) CAR T-cells in patients with recurrent glioblastoma (18). Analyses of tumor samples 14 days after administration, demonstrated that CAR T-cells were detected in the tumor and the expression of the transgene in tumor samples was higher than in the circulation, suggesting that the CAR T-cells effectively migrated into the tumor. However, circulating and intra-tumoral CAR T-cells rapidly declined in the weeks after administration. In contrast to the locoregional CNS inflammation following intra-CNS administration of Her2 CAR T-cells, analysis of the TME following a single intravenous administration of EGFRvIII CAR T-cells demonstrated increased expression of immunosuppressive markers and mediators, including IDO1, FoxP3, IL10, PD-L1 and TGF-β. Whether combination of CAR T-cell therapy with other therapeutic modalities, such as immune checkpoint inhibitors or small molecules (e.g., targeting IDO1) may improve these detrimental effects is currently unknown.

In addition to the studies discussed above, many other early clinical studies evaluated safety and clinical activity of various CAR T-cell products, e.g., mesothelin CAR T-cells (23-27), CD133 CAR T-cells (28,29), EGFR CAR T-cells (30,31), CEA CAR T-cells (32). In general, the treatment is safe with manageable toxicities. Unfortunately, clinical responses of most CAR T-cell products have so far been modest or absent, highlighting the need for further innovations directed at CAR T-cell persistence, CAR T-cell function and modulation of the tumor microenvironment, as discussed below (33).

Challenges for CAR T-cell therapy in solid tumors and future directions

Despite encouraging responses described in more recent CAR T-cell trials in solid tumors, many challenges to enhance (long-term) clinical activity remain. Next, we discuss several major limitations and possible solutions to improve the efficacy of CAR T-cell therapy for solid tumors.

Target antigen

Target antigen selection of the CAR is crucial for efficacy and safety. The optimal target antigen is highly and uniformly expressed on cancer cells and absent on healthy cells. Most target antigens for CAR T-cells described so far are tumor-associated antigens (TAA), which are upregulated, but not exclusively expressed on malignant cells (34). Expression on healthy cells might cause 'on-target, off-tumor toxicity' (OTOT) with variable clinical manifestations, depending on the expression profile and level of the target antigen. For instance, in patients treated with claudin18.2 CAR T-cells, one grade 3 and five grade 1–2 mucosal toxicities were observed, which were potentially related to expression of claudin18.2 on

normal gastric mucosal cells (9). OTOT was also observed with CAR T-cells targeting carbonic anhydrase IX (35), carcinoembryonic antigen-related cell adhesion molecule 5 (36), and erb-b2 receptor tyrosine kinase 2 (37) underlining the importance of target antigen selection.

One important mechanism for CAR T-cell resistance is antigen escape by cancer cells, because the target antigen might not be essential for tumor development and/or growth. For example, decrease in expression was described in a study targeting interleukin-13 receptor alpha 2 (IL13Rα2) in glioblastoma patients (38). A possible solution is targeting of two antigens, which showed better anti-tumor activity in pre-clinical studies with HER2- and IL13Rα2-targeting CAR T-cells compared to single antigen specific CAR T-cells (39). In addition to antigen escape, including a logic gate ('AND', 'OR', 'NOT', 'IF') might also reduce OTOT, since activation of the CAR T-cell is only triggered when there is binding to both antigens, as recently reviewed by Flugel *et al.* (34).

CAR T-cell expansion and persistence

Several clinical studies investigated expansion and persistence of CD19 CAR T-cells in patients with hematological diseases. In a study treating chronic lymphocytic leukemia patients with CD19 CAR T-cells, median peak expansion (C_{max}) of CAR T-cells in peripheral blood was 58.570 copies/µg genomic DNA in complete responders and in non-responders 205 copies/µg genomic DNA (40). In addition, CAR T-cell persistence was also significantly higher in complete than in non-responders. Although other studies confirmed this correlation (41), permanent persistence was not correlated with response in other studies (42,43). The underlying disease and the use of different CAR constructs might have contributed to these different observations.

In patients with solid tumors, expansion and persistence of CAR T-cells were in general described to be lower than in patients with hematological malignancies. For example, claudin18.2 CAR T-cells showed a median C_{max} value of 6.713 copies/µg genomic DNA (9), which tended to be higher in responding versus non-responding patients (10.553 versus 4.980 copies/µg genomic DNA). Interestingly, in this study part of the patients received a second infusion, which resulted in a significantly lower C_{max} of 1.151 copies/µg genomic DNA. Low CAR T-cell expansion after one infusion was seen in other studies using CARs targeting antigens including HER2, EGFRvIII or mesothelin (18,27,44). However, a cross-trial comparison of CAR T-cell expansion and persistence is difficult, at least partly due to different lymphodepleting chemotherapy regimens (cyclophosphamide alone versus cyclophosphamide/fludarabine combination), which strongly affects expansion and persistence of the CAR T-cells.

Recently, several strategies to improve expansion and persistence of CAR T-cells in solid tumor patients were described. Reinhard *et al.* used an RNA-based vaccine approach to enhance expansion and engraftment in pre-clinical models with claudin6 targeting CAR T-cells (13). This approach is currently under investigation in a phase I/II clinical trial in patients with metastatic solid tumors (NCT04503278). Another approach to efficiently expand CAR T-cells, is engineering IL-2/IL-2 receptor β orthogonal pairs, while avoiding toxic effects of systemic IL-2 administration (45,46).

An important factor influencing T-cell persistence is the differentiation state of the cell product. $CD45RO^+CCR7^-CD62L^-$ effector memory T-cells (T_{EM}) have the capacity to immediately mediate effector functions, whereas $CD45RO^+CCR7^+CD62L^+$ central memory T-cells (T_{CM}) have an increased capacity for proliferation and persistence (47). In addition, $CD45RA^+CCR7^+CD62L^+$ stem cell memory T-cells (T_{SCM}) were described with the highest potential for expansion (48) and therefore seem be attractive for adoptive T-cell therapies (49). In line with this, patients responding to CD19 CAR T-cells showed higher frequencies of $CD27^+CD45RO^-CD8^+$ before cell infusion and those cells had memory-like characteristics (40). Another study looking at 2 patients with CD19 CAR T-cells persisting more than 10 years found a population of $CD4^+CAR^+$ T-cells with an upregulation of cytotoxicity associated genes like granzyme K and A (50). To manipulate the CAR T-product with favorable attributes of memory-like features, knockout of genes interfering with terminal T-cell differentiation and exhaustion was explored as potential strategy. For example, PR domain zinc finger protein 1 (PRDM1) knockout enhanced the formation of less differentiated CAR T-cells, which ultimately resulted in enhanced anti-tumor activity in preclinical models (51). Since the costimulatory domain within the CAR construct might influence T-cell expansion and fitness of the cells, Daniels *et al.* constructed a library of around 2,300 different constructs, based on 13 signaling motifs (52). Using this innovative strategy, it was demonstrated that the costimulatory mediators tumor necrosis factor receptor-

associated factors (TRAFs) and phospholipase C gamma 1 (PLCγ1) enhanced the cytotoxicity and stemness of CAR T-cells.

In recent years, the field of synthetic immunology is rapidly growing, where user-defined input signals are used to control specific cellular outputs. One example of such an approach is the synthetic Notch receptor, where tumor recognition of the modified T-cell leads to local production of IL-2 leading to T-cell expansion and cell killing (53). Other studies described beneficial effects of CAR T-cells producing IL-12 (54), IL-18 (55) or IL-23 (56). A major challenge of this fast technical development in CAR T-design is to select the best constructs to move further for clinical application in patients.

Immunosuppressive tumor microenvironment

The immunosuppressive TME consists of many different cell types, such as regulatory T-cells (T_{reg}), tumor-associated macrophages (TAM), myeloid-derived suppressor cells (MDSC), and cancer-associated fibroblasts (CAF). The first challenge for CAR T-cells is to enter solid tumors, because of its physical barrier, which includes expression of adhesion molecules, chemokines and an abnormal extracellular matrix (57,58). Several approaches by armoring CAR T-cells with chemokines to enhance trafficking and infiltration in the tumor were described in pre-clinical models (59,60). For example, CAR T-cells that expressed IL-7 and chemokine (C-C motif) ligand 19 (CCL-19) showed improved infiltration and cell survival within the tumor (60). Results from a phase 1b clinical trial using IL-7 and CCL19 armored CD19 CAR T-cells in large B-cell lymphoma patients demonstrated promising durable responses (61).

After entering the tumor, the next challenge to overcome for CAR T-cells is to resist the immunosuppressive TME. Exhaustion of CAR T-cells with a $CD8^+$ T-to-NK-like T-cell transition due to the immunosuppressive TME has been described as a mechanism of treatment failure in solid tumors (62). Interfering with the transcription factors ID3 and SOX4 has shown pre-clinical evidence to prevent CAR T-cell dysfunction. As discussed above, modulation of TGF-β signaling is another strategy to improve CAR T-cell function. Transducing CAR T-cells with a TGF-β dominant-negative receptor, which lacks the intracellular signaling domain of the TGF-β receptor, dampens the suppressive effects of this cytokine on CAR-T cell activity (63,64). However, clinical data using this approach point to possible toxicities (15) and due to this toxicity, the clinical trial was stopped. This clearly indicates that cell engineering has to be performed with caution.

Next to inhibitory cytokines, interaction with checkpoint molecules is another mechanism of T-cell inhibition within the TME. Programmed cell death protein 1 (PD-1) was shown to be a relevant inhibitory molecule also in CAR T-cell therapy (65) and genetic inhibition of this pathway using PD-$1^{-/-}$ CAR T-cells might enhance anti-tumor activity (26). In addition to manipulation of the cell product, combining CAR T-cells with checkpoint inhibitors has also been investigated. In a phase I trial in 18 patients with metastatic mesothelioma, regional application of mesothelin targeting CAR T-cells in combination with anti-PD-1 resulted in 2 complete metabolic responses (24). These encouraging results warrants further investigations in larger patient cohorts.

Directly targeting of immunosuppressive cells of the TME has been another approach to enhance CAR T-cell activity. Fibroblast activation protein α (FAP) is expressed on CAF and targeting FAP with CAR T-cells has been explored in pre-clinical models (66). However, CAR T-cell treatment induced weight loss and bone marrow hypocellularity, caused by expression of FAP on healthy bone marrow stromal cells. This underlines the difficulty to specifically target certain cell populations within the TME without toxicity on healthy stromal cells.

Another aspect within the TME is the deprivation for nutritional factors like glucose. Importantly, glycolysis is the main energy source for effector T-cells (57). Moreover, accumulation of lactic acid within the tumor suppresses T-cell function (67). The application of IL-7 and/or IL-15 in the manufacturing process positively drives CAR T-cells towards a T_{CM}/T_{SCM} phenotype by interfering with metabolic pathways (47). IL-15 for example reduces mammalian target of rapamycin complex 1 (mTORC1) activity in $CD8^+$ T-cells and improves mitochondrial fitness (68). Similarly, interacting with the WNT (69), phosphoinositide 3-kinase (PI3K) (70) or protein kinase B (AKT) (71) pathway by pharmacological inhibition keeps CAR T-cells in a more T_{CM}/T_{SCM} like state with favorable anti-tumor activity. Whether pharmacological modulation of the metabolic profile of CAR T-cells during the manufacturing process will be sufficient to also steer an immunological phenotype after adoptive transfer remains to be determined. A possible solution might be stable genetic manipulation with CRISPR.

Conclusions

Recent clinical trials show promising clinical activity of advanced CAR T-cell products in heavily pre-treated patients with refractory solid tumors. This review focused on the most emerging developments with CAR T-cells in solid tumor patients, however, since this is a rapidly moving field, not all aspects could be covered here. Although many challenges need to be addressed, the evolving technological advances in the field will undoubtedly increase the role of CAR T-cell therapy in solid tumors oncology in the years to come.

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Footnote

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