

SPECIAL FEATURE REVIEW

Chimeric antigen receptors designed to overcome transforming growth factor- β -mediated repression in the adoptive T-cell therapy of solid tumors

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Adoptive cell therapy with chimeric antigen receptor (CAR)-engineered T cells produced lasting remissions in the treatment of advanced, so far refractory B-cell malignancies; however, the elimination of solid tumors remains so far elusive. The low efficacy of CAR T cells is thought to be due to the immune-repressive milieu within the tumor lesion, predominantly mediated by transforming growth factor- β (TGF- β) that represses effector T cell activities and drives differentiation towards regulatory T cells (Tregs). Seeking to boost antitumor immunity, TGF- β is currently targeted by different means in pre-clinical studies. While a recent clinical trial showed the utility of shielding CAR T cells from TGF- β repression, further strategies in counteracting TGF- β in the adoptive cell therapy warrant exploration. We here discuss the most recent advances in the field and draw future developments to make CAR T cell therapy more potent in the treatment of solid cancer.

Keywords: adoptive T cell therapy, chimeric antigen receptor, immunosuppression, immunotherapy, TGF- β

INTRODUCTION

The outstanding success of chimeric antigen receptor (CAR)-redirected T cell therapy has been noticeably achieved since its introduction in the treatment of hematologic malignancies, such as lymphoma or leukaemia; complete and lasting remissions were induced after transfer of CD19-specific CAR T cells. However, unfortunately the same cannot be noted for the treatment of solid tumors.¹ In the case of a solid tumor and in comparison with hematological cancer, CAR T cells have to infiltrate the stromal elements to reach and eliminate the recognised cancer cell. While a number of targetable tumor antigens on solid

cancer cells are known, the current pre-clinical research and first clinical observations revealed low antitumor efficacy of the specifically redirected CAR T-cell therapies.

In the event of CAR T cells successfully locating cognate cancer cells within the solid tumor lesion, they turn into dysfunctional cells. There are three known significant reasons for this. Firstly, the liberated intratumoral environment is presented around the tumor cells with hypoxia, oxidative stress, acidic pH and low levels of nutrients providing unsuitable conditions for a sustained T-cell antitumor response.^{2,3} Secondly, it is due to the presence of suppressive immune cells such as regulatory T cells (Tregs), myeloid-derived

suppressor cells (MDSCs), M2-polarised tumor-associated macrophages (TAMs) and N2-polarised tumor-associated neutrophils (TANs). These suppressive cells hold responsibility for the production of T cell inhibitory soluble factors and inhibitory cytokines. Among these inhibitory cytokines is transforming growth factor- β (TGF- β) produced by tumor cells, Tregs, MDSCs, M2-polarised TAMs and N2-polarised TANs accumulating to substantial concentrations within the tumor lesion.^{4,5} TGF- β is a key regulator of immune homeostasis; however, the presence of this cytokine is considered a major reason for the lack of success in the immunotherapy for solid tumors. This is based on the following observations: (1) human T cells are susceptible to TGF- β -mediated immune suppression; (2) elevated levels of TGF- β occur in a variety of solid tumors; and (3) TGF- β production correlates with poor prognosis. One other significant reason believed to be upholding the success of tumor treatment is the induced regulatory mechanisms on the infiltrating, activated CAR T cells, including upregulation of cytoplasmic and surface inhibitory receptors such as PD-1 or CTLA-4^{1,6}, making these cells susceptible to repression by tumor-expressed inhibitory ligands. TGF- β directly inhibits T cell activity through binding to the TGF- β receptors TGFBR1 and TGFBR2. TGF- β binding induces hetero-dimerization of the respective receptors and phosphorylation of the major TGF- β signal mediators SMAD2 and SMAD3. Phosphorylated SMADs induce a suppressive transcriptional programme that results in reduced cytokine production, cytotoxicity and T-cell amplification upon antigen engagement. TGF- β can also bind to a hybrid TGF- β 2/interleukin receptor.⁷ TGF- β moreover drives T-cell differentiation into regulatory T cells.⁸ that, in turn, produce TGF- β and further promote immune repression and tumor tolerance.

IMMUNOSUPPRESSION BY TGF- β IS THOUGHT TO BE THE MAJOR CAUSE OF UNSUCCESSFUL ANTITUMOR ACTIVITY

Extensive research has gone into understanding the crucial role of TGF- β in tumor progression, metastasis and treatment. Within these effects, TGF- β is responsible for the regulation of cancer cell amplification, the contribution to epithelial-to-mesenchymal transition (EMT), the ability to compromise immune response by the suppression of pro-inflammatory immune cells, the conversion

of fibroblasts to myofibroblasts and the increased production of extracellular matrix (ECM) in the tumor. Under physiological conditions, a continuous basal release of TGF- β enables sustainable normal tissue homeostasis. However, under a change of conditions, for example tissue injury, the local TGF- β secretion sourced from stromal cells and blood platelets increases rapidly in order to facilitate wound repair and to prevent uncontrolled regenerative cell proliferation and inflammation. This situation is commonly repeated in pre-malignant tumors, and TGF- β is secreted in the microenvironment with the intention to control cell proliferation and cancer progression. However, accumulated TGF- β is utilised by the cancer cells for the promotion of their malignant properties. The local release of TGF- β impacts the tumor microenvironment, which is advantageous to amplification, invasion and metastasis of cancer cells.⁹ Therefore, the interest of research about this cytokine is high in order to prevent immunosuppression.

Transforming growth factor- β is a multifunctional polypeptide that holds importance within proliferation, differentiation, angiogenesis, embryonic development and wound healing along with many other functions within different cell types.¹⁰ TGF- β comes along in three known isoforms, TGF- β I, TGF- β II and TGF- β III, and is home to a vast superfamily with 33 known human family participants, including bone morphogenetic proteins (BMPs), activins and inhibins, growth and differentiation factors (GDFs) and¹¹ the members of the family are evolutionary conserved proteins, and there is 70-80% homology among the TGF- β isoforms. Among the isoforms, TGF- β I is responsible for the largest impact on the regulation of the immune response by binding to the specific receptors on the respective immune cells.

There are three known types of TGF- β receptors responsible for initiating downstream signalling, and these are TGF- β RI, TGF- β RII and TGF- β RIII. There are seven known TGF- β RI, five known TGF- β RII and two known TGF- β RIII identified thus far. TGF- β Is include activin receptor-like kinases 1-7 (ALK1-7), TGF- β RIIs contain bone morphogenetic protein receptor-2 (BMPRII) and activin receptor 2 (ACTR2). Finally, β -glucan and endoglin belong to TGF- β RIII, and this is mainly responsible for functioning as co-receptors to enhance activin signalling. In the majority of tissues, TGF- β functions through the heteromeric complex formation between two TGF- β RI and two TGF- β RII

molecules. Although both receptors inhibit Ser/Thr kinase activity, TGF- β RIIs' responsibility is to function as the activator, whereas TGF- β RI holds the signalling propagating component.¹² The TGF- β and TGF- β -like cytokines mediate downstream intracellular signalling via the SMADs family of proteins, consisting of eight structurally related members in humans.^{13,14} There are three known functionally classified groups of SMADs: the receptor-activated SMADs (R-SMADs), which include SMAD1, 2, 3, 5 and 8; the common mediator SMAD (Co-SMAD), SMAD4; and the inhibitory SMADs (I-SMADs), which include SMAD6 and 7. The TGF- β signalling pathway can be categorised, one being the canonical or SMAD-dependent and the second, the non-canonical or SMAD-independent pathway.

Because of the antagonistic function within immune cells, TGF- β upholds immunosuppressive effects on all arms of the immune system.⁵ Therefore, as a result the initial pro-inflammatory antitumor immune response of activated CD4⁺ and CD8⁺ T cells is compromised, resulting in failure to identify and kill cancer cells. More so, TGF- β affects the function of natural killer (NK) cells, macrophages, neutrophils, dendritic cells (DCs), mast cells and B cells.¹⁵ More specifically, a tumor microenvironment rich in TGF- β is a potent suppressor of T-cell proliferation, which both reduces their effector function and inhibits the maturation of T helper cells.^{16,17} TGF- β within the tumor microenvironment also has the ability to induce macrophage polarisation from type M-1 to an M-2 phenotype, which hinders suppression of monocyte-mediated cell death, reduces effector functions and increases chemotaxis.¹⁸ Also, TGF- β induces an N2 neutrophil phenotype; this along with macrophages has the ability to reduce effector functions along with an increased secretion of pro-inflammatory cytokines.¹⁹ Moreover, high levels of TGF- β increase entry into apoptosis of B cells and inhibit the maturation of DCs and NK cells, and there is also the possibility of inducing chemotaxis of mast cells. Finally, these combined immunosuppressive effects of TGF- β affect the ability of the host to counteract tumor progression and thereby create a barrier to immunotherapy.

STRATEGIES TO OVERCOME TGF- β REPRESSION

Research has indicated the suppressive tumor microenvironment inhibits CAR T cell functions,

preventing the execution of antitumor reactions and the induction of a pro-inflammatory immune response. The need to overcome immune suppression by counteracting TGF- β signalling in order to provide a more supportive microenvironment and to increase the success of effector T cells in an antitumor attack has increased because of a better understanding of the effect of TGF- β directly on CAR T cells and indirectly on other immune cells within the microenvironment. We here outline, describe and compare the current suppressive techniques using CAR T cells in the treatment of solid cancer lesions (Figure 1). The details of each technique along with a comparison will lead to an understanding of the differences, similarities, limitations, advantages and disadvantages of each technique. The review discusses what the future potentially holds for CAR T cell therapy in association with TGF- β expressing solid tumors.

CD28 signalling CAR

It has been previously shown that CAR T cell repression through TGF- β can be overcome by CD28 co-stimulation provided by a CD28- ζ CAR upon engagement of cognate antigen while 4-1BB- ζ CAR and first-generation CD3 ζ CAR T cells suffered suppression.²⁰ In order to identify how CD28- ζ CAR T cells were able to overcome TGF- β , Golumba-Nagy *et al.* (2018)²¹ identified the importance of LCK activation and the subsequent IL-2 release along with the autocrine IL-2 receptor signalling, all crucial components in overcoming TGF- β repression. Since IL-2 released by activated CD28- ζ CAR T cells would sustain tumor-infiltrating Treg cells, which are a major source of TGF- β , and thereby furthermore augment immune repression, research explored the possibility of replacing IL-2 with other γ -cytokines and introducing a hybrid receptor that binds the γ -cytokine and transmits the IL-2 receptor signal, thereby mimicking IL-2 signalling. CAR-induced IL-2 release can be prevented by deleting the LCK binding domain within the intracellular CD28 signalling chain.²² Using such IL-2-deficient CAR, an additionally engineered autocrine loop with the release of transgenic IL-7, replacing IL-2 and IL-2 receptor β chain signalling upon IL-7 binding to the extracellular IL-7 receptor α chain, has the ability to improve antitumor efficiency of CAR T cells in a TGF- β environment independently of IL-2.²¹ With the artificial autocrine loop, replacing

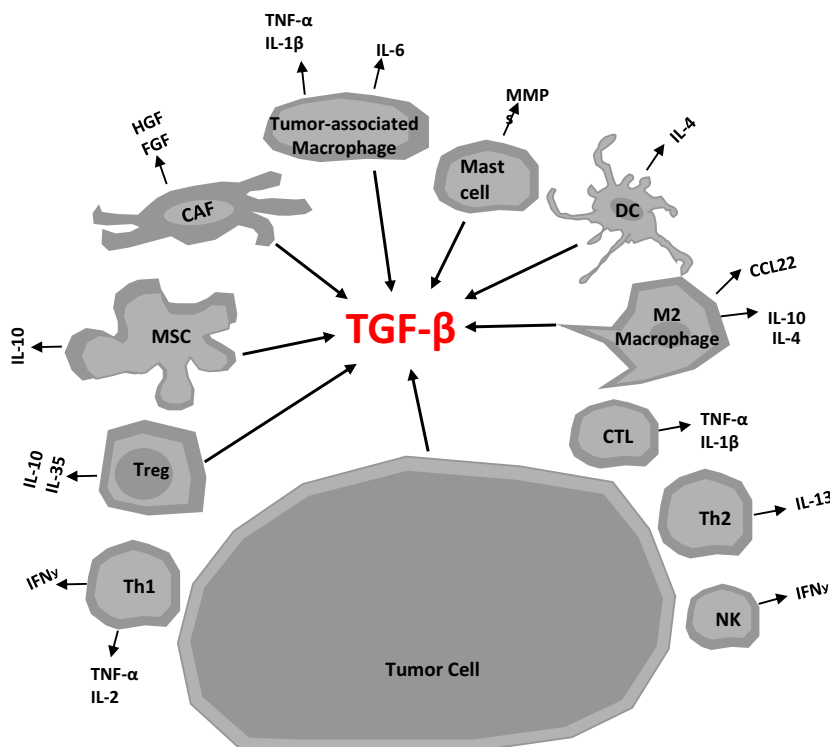


Figure 1. Schematic illustration identifying the major sources of transforming growth factor- β (TGF- β) within the tumor microenvironment. Major cell compartments producing TGF- β in a solid tumor lesion. The figure also shows a visual representation of other cytokines secreted in tumor microenvironment from various cells. CAF, cancer-associated fibroblast; CTL, cytotoxic lymphocytes; DC, dendritic cell; MSC, mesenchymal stem cells; NK, natural killer cell; Th1, T helper 1 cell; Th2, T helper 2 cell; Treg, T regulatory cell.

IL-2 with IL-7 and a hybrid receptor binding IL-7 and signalling through IL-2 receptor, T cells become activated upon binding to antigen via the CAR, and amplify and execute cytotoxicity along with releasing of pro-inflammatory cytokines such as IFN- γ (Figure 2).

Conclusive findings from this study include the suggestion that a co-release of IL-7 by CAR T cells is the most likely safer clinical method. The reason for this is that a systematic administration of this cytokine in patients with cancer for the treatment of lymphopenia did not show any severe side effects, but indeed provided a beneficial increase in CD4⁺ and CD8⁺ T-cell numbers that resulted in a decrease in Treg cells.²³ The hybrid IL-7R α /IL-2R β receptor is advantageous in the way it enables constitutively the expression of the receptor and translates extracellular IL-7 binding into intracellular IL-2 signalling. Therefore, CAR T cells with an IL-7 release and hybrid IL-7R α /IL-2R β receptor portrayed improved survival over a continuous period.²¹ A technical disadvantage of the strategy is engineering the T cell with an IL-7 expression cassette together with the hybrid

IL-7R α /IL-2R β receptor and the respective CAR. T cell modification with a high-capacity vector will be required in the near future to conform to good manufacturing practices.

Along this line, Boyerinas *et al.* (2017) reported the co-expression of TGFBR2 and TGFBR1 receptor variants where the TGF- β -binding domain of each receptor is fused to the intracellular signalling domains of a T cell-stimulating interleukin receptor that induce STAT transcription factor signalling pathways. Using IL-12 receptor signalling domains in such chimeric receptors, STAT4 signalling, and finally increase in IFN- γ release, is induced, resulting in superior T-cell persistence and expansion in the presence of TGF- β .⁷

Co-expression of the dnTGF- β receptor

In contrast to CD28- ζ CAR, T cells with the 4-1BB- ζ CAR are sensitive to repression by TGF- β , which makes an alternative strategy necessary. The strategy established by Kloss *et al.* (2018)²⁴ was that co-expressing a dominant-negative (dn) TGF- β RII along with a PSMA-specific CAR in T cells

Technique 1 modified CD28 CAR and IL-2 receptor signalling	Technique 2 dnTGF- β receptor	Technique 3 Constitutively Active Akt (caAkt)	Technique 4 TGF- β CAR
<p>Overall Aim: replace CAR induced IL-2 release with IL-7 and provide IL-7 induced IL-2 receptor signalling</p> <p>Mode of action: IL-7 induced IL-2R signalling provides TGF-β resistance in a CAR triggered fashion</p> <p>Genetic constructs: CD28-ζ CAR with deleted LCK binding site; induced transgenic IL-7 release; transgenic IL7Ra/IL-2Rβ receptor with IL-7 binding and IL-2R signalling</p> <p>Advantages: CAR dependent TGF-β protection; no Treg support due to lack of CAR induced IL-2; regulated, CAR induced IL-7/IL-2R auto-stimulatory loop</p> <p>Limitations: requires genetic modification with 3 constructs</p>	<p>Overall Aim: Block TGF-β by a dominant negative TGF-βRII receptor</p> <p>Mode of action: decoy receptor</p> <p>Genetic construct: transgenic TGF-β receptor without intracellular signalling moiety</p> <p>Advantage: dnTGF-βRII can be co-expressed with any tumor targeting CAR</p> <p>Limitations: as a decoy receptor acts independently of tumor targeting and does not provide T cell stimulation</p>	<p>Overall Aim: provide Akt signalling to promote T cell proliferation, effector functions and survival</p> <p>Mode of action: recruitment of Akt and PI3K to the signalling membrane complex inducing constitutive T cell activation</p> <p>Genetic construct: transgenic, modified constitutively active Akt</p> <p>Advantages: sustains T cell activation in the presence of a variety of repressor signals</p> <p>Limitations: constitutively active independently of tumor targeting</p>	<p>Overall Aim: convert TGF-β binding into T cell stimulation</p> <p>Mode of action: decoy receptor providing CD28-ζ mediated T cell stimulation in the presence of TGF-β</p> <p>Genetic construct: CAR that binds TGF-β and signals through CD28-ζ</p> <p>Advantages: provides T cell stimulation; protects neighbouring immune cells from suppressive effects</p> <p>Limitations: active in any tissue with soluble or membrane bound TGF-β</p>

Figure 2. Techniques to overcome transforming growth factor- β -mediated repression of chimeric antigen receptor T cells in solid tumors.

results in enhanced infiltration, proliferation, persistence and efficiency in the presence of TGF- β .²⁴ The dnTGF- β RII thereby acts as a decoy receptor binding TGF- β without downstream signalling. The study also identified a distinct transcriptional programme in the T cells expressing PSMA CAR and the dnTGF- β RII in the presence of TGF- β , indicating a successful

protection from TGF- β -mediated repression of T cell effector functions. This is moreover sustained by the enhanced secretion of pro-inflammatory cytokines by dnTGF- β RII CAR T cells that are expected to enhance innate and acquired immunity and finally antitumor response. The cytokine profile demonstrates a balanced TH2 phenotype with IL-4, IL-5 and IL-13 expression, as

well as TH1 phenotype with IL-2, IFN- γ and chemokines MIP1- α , MIP1- β and RANTES. Consequently, such engineered CAR T cells show a prolonged amplification in the presence of TGF- β . However, such cytokine-driven proliferation of CAR T cells can result in lymphoproliferative syndromes as observed in pre-clinical mouse models²⁵; the actual risk in a clinical situation still needs to be explored. A clinical trial has begun to infuse dnTGF- β RII and PSMA-specific 4-1BB- ζ CAR T cells in a first-in-human study in patients with refractory castration-resistant metastatic prostate cancer (ClinicalTrials.gov: NCT03089203) with the aim to sustain and augment CAR T cell proliferation and effector function within the solid tumor lesions.

One significant difference between this technique and the CD28 CAR is that CARs with any signalling chain can be used within this technique like 4-1BB- ζ and CD3 ζ signalling CARs. The study reports success in the way that the PSMA-specific, TGF- β -insensitive CAR T cells exhibit a proliferative advantage over wild-type PSMA CAR T cells that fully transmit TGF- β signalling, suggesting a possibility of clinical success because of the known difficulty of CAR T cell expansion in patients with solid tumors.²⁶

Constitutively active Akt (caAkt)

The serine/threonine kinase Akt/protein kinase-B regulates T cell amplification, effector functions and survival; the constitutively active Akt was hypothesised to counteract a variety of tumor-associated repressive mechanisms. The hypothesis is based on the crucial role of Akt as a major component of the phosphatidylinositol 3-kinase (PI3K) family and thereby acts downstream of both the TCR and the cytokine receptor signalling pathways.²⁷ On the one hand, CD28 signalling recruits PI3K and Akt to the membrane and activates tyrosine kinases required for T-cell activation. On the other hand, Akt repression is a crucial step in the signalling pathway of multiple inhibitory receptors including PD-1 and CTLA-4 by counteracting tyrosine phosphatases. The activation balance of the PI3K/Akt pathway is a crucial determinant to a number of inhibitors including TGF- β . Consequently, engineered T cells with constitutively active Akt, also named M μ - Δ Akt and being dual-acetylated and targeted to the lipid rafts, showed sustained NF- κ B expression and increased amplification, cytokine release and

resistance to apoptosis. Along with the highly activated state, caAkt T cells showed superior persistence and cytolytic activities in the presence of TGF- β ⁺ tumor cells and resistance to repression by cytokine deprivation, TGF- β and the presence of Treg cells.²⁷ With respect to safety, caAkt-engineered T cells did not amplify autonomously, but were still dependent on cytokine and antigen stimulation; however, they showed prolonged survival and elongated telomers.²⁷ While caAkt can compensate for subthreshold activation signals, caAkt did not furthermore increase T-cell functions upon adequate stimulation.

Transforming growth factor- β in the presence of stimulatory signals promotes the generation of inducible Treg cells; Akt is a strong repressor of the Treg phenotype.²⁸ Consequently, caAkt T cells showed a low frequency of conversion to Treg cells in the presence of TGF- β , which, in the context of prolonged survival, provides improved elimination of cognate cancer cells in the long-term.

TGF- β CAR T cells

Recently, Chang *et al.* (2018)²⁹ reported a CAR that binds TGF- β by a scFv-binding domain and stimulates the engineered T cell through CD28- ζ signalling, thereby converting the binding of an inhibitory ligand into a T cell-activating signal. The so-called 'switch' CARs were previously reported for PD-1³⁰ and others. TGF- β CAR T cells amplify and secrete pro-inflammatory TH1 cytokines in the presence of TGF- β . More importantly with respect to shaping the immune environment, such TGF- β switch CAR T cells can also protect neighbouring immune cells from repression; tumor-targeted CD8⁺ T cells execute their cytolytic activities against tumor cells in the presence of TGF- β .³¹ Moreover, CD4⁺ T cells do not convert into Treg cell phenotype. An advantage of the TGF- β CAR lies in the ability to induce T cell effector functions that interface with other immune cells in the near vicinity because of at least two mechanisms. Firstly, the expression of a TGF- β -binding receptor can sequester TGF- β and thereby can reduce TGF- β -mediated suppression of cytolytic CD8⁺ T cells. The effect is also obtained with dnTGF- β RII-engineered T cells³² and with soluble TGF- β binding proteins. Secondly, the activated TGF- β CAR T cells release stimulatory cytokines that help bystander immune cells to become activated and resist TGF- β repression.

UNRESOLVED ISSUES AND FUTURE ASPECTS

The TGF- β pathway is amenable to pharmacological blockade by small molecule inhibitors; however, a systemic TGF- β blockade is likely associated with a variety of side effects given the central role of TGF- β in regulating immune homeostasis. A blockade of TGF- β specifically in the redirected T cell is preferable. Basically, engineering T cells with the dnTGF- β R11 or with the TGF- β CAR was explored; the common mechanism is the sequestration of soluble TGF- β by binding. The lower levels of TGF- β in the tumor tissue likely reduce the TGF- β -mediated promotion of tumor metastasis by the induction of epithelial–mesenchymal transition.³³ With the release of pro-inflammatory cytokines and activating bystander immune cells, including tumor-infiltrating T cells (TILs), NK cells or macrophages, the TGF- β CAR seems to be superior to the dnTGF- β R11, which sequesters TGF- β but does not transmit an activating signal; the latter can be provided by co-expressing a CAR recognising the respective cancer cell.

The techniques available to make CAR T cells resistant to TGF- β showed feasibility, safety and efficacy in pre-clinical models and moreover implied that they can be used to promote adoptive CAR T cell therapy of advanced solid tumors. Given the fact that elevated serum levels of TGF- β are found in a broad variety of human solid tumors,^{15,34} the TGF- β blockade potentially represents a more general approach to enhance the efficacy of redirected immune cells against tumors. As long as the safety of the approach has not been fully documented in clinical trials, caution should be taken since T cell lymphomas developed in mouse models in which the dnTGF- β R11 was expressed under control of the CD2 or CD4 promoter.^{35–37} A clinical application, in contrast to the mouse model, should therefore more tightly control the dnTGF- β R11 expression in time and only when the CAR T cell enters the tumor tissue. In line with this concept, the CAR T cell modification with a TGF- β -antagonizing construct may be more tailored, for instance with a CAR-inducible dnTGF- β R11 expression regulated by an NFAT-IL-2 promoter element. In a recent study,²¹ the release of transgenic IL-7 was increased upon CAR engagement of antigen allowing TGF- β resistance preferentially upon

tumor entry. Alternatively, interference with the TGF- β signalling pathway can be performed by adding a small interfering RNA³⁸ and the RNA may be produced from a CAR-inducible transgenic construct by the CAR T cell itself. Gene editing, in contrast, would result in permanent silencing of the specific genes.

The application of a dominant-negative TGF- β type-2 receptor with a purpose to inhibit TGF- β signalling and allow effector T cells to resist otherwise inhibitory concentrations of TGF- β , which leaves their proliferation, cytokine secretion and cytolytic functions unchanged was introduced in a clinical aspect in a study conducted by Bollard *et al.* (2018).³⁹ DNR11-modified T cells were infused into patients with Epstein–Barr virus (EBV)-associated lymphomas. The reason being is that up to 40% of Hodgkin lymphomas (HLs) inhibit EBV genomes and express the type 2 latency proteins, latent membrane protein (LMP)-1, LMP-2, BARF1 and EBNA1. The study reports the effects of the administration of such engineered T cells to express a DNR11 (DNR11-LSTs) to eight patients with relapsed EBV-positive HL.³⁹ Significantly, the study reports that after infusion, DNR11-LSTs expanded and persisted for up to ≥ 4 years after infusion. It was recorded that no patient had previously undergone lymphodepleting chemotherapy before infusion; however, DNR11-LSTs alone produced therapeutic effects, such as tumor clinical responses in two of the seven patients with active disease. Additionally, there was one recorded clinical response after CD35-targeted radio immunotherapy, one PR for a total of 19 months and stable disease in three patients for 4, 10 and 13 months, and no toxicity was recorded in association with DNR11-LSTs.³⁹ Overall, the findings from this report demonstrate the safety of the technique but also emphasise the importance of strategies to overcome tumor immune evasion by TGF- β . From this, it is clear to underline the feasibility and therapeutic potential of counteracting a single but common tumor immune editing mechanism, although future applications may include exploiting this strategy to enhance the immunotherapy of many other tumors that exploit TGF- β for survival.

The approach in using modified CAR T cells with an inducible resistance mechanism potentially avoids deleterious systemic side effects, which is likely occurring during adjuvant therapies using cytokine supplementation, small drug

inhibitors or blockade of T-cell checkpoints. A tightly regulated and fully efficient induction of TGF- β resistance needs to be implemented within the CAR-modified T cell, making them more efficacious when entering the hostile tumor tissue. The same applies for TCR-modified T cells or any immune effector cell in general used for the treatment of solid tumors.

Transforming growth factor- β is certainly one of the most dominant suppressive factors in the tumor tissue; however, other repressive mechanisms towards infiltrating T cells are active independently of TGF- β and single genetic modifications of T cells may have limited effect. A number of suppressive mechanisms need to be additionally addressed, for example by blocking PD-1 and CTLA-4 or by co-expressing dnPD-1 or using a PD-1 switch receptor that provides a CD28 signal when engaging PD-1L. Co-expression of IDO or PKA inhibitors or of CD40L may also be required to shield CAR T cells from repression. Taken all known mechanisms together, TGF- β is certainly a major suppressive cytokine, but not the only one. Multiple inhibitory factors impact the PI3K/Akt pathway; increase in Akt levels, constitutively or inducible upon CAR signalling, may result in a more effective PI3K/Akt pathway response, resulting in a balance counteracting for a broad range of tumor-associated repressive mechanisms by regulating diverse signalling pathways associated with T cell activation,²⁷ thereby replacing the need for other multiple manipulations to confer effector CAR T cell resistance. An inducible active Akt driven by CAR signalling either by increase in transgenic Akt levels or the possibility to create higher levels of active Akt within the pathway would improve the safety of such modified T cells in order to reduce the risk of signalling independently of T cell amplification.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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