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TRUCKS, the fourth-generation CAR T cells: Current developments and clinical translation

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Abstract

The overriding goal of adoptive cell therapy with chimeric antigen receptor (CAR) redirected T cells in oncology is to eliminate cancer cells from infiltrated tissues. Clinical trials document that this form of immunotherapy can induce lasting remissions of hematologic malignancies; however, the successes could not yet be transferred to the treatment of solid tumors. In this situation, modulating the immune regulation within the solid tumor tissue is thought to be a key point. In order to induce a pro-inflammatory milieu CAR T cells were additionally engineered to release a transgenic cytokine upon CAR signaling in the targeted tumor tissue. Such TRUCKs ("T cells redirected for antigen-unrestricted cytokine-initiated killing"), also called "4th generation" CAR T cells, combine the direct antitumor attack of the CAR T cell with the immune modulating capacities of the delivered cytokine. Through CAR-induced release, the cytokine is ideally deposited in the targeted tissue alleviating systemic side effects. The TRUCK concept is currently explored using a panel of cytokines, including IL-7, IL-12, IL-15, IL-18, IL-23, and combinations thereof, and is entering early phase trials. Future developments will expand the application to a broader panel of released proteins converting CAR T cells to "living factories" of therapeutically active, locally deposited products with the potential to eliminate some clinical deficits of the currently used CAR T cells in the field of solid tumors.

KEYWORDS

adoptive cell therapy, CAR, chimeric antigen receptor, T cell

1 | INTRODUCTION

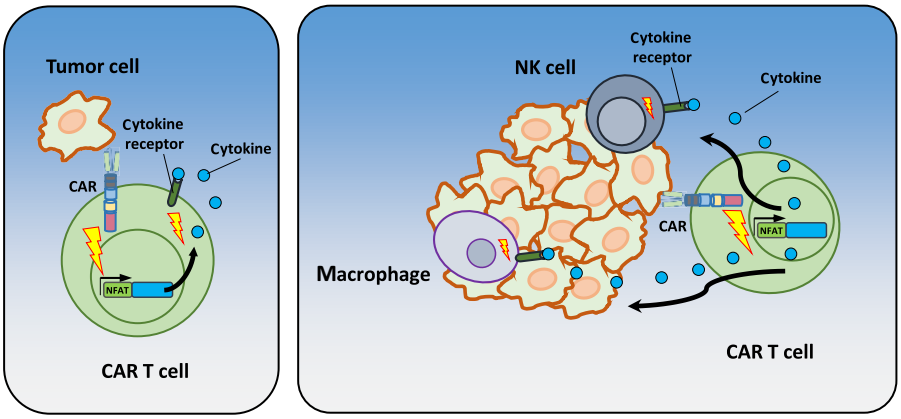
Multiple preclinical and clinical evidences indicate that adoptive transfer of antigen-specific T cells can control cancer in the long term. To redirect T cells specifically against a defined antigen expressed by cancer cells, patient's T cells are engineered with a T-cell receptor (TCR) or an artificial chimeric antigen receptor (CAR) recognizing the respective antigen. Accordingly, binding of the TCR to a peptide antigen presented by the major histocompatibility complex (MHC) initiates signaling through the TCR/CD3 complex that is enhanced by costimulatory receptor signaling. CAR uses the same CD3/costimulatory signaling pathway, however, binds to cognate antigen by a single-chain variable fragment (scFv) antibody independently of the MHC. By redirecting CAR T cells against CD19, so far refractory B-cell lymphoma and leukemia could be induced to long-term remissions and cure in a substantial number of cases.[1-3] The therapeutic

success of CAR T-cell therapy could not be transferred to the treatment of solid tumors, although some indications to clinical efficacy were obtained. For instance, the treatment of CEA$^+$ colorectal cancer with specific CAR T cells leads to stable disease in 7/10 patients, two of them with 30 weeks in stable disease and two patients experienced tumor shrinkage.[4] Glioblastoma was controlled upon installation of Her2-specific CAR T cells into the cavity after surgery inducing remissions in three of 17 patients and stable diseases in four of 17 patients.[5] Treatment with CAR T cells specific for GD2 induced complete remissions in three of 11 patients suffering from GD2$^+$ neuroblastoma; persistence of those CAR T cells beyond 6 weeks was associated with superior clinical outcome.[6] Epidermal growth factor receptor variant-III (EGFRvIII) specific CAR T cells traffic to regions of active glioblastoma, however, regulatory T cells (Tregs) also infiltrated the tumor repressing the antitumor response.[7] The observation suggests that CAR T-cell-mediated inflammation in the targeted tissue induces a broad compensatory immunosuppressive response that leads to repression and finally exhaustion of CAR T cells.

In this situation, modulating the immune regulatory milieu within the tumor tissue is thought to be key for improving CAR T-cell efficacy. A recently developed strategy utilizes CAR T cells for tumor targeting that are additionally engineered with a transgenic cytokine that is released upon CAR engagement of target within the targeted tumor (Figure 1). The strategy was developed to overcome the insufficient production of pro-inflammatory cytokine(s) by those T cells that accumulate in the tumor. CAR T cells engineered with CAR inducible release of transgenic IL-12 and IL-18 were successfully used in preclinical models[8-12]; T cells engineered with other cytokines are currently explored as well. TCR redirected T cells were likewise engineered for transgenic cytokine release.[13] CAR T cell mediated local delivery of transgenic IL-18 or IL-12 which induces acute inflammation in the tumor stroma and leads to a comprehensive antitumor response that is not achieved by coadministration of the respective cytokine to tumor-bearing mice.[10-12] Such TRUCKs ("T cells redirected for antigen-unrestricted cytokine-initiated killing"), also called "4th generation" CAR T cells, can provide a multifunctional treatment to the CAR targeted tissue which was so far not achieved by conventional CAR T cells.

2 | MATERIAL AND METHODS

The TRUCK concept is based on T cells that target the cognate tumor tissue through their engineered CAR or TCR and deliver a transgenic protein of interest, in particular a cytokine, to the targeted tissue upon specific signaling. For that, T cells with a transgenic CAR or TCR are additionally engineered with an inducible expression cassette for the protein of interest driven by the 6xNFAT-responsive elements and the IL-2 minimal promoter (Figure 2). Accordingly, TCR- or CAR-mediated signaling through the CD3-ZAP70 cascade, amplified by costimulation, results in the activation of the transcription factor NFAT that in turn activates the NFAT/IL-2 minimal promoter and finally initiates the expression of the transgenic protein. CAR and TCR signaling likewise induce NFAT/IL-2 driven transcription and finally the production and release of the transgenic protein; the promoter remains silent without adequate T-cell stimulation. CAR T cells producing a transgenic cytokine can basically provide stimulation in an autocrine fashion to sustain survival and amplification or in a paracrine fashion to modulate the immune cell environment. CAR T cells depositing the transgenic



CAR T cell releasing an *autocrine* acting transgenic cytokine, e.g., IL-7, IL-15, IL-18

CAR T cell releasing a *paracrine* acting transgenic cytokine, e.g., IL-12

FIGURE 1 The TRUCK concept. T cells are equipped with a tumor-specific chimeric antigen receptor (CAR) and a constitutive or NFAT-inducible expression cassette coding for a transgenic protein, preferentially but not exclusively, a cytokine. After CAR T cell binding to the CAR cognate antigen on the tumor cell, CAR signaling leads to NFAT phosphorylation, migration to the nucleus and induction of the NFAT-responsive/IL-2 minimal promoter that drives transgene expression. On the other hand, NFAT phosphorylation is also initiated by TCR/CD28 signaling making the concept translatable to T cells with transgenic TCR. In case of a cytokine as transgenic cell product, the cytokine can act in cis in an autocrine fashion to sustain survival and amplification of the CAR T cell, or in trans to modulate the immune cell environment, for instance to activate NK cells and to repolarize macrophages

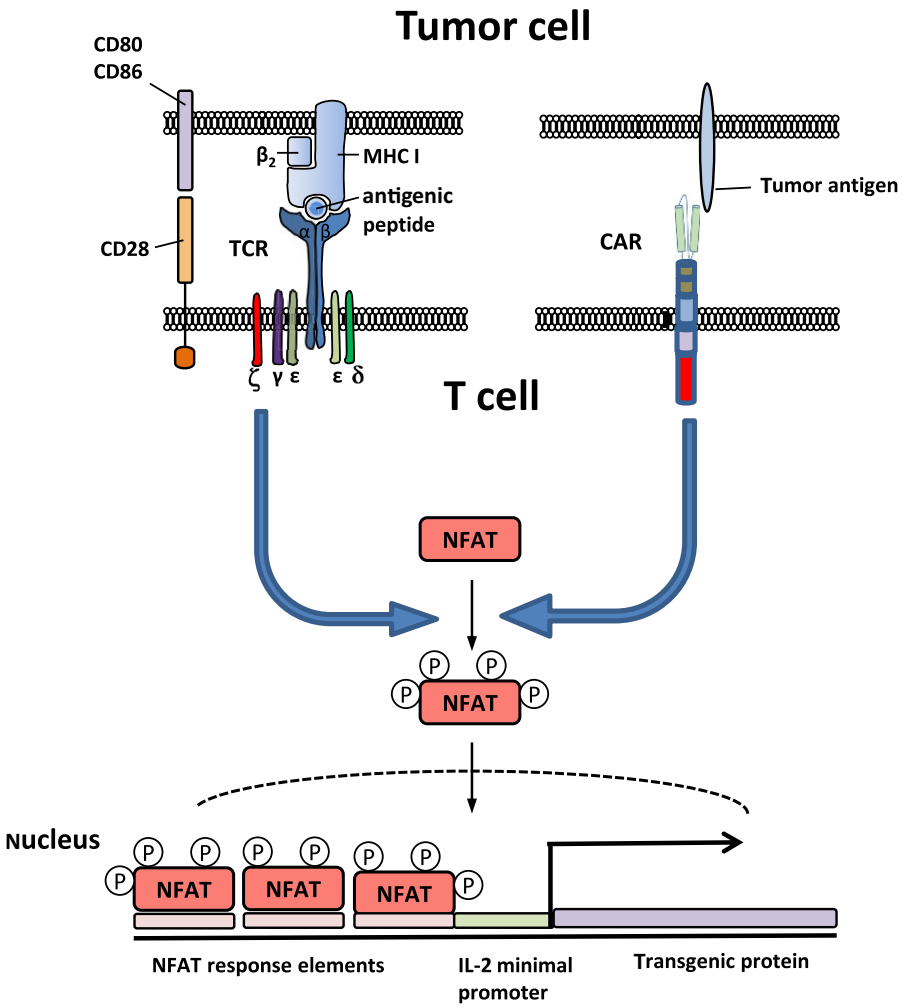


FIGURE 2 Both TCR and chimeric antigen receptor (CAR) signaling can induce the expression of a transgenic protein product in engineered T cells. CAR engagement of cognate antigen on tumor cells induces CD3 ζ-mediated downstream signaling in T cells and finally the activation of NFAT by phosphorylation that migrates into the nucleus and binds to the synthetic $(NFAT)_3$ response elements-IL-2 minimal promoter of the transgenic expression cassette; alternatively, $(NFAT)_6$ response elements-IL-2 minimal promoter constructs are used. As a consequence the transgenic protein is expressed by CAR-stimulated T cells; expression ceases upon withdrawal from CAR signaling. The same activation signal is generated upon TCR engagement of the respective antigen presented by the MHC; again, the NFAT response element-IL-2 minimal promoter drives expression of the transgenic protein. The TRUCK concept is thereby applicable to both TCR and CAR engineered T cells for use in adoptive cell therapy

therapeutic protein in a CAR-targeted lesion have the advantage to treat otherwise not accessible tumor lesions and to achieve therapeutic levels of the transgenic product at the site of CAR T-cell activation while reducing systemic toxicity. Some pro-inflammatory cytokines including IL-7, IL-15, IL-12, and IL-18 are currently explored (8-20) in the context of the TRUCK strategy (Figure 3).

3 | RESULTS AND CONCLUSIONS

3.1 | CAR T cells delivering IL-12 activate an innate immune response

From the aspect of safety, inducible release of the transgenic protein in the targeted tissue upon CAR signaling is of substantial relevance when the protein causes toxicity upon systemic administration. For instance, this is the case for IL-12 that is a highly potent inflammatory amplifier, however, induces severe toxicities preventing systemic application. The main sources of physiological IL-12 are monocytes, macrophages, and dendritic cells in response to microbial infections. IL-12 enhances T- and NK-cell cytotoxicity and induces their IFN-γ release, affects NK-cell amplification, and drives the Th1 response.[21,22] IFN-γ in turn activates monocytes and macrophages to further increase IL-12 production[23] helping to control local pathogen infections.[24] Anti-CD19 CAR T cells with constitutive IL-12 release produced a more robust response against $CD19^+$ leukemia[25] and did not require preconditioning of the host.[16] Interestingly, IL-12 releasing CAR T cells became less sensitive to repression by Treg cells.[16]

CAR T cells engineered to constitutively release IL-12 showed superior activities against orthotopic ovarian cancer xenografts

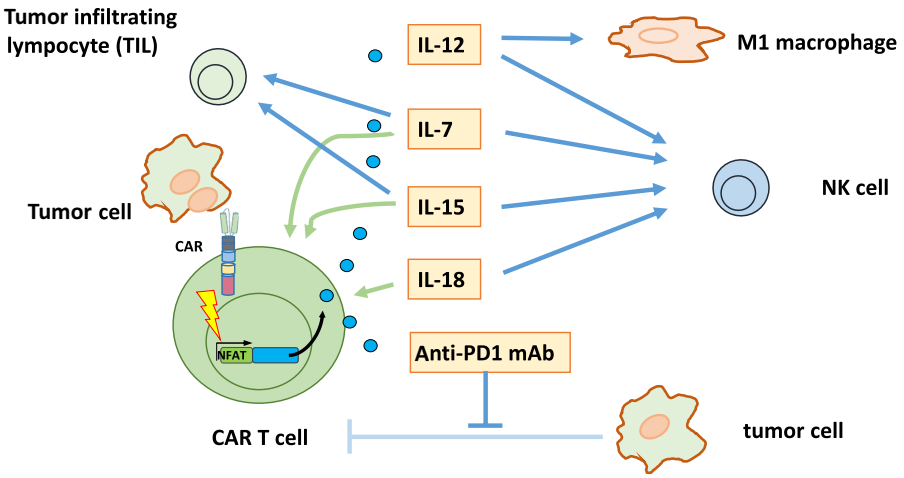


FIGURE 3 Transgenic proteins released by chimeric antigen receptor (CAR) T cells upon activation impact tumor environment. CAR T cells releasing transgenic cytokines when engaging the tumor tissue can activate tumor infiltrating T cells (TILs) and NK cells to prolong their persistence and amplification and to improve killing; IL-12 can also repolarize macrophages toward an M1 phenotype. These cytokines also act on the CAR T cells themselves prolonging their activation, improving their killing capacities, and protecting from activation-induced cell death. The TRUCK strategy can also be used to produce antibodies upon CAR signaling, for instance, a PD-1 blocking antibody that prevents T-cell suppression by tumor cells. CAR T cells can also be engineered to release other antibodies or proteins to modify the tumor environment

with increased survival of tumor bearing mice, prolonged T-cell persistence, and higher systemic IFN-γ levels.[9]

Constitutive IL-12 release, however, goes along with the risk of super-physiological high IFN-γ levels and consequently uncontrollable NK cell and macrophage activation and finally hematopoietic, intestinal, hepatic, and pulmonary failures[26] giving a strong rationale to express IL-12 under control of CAR signaling for local deposition in the targeting tissue based on at least two reasons.

(i) IL-12 modified T cells showed improved efficacy of toward large established tumors, that is, a single dose of 10^4 IL-12 modified T cells was therapeutically effective against established tumors compared to 2×10^7 T cells without IL-12.[27] High doses of systemically applied IL-12 did not recapitulate the effect indicating a clear therapeutic benefit of locally produced and deposited IL-12.
(ii) Frequently during tumor progression, some cancer cells in a tumor lesion lose antigen and MHC expression on their cell surface which makes them invisible to natural cytotoxic T cells as well as CAR-engineered T cells. The activation of the innate immune response is potentially a strategy to eliminate those antigen-loss cancer cells and to achieve lasting tumor reduction. To avoid systemic toxicities, CAR T cells with specificity for a tumor antigen were engineered with a NFAT/IL-2 promoter driven expression cassette to release inducible IL-12 (iIL-12) upon CAR engagement.[8] Once the CAR engages antigen, iIL-12 CAR T cells produce IL-12 providing constantly high cytokine levels to the targeted organ; IL-12 production ceases when CAR T cells no longer are in contact to their cognate antigen representing a safe "switch-off" when leaving the target tissue.

In an immune competent mouse model, systemic application of iIL-12 CAR T cells resulted in the elimination of antigen-loss cancer cells in a transplanted, antigen-heterogeneous tumor lesion consisting of both antigen-positive and -negative cancer cells.[8] The model thereby mimics a clinically relevant situation of a tumor with a substantial number of cancer cells lacking the targeted antigen. The therapeutic effect of IL-12 is generally attributed to orchestrating the Th1-type immune response against cancer by increasing the IFN-γ production by NK and T cells and to enhancing the cytolytic activity of $CD8^+$ T cells[28] and of a subset of $NKp46^+$ cells,[29] to counteracting angiogenesis through IFN-γ inducible genes, and to strengthening the lymphocyte-endothelial cell cross talk. Accordingly, application of iIL-12 CAR T cells activated macrophages in the tumor lesions and eliminated antigen-loss cancer cells.[8] Other innate cells, including NK and NK-T cells[30,31] are also IL-12 targets contributing to eliminate antigen-loss tumor cells through secreted TNF-α. At least in a preclinical model iIL-12 CAR T cells combine the antigen-redirected cytolytic T cell attack with an antigen-independent antitumor response through the activation of tumor resident innate immune cells.

In a mouse model of $CD19^+$ thymoma CD19-specific CAR T cells with transgenic IL-12 secretion induced efficient tumor eradication. The therapeutic effect was dependent on both $CD4^+$ and $CD8^+$ T cells and required further autocrine stimulation by IL-12 and IFN-γ, which in turn made the cells resistant to Treg cell suppression.[16]

Kueberuwa et al confirmed these observations in an additional $CD19^+$ tumor model; constitutive production of IL-12 by CD19-specific CAR T cells can eliminate established systemic lymphomas without prior lymphodepletion. Tumor control appears to be due to the combined action of direct cell killing, induction of a robust memory cell development and the induction of a host immune cell response against the cancer.[32]

Another example demonstrating the benefit of IL-12 in the elimination of solid tumors are CAR T cell targeting glypican-3 expressed

by hepatocellular carcinoma and releasing inducible IL-12.[33] Such IL-12 TRUCKs showed augmented the antitumor effect when encountering large tumor burdens which is superior to CAR T cells without IL-12 release. Tumor deposited IL-12 increased IFN-γ production, favored T-cell infiltration and persistence and decreased regulatory T cells in tumors.

T cell released IL-12 furthermore increased proliferation, decreased apoptosis, and increased cytotoxicity of CAR T cells when applied to immunosuppressive ascites tumors.[34] In a similar way, MUC-16-specific CAR T cells with IL-12 release could overcome the immunosuppression by the tumor microenvironment in mice bearing ovarian cancer xenografts resulting in increased survival rates, longer CAR T cell persistence, and higher systemic IFN-γ serum levels.[9] In order to link the IL-12 production to CAR T-cell activation and to eliminate PD-1 expression, the IL-12 transgene was placed under the control of the PDCD1 promoter by homology directed repair thereby generating PD-1/PD-L1-resistant CAR T cells with the activation-mediated IL-12 release.[35]

Taken together, IL-12 releasing CAR T cells have the capacity to change the immunosuppressive environment in the tumor lesion toward a Th1 polarized situation due to an increased Th1 cytokines and reduced IL-4 and IL-5 levels. On the other hand, IL-12 contributes to immune repression through released IL-10[36] which can be blocked by coadministrating soluble IL-10 receptor or a neutralizing IL-10 antibody. Coadministration of other cytokines, such as IL-2 and IL-18[37] may furthermore improve the antitumor activity of IL-12 CAR T cells in a specific fashion. Other cytokines of the IL-12 family may be alternatives for IL-12 TRUCK T cells. Both, IL-23 and IL-27 affect the IFN-γ production of NK cells and polarize the T-cell responses,[38] however, IL-23 promotes tumor progression[39] and thereby seems to be a less favorite cytokine in this respect.

3.2 | TRUCKs delivering IL-18

Current studies are exploring CAR T cells with transgenic IL-18 in order to improve the cytolytic response and to promote a proinflammatory immune reaction in the targeted tumor lesion. IL-18 promotes both Th1 and Th2 responses and, in concert with T-cell-secreted IFN-γ, accumulating IL-18 recruits and activates innate immune cells[40,41] and enhances IFN-γ production by NK cells and macrophages. Produced as an inactive precursor by macrophages, DCs and fibroblasts, IL-18 is converted into the mature form by Casp1 cleavage that binds to the heterodimeric IL-18Rα/IL-18Rβ receptor. IL-18 induced, exaggerated Th1 responses are prevented by binding to the IL-18 binding protein (IL-18 BP) that is constitutively produced by monocytes and macrophages as part of a negative feedback loop.[42,43] Upon binding to the respective receptor, IL-18 primarily signals through MyD88 and TRAF6 adapters resulting in MAPK and NFκB activation; this is unlike to most other proinflammatory cytokines that signal via the Jak/STAT pathway.[44] CAR T cells with constitutive IL-18 released increased IFN-γ levels and amplified upon triggering through the endogenous TCR in a xenograft mouse model.[10] In the B16F10 model with engineered $CD19^+$ melanoma cells IL-18 CAR T cells with CD19 specificity induced deeper B-cell aplasia, increased their antitumor efficacy and improved their amplification compared with CD19-specific CAR T cells without IL-18.[10] CAR T cells with CAR inducible IL-18 were more potent against advanced solid tumors in an immunocompetent mouse model with syngeneic pancreatic tumors than T cells without IL-18.[11] Systematic screening revealed that IL-18 polarizes CAR T cells toward T-bet^{high} $FoxO1^{low}$ effector cells mediating an acute inflammatory response in a fully immune competent host without prior preconditioning. Treatment with IL-18 CAR T cells was accompanied by increase in the number of M1 macrophages and activated NK cells in the targeted tumor tissue, both contributing to successful immune destruction.[11] The improved antitumor activities were confirmed other models including hematopoietic and solid tumors.[12] Noteworthy, IL-18 CAR T cells gain their activities by autocrine stimulation and transmit the effect to host T cells in a paracrine fashion.

Similarly as CAR engineered T cells, TCR redirected, IL-18 releasing T cells showed improved and persistent activities against melanoma.[13] In contrast to IL-12, IL-18 delivery seems to be safe since IL-18 administration to cancer patients did not cause life-threatening side effects even at high concentrations[45]; no adverse events were observed in mouse models with CAR T cells engineered to release IL-18. On the other hand, adoptive transfer of TCR T cells with inducible IL-12 into melanoma-bearing mice resulted in severe, edema-like toxicity accompanied by enhanced IFN-γ and TNF-α levels in the peripheral blood and reduced numbers of engineered T cells.[13] In accordance with improved in vitro amplification capacities, CAR T cells with constitutive IL-18 release showed superior in vivo expansion and persistence improving long-term survival of mice with hematological and solid malignancies.[12] In these models, IL-18 recruited and activated macrophages and induced an acute inflammation in the tumor tissue, both contributing to a productive antitumor response.

3.3 | TRUCKs with IL-15 release

An alternative strategy to improve T-cell activation and amplification is the transgenic release of IL-15 that suppresses T-cell apoptosis through BCL upregulation.[46] CD19-specific CAR NK cells with transgenic IL-15 release efficiently eliminated $CD19^+$ cell lines in vitro and increased the survival of mice with a xenograft Raji lymphoma compared with CAR NK-cell treatment without IL-15 release.[18] CAR T cells engineered with membrane-anchored IL-15 were also effective in the treatment of $CD19^+$ B-cell malignancies.[17] CAR T cells with membrane IL-15 showed long-term persistence, a $CD45RO^-CCR7^+CD95^+$ T stem cell memory (TSCM)-like phenotype and superior in vivo antitumor activity making IL-15 anchored CAR T cells to candidates for clinical exploration in other malignant diseases. Along this line, anti-GD2 CAR T cells with constitutive IL-15 release were reported to express a more stem-cell like phenotype and enhanced survival and antitumor response upon repetitive stimulation with tumor cells.[47]

3.4 | TRUCKs engineered to overcome TGF-β repression

CAR T cells can also be engineered to release of autostimulatory cytokines on order to counteract their repression in the tumor environment. T-cell responses are frequently repressed by TGF-β[15] that is present in high levels in a variety of solid tumors and goes along with poor prognosis. TGF-β also drives T-cell differentiation into regulatory T cells[48] that, in turn, produce TGF-β and further promote immune repression. CD28-ζ CAR T cells release IL-2 upon activation that counteracts TGF-β-mediated repression,[49] but also sustains Treg-cell survival and amplification, again suppressing the CAR T-cell response. By deleting the LCK binding domain within the intracellular CD28 signaling chain, IL-2 release upon CAR signaling is reduced, however, making T cells sensitive to TGF-β.[15] In this situation, Golumba-Nagy et al[15] explored replacing IL-2 by other γ-cytokines like IL-7 or IL-15 and introducing a hybrid receptor that binds the γ-cytokine and transmits the IL-2 receptor signal. T cells with CAR IL-2 deficiency were additionally engineered with an autocrine loop composed of transgenic IL-7 release, replacing IL-2, and providing IL-7 binding to the recombinant IL-7 receptor α-chain that is linked to the intracellular IL-2 receptor signaling β-chain. The hybrid IL-7Rα/IL-2Rβ receptor has the advantage of translating extracellular IL-7 binding into intracellular IL-2 signaling conferring resistance to TGF-β. Such IL-7 TRUCKs with a hybrid IL-7/IL-2R receptor become activated upon binding to antigen by the CAR, amplify, and execute cytolysis along with releasing proinflammatory cytokines in a so far repressive environment.

TGF-β resistance was also converted to Epstein-Barr virus (EBV)-specific, cytolytic T cells that were engineered to constitutively release IL-12 to the targeted EBV$^+$ Hodgkin's lymphoma lesion[50] which resulted in increased Th1 and reduced Th2 cytokines, like IL-4 and IL-5, finally providing resistance to TGF-β-mediated repression.

3.5 | Clinical implications

Previous clinical trials explored various strategies to deliver IL-12 locally to the tumor lesion, including the transfer of IL-12 gene-transduced tumor cells,[51] fibroblasts,[52] or dendritic cells.[53] IL-12 production at the tumor environment was associated with substantial macrophage infiltration, vessel damage and tumor necrosis.[37] Upon intratumoral injections, response rates were about 43%-56% in cutaneous T-cell lymphoma, Kaposi's sarcoma and mycosis fungoides; in other tumor entities, however, the efficacy was minimal underlining the need to increase cytokine levels or to combine IL-12 with other antitumor strategies. For instance, IL-12 was delivered together with GM-CSF on microspheres[54] in order to improve the antitumor activities. Compared to applying the cytokine, TRUCKs are "living factories" with the advantage to amplify and to continuously deliver the transgenic cytokine over an extended period as long as the T cell stays activated and persists in the targeted tumor lesion.

Based on mouse models,[28] Rosenberg and colleagues modified tumor-infiltrating lymphocytes (TILs) to express IL-12 for the treatment of patients suffering from metastatic melanoma (NCT01236573). Patients received a nonablative lymphocyte-depleting regimen followed by the infusion of CD8$^+$ TILs modified with IL-12 and ex vivo expanded. The IL-12 expression was under control of a NFAT-inducible promoter providing cytokine release upon TCR-mediated T-cell activation. The study ultimately had to be discontinued due to severe toxicity in most patients likely due to extensive IL-12 release.[55] It remains an open issue whether the use of CAR T cells with predefined specificity for a tumor associated antigen instead of polyclonal TILs would also lead to same systemic toxicities.

An ongoing trial (NCT03542799) explores EGFR-specific CAR T cells with engineered IL-12 release for the treatment of metastatic colon cancer. A TRUCK with a third-generation CAR with 4-1BB and CD28 costimulation along with the primary CD3ζ signal and NFAT driven, inducible IL-12 release is used. As a phase I trial, the aim is to determine the maximum tolerated dose and the safety and feasibility in cancer patients.

In a phase I trial, CAR T cells were directed against MUC16^{ecto} that is highly expressed on most epithelial ovarian carcinomas but at low levels on normal tissues; CAR T cells were further modified to secrete IL-12 to enhance cytotoxicity and persistence and to modulate the tumor microenvironment.[56] In this trial, 4-1BB-CD28-ζ CAR T cells with transgenic IL-12 release were injected intraperitoneally near the tumor site. The rationale is that secreted IL-12 is expected to enhance CAR T-cell persistence and to attract a Th1 immune cell infiltrate. For safety purposes, a suicide gene has been incorporated into the CAR T cells to mitigate any on-target, off-tumor or other unforeseen toxicity.

To treat Nectin4-positive advanced solid tumors, CAR T cells are going to be applied targeting Nectin4 and FAP on tumor stroma by the CAR and releasing IL-7 or IL-12 upon CAR engagement of target (NCT03932565).

3.6 | Future developments of the TRUCK strategy

The primary aim of the TRUCK strategy is to produce, either by inducible or by constitute release, and deposit a transgenic protein in the targeted tissue. By releasing a cytokine in the targeted tissue, systemic side effects are reduced, however, may occur upon entry into circulation. Linking the cytokine to an antibody or antibody fragment (chimeric antibody-cytokine fusion protein) for tumor targeting, the cytokine may become more "sticky" to the targeted tissue.

Another future aspect of transgenic cytokine delivery by CAR T cells is the release of two cytokines in order to combine their capacities. Both IL-7 and IL-15 are the common cytokine receptor γ–chain family cytokines. IL-7 plays a central role in the development

and maintenance of T cells, whereas IL-15 induces the differentiation and proliferation of T and NK cells and enhances the cytolytic activity of $CD8^+$ T cells. The activities may be combined by cosecretion of both individual cytokines or by linking both cytokines together to create a recombinant IL-7/IL-15 hybrid cytokine linked by a flexible peptide linker as previously reported for mouse cytokines.[57] Combining both cytokines aims at improving half-life of the respective cytokines and finally antitumor activities. Moreover, combining IL-7 and IL-15 may evoke additional effects on immune cells as compared with the individual factors. For instance, treatment with the IL-7/IL-15 fusion protein decreased Treg-cell infiltrations in tumors and more effectively improved NK-cell activities, whereas IL-7 and/or IL-15 treatment did not or far less.[57]

Within the technical framework of TRUCKs, it is conceivable to engineer T cells to release any protein with therapeutic potential or functional capacities. Among these proteins may be cancer-directed monoclonal antibodies to mediate antibody-dependent cellular cytotoxicity or sustain cellular responses and anticancer efficacy. Other favorite candidates for therapeutic proteins are immune checkpoint inhibitors that are clinically used with great success, in particular monoclonal antibodies against CTLA-4 like Ipilimumab and against PD-1 like Nivolumab. Such antibodies can also be produced by engineered T cells and released into the environment after CAR signaling. We assume that the approach will have the advantage of accumulating antibodies in the tumor tissue to high concentrations sufficient to prevent T-cell suppression. The strategy is currently explored in an early phase trial for the treatment of advanced solid tumors with EGFR-specific CAR T cells engineered to release CTLA-4 and PD-1 antibodies (NCT03182816).

Any other protein can be released by engineered TRUCK cells. It is conceivable that pro-drug converting enzymes are secreted by TRUCK T cells into the tumor tissue upon targeted activation. In this example, T cell released cytosine deaminase can cleave the chemotherapeutic prodrug 5-fluorocytosine (5-FC) into its active form 5-fluorouracil (5-FU) in the tumor area, thereby locally converting an inactive into a cytotoxic drug that kills tumor cells independently of T-cell recognition.

More recently, T cells were engineered with the IL-23 p40 receptor chain that associates with the endogenous IL23a p19 receptor to use released IL-23 in an autocrine fashion.[58] CAR T cells with engineered IL-23 receptor showed superior reactivity in various tumor models and attenuated side effects putting IL-23 in the forefront of sustaining cytokines for an improved CAR T-cell response.

Beyond cancer therapy, cytokine releasing engineered T cells have therapeutic potential for the treatment of a broad variety of other diseases. For the treatment of autoimmune diseases, engineered T cells can be envisioned to produce immune suppressive cytokines, including TGF-β or IL-10. However, this would require a protective shield in order to prevent engineered T-cell repression by the release of its own transgenic cytokine.

ETHICS STATEMENT

The authors confirm that the ethical policies of the journal, as noted on the journal's author guidelines page, have been adhered to and the appropriate ethical review committee approval has been received for their own cited work.

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