

Review Article

Chimaeric antigen receptor T-cell therapy for tumour immunotherapy

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Chimaeric antigen receptor (CAR) T-cell therapies, as one of the cancer immunotherapies, have heralded a new era of treating cancer. The accumulating data, especially about CAR-modified T cells against CD19 support that CAR T-cell therapy is a highly effective immune therapy for B-cell malignancies. Apart from CD19, there have been many trials of CAR T cells directed other tumour specific or associated antigens (TSAs/TAAs) in haematologic malignancies and solid tumours. This review will briefly summarize basic CAR structure, parts of reported TSAs/TAAs, results of the clinical trials of CAR T-cell therapies as well as two life-threatening side effects. Experiments *in vivo* or *in vitro*, ongoing clinical trials and the outlook for CAR T-cell therapies also be included. Our future efforts will focus on identification of more viable cancer targets and more strategies to make CAR T-cell therapy safer.

Introduction

Recently, cancer immunotherapies received a high degree of attention, which mainly contained the treatments for programmed death 1 (PD-1), programmed death ligand 1 (PD-L1), cytotoxic T lymphocytes-associated antigen 4 (CTLA-4) and chimaeric antigen receptors (CARs) [1]. Studies of (CAR)-specific T cells were viewed with exceptional interest for clinical development.

CAR T-cell therapy was a mode of adoptive T-cell therapy, which also contained tumour-infiltrating lymphocytes (TILs) and TCR engineered T cells (TCRT). However, TILs were reproducibly detectable only in a minority of cancers [2]. Also, the human leucocyte antigen (HLA) restricted nature of TCR recognition limited the application of TCRT to specific HLA repertoires. Fortunately, CAR T-cell therapy satisfied the need to explore new and efficacious adoptive T-cell therapy. CAR was a synthesized transmembrane protein, redirecting the target antigens expressed in tumour cells through genetic reprogramming. This gene transfer technology could efficiently introduce genes encoding CARs into immune effector cells [3]. Once transferred, engineered T cells provided specific antigens binding in a non-major histocompatibility complex (MHC) restricted manner, and were capable of recognizing tumour independently of HLA molecules. Therefore, compared with the TCRT therapy, the CARs might recognize a far greater range of potential cellular targets and could be applied to a broad range of patients irrespective of HLA phenotype [3-5]. These advantages, namely MHC-independent and tumour-specific were also carried by another approach, bi-specific T-cell engagers (BiTEs). BiTEs were a subclass of bi-specific antibodies (bsAbs). BiTEs did not need conventional MHC recognition when they induced T-cell activation through dual antigen binding. They were specific for CD3 on one arm and a tumour antigen on the second in order to bring T cells and malignant cells into close proximity [6]. However, Stone et al. [7] compared the *in vitro* sensitivity of these two strategies and found that CAR-expressing T cells were more sensitive than BiTE-treated T cells to low numbers of antigens per cell [7]. This indicated that CARs might be considered to be used in preference to BiTEs when epitope densities were low.

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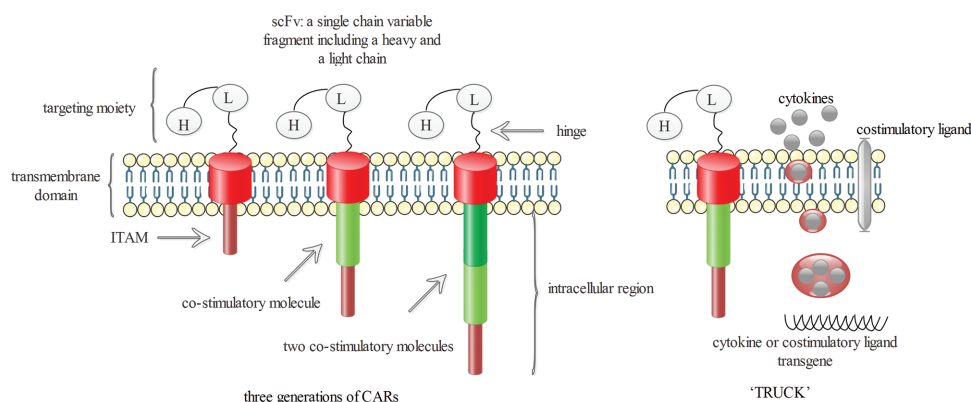


Figure 1. CAR T cells were classified into three generations based on intracellular signalling domains

First-generation CARs contained only one signalling domain. To provide the needed co-stimulatory receptors, CD28 or 4-1BB were integrated into the second-generation CARs. Third-generation CARs had two co-stimulatory domains, typically included both CD28 and 4-1BB or CD134 (OX40). Besides this, the concept of the 'TRUCK' was raised. They were produced through the introduction of additional genes, including those encoding T-cell-co-stimulatory ligands (4-1BBL) or pro-inflammatory cytokines (interleukin (IL)-12).

In decades, CAR T-cell therapy generated a great deal of enthusiasm in the field of cancer treatment. It made gratifying achievements for the treatment of haematologic malignancies like leukaemia [8] and lymphoma [9], as well as solid tumours such as neuroblastoma [10-12] and glioblastomas [13,14].

In this review, we will summarize current achievements and challenges of the CAR T-cell therapy and focus on the strategies to maximize the potential of this therapy.

Structures, advantages and disadvantages of each generation of CAR

Over the last decades, a lot of attempts were made to construct the structures of CARs. Generally, CARs contained a targeting moiety, a transmembrane domain and an intracellular region. Specifically, a single-chain variable fragment (scFv) linked to a hinge region made up the targeting moiety, and the intracellular region was an immunoreceptor tyrosine-based activation motif (ITAM) which comprises either a region of CD3 ζ chain or Fc ϵ RI γ [4,5].

Depending on the differences of intracellular signalling domains, CAR T cells were classified as first, second and third generation [15,16] (Figure 1). The first-generation CAR T cells just consisted of scFv and ITAM lacking co-stimulatory signalling. So, the activation and the proliferation of T cells were at a low level, leading to a short time of T-cell-killing and anti-tumour efficacy [17]. To address this limitation, the second-generation CAR T cells were designed, which expressed co-stimulatory molecules (CMs) in the intracellular domain. Concretely, they encompassed one CM such as CD28 and CD137 (4-1BB) [3,15]. The second generation showed strikingly enhanced expansion and persistence of T-cell activation, growth and survival [18]. In order to improve the efficacy, the third generation was developed based on the second generation. They had two CMs among CD28, CD27, 4-1BB and the others [3,16]. Inducted CMs into the CARs construction resulted in enhanced activation, proliferation and elevated survival of T cells so that the CAR T cells could exhibit more tumour cell-killing efficacy [16]. However, because of the presence of multiple intracellular signalling caused by the CMs of the second or third generation, an abundance of cytokines might be released and they would have resulted in cytokine storm, which was life threatening [19].

Besides, the concept of the fourth-generation CAR-modified T cells, which was also known as 'TRUCK' T cells, was raised by some studies [20]. The fourth-generation CAR T cells with additional genetic modification were able to express proliferative T-cell-co-stimulatory ligands (4-1BBL) or pro-inflammatory cytokines (IL-12) (Figure 1) [3]. Once recognizing the TSAs/TAAAs on the tumour cells, the fourth-generation CAR T cells released a large number of perforins, granzymes and tumour necrosis factors (TNFs), which eventually led to apoptosis of tumour cells. Compared with the first three generations, the 'TRUCK' T cells had more advantages on affecting local suppressive cells and were able to cause more anti-tumour destruction [21].

Table 1 Published results from clinical trials of CAR T cells targeting CD19 and CD20 in haematologic malignancies

Antigens	References	Diseases	Responses to CAR T cells	Main side effects
CD19	[64]	Two FL	Two NR	None
CD19	[18]	Six NHL	Two SD, four PD	None
CD19	[75]	Eight CLL, one ALL	Two SD, one reduction in lymphadenopathy, three no objective response, one B-cell aplasia, one PD, one NE	Fever
CD19	[76]	Three CLL	Two CR, one PR	TLS, SIRS, B-cell aplasia
CD19	[19]	Three FL, four CLL, one SMZL	One CR, five PR, one PD, one NE	SIRS, B-cell aplasia
CD19	[54]	Two ALL	Two CR	SIRS, CNS toxicity
CD19	[77]	Four ALL, four CLL	Two CCR, one CR, one PR, one SD, three PD	None
CD19	[78]	Four CLL, Four MCL, two DLBCL	One CR, one PR, six SD, two PD	TLS, SIRS, fever
CD19	[53]	16 ALL	14 CR	SIRS, neurotoxicity
CD19	[9]	Nine DLBCL, four CLL, two indolent lymphomas	Eight CR, four PR, one SD, two NE	SIRS, CNS toxicity
CD19	[79]	One DLBCL, 20 ALL	14 CR, three SD, four PD	SIRS
CD19	[8]	30 relapsed and refractory ALL	27 CR, three NR	SIRS
CD19	[80]	Nine relapsed and refractory ALL	Two MRD, two CR, three PD, one CNS1, one haematological improvement and reduction in blast counts of bone marrow	CRS, neurotoxicity
CD19	[81]	14 relapsed and refractory CLL	Four CR, four PR, six NR	CRS, B-cell aplasia
CD19	[82]	One MM	One CR	B-cell aplasia
CD20	[83]	Seven FL	Two CR, one PR, four SD	Fever
CD20	[64]	Two DLBCL	Two in remission continually after autologous haematopoietic stem cell transplantation	Cytopenias
CD20	[27]	Two MCL, one FL	Two without evaluable disease remained free of progression, one PR	Fever, cytopenias
CD20	[84]	Seven DLBCL	One CR, three PR, one SD, one PD, one NE	TLS, CRS

CCR, continuous complete response; CLL, chronic lymphocytic leukaemia; CNS, central nervous system; CNS1, no detectable leukaemia in the cerebrospinal fluid; CR, complete response; CRS, cytokine release syndrome; FL, follicular lymphoma; MCL, mantle cell lymphoma; MM, multiple myeloma; MRD: minimal residual disease; NE, not evaluable; NHL, non-Hodgkin's lymphoma; NR, no responses; PD, progressive disease; PR, partial response; SD, stable disease; SIRS, systemic inflammatory response syndrome; SMZL, splenic marginal zone lymphoma; TLS, lysis syndrome.

TSAs/TAAs for CAR T-cell therapy

A multitude of CARs targeting an array of TSAs/TAAs have been reported for their remarkable anti-tumour effect *in vitro* or *in vivo*, including targeting cell surface tumour antigens in haematologic malignancies and solid tumours [15]. Generally, antigens recognized by CARs needed to be expressed on the surface of the tumour, making it an important disadvantage. However, interestingly, certain article showed that intracellular tumour antigens might also be recognized using the cell receptor-mimic antibody (TCRm) CAR, which derived from the ESK1 TCRm monoclonal antibody (mAb) [22]. Moreover, tumour angiogenesis was an ideal choice of the targets for CAR T-cell therapy as well [23,24]. All these possibilities made CAR T-cell therapy a powerful tool for cancer treatments.

Cell surface tumour antigens in haematologic malignancies CD19

In clinical trials, CD19 was most widely used as a target tumour antigen of haematological cancers (Table 1). CD19 was considered as an ideal target for B-cell malignancies because of its high and uniform expression on B cells [25]. In 2014, Kochenderfer et al. [9] published the first report on successful treatment of diffuse large B-cell lymphoma (DLBCL), and demonstrated the feasibility and validity of anti-CD19 CAR T cells treating chemotherapy-refractory B-cell malignancies. The present study concluded 15 patients, eight achieved complete remissions (CRs). The result indicated anti-CD19 CAR T-cell therapy was a potentially powerful new treatment for B-cell malignancies. In the

same year, another clinical trial showed that CR was achieved in 27 patients among a total of 30 children and adults with relapsed or refractory acute lymphoblastic leukaemia (ALL) [8]. These anti-CD19 CAR T-cell therapy overcame limitations of conventional therapies and induced remission in patients with refractory disease and the effectiveness provided strong support for further development of this approach.

CD20

CD20 (Table 1) was an activated glycosylated phosphoprotein expressed on the surface of B-lymphocytes [26]. Till et al. [27] conducted a pilot clinical trial aiming to test the effect of a third-generation CD20-specific CAR on patients with relapsed indolent B-cell and mantle cell lymphomas. The treatment was well tolerated and the clinical results of this therapy were promising with one patient having an objective partial response and two remaining free of progression for 12 and 24 months. This year, Watanabe et al. [26] concluded a research that anti-CD20 CAR T-cell therapy might also be an applicable option for the treatment of CD20-positive lymphoid malignancies. What's more, the report found out a threshold of the antigen density. The threshold was sufficient for practical effectiveness, meanwhile, it might not result in adverse effects. Thus, antigen density seemed an ideal point for further investigation to reduce side effects.

CD30

CD30 was a member of the TNF receptor superfamily [28]. The malignant cells in a broad variety of Hodgkin's lymphoma (HL) and NHL selectively express CD30, which was considered as an alternative target antigen [29,30]. A phase I dose escalation study summed up that eight of nine patients with relapsed/refractory CD30 + HL or NHL treated by CAR CD30-T cells had either relapsed or progressed, showing objective anti-tumour responses [31]. However, lymphocytes and haematopoietic stem and progenitor cells (HSPCs) also expressed CD30 after activation. As for this concern, a recent research provided evidence that therapy with anti-CD30 CAR T cells derived by HRS3scFv displayed a superior therapeutic index in the treatment of CD30 + malignancies without attacking healthy activated lymphocytes and HSPCs [30]. All of these demonstrated that anti-CD30 CAR T-cell therapy could be alternative therapeutic strategy for patients with resistant/relapsed lymphomas.

CD33

CD33 was a myeloid differentiation antigen unexpressed on pluripotent haematopoietic stem cells or inside the haematopoietic system. It could be displayed on some normal B cells, activated T cells and natural killer (NK) cells, validated as an AML target [32]. A second-generation CD33-specific CAR was generated and was proved to be effective for acute myeloid leukaemia. In the present study, leukaemia cell lines and primary tumour cells were efficiently killed *in vitro* by CAR T cells. Furthermore, the number of tumour cells was lower in mice treated with anti-CD33 CAR T cells than in control-treated mice. It showed that the anti-CD33 CAR T cells were also effective *in vivo* [33]. Therefore, anti-CD33 CAR T-cell treatment was highly effective in preventing AML development.

CD123

CD123 was an attractive surface target highly expressed in leukaemic stem cells and leukaemic blasts but lowly expressed in normal HSPCs [34]. Mardiros et al. [35] found that their CD123 CAR T cells exhibited potent effector activity *in vitro* as well as anti-leukaemic activity *in vivo* against a xenogeneic model of disseminated AML. Another animal experiment supported that CAR T-cell therapy was a viable therapy for AML by targeting of CD123 via CAR-engineered T cells [36]. Therefore, all of these results suggested that CD123 CAR T-cell therapy was a promising immunotherapy and CD123 CAR T cells were potent candidates for future treatment of AML.

Cell surface tumour antigens in solid tumours

Prostate specific membrane antigen (PSMA)

PSMA was a 750-amino acid type II membrane-bound glycoprotein and abundantly expressed on the endothelium of many solid tumours, dramatically in prostate cancer [37]. A preclinical model was proposed that a second-generation anti-hPSMA CAR T cells exhibited evident and specific anti-tumour activity against a prostate tumour model, both *in vitro* and *in vivo* [38]. An experiment developed a second-generation anti-PSMA CAR T cells for improving the efficacy of first generation. The results described that the second-generation secreted more cytokines and proliferated more vigorously *in vitro* than the first generation. What's more, the second-generation appeared to be with higher potency on suppressing prostate tumour growth in animal models [39]. Both results provided the basis for advancing such approach towards clinical application.

Epidermal growth factor receptor variant type III (EGFRvIII)

EGFRvIII, a neo-antigen expressed in approximately 30% of glioblastomas and was correlated with poor prognosis [40]. According to Miao et al. [13], they established intracranial D-270 MG tumours, and showed that EGFRvIII CAR T cells had the capacity to suppress tumour growth and enhance survival of mice. Another report demonstrated that mice with glioma were successfully treated with a third-generation EGFRvIII CAR T cells. Significantly, the results endorsed clinical translation of this CAR in patients with brain tumours expressing EGFRvIII. [14]. Because of its highly specific and promising therapeutic efficiency, the results of clinical trials using EGFRvIII CAR T cells to treat glioblastoma attracted extensive attention.

Disialoganglioside (GD2)

GD2 was overexpressed among paediatric and adult solid tumours, such as neuroblastoma, retinoblastoma, glioma, Ewing's family of tumours and many other solid tumours [41]. A study tested T cells carrying the anti-GD2 CAR. The results displayed anti-cancer killing activity both in neuroblastoma cells *in vitro* and *in vivo* xenograft studies. More importantly, clinical testing of the approach was warranted in neuroblastoma and other GD2-positive malignancies due to the promising results of the study [10]. What's more, two trials also support the anti-tumour effects of the CAR T cells specific for the GD2. One of the trials reported that four patients with neuroblastoma had evidence of tumour necrosis, including a sustained complete remission. And there were no adverse events of the therapy seen in the total 11 subjects followed for up to 24 months. The second one demonstrated three patients achieving complete remission among 11 patients with active disease of neuroblastoma and observed long-term low-level presence of CAR expressing T cells was associated with longer survival [11,12]. GD2-targeting CARs, therefore, afforded us an alternative method for treatments of neuroblastoma.

Intracellular tumour antigens

Wilms tumour 1 (WT1)

WT1 was overexpressed in many cancers, including haematologic malignancies, like acute and chronic leukaemias and numerous solid tumours. A study designed WT1 28z CAR T cell, the first one against a human intracellular protein, WT1. WT1 28z T cells were specific for the WT1-HLA-A*02:01 complex and the outcome provided the proof-of-concept that CAR T cells could not only target the protein expressed on the cell surface of the tumour, but also target at intracellular antigens [22].

CAR T cells targeting tumour angiogenesis

Vascular endothelial growth factor receptor 2 (VEGFR-2)

Tumour angiogenesis could also be a target for CAR T cells, except TSAs/TAAAs. Some approaches, using VEGFR-2 CAR, aimed at targeting the tumour vasculature rather than the tumour cells because VEGFR-2 was overexpressed in tumour vasculature and was related to tumour progression and metastasis [42]. Chinnasamy et al. [23] developed a method to target tumour vasculature and the result was that the growth of five different types of established, vascularized syngeneic tumours was significantly inhibited by VEGFR-2 CAR-engineered mouse T cells plus exogenous IL-2 and the survival of mice was prolonged. Their late-stage study further displayed that co-administration of anti-VEGFR2 CAR T cells along with cells expressing a tumour-specific TCR could lead to a synergistic anti-tumour effect. Meanwhile, tumour-free survival of mice with established cancers was prolonged. All of these approaches targeting tumour angiogenesis opened new possibilities for the treatment of a wide variety of cancer types [24].

Other antigens in haematologic malignancies and in solid tumours were also well studied *in vitro* or *in vivo*, such as CD138 in MM [43], natural killer group 2 member D (NKG2D) in leukaemia and carcinoembryonic antigen (CEA) in colorectal cancer (Tables 2 and 3) [44].

Two serious side effects of CAR T-cell therapy and corresponding strategies

Although positive anti-tumour effects of the CAR T-cell therapies were mentioned above, some therapies targeting other TSAs/TAAAs showed obvious toxicities rather than clinical benefits (Figure 2). A case of 'on-target, off-tumour' toxicity was reported that one patient receiving a third generation of erbB-2-targeted CARs died on 5th day after therapy. The patient experienced respiratory distress within 15 min after cell infusion. Investigators speculated that the reason was that lung epithelial cells also expressed low level of erbB-2 recognized and damaged by CAR T cells with released mounts of cytokines [45]. In order to overcome this toxicity, it is crucial to find more specific antigens

Table 2 CAR T-cell therapies targeting other antigens in haematologic malignancies

Antigens	Diseases	<i>In vitro</i> , <i>in vivo</i> , in preclinical or in clinical trials	NCT ID or references
TRAIL receptor 1	Lymphoma	<i>In vitro</i>	[85]
Kappa	Lymphoma	Clinical trial	NCT00881920
CD22	FL, ALL, NHL	Clinical trial	NCT02315612
HA-1 H	Leukaemia	<i>In vitro</i>	[86]
NKG2D	Leukaemia	Clinical trial	NCT02203825
FAP	B-cell CLL	Clinical trial	NCT01722149
ROR1	CLL	Clinical trial	NCT02194374
CD138	MM	<i>In vitro</i>	[43]
	MM	Clinical trial	NCT01886976
NY-ESO-1	MM	<i>In vivo</i>	[87]
Lewis Y	MM	Clinical trial	NCT01716364

FAP, fibroblast activation protein; NY-ESO-1, New York-oesophageal-1; ROR1, receptor tyrosine kinase-like orphan receptor 1; TRAIL receptor 1, TNF-related apoptosis-inducing ligand (TRAIL) receptor 1.

Table 3 CAR T-cell therapies targeting other antigens in solid tumours

Antigens	Diseases	<i>In vitro</i> , <i>in vivo</i> , in preclinical or in clinical trials	NCT ID or references
HER2	Osteosarcoma	<i>In vitro</i>	[88]
	Breast cancer	<i>In vitro</i>	[89]
	Sarcoma	Clinical trial	NCT00902044
	Metastatic cancer	Clinical trial	NCT00924287
	Glioblastoma	Clinical trial	NCT01109095
CEA	Solid tumours	Clinical trial	NCT01935843
	Colorectal cancer	<i>In vivo</i>	[44]
	Colorectal cancer	Clinical trial	NCT00673322
	Breast cancer	Clinical trial	NCT00673829
	Liver metastases	Clinical trial	NCT01373047
CSPG4	Metastatic cancers	Clinical trial	NCT01723306
	Melanoma, breast carcinoma	<i>In vivo</i>	[90]
EphA2	Glioblastoma	<i>In vivo</i>	[91]
FR	Ovarian cancer	<i>In vivo</i>	[92]
IL-11R α	Osteosarcoma	<i>In vivo</i>	[93]
IL-13R α 2	Glioblastoma	Preclinical trial	[94]
	Malignant glioma	Clinical trial	NCT02208362
	Refractory brain neoplasm		
IL-13R	Recurrent brain neoplasm		
	Glioma	Preclinical trial	[95]
CD171	Neuroblastoma	Clinical trial	NCT02311621
EGFR	Advanced EGFR-positive solid tumours	Clinical trial	NCT01869166
	Advanced glioma	Clinical trial	NCT02331693

CSPG-4, chondroitin sulfate proteoglycan-4; EGFR, epidermal growth factor receptor; EphA2, Eph tyrosine kinase receptor A2; FR, folate receptor; HER2, human epidermal growth factor receptor 2.

expressed on tumour cells. However, majority of the antigens also keep low density on normal tissues [46]. In considering the difficulty to find truly TSAs or TAAs, Kloss et al. [47] presented a ‘dual-targeting’ strategy. The approach transduced T cells with a CAR that provided suboptimal activation and a chimaeric co-stimulatory receptor (CCR) (Figure 3). The suboptimal activation was upon binding of one antigen whereas the CCR was used to recognize a second antigen. T cells engineered in this manner would recognize the tumour, whereas tissues expressing either antigen alone would not activate them [47]. The efficiency of T-cell activation is ineffective in the absence of simultaneous CCR recognition of the second antigen. Similarly, T cells do not react against issues only expressing the CCR because

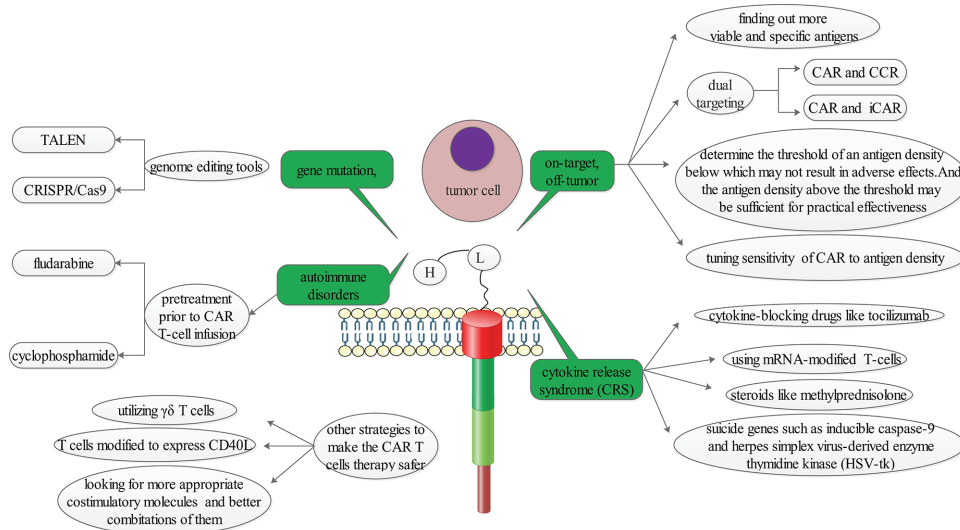


Figure 2. Strategies that could solve the problems including ‘on-target, off-tumour’ toxicity, ‘CRS’, ‘gene mutation’ and ‘autoimmune disorders’ and that might generate better CAR T products were taken into account

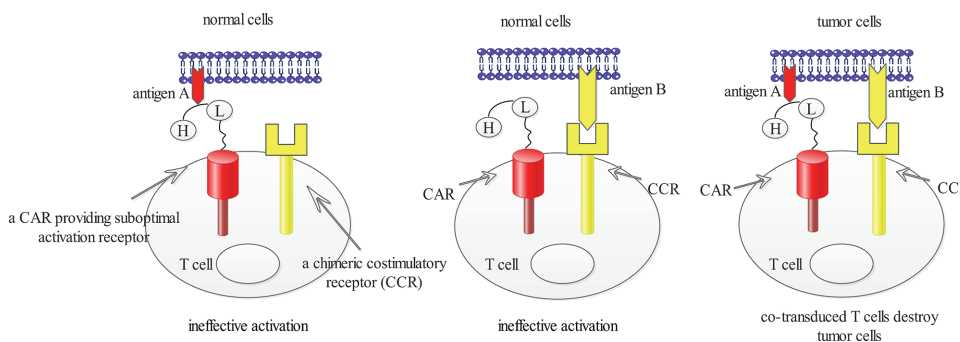


Figure 3. Normal cells that expressed only one antigen did not fully activate T cells

T cells expressing both CCR and suboptimal activation receptor were sufficiently activated by A+B+ target cells.

T cells would not be activated, lacking the CAR. Another alternative strategy is to employ both activating and inhibitory CAR (iCAR) that operated as logic gates (Figure 4). The iCAR delivered a dominant inhibitory signal such as PD-1 or CTLA-4 to achieve antigen-specific suppression of T-cell cytotoxicity, and the activating CAR was capable of full T-cell activation. Tumour cells expressing only the activating ligand could operate T-cell function, whereas normal cells expressing both antigens could inhibit T-cell function [48]. These approaches thereby could improve tumour selectivity.

Additionally, antigen density seemed an ideal point regarding reduction of side effects. Watanabe et al. [26] found out a threshold for antigen selection, below which might not result in adverse effects, whereas that displayed practical effectiveness above the threshold. On the other hand, the possibility of generating two CARs that differ in affinity was raised. CAR T cells in the present study were described to have the ability to distinguish malignant from normal cells based on antigen density. In the present study, EGFR-specific CAR T cells bearing low-affinity nimotuzumab-CAR selectively targeted cells overexpressing EGFR. However, they displayed diminished effector function when the density of EGFR decreased. In contrast, activation of T cells with high-affinity cetuximab-CAR showed no change [46]. Similar findings were reported including EGFRvIII-specific CAR and ROR1-specific CAR [49,50]. These methods of tuning sensitivity of CAR to antigen density made it possible to maintain anti-tumour activity without recognition of normal tissue. Many other tumours overexpressed TAAs relative to normal tissue as well. Therefore, these methods could be applied to other malignancies.

Another main safety concern was CRS. CRS was characterized by fever, hypoxia and hypotension, and potentially led to organ failure by producing several pro-inflammatory cytokines [51]. Cytokine-blocking drugs like tocilizumab

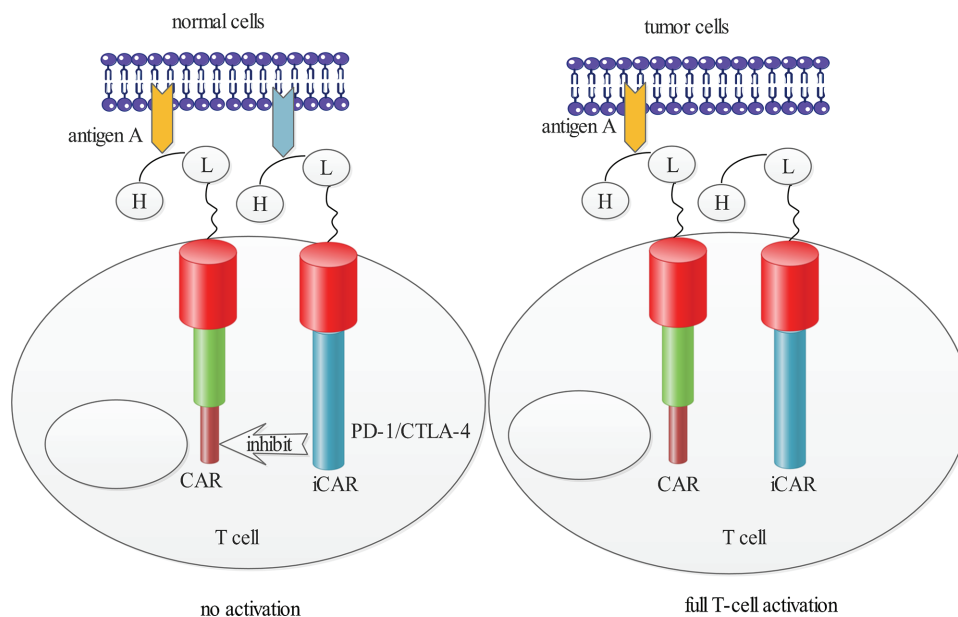


Figure 4. Normal cells could inhibit T-cell function when a CAR capable of full T-cell activation was co-expressed with an iCAR delivering PD-1 or CTLA-4

However, tumour cells fully activated T cells in the absence of the target for inhibitory signal.

that could block IL-6 signalling pathway and steroids like methylprednisolone were proved to be valid countermeasures [52-54]. Moreover, suicide genes such as inducible caspase-9 and herpes simplex virus-derived enzyme thymidine kinase (HSV-tk) were studied [55-57]. Also, the use of mRNA-modified T cells in CAR applications might be an ideal approach. A report that was the first preclinical one developed mRNA-modified CART33 cells and showed potent, but transient, *in vitro* and *in vivo* activity [58]. This solution would not permanently express CD33-specific CART cells, so it minimized the risk of long-term toxicity.

Challenges and outlook for CAR T-cell therapy

Despite the two tough toxicities, other problems such as minimizing the risk of gene mutation, reducing autoimmune disorders and generating better CAR T products remained challenges for the CAR T-cell therapy. In order to make the CAR T-cell therapies safer, more strategies need to be considered (Figure 2).

As known, modified T cells carried the CAR gene, which is introduced by a retrovirus. However, the target gene is randomly inserted into the genome, so the oncogenes would be activated if the insertion site is located in the proto-oncogene area. It might lead to the high risk of gene mutation and tumorigenicity after T cells reinfused to the body. It was reported that TAL effector nuclease (TALEN) and CRISPR/Cas9 were powerful genome editing tools for safe insertion. Therapeutic transgenes could be inserted to 'genomic safe harbours' – chromosomal locations where transgenes would not perturb endogenous gene activity and promote cancer [59-61].

Moreover, T cells after gene modification simultaneously carried endogenous TCR and exogenous CAR, both of which might be recombinant resulting in autoimmunity [62]. Fortunately, evidence indicated that pre-treatment with fludarabine and cyclophosphamide, prior to CAR T-cell infusion was helpful to improve the efficiency of cell immunotherapy [63]. However, the study of the immunogenicity would still be in the process and of great significance for patient's prognosis and the outcome.

In nearly all studies to date, T cells bearing $\alpha\beta$ receptors were used [9,64]. However, $\alpha\beta$ T cells require specific TAAs and appropriate CMs for activation. Tumour cells would be resistant to $\alpha\beta$ T-cell-mediated cytotoxicity when there is loss of TAA expression or absence of CMs [65]. Meanwhile, success with solid tumours was limited [45,66,67]. So, generating better CAR T products seemed to be of great significance. In this regard, biological characteristics and unique functions of $\gamma\delta$ T cells that could apply CAR T-cell therapy for solid tumours were highlighted [68]. Rischer et al. [69] suggested that $\gamma\delta$ T cells might serve as potent and specific anti-tumour effector cells because they demonstrated that $\gamma\delta$ CAR T cells showed cytotoxicity against tumour cell targets. Moreover, Deniger et al. [70] showed that $\gamma\delta$ CAR T cells not only killed CD19+ tumour cell lines *in vitro*, but also inhibited tumour growth

in a mouse xenograft model. More recently, another potential advantage of $\gamma\delta$ T cells was recommended. Mirzaei et al. [68] suggested that utilizing engineered allogeneic donor-derived $\gamma\delta$ T cells that expressed CAR transgene could be theoretically used as an off-the-shelf product because they were not restricted to MHC. All these offered the hypothesis that $\gamma\delta$ -derived CAR T-cell product would be a promising therapeutic strategy to improve anti-tumour immune responses.

Also, Curran et al. [71] made T cells genetically modified to constitutively express CD40L with the ability to enhance T-cell proliferation and tumour cell immunogenicity. This approach not only increased CD19-specific CAR/CD40L T cells efficiency but also has profound effects on the tumour micro-environment [71]. Additionally, the incorporation of CMs used in the second- and third-generation CARs could strengthen CAR T-cell activation [3,15,16]. So, we could make efforts to find better combinations of them, which indicated that new generation of CAR T cells might be applied to cancer treatment in the near future.

Conclusions

CAR T-cell therapy is currently perceived as one of the most promising therapeutic approaches for cancer treatment because of significant outcomes in various studies [5,72]. Instead of traditional drug and radiation therapy, CAR T-cell therapy may reduce or even replace some bone marrow transplantations for haematologic malignancies, avoiding high cost of long-term hospitalization and high risk of bone marrow transplantations. However, the application of CAR T-cell therapy still requires efforts and multiple exploratory studies to limit or contain the side effects. Furthermore, cell preparation, functional testing, evaluation of therapeutic efficacy and so on are also required systematic knowledge and norms.

Additionally, some experiments demonstrated that CAR-transduced cytokine-induced killer (CIK) and NK cells were effective [73,74]. To assess the validity, the safety of these approaches appears to be important and necessary. So, we can expect that CIK and NK cells may also be well involved in more clinical CAR therapies in the future.

We believe that with the growing understanding of technology foundations and clinical researches, CAR T-cell therapy will have a promising role in tumour immunotherapy.

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Competing interests

The authors declare that there are no competing interests associated with the manuscript.

Abbreviations

ALL, acute lymphoblastic leukaemia; AML, acute myeloblastic leukaemia; BiTE, bi-specific T-cell engager; CAR, chimaeric antigen receptor; CEA, carcinoembryonic antigen; CLL, chronic lymphocytic leukaemia; CM, co-stimulatory molecule; CTLA-4, cytotoxic T lymphocytes-associated antigen 4; EGFR, epidermal growth factor receptor; HL, Hodgkin's lymphoma; HLA, human leucocyte antigen; HSPC, haematopoietic stem and progenitor cell; iCAR, inhibitory CAR; IL, interleukin; ITAM, immunoreceptor tyrosine-based activation motif; MCL, mantle cell lymphoma; MHC, major histocompatibility complex; MM, multiple myeloma; NHL, non-Hodgkin's lymphoma; NKG2D, natural killer group 2 member D; NK, natural killer; PSMA, prostate specific membrane antigen; ROR1, receptor tyrosine kinase-like orphan receptor 1; TALEN, TAL effector nuclease; TCR, T cell receptor; TCRT, TCR engineered T cells; TIL, tumour-infiltrating lymphocyte; TNF, tumour necrosis factor; TSA, tumour specific antigens; TSS, tumour associated antigens.

References

- 1 Couzin-Frankel, J. (2013) Cancer immunotherapy. *Science* **342**, 1432–1433
- 2 Ruella, M. and Kalos, M. (2014) Adoptive immunotherapy for cancer. *Immunol. Rev.* **257**, 14–38
- 3 Brentjens, R.J. and Curran, K.J. (2012) Novel cellular therapies for leukemia: CAR-modified T cells targeted to the CD19 antigen. *Hematology/American Society of Hematology. Education Program* **2012**, 143–151
- 4 Heiblig, M., Elhamri, M., Michallet, M. and Thomas, X. (2015) Adoptive immunotherapy for acute leukemia: new insights in chimeric antigen receptors. *World J. Stem Cells* **7**, 1022–1038
- 5 Firor, A.E., Jares, A. and Ma, Y. (2015) From humble beginnings to success in the clinic: chimeric antigen receptor-modified T-cells and implications for immunotherapy. *Exp. Biol. Med.* **240**, 1087–1098
- 6 Suryadevara, C.M., Gedeon, P.C., Sanchez-Perez, L., Verla, T., Alvarez-Breckenridge, C., Choi, B.D. et al. (2015) Are BiTEs the “missing link” in cancer therapy? *Oncoimmunology* **4**, e1008339

- 7 Stone, J.D., Aggen, D.H., Schietinger, A., Schreiber, H. and Kranz, D.M. (2012) A sensitivity scale for targeting T cells with chimeric antigen receptors (CARs) and bispecific T-cell engagers (BiTEs). *Oncimmunology* **1**, 863–873
- 8 Maude, S.L., Frey, N., Shaw, P.A., Aplenc, R., Barrett, D.M., Bunin, N.J. et al. (2014) Chimeric antigen receptor T cells for sustained remissions in leukemia. *N. Engl. J. Med.* **371**, 1507–1517
- 9 Kochenderfer, J.N., Dudley, M.E., Kassim, S.H., Somerville, R.P., Carpenter, R.O., Stetler-Stevenson, M. et al. (2015) Chemotherapy-refractory diffuse large B-cell lymphoma and indolent B-cell malignancies can be effectively treated with autologous T cells expressing an anti-CD19 chimeric antigen receptor. *J. Clin. Oncol.* **33**, 540–549
- 10 Prapa, M., Calderer, S., Spano, C., Bestagno, M., Golinelli, G., Grisendi, G. et al. (2015) A novel anti-GD2/4-1BB chimeric antigen receptor triggers neuroblastoma cell killing. *Oncotarget* **6**, 24884–24894
- 11 Pule, M.A., Savoldo, B., Myers, G.D., Rossig, C., Russell, H.V., Dotti, G. et al. (2008) Virus-specific T cells engineered to coexpress tumor-specific receptors: persistence and antitumor activity in individuals with neuroblastoma. *Nat. Med.* **14**, 1264–1270
- 12 Louis, C.U., Savoldo, B., Dotti, G., Pule, M., Yvon, E., Myers, G.D. et al. (2011) Antitumor activity and long-term fate of chimeric antigen receptor-positive T cells in patients with neuroblastoma. *Blood* **118**, 6050–6056
- 13 Miao, H., Choi, B.D., Suryadevara, C.M., Sanchez-Perez, L., Yang, S., De Leon, G. et al. (2014) EGFRvIII-specific chimeric antigen receptor T cells migrate to and kill tumor deposits infiltrating the brain parenchyma in an invasive xenograft model of glioblastoma. *PLoS ONE* **9**, e94281
- 14 Choi, B.D., Suryadevara, C.M., Gedeon, P.C., Herndon, II, J.E., Sanchez-Perez, L., Bigner, D.D. et al. (2014) Intracerebral delivery of a third generation EGFRvIII-specific chimeric antigen receptor is efficacious against human glioma. *J. Clin. Neurosci.* **21**, 189–190
- 15 Kim, M.G., Kim, D., Suh, S.K., Park, Z., Choi, M.J. and Oh, Y.K. (2016) Current status and regulatory perspective of chimeric antigen receptor-modified T cell therapeutics. *Arch. Pharm. Res.* **39**, 437–452
- 16 Pircher, M., Schirrmann, T. and Petrusch, U. (2015) T cell engineering. *Prog. Tumor Res.* **42**, 110–135
- 17 Gross, G., Waks, T. and Eshhar, Z. (1989) Expression of immunoglobulin-T-cell receptor chimeric molecules as functional receptors with antibody-type specificity. *Proc. Natl. Acad. Sci. U.S.A.* **86**, 10024–10028
- 18 Savoldo, B., Ramos, C.A., Liu, E., Mims, M.P., Keating, M.J., Carrum, G. et al. (2011) CD28 costimulation improves expansion and persistence of chimeric antigen receptor-modified T cells in lymphoma patients. *J. Clin. Invest.* **121**, 1822–1826
- 19 Kochenderfer, J.N., Dudley, M.E., Feldman, S.A., Wilson, W.H., Spaner, D.E., Maric, I. et al. (2012) B-cell depletion and remissions of malignancy along with cytokine-associated toxicity in a clinical trial of anti-CD19 chimeric-antigen-receptor-transduced T cells. *Blood* **119**, 2709–2720
- 20 Chmielewski, M. and Abken, H. (2015) TRUCKS: the fourth generation of CARs. *Expert Opin. Biol. Ther.* **15**, 1145–1154
- 21 Dai, H., Wang, Y., Lu, X. and Han, W. (2016) Chimeric antigen receptors modified T-Cells for cancer therapy. *J. Natl. Cancer Inst.* **108**, djv439
- 22 Rafiq S, D.T., Brentjens, R.J., Scheinberg, D.A. and Liu, C. (2014) Engineered T cell receptor-mimic antibody, (TCRm) chimeric antigen receptor (CAR) T cells against the intracellular protein Wilms tumor-1 (WT1) for treatment of hematologic and solid cancers. *Blood* **124**, 2155
- 23 Chinnasamy, D., Yu, Z., Theoret, M.R., Zhao, Y., Shrimali, R.K., Morgan, R.A. et al. (2010) Gene therapy using genetically modified lymphocytes targeting VEGFR-2 inhibits the growth of vascularized syngenic tumors in mice. *J. Clin. Invest.* **120**, 3953–3968
- 24 Chinnasamy, D., Tran, E., Yu, Z., Morgan, R.A., Restifo, N.P. and Rosenberg, S.A. (2013) Simultaneous targeting of tumor antigens and the tumor vasculature using T lymphocyte transfer synergize to induce regression of established tumors in mice. *Cancer Res.* **73**, 3371–3380
- 25 Frigault, M.J. and Maus, M.V. (2016) Chimeric antigen receptor-modified T cells strike back. *Int. Immunol.* **28**, 355–363
- 26 Watanabe, K., Terakura, S., Martens, A.C., van Meerten, T., Uchiyama, S., Imai, M. et al. (2015) Target antigen density governs the efficacy of anti-CD20-CD28-CD3 zeta chimeric antigen receptor-modified effector CD8+ T cells. *J. Immunol.* **194**, 911–920
- 27 Till, B.G., Jensen, M.C., Wang, J., Qian, X., Gopal, A.K., Maloney, D.G. et al. (2012) CD20-specific adoptive immunotherapy for lymphoma using a chimeric antigen receptor with both CD28 and 4-1BB domains: pilot clinical trial results. *Blood* **119**, 3940–3950
- 28 Savoldo, B., Rooney, C.M., Di Stasi, A., Abken, H., Hombach, A., Foster, A.E. et al. (2007) Epstein Barr virus specific cytotoxic T lymphocytes expressing the anti-CD30zeta artificial chimeric T-cell receptor for immunotherapy of Hodgkin disease. *Blood* **110**, 2620–2630
- 29 Di Stasi, A., De Angelis, B., Rooney, C.M., Zhang, L., Mahendravada, A., Foster, A.E. et al. (2009) T lymphocytes coexpressing CCR4 and a chimeric antigen receptor targeting CD30 have improved homing and antitumor activity in a Hodgkin tumor model. *Blood* **113**, 6392–6402
- 30 Hombach, A.A., Gorgens, A., Chmielewski, M., Murke, F., Kimpel, J., Giebel, B. et al. (2016) Superior therapeutic index in lymphoma therapy: CD30 CD34 hematopoietic stem cells resist a chimeric antigen receptor (CAR) T cell attack. *Mol. Ther.* **24**, 1432–1434
- 31 Ramos, C.A., Heslop, H.E. and Brenner, M.K. (2016) CAR-T cell therapy for lymphoma. *Annu. Rev. Med.* **67**, 165–183
- 32 Walter, R.B. (2014) The role of CD33 as therapeutic target in acute myeloid leukemia. *Expert Opin. Ther. Targets* **18**, 715–718
- 33 O’Hear, C., Heiber, J.F., Schubert, I., Fey, G. and Geiger, T.L. (2015) Anti-CD33 chimeric antigen receptor targeting of acute myeloid leukemia. *Haematologica* **100**, 336–344
- 34 Mardiros, A., Forman, S.J. and Budde, L.E. (2015) T cells expressing CD123 chimeric antigen receptors for treatment of acute myeloid leukemia. *Curr. Opin. Hematol.* **22**, 484–488
- 35 Mardiros, A., Dos Santos, C., McDonald, T., Brown, C.E., Wang, X., Budde, L.E. et al. (2013) T cells expressing CD123-specific chimeric antigen receptors exhibit specific cytolytic effector functions and antitumor effects against human acute myeloid leukemia. *Blood* **122**, 3138–3148
- 36 Gill, S., Tasian, S.K., Ruella, M., Shestova, O., Li, Y., Porter, D.L. et al. (2014) Preclinical targeting of human acute myeloid leukemia and myeloablation using chimeric antigen receptor-modified T cells. *Blood* **123**, 2343–2354
- 37 Santoro, S.P., Kim, S., Motz, G.T., Alatzoglou, D., Li, C., Irving, M. et al. (2015) T cells bearing a chimeric antigen receptor against prostate-specific membrane antigen mediate vascular disruption and result in tumor regression. *Cancer Immunol. Res.* **3**, 68–84
- 38 Zuccolotto, G., Fracasso, G., Merlo, A., Montagner, I.M., Rondina, M., Bobisse, S. et al. (2014) PSMA-specific CAR-engineered T cells eradicate disseminated prostate cancer in preclinical models. *PLoS ONE* **9**, e109427

- 39 Ma, Q., Gomes, E.M., Lo, A.S. and Junghans, R.P. (2014) Advanced generation anti-prostate specific membrane antigen designer T cells for prostate cancer immunotherapy. *Prostate* **74**, 286–296
- 40 Ruella, M. and Levine, B.L. (2016) Smart CARs: optimized development of a chimeric antigen receptor (CAR) T cell targeting epidermal growth factor receptor variant III (EGFRvIII) for glioblastoma. *Ann. Transl. Med.* **4**, 13
- 41 Suzuki, M. and Cheung, N.K. (2015) Disialoganglioside GD2 as a therapeutic target for human diseases. *Expert Opin. Ther. Targets* **19**, 349–362
- 42 Chinnasamy, D., Yu, Z., Kerkar, S.P., Zhang, L., Morgan, R.A., Restifo, N.P. et al. (2012) Local delivery of interleukin-12 using T cells targeting VEGF receptor-2 eradicates multiple vascularized tumors in mice. *Clin. Cancer Res.* **18**, 1672–1683
- 43 Jiang, H., Zhang, W., Shang, P., Zhang, H., Fu, W., Ye, F. et al. (2014) Transfection of chimeric anti-CD138 gene enhances natural killer cell activation and killing of multiple myeloma cells. *Mol. Oncol.* **8**, 297–310
- 44 Blat, D., Zigmund, E., Alteber, Z., Waks, T. and Eshhar, Z. (2014) Suppression of murine colitis and its associated cancer by carcinoembryonic antigen-specific regulatory T cells. *Mol. Ther.* **22**, 1018–1028
- 45 Morgan, R.A., Yang, J.C., Kitano, M., Dudley, M.E., Laurencot, C.M. and Rosenberg, S.A. (2010) Case report of a serious adverse event following the administration of T cells transduced with a chimeric antigen receptor recognizing ERBB2. *Mol. Ther.* **18**, 843–851
- 46 Caruso, H.G., Hurton, L.V., Najjar, A., Rushworth, D., Ang, S., Olivares, S. et al. (2015) Tuning sensitivity of CAR to EGFR density limits recognition of normal tissue while maintaining potent antitumor activity. *Cancer Res.* **75**, 3505–3518
- 47 Kloss, C.C., Condomines, M., Cartellieri, M., Bachmann, M. and Sadelain, M. (2013) Combinatorial antigen recognition with balanced signaling promotes selective tumor eradication by engineered T cells. *Nat. Biotechnol.* **31**, 71–75
- 48 Fedorov, V.D., Themeli, M. and Sadelain, M. (2013) PD-1- and CTLA-4-based inhibitory chimeric antigen receptors (iCARs) divert off-target immunotherapy responses. *Sci. Transl. Med.* **5**, 215ra172
- 49 Johnson, L.A., Scholler, J., Ohkuri, T., Kosaka, A., Patel, P.R., McGettigan, S.E. et al. (2015) Rational development and characterization of humanized anti-EGFR variant III chimeric antigen receptor T cells for glioblastoma. *Sci. Transl. Med.* **7**, 275ra22
- 50 Hudecek, M., Lupo-Stanghellini, M.T., Kosasih, P.L., Sommermeyer, D., Jensen, M.C., Rader, C. et al. (2013) Receptor affinity and extracellular domain modifications affect tumor recognition by ROR1-specific chimeric antigen receptor T cells. *Clin. Cancer Res.* **19**, 3153–3164
- 51 Geldres, C., Savoldo, B. and Dotti, G. (2016) Chimeric antigen receptor-redirectioned T cells return to the bench. *Semin. Immunol.* **28**, 3–9
- 52 Maude, S.L., Barrett, D., Teachey, D.T. and Grupp, S.A. (2014) Managing cytokine release syndrome associated with novel T cell-engaging therapies. *Cancer J.* **20**, 119–122
- 53 Davila, M.L., Riviere, I., Wang, X., Bartido, S., Park, J., Curran, K. et al. (2014) Efficacy and toxicity management of 19-28z CAR T cell therapy in B cell acute lymphoblastic leukemia. *Sci. Transl. Med.* **6**, 224ra25
- 54 Grupp, S.A., Kalos, M., Barrett, D., Aplenc, R., Porter, D.L., Rheingold, S.R. et al. (2013) Chimeric antigen receptor-modified T cells for acute lymphoid leukemia. *N. Engl. J. Med.* **368**, 1509–1518
- 55 Di Stasi, A., Tey, S.K., Dotti, G., Fujita, Y., Kennedy-Nasser, A., Martinez, C. et al. (2011) Inducible apoptosis as a safety switch for adoptive cell therapy. *N. Engl. J. Med.* **365**, 1673–1683
- 56 Ciceri, F., Bonini, C., Stanghellini, M.T., Bondanza, A., Traversari, C., Salomoni, M. et al. (2009) Infusion of suicide-gene-engineered donor lymphocytes after family haploidentical haemopoietic stem-cell transplantation for leukaemia (the TK007 trial): a non-randomised phase I-II study. *Lancet Oncol.* **10**, 489–500
- 57 Wang, W. and Wang, Y. (2012) Equipping CAR-modified T cells with a brake to prevent chronic adverse effects. *Curr. Gene Ther.* **12**, 493–495
- 58 Kenderian, S.S., Ruella, M., Shestova, O., Klichinsky, M., Aikawa, V., Morrissette, J.J. et al. (2015) CD33-specific chimeric antigen receptor T cells exhibit potent preclinical activity against human acute myeloid leukemia. *Leukemia* **29**, 1637–1647
- 59 Sun, N., Liang, J., Abil, Z. and Zhao, H. (2012) Optimized TAL effector nucleases (TALENs) for use in treatment of sickle cell disease. *Mol. Biosyst.* **8**, 1255–1263
- 60 Yang, H., Wang, H., Shivalila, C.S., Cheng, A.W., Shi, L. and Jaenisch, R. (2013) One-step generation of mice carrying reporter and conditional alleles by CRISPR/Cas-mediated genome engineering. *Cell* **154**, 1370–1379
- 61 Sadelain, M., Papapetrou, E.P. and Bushman, F.D. (2011) Safe harbours for the integration of new DNA in the human genome. *Nat. Rev. Cancer* **12**, 51–58
- 62 Chhabra, A. (2011) TCR-engineered, customized, antitumor T cells for cancer immunotherapy: advantages and limitations. *Scientific World J.* **11**, 121–129
- 63 Ninomiya, S., Narala, N., Huye, L., Yagyu, S., Savoldo, B., Dotti, G. et al. (2015) Tumor indoleamine 2,3-dioxygenase (IDO) inhibits CD19-CAR T cells and is downregulated by lymphodepleting drugs. *Blood* **125**, 3905–3916
- 64 Jensen, M.C., Popplewell, L., Cooper, L.J., DiGiusto, D., Kalos, M., Ostberg, J.R. et al. (2010) Antitransgene rejection responses contribute to attenuated persistence of adoptively transferred CD20/CD19-specific chimeric antigen receptor redirected T cells in humans. *Biol. Blood Marrow Transplant.* **16**, 1245–1256
- 65 Schultze, J.L. and Nadler, L.M. (1999) T cell mediated immunotherapy for B cell lymphoma. *J. Mol. Med.* **77**, 322–331
- 66 Kershaw, M.H., Westwood, J.A., Parker, L.L., Wang, G., Eshhar, Z., Mavroukakis, S.A. et al. (2006) A phase I study on adoptive immunotherapy using gene-modified T cells for ovarian cancer. *Clin. Cancer Res.* **12**, 6106–6115
- 67 Lamers, C.H., Sleijfer, S., van Steenberghe, S., van Elkakker, P., van Krimpen, B., Groot, C. et al. (2013) Treatment of metastatic renal cell carcinoma with CAIX CAR-engineered T cells: clinical evaluation and management of on-target toxicity. *Mol. Ther.* **21**, 904–912
- 68 Mirzaei, H.R., Mirzaei, H., Lee, S.Y., Hadjati, J. and Till, B.G. (2016) Prospects for chimeric antigen receptor (CAR) gammadelta T cells: a potential game changer for adoptive T cell cancer immunotherapy. *Cancer Lett.* **380**, 413–423
- 69 Rischer, M., Pscherer, S., Duwe, S., Vormoor, J., Jurgens, H. and Rossig, C. (2004) Human gammadelta T cells as mediators of chimaeric-receptor redirected anti-tumour immunity. *Br. J. Haematol.* **126**, 583–592

- 70 Deniger, D.C., Switzer, K., Mi, T., Maiti, S., Hurton, L., Singh, H. et al. (2013) Bispecific T-cells expressing polyclonal repertoire of endogenous gammadelta T-cell receptors and introduced CD19-specific chimeric antigen receptor. *Mol. Ther.* **21**, 638–647
- 71 Curran, K.J., Seinstra, B.A., Nikhamin, Y., Yeh, R., Usachenko, Y., van Leeuwen, D.G. et al. (2015) Enhancing antitumor efficacy of chimeric antigen receptor T cells through constitutive CD40L expression. *Mol. Ther.* **23**, 769–778
- 72 Teachey, D.T., Lacey, S.F., Shaw, P.A., Melenhorst, J.J., Maude, S.L., Frey, N. et al. (2016) Identification of predictive biomarkers for cytokine release syndrome after chimeric antigen receptor T-cell therapy for acute lymphoblastic leukemia. *Cancer Discov.* **6**, 664–697
- 73 Chen, X., Han, J., Chu, J., Zhang, L., Zhang, J., Chen, C. et al. (2016) A combinational therapy of EGFR-CAR NK cells and oncolytic herpes simplex virus 1 for breast cancer brain metastases. *Oncotarget* **7**, 27764–27767
- 74 Tettamanti, S., Marin, V., Pizzitola, I., Magnani, C.F., Giordano Attianese, G.M., Cribioli, E. et al. (2013) Targeting of acute myeloid leukaemia by cytokine-induced killer cells redirected with a novel CD123-specific chimeric antigen receptor. *Br. J. Haematol.* **161**, 389–401
- 75 Brentjens, R.J., Riviere, I., Park, J.H., Davila, M.L., Wang, X., Stefanski, J. et al. (2011) Safety and persistence of adoptively transferred autologous CD19-targeted T cells in patients with relapsed or chemotherapy refractory B-cell leukemias. *Blood* **118**, 4817–4828
- 76 Porter, D.L., Levine, B.L., Kalos, M., Bagg, A. and June, C.H. (2011) Chimeric antigen receptor-modified T cells in chronic lymphoid leukemia. *N. Engl. J. Med.* **365**, 725–733
- 77 Cruz, C.R., Micklethwaite, K.P., Savoldo, B., Ramos, C.A., Lam, S., Ku, S. et al. (2013) Infusion of donor-derived CD19-redirected virus-specific T cells for B-cell malignancies relapsed after allogeneic stem cell transplant: a phase 1 study. *Blood* **122**, 2965–2973
- 78 Kochenderfer, J.N., Dudley, M.E., Carpenter, R.O., Kassim, S.H., Rose, J.J., Telford, W.G. et al. (2013) Donor-derived CD19-targeted T cells cause regression of malignancy persisting after allogeneic hematopoietic stem cell transplantation. *Blood* **122**, 4129–4139
- 79 Lee, D.W., Kochenderfer, J.N., Stetler-Stevenson, M., Cui, Y.K., Delbrook, C., Feldman, S.A. et al. (2015) T cells expressing CD19 chimeric antigen receptors for acute lymphoblastic leukaemia in children and young adults: a phase 1 dose-escalation trial. *Lancet* **385**, 517–528
- 80 Dai, H., Zhang, W., Li, X., Han, Q., Guo, Y., Zhang, Y. et al. (2015) Tolerance and efficacy of autologous or donor-derived T cells expressing CD19 chimeric antigen receptors in adult B-ALL with extramedullary leukemia. *Oncoimmunology* **4**, e1027469
- 81 Porter, D.L., Hwang, W.T., Frey, N.V., Lacey, S.F., Shaw, P.A., Loren, A.W. et al. (2015) Chimeric antigen receptor T cells persist and induce sustained remissions in relapsed refractory chronic lymphocytic leukemia. *Sci. Transl. Med.* **7**, 303ra139
- 82 Garfall, A.L., Maus, M.V., Hwang, W.T., Lacey, S.F., Mahnke, Y.D., Melenhorst, J.J. et al. (2015) Chimeric antigen receptor T cells against CD19 for multiple myeloma. *N. Engl. J. Med.* **373**, 1040–1047
- 83 Till, B.G., Jensen, M.C., Wang, J., Chen, E.Y., Wood, B.L., Greisman, H.A. et al. (2008) Adoptive immunotherapy for indolent non-Hodgkin lymphoma and mantle cell lymphoma using genetically modified autologous CD20-specific T cells. *Blood* **112**, 2261–2271
- 84 Wang, Y., Zhang, W.Y., Han, Q.W., Liu, Y., Dai, H.R., Guo, Y.L. et al. (2014) Effective response and delayed toxicities of refractory advanced diffuse large B-cell lymphoma treated by CD20-directed chimeric antigen receptor-modified T cells. *Clin. Immunol.* **155**, 160–175
- 85 Kobayashi, E., Kishi, H., Ozawa, T., Hamana, H., Nakagawa, H., Jin, A. et al. (2014) A chimeric antigen receptor for TRAIL-receptor 1 induces apoptosis in various types of tumor cells. *Biochem. Biophys. Res. Commun.* **453**, 798–803
- 86 Inaguma, Y., Akahori, Y., Murayama, Y., Shiraiishi, K., Tsuzuki-Iba, S., Endoh, A. et al. (2014) Construction and molecular characterization of a T-cell receptor-like antibody and CAR-T cells specific for minor histocompatibility antigen HA-1H. *Gene Ther.* **21**, 575–584
- 87 Schuberth, P.C., Jakka, G., Jensen, S.M., Wadle, A., Gautschi, F., Haley, D. et al. (2013) Effector memory and central memory NY-ESO-1-specific re-directed T cells for treatment of multiple myeloma. *Gene Ther.* **20**, 386–395
- 88 Mata, M., Vera, J.F., Gerken, C., Rooney, C.M., Miller, T., Pfent, C. et al. (2014) Toward immunotherapy with redirected T cells in a large animal model: ex vivo activation, expansion, and genetic modification of canine T cells. *J. Immunother.* **37**, 407–415
- 89 Hu, W.X., Chen, H.P., Yu, K., Shen, L.X., Wang, C.Y., Su, S.Z. et al. (2012) Gene therapy of malignant solid tumors by targeting erbB2 receptors and by activating T cells. *Cancer Biother. Radiopharm.* **7**, 711–718
- 90 Geldres, C., Savoldo, B., Hoyos, V., Caruana, I., Zhang, M., Yvon, E. et al. (2014) T lymphocytes redirected against the chondroitin sulfate proteoglycan-4 control the growth of multiple solid tumors both in vitro and in vivo. *Clin. Cancer Res.* **20**, 962–971
- 91 Chow, K.K., Naik, S., Kakarla, S., Brawley, V.S., Shaffer, D.R., Yi, Z. et al. (2013) T cells redirected to EphA2 for the immunotherapy of glioblastoma. *Mol. Ther.* **21**, 629–637
- 92 Lanitis, E., Poussin, M., Hagemann, I.S., Coukos, G., Sandaltzopoulos, R., Scholler, N. et al. (2012) Redirected antitumor activity of primary human lymphocytes transduced with a fully human anti-mesothelin chimeric receptor. *Mol. Ther.* **20**, 633–643
- 93 Huang, G., Yu, L., Cooper, L.J., Hollomon, M., Huls, H. and Kleinerman, E.S. (2012) Genetically modified T cells targeting interleukin-11 receptor alpha-chain kill human osteosarcoma cells and induce the regression of established osteosarcoma lung metastases. *Cancer Res.* **72**, 271–281
- 94 Krebs, S., Chow, K.K., Yi, Z., Rodriguez-Cruz, T., Hegde, M., Gerken, C. et al. (2014) T cells redirected to interleukin-13Ralpha2 with interleukin-13 mutein-chimeric antigen receptors have anti-glioma activity but also recognize interleukin-13Ralpha1. *Cytotherapy* **16**, 1121–1131
- 95 Kong, S., Sengupta, S., Tyler, B., Bais, A.J., Ma, Q., Doucette, S. et al. (2012) Suppression of human glioma xenografts with second-generation IL13R-specific chimeric antigen receptor-modified T cells. *Clin. Cancer Res.* **18**, 5949–5960