Ther Adv Med Oncol
2020, Vol. 12: 1–13
DOI: 10.1177/1758835920962963

Potential strategies against resistance to CAR T-cell therapy in haematological malignancies

Qing Cai, Mingzhi Zhang and Zhaoming Li

***Abstract*:** Chimeric antigen receptor (CAR) T-cell therapy is a rapidly developing method for adoptive immunotherapy of tumours in recent years. CAR T-cell therapies have demonstrated unprecedented efficacy in the treatment of patients with haematological malignancies. A 90% complete response (CR) rate has been reported in patients with advanced relapse or refractory acute lymphoblastic leukaemia, while >50% CR rates have been reported in cases of chronic lymphocytic leukaemia and partial B-cell lymphoma. Despite the high CR rates, a subset of the patients with complete remission still relapse. The mechanism of development of resistance is not clearly understood. Some patients have been reported to demonstrate antigen-positive relapse, whereas others show antigen-negative relapses. Patients who relapse following CAR T-cell therapy, have very poor prognosis and novel approaches to overcome resistance are required urgently. Herein, we have reviewed current literature and research that have investigated the strategies to overcome resistance to CAR T-cell therapy.

Keywords: CAR T-cell therapy, drug resistance, haematological malignancies

Received: 3 May 2020; revised manuscript accepted: 7 September 2020.

Introduction

Chimeric antigen receptors (CARs) are synthetic tumour-specific receptors that are genetically reprogrammed *in vitro* using a patient's own T lymphocytes, which bind a tumour antigen in a major histocompatibility complex-independent manner, allowing T cells to recognise and kill antigen-expressing cancer cells. In the past few years, clinical trials using CAR T cells have demonstrated high rates of response in the treatment of patients with haematological malignancies, as well as increased duration of remission in patients with acute lymphoblastic leukaemia (ALL),[1,2] chronic lymphocytic leukaemia (CLL),[3] and partial B cell lymphomas.[4,5] CAR T-cell therapy has provided a new therapeutic option to patients with relapse/refractory haematological malignancies. Based on the results, the United States Food and Drug Administration (FDA) approved tisagenlecleucel in August 2017 for paediatric patients and young adults with B-cell ALL (B-ALL). Furthermore, in October 2017, the FDA approved CAR T-cell therapy for the treatment of B-cell lymphoma.[6] A current challenge in CAR T-cell therapy is that a portion of the patients achieving remission following CAR T-cell therapy subsequently undergo relapse. The mechanism of development of resistance to CAR T-cell therapy is not completely understood. Some patients have been reported to demonstrate antigen-positive relapse due primarily to shorter duration of persistence of CAR T cells, whereas others show antigen-negative relapses associated with lineage switching, acquired mutation and alternative splicing, epitope-masking and antigen downregulation.[7–15] The current review outlines the diverse strategies to overcome or reduce resistance to CAR T-cell therapy.

Basic structure and development of CAR T-cells

CAR T-cell therapy is a cellular therapy that redirects a patient's T cells to specifically target and destroy tumour cells. CARs are proteins expressed on the surface of T and natural killer (NK) cells,

Correspondence to:
Mingzhi Zhang
Department of Oncology, The First Affiliated Hospital of Zhengzhou University, 6th Floor, Building 10, No.1 Construction East Road, Zhengzhou, Henan Province 450052, China
mingzhi_zhang1@163.com

Zhaoming Li
Department of Oncology, The First Affiliated Hospital of Zhengzhou University, Zhengzhou, 450052, China
fcclizm@zzu.edu.cn

Qing Cai
Department of Oncology, the First Affiliated Hospital of Zhengzhou University, Zhengzhou, Henan province, China

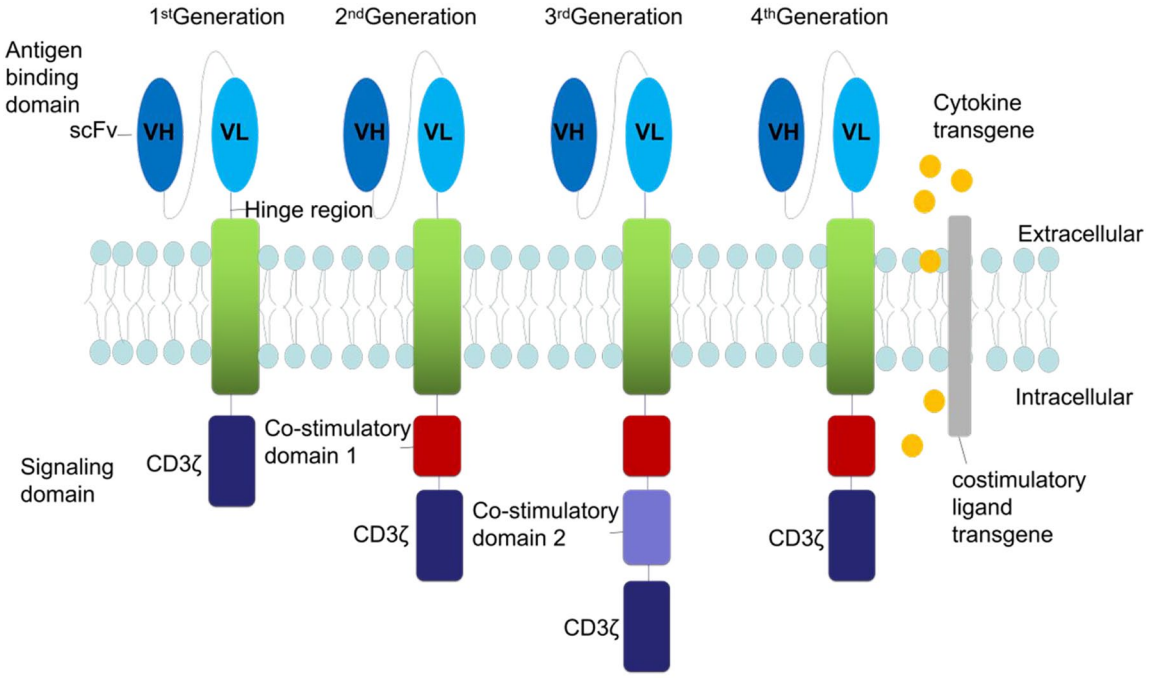

Figure 1. Schematic representation of CAR structure. CAR T cells are composed of three parts: (1) an scFv, (2) a transmembrane domain, and (3) a signal transduction domain of the TCR. First-generation CARs used a CD3ζ as the signal transduction domain of the TCR, whereas second-generation CARs include additional co-stimulatory signaling domains (CD28 or 4-1BB). Third-generation CARs consist of two distinct co-stimulatory domains, such as both CD28 and 4-1BB. Fourth-generation CARs are additionally armored with genes that enable, for example, the expression of cytokines.
CAR, chimeric antigen receptor; scFv, single-chain variable domain of an antibody; TCR, T-cell receptor.

which contain extracellular binding domains, a hinge region that mediates the linkage of extracellular to transmembrane domains, a transmembrane domain and an intracellular signaling domain (Figure 1).[16–20] In 1987, Kuwana *et al.* first proposed the concept of CAR and constructed a prototype of CAR-T cells that specifically recognised tumour-associated antigens.[21] In the first-generation CARs, the intracellular signaling domain comprised solely a CD3ζ chain, a component of the endogenous T-cell receptor (TCR).[22] These first-generation CARs showed minimal killing and persistence *in vivo* along with limited clinical benefits.[23–28] Second-generation CARs incorporated co-stimulation into the CD3ζ construct. Most investigators work with second-generation CARs, involving those that express the classical co-stimulatory molecules, namely the tumour necrosis factor (TNF) superfamily members 9 (4-1BB) and 4 (OX40).[29,30] However, some investigators have expanded their toolkit to include other types of co-stimulatory molecules into the CAR constructs, such as OX40, 4-1BBL, or inducible co-stimulator (ICOS).[31–33] Studies have reported that second-generation CAR T cells demonstrated potent expansion and cytokine secretion abilities, and persistence of anti-tumour T cells both *in vitro* and *in vivo*.[7,17,34–36] Third-generation CARs containing three or more co-stimulatory domains to boost T-cell activation signals, including CD28, 4-1BB, and CD3ζ, were developed to improve the design and enhance the activation of the second-generation CARs.[37–45] The fourth-generation CARs (T cells redirected for universal cytokine killing, TRUCKs) can secrete pro-inflammatory cytokines such as IL-12 into the tumour microenvironment,[46,47] which consequently improve the tumour eradication ability of these cells.[48–52]

Efficacy of CAR T cells in the treatment of haematological malignancies

Haematological malignancies are one of the most common cancers among patients in China. Presently, haematological malignancies remain incurable and have a high recurrence rate and mortality. In recent years, novel gene and targeted therapies have emerged for the treatment of patients with haematological malignancies;

Table 1. Summary of CAR T cells in the treatment of B-ALL, B-NHL and CLL.

Disease	Patient populations	Response and relapse	References
B-ALL	53 adults	44/53 (83%) achieved a CR, the median overall survival was 12.9 months.	Park *et al.*[2]
	30 paediatric and adults	27/30 (90%) achieved a CR, seven patients who had a complete remission subsequently had a relapse between 6 weeks and 8.5 months after infusion of CAR T cells.	Maude *et al.*[7]
	75 paediatric and adults	45/75 (60%) had a CR, the rate of overall survival was 90% at 6 months after infusion and 76% at 12 months after infusion.	Kochenderfer *et al.*[54]
	21 paediatric and adults	14/21 (66.7%) achieved a CR.	Lee *et al.*[53]
B-NHL	28 adults	6/14 DLBCL patients achieved a CR and 10/14 FL patients achieved a CR.	Schuster *et al.*[60]
	7 adults	4/7 (57%) achieved a CR. Three patients are in ongoing CR at 12 months post CAR T cells infusion.	Locke *et al.*[63]
	101 adults	CR rate was 54%. With a median follow-up of 15.4 months, with 40% continuing to have a complete response.	Ye *et al.*[66]
	15 adults	8/15 (53%) had a CR. Seven patients are in ongoing CR, ranging from 9 to 22 months post CAR T cells infusion.	Schuster *et al.*[60]; June and Sadelain[61]
	7 adults	2/7 achieved a CR, one patient attained a PR, another four patients exhibited SD. Two patients are in ongoing CR at 3 months and 13 months post CAR T cells infusion.	June and Sadelain[61]
CLL	14 adults	8/14 (58%) achieved an objective response, with 4/14 (29%) achieving a CR. CAR T cells persisted for >5 years in two patients with durable CRs.	Porter *et al.*[59]
	3 adults	2CR, 1PR; two of whom experienced long-lasting CR.	Porter *et al.*[59]

B-ALL, B-cell acute lymphoblastic leukaemia; B-NHL, B-cell non-Hodgkin lymphoma; CAR, chimeric antigen receptor; CLL, chronic lymphocytic leukaemia; CR, complete remission; DLBCL, diffuse large B-cell lymphoma; FL, follicular lymphoma; PR, partial remission; SD, stable disease.

however, clinical remission rates are limited. In 2013, the journal *Science* summarised the top 10 breakthrough technologies in the scientific community, with tumour immunotherapy topping the list. CAR T-cell therapy, as a special tumour immunotherapy, has demonstrated remarkable results in the treatment of patients with malignant tumours, especially lymphatic haematopoietic malignancies.

B-ALL

CAR T-cell therapy has emerged as a highly effective therapy for patients with relapsed or refractory B-ALL with previously limited treatment options. The therapy was reported to demonstrate complete responses (CRs) ranging from 60% to 90% (Table 1).[2,7,48–53] Relapse rates of approximately 30–50% were reported in patients with B-ALL, with the majority being CD19-negative relapses.[7]

In a phase II, single-cohort, 25-centre global study, 75 patients received an infusion of tisagenlecleucel and were followed up for at least 3 months; the overall remission rate was 81%.[54] A total of 45 patients (60%) had complete remission and 16 (21%) had complete remission with incomplete haematological recovery. Among the patients with complete remission, 17 experienced relapse before receiving additional anticancer therapy. Characterisation of CD19 status at the time of relapse showed that 1 patient had CD19-positive and 15 had CD19-negative recurrence, whereas six patients had unknown status. Turtle *et al.* conducted a clinical trial on 29 patients with B-ALL who received CAR T cells, and demonstrated a complete response (CR) rate of 93%. Among the patients with complete remission, nine had a relapse. Characterisation of CD19 status at the time of relapse showed that two patients had a CD19-negative relapse.[55]

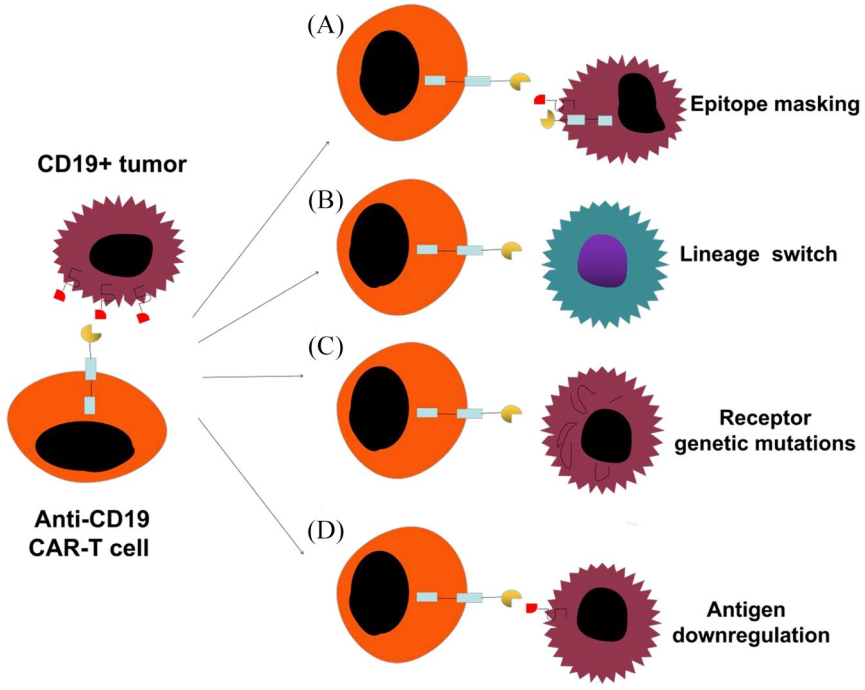

Figure 2. Mechanisms of resistance to CAR-T cell therapy. (A) Lentiviral modification of a single leukemic cell allowed for joint CAR19 and CD19 expression on their cell surface, effectively masking the CD19 epitope from CAR T cells. (B) Tumour cells can switch to a genetically related but phenotypically different disease. (C) Tumour cells, through genetic mutations, can either completely lose CD19 receptor expression or modify the CD19 receptor that lack the extracellular epitopes recognised by CAR T cells. (D) Tumour cells downregulate the surface target antigen to levels below those needed for CAR T cells activation.
CAR, chimeric antigen receptor.

B-cell non-Hodgkin lymphomas and CLL

Previous research has shown remarkable rates of complete and durable remission in patients with CLL[56–59] and B-cell non-Hodgkin lymphoma (B-NHL).[23,56–61] CAR T-cell therapy has been approved for the treatment of lymphoma in adults, with a lower remission rate of approximately 50–70%.[6,62–64] Furthermore, antigen loss has also been observed in such patients.[65,66] In a multicentre, phase II trial, 111 patients with histologically confirmed diffuse large B-cell lymphoma, primary mediastinal B-cell lymphoma, or transformed follicular lymphoma were enrolled, of which 101 received Axicabtagene Ciloleucel, an autologous anti-CD19 CAR T-cell therapy. The objective response rate observed in the patients was 82%, and the CR rate was 54%. At a median follow-up of 15.4 months, 42% of the patients continued to demonstrate a response, with 40% showing CR.[6] Another clinical trial enrolled 28 adult patients, wherein complete remission occurred in 6 out of 14 patients with diffuse large B-cell lymphoma and in 10 out of 14 patients with follicular lymphoma.[59,66] Porter *et al.* treated 14 adult patients with CLL using CD19 CAR T cells, and reported CR in 4 (29%) patients.[59]

Underlying mechanisms of resistance

Two main mechanisms have been recognised in relapse following CAR T-cell therapy, including antigen-negative and antigen-positive relapses.

Antigen-positive relapse

Antigen-positive relapse has been assumed to be due primarily to short persistence of CAR T cells[7]; however, it can also occur in association with a suppressive tumour microenvironment.[67] The reasons for loss of CAR-T cell persistence are complex and might be difficult to determine in individual patients. According to a trial by Park *et al.*, shorter duration of persistence of the CD19-CD28-ζ CAR T cells employed by Memorial Sloan Kettering Cancer Center (MSKCC) could partially explain the low rate of CD19-positive relapse.[2] In another study, although the CR rates in the cohort were robust, early relapse was noted in a subset of patients, associated with loss of

CAR T cells in the blood due to an anti-CAR T-cell immune response to the epitopes in the murine single-chain variable fragment (scFv). This mechanism was reported to contribute to the early loss of CAR T cells in some patients after the use of a CAR containing a murine scFv.[68]

Antigen-negative relapse

The reason for antigen-negative relapse was unclear. Since the antigen-negative relapse has been considered a major barrier to CAR-T therapies, studies have uncovered multiple mechanisms responsible for the antigen-negative relapse, which are described below.

Epitope-masking. Ruella *et al.* described a rare case of a 20-year-old man with B-ALL who had suffered three chemotherapeutic relapses. He was enrolled in a phase I trial [ClinicalTrials.gov identifier: NCT01626495] to evaluate the safety, feasibility, and engraftment of CD19-targeted CAR T cells (CTL019) in paediatric and young adult patients with B-ALL.[10] The patient was in complete remission at day 28 after infusion. However, he experienced relapse after 9 months of the CAR T-cell therapy with CD19-negative disease. Ruella *et al.* probed this patient's CD19-negative relapse, ruling out CD19 mutations and splicing variants, and found that the anti-CD19 CAR had been introduced into a single leukaemic B cell during manufacturing of the CD19 CAR T cell. This tumour clone was infused into the patient alongside the CAR T-cell therapy and eventually expanded, which led to disease progression and death. The study reported that resistance to CTL019 occurred not due to loss of target by the leukaemic blasts, but due to the CAR molecule bound to adjacent CD19, which effectively masked the CD19 epitope from CAR T cells in the patient. Ruella and his team were able to create a model of this phenomenon, known as 'epitope masking' (Figure 2A), both *in vitro* and *in vivo*.

Lineage switch. Lineage switch occurs when a patient experiences relapse with a genetically related but phenotypically different malignancy (Figure 2B), which might be a mechanism for antigen loss after CAR T-cell therapy observed in clinical trials.[9,69–72] Evans *et al.* reported relapse after CD19 CAR T-cell therapy in a patient with CLL with Richter's transformation. The patient demonstrated a plasmablastic lymphoma, which is inherently CD19-negative.[73] A group of researchers from Seattle reported that two of the seven patients with B-ALL harbouring rearrangement of the mixed lineage leukaemia gene experienced relapse with CD19-negative AML following treatment with CD19 CAR T cells.[9]

Receptor genetic mutations. Acquired mutations and alternatively spliced CD19 alleles in the malignant B cells are other mechanisms for CD19-negative relapse following CD19-targeted CAR T-cell therapy (Figure 2C). Sotillo *et al.* found that exon 2 of CD19 was frequently spliced, leading to the disappearance of the CD19 epitope, which is recognised by CAR T cells.[8] In addition, they observed the variants Δexon-5, 6, which lack the transmembrane domain of CD19, thereby leading to loss of surface expression. Furthermore, they observed that the CD19 protein was present in some patients; however, it was truncated due to lack of the epitope required to trigger CAR T cell recognition for lysis. The Orlando trail identified mutations in CD19 in all 12 specimens at the time of relapse.[74] Mutations were found throughout exons 2–5 of CD19. Encoding of the transmembrane domain begins at exon 5; therefore, variants in exons 2–5 have been predicted to lead to a truncated protein lacking membrane anchorage. Each patient in the study had at least one unique frameshift insertion or deletion and, in some cases, missense single nucleotide variants were also observed, and alternative splicing occurred with rare frequency. Therefore, acquired mutations and alternative splicing could be other possible mechanisms responsible for antigen-negative relapse.[75,76]

Antigen downregulation. Partial antigen loss may be considered a mechanism for resistance to CAR T-cell therapy due to antigen downregulation (Figure 2D).[11–15] During the course of antigen recognition, natural TCRs produce a highly organised immune synapse that can recognise an antigen at a very low density.[77,78] However, the immune synapse created during antigen recognition by CARs is less organised than that by a natural TCR.[79] These distinctions are likely to significantly affect the quality of responses induced in T cells expressing CARs. Fry *et al.* observed that relapses in patients treated with a CD22 CAR were associated with diminished and variable CD22 site density on B-ALL cells.[11] The investigators further demonstrated in animal studies that differential levels of CD22 on leukaemic cells could have a dramatic impact on the anti-cancer efficacy. Another study using a CD20 CAR demonstrated that a threshold level of around 200

antigen molecules per target cell was required to induce lysis, while approximately 10-fold higher numbers of molecules were needed to stimulate cytokine production.[13] Therefore, the signal strength and effector function of CARs might be limited by density of the tumour antigens.

Overcoming resistance to CAR T-cell therapy

Improving CAR T-cell design

Selection of effector T cells. Effector T cells are the main processing plant for the biological activity of CARs, and play a crucial role in the anti-tumour effect and duration of action of CARs. Accurate detection and isolation of the most potential subpopulations of T cells before *in vitro* expansion can improve the outcomes of CAR T-cell immunotherapy. The cytotoxic activity and proliferative capacity of effector memory T cells are superior to that of central memory T cells (TCM) *in vitro*; however, TCMs have the potential to induce immune memory, as well as exert a longer lasting anti-tumour activity.[80] Stem cell memory T cells (TSCM) have the property of persistent self-renewal; therefore, these cells have the potential for high proliferation and persistence.[81] Thus, the process of inducing the conversion of CAR T cells into TCMs and TSCMs could be an alternative method to prevent antigen-positive recurrence by enhancing the response and persistence of the cells.

Antigen density. Numerous studies have demonstrated that antigen density on tumour cells correlates with the efficacy and remission durability of CAR T cells in patients with leukaemia and lymphoma.[13,15,82–85] A recent research demonstrated that upregulation of CD22 on the cell surface improved CAR T cell functionality and long-term persistence.[82] Moreover, Bryostatin 1, a drug that is being administered safely to humans, can increase the expression of CD22 in leukaemia and lymphoma cell lines, resulting in longer duration of *in vivo* response. Another research found that γ-secretase inhibitors could markedly increase surface levels of BCMA on myeloma cells, thereby improving tumour recognition by CAR T cells *in vitro* and enhancing anti-tumour efficacy of BCMA-targeted CAR T-cell therapy.[86]

Selection of co-stimulatory molecules. The co-stimulatory molecules in the intracellular signalling region of the CAR T cells play an important role in regulating T cell expansion, duration, and anti-tumour effects; however, the biological activities of individual costimulatory molecules are different. Common costimulatory molecules include CD28, 4-1BB, OX40, ICOS, and CD27, of which CD28 and 4-1BB can effectively promote the secretion of IL-2 and IFN-γ. Studies have shown that 4-1BB can effectively promote the expansion of memory T cells and reduce the depletion of persistent CAR T signals.[45] Thus, CAR T cells incorporating a 4-1BB costimulatory molecule might lead to a reduced antigen-positive relapse. Hombach *et al.* proved that CAR T cells containing CD28 costimulatory molecules were more effective than those containing CD28-OX40, because CD28-OX40 are capable of promoting activation-induced cell death and reduce anti-tumour activity.[87] Other studies have shown that CAR T cells with two co-stimulants (such as CD28 and 4-1BB) were more effective in improving the survival and cytotoxicity of T cells than CAR T cells with a single co-stimulatory molecule. The above studies indicate that co-stimulatory molecules greatly affect the efficacy of CAR T-cell therapy; however, further *in vitro* and *in vivo* research is necessary to determine the optimal type and number of co-stimulatory molecules.

Fully human CARs. Presently, clinical trials commonly use the CAR scFv segment of murine origin, which has high affinity and immunogenicity. CAR T cells with high affinity have poor ability to distinguish tumour cells with high levels of target antigen from normal cells with low expression. Furthermore, the human body will reject CARs with high immunogenicity, considering them foreign bodies. Reducing immunogenicity of CARs using fully human scFvs could improve the persistence of CAR T cells and their functions against tumour cells.[88–90] Sommermeyer *et al.* reported that CARs constructed from fully human CD19-specific scFvs exhibited superior function *in vitro* and *in vivo* compared with the FMC63 CAR utilised in clinical trials.[91] Specifically, fully human CD19-specific scFvs were more effective in lysing CD19+ target cells, produced higher levels of cytokines, and proliferated more after activation compared with murine scFv.

Armoured CAR T cells. Armoured CAR T cells are modified to co-express cytokines and co-stimulatory molecules in order to enhance the anti-tumour immune response by converting a suppressive tumour microenvironment into a proinflammatory one.[92] For example, CAR T cells armoured to

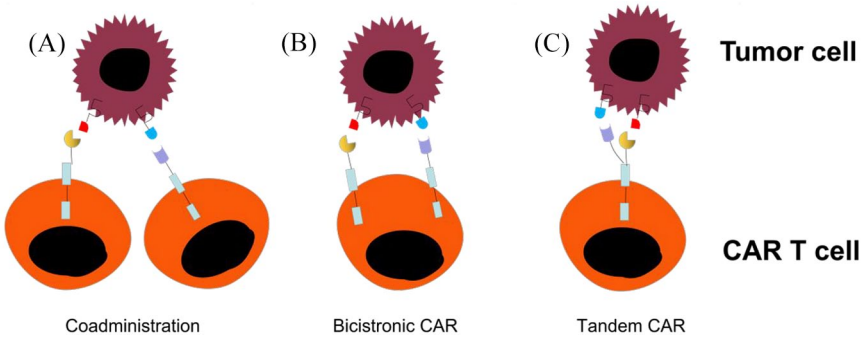

Figure 3. Targeting more than one antigen receptor approaches. (A) Coadministration-producing two separate CAR-T cell products and infusing together or sequentially. (B) Bicistronic CAR-using a single vector that encodes two or three different CARs on a single T cell. (C) Tandem CAR-encoding two CARs on same chimeric protein using a single vector.
CAR, chimeric antigen receptor.

secrete IL-12 enhance the cytotoxic activity of CD8+ T and NK cells and stimulate a Th1 helper T cell response.[93] CD40L expressed in armoured CAR T cells increased the cytotoxicity of these cells *in vitro* and prolonged survival of lymphoma-bearing mice. CAR T cells armoured with 4-1BBL has been reported to exert immunostimulatory effects.[94] However, the effectiveness of this approach needs further clinical translation as the results have been predominantly proven on pre-clinical models.

Universal CAR T cells. Universal CAR T cells are used in genome-editing technologies such as zinc finger nuclease, transcription activator-like effector nuclease (TALEN) and CRISPR-Cas9 to knock out TCR, human leukocyte antigen and other related signaling pathway genes on donor T cells,[95] thereby reducing the risk of graft-*versus*-host disease and immune rejection. Furthermore, simultaneously knocking out immune checkpoints such as programmed death-1 (PD-1) has been shown to enhance the function of CAR-T cells.[95–98] Waseem *et al.* used TALEN to knock out TCR and CD52 on CAR T cells targeting CD19 for the treatment of patients with refractory ALL, and reported improvement in the condition of the patients within 28 days after treatment. The results proved the safety and effectiveness of universal CAR T-cell immunotherapy for the first time.[97] Therefore, use of universal CAR T cells might be another possible strategy to overcome resistance to CAR T-cell therapy.

Multi-targeted CAR T cells. Strategies to overcome the relapse rate due to antigen loss following CAR T-cell therapy can be combined with the following approaches: (a) T-cell products that are separately transduced for different CARs can be infused together or sequentially; (b) use of a single vector that encodes two or three different CARs on a single T cell (bicistronic CAR); or (c) encode two CARs on the same chimeric protein using a single vector (tandem CAR) (Figure 3). Majzner *et al.* described these different approaches in a review.[12] Many of these approaches are currently being investigated in clinical trials on patients with haematological malignancies.[11,99–101] A recent study investigated the clinical efficacy of bispecific tandem CAR T cells directed against both CD19 and CD20 antigens in patients with relapsed/refractory (R/R) B-cell NHL.[102] In addition, Ruella *et al.* reported that dual CAR CD19 and CD123 overcame both antigen escape and lineage switch.[103] Several clinical trials are underway to test multi-specific CAR T cells; however, the effectiveness of these approaches remains to be established.

Improvement of tumour immune microenvironment

Improving the tumour immune microenvironment can greatly improve the immune efficacy of CAR T cells and reduce the adverse events. However, due to the complexity of the tumour microenvironment and the diversity of regulatory mechanisms, clinical efficacy cannot be achieved by monotherapy. The main regulatory mechanisms of the immune microenvironment can be combined with the following comprehensive treatments.

Studies have proved that addition of an agent blocking the PD-1 immunosuppressive pathway

(anti-PD-1) greatly improved the efficacy of CAR T cells by inhibition of the interaction between PD-1 and its ligands PD-L1/PD-L2.[104,105]

Similarly, chemotherapy and radiotherapy can improve immunosuppression by inducing apoptosis of or specifically removing regulatory T cells (Tregs). Moreover, eradication of Tregs can enhance the cell response and increase levels of CAR T cells.[106] One study found that chemotherapy based on low-dose cyclophosphamide could effectively eliminate Tregs and exert immunomodulatory effects. A combined immunotherapeutic approach has been reported to improve the prognosis.[107]

The cytokines TGF-β and IL-10 are the major immunosuppressive factors, and downregulate the expression of TGF-β and IL-10 receptors on T cells by genetic engineering methods, to improve the efficacy of CAR T cells. In addition, activation factors such as IL-2, IL-12, and IL-15 can promote the immune function of effector T cells by creating a microenvironment that is conducive to the survival and efficacy of T cells, resulting in more effective anti-tumour effects by inducing the secretion of activating factors by CAR T cells.[47,108,109]

Combination therapy

Combining CAR T-cell therapy with other agents, such as Bruton tyrosine kinase inhibitors, may reduce recurrence after infusion and improve long-term survival. Fraietta *et al.* reported that treatment with ibrutinib could significantly increase the implantation and expansion of CAR T cells in patients with CLL, and enhanced its targeted cytotoxic activity.[110] The outcomes could be attributed to downregulation of immunosuppressive receptors and improvement in the proliferation and activation functions of T cells by ibrutinib.[110,111] On the other hand, differentiation of Th2 cells could have been inhibited and immune response of Th1 cells could have been promoted by inhibiting the activity of IL-2 mediated T cell kinase.[112] Other studies have shown superior effectiveness of the combined therapeutic approach than ibrutinib[113,114] or CAR T-cell monotherapy,[115] thus providing a new research direction to address the issue of resistance to CAR T-cell therapy.

Conclusion

In conclusion, advancements in our understanding of the mechanisms of resistance to CAR T-cell therapy are leading to new insights regarding this treatment. Novel strategies are being developed to overcome the resistance and improve clinical outcomes in patients with relapsed and refractory haematological malignancies. Various treatment approaches, such as targeting more than one antigen receptor, armoured CAR T-cells, fully human CAR T cells, CAR NK-cell therapy, and combination therapies with other immunotherapeutic agents are being explored to overcome the issue of resistance. However, the effectiveness of the aforementioned treatments remains unclear. Thus, further research is needed to maximise the duration of responses while minimising the risk of relapse.

Conflict of interest statement

The authors declare that there is no conflict of interest.

Funding

The authors disclosed receipt of the following financial support for the research, authorship, and/or publication of this article: This study was supported by National natural Science Foundation of China (U1904139), Department of Science and Technology of Henan province (182102310114).

ORCID iD

Mingzhi Zhang https://orcid.org/0000-0002-4265-944X

References

1. Sadelain M, Brentjens R, Rivière I, *et al.* CD19 CAR therapy for acute lymphoblastic leukemia. *Am Soc Clin Oncol Educ Book* 2015: e360–e363.
2. Park JH, Riviere I, Gonen M, *et al.* Long-term follow-up of CD19 CAR therapy in acute lymphoblastic leukemia. *N Engl J Med* 2018; 378: 449–459.
3. Turtle CJ, Hay KA, Hanafi L-A, *et al.* Durable molecular remissions in chronic lymphocytic leukemia treated with CD19-specific chimeric antigen receptor-modified T cells after failure of ibrutinib. *J Clin Oncol* 2017; 35: 3010–3020.
4. Locke FL, Ghobadi A, Jacobson CA, *et al.* Long-term safety and activity of axicabtagene ciloleucel in refractory large B-cell lymphoma (ZUMA-1): a single-arm, multicentre, phase 1-2 trial. *Lancet Oncol* 2019; 20: 31–42.
5. Chavez JC, Bachmeier C and Kharfan-Dabaja MA. CAR T-cell therapy for B-cell lymphomas:

clinical trial results of available products. *Ther Adv Hematol* 2019; 10: 153133357.

6. Neelapu SS, Locke FL, Bartlett NL, *et al.* Axicabtagene ciloleucel CAR T-cell therapy in refractory large B-cell lymphoma. *N Engl J Med* 2017; 377: 2531–2544.
7. Maude SL, Frey N, Shaw PA, *et al.* Chimeric antigen receptor T cells for sustained remissions in leukemia. *N Engl J Med* 2014; 371: 1507–1517.
8. Sotillo E, Barrett DM, Black KL, *et al.* Convergence of acquired mutations and alternative splicing of CD19 enables resistance to CART-19 immunotherapy. *Cancer Discov* 2015; 5: 1282–1295.
9. Gardner R, Wu D, Cherian S, *et al.* Acquisition of a CD19-negative myeloid phenotype allows immune escape of MLL-rearranged B-ALL from CD19 CAR-T-cell therapy. *Blood* 2016; 127: 2406–2410.
10. Ruella M, Xu J, Barrett DM, *et al.* Induction of resistance to chimeric antigen receptor T cell therapy by transduction of a single leukemic B cell. *Nat Med* 2018; 24: 1499–1503.
11. Fry TJ, Shah NN, Orentas RJ, *et al.* CD22-targeted CAR T cells induce remission in B-ALL that is naive or resistant to CD19-targeted CAR immunotherapy. *Nat Med* 2018; 24: 20–28.
12. Majzner RG and Mackall CL. Tumor antigen escape from CAR T-cell therapy. *Cancer Discov* 2018; 8: 1219–1226.
13. Watanabe K, Terakura S, Martens AC, *et al.* Target antigen density governs the efficacy of anti-CD20-CD28-CD3 ζ chimeric antigen receptor-modified effector $CD8^+$ T cells. *J Immunol* 2015; 194: 911–920.
14. Hombach AA, Görgens A, Chmielewski M, *et al.* Superior therapeutic index in lymphoma therapy: $CD30^+$ $CD34^+$ hematopoietic stem cells resist a chimeric antigen receptor T-cell attack. *Mol Ther* 2016; 24: 1423–1434.
15. Walker AJ, Majzner RG, Zhang L, *et al.* Tumor antigen and receptor densities regulate efficacy of a chimeric antigen receptor targeting anaplastic lymphoma kinase. *Mol Ther* 2017; 25: 2189–2201.
16. Levine BL, Miskin J, Wonnacott K, *et al.* Global manufacturing of CAR T cell therapy. *Mol Ther Methods Clin Dev* 2017; 4: 92–101.
17. Finney HM, Lawson AD, Bebbington CR, *et al.* Chimeric receptors providing both primary and costimulatory signaling in T cells from a single gene product. *J Immunol* 1998; 161: 2791–2797.
18. Gross G, Waks T and Eshhar Z. Expression of immunoglobulin-T-cell receptor chimeric molecules as functional receptors with antibody-type specificity. *Proc Natl Acad Sci U S A* 1989; 86: 10024–10028.
19. Till BG, Jensen MC, Wang J, *et al.* CD20-specific adoptive immunotherapy for lymphoma using a chimeric antigen receptor with both CD28 and 4-1BB domains: pilot clinical trial results. *Blood* 2012; 119: 3940–3950.
20. Chu J, Deng Y, Benson DM, *et al.* CS1-specific Chimeric Antigen Receptor (CAR)-engineered natural killer cells enhance in vitro and in vivo antitumor activity against human multiple myeloma. *Leukemia* 2014; 28: 917–927.
21. Kuwana Y, Asakura Y, Utsunomiya N, *et al.* Expression of chimeric receptor composed of immunoglobulin-derived V regions and T-cell receptor-derived C regions. *Biochem Biophys Res Commun* 1987; 149: 960–968.
22. Sermer D and Brentjens R. CAR T-cell therapy: full speed ahead. *Hematol Oncol* 2019; 37(Suppl. 1): 95–100.
23. Till BG, Jensen MC, Wang J, *et al.* Adoptive immunotherapy for indolent non-Hodgkin lymphoma and mantle cell lymphoma using genetically modified autologous CD20-specific T cells. *Blood* 2008; 112: 2261–2271.
24. Harding FA, McArthur JG, Gross JA, *et al.* CD28-mediated signalling co-stimulates murine T cells and prevents induction of anergy in T-cell clones. *Nature* 1992; 356: 607–609.
25. Lenschow DJ, Walunas TL and Bluestone JA. CD28/B7 system of T cell costimulation. *Annu Rev Immunol* 1996; 14: 233–258.
26. Jensen MC, Popplewell L, Cooper LJ, *et al.* 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* 2010; 16: 1245–1256. .
27. Park JR, Digiusto DL, Slovak M, *et al.* Adoptive transfer of chimeric antigen receptor re-directed cytolytic T lymphocyte clones in patients with neuroblastoma. *Mol Ther* 2007; 15: 825–833.
28. Pule MA, Savoldo B, Myers GD, *et al.* Virus-specific T cells engineered to coexpress tumor-specific receptors: persistence and antitumor activity in individuals with neuroblastoma. *Nat Med* 2008; 14: 1264–1270.

29. Jensen MC and Riddell SR. Design and implementation of adoptive therapy with chimeric antigen receptor-modified T cells. *Immunol Rev* 2014; 257: 127–144.

30. van der Stegen SJC, Hamieh M and Sadelain M. The pharmacology of second-generation chimeric antigen receptors. *Nat Rev Drug Discov* 2015; 14: 499–509.

31. Croft M. The role of TNF superfamily members in T-cell function and diseases. *Nat Rev Immunol* 2009; 9: 271–285.

32. Guedan S, Chen X, Madar A, *et al.* ICOS-based chimeric antigen receptors program bipolar TH17/TH1 cells. *Blood* 2014; 124: 1070–1080.

33. Hombach AA, Heiders J, Foppe M, *et al.* OX40 costimulation by a chimeric antigen receptor abrogates CD28 and IL-2 induced IL-10 secretion by redirected $CD4^+$ T cells. *Oncoimmunology* 2012; 1: 458–466.

34. Maher J, Brentjens RJ, Gunset G, *et al.* Human T-lymphocyte cytotoxicity and proliferation directed by a single chimeric TCRζ/CD28 receptor. *Nat Biotechnol* 2002; 20: 70–75.

35. Savoldo B, Ramos CA, Liu E, *et al.* CD28 costimulation improves expansion and persistence of chimeric antigen receptor-modified T cells in lymphoma patients. *J Clin Invest* 2011; 121: 1822–1826.

36. Yvon E, Del VM, Savoldo B, *et al.* Immunotherapy of metastatic melanoma using genetically engineered GD2-specific T cells. *Clin Cancer Res* 2009; 15: 5852–5860.

37. Enblad G, Karlsson H and Loskog AS. CAR T-cell therapy: the role of physical barriers and immunosuppression in lymphoma. *Hum Gene Ther* 2015; 26: 498–505.

38. Carpenito C, Milone MC, Hassan R, *et al.* Control of large, established tumor xenografts with genetically retargeted human T cells containing CD28 and CD137 domains. *Proc Natl Acad Sci U S A* 2009; 106: 3360–3365.

39. Chen F, Fan C, Gu X, *et al.* Construction of anti-CD20 single-chain antibody-CD28-CD137-TCRζ recombinant genetic modified T cells and its treatment effect on B cell lymphoma. *Med Sci Monit* 2015; 21: 2110–2115.

40. Wilkie S, Picco G, Foster J, *et al.* Retargeting of human T cells to tumor-associated MUC1: the evolution of a chimeric antigen receptor. *J Immunol* 2008; 180: 4901–4909.

41. Zhao Y, Wang QJ, Yang S, *et al.* A herceptin-based chimeric antigen receptor with modified signaling domains leads to enhanced survival of transduced T lymphocytes and antitumor activity. *J Immunol* 2009; 183: 5563–5574.

42. Wang J, Jensen M, Lin Y, *et al.* Optimizing adoptive polyclonal T cell immunotherapy of lymphomas, using a chimeric T cell receptor possessing CD28 and CD137 costimulatory domains. *Hum Gene Ther* 2007; 18: 712–725.

43. Chang L, Chang WC, McNamara G, *et al.* Transgene-enforced co-stimulation of $CD4^+$ T cells leads to enhanced and sustained anti-tumor effector functioning. *Cytotherapy* 2007; 9: 771–784.

44. Wang J, Press OW, Lindgren CG, *et al.* Cellular immunotherapy for follicular lymphoma using genetically modified CD20-specific $CD8^+$ cytotoxic T lymphocytes. *Mol Ther* 2004; 9: 577–586.

45. Zhong X-S, Matsushita M, Plotkin J, *et al.* Chimeric antigen receptors combining 4-1BB and CD28 signaling domains augment PI_3kinase/AKT/Bcl-X_L activation and $CD8^+$ T cell-mediated tumor eradication. *Mol Ther* 2010; 18: 413–420.

46. Kochenderfer JN, Dudley ME, Feldman SA, *et al.* 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* 2012; 119: 2709–2720.

47. Chmielewski M, Kopecky C, Hombach AA, *et al.* IL-12 release by engineered T cells expressing chimeric antigen receptors can effectively Muster an antigen-independent macrophage response on tumor cells that have shut down tumor antigen expression. *Cancer Res* 2011; 71: 5697–5706.

48. Pegram HJ, Lee JC, Hayman EG, *et al.* Tumor-targeted T cells modified to secrete IL-12 eradicate systemic tumors without need for prior conditioning. *Blood* 2012; 119: 4133–4141.

49. Kerkar SP, Goldszmid RS, Muranski P, *et al.* IL-12 triggers a programmatic change in dysfunctional myeloid-derived cells within mouse tumors. *J Clin Invest* 2011; 121: 4746–4757.

50. Kerkar SP, Muranski P, Kaiser A, *et al.* Tumor-specific $CD8^+$ T cells expressing interleukin-12 eradicate established cancers in lymphodepleted hosts. *Cancer Res* 2010; 70: 6725–6734.

51. Pritchard MT, Wolf SF, Kraybill WF, *et al.* The anti-tumor effect of interleukin-12 is enhanced by mild (fever-range) thermal therapy. *Immunol Invest* 2005; 34: 361–380.

52. Zhang L, Kerkar SP, Yu Z, *et al.* Improving adoptive T cell therapy by targeting and controlling IL-12 expression to the tumor environment. *Mol Ther* 2011; 19: 751–759.
53. Lee DW, Kochenderfer JN, Stetler-Stevenson M, *et al.* T cells expressing CD19 chimeric antigen receptors for acute lymphoblastic leukaemia in children and young adults: a phase 1 dose-escalation trial. *Lancet* 2015; 385: 517–528.
54. Kochenderfer JN, Dudley ME, Carpenter RO, *et al.* Donor-derived CD19-targeted T cells cause regression of malignancy persisting after allogeneic hematopoietic stem cell transplantation. *Blood* 2013; 122: 4129–4139.
55. Turtle CJ, Hanafi L-A, Berger C, *et al.* CD19 CAR-T cells of defined $CD4^+$:$CD8^+$ composition in adult B cell ALL patients. *J Clin Invest* 2016; 126: 2123–2138.
56. Porter DL, Levine BL, Kalos M, *et al.* Chimeric antigen receptor-modified T cells in chronic lymphoid leukemia. *N Engl J Med* 2011; 365: 725–733.
57. Fraietta JA, Lacey SF, Orlando EJ, *et al.* Determinants of response and resistance to CD19 chimeric antigen receptor (CAR) T cell therapy of chronic lymphocytic leukemia. *Nat Med* 2018; 24: 563–571.
58. Kalos M, Levine BL, Porter DL, *et al.* T cells with chimeric antigen receptors have potent antitumor effects and can establish memory in patients with advanced leukemia. *Sci Transl Med* 2011; 3: 95ra73.
59. Porter DL, Hwang W-T, Frey NV, *et al.* Chimeric antigen receptor T cells persist and induce sustained remissions in relapsed refractory chronic lymphocytic leukemia. *Sci Transl Med* 2015; 7: 303ra139.
60. Schuster SJ, Svoboda J, Chong EA, *et al.* Chimeric antigen receptor T cells in refractory B-cell lymphomas. *N Engl J Med* 2017; 377: 2545–2554.
61. June CH and Sadelain M. Chimeric antigen receptor therapy. *N Engl J Med* 2018; 379: 64–73.
62. Kochenderfer JN, Dudley ME, Kassim SH, *et al.* 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* 2015; 33: 540–549.
63. Locke FL, Neelapu SS, Bartlett NL, *et al.* Phase 1 results of ZUMA-1: a multicenter study of KTE-C19 anti-CD19 CAR T cell therapy in refractory aggressive lymphoma. *Mol Ther* 2017; 25: 285–295.
64. Kochenderfer JN, Somerville R, Lu T, *et al.* Long-duration complete remissions of diffuse large B cell lymphoma after anti-CD19 chimeric antigen receptor T cell therapy. *Mol Ther* 2017; 25: 2245–2253.
65. Shalabi H, Kraft IL, Wang H-W, *et al.* Sequential loss of tumor surface antigens following chimeric antigen receptor T-cell therapies in diffuse large B-cell lymphoma. *Haematologica* 2018; 103: e215–e218.
66. Ye B, Stary CM, Li X, *et al.* Engineering chimeric antigen receptor-T cells for cancer treatment. *Mol Cancer* 2018; 17: 32.
67. Ghorashian S, Pule M and Amrolia P. CD19 chimeric antigen receptor T cell therapy for haematological malignancies. *Br J Haematol* 2015; 169: 463–478.
68. Hay KA and Turtle CJ. Chimeric antigen receptor (CAR) T cells: lessons learned from targeting of CD19 in B-cell malignancies. *Drugs* 2017; 77: 237–245.
69. Rayes A, McMasters RL and O'Brien MM. Lineage switch in MLL-rearranged infant leukemia following CD19-directed therapy. *Pediatr Blood Cancer* 2016; 63: 1113–1115.
70. Zoghbi A, Stadt UZ, Winkler B, *et al.* Lineage switch under blinatumomab treatment of relapsed common acute lymphoblastic leukemia without MLL rearrangement. *Pediatr Blood Cancer*. Epub ahead of print 28 April 2017. DOI: 10.1002/pbc.26594.
71. Nagel I, Bartels M, Duell J, *et al.* Hematopoietic stem cell involvement in BCR-ABL1-positive ALL as a potential mechanism of resistance to blinatumomab therapy. *Blood* 2017; 130: 2027–2031.
72. Jacoby E, Nguyen SM, Fountaine TJ, *et al.* CD19 CAR immune pressure induces B-precursor acute lymphoblastic leukaemia lineage switch exposing inherent leukaemic plasticity. *Nat Commun* 2016; 7: 12320.
73. Evans AG, Rothberg PG, Burack WR, *et al.* Evolution to plasmablastic lymphoma evades CD19-directed chimeric antigen receptor T cells. *Br J Haematol* 2015; 171: 205–209.
74. Orlando EJ, Han X, Tribouley C, *et al.* Genetic mechanisms of target antigen loss in CAR19 therapy of acute lymphoblastic leukemia. *Nat Med* 2018; 24: 1504–1506.
75. Fischer J, Paret C, El Malki K, *et al.* CD19 isoforms enabling resistance to CART-19

immunotherapy are expressed in B-ALL patients at initial diagnosis. *J Immunother* 2017; 40: 187–195.

76. Bagashev A, Sotillo E, Tang C-HA, *et al.* CD19 alterations emerging after CD19-directed immunotherapy cause retention of the misfolded protein in the endoplasmic reticulum. *Mol Cell Biol* 2018; 38: e00383-18.

77. Valitutti S and Lanzavecchia A. Serial triggering of TCRs: a basis for the sensitivity and specificity of antigen recognition. *Immunol Today* 1997; 18: 299–304.

78. Sykulev Y, Joo M, Vturina I, *et al.* Evidence that a single peptide-MHC complex on a target cell can elicit a cytolytic T cell response. *Immunity* 1996; 4: 565–571.

79. Davenport AJ, Cross RS, Watson KA, *et al.* Chimeric antigen receptor T cells form nonclassical and potent immune synapses driving rapid cytotoxicity. *Proc Natl Acad Sci U S A* 2018; 115: E2068–E2076.

80. Klebanoff CA, Gattinoni L, Torabi-Parizi P, *et al.* Central memory self/tumor-reactive $CD8^+$ T cells confer superior antitumor immunity compared with effector memory T cells. *Proc Natl Acad Sci U S A* 2005; 102: 9571–9576.

81. Gattinoni L, Klebanoff CA and Restifo NP. Paths to stemness: building the ultimate antitumour T cell. *Nat Rev Cancer* 2012; 12: 671–684.

82. Ramakrishna S, Highfill SL, Walsh Z, *et al.* Modulation of target antigen density improves CAR T-cell functionality and persistence. *Clin Cancer Res* 2019; 25: 5329–5341.

83. Weijtens ME, Hart EH and Bolhuis RL. Functional balance between T cell chimeric receptor density and tumor associated antigen density: CTL mediated cytolysis and lymphokine production. *Gene Ther* 2000; 7: 35–42.

84. Turatti F, Figini M, Balladore E, *et al.* Redirected activity of human antitumor chimeric immune receptors is governed by antigen and receptor expression levels and affinity of interaction. *J Immunother* 2007; 30: 684–693.

85. James SE, Greenberg PD, Jensen MC, *et al.* Antigen sensitivity of CD22-specific chimeric TCR is modulated by target epitope distance from the cell membrane. *J Immunol* 2008; 180: 7028–7038.

86. Pont MJ, Hill T, Cole GO, *et al.* γ-secretase inhibition increases efficacy of BCMA-specific chimeric antigen receptor T cells in multiple myeloma. *Blood* 2019; 134: 1585–1597.

87. Hombach AA, Rappl G and Abken H. Arming cytokine-induced killer cells with chimeric antigen receptors: CD28 outperforms combined CD28-OX40 "super-stimulation". *Mol Ther* 2013; 21: 2268–2277.

88. Song DG, Ye Q, Poussin M, *et al.* A fully human chimeric antigen receptor with potent activity against cancer cells but reduced risk for off-tumor toxicity. *Oncotarget* 2015; 6: 21533–21546.

89. Lanitis E, Poussin M, Hagemann IS, *et al.* Redirected antitumor activity of primary human lymphocytes transduced with a fully human anti-mesothelin chimeric receptor. *Mol Ther* 2012; 20: 633–643.

90. Johnson LA, Scholler J, Ohkuri T, *et al.* Rational development and characterization of humanized anti-EGFR variant III chimeric antigen receptor T cells for glioblastoma. *Sci Transl Med* 2015; 7: 275ra22.

91. Sommermeyer D, Hill T, Shamah SM, *et al.* Fully human CD19-specific chimeric antigen receptors for T-cell therapy. *Leukemia* 2017; 31: 2191–2199.

92. Jaspers JE and Brentjens RJ. Development of CAR T cells designed to improve antitumor efficacy and safety. *Pharmacol Ther* 2017; 178: 83–91.

93. Kerkar SP, Leonardi AJ, van Panhuys N, *et al.* Collapse of the tumor stroma is triggered by IL-12 induction of Fas. *Mol Ther* 2013; 21: 1369–1377.

94. Stephan MT, Ponomarev V, Brentjens RJ, *et al.* T cell-encoded CD80 and 4-1BBL induce auto- and transcostimulation, resulting in potent tumor rejection. *Nat Med* 2007; 13: 1440–1449.

95. Ren J, Liu X, Fang C, *et al.* Multiplex genome editing to generate universal CAR T cells resistant to PD1 inhibition. *Clin Cancer Res* 2017; 23: 2255–2266.

96. Torikai H, Reik A, Liu PQ, *et al.* A foundation for universal T-cell based immunotherapy: T cells engineered to express a CD19-specific chimeric-antigen-receptor and eliminate expression of endogenous TCR. *Blood* 2012; 119: 5697–5705.

97. Qasim W, Zhan H, Samarasinghe S, *et al.* Molecular remission of infant B-ALL after infusion of universal TALEN gene-edited CAR T cells. *Sci Transl Med* 2017; 9: eaaj2013.

98. Liu X, Zhang Y, Cheng C, *et al.* CRISPR-Cas9-mediated multiplex gene editing in CAR-T cells. *Cell Res* 2017; 27: 154–157.

99. Amrolia PJ, Wynn R, Hough R, *et al*. Simultaneous targeting of CD19 and CD22: phase I study of AUTO3, a bicistronic chimeric antigen receptor (CAR) T-Cell therapy, in pediatric patients with relapsed/refractory B-cell Acute Lymphoblastic Leukemia (r/r B-ALL): amelia study. *Blood* 2018; 132(Suppl. 1): 279.

100. Qin H, Ramakrishna S, Nguyen S, *et al*. Preclinical development of bivalent chimeric antigen receptors targeting both CD19 and CD22. *Mol Ther Oncolytics* 2018; 11: 127–137.

101. Qin H, Nguyen SM, Ramakrishna S, *et al*. Novel CD19/CD22 bicistronic chimeric antigen receptors outperform single or bivalent cars in eradicating $CD19^{+}CD22^{+}$, $CD19^{-}$, and $CD22^{-}$ pre-B leukemia. *Blood* 2017; 130(Suppl. 1): 810.

102. Shah NN, Zhu F, Taylor C, *et al*. A phase 1 study with point-of-care manufacturing of dual targeted, tandem anti-CD19, anti-CD20 chimeric antigen receptor modified T (CAR-T) cells for relapsed, refractory, non-Hodgkin lymphoma. *Blood* 2018; 132: 4193.

103. Ruella M, Barrett DM, Kenderian SS, *et al*. Dual CD19 and CD123 targeting prevents antigen-loss relapses after CD19-directed immunotherapies. *J Clin Invest* 2016; 126: 3814–3826.

104. John LB, Devaud C, Duong CP, *et al*. Anti-PD-1 antibody therapy potently enhances the eradication of established tumors by gene-modified T cells. *Clin Cancer Res* 2013; 19: 5636–5646.

105. Freeman GJ, Long AJ, Iwai Y, *et al*. Engagement of the PD-1 immunoinhibitory receptor by a novel B7 family member leads to negative regulation of lymphocyte activation. *J Exp Med* 2000; 192: 1027–1034.

106. Yao X, Ahmadzadeh M, Lu Y-C, *et al*. Levels of peripheral $CD4^{+}FoxP3^{+}$ regulatory T cells are negatively associated with clinical response to adoptive immunotherapy of human cancer. *Blood* 2012; 119: 5688–5696.

107. Lutsiak MEC, Semnani RT, De Pascalis R, *et al*. Inhibition of $CD4^{+}25^{+}$ T regulatory cell function implicated in enhanced immune response by low-dose cyclophosphamide. *Blood* 2005; 105: 2862–2868.

108. Nishio N and Dotti G. Oncolytic virus expressing RANTES and IL-15 enhances function of CAR-modified T cells in solid tumors. *Oncoimmunology* 2015; 4: e988098.

109. Wang LX, Westwood JA, Moeller M, *et al*. Tumor ablation by gene-modified T cells in the absence of autoimmunity. *Cancer Res* 2010; 70: 9591–9598.

110. Fraietta JA, Beckwith KA, Patel PR, *et al*. Ibrutinib enhances chimeric antigen receptor T-cell engraftment and efficacy in leukemia. *Blood* 2016; 127: 1117–1127.

111. Kondo K, Shaim H, Thompson PA, *et al*. Ibrutinib modulates the immunosuppressive CLL microenvironment through STAT3-mediated suppression of regulatory B-cell function and inhibition of the PD-1/PD-L1 pathway. *Leukemia* 2018; 32: 960–970.

112. Dubovsky JA, Beckwith KA, Natarajan G, *et al*. Ibrutinib is an irreversible molecular inhibitor of ITK driving a Th1-selective pressure in T lymphocytes. *Blood* 2013; 122: 2539–2549.

113. Winqvist M, Asklid A, Andersson PO, *et al*. Real-world results of ibrutinib in patients with relapsed or refractory chronic lymphocytic leukemia: data from 95 consecutive patients treated in a compassionate use program. A study from the Swedish chronic lymphocytic leukemia group. *Haematologica* 2016; 101: 1573–1580.

114. Burger JA, Keating MJ, Wierda WG, *et al*. Safety and activity of ibrutinib plus rituximab for patients with high-risk chronic lymphocytic leukaemia: a single-arm, phase 2 study. *Lancet Oncol* 2014; 15: 1090–1099.

115. Mueller KT, Maude SL, Porter DL, *et al*. Cellular kinetics of CTL019 in relapsed/refractory B-cell acute lymphoblastic leukemia and chronic lymphocytic leukemia. *Blood* 2017; 130: 2317–2325.