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# CD19-Chimeric Antigen Receptor T Cells for Treatment of Chronic Lymphocytic Leukaemia and Acute Lymphoblastic Leukaemia

C. L. Lorentzen*,† & P. T. Straten†

*University of Copenhagen, Copenhagen, Denmark; and †Center for Cancer Immune Therapy (CCIT), Department of Hematology, 65Q9 Copenhagen University Hospital, Herlev, Denmark

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*Correspondence to*: C. L. Lorentzen, Center for Cancer Immune Therapy (CCIT), Department of Hematology, 65Q9 Copenhagen University Hospital, Herlev, 75 Herlev Ringvej, DK-2730 Herlev, Denmark. E-mail: cathrinelorentzen88@gmail.com

## Abstract

Adoptive cell therapy (ACT) for cancer represents a promising new treatment modality. ACT based on the administration of cytotoxic T cells genetically engineered to express a chimeric antigen receptor (CAR) recognizing CD19 expressed by B cell malignancies has been shown to induce complete lasting responses in patients with chronic lymphocytic leukaemia (CLL) and acute lymphoblastic leukaemia (ALL). So far, eleven clinical trials including 99 CLL and ALL patients treated with CAR T cells targeting CD19 have been published, and the results from these trials are promising with impressive clinical responses in heavily pretreated patients. Thus, CAR T cell therapy has induced complete responses in both CLL and ALL, and surprisingly, current results indicate that patients with ALL are more prone to respond than are CLL patients. Importantly, the majority of CAR cell studies have observed severe therapy-associated toxicities, which needs attention. Herein we review current data and discuss key aspects of this powerful approach to treat and potentially cure B cell malignancies.

## Introduction

Adoptive T cell therapy has emerged to be a promising immune-based cancer treatment. Tumour infiltrating lymphocytes (TILs) have been limited in application to melanoma patients, whereas antigen-specific chimeric antigen receptor (CAR) T cells provide a new strategy for the treatment of patients with B cell leukaemia and B cell lymphomas [1]. CARs are fusion proteins composed of both B cell and T cell receptor fragments, thus combining the antigen recognition of an antibody with the cytotoxic capacity of the T cell [2]. The first CAR T cell receptor was generated in 1989 [3]. Since then, the CAR construct has evolved and CARs now include various intracellular domains [1]. An attractive CAR cell target when treating B cell malignancies is CD19 since this antigen is expressed solely on B cells [4].

Chronic lymphocytic leukaemia (CLL) is the most common type of adulthood leukaemia [5]. The overall 5-year survival rate in the United States (US) is 79.2%, but the CLL survival variation is large ranging from a few months to a regular life expectancy. Symptomatic CLL treatment is available, but patients are rarely cured [5, 6]. Acute lymphoblastic leukaemia (ALL) is the most frequent childhood malignancy. Although the 5-year survival rate of children aged 0–14 years is 91.7% in the United States [5], the current chemotherapy treatment induces both short- and long-term toxicities [7, 8]. In addition, the 5-year survival rates for adult ALL patients aged 40–64 years are 28.2% [5] and the patients who do not achieve chemotherapy-induced complete remission (CR) have less than 10% chance of survival [7]. These findings indicate that new methods are needed to improve the survival of CLL and ALL patients and CAR cell therapy is a new approach [9].

Currently, 11 clinical trials enrolling CLL and ALL patients for CD19-CAR T cell treatment have been published (Table 1).

## The definition of CAR T cells

CAR T cells are tumour-specific T cells with a receptor composed of an antigen-binding domain derived from a B cell receptor fused to the intracellular signalling domain of a TCR. When a ligand binds to a CAR, a signalling cascade is activated, thereby initiating T cell activation and proliferation. CAR cells are produced by an ex vivo transfer of genes encoding the CAR which provide the cells with the specificity of an antibody and the target destruction power of a T cell [2,10].

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**Table 1** Published CD19-CAR T cell trials for ALL and CLL.

| Reference | Disease | Patients (N) | Construct | Conditioning therapy | IL-2 (*in vivo*) | T cell source | Gene transfer | CD19-CAR T cell dose infused | Outcome | Longest duration |
|---|---|---|---|---|---|---|---|---|---|---|
| 2011, Porter[p] [56] U. Penn<br>2011, Kalos [35] U. Penn | CLL<br>CLL | 1<br>3 | 2. generation scFv-4-1BB-CD3ζ | BEN ± RTX or PEN/CTX | – | Autologous | Lentiviral vector | 1.46 × $10^5$/kg–1.6 × $10^7$/kg split dose over 3 days | 2 × CR<br>1 × PR | CR (11 months+) |
| 2011, Brentjens [52] MSKCC | CLL<br>ALL | 8[a]<br>2[b,q] | 2. generation scFv-CD28-CD3ζ | 3 CLL: no conditioning.<br>5 CLL: CTX<br>1 ALL: CTX | – | Autologous | Retroviral vector | 3 CLL: 1.2–3.0 × $10^7$/kg<br>4 CLL: 0.4–1.0 × $10^7$/kg<br>1 ALL: 0.3–3.0 × $10^7$/kg split dose over 2 days | 4 CLL: NR<br>1 CLL: PR<br>2 CLL: SD<br>1 ALL: CR[q] | PR (3 months)<br>1 ALL patient with CR underwent allo-HSCT 8 weeks after CAR cell infusion |
| 2012, Konchenderfer [53] NCI | CLL | 4[c,l] | 2. generation scFv-CD28-CD3ζ | CTX and FLU | 7.2 x$10^5$ IU/kg IV every 8 h day 0–5 | Autologous | Retroviral vector | 0.3–2.8 × $10^7$/kg | 1 × CR<br>1 × SD<br>2 × PR | CR (15 months+)[l] |
| 2013, Brentjens [58] MSKCC | ALL | 5[d,q] | 2. generation scFv-CD28-CD3ζ | CTX | – | Autologous | Retroviral vector | 1.5–3 × $10^6$/kg (split dose day 1 and 2) | 4 × CR[q]<br>1 × cCR | CR (13 weeks)<br>4 with CR underwent allo-HSCT 43-121 days after CAR cell infusion |
| 2013, Grupp [60] U. Penn | ALL | 2 | 2. generation scFv-4-1BB-CD3ζ | 1: none<br>1: EPI-CTX | – | Autologous[h] | Lentiviral vector | 0.14-1.2 × $10^7$/kg | 2 × CR | CR (180 days+) |
| 2013, Cruz [44] BCM | ALL<br>CLL | 4<br>4 | 2. generation scFv-CD28-CD3ζ | None, they were all allo-HSCT | – | Allogeneic, donor derived | Retroviral vector | Dose escalating schedule: 1.5 –12 × $10^7$/$m^{2o}$ | 2 ALL: cCR<br>1 ALL: CR<br>1 ALL: NR<br>1 CLL: PR<br>1 CLL: SD<br>2 CLL: NR | 1 ALL patient: cCR (8 months+) |
| 2013, Kochenderfer [51] NCI | CLL | 4[e] | 2. generation scFv-CD28-CD3ζ | None, they were all allo-HSCT | – | Allogeneic, donor derived | Retroviral vector | 0.4 × $10^6$ – 2.4 × $10^6$/kg | 1 × SD<br>2 × NR<br>1 × CR | CR (9 months+) |
| 2014, Davila [59] MSKCC | ALL | 16[d, q] | 2. generation scFv-CD28-CD3ζ | Physician's choice salvage chemotherapy and CTX | – | Autologous[i] | Retroviral vector | 1.5–3 × $10^6$/kg[k] 1/3 dose at day 1, 2/3 dose at day 2. | 12 × CR[j]<br>2 × cCR<br>2 × NR | CR (13 weeks)<br>7 with CR underwent allo-HSCT 43-121 days after CAR cell infusion |

**Table 1** Continued.

| Reference | Disease | Patients (N) | Construct | Conditioning therapy | IL-2 (*in vivo*) | T cell source | Gene transfer | CD19-CAR T cell dose infused | Outcome | Longest duration |
|---|---|---|---|---|---|---|---|---|---|---|
| 2014, Kochenderfer [6] NCI | CLL | 4[f,l] | 2. generation scFv-CD28-CD3ζ | CTX and FLU | – | Autologous | Retroviral vector | Reduced from 5 to $1 \times 10^6$/kg due to toxicity. CLL patients received $1–4 \times 10^6$/kg | 3 × CR 1 × PR | CR (23 months+) |
| 2014, Lee [7] NCI | ALL | 20[g] | 2. generation scFv-CD28-CD3ζ | CTX and FLU | – | Autologous[m] | Retroviral vector | $1–3 \times 10^6$/kg. Defined the maximum tolerated dose as $1 \times 10^6$/kg | 14 × CR[n] 3 × SD 3 × PD | CR (5 months) 10 with CR underwent allo-HSCT 45–82 days after CAR cell infusion |
| 2014, Maude [61] U. Penn | ALL | 30 | 2. generation scFv-4-1BB-CD3ζ | 3: None[t] 27: Physician's choice salvage chemotherapy | – | Autologous[r] | Lentiviral vector | $0.76 – 20.6 \times 10^6$/kg over 1–3 days | 27 × CR[s] 3 × NR | CR (24 months+) 3 with CR underwent allo-HSCT after CAR cell infusion |

Institutions: BMC, Baylor College of Medicine. MSKCC, Memorial Sloan-Kettering Cancer Center. NCI, National Cancer Institute. U. Penn, University of Pennsylvania.
Allo-HSCT, Allogeneic hematopoietic stem cell transplant; BEN, Bendamustine; cCR, Continued complete remission (no detectable disease before or after CAR cell infusion); CR, Complete remission; CTX, Cyclophosphamide. EPI, Etoposide; FLU, Fludarabine; NR, No response; PEN, Pentostatin; PD, Progressive disease; PR, Partial remission; RTX, Rituximab; SD, Stable disease.
[a]One CLL patient died within 48 h after CAR cell infusion. [b]One ALL patient did not receive CAR cells because of intervening complications. [c]Eight patients in total, four with CLL. [d]The 2014 trial include five patients from the 2013 trial. [e]10 patients in total, four with CLL. [f]15 patients in total. Malignancy response was evaluated in 13 patients, four with CLL.[g]21 patients in total, 20 with ALL. [h]One patient underwent an allo-HSCT with umbilical cord blood before CAR cell infusion. [i]Four patients had undergone an allo-HSCT. [j]Two patients had minimal residual disease. [k]One patient only received 16% of the prescribed dose. [l]One patient from the 2012 Kochenderfer trial is re-treated in the 2014 Kochenderfer trial. [m]Seven patients had undergone allo-HSCT. [n]One patient had CR with incomplete count recovery, one patient had CR with <5% marrow blasts, and the others had CR with <0.01% marrow blasts. [o]Based on total cell numbers and not on CD19-CAR T cells. [p]A case report enrolled in the 2011 Kalos trial. [q]The ALL patient in Brentjens 2011 trial is included in the Brentjens 2013 trial and in the 2014 Davila trial. [r]18 patients had undergone allo-HSCT. [s]Three patients had minimal residual disease. [t]The patients had persistent cytopenia.

Adoptive T cell therapy has been limited by many barriers; one of them being the requirement for antigen presentation by major histocompatibility complexes (MHCs). Tumour cells often downregulate surface HLA molecules, thus making CAR T cells favourable in cancer treatment, as they target tumour cells in a MHC-independent manner [2,9,10].

In 1989, Gross, Waks and Eshhar generated and expressed a chimeric T cell receptor on murine cells [3], and in 1993, Eshhar *et al.* introduced new intracellular signalling domains to improve the CAR T cell construct [2]. Since then, CAR design has evolved but the basic composition of the chimeric receptors remains. They are composed of a single-chain variable fragment (scFv), a hinge region, a trans membrane domain and an endodomain [1] (Fig. 1). ScFvs are a combination of antibody heavy- and light-variable regions ($V_{Ls}$ and $V_{Hs}$) joined by a flexible linker. The scFvs possess the specificity and affinity of the natural Fab regions of an antibody [2]. The scFvs derive from murine or humanized antibodies, and each scFv is constructed to target a specific surface molecule such as CD19 on human B cells [1].

The basic CAR design consists of many changeable components. By altering these components in various ways, such as changing the epitope-recognition part, cell function can be optimized. Data show that the epitope position on a membrane-bound antigen determines the power of T cell activation to a higher extent than the binding efficacy of the scFv itself and that a proximal epitope activates T cells most efficiently [11]. In addition, inclusion of a flexible extracellular spacer region, a hinge region, improves CAR cell activation when targeting a distal epitope [9]. CAR constructs also differ in flexibility and length of the hinge region conjoining the scFv to the transmembrane region. Several intracellular signalling domains, alone or combined, have been used to improve the results of CAR T cell treatment. These intracellular signalling domains have been added or combined over the past years, and several generations of CAR cells have been denominated according to the signalling domains [1,10].

## CAR signalling domains

Originally, CAR T cells incorporated scFvs combined to the $\alpha$ and $\beta$ chains of a TCR signalling domain [3]. The CAR construct has evolved, and now, the scFvs typically conjoin to a CD molecule providing the cell signalling. Currently, four CAR T cell generations have been produced (Fig. 1). The first CAR constructs (first-generation CARs) include a single intracellular signalling domain, and from the early 1990s, CD3ζ has been the signalling domain of choice [12, 13]. First-generation CAR cells were found to have little tumour eradication effect on cells not presenting costimulatory ligands [14]. Therefore, several research groups began constructing CAR T cells including a costimulatory domain (second-generation CARs) finding that this CAR cell generation had a greater antitumour activity than first-generation cells (Fig. 1) [13, 15–17]. CD28, 4-1BB and OX40 have been included as costimulatory domains, and several studies have compared the therapeutic effects of CD28/CD3ζ (28-ζ) CAR cells with 4-1BB-ζ CAR cells [18, 19], but the results are inconclusive. Studies have later demonstrated that incorporating more than one intracellular costimulatory CAR domain such as CD28.OX40-ζ and CD28.1-4BB-ζ generated an even more effective antitumour response (third-generation CARs) [18, 20] (Fig. 1)However, CD28.4-1BB-ζ third-generation CAR cells did not show improved anti-leukaemic efficacy over 4-1BB-ζ CAR cells in a NOD-SCID mice study [21]. Fourth-generation CARs (TRUCKs) are CAR cells capable of secreting cytokines and expressing cell surface tags such as costimulatory ligands [22, 23] (Fig. 1). Murine CAR T cells secreting IL-12 induce effective tumour eradication

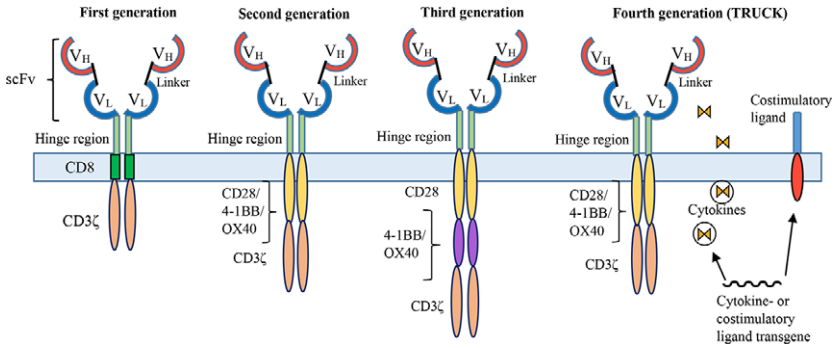


**Figure 1** An illustration of CAR generations. First-generation CARs typically consist of a scFv (single-chain variable fragment), a hinged region, a transmembrane domain (CD8) and a single signalling domain. Second-generation CARs include an additional intracellular, transmembrane signalling domain, for example CD28 or 4-1BB. Third-generation CARs contain two intracellular signalling domains, and fourth-generation CARs (TRUCKs) include the incorporation of genes encoding cytokines, for example IL-2, produced during CAR cell signalling or the incorporation of genes encoding a costimulatory ligand.

[24]. In addition, IL-15-producing CAR cells had a greater expansion and a higher anti-tumour effect compared to CD28-ζ CAR cells in a murine model [25].

## CAR T cell targets

CAR T cells are only capable of targeting surface molecules [10]. In theory, there are numerous potential CAR targets, but so far, very few antigens that are solely tumour specific have been discovered. If the target antigen is not tumour cell specific, the CAR cells might cause an autoimmune reaction, an on-target, off-tumour effect, that could be fatal to the patient [26]. CAR T cell treatment of a patient with an ERBB2 tumour resulted in pulmonary damage and eventually death. A lung tissue examination revealed the expression of ERBB2 [27].

CD19 is an attractive CAR surface antigen target as it is expressed only on B cells and B lymphoid progenitors but not on progenitor stem cells. In addition, the CD19 expression is maintained on neoplastic transformed B cells [4]. Another popular CAR target molecule is the B cell surface antigen CD20 expressed in more than 90% of B cell lymphomas [28]. Furthermore, CAR cell constructs targeting ROR-1, Ig κ light chain and CD33 show promising results [23]. CAR T cells targeting more than one antigen, such as fourth-generation CARs with costimulatory ligands, allow CAR T cells to target a combination of antigens individually expressed in healthy tissues but coexpressed on tumour cells [26].

## CAR T cell production

CAR T cells are a product of gene-transfer technology methods. Currently, the cells are produced in special facilities under Good Manufacturing Practice rules, and in average, the CAR T cell production takes 10–14 days [9]. The typical CAR cell manufacturing starts with leukapheresis of the patient where peripheral blood mononuclear cells (PBMCs) are isolated. The T cell fraction is stimulated and activated through different approaches including CD3 antibodies (OKT3), CD28 antibodies and IL-2 stimulation [9, 29, 30]. A recent murine study demonstrated an increased T cell expansion using an IL-12 and IL-15 stimulating culture [31]. Human T cell activating CD3/CD28 magnetic beads provide a new method that facilitates the isolation process and activation process [32–34]. After a few days, the T cells are activated and incubated with a retroviral vector encoding the CAR genes, and spinoculation has been one of the most utilized transduction methods. Recombinant polypeptide of human fibronectin fragments increases transduction efficacy. T cells are often transduced twice and expanded in a medium containing IL-2. When CAR T cells have expanded to the desired levels, they are washed and infused back into the patient [9, 32].

Both γ-retroviral and lentiviral vectors are used for transduction, but it is not yet possible to assess the advantage of one viral vector system over another [9, 35, 36].

Plasmid mRNA is an alternative to viral vector transduction. The mRNA rapidly degrades within the cell without integrating into the host cell genome. Large-scale production is fast and the mRNA is incorporated into the T cells through electroporation. T cells only express mRNA-CARs for a few days, but this transduction form could prove to be useful for early research when testing possible new antigen targets [37–39].

Transposon systems constitute an alternative method of gene transfer. Transposons are easily and inexpensively manufactured in large amounts compared to viral vectors. In addition, recipient cells do not need prior activation to transposon transfer which reduces the duration of CAR cell culture time [40]. Furthermore, many industrial groups are capable of producing DNA plasmids in large amounts in contrast to the few existing facilities with the expertise of viral vector production. The *Sleeping Beauty* transposon system and the *PiggyBac* transposon systems have been used in recent CAR T cell research studies [41–43].

Virus-specific T (VST) cells transduced with CAR-encoding genes display both antiviral and antitumour activity. The CAR VST cells could be an attractive treatment for immunosuppressed patients at risk of fatal viral infections with especially adenovirus (AdV), cytomegalovirus (CMV) and Epstein–Barr virus (EBV) [44, 45]. In theory, this viral stimulation will expand the CAR cells *in vivo* [44–46].

CAR T cell production is still a time-consuming process. A few years ago, CAR T cells were mainly produced using 'open' methods such as plates and flasks with a potential high risk of environmental contamination. By producing the cells in a more closed system, such as tissue culture bags, the contamination risk has decreased [32]. The closed systems also reduce the requirement for expensive clean rooms and speed up the CAR T cell production [9].

## CAR T cell phenotypes

Many of the early CAR T cell trials observed little or no expansion of the infused CAR cells *in vivo,* and the results were linked to the lack of persistency. By examining CAR T cell phenotypes, the importance of producing CAR T cells capable of proliferating was discovered [33].

Naïve T cells are able to differentiate into effector cells and two kinds of memory cells: central memory ($C_M$) cells and effector memory ($E_M$) cells. $E_M$ T cells are further divided into four distinct populations (Table 2). Studies show that $C_M$ T cells are more capable of proliferation and self-renewal than $E_M$ T cells which make $C_M$ T cells the most favourable CAR T cell phenotype [33, 47]. Memory stem cells ($SC_M$) are recently discovered members of the stem cell family. They have a greater self-renewal capacity

**Table 2** T cell phenotypes.

| T cell phenotype | CD molecules |
|---|---|
| Naïve T cells | $CD45RA^+$, $CCR7^+$, $CD27^+$, $CD28^+$ |
| $C_M$ T cells | $CD45RA^-$, $CCR7^+$, $CD27^+$, $CD28^+$ |
| $E_M$ T cells | $CD45RA^-$, $CCR7^-$ |
| $E_{M1}$ T cells | $CD45RA^-$, $CCR7^-$, $CD27^+$, $CD28^+$ |
| $E_{M2}$ T cells | $CD45RA^-$, $CCR7^-$, $CD27^+$, $CD28^-$ |
| $E_{M3}$ T cells | $CD45RA^-$, $CCR7^-$, $CD27^-$, $CD28^-$ |
| $E_{M4}$ T cells | $CD45RA^-$, $CCR7^-$, $CD27^-$, $CD28^+$ |
| Effector T cells | $CD45RA^{+/-}$, $CCR7^-$, $CD27^-$, $CD28^-$ |
| $SC_M$ T cells | $CD45RA^+$, $CCR7^+$, $CD27^+$, $CD28^+$ |

$C_M$, Central memory; $E_M$, Effector memory; $SC_M$, Stem cell memory. T cell phenotypes determined by surface molecules CD45RA, CCR7, CD27, and CD28.

than $C_M$ T cells and are capable of generating $C_M$ T cells, $E_M$ T cells and effector T cells [48, 49].

T cell phenotypes express different surface molecules such as CD45RA, CCR7, CD27 and CD28, and the combination of these CD molecules defines the individual T cell subset (Table 2) [33, 47, 48, 50]. Seven CLL and ALL trials have examined the CAR T cell phenotypes (Table 1) [6, 7, 35, 44, 51–53]. Two trials found that the CAR T cells acquired more differentiated phenotypes from the time of infusion to peak CAR T cell blood levels. The $E_M$ phenotype expanded and the $C_M$ phenotype decreased [6, 51]. Four groups examined the CAR T cell phenotypes either before or after cell infusion, and three of them observed that the majority of the pre-infused CAR T cells were $E_M$ cells [44, 52, 53]. A single trial found that the predominant phenotype changed from an $E_M$ type to a $C_M$ type after infusion [35].

## CD19-CAR T cells for CLL and ALL treatment

So far, various academic medical centres have published the results of 11 clinical trials and a single case report regarding adoptively transferred CD19-CAR T cells in patients with ALL and CLL. The publications include the treatment results of 26 CLL patients and 72 ALL patients.

### Treating CLL with CD19-CAR T cells

The first CD19-CAR T cell trials involved patients with CLL and non-Hodgkin lymphoma [16, 35, 54–56]. These indolent B cell malignancies were chosen for the initial trials because the graft-versus-leukaemia effect was more noticeable in these cancers and because the slow disease progression provided more time for CAR cell production [57]. The CLL trails demonstrated occasional responses of long-term CR (Table 1).

In 2011, Kalos *et al.* published the results of a trial enrolling three patients with advanced chemotherapy-refractory CLL. Two of the three patients receiving CD19-CAR T cell treatment achieved CR, and one patient had PR. The patients were treated with autologous, lentiviral-transduced, 4-1BB second-generation CAR cells, and they all received conditioning chemotherapy (Table 1) [35]. The same year, Brentjens *et al.* published the results of eight patients with CLL. The patients were treated with autologous, retroviral-transduced, CD28 second-generation CD19-CAR T cells. Three patients treated without conditioning therapy showed no clinical response (NR) to the CAR treatment. Of the cyclophosphamide (CTX)-treated patients, one died, two had stable disease (SD), and one patient exhibited PR with a reduction in peripheral lymphadenopathy (Table 1) [52].

The following year, Kochenderfer *et al.* reported the results of four patients with advanced, progressive CLL treated with autologous, retroviral-transduced, CD28 second-generation CD19-CAR T cells. The patients all received IL-2 injections and conditioning therapy. One patient achieved CR, one had SD, and two patients achieved PR (Table 1) [53].

In 2013, Cruz *et al.* showed that CAR VST cells could safely be administered to post-allo-HSCT patients without exacerbating GVHD. The patients received donor-derived, virus-specific, retroviral-transduced, CD28 second-generation CD19-CAR T cells without preconditioning therapy. None of the CLL patients had viral infections that could expand the CAR VSTs. Two patients had NR, one had SD, and one patient achieved PR (Table 1) [44]. Kochenderfer *et al.* published the results of four allo-HSCT relapsed CLL patients that same year. The patients received donor-derived, retroviral-transduced, CD28 second-generation CD19-CAR T cells without preconditioning therapy. One patient achieved CR, one had SD for 3 months, and two had PD (Table 1) [51].

In 2014, Kochenderfer *et al.* treated four CLL patients with autologous, retroviral-transduced, CD28 second-generation CD19-CAR T cells. They received the same conditioning chemotherapy. Three patients achieved CR and one had PR (Table 1) [6].

Of the CD19-CAR T cell CLL trials listed above, seven (27%) of 26 evaluable patients obtained CR (Table 1).

### Treating ALL with CD19-CAR T cells

The antitumour responses observed in ALL-resembling murine models led to the initiation of clinical trials for ALL patients receiving CD19-CAR T cell treatment. Brentjens *et al.* published the first trial in 2011. A single ALL patient receiving CD19-CAR T cells achieved CR (Table 1) [52]. In 2013, the same group demonstrated that CAR cell treatment of five ALL patients resulted in CR in all five (Table 1) [58]. The results are described in the 2014 Davila *et al.*'s publication [59]. In 2013, Grupp *et al.* treated two children with relapsed and refractory ALL with lentiviral-transduced, 4-1BB second-generation CAR cells. One patient received autologous CAR T cells, and the

other received conditioning chemotherapy and donor CAR T cells from her previous allogeneic umbilical cord blood donor. Both patients achieved CR, but one relapsed with $CD19^-$ blasts after 2 months [60]. In 2013, Cruz *et al.* administered donor-derived, retroviral-transduced, CD28 second-generation, CD19-CAR VST cells to four previous allo-HSCT ALL patients without preconditioning therapy. Three patients had viral infections that could expand the CAR VST cells. In an AdV-infected patient, the expansion of AdVST cells was not associated with an increase in CD19-CAR T cells. However, two $EBV^+$ patients had an increase in both EBV-specific precursors and CAR signals indicating an expansion of the CAR VST cells. Two patients had continued CR (cCR) as they obtained CR before CAR T cell infusion, but were at high risk of relapsing. One patient had CR for 3 months and one had NR to the CAR T cells (Table 1) [44].

In 2014, Davila *et al.* [59] published the results of 16 patients with refractory or relapsed ALL. Five of the patients were described in 2013 [58] and one patient in 2011 as well [52]. The patients were treated with preconditioning chemotherapy and CTX and were infused with autologous, retroviral-transduced, CD28 second-generation CD19-CAR T cells. Twelve patients achieved CR and two patients had cCR. Two patients had NR (Table 1) [59]. In 2014, Lee *et al.* published a trial including 20 children and young adults with ALL treated with retroviral-transduced, CD28 second-generation CD19-CAR T cells. Seven patients had previously undergone an allo-HSCT. Of the 20 patients, 14 achieved CR. Three patients had SD and three patients had PD (Table 1) [7]. Maude *et al.* [61] recently published a trial including 30 ALL patients treated with lentiviral-transduced, 4-1BB second-generation CAR cells. Eighteen patients had relapsed disease after allo-HSCT, 27 achieved CR, and three had NR (Table 1).

So far, seven clinical trials have published the results of 72 ALL patients receiving CD19-CAR T cells. The treatment induced CR in 60 (83%) of 72 patients including minimal residual disease ($MRD^+$) and cCR patients (Table 1).

## CD19-CAR T cell efficacy in CLL versus ALL treatment

When interpreting the clinical results, it must be kept in mind that still very few patients have been treated with CAR T cells and that most trials use lymphocyte-depleting chemotherapy which in itself has an antimalignant effect. Furthermore, the CLL and ALL trials use different CAR constructs with various costimulatory domains. Other differences include the use of IL-2 *in vivo*, choices of transduction vectors, infused T cell dosages and the usage of preconditioning chemotherapy. These differences make it difficult to compare the trials, especially as they often vary in more than one way. However, the two most recently published trials have similar trial and CAR designs [6, 7] (Table 1). Across trial and CAR design, there is tendency that ALL patients have higher response rates to CAR T cell treatment than CLL patients. The two only trials enrolling both ALL and CLL patients observed a better antitumour response in ALL patients, even though three CLL patients were included in a cohort not receiving lymphodepleting therapy, thereby possibly impairing their chances of obtaining CR (Table 1) [52].

The ALL patients achieved CR in 83% of the cases, whereas only 27% of the CLL patients attained CR. Yet, it must be considered that 72 ALL patients compared to only 27 CLL patients have been treated. If, however, CD19-CAR T cell treatment actually does have a higher response rate in ALL patients, what might be the reason?

One explanation could be the inhibitory tumour environment of CLL. This indolent cancer is associated with immunodeficiency due to a combination of impaired natural killer (NK) cell function and T cell function as well as increased numbers of regulatory T cells (Tregs) capable of suppressing non-Tregs [62] and potentially CAR T cells as well. In addition, monocyte-derived cells are capable of attracting malignant B lymphocytes to lymphoid tissues and protect them from apoptosis [63].

The differences in tumour burden size between ALL and CLL patients could also affect CAR cell efficacy. CLL patients often have larger tumour burdens than ALL patients at the time of CAR cell infusion [29], and data from two trails suggest an inverse correlation between tumour burden size at the time of CAR cell infusion and the clinical response of the CAR cells *in vivo* [35, 52, 64]. Nevertheless, large tumour burdens are not insensitive to CAR T cell treatment and the size of tumour alone cannot predict the outcome [64]. In fact, some data suggest that patients with the highest B cell counts at the time of CAR T cell infusion have the most impressive antimalignancy responses, thus indicating that the B cells promote expansion and survival of the CAR T cells. Two other recently published trials found no association between the size of tumour burden and clinical outcome [59].

The age difference between the ALL and CLL patients might also play a role. The average age of ALL patients enrolled in the CD19-CAR T cell trials listed in Table 1 is 27 years, not including one ALL trial only listing the median age [61]. The average age of the CLL patients is 61 years. This age difference is expectable as the two subtypes of leukaemia are diagnosed at different stages of life [7, 62]. It would be reasonable to assume that young patients could have a better chance of withstanding the side effects of CAR T cell treatment favouring ALL patient survival, but the treatment-related serious events do not seem to correlate with age (Table 3). With traditional therapy, the US 5-year survival rate is 91.7% for ALL patients under 14 years of age but only 41.8% in

**Table 3** Toxicities in published CD19-CAR T cell trials for ALL and CLL.

| Reference | Disease | Patients (N) | Conditioning therapy | IL-2 (*in vivo*) | Elevated proteins and soluble receptors | Steroid, anti-TNFα and anti-IL-6R therapy | Toxicities (CAR cell related) |
|---|---|---|---|---|---|---|---|
| 2011, Porter[k] | CLL | 1 | BEN ± RTX or | – | IL, 6, IL-8, IL-1, | 1 patient: | Fever, rigor, dyspnoea, cardiac dysfunction, hypotension |
| [56] U. Penn | CLL | 3 | PEN/CTX | | IFN-γ, MIP-1α, MIP-1β, | corticosteroid | and acute kidney injury due to tumour lysis |
| 2011, Kalos | | | | | MCP-1, CXCL9, CXCL10, | on day 18 due to | syndrome |
| [35] U. Penn | | | | | IL-1Rα, IL-2Rα | toxicity | |
| 2011, Brentjens | CLL | 8[a] | 3 CLL: no | – | GM-CSF, IFN-γ, TNF-α, | None | Rigor, chills, fever, hypotension, chest pain, |
| [52] MSKCC | ALL | 2[b,l] | conditioning | | MIP-1α, MIP-1β, | | diarrhoea, hyponatremia |
| | | | 5 CLL: CTX | | IL-2, IL-4, IL-10 | | 1 patient[a] had persistent fevers, hypotension, |
| | | | 1 ALL: CTX | | | | dyspnoea and renal failure |
| 2012, | CLL | 4[c,i] | CTX and FLU | $7.2 \times 10^5$ | IFN-α, TNF, IL-2 | None | Hypotension, acute renal failure, hypoxaemia, |
| Konchenderfer | | | | IU/kg | | | hyperbilirubinemia, capillary leak syndrome, fever, |
| [53] NCI | | | | every 8 h; | | | fatigue, obtundation, anorexia, elevated liver enzymes |
| | | | | days 0–5 | | | and electrolyte abnormalities |
| 2013, Brentjens | ALL | 5[d,l] | CTX | – | CXCL10, IFN-γ, IL-2, IL-6 | 2 patients: | Fever, hypotension and transient metal status |
| [58] MSKCC | | | | | | DEX due | changes |
| | | | | | | to toxicity | |
| 2013, Grupp | ALL | 2 | 1: none | – | IL-1Rα, IL-2R, IL-2, IL-6, | 1 patient: | Fever, myalgia, confusion, hypotension and acute |
| [60] U. Penn | | | 1: EPI-CTX | | IL-10, TNF-α, IFN-γ | glucocorticoid, | vascular leak syndrome requiring pressor support, |
| | | | | | | TOC and ETA | acute respiratory distress syndrome requiring intubation |
| | | | | | | | and elevated liver enzymes |
| 2013, Cruz | ALL | 4 | None, they were all | – | No elevation of the | None | None |
| [44] BCM | CLL | 4 | allo-HSCT | | measured IL-6, | | |
| | | | | | TNF-α or IFN-γ | | |
| 2013, | CLL | 4[e] | None, they were all | – | IFN-γ[h], IL-10, IL-6 | None | Fever, fatigue, hypotension, decreased cardiac ejection |
| Kochenderfer | | | allo-HSCT | | | | fraction, anaemia, neutropenia, headache, dyspnoea |
| [51] NCI | | | | | | | and hypophosphatemia |
| 2014, Davila | ALL | 16[d,l] | Physician's choice | – | IFN-γ, IL-6, Flt-3L, | 3 sCRS patients: | Hypotension, fatigue, fever, neutropenia, atrial |
| [59]MSKCC | | | of salvage | | IL-5, IL-10, GM-CSF | high-dose steroids. | fibrillation, altered mental status, delirium, |
| | | | chemotherapy | | | 3 other sCRS | chills, sinus tachycardia and respiratory |
| | | | and CTX | | | patients: TOC | failure |
| 2014, | CLL | 4[f,i] | CTX and FLU | – | IFN-γ, IL-6, TNF | 2 patients: TOC | Fever, hypotension, confusion, headache, dyspnoea, |
| Kochenderfer | | | | | | | upper extremity thrombosis, urinary tract infection and |
| [6] NCI | | | | | | | increased creatinine levels |
| 2014, Lee | ALL | 20[g] | CTX and FLU | – | IFN-γ, IL-6, TNF-α, | 2 patients: TOC 2 | The most common grade 3 and grade 4 |
| [7] NCI | | | | | GM-CSF, IL-2, IL-10 | patients: TOC and | toxicities[j]: fever, neutropenia, hypotension, |
| | | | | | | corticosteroid | anaemia, low platelet-, lymphocyte- and |
| | | | | | | | white blood cell counts, increased liver enzymes, |
| | | | | | | | hypokalaemia and hypophosphataemia. 6 patients |
| | | | | | | | had neurotoxicity grades 1–3 |
| | ALL | 30 | | – | IFN-γ, IL-6, IL-2R, ferritin | | |

Table 3 Continued.

| Reference | Disease | Patients (N) | Conditioning therapy | IL-2 (*in vivo*) | Elevated proteins and soluble receptors | Steroid, anti-TNFα and anti-IL-6R therapy | Toxicities (CAR cell related) |
|---|---|---|---|---|---|---|---|
| 2014, Maude [61] U. Penn | | | 3: None[m]<br>27: Physician's choice of salvage chemotherapy | | | 9 patients: TOC[n] 6 patients: TOC and glucocorticoid | Mild-to-moderate CRS, coagulopathy, delirium, aphasia, confusion and hallucinations |

Institutions: BCM , Baylor College of Medicine; MSKCC, Memorial Sloan-Kettering Cancer Center; NCI , National Cancer Institute; U. Penn , University of Pennsylvania.
BEN , Bendamustine; CRS, Cytokine release syndrome; CTX , Cyclophosphamide. DEX , Dexamethasone; ETA , Etanercept (anti-TFNα). FLU , Fludarabine; PEN , Pentostatin; RTX , Rituximab; sCRS , Severe cytokine release syndrome; TOC , Tocilizumab (anti-IL-6R).
[a]1 CLL patient died within 48 h after CAR cell infusion. [b]One ALL patient did not receive CAR cells because of intervening complications. [c]Eight patients in total, four with CLL. [d]The 2014 trial include the five patients from the 2013 trial. [e]10 patients in total, four with CLL. [f]15 patients in total. Malignancy response was evaluated in 13 patients, four with CLL. [g]21 patients in total, 20 with ALL. [h]Increased in three patients. One patient had high levels before CAR T cell infusion. [i]One patient from the 2012 Kochenderfer trial is re-treated in the 2014 Kochenderfer trial. [j]Toxicity grading system (1–4) where one is a mild reaction with no interventions needed and four is lifethreatening consequences [7]. [k]A case report included in the 2011 Kalos trial. [l]The ALL patient in Brentjens 2011 trial is included in the Brentjens 2013 trial and in the 2014 Davila trial. [m]The patients had persistent cytopenia. [n]Four patients received a second dose.

the 20- to 39-year-olds and even lower in post-allo-HSCT patients. CLL patients are older than 50 years of age at the time of diagnosis in 95% of cases, and the overall 5-year surviving rate is 79.2% [5]. CAR T cell therapy has proved to be effective in both young and older ALL patients, which was demonstrated by a recently published article in which 14 of 16 patients achieved CR, their age ranging from 23 to 74 years [59], and as both older ALL and CLL patients have achieved CR, age alone cannot predict antitumour effect [6, 59] (Table 1).

$CD19^-$ blasts are known to emerge in ALL patients, and the $CD19^-$ blast has been observed in CD19-CAR cell-treated patients [60]. The lack of CD19 could make CD19-CAR T cells less useful for ALL treatment in some cases. Three ALL trials have observed six patients relapsing with $CD19^-$ blasts [7, 60, 61]. Two patients presented $CD19^-CD22^+$ blasts, and since the CD22 and CD19 antigens have similar tissue distributions, a CD22-CAR cell could be part of an effective antileukaemic treatment either alone or combined with a CD19-CAR [65].

## Toxicity associated with CAR T cells

All but one CD19-CAR trial have reported about CAR cell-related toxicities. The most common toxicities are fevers, hypotension, dyspnoea, fatigue and confusion (Table 3). The majority of toxicities are reversible without medical intervention, but significant adverse toxicities including renal failure, respiratory distress syndrome and sepsis requiring acute medical support have been described (Table 3). A single death associated with CD19-CAR T cell infusion has been reported [52]. All trials listed in Table 3 have measured elevated serum cytokine levels after CAR T cell infusion, and the cytokines have been observed to correlate with the severity of cytokine release syndrome (CRS) [7, 53]. CRS is a clinical response to elevated cytokine levels and include symptoms such as hypotension, fevers, neurological changes and hypoxia [62]. The majority of elevated serum cytokines are most likely produced by the CD19-CAR T cells [53], but IL-6 production has also been suggested to derive from apoptotic B cells or activated macrophages recruited to the lysed tumour cells [1]. Davila *et al.* defined the criteria for severe CRS (sCRS) and found that the levels of seven cytokines including IFN-γ, IL-6 and GM-CSF correlate with the size of tumour burden and with sCRS development. They also observed that CRP levels can predict the risk of developing sCRS [59].

Macrophage activation syndrome (MAS) has also been observed after CAR cell treatment. It is characterized by persistent fevers, hepatosplenomegaly and cytopenia due to inflammation. Serum levels including ferritin, bilirubin and triglycerides are elevated [60, 66].

The neurotoxicities described in some trials (Table 3) have led to investigation of the cerebrospinal fluid (CSF) of symptomatic patients [6, 7, 59]. One group revealed that

CSF contained detectable CD19-CAR T cells in some but not all ALL patients with neurological symptoms [59]. Another group examined the CSF of ALL patients and found that the patients who developed neurotoxicity had higher detectable levels of CD19-CAR T cells in the CSF than the patients who did not [7]. A recent published trial did not find a correlation between CNS disease and CAR T cell-induced encephalopathy [61]. The expression of CD19 in brain tissue has not been documented [6].

Several patients with CAR-related toxicities have received corticosteroids or anti-IL-6R therapy such as tocilizumab (TOC) (Table 3). Whether corticosteroids and anti-IL-6R therapy interact with CAR T cell expansion and clinical response still needs to be answered. Three patients treated with high-dose steroids had a reduction in CD19-CAR T cells, and they relapsed after achieving CR. Three other patients with TOC had no CAR cell ablation [59]. Another corticosteroid-treated patient had a modest increase in cytokine levels and achieved PR, whereas the rest achieved CR without steroid treatment [35]. However, two patients receiving corticosteroids and TOC had a similar CAR T cell response as two patients treated with TOC alone [7] and a patient receiving glucocorticoids, TOC and anti-TNFα had a higher antitumour efficacy compared to a patient not receiving cytokine-diminishing treatment [60]. These observations indicate that TOC treatment does not affect CAR T cell efficacy, whereas the consequences of corticosteroids are more unclear.

Hypogammaglobulinemia is an expected on-target, off-tumour complication due to B cell depletion, and this phenomenon correlates with ongoing CAR T cell activity [66]. The majority of CAR-treated patients developed B cell aplasia, and a few patients even developed long-term B cell depletion persisting after CAR T cell levels had declined, indicating a functional persistence of CAR cells below the measurable limits [53, 61]. Hypogammaglobulinemia is often treated with Ig infusions to avoid the risk of infections. However, the actual consequences of long-term hypogammaglobulinemia are debated [8, 32, 67].

## How to improve efficacy and safety

The relationship between the infused CAR T cell dose and the T cell expansion *in vivo* is still not clear. The CD19-CAR T cell trials listed in Table 1 have infused cell doses ranging from $1.46 \times 10^5$ to $3.0 \times 10^7$ cells/kg. A CLL patient infused with the lowest dose of CAR T cells still obtained CR [35]. A recently published trial aimed to define the maximum tolerated dose of CD19-CAR T cells, and the dose was defined as $1 \times 10^6$ cells/kg. However, this dose could be prone to fallacy as the CAR T cells expand variably *in vivo* [7].

Exogenous IL-2 injections were used in early trials to stimulate T cell expansion, but possible disadvantages are described. CLL patients developed fever and diarrhoea following IL-2 infusions, and IL-2 probably contributed to the toxicity [53]. In addition, previous studies showed that exogenous IL-2 can stimulate Tregs which potentially suppress CAR T cells [21, 35].

The majority of CD19-CAR trials have used conditioning therapy prior to CAR T cell infusion (Table 1). Chemotherapy-induced lymphopenia creates a space for expansion of the infused CAR T cells partly because of inhibitory Treg ablation [57, 68]. A murine model demonstrated that increasing conditioning chemotherapy augments CAR T cell persistence and antitumour efficacy [69]. The lack of prior conditioning could have influenced the outcome of CLL patients treated in a cohort without CTX. They had low T cell persistence compared to the CTX-treated cohort [52]. In contrast, a recent trial observed that patients who achieved CR after chemotherapy had the same clinical outcome as the patients who did not, thereby questioning the need for chemotherapy prior to CAR cell infusions [59]. At this time, six of 19 patients have achieved CR without preconditioning regimens (Table 1). By avoiding preconditioning chemotherapy, patients that are too weak for the current CAR cell therapy could have a chance of receiving CAR cell treatment [70].

The importance of CAR T cell long-term persistence is debated. Some degree of CAR cell persistence is needed for an antitumour response, exemplified by three trials in which patients with short T cell persistence were unresponsive to CAR cell treatment or had early relapse [44, 52, 61]. In contrary, a research group found that patients with greater CAR cell persistence did not have better antimalignancy responses [53]. CAR cell persisting for a few months might be adequate for a potent antitumour effect, and it is suggested that the peak of circulating CAR cells correlates with antitumour response [7]. Many ALL patients with CR undergo allo-HSCT following CAR treatment making the importance of CAR T cell persistency hard to determine (Table 1), and because of the limited follow-up time, the long-term consequences of self-sustaining CAR T cells are not known [71]. In the most recent ALL trail published, only three of 30 patients received an allo-HSCT with a follow-up period of 2 years. This study also found a correlation between peak levels of CD19-CAR cells and antitumour responses [61]. Immunological mechanisms could play a role in CAR T cell clearance as T cells reacting to autologous CAR cells have been described [7, 54].

CAR T cells are antigen specific. However, they can cross-react with identical epitopes on different antigens or react with undiscovered target antigens in healthy tissue [72]. By incorporating a suicide gene into CAR T cells, they begin expressing a receptor that induces apoptosis when activated, but this also eliminates the therapeutic effects [25]. Inhibitory CAR (iCAR) cells with CTLA-4 receptors provide antigen-specific inhibitory signals when

encountering their ligand on APCs [26]. These safety mechanisms could also prevent long-term B cell aplasia [66].

Initiating CAR cell treatment at an early stage of disease would probably result in fewer associated toxicities due to lower tumour burdens. Repeated CAR T cell infusion could be beneficial to the patients with contraindications to allo-HSCT. One study found no clinical responses in three patients receiving two CAR cell infusions [7]. However, a patient achieving CR after three CAR cell infusions is recently described [61].

## Discussion and conclusions

Eleven clinical trials and one case report have published the results of 98 CLL and ALL patients treated with CD19-CAR T cells, and many of these patients have obtained CR (Table 1). However, the trials are difficult to compare because of differences in the use of conditioning chemotherapy, CAR T cell production methods, CAR cell constructs, toxicity management and the size of tumour burdens. These parameters make it challenging to identify which aspects are critical to CAR cell efficacy.

Based on available data, the CR rate appears to be higher in ALL than in CLL patients. Various explanations such as variance in tumour environments, the size tumour burdens and age differences between the patients have been proposed, but this issue remains unresolved. To identify the effect of changes made in the clinical protocols, systematic clinical testing is required.

Many additional questions remain. To this end, it is not known which CAR construct will induce the best antitumour response without increasing the risk of side effects. Similarly, the optimal method for gene transfer remains unknown as does the optimal phenotype and cell numbers of CAR T cells. Moreover, it is not known whether conditioning chemotherapy is necessary and how toxicities could be handled without interfering with CAR cell expansion.

Concerning the antigen-loss variants seen so far, future studies using bivalent CAR cells targeting two antigens could possibly lower the risk of such escape. Again, however, this needs to be tested in clinical trials.

Identification of cancer-specific (or lineage-specific) antigens is necessary to avoid on-target, off-tumour effects; however, characterization of these antigens is complicated, even more in malignancies outside the B cell lineage [7, 26].

A final hurdle is the financial and technical challenges of producing specific single-patient products, and a future solution could be the production of universal T cells based on HLA gene knockdown [1].

So far, published trials indicate that CD19-CAR T cells are effective when adoptively transferred to patients with B cell malignancies and that these cells will be part of a future treatment for haematological cancers.

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