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CAR T-cells for T-cell acute lymphoblastic leukemia

Marie Emilie Dourthe^{a,b}, André Baruchel^{a,c,*}

^a Pediatric Hematology-immunology department, Robert Debré University Hospital, Assistance Publique-Hôpitaux de Paris (APHP) and Université Paris Cité, France

^b Université Paris Cité, Institut Necker-Enfants Malades (INEM), Institut national de la santé et de la recherche médicale (Inserm) U1151, Assistance Publique-Hôpitaux de Paris (APHP), Necker Enfants-Malades Hospital, Paris, France

^c Université Paris Cité, Institut de Recherche Saint-Louis, Paris EA-3518, France

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ABSTRACT

T-cell acute lymphoblastic leukemia (T-ALL) has a dismal prognosis in case of relapsed or refractory disease. Contrary to B-ALL, few immunotherapies are available for T-ALL. Use of autologous CAR T-cells is challenging due to shared antigen between leukemic and normal T-cells responsible for fratricide and T-cell aplasia in case of persistence of CAR T-cells. Moreover, risk of contamination of the apheresis product by lymphoblasts remains an issue. To counteract these challenges several methods are used to edit T-cell such as protein expression blocker, CRISPR/Cas9 and base-editing. Other possibility is to use autologous T-cells naturally selected *in vitro* or donor-derived T-cells allowing gene edition and reduction of the risk of graft vs host disease. Encouraging results are obtained in preclinical and clinical studies for early response rate but several questions remain. Is the persistence of these cells requiring for maintaining the remission? Is it feasible to recover a target-negative T-cell population without risk of profound immunosuppression? Has an allogeneic stem cell transplantation to be planned for patients after CAR T-cells infusion? What about the risk of engineered T-cells in the long term?

1. Introduction

T-cell acute lymphoblastic leukemia (ALL) is a rare hematological malignancy representing less than 15–20% of all ALLs in children/adolescents and 25–30% in adults. Despite improvement of outcome of patients treated for *de novo* T-ALL with intensive polychemotherapy and allogeneic stem cell transplantation (allo HSCT) in some cases, prognosis of relapsed or refractory (R/R) disease remains dismal. Currently, less than 30% of patients who relapse will be cured. Recent works had identified some negative genetic biomarkers in patients with induction failure [1]. However, most of lesions are not currently amenable to targeted therapy. Moreover, the diversity of genetic lesions seen in T-ALL makes identification of effective therapies challenging. Thus, there is an urgent medical need for development of new therapies targeting ubiquitous T-ALL antigens independently of their deregulated pathway.

In B-ALL several antigens (mainly CD19 and CD22) could be targeted and immunotherapy represents a major revolution in treatment of R/R disease [2–4].

CAR T-cells targeting CD19 antigen is a promising cancer immunotherapy obtaining excellent response rate in patients heavily pretreated

for multiple relapses or chemo-resistant B cell leukemia and lymphoma. This treatment based on genetically modified autologous T-cells is considered as a living drug and allowed to obtain both remission and even long-term relapse free survival [5,6].

Contrary to B-ALL few immunotherapies are clinically used in T-ALL due to several hurdles. Three main barriers are specifically described in T-ALL for development of CAR T-cells: product contamination with T malignant lymphoblast, fratricide and T-cell aplasia. To counteract these challenges and allow CAR T-cells efficacy in T-ALL several methods are used in preclinical and clinical studies. Here, we reviewed different solutions to face these obstacles and described ongoing clinical studies evaluating safety and efficacy of CAR T-cells targeting promising antigen in T-ALL.

2. How to overcome obstacles to CAR T-cells in T-ALL

The different methods used to overcome obstacles on using CAR T-cells in T-ALL are summarized in Table 1 and Fig. 1.

* Corresponding author at: Pediatric Hematology-immunology department, Robert Debré University Hospital, Assistance Publique-Hôpitaux de Paris (APHP) and Université Paris Cité, France.

E-mail address: andre.baruchel@aphp.fr (A. Baruchel).

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Table 1
Methods used to overcome obstacles to CAR T-cells in T-ALL.

| | |
|-----------------------------|--|
| Avoid fratricide | <ul style="list-style-type: none"> Downregulation of antigen expression on CAR T-cells surface by: <ul style="list-style-type: none"> CRISPR/Cas9 Protein expression blocker Cytosine Base Editing |
| Avoid GVHD | <ul style="list-style-type: none"> Naturally selected targeted antigen T-cells Use of T-cells from donor of allo HSCT for CAR manufacturing Suppression of TCR $\alpha\beta$ expression by gene editing Use of non $\alpha\beta$ T-cells <ul style="list-style-type: none"> NK, NKT-cells, macrophage MAIT cells $\gamma\delta$ T-cells |
| Avoid T-cell aplasia | <ul style="list-style-type: none"> Identification of specific targets of T-cells: <ul style="list-style-type: none"> CD1a TRBC1 CCR9 Regulation of persistence of CAR T-cells: <ul style="list-style-type: none"> Transient transfection Suicide gene Logic gates |

2.1. Apheresis/manufacturing failure

Two main situations could lead to manufacturing failure. First, circulating malignant T-cells could be present in peripheral blood of patients with T-ALL. Thus, in case of apheresis, there is a risk of contamination by malignant T-cells that could be harvested and transduced by CAR construct. This phenomenon was already described in B-ALL by Ruella et al. that led to a case of CAR-transduced B cell leukemia relapse [7]. Second, patients with R/R T-ALL could have received

several lines of chemotherapy or have aggressive disease hindering the collection of a sufficient number of T-lymphocytes with adequate fitness to manufacture CAR T-cells. These limitations could be overcome by the use of modified allogeneic T-cells.

2.2. Overcome fratricide

Ideal target antigen must be specific of the tumoral cell. It had to be expressed in all cancer cells and not or low expressed in normal T cells and other tissues [8]. Shared antigenicity of the target and the CAR T-cells may lead to self-killing of CAR T-cells named fratricide [9]. Thus, CAR T-cells may target each other during manufacturing and after administration *in vivo* interfering their expansion *in vitro* and their proliferation and persistence *in vivo*. However, most of targets used for development of CAR T-cells in T-ALL are pan-T cell antigens (CD3 [10], CD4 [11], CD5 [12], CD7 [13]) or are expressed on activated T cells (CD38).

This situation could be avoided during manufacturing in situations in which an antigen is downregulated after activation of CAR T-cells or if we can harness antigen negative T-cells. These subpopulations of antigen negative T-cells could be naturally selected or through genetic manipulations by preemptive cell-engineering steps. Several solutions have been explored, mainly for CD7, an antigen widely expressed in T-ALL. CD7 is a transmembrane glycoprotein widely expressed in progenitors and mature T and NK-cells but without a pivotal role in T-cell development or function. It is expressed in 95% of T-ALL cases.

First, Dario Campana's team reported on the blockade of CD7 expression by using a protein expression blocker (PEBL) based on an anti-CD7 single-chain variable fragment coupled with an intracellular retention domain [13]. Transduction of anti CD7 PEBL led to abrogation of CD7 expression in virtually all T-cells by blocking protein in endoplasmic reticulum (ER) or Golgi apparatus, impairing cell surface expression of CD7. The expression of PEBL did not impair T cell proliferation and cytotoxicity. The modified T-cells were more efficient than CD7+ T cells spared by fratricide and demonstrated strong anti-tumoral

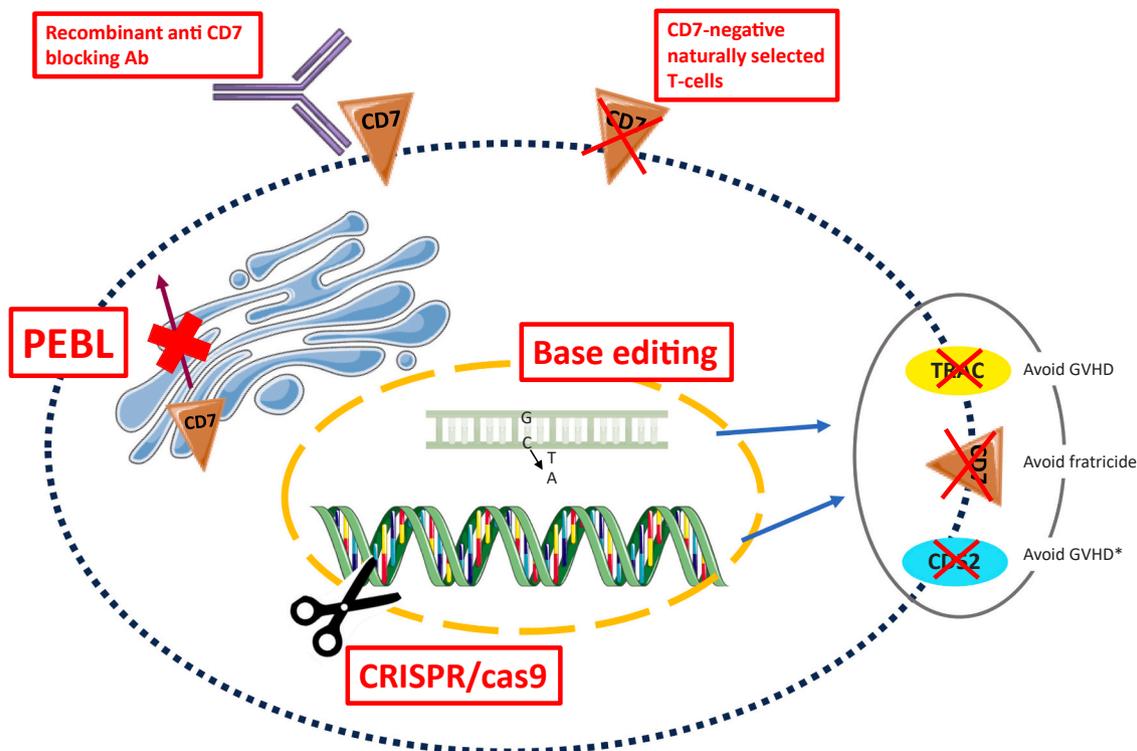


Fig. 1. graphic representation of methods to avoid fratricide and GVHD in CAR T-cells targeting CD7. *Allow to administrate alemtuzumab to avoid GVHD. Abbreviations: GVHD, graft vs host disease; PEBL, protein expression blocker.

activity in patient derived T-ALL xenograft. A phase I is ongoing with these CAR T-cells in children and adults with relapse/refractory T-ALL in Singapore (CARTALL, NCT05043571)

The CD7 gene can be disrupted by using CRISPR/Cas9 technology prior to CAR expression. Works of Gomes-Silva et al. showed that this method minimizes fratricide and allows expansion of CD7^{ko} CAR CD7 with robust anti-tumor activity [14]. In the same way, Cooper et al. reported on the efficacy of UCART7, healthy donor derived T cells fratricide resistant by inhibiting CD7 expression and TRAC by CRISPR/Cas9 [15]. UCART7 obtained an efficacy in murine model and a phase I/II study (WU-CART-007) is ongoing to evaluate efficacy in human T-ALL [16]. However, safety concerns remain in CRISPR/Cas9 editing, notably for the risk of chromosome loss. A modified cell manufacturing process was employed in a first-in-human clinical trial to reduce this risk [17]. To edit CD7 gene, Diorio et al. reported on an alternative approach using cytosine base editing (CBE) [18]. Cytosine base editors allowed to install point mutations in T cells with more than 90% of efficiency to silence gene expression without creating double break strands reducing undesired editing as compared with CRISPR/Cas9. They developed 7CAR8, CD7 directed allogeneic CAR T-cells. This technic of base editing does not impact T-cells proliferation nor create non desired gene edition. They demonstrated a high efficiency of 7CAR8 in several *in vitro* and *in vivo* models and suggested a high potential for clinical translation.

Interim phase I data on the use of base-edited CAR7 T cells have been recently reported [19]. Base editing by cytidine deamination guided by CRISPR allows precise C→U→T conversion that can be directed to create premature stop codon or disrupt splice sites without induction of double strand breaks. The authors electroporated in healthy donor T cells 3 single-guide RNAs targeting TRBC1, TRBC2, CD7 and CD52 with a codon-optimized cytidine base editor mRNA. BE-CAR7 showed potent activity with remission induced in 2 out of 3 patients. This strategy is used as a bridge to allo HSCT since this procedure ensure donor-derived immune reconstitution and thus limits the persistence of engineered cells.

To minimize CAR T-cells mediated fratricide, Pan et al. reported the clinical use of a CD7 targeting CAR construct using intra block technology preventing CD7 cell surface expression [13,20]. The vector contains a CD7-binding domain fused with an endoplasmic reticulum retention signal sequence. These T-cells were issued from healthy donor either previous stem cell transplantation donors or new donors. The results of the phase I study described a complete remission for 18 out of 20 infused patients. After a median follow up of 6.3 months 15 patients remained in remission. Notably, these CAR T-cells were detectable at 6 months and CD7 negative normal T cells with preserved *in vitro* anti-viral functions emerged after infusion. These results suggested an alleviation of immunodeficiency risk after CD7 CAR T-cells.

More recently, a trial evaluated the efficacy of an autologous nanobody-derived fratricide-resistant CD7 CAR-T cells in patients with R/R T-ALL/LBL [21]. The CD7 blockade strategy consisted in using tandem CD7 nanobody VHH6 coupled with an ER/Golgi-retention motif peptide to intracellularly fasten CD7 molecules without impairing CAR-mediated cell function. In phase clinical phase I study for patients with R/R T-ALL or T-LBL a complete remission rate was 7/8 patients at 3 months after infusion. Most of patients had only grade 1 or 2 CRS with no T cell hypoplasia.

In parallel, another team developed naturally selected CD7 CAR-T cells (NS7CAR) manufactured without additional genetic manipulations from patient or donor -derived T-cells [22]. The 7CAR lentiviral transduction of bulk T cells (NS7CAR) or sorted CD7 negative T cells (Neg7CAR) were performed 2 days after CD3/CD28 activation. They compared these 2 populations with CD7-ablated 7CAR-T (KO7CAR) derived by electroporating bulk T cells with CD7-targeting Cas9 RNP. The NS7CAR cells had a lower expansion than Neg7CAR and KO7CAR but a significant higher expression of the 7CAR molecule. Although NS7CAR T-cells expressed CD7 mRNA and protein, they remain CD7

negative per FCM and escaped fratricidal cytotoxic killing by antigen masking and intracellular sequestration. Concerning T cells characteristics NS7CAR contained more CD8+ subsets and an increased central memory phenotype compared to other groups. *In vitro* anti leukemic activity of NS7CAR and KO7CAR had comparable cytotoxicity against T-ALL cell lines contrary to Neg7CAR cells that have a lesser extent. Consistent with these results both NS7CAR and KO7CAR led to reduce leukemia burden in a mouse model engrafted with CCRF-CEM cells. Due to this favorable result, a phase I study was reported on 20 patients with R/R T-ALL or T-LBL (median age 22 years) who received NS7CAR-T cells produced from patients (n=18) or their allo-HSCT donors. (n=2). NS7CAR were well tolerated with only one grade 3 CRS and 2 neurotoxicity grade 1. Fourteen patients received allo HSCT. At day 28 16/17 patients obtained MRD negative complete remission. After a median follow up of 142 days promising duration of remission was obtained. With a median follow up of 210 days post transplantation the 7/10 CR patients maintained MRD negative status. In parallel, patient experienced dramatic decrease of malignant and normal CD7+ cells in peripheral blood at a median time of 7 days post NS7CAR infusion. T/B/NK cells recovered to the baseline levels but decreased shortly after allo-HSCT. An adequate recovery of normal CD7+ T cells after allo HSCT was observed [22].

At last, a new strategy to avoid fratricide was demonstrated consisting in using recombinant anti CD7 blocking antibodies. During the preparation phase anti CD7 CAR T-cells are cultured with antibodies to block the CD7 antigen on the surface of T-cells. Thus, CD7 CAR T-cells had higher viability and proliferative capacity *in vitro* [23]. Even if these CAR T-cells had cytotoxic effect *in vitro*, *in vivo* data on murine models are missing.

Some antigens, despite their expression on T lymphocytes seem to induce fratricide to a lesser extent. That is the case for CD5, a glycoprotein expressed on lymphocyte precursors, mature T-cells and approximately 80% of T-ALLs. In T-ALL xenograft models, CD5 CAR T-cells expansion is delayed by 2–3 days due to fratricide but subsequent expansion and proliferation are not impaired. Resistance to fratricide is explained by downregulation of CD5 antigen (by masking or internalization) and elevated levels of molecules against cytotoxic pathways (perforin/granzyme and Fas/FasL) avoiding apoptosis of CAR T-cells. A clinical trial evaluating the efficacy of anti CD5 CAR T-cells in children and adults with T-ALL and LBL reported initial result in four out of nine patients with response with 3 complete remissions. No severe toxicity and no complete T cell aplasia occurred [24].

To overcome fratricide without gene modification, identification of an antigen selectively expressed on T-ALL blasts but not normal T cells is critical. Two main targets were already used and reported in preclinical studies.

First, CD1a is expressed by cortical T-ALL (CoT-ALL), major subgroup of T-ALL, and practically absent in normal tissues with the exception of developing cortical thymocytes and Langerhans cell. CD1a-specific CAR T-cells exhibit robust cytotoxicity against CD1a coT-ALL cell lines and primary coT-ALL cells, both *in vitro* and *in vivo* [25]. This target avoids fratricide and allows a long-term persistence and functionality. Despite the rarity of this subtype of T-ALL a phase I is ongoing for children and adults in Spain (NCT05679895).

Another potential identified target to avoid fratricide is CCR9, a transmembrane G-coupled receptor for the ligand CCL25. It is expressed in mice in gut intraepithelial T-cells but only in less than 5% of normal circulating T- and B-cells. Maciocia et al. demonstrated that CCR9 is expressed on a high proportion of R/R T-ALL [26]. Anti CCR9 CAR T-cells demonstrated a strong antitumor activity *in vitro* and *in vivo* in multiple models of T-ALL without fratricide. A phase I is to be opened in UK for adult T-ALL.

Finally, a preclinical study validated the viability of a very innovative method based on personalized tumor specific CAR T-cells [27]. All leukemic cells from each patient have a unique TCR at the cell surface. Huang *et al.* proposed a pipeline based on epitope screening technology

aimed at isolating unique CDR3 regions from patients with leukemia. They designed a personalized CDR3 scFv against TCR from cell lines and patients that were cloned into lentiviral vector encoding 2nd generation CARs (JCAR). They demonstrated the efficacy of this complex approach *in vitro* and in a xenograft model.

2.3. Overcome T cell aplasia

In the B-ALL field, the most used target for CAR T-cells is CD19. On-target off-tumor effect is the B-cell aplasia that is easily managed by immunoglobulin replacement. A potential T-cell aplasia occurring after CAR T-cells targeting pan T antigen represents a greater challenge due to the absence of T-cell replacement strategies except from subsequent allo-HSCT.

Different solutions could be raised to overcome T cell aplasia. First, choosing a highly specific target is the basic measure. An innovative approach is to select TRBC1 or TRBC2 alleles at the TCR beta constant region, that spare a substantial proportion of normal T cells. Indeed, each TCR selects a TCR beta chain constant region encoded by either TRBC1 or TRBC2 for expression in a mutually exclusive manner. Hence, normal T-cells population expressed a mix of TRBC1 and TRBC2 contrary to leukemic cells that expressed only the same constant region of TCR beta either TRBC1 or TRBC2. Based on this concept, Maciocia et al. reported on the use of anti TRBC1 CAR T-cells *in vitro* and in a disseminated model of leukemia in mouse model *in vivo* [28]. This infusion led to reduce leukemia infiltration and improve survival of mice injected with JURKAT T-cells which expressed TRBC1-TCR. With this approach the authors hypothesize that patient could retain approximately two thirds of their normal T-cells with preservation of their normal polyclonal antiviral activity. Moreover, the potential of “on target, off tumor” activity is negligible due to the exclusive expression of TRBC1 on mature T and NKT-cells.

This strategy could be used only for mature T cell malignancies or for the few T-ALLs expressing the T-cell receptor at the cell surface, ie 15–20% [29].

Second, regulating the CAR T-cells persistence and cytotoxicity with different methods. These approaches could eliminate quickly CAR T-cells in case of adverse events such as prolonged T-cell aplasia [30]. Three main types are reported: 1. Passive control, like transient transfection or affinity tuning; 2. Inducible control, by co-administration of a drug that activates a suicide gene; 3. Logic gates (AND gate) technology consisting in targeting different antigens simultaneously: CAR T cells are functional only if both are recognized. For this method, a synNotch receptor is introduced in CAR T-cells [31]. This receptor releases a transcription factor after binding an antigen. This transcription factor activates the expression of another CAR targeting a different antigen.

Third, to avoid the risk of profound T cell loss that mirror Severe Combined Immune Deficiency, patient could receive allo-HSCT after CAR T-cells infusion. However, surprisingly data from early phase clinical trial showed recovery of T-cell without expression of the targeted antigen and able to have anti infectious functionality, mitigating this challenge [20]. At last, it is necessary to evaluate the availability of an allogeneic donor after CAR T-cells infusion in case of profound T-cell aplasia.

2.4. Overcome GVHD with allogeneic CAR T-cells

To mitigate challenges of apheresis failure and fratricide, the field of allogeneic CAR T-cells is in constant development. A feared complication is the Graft versus host disease (GVHD), a well known adverse event that can occur after allo HSCT when donor-derived T-cells attack host tissues.

$\alpha\beta$ T-cells are crucial for GVHD. This population represents more than 95% of circulating T-cells. Several possibilities are in development developing to counteract this risk with allogeneic CAR T-cells: using T-

cells from donor of allogeneic stem cell in patients who previously received allo HSCT; using non $\alpha\beta$ T cells; genome editing to suppress $\alpha\beta$ TCR.

1. A meta-analysis has been recently published reporting on donor-derived CAR T-cells in patients who already received allo HSCT before CAR T-cells. Sixteen clinical studies evaluated the safety and efficacy of DD CAR T-cells targeting CD19 in B-ALL with promising results. 203 patients were included with high response rate of 80% after CAR T-cells infusion and only 4% of GVHD. These results suggest that it could be transposed for T-ALL [32].
2. Use of non $\alpha\beta$ T-cells suggests the feasibility to use cells with cytotoxic capacity, easy to transduce and available with good expansion. For this objective, several preclinical and few clinical studies evaluated NK cells as source of cells for manufacturing. *In vitro* studies showed high cytotoxicity of CAR NK cells but a low expansion capacities *ex vivo*, that limits their use in clinical settings [33]. You et al. demonstrated that CD7-directed NK cells had cytotoxicity *in vitro* and *in vivo* against T-ALL with an improvement of survival in mouse model [34]. Comparable results were obtained with CD5 directed CAR NK92 cells [35].

Other cells as NKT cells, MAIT or macrophage cells could be used as source of universal allogeneic CAR T-cells [36,37]. At last, $\gamma\delta$ T cells could represent an interesting source of allogeneic cells as able to expand and to have cytotoxic activity. Notably, they migrate to tissues where autologous CAR T-cells have difficulty to access.

3. Several methods to inactivate $\alpha\beta$ TCR are also developed. The objectives are to create double strand breaks on DNA on a specific localization by introducing a nuclease. Challenge is in avoiding the off-target cleavage. To avoid alloreactivity the easier approach is thus to delete the constant region of α chain (TRAC). The 1st study reporting on the zinc finger technology to delete TRAC gene was published in 2012 [38]. This technic did not compromise the efficacy of CAR T-cells. Other methods are developed: using TALEN nuclease (activator like effector nuclease technology) that could edit simultaneously TRAC and CD52 genes [39–42]. These allogeneic T-cells deficient for TRAC and CD52 were not responsible for GVHD in murine model and were resistant to alemtuzumab, monoclonal antibody targeting CD52, used to eliminate T-cells from host. More recently, CRISPR system were developed to generate universal CAR T-cells with the risk to create multiple cleavage in off target sites. This genome editing is preceded or followed by CAR construct transduction. Strategies to integrate CAR in the TRAC locus by CRISPR/Cas9 was also used to decrease the risk of insertional mutagenesis and obtain simultaneous expression of CAR in cells with inactivation of TCR [43]. These CAR T-cells demonstrated a higher anti tumoral activity in a murine model of CD19 targeting in B-ALL.

3. Targets in ongoing and future clinical trials

The main reported clinical trials on CAR T-cells in T-ALL are summarized in Table 2.

- CD7

To avoid fratricide during CAR T-cells expansion, CD7 surface expression must be abrogated, either by gene edition or by selecting naturally occurred CD7 negative T cells as a source for CAR T generation. Several groups reported the efficacy of CD7 specific CAR in preclinical models of T-cell malignancies. They all considered the abrogation of surface expression of CD7, by methods already described above in this review.

An off-the-shelf allogeneic CAR T-cells with disruption of TCR α and CD7 by CRISPR/Cas9 system, named GC027 was used for 2 cases of T-ALL patients [44]. Patient 1, 24 years old, with a relapsed disease, received a single dose of GC027 after lymphodepleting chemotherapy containing fludarabine, cyclophosphamide,

Table 2
Main reported clinical trials of CAR T-cells in T-ALL.

| References | Targets allo/auto T-cells | Gene/protein editing | Infused pts (n) | Toxicity | Response | Allo HSCT after CAR T-cells |
|----------------------------------|-------------------------------|--|-----------------|---|---|-----------------------------|
| Zhang et al. Nanobody-derived | CD7 auto CAR T-cells | Tandem CD7 nanobody VHH6 coupled with an endoplasmic reticulum/Golgi-retention motif peptide | 8 | CRS G1–2 87.5% | CR n=7 | no |
| Lu et al. NS7CAR | CD7 auto CAR T-cells | no | 53 | CRS G3+ n=6 Neurotoxicity n=2 | CR n=46 18-month OS: 75% 18-month EFS:53.1% CR 90% | 14 |
| Pan et al. | CD7 Donor-derived CAR T-cells | CD7 protein expression blocker | 20 | CRS G3 n=2 Early GVHD n=8 | 1-year PFS: 41.6% 1-year OS: 54% | 8 |
| Li et al. GC027 | CD7 Allo CAR T-cells | CRISPR/Cas9 TCR α /CD7 | 2 | CRS G3 n=2 | CR n=2 1 remission at 1 y 1 relapse at D48 | No |
| Huang et al. RD13–01 | CD7 Allo CAR T-cells | CRISPR/Cas9 TRAC/CD7/HLA-II | 10 | CRS G3 n=1 Neurotoxicity n=1 | CR n=8 | No data |
| Chiesa et al. BE-CAR7 | CD7 Allo CAR T-cells | CRISPR/Cas9 TCR/CD52/CD7 | 3 | CRS G2 n=3 ICANS G1 n=2 | CR n=2 | No data |
| Hill et al. | CD5 Auto CAR T-cells | no | 9 | CRS G1–2 n=3 Neurotoxicity n=1 | CR n=3 | 1 |
| Pan et al. | CD5 Donor-derived CAR T-cells | CRISPR/Cas9 CD5 | 5 | CRS G1–2 n=4 GVHD G1 n=1 | CR n=5 | No data |

Abbreviations: allo, allogeneic; auto, autologous; CR, complete remission; CRS, cytokine release syndrome; GVHD, graft vs host disease; OS, overall survival; PFS, progression free survival; pts, patients

prednisone and melphalan. Blasts BM before lymphodepleting chemotherapy was 25%. Patient 2, 39 years old, with a refractory disease (BM and CNS involvement) received a single dose of GC027 after lymphodepleting chemotherapy containing fludarabine, cyclophosphamide and prednisone. Grade 3 CRS occurred in both patients. Both achieved complete response (CR) in bone marrow (BM) and central nervous system (CNS). None of the 2 patients had available donors for HSCT. Peak of CAR T-cells expansion were observed between day 8 and 11. No CD7 related on target/off tumor toxicity was observed. No CAR T-cells were detected beyond day 30 and gradual recovery of CD7+ was observed. Patient 1 remains in ongoing remission for over 1 year. Patient 2 had relapsed 48 days after infusion.

A phase I clinical trial of RD13–01 infusion in 10 enrolled patients was reported (NCT04620655) [45]. RD13–01 consisted in T-cells edited for CD7/TRAC/RFX5-related genes by CRISPR/Cas9. To compensate the lack of CD7 they add CD132 to increase the production of IL-2. Eight patient achieved CR, 7 of whom were MRD negative. One patient developed grade 3 CRS and one patient grade 3 neurotoxicity. Long term follow up is lacking.

Base editing was used to remove TCR, CD52 and CD7 to produce allogeneic BE-CAR7 T-cells in UK. A phase I study was initiated to treat 10 children after lymphodepleting chemotherapy based on fludarabine cyclophosphamide and alemtuzumab (ISRCTN15323014). After remission patients have to receive allogeneic stem cell transplantation to deplete BE-CAR7 T-cells and allow immune reconstitution. Only 3 patients had already received BE-CAR7 T-cells with grade 2 CRS (n=2) and grade 1 ICANS (n=1) without GVHD. Two out of 3 patients obtained CR at 1 month after infusion [42].

Concerning the method of protein blocker, results of 3 clinical studies are ongoing/reported. Zhang et al. reported on the results of a CD7 CAR T-cells derived from autologous nanoantibodies [21]. Eight patients were enrolled and 7 patients achieved CR after 3 months of CAR T-cells infusion. No T-cell aplasia and no neurotoxicity occurred. Most patients experienced only grade 1 or 2 CRS. Pan et al. reported the result of the phase I clinical trial of donor-derived CA7 CAR T-cells with the same technology of protein blocker in 20 patients. Grade 3 + occurred on only 10% of patients and neurotoxicity was self-limiting. Early GVHD occurred in 60% of the subjects. CAR T-cells proliferate effectively in all patients without evidence of rejection. 90% of patients obtained CR [20]. Results of the interim analysis of the phase II clinical trial of donor-derived CD7 CAR T-cells in 20 patients was reported during ASH 2022. The best overall response rate at 3 months was 90% with 2 patients who developed grade 3 or higher CRS. Importantly, 8 patients developed grade 1–2 GVHD. All these sides effects were reversible. After a median follow-up of 17.2 months the 1-year PFS is 41.6% and the 1-year OS is 54%. Twelve out of 18 responders received no further treatment: 3 remained in remission, 7 patients relapsed and 2 died of infection. Eight patients proceeded in allo HSCT. Five remain in remission [46].

The long-term results of the phase I/II clinical trial evaluating NS7CAR was reported for 53 patients [22]. Forty six out of 48 patients achieved MRD negative CR. The 18-month OS and EFS were respectively 75% and 53.1%. Five patients had grade 3 and one patient grade 4 CRS. Two patients had grade 2 neurotoxicity. Following NS7CAR 14 patients received allo HSCT (10 consolidative, 4 salvage) with no relapses to date. Of the 6 patients who did not receive a transplant, 4 remained in CR at a median time of 54 (32–180) days.

In parallel, an ongoing phase I clinical trial (NCT03690011) is led evaluating the effects of unedited autologous CD7 CAR T-cells [47]. The related preclinical study reported on the feasibility of ex vivo expansion of CD7 CAR T-cells without gene editing but by using dasatinib and ibritinib to prevent fratricide and allow CAR-T expansion. The unedited CD7 CAR T-cells persisted long term with a sustained anti leukemic activity in 2 mouse xenografts models of T-ALL of human T-ALL by self-selecting for CD7 negative CAR T-cells with a similar transcriptome ton control CD7 edited CAR T-cells.

UCART7, universal off-the-shelf allogeneic CD7 directed CAR T-cells (UCART7) that target CD7+ T cell malignancies demonstrated efficacy in human T-ALL cell lines and primary T-ALL in vitro and *in vivo* [15]. To avoid fratricide and GvHD TRAC and CD7 are disrupted by CRISPR/Cas9 genome editing. A phase I/II study is ongoing to evaluate efficacy and safety of UCART7 in children >12 years and adults with T-ALL (WU-CART-007, NCT04984356).

- CD5

CD5 is a pan T-cells surface marker present in ~85% of T-cells malignancies. Hill *et al.* reported on the results of a phase I dose escalation study of CD5 directed CAR T-cells for R/R T-ALL and lymphoma [24]. This CAR T-cells product contains the CD28 co-stimulatory domain. Four patients with T-ALL and 5 patients with T non-Hodgkin lymphoma received a single infusion after lympho-depleting chemotherapy containing fludarabine and cyclophosphamide. Four of 9 evaluable patients obtained an objective response with a complete response in 3 patients. CRS occurred in 3/9 patients, all grade 1–2. One patient developed grade 2 neurotoxicity. Two patients had prolonged cytopenias at 6 weeks.

A Phase I clinical study is ongoing evaluating a donor-derived CD5 CAR T-cells that resist fratricide by deletion of CD5 gene in patients who relapsed after CD7 CAR T-cells in CD7 negative T-ALL [48]. Five patients received donor-derived CAR T-cells and experience CR at 1 month after infusion. Four patients experienced CRS grade 1–2. Only one patient experienced GVHD grade 1. Patients positive CD5 T-cells were depleted while CD5 negative T-cells increased at 1 month.

- TRBC1

A phase I/II study (AUTO4, NCT03590574) is actually evaluating the safety and efficacy of anti TRBC1 CAR T-cells in adult patients with relapsed or refractory TRBC1 positive selected T-non-Hodgkin lymphoma. The results were reported for 10 patients at 4 dose levels. CRS of any grade occurred in 4 patients without neurotoxicity. Seven patients achieved complete metabolic response [29].

- CD1a

Recently, a second generation CD1a CAR T-cells with 4–1BB co-stimulatory domain showing no fratricide effects and long-term persistence was developed [25]. Specific cytotoxicity of CD1a CAR T-cells was demonstrated against CD1a T-ALL cell lines and primary blasts. A phase I clinical trial is presently ongoing (NCT05745181).

4. Conclusion

Due to few targetable genetic lesions and high diversity of mutational landscape of T-ALL, identification of effective agents is challenging. Targeting ubiquitous T-ALL antigens such as CD7 independently of genetic biomarkers could mitigate these limitations. In this line, harnessing the immune system against leukemic T-cells could be a promising option. Shared expression of targets between normal and tumoral cells remains the major hurdles to the development of CAR T-cells. However, several methods are in development to counteract fratricide, T-cell aplasia and GVHD *in vitro* and *in vivo* [9].

Several questions and opportunities remain concerning ongoing clinical studies about type and place of autologous or allogeneic CAR T-cells [20,22].

First, in case of autologous product, is the long-term persistence of CAR T-cells required for maintaining remission in patients so critical as

for B-ALL? If preliminary results reported on the emergence of target-negative T-cells with antiviral function are confirmed, autologous CAR T-cells could be a standalone treatment. However, no data are already reported on the risk of infection later during the follow up of patients. Moreover, overall response rate is obtained for most of patients but longer follow up is needed.

Second, allogeneic CAR T-cells is considered as a bridge to allo HSCT notably for refractory disease. They could be regularly reinfused to maintain immunosurveillance and thus remission. Identifying patients who need consolidation by allo HSCT to maintain response independently of immune reconstitution remains a major challenge as for B-ALL [49].

Third, other results are awaited concerning CD5 CAR T-cells notably since this target could limit the risk of fratricide and reduce the requirement of gene editing. Other targets demonstrated potential anti leukemic effects as CCR9 or CCR8 for example [26,50]. CD38 as an antigen already targeted by a monoclonal antibody which shown clinical response could be used for CAR T-cells in T-ALL [51]. However, despite promising results *in vitro* and in preclinical models, its large expression on hematopoietic progenitor could lead to limiting toxicities in patients [52]. Are these targets potentially translated in clinical settings and with which limits and adverse effects are persisting questions. At last, as in B-ALL or B-NHL [53], dual targeting could be an option for T-ALL to limit on target-off tumor effect and preclinical studies are needed to evaluate the efficacy of this therapeutic approach.

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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