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Genome editing approaches for universal chimeric antigen receptor T cells

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ABSTRACT

Autologous chimeric antigen receptor (CAR) T cell therapy has revolutionised the management of certain B-cell malignancies. However, as bespoke therapies, challenges include complex manufacturing logistics and risks ranging from suboptimal harvests to inadvertent transduction and masking of blast populations. Premanufactured, ready -to -use allogeneic CAR T cells could mitigate some of these hurdles if barriers created by HLA (Human leukocyte antigen) mismatching can be addressed. Genome editing to disrupt TCRαβ (T-cell receptor αβ) expression has been shown to be effective in addressing alloreactivity and avoiding graft versus host disease (GVHD). Platforms including transcription activator-like effector nucleases (TALENs), homing endonucleases and clustered regularly interspersed short palindromic repeats (CRISPR) / Cas9 have allowed multiplex editing of TCR genes in combination with CD52, the target antigen of alemtuzumab, as a strategy to evade lymphodepletion used to prevent host v graft rejection effects. Alternative approaches have targeted pathways to prevent HLA expression on donor T cells, and have also allowed targeted insertion of CAR genes, including placing transgene expression under the control of endogenous transcriptional machinery. These tools have rapidly progressed to clinical trials, and applications have extended beyond B-cell malignancies, showing promising early results in other settings, including relapsed/refractory(r/r) T-cell leukaemia. Short term immunological effects and toxicities have been generally manageable, and long-term monitoring is ongoing to help build confidence in safety over time.

1. Background

1.1. Genome editing

Advances in genome editing techniques have provided new possibilities for treating diseases not amenable to conventional therapies. The majority of nuclease-based editing techniques rely on the precise recognition of a specific DNA sequence, followed by the creation of targeted double-stranded break (DSB). Zinc finger nucleases (ZFN) [1] and TALENs [2] both employ protein recognition domains fused to Fok-1 nuclease and are highly efficient but their application has been constrained by limited targeting opportunities. Initial proof of concept clinical studies of TALEN mediated disruption in T cells targeted the T cell receptor alpha-chain constant (TRAC) gene to disrupt allo-reactivity from donor derived T cells modified with a lentiviral vector CAR to generate CAR19 T-cells against B-acute lymphoblastic leukaemia (B-ALL) [3]. Similar approaches using propriety homing endonucleases have also been deployed for the generation of other universal donor CAR T cells. Alternatively, CRISPR/Cas9 iterations use a PAM dependent, RNA-guided delivery system to direct Cas9 mediated DNA cleavage, and these platforms are highly adaptable and accessible, including for multiplexed effects at several loci, and site-specific gene insertion effects. Derivations include base editors with deactivated Cas9 fused to cytidine deaminase and a uracil glycosylase inhibitor that can introduce precise cytidine to thymidine (C- T) conversions without DNA breaks. They are already being used to create stop codons or disrupt splice donor/-acceptor sites for knockout effects without DNA breaks [4]. Alternative adenine deaminase base editors can introduce single A>G changes [5], and most recently, prime-editing has been described and employs a reverse transcriptase enzyme alongside prime editing guide (PEG) RNAs for larger edits without DSBs [6–8].

1.2. Allogeneic v Autologous cell therapy

Autologous CARs have been studied for more than a decade now and successfully translated into clinic with food and drug administration (FDA) approval of anti-CD19 CAR T cell products such as tisagenlecleucel and other autologous products [9–18]. However, these

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personalised therapies have inherent limitations such as the requirement of complex logistics, expertise and infrastructure, leading to high cost and low availability in resource limited settings. There is a risk of disease progression due to delays in manufacturing processes and there is a risk of production failure in patients after multiple lines of prior chemotherapy or haematopoietic stem cell transplantation (HSCT). Engineered allogeneic donor T-cells could mitigate these constraints and provide on-demand availability, at reduced cost, and without the risk of blast transduction (Fig. 1). Donor CAR T cell banks may also allow use of combination of CAR T cells directed against different targets to mitigate risks of target escape. However, there are notable challenges to overcome before these allo-T cells can be fully exploited.

2. HLA barriers

HLA mismatch between the host and recipient can result in recipient mediated graft rejection or donor mediated GVHD through recognition of non-self-antigens by the TCR/CD3 complex. The TCR complex consists of either an α- and β-chain or a γ- and δ-chain in association with four subunits of the CD3 complex. αβ T-cells are the predominant mediators of adaptive immunity, but alloreactive TCRs can also direct GVHD. Unlike in the autologous setting, HLA-mismatched donor T cells must evade host-mediated immunity and deliver antileukemic effects without causing GVHD. These barriers can be overcome by manipulating T-cells to prevent expression of TCR αβ and HLA molecules, and/or by rendering cells insensitive to lymphodepleting drugs such as alemtuzumab (anti-CD52 monoclonal antibody) (Fig. 2).

Gene editing in this context was first modeled in primary human T cells using ZFN to knock out TCR α chain constant (TRAC) or TCR β chain constant region 1 and 2(TRBC1/2) [1]. Stringent depletion can be achieved by removal of remaining αβ T cells by using commercially available magnetic beads with anti- αβ TCR antibodies, originally developed in the context of HSCT, and aiming for residual TCRαβ T cells $<5\times10^4$/kg [19]. Conditioning with fludarabine and cyclophosphamide is sufficient to provide homeostatic proliferative advantage to infused T cells [20,21], but in the allogeneic context more intense lymphodepletion is required. This might be achievable using higher doses of chemotherapy, but lessons from transplantation suggest a need for deeper and more prolonged lymphodepletion. One approach has been to disrupt CD52 expression on incoming T-cells rendering them resistant to anti CD52 monoclonal antibody (alemtuzumab) [2], and then incorporating alemtuzumab into the lymphodepletion regimen alongside fludarabine and cyclophosphamide. Another approach has modelled T cell resistance to fludarabine by disruption of deoxycytidine kinase to inhibit production of triphosphates responsible for cellular toxicity in preclinical experiments [22]. The consequences of using augmented lymphodepletion and more prolonged immunosuppression include protracted cytopenia and severe viral infections, which have to be managed and included in risk: benefit determinations.

A first in human approach for B-ALL disrupted both TRAC and CD52 using TALENs [3]. Two infants, who had relapsed after allo-SCT, achieved molecular remission within 28 days of infusion and exhibited sustained long-term remission after a second allo SCT (Table 1) [3]. Subsequent trials have examined the approach in both children and adults [23,24]. Interim data on 7 children and 14 adults reported grade 3–4 CRS in 3 patients, GVHD in 2 patients, and grade 4 cytopenia in 6 patients. There were 2 deaths reported, one due to neutropenic sepsis and the other due to pulmonary haemorrhage. Sixty-seven percent achieved either complete remission (CR) or complete remission with incomplete count recovery (CRi) at day 28 [23]. Completed data from the adult study on 25 patients reported an overall response rate of 48% at a median follow up of 12.8 months and a median progression free survival of 2.1 months. Grade 3 cytokine release syndrome (CRS) was observed in 24% patients, while grade 3 infections were seen in 28%. Out of the 14 deaths, 9 were due to progressive disease and 5 from infection and other causes [24]. A similar approach (TT52CAR19 study) using CRISPR-Cas9 guides linked to CAR19 expression and mRNA coding for Cas9 for multiplexed DNA editing of TRAC and CD52 genes has also been applied to children with relapsed refractory B-ALL [25]. Out of 6 patients, 4 achieved complete remission and proceeded to transplant.

Presence of pre-existing donor directed anti-HLA antibodies can result in rapid clearance of the infused product. Therefore, all trial patients were screened for pre-existing antibodies directed against the CAR T cell HLA type. Disrupting HLA molecules to evade host mediated rejection could offer a solution and provide alternatives to intense lymphodepletion currently needed in for allo-CAR cells. In this context, B2M gene disruption using CRISPR/Cas9 by CRISPR Therapeutics has been investigated for HLA class 1 disruption [30,33]. Issues such as 'missing-self' effects against HLA-I negative cells mediated by natural killer (NK) cells [34] may be relevant. Contingencies include the inclusion of non-polymorphic HLA-E or HLA-G molecules, to protect cells from NK -mediated cell lysis (Fig. 2) [35]. It also remains uncertain whether the removal of HLA class I alone is sufficient to prevent host-mediated rejection or if additional measures are needed to address HLA class II-mediated immunity. Activated T-cells express high levels of HLA class II and disruption of critical transcription regulators such as histocompatibility complex class II transactivator (CIITA) [36] and regulatory factor X complex (RFX) [37] has been developed.

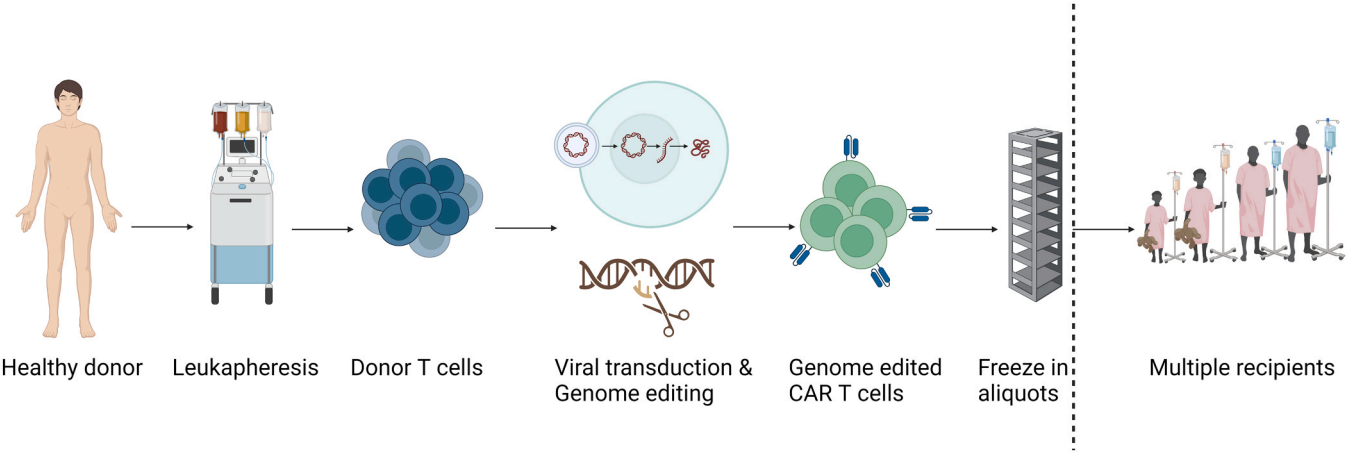

Fig. 1. Summary of procedures for manufacture of allogeneic CAR T cells. T-cells harvested from the healthy donor are used to generate CAR T cells by viral transduction and genome editing. These genome edited CAR T cells can be banked in aliquots as a 'ready to use' products in multiple recipients.

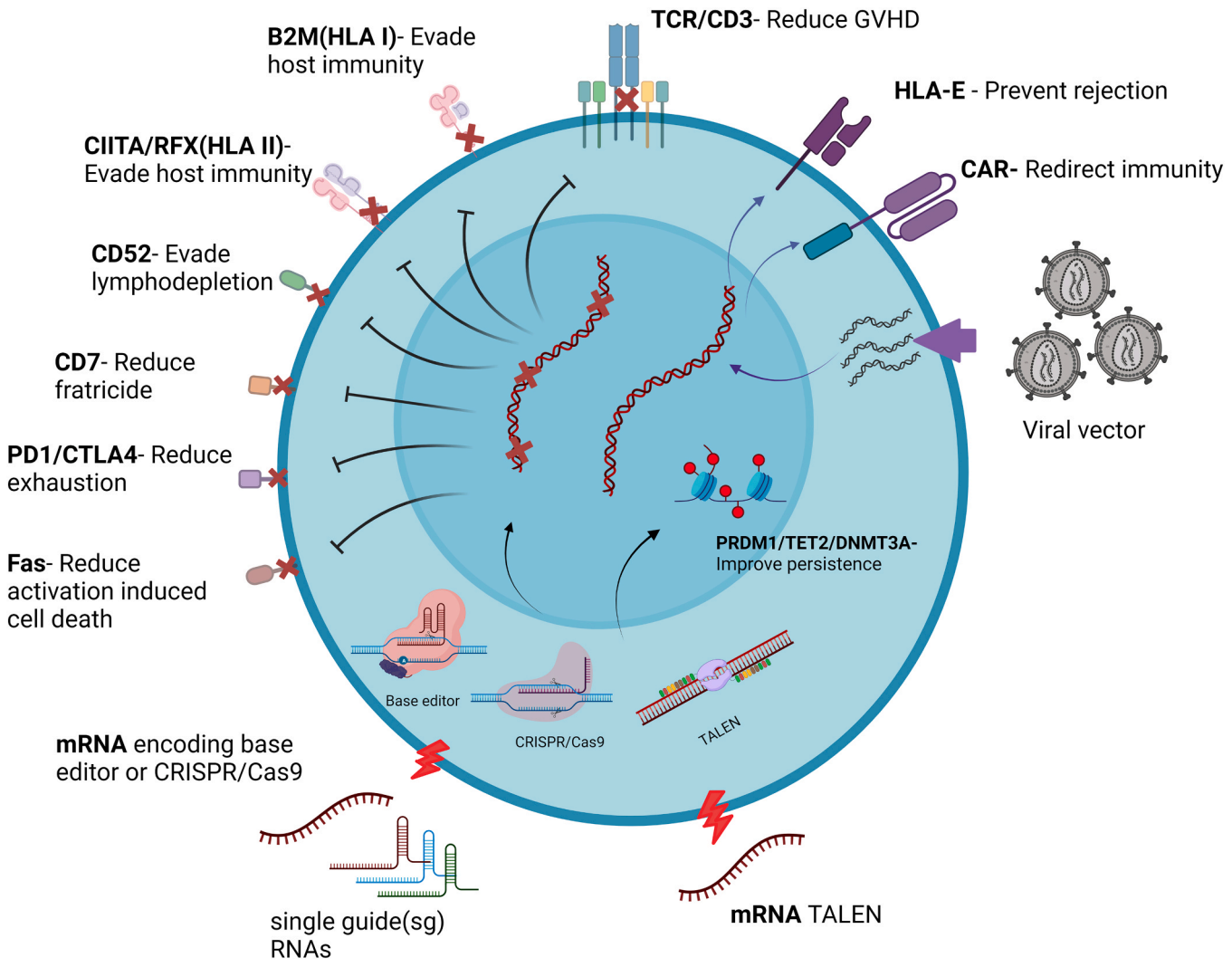

Fig. 2. Summary of opportunities for T-cell editing. Allogeneic CAR T cells can be generated after disruption of TCR/CD3 complex to circumvent GVHD, removal of HLA molecules to prevent host mediated rejection or disrupt CD52 to evade lymphodepletion with alemtuzumab. Additionally, genome editing can overcome fratricide by removal of shared antigens and help improve CAR T persistence and proliferation by disruption of checkpoint inhibitors, apoptosis genes or epigenetic modifiers. Addition of HLA-E may be useful in preventing NK cell mediated elimination of cells devoid of HLA class I.

3. Genome editing to improve persistence and activity

Most clinical CAR T trials have used gamma-retroviral or lentiviral mediated transduction of T-cells for the desired CAR expression. Although this is an efficient process, genome integration effects can include variegated transgene expression and may drive proliferation and promote persistence. A more physiological approach may be through site-specific transgene insertion of the CAR into loci offering dynamic transcriptional regulation through endogenous promoters and regulatory machinery. In this context, adeno-associated virus (AAV) has been used to deliver homology flanked CD19-specific CAR templates to the TRAC locus of genome edited T cells, with claims of more uniform expression in populations of CART cells [38]. These TRAC sited CAR T cells demonstrated delayed terminal differentiation and retained memory phenotype with reduced exhaustion phenotype and potential of enhanced therapeutic potency [38]. In a phase I study, CRISPR therapeutics is testing allo-CAR19 inserted at the TRAC locus using AAV and an additional B2M edit using CRISPR/Cas9 [30]. In preliminary results, out of the 32 patients with relapsed/refractory large B-cell lymphoma, complete remission was achieved in 5 patients at 6 months with 2 patients in CR for >2 years (Table 1). There was no GVHD, 2 patients had grade ≥3 ICANS (Immune effector cell-associated neurotoxicity syndrome), 4 had ≥3 infections including 1 who succumbed to HHV6 encephalitis [30]. Precision Biosciences have also reported allo-CAR19 in r/r B-cell malignancies where the CAR was sited into the TRAC locus after editing with a TRAC-specific ARCUS nuclease (derived from the nuclease I-CreI). Of a cohort of 18 patients who received enhanced lymphodepletion and were evaluable, 8 achieved a CR which was sustained for >6 months in 3 patients with 1 patient receiving consolidative transplant. Except for one death due to infection, it was generally well tolerated [29].

Alternatives to AAV mediated CAR delivery using non-viral techniques for site-specific insertion of transgenes are also in development [39–41]. There are several challenges for this approach including the concentration of high quality double stranded DNA required. Preclinical studies have showed that co-electroporation of CRISPR/Cas9 RNP with long linear homology directed repair (HDR) DNA templates has reduced toxicity usually associated with dsDNA delivery [42]. Several strategies are currently under investigation to promote HDR and to select HDR compared to non-homologous end joining (NHEJ) pathways of repair [43].

Tonic antigenic exposure promotes CAR T cell exhaustion and cell death with consequent poor disease control. Although still being elucidated in the context of CAR T cells, negative T cell regulators like PD-1,

Table 1
Trials of genome edited T cells against B-cell malignancies with published data.

Sponsor/study	Indication	Product	Edits	Patient (n)	Safety	Efficacy
Great Ormond Street Hospital Special's License	B-ALL	UCART19	- LV CAR19 - TALEN ko of TRAC & CD52	2	Grade 2 GVHD 50%	CR 100%[3]
Allogene NCT04416984 NCT03939026	LBCL	ALLO-501A ALLO-501	- LV CAR19 - TALEN ko of TRAC & CD52	47	Grade ≥3 CRS 2%; Grade ≥3 infections 24%	CR 50%[26]
Allogene NCT04093596	MM	Allo-715	- LV anti BCMA - TALEN ko of TRAC & CD52	21(DL3)	Grade ≥3 CRS 2% Grade ≥3 infections 29%	ORR 64%(FCA39)&80% (FCA60) [27]
Cellectis NCT04150497	B-ALL	UCART22	- LV CAR22 - TALEN ko of TRAC & CD52	9	No Grade 3 CRS or infection	No CR[28]
Precision Bio NCT03666000	B-ALL/ NHL	PBCAR0191	- AAV site specific inserted CAR19 Arcus ko of TRAC	21	Grade ≥3 CRS 6%; Grade ≥3 infections 31%−80%	CR/CRi 62% 80%[29]
CRISPR Tx NCT04035434	LBCL	CTX110	- AAV site specific inserted CAR19 - CRISPR/Cas9 TRAC & B2m	32	Grade ≥3 infections 12.5%; Grade ≥3 ICANS 14.3%	CR 34.4%[30]
Great Ormond Street Hospital NCT04557436	B-ALL	TT52CAR19	CRISPR/Cas9 TRAC, CD52 LV CAR19	6	Grade ≥3 ICANS 17%	CR/CRi66% [25]
Servier/Allogene NCT02746952	B-ALL	UCART19	- LV CAR19 - TALEN ko of TRAC & CD52	25	Grade ≥3 CRS 24%; Grade ≥3 ICANS 4%; Grade ≥3 infections 28%	CR/CRi 48%[24]
Zhejiang University Nanjing Bioheng Biotech	B-ALL	CTA 101	CRISPR/Cas9 ko of TRAC, CD52 LV CAR19/22	6	Grade ≥3 CRS 16%; Grade ≥3 infections 50%	CR/CRi83%[31]
Zhejiang University Bioray Laboratories NCT04213469	B-NHL	PD1–19bbz	Virus free CRISPR/Cas9 CD19 integration to PD1 locus	21	No Grade 3 CRS or infection	CR 85.7%[32]

Ko, Knockout; MM, Multiple myeloma; BCMA, B-cell maturation antigen; DL, Dose level; ORR, Overall response rate; FCA 39/60, Fludarabine, Cyclophosphamide, ALLO 647(39 mg or 60 mg); B-NHL, B non-Hodgkin lymphoma.

CTLA-4 and LAG-3 might have a role as evidenced by enhanced CAR T functions with PD-1 inhibitors [44]. Examples include multiplexed gene editing of TCR, B2M and PD1 using CRISPR/Cas9 to generate CAR against prostate stem cell antigen (PSCA) which showed enhanced antitumor effects in a preclinical model [45]. Similarly, epigenetic factors which may be altered in exhausted T-cells have been manipulated. Genome editing to disrupt PRDM1 [46], TET2 [47] and DNMT3A [48] have helped maintain early memory phenotype and improve persistence.

4. Genome editing of T cells to overcome targeting of shared antigens

B-cells generally exhibit expression of CD19 in both normal and blast populations, but after CAR therapy, there are effective strategies to address B-cell deficiency. Application of a similar approach against T cell cancers is more challenging. If antigens expressed on leukemic T cells are shared with normal T-cell populations, CAR transduction of the T cell against these antigens could lead to self-targeting/fratricide. Genome editing can be utilized to circumvent this issue by disrupting shared antigens before CAR transduction of the T-cell. Targets have included TCR/CD3 complex, CD1a, CD5 and CD7. TALEN mediated disruption of TRAC has been used to disrupt CD3 complex before viral transduction of T-cells with an anti-CD3 CAR [49]. Fratricide has been shown to be more limited in case of anti-CD5 CAR [50] probably due to activation induced internalization of the CD5 receptor [51]. However, CD5 is only expressed in about 80% of T-ALL cases [52,53] and is crucial for T-cell activation and differentiation [54]. CD7 is an attractive target as it is expressed at high levels in over 95% of T-ALL cases including early T-cell precursor (ETP)-ALL (CD7 is one of the earliest markers for T-cell development) [53] and its loss does not appear to affect T-cell maturation and function [55,56]. Successful preclinical modelling of CAR7 T cells was first shown with protein expression restriction using endoplasmic reticulum/golgi retention signal sequences [57,58] and then with CRISPR/Cas9 editing of CD7, alone or with TRAC [59]. Multiplexed editing of TRBC and CD7 by cytidine deaminase by using base editing has also been achieved [60]., including versions with PD1 and/or CD52 disruption [61]. This has led to a phase 1 clinical trial of the base edited CAR7 trial in children [60]. Activated T-cells from a healthy donor were electroporated with cytosine deamination base editor mRNA and sgRNAs targeting TRBC, CD52, and CD7. The BE3 mRNA coded for a catalytically impaired cas9 nickase fused to a rat-derived single-stranded DNA aminase and uracil glycosylase to induce conversion of C to U at single base pair region within a 5-bp window. The targeted conversion gave rise to premature stop codon and/or disrupt splice sites resulting in highly specific knock out effects. The cells were then transduced with CAR7 using a 3rd generation lentivirus. There were no translocations identified using digital droplet PCR and karyotyping of the BE-CAR7 revealed normal results. Encouraging experience on the first 3 patients treated with base edited CD7 CAR T as part of a phase 1 clinical trial have been reported(Table 2) [60].

Another trial [63] has reported allogeneic gene edited CAR7 T cells in 12 cases of CD7 positive hematological malignancies (11 T-lymphoblastic leukaemia/ lymphoma + 1 acute myeloid leukaemia) [63]. In addition to CRISPR/Cas9 mediated knock out of CD7, TCR/CD3 and RFX5 genes, a NK cell inhibitory receptor was generated with EC1-EC2 extracellular and transmembrane domains of E-cadherin (Ecad) fused to a CD28 intracellular domain in order to inhibit NK mediated missing self-recognition. Out of 11 evaluable patients, objective responses were observed in 9 and complete responses in 7 patients. Three patients were bridged to transplant and at 10.5 months, 4 patients remained in complete remission [63]. Additionally, CTX130, a CD70-targeting allogeneic CAR T cells is being evaluated for safety and efficacy as a multicentric study for adults with-cell lymphoma [33]. These cells were modified using CRISPR/Cas9 to disrupt TRAC, B2M and CD70. Interim data on 15 patients reported 29% complete remission with acceptable safety profile [33].

5. Safety and long-term monitoring

5.1. Risks associated from T cell effector activity

Complications related to the antigen receptor binding by CAR T cells may be due to on-target off tumor effects or off-target effects. To date,

Table 2
Trials of genome-edited T cells against T-cell malignancies with published data.

Sponsor/study	Indication	Product	Edits	Patient (n)	Safety	Efficacy
Great Ormond Street Hospital NCT05397184	T-ALL	BE-CAR7	- LV CAR7 - BE TRBC ½, CD7, CD52	3	Grade ≥3 CRS 33% Grade ≥3 infections 66%	CR/CRi 100% [60]
Beijing Lu Daopei Institute of Hematology NCT04620655	T-ALL/TLBL	CD7 UCAR-T cells	- RD13–01 CAR7, NKi ligand - CRISPR/Cas9 CD7, TRAC, RFX5	10	Death due to bacterial infection 1 Grade ≥3 CRS 10% Grade ≥3 ICANS 10%	CR 80%[62]
Zhejiang University School of Medicine NCT04538599	T-ALL/TCL/AML (CD7+)	RD13–01	- RD13–01 RV CAR7, NKi ligand - CRISPR/Cas9 TCR/CD3, CD7, RFX5	12	Death due to EBV related DLBCL- 1	CR 64%[63]
Gracell ChiCTR1900025311	T-ALL/T-LBL	GC027	- LV CAR7 - CRISPR TRAC CD7	12	Death due to pneumonia- 1 Grade ≥3 CRS 67%	CR 92%[64]
CRISPR Tx NCT04502446	TCL	CTX130	- CD70 AAV-site specific - CRISPR/Cas9 TRAC, B2m	15	Grade ≥3 infections 7%	CR 29%[33]

LBL, Lymphoblastic lymphoma; NKi, NK cell inhibitor; TCL, T-cell lymphoma; DLBCL, Diffuse large B cells lymphoma; AML, Acute myeloid leukaemia.

CRS and ICANS from allo T cells have been comparable to autologous CART products [24]. GVHD experience of alloreactivity in clinical trials has been modest and effects readily manageable [25,65]. Additionally, CAR effects on the bystander cells especially marrow precursors may be linked to cytopenia, including prolonged neutropenia and lymphopenia, which are mitigated by donor derived reconstitution after allo-SCT.

5.2. Risk associated with viral vectors

Risk of genotoxicity and transformation from integrating vector is lower in mature T-cells compared to the haematopoietic stem cells [66, 67]. However, recently alerts were issued [68] in relation to T-cell malignancies after autologous CAR T cell therapy targeting BCMA (B cell maturation antigen) or CD19 [69–71]. Currently the frequency of such events appear to be low, with around 22 cases documented following approximately 34,400 infusions [72]. T cell lymphoma has ben reported in an autologous CAR19 trial where piggyBac transposons were used for gene transfer [73]. Although comprehensive investigations into the underlying mechanisms of these lymphomas did not identify a common underlying mechanism, analysis of the first patient revealed a high number of integration sites but no insertion into the known oncogenes. The malignant cells exhibited global changes in gene expression primarily related to significantly increased copy number variations. These reports highlight the importance of long-term monitoring of patients after gene modified cell therapies.

5.3. Risk associated with genome editing

Following multiplexed editing, karyotype changes were found in around 2–5% of cells after genome editing with TALENs [2,3]. Similarly, application of CRISPR/Cas9 to remove TCR and programmed death-1 (PD-1) in autologous T cells resulted in detectable translocation between edited sites [74] and additional investigations reported frequent aneuploidy and effects such as truncation of chromosomes [75]. Adverse clinical effects have not been attributed to such effects, although one subject exhibited inversion of chromosome 14 and clonal proliferation of these allogeneic CART cells leading to temporary halt in the ALLO-501A trial although subsequent investigation suggested a process related to RAG mediated recombination rather than direct TALEN effects [76]. As base editing uses site specific single base pair conversion without introducing a DSB, it should attenuate the resultant chromosomal breaks and translocations. Predicted translocation frequency after base editing has been very low or undetectable compared to CRISPR modified T-cells [77,78]. but long-term monitoring will help detect unexpected effects.

6. Summary

Genome editing offers the possibility of improved CAR T cell therapies with a wider range of applications. Improved site-specific gene insertion, disruption of checkpoint inhibitors or epigenetic modifiers is being investigated to improve function and persistence. Encouraging outcomes from trials of 'ready to use' universal donor products' in difficult to treat relapsed leukemia have been reported and with further advancements, broader utilization earlier in treatment protocols can be anticipated.

Authorship

AKM and W.Q. wrote the manuscript.

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