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BANKED ALLOGENEIC IMMUNE EFFECTOR CELLS

Genome-edited allogeneic donor "universal" chimeric antigen receptor T cells

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αβ T cell receptor (TCRαβ) T cells modified to express chimeric antigen receptors (CAR), are now available as authorized therapies for certain B-cell malignancies. However the process of autologous harvest and generation of patient-specific products is costly, with complex logistics and infrastructure requirements. Premanufactured banks of allogeneic donor–derived CAR T cells could help widen applicability if the challenges of HLA-mismatched T-cell therapy can be addressed. Genome editing is being applied to overcome allogeneic barriers, most notably, by disrupting TCRαβ to prevent graft-versus-host disease, and multiple competing editing technologies, including CRISPR/Cas9 and base editing, have reached clinical phase testing. Improvements in accuracy and efficiency have unlocked applications for a wider range of blood malignancies, with multiplexed editing incorporated to target HLA molecules, shared antigens and checkpoint pathways. Clinical trials will help establish safety profiles and determine the durability of responses as well as the role of consolidation with allogeneic transplantation.

Introduction

Autologous gene–modified T cells, including chimeric antigen receptor (CAR) T cells have been widely investigated through clinical trials, leading to approvals from the US Food and Drug Administration for new cell-based therapies against hematological malignancies. Anti-CD19 CAR T-cell therapy, tisagenlecleucel, was approved for refractory or relapsed (R/R) B-cell acute lymphoblastic leukemia (B-ALL) and non-Hodgkin lymphoma (NHL)[1–4] and axicabtagene ciloleucel for B-cell follicular lymphoma and NHL.[5–7] Brexucabtagene autoleucel has been authorized for B-ALL and mantle cell lymphoma,[8,9] and lisocabtagene maraleucel for B-cell NHL.[10] For multiple myeloma, idecabtagene vicleucel and ciltacabtagene autoleucelare are available against R/R multiple myeloma.[11–14] A variety of additional autologous therapies are under commercial development or are being provided by academic centers.[15,16] However, the infrastructure, logistics, and expertise required to generate these bespoke cell therapies are costly and difficult to replicate, especially in resource-scarce settings. Wider applications and routes to broader access at a reduced cost are under investigation through genome engineering of healthy donor-derived T cells, using genetic modification to overcome barriers associated with HLA-mismatched cell therapies. Ultimate ambitions aim to establish readymade universal cell banks for "off-the-shelf" affordable and accessible cell therapy without the risk of manufacturing delays, product failures,[17] or accidental transduction of blasts.[18] Premanufactured CAR T cell banks should also enable optimally timed therapeutic interventions earlier in the course of disease evolution as an alternative to intense chemotherapy. Critical issues to address in the allogeneic non-matched setting include the risk of graft-versus-host disease (GVHD) from the donor-derived T cells and host-mediated rejection from innate and adaptive humoral or cellular immune compartments. Third party, donor-derived, virus-specific T cells infused after allogeneic stem cell transplantation (SCT) can deliver immunity without significant GVHD,[19] and such populations have been used as a starting material for CAR therapy.[20,21] Alternatively, endogenous T cell receptor (TCR) expression can be disrupted by using antibody-derived protein expression blockers[22] RNA interference,[23] or genome editing. The latter, in combination with immunosuppressive conditioning of the host, has perhaps the greatest potential to provide strategic solutions for further improvements in allogeneic CAR T-cell therapies.

Genome editing technologies

Protein-based DNA-recognition domains fused to nuclease enzymes, including zinc finger nucleases, homing endonucleases, and transcription activator-like effector nucleases (TALENs) have been investigated for the engineering of allogeneic CAR T cells for more than a decade (Figure 1). These platforms were constrained by limited targeting opportunities, although were suitable for disruption of TCR-related genes such as the TCR α-chain constant (TRAC) domain gene for initial proof of concept and modeling studies of CAR19-engineered T cells[24,25] and T cells modified to express recombinant TCRs.[26] Preclinical studies using TALENs for the multiplexed editing of CAR19 T cells demonstrated how messenger RNA (mRNA)–based delivery of the editors could be combined with the lentiviral expression of a CAR to generate allogeneic CAR19 T cells devoid of endogenous TCR.[27] Subsequent variations included homing endonuclease–edited iterations with a site-specific insertion of a CAR transgene at the TRAC locus.[28]

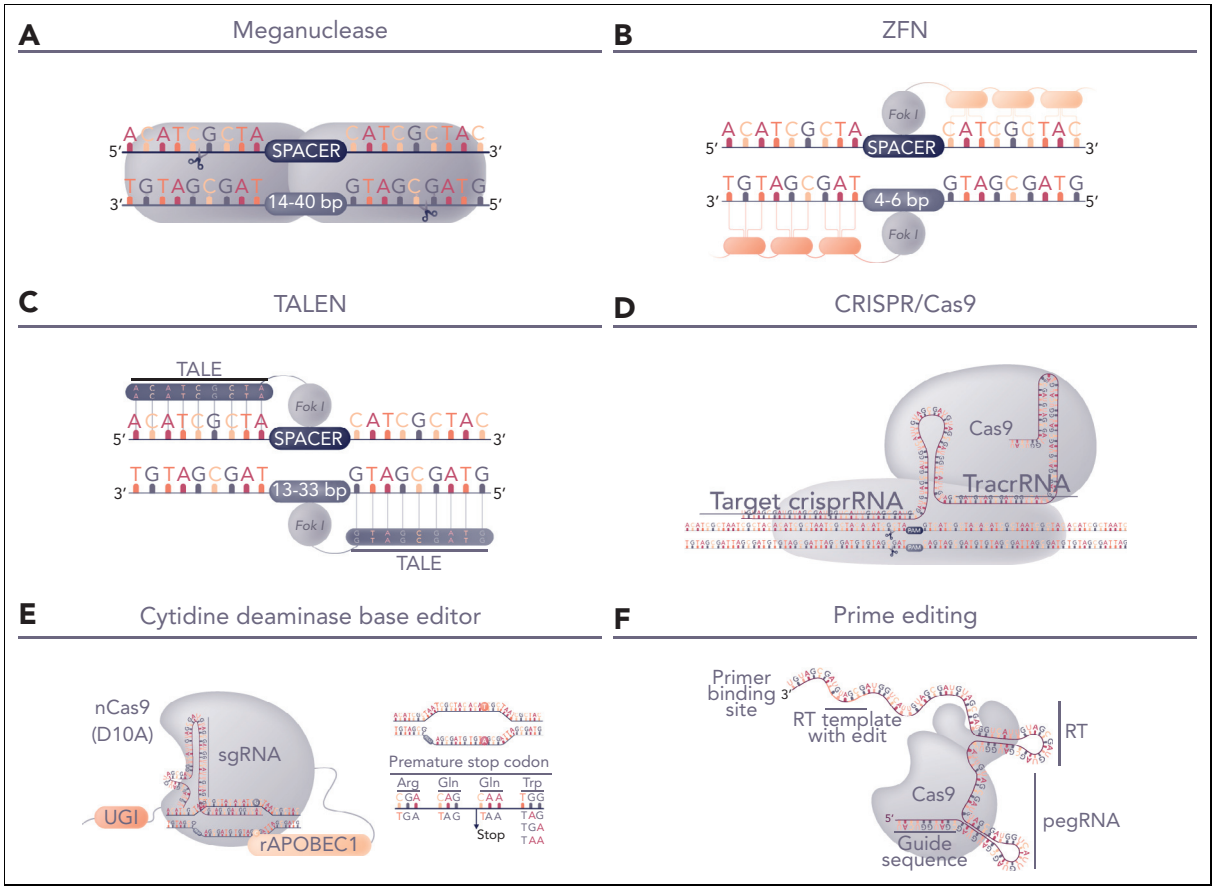

Figure 1. Genome-editing platforms for T-cell modification. (A-D) Editing tools for highly specific dsDNA cleavage comprise either protein-based DNA recognition molecules including (A) homing endonucleases (meganuclease), (B) zinc finger nucleases, (C) TALENs, (D) RNA-guided nucleases exemplified by CRISPR/Cas9. After DNA cleavage, repair by nonhomologous end joining offers the prospect of gene-knockout with indel signatures and alternative homologous repair based repair, which can be exploited for site-specific transgene insertion. The latter requires delivery of template DNA flanked by homology arms and allows placement of CAR genes under the transcriptional control of endogenous transcriptional machinery. Alternatively, (E) base editors use nickase-restricted Cas9 variants, fused to cytidine deaminase or adenosine deaminase for highly targeted C>T or A>G base conversion. Cytidine base editor (CBE) has been used for multiplexed gene knockout, avoiding translocations usually encountered following nuclease activity. (F) Emerging prime editing offers the prospect of gene editing through localized template repair by fusing reverse transcriptase to deactivated Cas9. dsDNA, double-stranded DNA.

Early hurdles of efficiency and reagent-mediated toxicity were addressed and translational clinical applications of TALENs were first investigated in patients with B-ALL (Table 1).[29] Subsequently, the development of RNA-guided systems based on clustered regularly interspaced short palindromic repeats (CRISPR)/Cas9 for cell engineering has provided an ever larger toolbox[30–33] for laboratory and preclinical T-cell engineering studies.[34–37] Alternatives to precise DNA cleavage and gene insertion by CRISPR/Cas9 include disruption through targeted base conversion using CRISPR-guided cytidine deamination[38] to introduce premature stop codons[39] or carefully targeted adenosine deamination[40] to modify critical splice sites. Most recently, prime editing has been added to the toolbox and incorporates an impaired Cas9 fused to murine reverse transcriptase and a prime-editing guide RNA for localized insertion, deletion, or base conversion without dsDNA breaks.[41] Although iterations of this platform are still evolving,[42,43] translational applications of other tools have been advanced to clinical phase applications and human studies, and T-cell therapies have offered a well-circumscribed arena for investigation, with the safeguards of ex vivo engineering and characterization as well as clear therapeutic objectives and defined safety readouts.

Genome editing to prevent alloreactivity

GVHD is mediated by antigen-specific αβ T-cell receptors (TCRαβs), and cell surface expression can be disrupted at the genomic level by targeting genes associated with the expression of the multimeric TCRαβ/CD3 complex (Figure 2). Notably, the TRAC locus has been targeted, and with the gene exhibiting allelic exclusion, disruption of the active allele is sufficient to efficiently prevent TCRαβ expression. Alternatively, editing the T cell receptor β chain constant (TRBC1/2) locus can result in the disruption of the β chain, and in either case, residual TCRαβ cells can be stringently depleted using commercially available magnetic bead depletion systems, resulting in T-cell products with redirected specificity through CAR (or recombinant TCR expression) and a greatly reduced alloreactivity. Similar TCRαβ disruption has also been reported using RNA interference,[23,44] and by the restriction of components of the multimeric receptor complex at the protein level,[22] as alternatives to genome editing. Efficient and stable disruption, combined with stringent removal of residual TCRαβ cells, has proven to be a successful mitigation against GVHD, and in human studies, (Table 1) the adoption of dose limits extrapolated from haploidentical allo-SCT for TCRαβ carriage of $<5 \times 10^4$/kg has proven to be robust.[45,46] The additional issue of

Table 1. Trials of genome-edited T cells with published or interim clinical data available

Study	Investigational product	Modifications	Indication	Lymphodepletion	Number	Toxicity	Outcomes
Great Ormond Street Hospital Special's License	UCART19	LV-CAR19 TALEN ko of TRAC & CD52	B-ALL	F,C, AntiCD52	2	Grade2 GVHD	CR 100%[29]
Servier/Allogene NCT02808442 NCT02746952	UCART19	LV-CAR19 TALEN ko of TRAC & CD52	B-ALL	F,C, ± AntiCD52	21	Grade 3+ CRS 15%; Grade 3+ infections 39%	CR 67%[68]
Allogene NCT04416984 NCT03939026	ALLO-501A ALLO-501	LV-CAR19 TALEN ko of TRAC & CD52	LBCL	F,C, AntiCD52	47	Grade 3+ CRS: 2%; Grade 3+ infections 24%	CR 50%[113]
Allogene NCT04093596	Allo-715	LV-anti BCMA TALEN ko of TRAC & CD52	MM	F,C, AntiCD52	26 (DL3,4)	Grade 3 infections 13%	ORR 61%[114]
Cellectis NCT04150497	UCART22	LV-CAR22 TALEN ko of TRAC & CD52	B-ALL	F,C, ± AntiCD52	9	No Grade 3 CRS or infection	No CR[115]
Precision Bio NCT03666000	PBCAR0191	Arcus ko TRAC AAV site specific inserted CAR19	B-ALL NHL	F, C	27	Grade 3+ CRS 6%; Grade 3+ infections 31%-80%	CR/CRi 62%-80%[72,73]
CRISPR Tx NCT04035434	CTX110	CRISPR/Cas9 TRAC & B2m AAV site specific inserted CAR19	LBCL	F,C	24 DL2+	Grade 3+ infections 9%; Grade 3+ ICANS 4%	CR 38%[74]
Great Ormond Street Hospital NCT04557436	TT52CAR19	CRISPR/Cas9 TRAC, CD52 LV CAR19	B-ALL	F,C, AntiCD52	6	NCT04557436, TT52CAR19' Grade 3+ ICANS 17%	CR/CRi 66%[70]
Beijing Chinese PLA General Hospital NCT03166878	U-Car	CRISPR/Cas9 TRAC, B2m LV CAR19	DLBCL	F,C	2		No CR[71]
Zhejiang University Nanjing Bioheng Biotech	CTA 101	CRISPR/Cas9 TRAC,CD52 LV CAR19/22	B-ALL	F,C, AntiCD52	6	Grade 3 CRS 16% Grade 3 infections 50%	CR/CRi 83%[116]
Gracell ChiCTR1900025311	GC027	CRISPR TRAC CD7 LV-CAR7	T-ALL	F,C ±Mel	6	Grade 3 CRS 100% Grade 3 infections 50%	CR/CRi 83%[84]
CRISPR Tx NCT04502446	CTX130	CRISPR/Cas9 TRAC, B2m, CD70 AAV-site specific	TCL	F,C	15	Grade 3+ infections 7%;	CR 29%[75]

C, cyclophosphamide; DLBCL, diffuse large B-cell lymphoma; F, fludarabine; ICANS, immune cell associated neurotoxicity syndrome; LV, lentivirus; Mel, melphalan; TCL, T-cell lymphoma; ko, knockout.

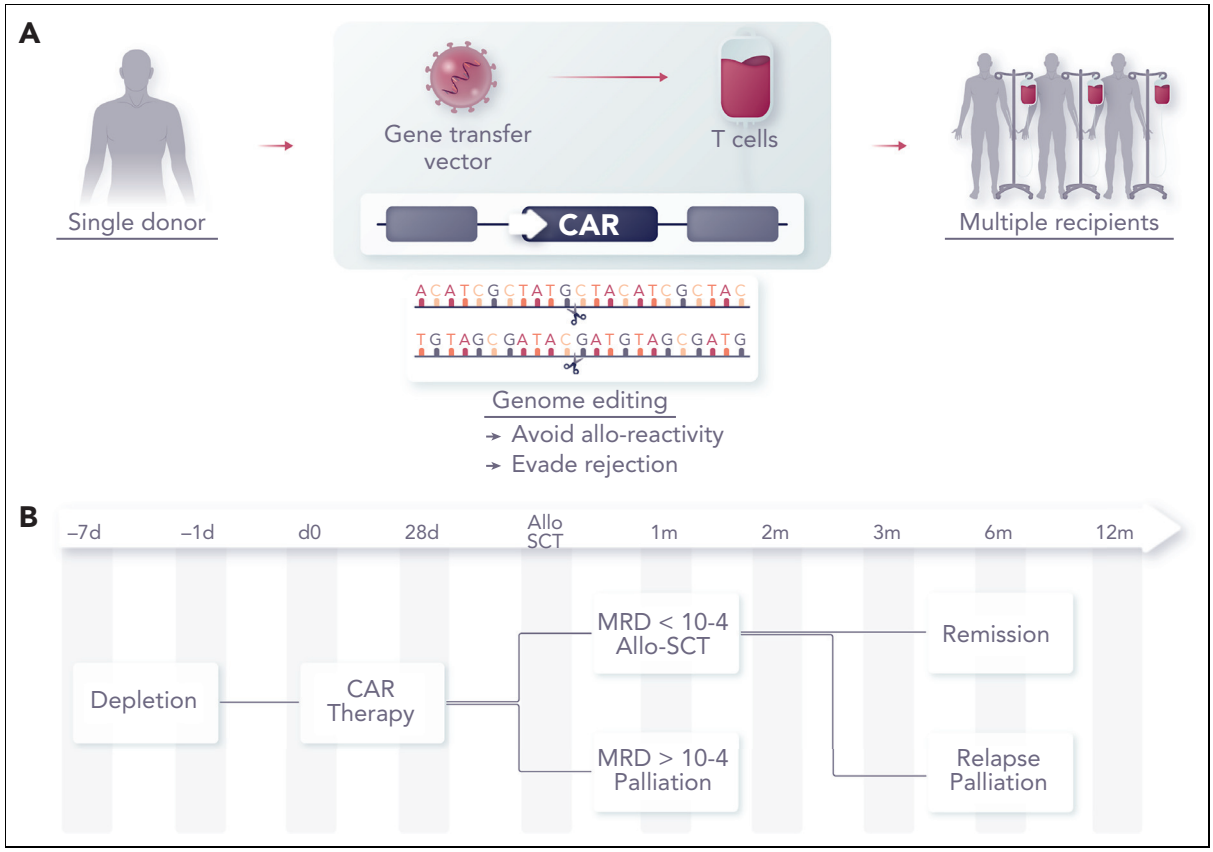

Figure 2. Concept of banked universal CAR T cells. (A) CAR T cells can be generated from healthy allogeneic donors for use in multiple recipients after genome editing to remove endogenous TCRαβ to prevent GVHD, disruption of HLA to reduce rejection or removal of CD52 to allow cells to persist in the presence of alemtuzumab, a serotherapy used as a part of augmented lymphodepletion. (B) Therapeutic effects sufficient to induce molecular remission are achievable within a period of 2 to 4 weeks, offering a bridge of consolidation with allogeneic SCT.

how long allogeneic cells persist before host-mediated rejection or clearance with chemotherapy ahead of allo-SCT is also a relevant consideration, with complications akin to transfusion-associated GVHD being possible if residual TCRαβ T cells expand and mediate uninhibited multiorgan alloreactivity.[47]

Editing to evade host-mediated rejection

Donor-derived allogeneic T cells are susceptible to host-mediated rejection after immune recognition, particularly in the context of a mismatched HLA. Preexisting anti-HLA antibodies could meditate rapid clearance of mismatched donor cells, and thus, screening and exclusion of previously transplanted or heavily transfused patients with anti-HLA antibodies is warranted. Alternative banks, with different HLA typing, may allow the circumvention of specific anti-HLA antibodies directed against a particular cell batch. Addressing cell-mediated responses by the host T and natural killer cells is generally managed by the administration of preparative conditioning chemotherapy ahead of cell infusion. In the autologous setting, host lymphodepletion using combinations of fludarabine and cyclophosphamide is thought to promote the expansion of infused CAR T cells through reduced homeostatic competition.[48,49] More intense dosing regimens in the allogeneic setting aim to subdue host cellular responses against mismatched allogeneic T cells as well as provide a homeostatic advantage. However, greater lymphodepletion has increased the risk of complications associated with T-cell immunodeficiency and may extend to wider and more protracted cytopenia. Another strategy has been to include serotherapy based on an anti-CD52 conditioning antibody such as alemtuzumab, which is licensed for immunomodulation in multiple sclerosis[50] and used widely in allo-SCT. In this context, the genetic disruption of CD52 as well as TRAC, allows allogeneic T cells to evade serotherapy effects while the host compartment has depleted.[27] Conventionally, effects of alemtuzumab are generally borne for a period of 3 to 4 weeks, the period during which reactivation of viruses is common and can be problematic in the context of prolonged T- and B-cell immunodeficiency.[51] Alternative serotherapy-based strategies could employ anti-CD3 monoclonal antibodies for energizing or depleting host CD3+ T cells while exploiting resistance of infused cells devoid of TCRαβ/CD3 on their surface.[52] In addition, resistance to the lymphodepleting chemotherapy agent, fludarabine, through the disruption of deoxycytidine kinase in CAR T cells has also been investigated in preclinical studies,[53] although it has not been assessed in clinical testing phases. There may also be applications of cellular therapies to counter host-mediated responses, for example, by using T cells expressing alloimmune defense receptors[54] or signaling chimeric HLA molecules[55] to target host alloreactive cells or more broadly by using CAR T cells through augmented lymphodepletion; a study investigating tandem anti-CD7 and CAR19 is currently underway.[56]

Another approach involves the removal of HLA molecules from the infused cells to promote immunological stealth, and

achieving the disruption of HLA class I in T cells in models has been relatively straightforward through the targeting of the β_2 microglobulin (B2m) gene locus.[57] Whether the disruption of interactions between host CD8 T cells and donor HLA class I is sufficient to prevent rejection is under investigation in several studies (Table 1). It may be necessary to simultaneously prevent interactions between host CD4 T cells and HLA class II on the surface of activated incoming CAR T cells, and editing of HLA class II-associated transcription factors has also been modeled.[58] There is also the possibility of responses mediated by natural killer cells against donor T cells lacking HLA expression as part of the missing-self immunity, and there are strategies proposed to mitigate against this through the expression of nonpolymorphic HLA molecules[59] or by selectively retaining more conserved HLA molecules.[60] Finally, risks associated with the evasion of immune surveillance by HLA-depleted CAR T cells, for instance, after the infection with T cell trophic viruses, have to be considered and factored into study designs, as done for other hypoimmunogenic cell therapies in development.[61]

Advanced editing for site-specific transgene insertion

Targeted CAR transgene integration might ameliorate vector-mediated variegation effects (discussed below) and may promote more physiologically regulated cell responses.[62] In the context of engineering T cells with recombinant TCRαβ, orthotopically sited TCRα and TCRβ chains supported TCRαβ down-regulation after antigenic stimulus, in a fashion similar to normal physiological responses mediated by T cells.[63] Such control may be critical in preventing activation-induced cell death or exhaustion-related dysfunction. With respect to CAR transgenes, effective physiological control may have been ceded, given the inclusion of potent costimulatory domains, but animal studies have suggested that the CAR gene insertion at the TRAC locus under the control of endogenous TCR transcriptional machinery can provide improved cytotoxic activity and, in carefully calibrated models, may support reduced exhaustion.[62] Non-integrating Adeno-associated viruses (AAV) were used to deliver homology-flanked templates for CAR insertion in such models, but advances in non-viral engineering processes have allowed CRISPR/Cas9 ribonucleoprotein (RNP)–mediated delivery of recombinant template DNA for similarly efficient homology-directed site-specific recombinant TCR[63,64] or CAR integration.[65,66] (Figure 3) Direct comparisons in human trials will be required to determine how targeted CAR integration and expression under the transcriptional control of endogenous promoters compares with conventional vector-mediated (non-targeted) transduction, and whether the inclusion of strong heterologous promoter elements influence CAR T-cell expansion, replicative capacity, and anti-tumor activity.

Clinical trials of genome-edited CAR T cells

Formal trials of universal CAR19 (UCAR19) T cells in children (PALL study) and adults (CALM study) followed successful therapy in 2 infants with R/R B-ALL who had relapsed after a first allo-SCT.[29] The prognosis for such children is known to be poor, with around 15% event–free survival in European cohorts of children who relapse after allo-SCT in B-ALL,[67] and in both cases, the manufacture of autologous CAR19 T cells had not been possible. Both infants were conditioned with a combination of fludarabine, cyclophosphamide, and alemtuzumab and received a single dose of UCAR19 T cells. Bone marrow examination after 28 days revealed molecular remission on performing flow cytometry and molecular analysis. Interestingly, there was no notable CRS or ICANS, although the first infant developed skin GVHD over the coming weeks and required systemic steroid therapy. Subsequent second transplants after reduced-intensity conditioning using their original stem cell donors consolidated full donor chimerism, and both patients enjoyed complete immune recovery, including restoration of B-cell compartments and humoral immunity. Thus, time-limited–anti-leukemia effects of allogeneic CAR19 T cells had been sufficient to ensure remission, with deletion and non-persistence of infused cells after the conditioning for transplant was verified. Subsequent phase 1 trials were sponsored by Servier & Allogene, with trial sites in Europe and North America, whereby the pediatric study was structured as a bridge to allo-SCT, whereas the CALM study included optional allo-SCT. Interim analysis in 2020 reported that 7 children and 14 adults had been treated, with supportive data on safety and efficacy effects. CRS was common (91%), although more severe grade 3 to 4 CRS was reported in only 3 patients. GVHD only occurred in 2 patients, but 6 patients had grade 4 cytopenia. Two treatment-related deaths occurred; 1 caused by neutropenic sepsis in a patient with concurrent CRS and 1 by pulmonary hemorrhage in a patient with persistent cytopenia. Complete remission (CR) or CR with incomplete count recovery (CRi) by day 28 was 67% and the effects were poor in 4 subjects who omitted alemtuzumab.[58,68]

Subsequently, CRISPR/Cas9 technology was incorporated into a lentiviral configuration for the coupled expression of multiplexed single guide RNAs, and CAR19. Placement of specific guide RNAs under the control of polymerase III promoters into the vector 3′ long terminal repeat (LTR) sidestepped the need to manufacture guide RNA, and one round of lentiviral exposure ahead of Cas9 mRNA delivery by electroporation and magnetic column processing yielded CAR+TCR-CD52-CAR T cells.[37] Cells were screened for karyotype aberrations and possible translocations between edited sites as well as guide-dependent off-target effects preidentified by digenome sequencing.[69] Children in the United Kingdom without autologous CAR options were treated and followed a similar treatment protocol with fludarabine, cyclophosphamide, and alemtuzumab lymphodepletion after a single dose of cells. Four of the first 6 treated patients achieved remissions by day 28 and proceeded to allo-SCT. Two children later relapsed and 2 were in ongoing remission.[70] An expected consequence of using intensified lymphodepletion, including alemtuzumab, was the frequency of viral reactivations and the depth of cytopenia in the first 3 to 4 weeks after therapy, but the strategy may also have dampened the severity of CRS through depletion of monocytes and macrophages, which may otherwise have generated interleukin-6 and other cytokines. Direct outcome comparisons with licensed autologous therapies are difficult in the absence of controlled studies, but in the context of refractory pediatric B-ALL, long-term disease-free survival in children who received augmented lymphodepletion appears broadly comparable for the small number treated to date.

Guo et al,[71] in Beijing, China, applied allogeneic CAR19 T cells generated by lentiviral transduction and electroporation of CRISPR/Cas9 RNP complexes targeting TRAC and B2m.

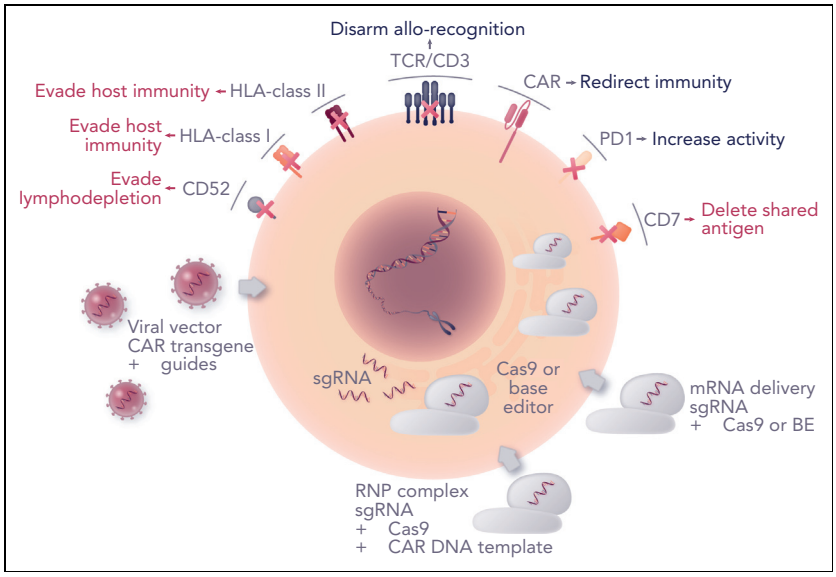

Figure 3. Advanced engineering strategies. Combining CAR transfer with genome editing offers the prospect of enhanced CAR T cells. Viral vector CAR transduction has been combined with co-expressed guide RNAs and electroporation of mRNA coding for Cas9 or base editor (BE). Alternatively, Cas9/guide RNP complexes offer efficient editing, and a route to site-specific transgene insertion by homologous recombination if a suitable CAR template is provided. Other advances include the ability to remove shared antigens such as CD7 that would otherwise result in T-cell fratricide during manufacture, and simultaneous manipulation of checkpoint pathways to address exhaustion and promote activity.

The products were enriched by CD3-mediated depletion and infused into 2 adult patients with R/R diffuse large B-cell lymphoma after the lymphodepletion with fludarabine and cyclophosphamide.[71] There was evidence of expansion and biological activity without GVHD, although the disease progressed in both the subjects.

Precision Biosciences have used a proprietary homing endonuclease technology, Arcus, in the early-phase trials of allogeneic CAR19 T cells. Initially, T cells had a single TRAC edit and a sited CAR insertion (with a promoter cassette) and were depleted of residual TCRαβ for the use in patients with R/R B-ALL. Results from PBCAR0191 in 15 subjects noted no instances of GVHD and evidence of remissions by day 28 in 9 subjects, with subsequent allogeneic SCT in 4 subjects.[72] In other patients, enhanced lymphodepletion with higher doses of fludarabine (120 mg/m^2 in total) and cyclophosphamide (3000 mg/m^2 in total) was investigated to improve expansion and persistence in subjects with CD19$^+$ NHL or ALL.[73] Although 70% of the responses were noted by day 28, by 6 months, the majority had relapsed. Further iterations (PBCAR19B) are now clinical investigation following the incorporation of an anti-B2m short hairpin RNA to disrupt HLA class I and a non-polymorphic HLA-E transgene to address the issue of host-mediated rejection (NCT04649112).

CRISPR Therapeutics is testing universal donor T cells with a CAR19 homology–flanked cassette integrated into the TRAC locus using AAV, with a second edit at the B2m site using CRISPR/Cas9. Preliminary results from patients with NHL reported a 38% remission rate with no GVHD and 1 case of severe neurotoxicity attributed to viral reactivation, but longer-term outcomes are awaited.[74] A similar approach with additional disruption of CD70 is being tested in patients with T-cell lymphoma using anti-CD70 CAR inserted at the TRAC locus (discussed below).[75]

Genome editing to address fratricide between CAR T cells

When using CAR-T strategies against T-cell malignancies, we have to consider factors that were more easily resolved with anti-B-cell CAR therapies. First, one of the consequences of successful anti-T-cell effects after the infusion is the likelihood of viral reactivations and other complications during a period of deep T-cell lymphopenia. Unlike in the B-cell setting, where B-cell aplasia can be addressed by regular immunoglobulin replacement therapy, there are no ready solutions to T-cell deficiency. The kinetics and quality of T-cell reconstitution may be problematic and could necessitate allogeneic SCT to ensure timely restoration of the T-cell compartment.

Second, the fratricide effects between T cells designed to express receptors against T-cell antigens may preclude the generation of effector cells and critically impair yields by the end of manufacture. Antigens expressed on T cells, including the TCRαβ/CD3 complex, CD5, and CD7 have been targeted by CARs following the steps to disrupt expression by inhibitory or restriction proteins[76] or by genome editing.[77] Genome editing using TALENs to target TRAC was used to disrupt the assembly of the cell surface TCRαβ/CD3 complex ahead of the expression of an anti-CD3ε CAR in T cells.[78] CRISPR/Cas9 editing of TRAC and CD7 for the generation of universal donor CAR7 T cells has also been described and could be used as an allogeneic non-matched donor therapy that avoids the need to harvest from heavily-treated patients and mitigates against the risk of transduction of leukemic blasts.[79] In addition, base-edited CAR7 T cells generated by multiplexed cytidine deamination disruption of T cell receptor β chain and CD7 have been described as a universal platform with advantages over Cas9 editing in terms of translocations after multiple edits.[80,81] As part of the extended multiplexed editing, PD1 disruption has been incorporated into CAR7-edited cells,[81] although the role and method of checkpoint pathway manipulation as well as the most appropriate targets and optimal timing require further elucidation for cellular therapies in general.[82]

Human applications of anti-CD7 CAR T-cell therapies have been reported with encouraging results against refractory T-cell malignancies, with generally manageable toxicity profiles. In China, anti-CD7 CAR T cells, manufactured from previous or new allo-SCT donors were generated using a vector

incorporating an endoplasmic reticulum retention element fused to a CD7-binder (rather than genome editing) to prevent fratricide. Eighteen of 20 patients with R/R T-ALL achieved complete remission and 7 patients proceeded to stem cell transplantation (NCT04689659).[83] Moreover, also in China, Gracell[84] has reported the application of donor-derived CRISPR/Cas9 CAR7 T cells edited at the TRAC and CD7 loci in T-ALL with 6 out of 6 treated patients achieving CR/CRi in interim reports. The company has also initiated studies of universal TRAC/CD7-disrupted T cells expressing dual moiety CARs against both CD7 and CD19, aiming to exploit the lymphodepleting effects of CAR7 to promote CAR19 effects against B-cell malignancy.[56] An initial trial of base-edited CAR7 T cells for T-ALL is underway in London for children with refractory leukemia, aiming to secure remission ahead of allo-SCT (ISRCTN15323014). These universal donor cells have 3 genes disrupted (TRBC, CD7, and CD52) ahead of lentiviral transduction for CAR7 expression.[85]

Finally, in the context of T-cell lymphoma, allogeneic CAR T-cell therapy, CTX130TM, targets CD70 and includes CRISPR/Cas9 disruption of TRAC,B2m, and CD70. Interim results from 15 evaluable patients have recently been reported with 29% complete remissions, no significant GVHD, and acceptable toxicity profiles in the initial trial phase.[75]

Cell sources and manufacturing yields

Adult donor-derived T cells have been most widely used for starting cells using steady-state leukapheresis, an efficient and cost-effective method of sourcing large numbers of healthy donor mononuclear cells.[86] Excess material can be cryopreserved and used in multiple production campaigns. Cells are generally activated without preseparation of T cells with anti-CD3 and anti-CD28 reagents, and T-cell expansion and dominance develop in the culture over several days. There may be advantages in isolating and transducing specific T-cell subsets, and this has been investigated in studies in an autologous setting.[87] The identification and targeting of populations with optimal expansion and effector function would provide a route to more efficient use of materials, including vector stocks. For banking purposes, yields after engineering and any enrichment steps, such as depletion of residual TCRαβ T cells are dependent on the efficiency of the gene-transfer and editing steps as well as expansion kinetics. Longer periods of ex vivo manipulation and proliferation may increase yields but may lead to exhaustion and loss off effector populations, although in general, each manufacturing campaign can readily produce and bank dozens of single-infusion doses.

Alternative starting material includes umbilical cord blood T cells which have distinct ontogeny because naïve phenotype may have anti-tumor advantages, although cell numbers are often limited compared with peripheral blood collections.[88–90] In the future, unlimited cells may be available through pluripotent stem cell engineering, as demonstrated in preclinical studies of CAR-engineered–T cell–derived induced pluripotent stem cells (iPS), including iterations with MHC disruption.[91–93] However, transcriptional studies determined that the lymphocytes subsequently generated are not true TCRαβ-T cells and may resemble innate TCRγδ T cells more. Replicating the complexity of differentiation and maturation of the adaptive T-cell compartment and delivering TCRαβ T cell-like function will require further development, but in the interim, the first clinical applications of products that are currently available are underway. A clonal iPS–derived T-cell line (FT819) with a CAR19 incorporating a modified signaling domain and inserted at the TRAC locus is being investigated in combination with interleukin-2 in subjects with R/R B-cell malignancies (NCT04629729) with the first patient receiving the dose in 2021.[94]

Safety and risks of allogeneic genome-edited T cells

Similar to the autologous setting, complications and toxicities of allogeneic edited cells can arise because of the antigen-binding properties of the CAR and may include on- and off-target effects, and issues such as CRS and neurotoxicity may arise in the days and weeks after infusion. In the longer term, perhaps unforeseen consequences related to gene modification and the use of integrating vectors could also arise in a manner similar to the autologous context. Existing long-term experience, over a period of 2 decades with gamma-retroviral and lentiviral transduction, provides confidence that the transformation risk in T-cell studies is low.[95,96] In contrast, gamma-retroviral gene addition to hematopoietic stem cells for the correction of various single-gene inherited immune disorders has been linked to malignant transformation.[97–99] Studies subsequently mapped risks to the promoter/enhancer elements of viral LTRs, specific insertion sites, and transactivation of protooncogenes.[98–101] Although much of these risks were obviated by adopting self-inactivating configurations,[102,103] recent reports of stem cell transformation in clinical lentiviral stem cell studies have again heightened concerns, although underlying predispositions and the role of chemotherapy may also be important.[104] In the T-cell arena, extended tracking in CAR trials has reported clonal dominance (but not transformation) arising as a consequence of lentiviral integration sites in trials of CAR19-[105] and CAR22-[106] engineered T cells, and there are data mapping integration sites from a large number of subjects, including the earliest treated subject from more than 10 years ago.[107] However, T-cell lymphoma has recently been reported in 2 patients receiving piggyBac transposon–modified CAR19 T cells. The underlying mechanisms have yet to be elucidated, but the experience highlights the risks of unexpected adverse events and the importance of careful long-term monitoring. Regarding genome editing, there have been extensive efforts to map on- and off-target nuclease effects and descriptions of chromosomal loss and gain, translocations, and other aberrations after cell manipulation. The quantification of predictable translocations after multiplexed nuclease-mediated–dsDNA cleavage has been reported for some products.[27] Karyotype and fluorescence in situ hybridization analysis using a probe close to the TRAC locus on chromosome 14 had previously found that around 5% of the metaphase spreads exhibited abnormal karyotypes after TALEN modification for universal CAR19 T cells.[29] Recently, such chromosome 14 abnormalities triggered a temporary regulatory hold after many concerns relating to a patient who had received Allogene Therapeutics' ALLO-501A CAR-19 T cells trial.[108] A similar frequency of karyotype aberrations was also reported after recombinant TCR lentiviral transfer in autologous T cells edited at TRAC and PD1 using CRISPR/Cas9, albeit without any overt consequences.[109] Subsequently, additional unbiased investigations using the same guide RNA sequences reported frequent aneuploidy and truncations of chromosomes

harboring the TRAC and PD1 target sites.[110] Although CRISPR/Cas9 editing at the PD1 locus has suggested that PD-1 operates as a haploinsufficient suppressor of T-cell lymphomagenesis in some animal studies,[111] there have been no reports of T-cell lymphomas in human trials involving PD1 editing.

For the lentiviral CRISPR/Cas9 product, TT52CAR19, in which duplex guide RNA cassettes are incorporated into lentiviral LTRs, karyotypes were reported normal at the end of production, but around 1% of cells carried predictable translocations quantified using droplet digital polymerase chain reaction.[70] The application of base editors should largely address translocation risk from dsDNA breakage, and several studies have reported direct comparisons between Cas9 editing and base editing for gene disruption.[80,112] Mapping sites of off-target base editor activity, whether guide-dependent or otherwise is challenging, and predicting the impact of any detected changes is difficult. The full impact of undesirable edits may perhaps only become apparent in the event of unexpected side events linked to populations of engineered cells, which would then be interrogated in detail. The role of vigilant monitoring, patient tracking, and sample retention over the long term is essential for early-stage therapeutic applications.

Summary

The ability to harvest, manipulate ex vivo, and then characterize and cryopreserve T cells has provided an attractive arena for early adoption of gene therapy technologies, including emerging genome editing platforms. Applications to generate 'off-the-shelf' CAR T-cell therapies that can be used without HLA matching are in clinical phase studies, and as the challenges of host immunity and rejection are addressed, wider deployment earlier in therapeutic hierarchies can be anticipated. In time, premanufactured, ready-to-use allogeneic CAR T cells have the potential to increase accessibility and provide reduced-cost alternatives to bespoke CAR therapies.

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Footnote

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