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Mini Review

Genome editing of therapeutic T cells

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ABSTRACT

The potential of engineered TCR$\alpha\beta$ T cells as potent mediators of leukemic clearance has been demonstrated in clinical trials, and authorised therapies are being deployed against B cell malignancies in particular. While most applications have relied on harvest and manipulation of autologous lymphocytes, the emerging application of genome editing technology has demonstrated that allogeneic TCR$\alpha\beta$ cells can be engineered to overcome Human Leukocyte Antigen (HLA) barriers and provides a route to more cost effective and widely accessible 'off-the-shelf' therapies. Genome editing also offers the prospect of addressing other hurdles such as shared-antigen expression and has been applied to direct site-specific transgene integration, for improved transcriptional regulation and function.

Introduction

T cells are attractive targets for gene therapy, being amenable to harvest, manipulation and re-infusion both in an autologous manner, and less frequently, in the allogeneic setting. T cells modified to express recombinant T cell receptors (rTCR$\alpha\beta$) and chimeric antigen receptors (CAR) are widely being investigated to treat malignancies [1]. These products are generally manufactured by ex-vivo transduction using gamma-retroviral or lentiviral vectors and the first autologous CAR products targeting CD19 have received marketing authorization for the treatment B cell derived leukemia and lymphoma [2]. Genome editing strategies offer the possibility of delivering pre-manufactured T cells suitable for multi-recipient use, and this would reduce costs and widen accessibility. If HLA barriers can be addressed, allogeneic CAR T cells from healthy donors offer a number of advantages compared to autologous therapies. Healthy donor T cells are likely to be 'fitter' and more tolerant of harvest and ex-vivo manipulation than autologous T cells from patients who have received intensive chemotherapy. Secondly, cells can be prepared in advance and characterised in detail. Formulations may be manipulated to optimize effective subset combination and infusions can be timed for pre-programmed treatment regimens. Healthy donor T cells also remain free from contamination with inadvertently transduced leukemic blasts, which could become 'masked' and escape CAR T cell mediated elimination [3].

There are three major hurdles allogeneic T cell strategies must overcome in order to mediate effective anti-tumor effects as effectively as autologous cells. Firstly, T cells express a diverse repertoire of T cell receptors that interact with polymorphic HLA molecules, and in the HLA mismatched setting, mediate graft versus host disease (GVHD) which can manifest with multisystem complications. Secondly, host immunity can recognize and react against mismatched HLA molecules expressed on infused allogenic CAR T cells, and this may be mediated by pre-existing antibodies or host T cells. Experience from mismatched allogeneic bone marrow transplantation has fashioned understanding of how to overcome HLA barriers with combinations for chemotherapy, radiotherapy and serotherapy and the attendant risks of infectious complications, marrow suppression and protracted cytopenia [4,5]. The inclusion of highly immunosuppressive regimens combining Fludarabine and Cyclophosphamide has become favourable in the autologous setting to promote homeostatic expansion and this can address the third hurdle of competition for cytokines and growth factors from the pre-existing T cell compartment. Overall, in the allogeneic setting, more intense depletion is preferred and has, for example, included the addition of serotherapy with Alemtuzumab, an anti-CD52 monoclonal antibody [6].

Gene therapy strategies to generate 'universal' TCR$\alpha\beta$ disrupted T cells that can overcome HLA barriers are being extensively investigated (Fig. 1). Approaches have included nuclease editing [7], small interfering RNA [8], & expression of inhibitory proteins [9]. One or both TCR$\alpha\beta$ chains have been targeted for genome editing using zinc finger nuclease, [10,11], TALENs, [7,12] meganuclease, [13] mega-TALEN, [14] and CRISPR/Cas [15,16]and base editing technologies. [17,18] The therapeutic potential of TCR depleted CAR19 T cells was established in 2015 when two infants with relapsed B-ALL achieved molecular remissions after received UCART19, TALEN edited CAR19 T cells devoid of TCR and CD52 [12]. Subsequent multi-center trials delivered by Servier and Allogene investigated the strategy further in children and adults, confirming the potential of allo-CAR T cells and highlighting the importance of sufficient lymphodepletion to allow mismatched cells to expand and mediate their anti-leukemic effects [19]. A similar strategy is now being

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Table 1
Clinical trials reporting genome edited CAR19 T cell experience against B cell malignancies.

Sponsor/study	Target edits	Platforms	Status
Great Ormond Street Hospital UCART19	TRAC & CD52	TALEN LV	Closed
Servier/Allogene UCART19 PALL & CALM NCT02808442 NCT02746952	TRAC & CD52	TALEN LV	Closed
Precision Bio NCT03666000	TRAC	Arcus® homing endonuclease AAV	Open
CRISPR Therapeutic CTX110 NCT04035434	TRAC & B_2M	CRISPR/Cas9 AAV	Open
Great Ormond Street Hospital TT52CAR19 NCT04557436	TRAC & CD52	CRISPR/Cas9 LV	Open

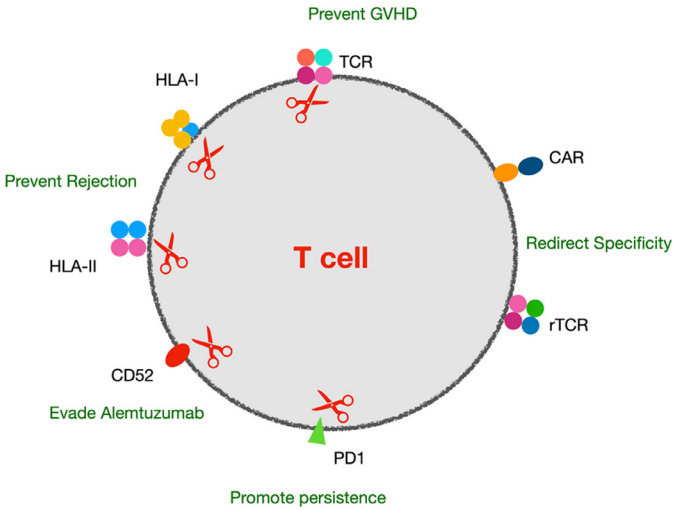

Fig. 1. Concept of T cell editing to improve specificity redirected therapies
T cells can be redirected to target specific antigens through the introduction of recombinant T cell receptors (rTCR) or chimeric antigen receptors (CAR), usually by viral vector transduction. Genome editing to disrupt expression of critical surface molecules is being applied to enhance activity and overcome HLA barriers. For example, checkpoint inhibitor pathway disruption through targeting of PD1 expression may promote persistence. Targeting of endogenous TCR can be used to prevent graft versus host disease in the allogeneic setting, and removal of HLA molecules should reduce host T cell mediated rejection. Alternatively, removal of CD52 allows T cells to survive in the presence of Alemtuzumab, a potent lymphodepleting antibody.

investigated using non-HLA matched healthy donor T cells edited using CRISPR/Cas9 in children with B-ALL who are ineligible for autologous lentiviral CAR19 therapy at Great Ormond Street Hospital in London. Precision Biosciences have used an alternative editing platform based on their proprietary homing endonuclease and AAV delivery to target CAR transgene integration the TRAC locus for first in human clinical trials [13]. Data from humanised immunodeficient mice had suggested that integration within the TRAC site may confer an element of transcriptional regulation and reduce the likelihood of exhaustion associated with vector mediated constitutive transgene expression [20]. Another approach has involved disruption of HLA class I expression on allogeneic donor CAR T cells by targeting of the non-polymorphic B2M chain has been combined with TRAC disruption by CRISPR/Cas9 and targeted AAV mediated insertion of CAR19 for treatment of B cell derived malignancies, with initial data recently reported by CRISPR Therapeutics (Table 1). Complete removal of class I HLA may in theory trigger 'missing-self' natural killer (NK) responses, and although this has yet to be determined in human studies, strategies to address the issue if found to be problematic include the expression of non-polymorphic HLA-E to inhibit NK activity. [21]

Table 2
Advanced genome editing approaches to address fratricide during production of T cells expressing anti-CD7 CAR to treat T cell malignancies.

Site	Subjects	CAR/Edit	Outcome
Baylor, Texas USA NCT03690011	T-ALL	LV CRISPR/Cas9 CD7	Pending
Graycell Chongqing China NCT04264078	T-ALL	LV CRISPR/Cas9 TRAC/CD7	Adult T-ALL 5 pts, remission without
Graycell ChiCTR190002531 ISRCTN19144142	T-ALL	LV CRISPR/Cas9 TRAC/CD7	Adult T-ALL 2 patients in remission

Genome editing to remove shared T cell antigens

CAR approaches to tackle T cell malignancies have been challenging because expression of surface antigens such as the TCR$\alpha\beta$/CD3 complex, CD2, CD5, and CD7 may result in compromising fratricidal effects during T cell production. The issue can be circumvented by removal of the relevant cell surface protein, either by protein inhibition or genetic disruption at the DNA level. For example, prevention of cell surface expression of CD3ε has been achieved through disruption of TRAC and prevention of assembly of the multimeric TCR/CD3 complex. [22] Thereafter, carefully timed transduction by lentiviral vector expressing anti-CD3ε CAR yields large numbers of anti-T cell CAR T cells which 'self-enrich' during culture. Using similar approaches, CD7 appears to be a promising target with anti-CD7 CAR T cells generated by expression of inhibitory proteins [23] or CRISPR/Cas9 [24,25] and by cytidine deaminase mediated base editing [26]. The first reports of clinical experience of anti-CD7 CAR T cell therapies are emerging [27], with encouraging remissions in the small number of individuals with refractory T-ALL treated to date (Table 2). A similar strategy has been proposed to treat another hematological malignancy, acute myeloid leukaemia, which may also express CD7 [28], and the more commonly targeted antigen CD33 is also being investigated for possible disruption by genome editing [29].

Emerging T cell data on safety profiles of genome editing

Experience with gamma-retroviral and lentiviral transduced T cells now extends over 20 years, and despite vector integration close to or within transcriptionally active genes, there have been no reports of malignant transformation of engineered T cells. In marked contrast, multiple trials where haematopoietic stem cells were transduced with gamma

retroviral vectors have been associated with proto-oncogene transactivation and malignant transformation [30-33]. Removal of enhancer elements within viral long terminal repeats, and a switch to lentiviral platforms has largely addressed these concerns, although vector integration associated proliferation or survival drive may be relevant. Examples of integration site driven clonal proliferation has been documented in both the HSC [34] and T cell context [35] and this may be relevant when comparing with alternative non-viral electroporation approaches that target site-specific integration of CARs or rTCR by homologous recombination following CRISPR/Cas9 double strand DNA cleavage [36].

As genome editing has entered clinical phase testing, effects at both desired target sites and possible off target sites have been under scrutiny [37]. Targeting of checkpoint pathways through disruption of the Pdcd1 locus which encodes the inhibitory PD-1 receptor has been an early target of CRISPR/Cas9 editing of tumor infiltrating lymphocytes (TILs) to promote anti-cancer effects in lung cancer [38]. PD-1 usually restricts antigen specific TCR responses during persistent stimulation and prevents autoimmunity developing and has been widely targeted pharmacologically by checkpoint inhibitors to promote antigen-driven effects in otherwise suppressive microenvironments. Identification of PD1 as a haplo-insufficient suppressor of T cell lymphomagenesis raised concerns that biallelic disruption by genome editing may result in malignant transformation of T cells [39]. However, initial reports have found no evidence of such events, and data indicated disruption of PD1 may have promoted survival and expansion of rTCR engineered T cells [38,40].

In the context of multiplexed editing, the translocation frequency between chromosomes, has been precisely quantified in studies using both TALEN and CRISPR/Cas9 technology, and up to 5% of metaphase spreads exhibited abnormal karyotypes [12,40]. To date, no adverse effects have been attributed to such populations and the likelihood of complications depends on precise sites of DNA cleavage and consequences of recombination events. It may be possible to predict potentially problematic translocations but more likely adverse issues will only be uncovered through careful interrogation of samples from clonally expanded T cells recovered after infusion. The application of base-editing technology to induce targeted single nucleotide base-conversion rather than nuclease mediated DNA breakage offers a solution to address the issue by relying on the disruption of splice sites or the creation of premature stop codons [41]. While there may still be low levels of DNA breakage and repair, almost complete elimination of translocations has been reported in T cells after multiplexed editing [17,26]. Deep characterization of guide RNA dependent and independent effects at off-target sites is challenging, especially across large numbers of rapidly proliferating T cells, and predicting risk attributable to aberrant sequence changes is likely to remain speculative until clinical experience and data emerges from early phase trials.

Conclusions

Genetically modified T cells have been amongst the first licenced cell therapy products and hold promise across a wide range of applications where manipulation of immunity provides therapeutic benefit. The emerging application of genome editing offers strategies to enhance beneficial effects and widen applications, including through the supply of pre-manufactured ‘off the shelf’ treatments. Careful and ongoing monitoring of longer terms effects, and diligent investigation of adverse events is warranted as therapies are deployed.

Disclosures

WQ has filed patents related to the production of engineered T cells; received research funding from companies developing T cell therapies (Cellectis, Servier, Bellicum); holds equity in Autolus Ltd

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