Abstract

Allogeneic chimeric antigen receptor T (CAR T) cells can offer advantages over autologous T cell therapies, including the availability of ‘fit’ cells for production, and elimination of risks associated with inadvertent transduction of leukemic blasts. However, allogeneic T cell therapies must address HLA barriers and conventionally rely on the availability of a suitable HLA-matched donor if graft-versus-host-disease and rejection effects are to be avoided. More recently, the incorporation of additional genome editing manipulations, to disrupt T cell receptor expression and address other critical pathways have been explored. Clinical trials are underway investigating non-HLA matched T cells expressing anti-CD19 CARs for the treatment of B cell acute lymphoblastic leukaemia (B-ALL) and anti-CD123 CAR for acute myeloid leukemia (AML). Such approaches continue to be refined and improved to widen accessibility and reduce the cost of T cell therapies for a wider range of conditions.

Word counts
Abstract 140.
Manuscript 2077
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Introduction

Chimeric antigen receptor (CAR) T cells are widely being investigated for their ability to treat certain malignancies, in particular leukemias and lymphomas.\textsuperscript{1} Allogeneic chimeric antigen receptor T cells offer possible advantages compared to autologous CAR T cell therapies. Firstly, donor T cells, free of exposure to multiple rounds of anti-leukemia therapy are likely to be ‘fitter’ and more tolerant of harvest and ex-vivo manipulation than autologous T cells. Secondly, the cells can be prepared in advance and be available for optimally timed delivery as part of a programmed treatment regimen. Scheduling can be informed by disease status and minimal residual disease (MRD) quantification to optimise therapeutic impact and reduce the risk of adverse effects. Thirdly, there is no risk of contamination from inadvertently transduced leukemic blasts, which may then exhibit ‘masking’ of target antigen if bound by the antigen binding domains of the introduced CAR, and thereby escape CAR-T cell recognition and elimination. Such a scenario has recently been reported in a trial of autologous CAR19 T cells.\textsuperscript{2} Finally, over time it is likely that the economic burden of allogeneic products, manufactured for multiple recipients in centralised batched production runs, will be reduced compared to bespoke autologous therapies generated for individual patients. In this review, generic issues relating to addressing HLA barriers, the risks of graft versus host disease (GVHD) and host mediated rejection in the application of allogeneic CAR-T cell therapies are discussed, and strategies considered for disruption of T cell receptor expression, HLA molecules and evasion of the effects of lymphodepleting drugs.

Addressing HLA barriers for allogeneic T cell therapies.

T cells express highly diverse heterodimeric αβ T cell receptors that interact with polymorphic HLA molecules, and are key mediators of self and non-self discrimination.\textsuperscript{3,4} HLA mismatches between donor and host are responsible for both host-versus-graft and graft-versus-host responses. Thus the host immune compartment will recognise and react against infused allogeneic CAR-T cells, recognising cell surface HLA class I and class II molecules. As with conventional unrelated donor allo-SCT, pre-existing antibodies can
Allogeneic CAR T cells mediate immune rejection if the host has been previously sensitised against HLA (for example by multiple transfusions), and thus screening for presence of relevant donor-specific anti-HLA antibodies is required. Acute rejection over a period of days or weeks may be mediated by a combination of humoral and cellular immunity, and is usually mitigated by lymphodepletion. The modality and intensity of host preparation balances the requirement for host immunosuppression against the risks of infectious complications, marrow suppression and protracted cytopenias. Popular chemotherapy regimens ahead of allo-SCT therapy include immunosuppressive combinations of Fludarabine and Cyclophosphamide, but can also include serotherapy such as the anti-CD52 monoclonal antibody, Alemtuzumab. The effects of Alemtuzumab against CD52 expressing immune cells, including T cells, can result lymphodepletion lasting a period of weeks, during which time viral reactivations can be problematic. The importance of depleting host T cells ahead of CAR-T cell therapy is not just an issue in the allogeneic setting. In the autologous setting similar lymphodepletion strategies, usually at reduced doses, are used to conjure an environment that reduces homeostatic competition for cytokines from existing T cells and this probably promotes engraftment and expansion of infused cells. Thus there is a strong rationale in for allo-CAR T cell therapies for preparative lymphodepletion to reduce rejection effects and minimise homeostatic competition. The optimal strength and duration of the immunosuppression required has still to be clearly defined.

Allogeneic CAR-T cells may mediate graft versus host disease (GVHD), especially in the HLA-mismatched setting. Dosing strategies in the allogeneic setting have to account for the risk of GVHD as well as on- and off target CAR mediated effects. Broader experience from allo-SCT, in particular haploidentical transplants, might inform dosing considerations in relation to thresholds for GVHD. Administration of >5x10⁶ /kg haploidentical T cells is considered a threshold for increased likelihood of GVHD in pediatric allo-SCT, and may provide a useful guide for the fully mismatched T cells. Unfortunately conventional strategies to mitigate against GVHD using systemic immunosuppression after infusion with agents such as Ciclosporin or Steroids are not readily applicable to the CAR setting where
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expansion, persistence and effector function are crucial. Other strategies to reduce the risk of GVHD have included engineering virus specific donor derived T cells in anticipation that the infusion of T cells with a narrowed repertoire of HLA-peptide specific receptors will have reduced alloreactivity.\(^9\)

Clinical experience of allogeneic hematopoietic stem cell donor derived allogeneic CAR T cells

Discussion here is focussed on cells harvested directly from healthy donors, rather than post-transplant ‘autologous’ collections of donor derived CAR T cells. Comparisons between different CAR19 trials and animal models are confounded by differences in CAR specifications, activation domains (41BB, CD28) and vector delivery systems (eg gamma retroviral, lentiviral, transposon). Nonetheless, animal modelling had reported that CD28-CD3ζ allo-reactive CAR19 T cells mediated anti-lymphoma effects in human:murine chimeras but were prone to exhaustion and deletion.\(^{10,11}\) Alternative modelling of ‘universal’ CAR19-41BB-CD3ζ T cells also found potent anti-leukemic effects but suggested that depletion of TCRαβ signalling may have enhanced persistence and reduced expression of PD1 exhaustion markers.\(^{12}\)

In patients, Kochenderfer et al treated relapsed leukemia after matched sibling or unrelated donor transplants with retrovirally transduced CAR19-CD28-CD3ζ T cells (without lymphodepletion) and encountered cytokine release syndrome (CRS) but not GVHD.\(^{13}\) Persistence was limited to under 28 days, and efficacy was limited. Similarly, exhaustion of virus specific T cells transduced with CAR19 probably contributed to lack of persistence in other studies reported by Cruz et al.\(^{9}\) Similar CAR configurations delivered by sleeping beauty transposon gene transfer were associated with GVHD in 3/19 subjects,\(^{14}\) and lentiviral CAR19-411B-CD3ζ modified T cells caused GVHD in two subjects.\(^{15}\) (Table 1) Of course, larger numbers of subjects will need to be treated to determine both the efficacy and risks of GVHD in the allogeneic transplant donor derived CAR-T setting, but the logistics of manufacturing and delivering therapy are similar to the autologous setting.
Strategies to generate ‘universal’ TCR disrupted CAR-T cells

TCRαβ expression can be disrupted by a variety of strategies including nuclease mediated genome editing,\textsuperscript{16} knockdown by small interfering RNA\textsuperscript{17}, and expression of inhibitory protein including antibody-derived single-chain variable fragments specific for CD3ε.\textsuperscript{18} Applications using a variety of DNA nucleases target the constant regions of one or both TCR chains have included zinc finger nuclease,\textsuperscript{19} meganuclease,\textsuperscript{20} TALEN,\textsuperscript{21,22} megaTalen\textsuperscript{23} and CRISPR/Cas9\textsuperscript{12,24,25} technologies. More sophisticated approaches have included targeted insertion of transgenes, including CAR19, into the TRAC locus for the possibility of improved regulation of gene expression\textsuperscript{20,26}. Clinical proof of concept demonstration of the potential of TCR depleted CAR19 T cells were established in two infants with relapsed B-ALL who received infusions of TALEN edited CAR19 T cells and were able to achieve molecular remissions sufficient to mandate successful allogeneic SCT.\textsuperscript{27} Ongoing multi-centre trials are investigating the strategy further in children and adults (discussed below). Several groups have described preclinical development of similar approaches using CRISPR/Cas9, including multiplexed iterations and versions coupling TCR knockout effects with CAR19 expression.\textsuperscript{12,24,25} Additional refinements for TCR disruption using targeted base conversion by deamination of cytidine to thymidine and the creation of premature stop codons, for possible gene disruption without DNA scission are discussed below.

Strategies to address host mediated rejection

An obvious approach to evade host responses to mismatched cells is to disrupt expression of HLA molecules on the cell surface. Lessons from individuals with inherited defects of MHC expression resulting in ‘bare-lymphocyte’ syndromes may be informative.\textsuperscript{28} Mutations in TAP1 or TAP2 (transporter associated with antigen presentation) or Tapasin can result in loss of class I expression\textsuperscript{29}, but it is disruption of B2M to prevent assembly of HLA-class I heterodimers that has been most widely explored by gene editing.\textsuperscript{24,25,30} Similarly targeting of regulatory factor X complex (RFX5, RFXB/ANK, and RFXAP), mimics inherited MHC class II
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deficiency and loss of class II expression on T cells. Strategies to address ‘missing self’
responses by host natural killer (NK) cells include the expression of non-polymorphic HLA
class I molecules such as HLA-E to provide inhibitory signals.31, 32 Disrupting HLA expression
may need to be extended to class II molecules as these are upregulated on activated T cells.
Caution may be required in considering how HLA depleted cells be removed if they become
virally infected, as in the absence of MHC, immune mediated clearance may not occur and
they could become reservoirs for infection. Alternative strategies aim to render infused T
cells resistant lymphodepleting chemotherapy or antibody therapy (Figure 1). An example of
the latter is the use of Alemtuzumab to target CD52. The antibody mediates in vivo effects
over a period of 2-3 weeks, and therefore T cells engineered to be devoid of cell surface
CD52 expression acquire a survival advantage in the presence of the drug. Multiplexed
TALEN mediated disruption of TRAC and CD52 has been modelled in human: murine
chimeras and is now undergoing clinical phase investigation as mentioned.21 The major
disadvantage of using such serotherapy is that the depth and duration of subsequent
lymphopenia is associated with viral reactivation and this can result in significant morbidity.
A similar approach envisages conferring resistance to lymphodepleting purine nucleoside
analogs through disruption of deoxycytidine kinase (dCK) which could allow cells to resist
Fludarabine33 although scheduling, dosing and duration required for such an approach
remain to be determined.

Clinical trials of universal CAR T cells
TALENs targeting the constant domain of the T cell receptor alpha constant (TRAC) domain
and simultaneously disrupting CD52, have been combined with lentiviral delivery of CAR19-
41BB-CD3ζ. Disruption of TRAC abrogates the risk of GvHD and loss of CD52 renders cells
resistant against Alemtuzumab following lymphodepletion. Two infants with relapsed
pediatric B-ALL after a first myeloablative allo-SCT achieved molecular remission after a
single infusion of TCR’CD52’CAR19+ T cells. Both then went on to successful second allo-SCT
after further conditioning and achieved long term remission.27 The vector configurations
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included a suicide gene element, RQR8, that aimed to confer sensitivity to the anti-CD20 antibody Rituximab but variable expression levels were encountered and the elimination strategy relied on transplant conditioning. Two clinical trials using similar universal, genome edited CAR19 T cells (UCART19) in children (NCT02808442) and adults (NCT02746952) are underway in Europe and US using banks of pre-manufactured T cells. A similar approach has also been initiated for Blastic plasmacytoid dendritic cell neoplasm (BPDCN) (NCT03203369) and relapsed acute myeloid leukemia (AML) (NCT03190278) with a view to securing remission ahead of allo-SCT using universal T cells targeting CD123. Additional targets under development include CD7, which is expressed on T cells and certain myeloid malignancies. Examples for combined CD7 and TRAC disruption using CRISPR/cas9 have been described and the approach could be of value against T cell leukemias or certain subsets of AML. Similarly, anti-CD3 CAR-T cells, produced following disruption of TCR-CD3 expression may be useful for T cell malignancies in the allogeneic setting ahead of allo-SCT.

Future prospects

Current vector systems and genome editing platforms already offer the prospect of efficient multiplexed genome editing, in combination with constitutive expression of one or more transgenes. These are being advanced through translational pipelines and can offer linked expression of CARs and safety suicide genes in case of adverse effects, with both elements expressed constitutively by vector encoded promotor machinery. Additional refinements include adoption of strategies for site-specific transgene integration, for examples into the TRAC locus, for physiological levels of expression. Several groups have reported targeted insertion of CAR transgenes at this site, and regulated CAR expression may mitigate against exhaustion effects. Approaches using double stranded DNA for template delivery alongside CRISPR/Cas9 have also recently been reported, and could offer more economical strategies for compared to current approaches that rely on viral vector delivery.
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It is anticipated that CAR-T cell banks will support delivery of more homogenous products, not just with defined T cell subset profiles, but also defined integration sites and additional modifications. It may be possible to support enhanced T cell expansion and persistence by targeting integration sites associated with clonal dominance in past patients who were successfully treated. Finally, direct chemical base modification offers is now being investigated whereby base editors (BE) comprising an inactive form of Cas9 mutant fused to a cytidine deaminase (eg APOBEC1) and a uracilglycosylase inhibitor (UGI) can provide targeted modifications without DNA breaks. Komor et al reported conversion of cytidine (C) to uracil (U) within a window of 13–17 nucleotides from a protospacer adjacent motif (PAM) sequence and this strategy can be deployed to target certain nucelotides to create stop codons (TAG, TAA, or TGA). The approach may allow targeted disruption of multiple genes simultaneously while minimizing the risks of translocation events associated with nuclease based genome engineering.

There are still notable hurdles to address, but there is strong academic and Pharma driven momentum for the provision of ready-made, off-the-shelf, efficacious T cell therapy without the risk of side-effects will surely reduce costs and widen applicability of cellular immunotherapy.
# Table 1. Clinical reports of allogeneic CAR-T cells

Published reports of allogeneic CAR T cells, the vector platform and configuration used and whether lymphodepletion was included.

<table>
<thead>
<tr>
<th>Design</th>
<th>Vector</th>
<th>CAR configuration</th>
<th>Lymphodepletion</th>
<th>Subjects Treated (N)</th>
<th>Efficacy</th>
<th>GVHD</th>
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<td>Post allo DLI</td>
<td>γRV</td>
<td>CD28-CD3ζ</td>
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<td>20</td>
<td>CR n=6</td>
<td>Acute</td>
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<tr>
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</table>
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Legend Figure 1
Concept of genome modified CAR T cells, expressing antigen specific CARs but also modified to prevent alloreactivity by disruption of T cell receptor alpha (TRAC) or beta chains (TRBC1/2). Simultaneous editing of targets to address host mediated rejection (HLA class I and possibly class II molecules) and/or elimination of molecules targeted by lymphodepletion agents such as Alemtuzumab (anti-CD52). In this example the CAR is delivered by viral vector for non-specific genomic integration, but emerging approaches have also demonstrated targeted insertion, for example into the TRAC locus.

Acknowledgments
Supported by National Institute for Health Research and Great Ormond Street Biomedical Research Centre.

Disclosures
WQ has received research and trial funding from Servier, Cellectis, Bellicum, Autolus, Miltenyi Biotec, holds equity in Autolus Therapeutics, Orchard Therapeutics, and has received advisory fees from Novartis.
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References

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Figure 1. Engineered ‘universal’ CAR T cell

- Target for Host
- Target for Alemtuzumab
- HLA-class I
- HLA-class II
- CD52
- Cas9/guideRNA
- TCR/CD3
- Mediates GVHD
- Anti-leukemic effects
- CAR
- Viral Vector