Expert Review of Hematology

ISSN: 1747-4086 (Print) 1747-4094 (Online) Journal homepage: http://www.tandfonline.com/loi/ierr20

Emerging applications of gene edited T cells for the treatment of leukemia

Christos Georgiadis & Waseem Qasim

To cite this article: Christos Georgiadis & Waseem Qasim (2017) Emerging applications of gene edited T cells for the treatment of leukemia, Expert Review of Hematology, 10:9, 753-755, DOI: 10.1080/17474086.2017.1350575

To link to this article: http://dx.doi.org/10.1080/17474086.2017.1350575

Accepted author version posted online: 04 Jul 2017.
Published online: 25 Jul 2017.

EXPERT REVIEW OF HEMATOLOGY, 2017
VOL. 10, NO. 9, 753–755
https://doi.org/10.1080/17474086.2017.1350575

Taylor & Francis
Taylor & Francis Group

EDITORIAL

Emerging applications of gene edited T cells for the treatment of leukemia

Christos Georgiadis and Waseem Qasim

Molecular and Cellular Immunology Unit, Institute of Child Health, London, UK

ARTICLE HISTORY Received 5 May 2017; Accepted 30 June 2017

KEYWORDS TALEN; CRISPR; Cas9; CAR; T cell immunotherapy; PD1; Gene editing

Increasing numbers of early phase clinical trials are underway to evaluate the application of T-cells engineered to express new receptor genes targeting leukemia antigens. There have been compelling reports of high remission rates, in particular, using chimeric antigen receptors (CARs) against CD19 in B-cell malignancies using lentiviral and gamma-retroviral gene-addition technology [1]. CARs generally comprise an extracellular domain derived from a monoclonal antibody single-chain variable fragment (scFv) and link, via a transmembrane moiety, to activation domains derived from combinations of CD28, 41BB, and CD3ζ[2]. The majority of such therapies have been generated in a bespoke manner using autologous peripheral blood mononuclear cell harvests, and this has required dedicated infrastructure and expertise, with each product taking around two weeks to manufacture. To date, only a limited number of centers worldwide have been able to produce and deliver gene-modified cells, and there are ongoing debates around centralized versus 'point-of-care' manufacturing as larger phase, pharmaceutical-led trials get underway. Automation may address some of these issues [3], but a second, and perhaps more critical aspect, relates to difficulties in harvesting sufficient functionally intact autologous cells from heavily treated, often lymphopenic patients. In some cases, where a human leukocyte antigen (HLA)-matched allogeneic donor is available, it may be possible to generate healthy donor-derived CAR19 T-cells [4], although their application introduces a risk of graft versus host disease (GVHD) [5], and the manufacturing process is still highly bespoke.

Emerging gene-editing tools offer solutions that are beginning to enter the clinical area, and T-cells have proven ideal targets for gene engineering, given the established processes for cell harvest and *ex vivo* manipulation. The ability to use banked CAR T-cells, generated from non-HLA matched donors in an 'off-the-shelf' manner has been an attractive prospect that has driven attempts to overcome HLA-barriers, both in terms of allo-reactivity from infused cells and host-mediated rejection [6–8]. T-cells encode highly specific heterodimeric αβ T-cell receptors, and these are the key mediators of major histocompatibility complex (MHC) recognition leading to GVHD. Strategies to disrupt T-cell receptor (TCR) expression have used a variety of reagents including RNA interference (RNAi) [9] and targeted gene disruption using directed DNA nucleases such as Zinc Finger Nucleases [6,7], Meganucleases [10], MegaTALs [11,12], and Transcription activator like effector nucleases (TALENs) [8,13,14]. The latter operate at the genomic level, directing engineered endonucleases to highly specific loci to create double stranded DNA cleavage. Cellular repair at such sites by Non Homologous End Joining (NHEJ) creates a variety of insertions and deletions (Indels), disrupting native sequences and, depending on the target site, resulting in defective or absent gene expression. The addition of Clustered regularly interspaced short palindromic repeat (CRISPR)-Cas9 reagents to the editing toolbox has further extended the range of genomic sites susceptible to DNA scission [15–17]. Improvements in the ability to deliver editing reagents into cells as RNA have been a key advance that has allowed translation and scalability to therapeutic strategies. This has included targeting of key genes in T-cells harvested and first transduced to express CAR19. Targeting the T-cell receptor alpha chain constant domain (TRAC) prevents assembling of functional cell surface TCRαβ, abrogating the risk of the GVHD. To address the risk of rejection, recipients can be immune-depleted using Alemtuzumab, a monoclonal antibody against CD52, while infused CAR T-cells are rendered resistant to depletion by disruption of CD52 expression using TALEN gene editing [8]. Experience with Alemtuzumab for conditioning ahead of allogenic transplantation suggests its effects can persist for several weeks and would usually impact heavily on infused T-cells [18]. Thus, CAR-T-cells devoid of CD52 and infused in the presence of *in vivo* Alemtuzumab had a survival advantage when $TCR^{-}CD52^{-}$ CAR19 T-cells were used in pediatric patients with leukemic relapse at Great Ormond Street Hospital in London [14]. Cells persisted and mediated potent anti-leukemic effects over several weeks but a key concern was whether carriage of residual TCRαβ T-cells could cause GVHD in profoundly lymphopenic hosts. While the cells had been subjected to CliniMacs TCRαβ depletion after TALEN-mediated knockout of TRAC and less than 1% of cells expressed CD3/TCR, these residual cells could still breach thresholds sufficient to cause GVHD. Based on experience from haploidentical transplantation [19], the threshold for such effects was estimated to be around 5×10^4 T-cells/kg, sufficient to allow therapeutic CAR T-cell dosing in the 10^6/kg range – a target for efficacy based on reports from the autologous setting [20]. The first infant treated was found to be in molecular remission within 28 days, although developed GVHD and required intervention with steroid therapy. Remission was followed by successful allogeneic transplantation, with eradication of persisting CAR19 cells and subsequent donor-derived immune reconstitution. This also addressed any theoretical concerns related to the

CONTACT Waseem Qasim W.Qasim@ucl.ac.uk Molecular and Cellular Immunology Unit, Institute of Child Health, UCL Great Ormond Street, London WC1N 1EH, UK

effects of the multiple genetic manipulations the cells had been subjected to. The risk of lentiviral mediated insertional mutagenesis in T-cells was considered extremely low given that there have been no reports of vector-mediated genotoxicity in trials using engineered T-cells. Furthermore, preclinical experiments had used sensitive quantitative polymerase chain reaction (qPCR) techniques to quantify the frequency of translocation events between chromosomes subjected to double strand DNA breaks to be <1/100 [8]. Karyotype and FISH analysis had detected abnormalities in <5% of metaphase spreads, and while such manifestations raised concerns of possible transformational consequences, the application of cells in a time-limited setting ahead of allogeneic stem cell transplantation was ideal. Phase 1 trials are now assessing the strategy in more depth in both children and adults with relapsed CD19+ B-cell malignancies.

A number of other leukemia target antigens are also under consideration, with US sites at MD Anderson and Weill Cornell recently approved to undertake trials of TCR depleted CAR therapy against CD123 (IL3-receptor) in acute myeloid leukemia as a bridging strategy to allogeneic transplantation. In addition, editing the TCR locus is also attractive for therapies based on recombinant αβTCR gene transfer, for example to target melanoma antigens or Wilms tumor-1 (WT1) antigen in hematological malignancy. In such a context, disruption of endogenous α or β TCR chains should reduce the risk of aberrant cross pairing with introduced chains and reduce competition of components of the multimeric CD3 complex required for effective cell surface expression and signaling. A proposed study to express TCR specific for NY-ESO-1 and disrupt expression of TCR α and β as well as the checkpoint receptor PD-1 with CRISPR/Cas9 will be undertaken at UPenn, UCSF, and MD Anderson for subjects with multiple myeloma, melanoma, or synovial sarcomas. The inclusion of PD-1 disruption to interfere with checkpoint pathways aims to reduce T-cell exhaustion and improve persistence, although could also risk invigorated responses and carries a risk of autoimmunity. Recently, researchers at Sechuan University in China have reported infusion of tumor infiltrating T-cells (TILs) in lung cancer patients that had been disrupted for PD1 using CRISPR/Cas9 and follow-up and safety data is awaited. Meanwhile, the reagents continue to evolve and improve, with multiple CRISPR platforms and variant Cas systems promising to unlock highly efficient, on-target effects, with minimal off-target consequences. Other laboratory developments are delivering further refinements, including the possibility of targeted CAR insertion into the TRAC locus thereby introducing an element of regulatory control from relevant transcriptional machinery while simultaneously disrupting TCR expression. Adeno-associated virus was recently used for targeted integration of promoterless CAR19 cassettes directly into the TRAC locus following either CRISPR/Cas9 [17] or I-CreI homing endonuclease [10] mediated DNA breakage, and this may reduce theoretical risks of vector-mediated insertional mutagenesis. This approach harnesses homologous recombination pathways rather than default NHEJ repair, and although scalability may be challenging, the products are expected to be more homogeneous.

Peering into the near-future, with early phase testing of TALEN gene-edited 'universal' CAR-T cells underway and application of CRISPR-edited cells anticipated, there should be valuable 'proof-of-concept' experience over the next 2–5 years. Ever improving sequencing capabilities, with ultra-deep characterization will help characterize and track genetic modifications. Thereafter, as safety data accumulates and refinements are incorporated, a wider range of therapeutic approaches, with combinational engineering is expected. Emerging alliances between gene-editing companies such as Cellectis, Editas Medicine, CRISPR Therapeutics, and Intellia Therapeutics and firms developing CAR-T cells such as Pfizer, Juno Therapeutics, Celgene, and Novartis should help resource carefully monitored clinical trials and accommodate the requisite long-term (15 year) follow-up arrangements required by regulatory authorities. With a dizzying myriad of possibilities, gene-editing is set to fashion the next generation of T-cell therapies.

Funding

The authors are supported by National Institute of Health Research, UK.

Declaration of interest

W Qasim has received research support Cellectis, Servier, Bellicum, Miltenyi Biotec and holds stock and consults for Autolus Ltd and Orchard Therapeutics. The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

References

Papers of special note have been highlighted as either of interest (•) or of considerable interest (••) to readers.

1. Riviere I, Sadelain M. Chimeric antigen receptors: a cell and gene therapy perspective. Mol Ther. 2017;25(5):1117–1124.
2. Sadelain M, Brentjens R, Riviere I. The basic principles of chimeric antigen receptor design. Cancer Discov. 2013;3(4):388–398.
3. Mock U, Nickolay L, Philip B, et al. Automated manufacturing of chimeric antigen receptor T cells for adoptive immunotherapy using CliniMACS prodigy. Cytotherapy. 2016;18(8):1002–1011.
• **Automation of CAR T-cell production.**
4. Kochenderfer JN, Dudley ME, Carpenter RO, et al. Donor-derived CD19-targeted T cells cause regression of malignancy persisting after allogeneic hematopoietic stem cell transplantation. Blood. 2013;122(25):4129–4139.
5. Jacoby E, Yang Y, Qin H, et al. Murine allogeneic CD19 CAR T cells harbor potent antileukemic activity but have the potential to mediate lethal GVHD. Blood. 2016;127(10):1361–1370.
6. Torikai H, Reik A, Pq L, et al. A foundation for universal T-cell based immunotherapy: T cells engineered to express a CD19-specific chimeric-antigen-receptor and eliminate expression of endogenous TCR. Blood. 2012;119(24):5697–5705.
• **Concept of T-cell receptor disrupted T-cells investigated.**
7. Provasi E, Genovese P, Lombardo A, et al. Editing T cell specificity towards leukemia by zinc finger nucleases and lentiviral gene transfer. Nat Med. 2012;18(5):807–815.
8. Poirot L, Philip B, Schiffer-Mannioui C, et al. Multiplex genome-edited T-cell manufacturing platform for "Off-the-Shelf" adoptive T-cell immunotherapies. Cancer Res. 2015;75(18):3853–3864.
• **TALEN engineering of T-cell receptor and CD52 disrupted T-cells.**
9. Bunse M, Bendle GM, Linnemann C, et al. RNAi-mediated TCR knockdown prevents autoimmunity in mice caused by mixed TCR

dimers following TCR gene transfer. Mol Ther J Am Soc Gene Ther. 2014;22(11):1983–1991.
10. MacLeod DT, Antony J, Martin AJ, et al. Integration of a CD19 CAR into the TCR alpha chain locus streamlines production of allogeneic gene-edited CAR T cells. Mol Ther J Am Soc Gene Ther. 2017;25(4):949–961.
11. Boissel S, Jarjour J, Astrakhan A, et al. megaTALs: a rare-cleaving nuclease architecture for therapeutic genome engineering. Nuclei Acids Res. 2014;42(4):2591–2601.
12. Osborn MJ, Webber BR, Knipping F, et al. Evaluation of TCR gene editing achieved by TALENs, CRISPR/Cas9, and megaTAL nucleases. Mol Ther J Am Soc Gene Ther. 2016;24(3):570–581.
13. Berdien B, Mock U, Atanackovic D, et al. TALEN-mediated editing of endogenous T-cell receptors facilitates efficient reprogramming of T lymphocytes by lentiviral gene transfer. Gene Ther. 2014;21(6):539–548.
14. Qasim W, Zhan H, Samarasinghe S, et al. Molecular remission of infant B-ALL after infusion of universal TALEN gene-edited CAR T cells. Sci Transl Med. 2017;9:374.
•• **First in human application of TALEN-edited cells.**
15. Ren J, Liu X, Fang C, et al. Multiplex genome editing to generate universal CAR T cells resistant to PD1 inhibition. Clin Cancer Res. 2017;23(4):2255–2266.
16. Ren J, Zhang X, Liu X, et al. A versatile system for rapid multiplex genome-edited CAR T cell generation. Oncotarget. 2017;8(10):17002–17011.
17. Eyquem J, Mansilla-Soto J, Giavridis T, et al. Targeting a CAR to the TRAC locus with CRISPR/Cas9 enhances tumour rejection. Nature. 2017;543(7643):113–117.
• **Site-specific insertion of CAR into T-cell receptor locus.**
18. Chakrabarti S, Hale G, Alemtuzumab WH. (Campath-1H) in allogeneic stem cell transplantation: where do we go from here? Transplant Proc. 2004;36(5):1225–1227.
19. Bertaina A, Merli P, Rutella S, et al. HLA-haploidentical stem cell transplantation after removal of alphabeta+ T and B cells in children with nonmalignant disorders. Blood. 2014;124(5):822–826.
20. Lee DW, Kochenderfer JN, Stetler-Stevenson M, et al. T cells expressing CD19 chimeric antigen receptors for acute lymphoblastic leukaemia in children and young adults: a phase 1 dose-escalation trial. Lancet. 2015;385(9967):517–528.