HHS Public Access

Author manuscript

Biol Blood Marrow Transplant. Author manuscript; available in PMC 2019 January 01.

Published in final edited form as:

Biol Blood Marrow Transplant. 2018 January ; 24(1): 27–31. doi:10.1016/j.bbmt.2017.10.017.

Building a Safer and Faster CAR: Seatbelts, Airbags and CRISPR

Miguel-Angel Perales[1,2], **Partow Kebriaei**[3], **Leslie S. Kean**[4,5,6], and **Michel Sadelain**[7]

[1]Department of Medicine, Adult Bone Marrow Transplant Service, Memorial Sloan Kettering Cancer Center, New York, NY

[2]Weill Cornell Medical College, New York, NY

[3]Department of Stem Cell Transplantation and Cellular Therapy, MD Anderson Cancer Center, Houston, TX

[4]The Ben Towne Center for Childhood Cancer Research, Seattle Children's Research Center, Seattle WA

[5]The Fred Hutchinson Cancer Research Center, Seattle WA

[6]Department of Pediatrics, University of Washington, Seattle WA

[7]Center for Cell Engineering and Immunology Program, Memorial Sloan Kettering Cancer Center, New York, NY, USA

Abstract

Therapeutic T cell engineering has recently garnered widespread interest owing to the success of CD19 (Chimeric Antigen Receptor) CAR therapy. CARs are synthetic receptors for antigen that redirect the specificity and reprogram the function of the T cells in which they are genetically introduced. CARs targeting CD19, a cell surface molecule found in most leukemias and lymphomas, have yielded high remission rates in patients with chemorefractory, relapsed disease, including acute lymphoblastic leukemia, chronic lymphocytic leukemia and non-Hodgkin lymphoma. The toxicities of this treatment include B cell aplasia, cytokine release syndrome (CRS) and neurotoxicity. Although reversible in most instances, these toxicities may require specific medical interventions, including transfer to intensive care to treat severe CRS. Guidelines for managing these toxicities are emerging. The recent report of a non-human primate model for CRS is poised to help advance the management of this syndrome. Finally, new engineering modalities, based on the use of targeted nucleases like CRISPR, may further enhance the efficacy and safety of CAR T cells.

CORRESPONDING AUTHOR: Miguel-Angel Perales, M.D., Adult Bone Marrow Transplantation Service, Department of Medicine, Memorial Sloan Kettering Cancer Center, 1275 York Avenue, Box 298, New York, NY 10065 USA, peralesm@mskcc.org, Fax: 212-717-3500, Phone: 212-639-8682.

CONFLICT OF INTEREST STATEMENT

M-A Perales has received consulting fees from Incyte, Merck, Novartis, Seattle Genetics and Servier. M. Sadelain is a scientific cofounder of Juno Therapeutics. The other authors declare the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Keywords

Chimeric antigen receptor T cells; CART; Immunotherapy; Adoptive T cell therapy; Adoptive immunotherapy; Hematopoietic stem cell transplantation; Cytokine Release Syndrome; Neurological Toxicity

Introduction

CD19-specific Chimeric Antigen Receptor (CAR) T-cell immunotherapy has shown significant antitumor activity in the treatment of high-risk relapsed and refractory leukemias and lymphomas, and has revolutionized the treatment landscape for patients with advanced lymphoid malignancies [1–19]. This cellular therapy has remarkable efficacy, even in highly chemotherapy-resistant tumors, with response rates up to 90% for patients with relapsed B-cell acute lymphoblastic leukemia (ALL) and greater than 60% for patients with relapsed non-Hodgkin's lymphoma (NHL) [10–15]. However the side effects and toxicities of CAR T-cell therapy, particularly in ALL patients with high tumor burden can be life-threatening [10, 20]. With the recent approval by the US FDA of CD19 CAR T cells for ALL and NHL there will be expanded use of these therapies, not just in centers that have been involved in the development of these treatments, but also in many centers with experience in hematopoietic stem cell transplantation (HCT) but that have previously not treated patients with CAR T cells. In this review, we will discuss general recommendations for the adoption of CAR T cells in the clinical setting, with a focus on the management of the two main complications, cytokine release syndrome (CRS) and neurotoxicity, and will also look to future developments in CAR T cells, including the use of CRISPR/Cas9 for gene editing to enhance both potency and safety. For a more detailed discussion of different CAR T cell targets and clinical results in various indications, the reader is referred to recently published reviews in this journal [21–23].

The CARS are in the showroom: Time to figure out how to drive safely

CAR T-cell therapy has revolutionized the treatment landscape for patients with advanced lymphoid malignancies, but manipulating the immune system is also associated with unique and potentially life-threatening toxicities. As CAR T therapies become more prevalent for a broader range of malignancies, it is critical that we continue to investigate the pathophysiology and patterns of toxicity, prepare our clinical units to recognize and effectively manage these unique toxicities, and finally investigate ways to modify CAR constructs to increase safety. Herein, we will briefly review these topics, mainly based on clinical studies utilizing second generation CD19 CAR T-cells, which include a co-stimulatory molecule such as CD28 or 4-1BB, in efforts to "drive safely."

All responding patients have had some degree of CRS that typically begins within the first week of T cell infusion, and is in response to the in vivo proliferation of the CAR T cells; the rates for severe CRS range widely from 13% to 43% [10–14, 24]. CRS is characterized by high fevers, and can progress to severe CRS with capillary leak and resulting hypotension and respiratory compromise. Macrophage activation syndrome may accompany severe CRS. Reported rates for severe inflammatory cytokines and standard laboratory markers of

inflammation, including ferritin, C-reactive protein, and LDH, are elevated [13, 25]. However, in contrast to the latter standard laboratory markers, marked elevation of the specific inflammatory cytokines interferon-γ, soluble interleukin 2 receptor (IL-2r), and interleukin 6 (IL-6), which appear central to the CRS process, can identify which patients will develop severe CRS [25–27]. Tumor burden, CAR T-cell dosage, and use of lymphodepleting chemotherapy impact the development of CRS, and while there is a correlation between development of CRS and response to CAR T cells [11, 12], there does not appear to a strong association between the degree of CRS and outcome [10]. Neurologic toxicities, which include encephalopathy and seizures, may occur early post CAR T-cell infusion in the setting of CRS, or less commonly weeks later. The mechanism of neurological events is unclear; it may be cytokine-mediated or due to direct CAR T-cell infiltration. Interestingly, detection of CAR T cells in the CSF did not correlate with development of neurologic toxicity in the study reported by Maude and colleagues [10]. Neurotoxicity is discussed in more detail below. Additionally, patients treated with CD19 CAR T cell therapy develop long-term B-cell aplasia which is a predicted on-target effect of anti-CD19 therapy. However, similar to patients treated with anti-CD20 antibody therapy, it has been shown that B-cell independent plasma cells contribute to long-lasting humoral immunity in these patients [28].

Currently, the mainstay of CRS therapy is tocilizumab, an anti-IL-6 receptor antagonist, which results in prompt resolution of CRS-related symptoms in the majority of patients [3]. Other IL-6 inhibitors, such as siltuximab, a chimeric monoclonal antibody that binds directly to IL-6, are under investigation. Corticosteroids, while effective in managing CRS, are toxic to the infused cells and may limit efficacy. However, corticosteroids, in contrast to IL-6 inhibitors, have been noted to be more efficacious for neurotoxicity, especially for late toxicity not associated with CRS. In addition to medications, accurate assessment of these unique toxicities, and education of healthcare personnel involved in the care of these patients is important in improving overall safety. The Lee grading system was first published in 2014, and is helpful in defining the severity of CRS and neurotoxicity, and subsequent management [20]. The MD Anderson group more recently published their grading system, which is based on the Lee system, and further expands on the approach to neurotoxicity grading and management [29]. Notably, none of these grading systems have been validated in a prospective fashion, but are useful first steps in helping to identify and manage toxicity. Furthermore, patients who develop severe CRS and/or neurotoxicity require multidisciplinary care including neurology and critical care consultants. Typically, transplant units are uniquely qualified to take care of such patients as they are familiar with cellular therapy toxicity. The Foundation for the Accreditation of Cellular Therapy (FACT) has developed Immune Effector Cells (IEC) standards to help hospitals adequately prepare to safely infuse cellular therapies, such as CAR T cells [30]. Furthermore, gathering and reporting toxicity will collectively add to the knowledge of using these therapies. The Center for International Blood and Marrow Transplant Research (CIBMTR) and the European Blood and Marrow Transplantation (EBMT) registries are working together to harmonize reporting forms.

Finally, modification of the CAR construct to include the inclusion of a suicide gene, such as iCaspase9 [31], or surface tag such as EGFR [13, 32], which would allow ablation of the

CAR T cells in the event of serious toxicity, provide added safety measures. Clinical experience with the iCaspase9 suicide gene has been reported in patients who received a haplo-identical HCT followed by iCaspase9 modified T-cell adback in efforts to improve immune reconstitution and disease control [33]. As expected, nearly 50% of patients developed acute GVHD, which resolved within 24 hours of receiving the dimerizing agent. Importantly, in one patient with GVHD and CRS, the CRS symptoms resolved within two hours of receiving the dimerizer [33].

Modeling CAR-T-mediated neurotoxicity in Non-Human Primates

The neurologic toxicities associated with CAR T cells are numerous, and include aphasias, visual and auditory hallucinations, as well as encephalopathy and seizures. The most severe of the neurologic complication is rapid onset cerebral edema. This complication is often unresponsive to medical measures, and has led to several patient deaths. While CRS can be ameliorated with steroids, and the above mentioned anti-IL-6 (situximab) and anti-IL6 receptor (IL-6R) antibodies (tocilizumab), these agents do not appear to diminish the incidence and severity of neurotoxicity [20, 34].

One of the major barriers to understanding the molecular pathobiology of CAR-T-mediated neurotoxicity has been the lack of animal models that can faithfully recapitulate the multiple clinical and immunologic aspects of this syndrome. The resulting lack of knowledge about the risk factors, causes, prevention and treatment paradigms for neurotoxicity and how they impact CAR T-cell efficacy represents a critical barrier to the field. To address these critical questions, a group at the Seattle Children's Research Center and the Fred Hutchinson Cancer Research Center has developed the first non-human primate (NHP) model of CRS and neurologic toxicity, using CD20 CAR T cells in rhesus macaques (RMs) [35]. This model has allowed them to rigorously interrogate the clinical and immunologic toxicities associated with B-cell directed CAR T-cell therapy.

To create this model, they generated an RhCD20-χHIV lentivirus using a codon-optimized Rhesus CD20 (RhCD20) sequence. They synthesized a second generation 4-1BB:zeta CD20CAR construct that encodes a Leu16 (murine anti-human CD20) scFv fused to a human IgG4 CH2-CH3 hinge, a CD28 transmembrane domain, a 4-1BB (CD137) co-stimulatory domain and CD3ζ, followed by a Thoseaasigna virus 2A (T2A) peptide and a truncated epidermal growth factor receptor (EGFRt). They incorporated this expression cassette into either a SIN-HIV-1- or a SIV-based lentiviral vector and prepared either χHIV- or SIV-based lentiviruses [36, 37]. They generated the CD20 CAR-expressing SIV lentivirus by four plasmid (the SIV Gag/Pol plasmid, SIV Rev/Tat plasmid, VSV-G envelope plasmid, and the CD20 CAR-expressing SIV-vector plasmid) co-transfections of 293T cells and concentrated lentivirus-containing supernatants by ultracentrifugation. Rhesus macaque T cells were then transduced and expanded with the resulting virus, and CD20-specific CAR T cells were then infused into recipients after pre-conditioning with cyclophosphamide, at a dose of 0.6–1.2 × 107 cells/kg. They observed CD20-specific CAR T cells expansion in NHPs, with peak CAR T-cell levels occurring 7 to 8 days following CAR T-cell infusion. They observed elevated serum levels of multiple cytokines and disproportionately high concentrations of several cytokines in the cerebrospinal fluid (CSF): Neurotoxicity was

associated with encephalitis, characterized by the accumulation of both CAR and non-CAR T cells in the CSF and brain parenchyma. These results thus suggest that neurotoxicity is associated with a complex program of immune activation, which includes multiple cellular and soluble mediators. This work has thereby identified two major elements that contribute to CAR T cell-mediated neurotoxicity: (1) an increase in multiple cytokines in the CSF compared to the serum and (2) the development of encephalitis, in which both CAR and non-CAR T cells accumulate in both the CSF and the brain.

The complexity of the inflammatory reaction that occurs during neurotoxicity challenges some of the previous theories concerning the mechanisms driving this process. These include the hypothesis that neurotoxicity in patients may be caused by occult CD19 antigen in the brain, resulting in antigen-specific CAR T infiltration. The observation that (1) a CD20 CAR construct also causes neurotoxicity; (2) that neither CD19 nor CD20 are expressed in the brain; and (3) that both CAR and non-CAR T cells infiltrate the brain, do not support this hypothesis. Both patient data and data from NHP CAR-T neurotoxicity model also underscore the likely ineffectiveness of specifically targeting IL-6 to ameliorate neurotoxicity, given the multiplicity of cytokines that are elaborated during this process. Just as neutralizing a single cytokine may be incapable of preventing or treating neurotoxicity, the results from the NHP CAR-T model also suggest that blocking a single integrin, such as $\alpha 4\beta 1$ with natalizumab, may also be insufficient to prevent neurotoxicity.

The NHP model of CAR-T-mediated neurotoxicity thus points to a multi-modal program of inflammation that results in a soluble-and cell-mediated neuro-inflammatory syndrome, which includes both the accumulation of pro-inflammatory cytokines in the CSF and significant expansion and activation of both CAR and non-CAR T cells in the brain parenchyma. The NHP model of CAR-T cell neurotoxicity represents a platform for detailed investigation of the mechanisms driving neurotoxicity after adoptive cellular therapy and for the testing of therapeutic strategies to eliminate the clinical complications of this syndrome.

Using CRISPR to build a more powerful CAR

CAR T cells are currently generated using randomly integrating vectors, including γ-retroviral vectors, lentiviral vectors and DNA transposons, to insert the CAR cDNA in the T cell genome [38]. These vectors have all been effective, but this approach results in variegated gene expression owing to chromosomal position effects. The emergence of targeted nucleases, including zinc-finger nucleases, transcription activator-like effector nucleases (TALENs) and CRISPR/Cas9, provides a new means to specifically disrupt endogenous genes or target transgene delivery to chosen locations [39–42]. CRISPR/Cas9 is a particularly versatile and operationally simple system, requiring the transient expression of a nuclease and a guide RNA to induce double-strand DNA breaks at the targeted site [43].

Taking advantage of this tool, Eyquem, Mansilla-Soto *et al.* inserted the CAR cDNA at different genomic locations, placing CAR transcription under the control of promoters of different strengths [44]. As expected, CAR expression in human peripheral blood T cells showed consistent and reproducible levels of cell surface expression, reflected in a narrow peak of expression by FACS analysis. In contrast, the conventionally generated T cells

showed variable expression, spanning 2 to 3 logs within a typical transduced cell population. The edited CAR T cell populations not only attained greater consistency in CAR expression, but displayed greater potency when the CAR coding sequence was inserted into one locus, the T cell receptor α locus (*TRAC*), when CAR expression was it under the transcriptional control of endogenous regulatory elements but not exogenous promoters. Remarkably, other integration sites or other promoters, which resulted in either lower or higher baseline CAR expression, did not display increased T cell potency in the NALM/6 stress test, a murine ALL model used to measure and compare T cell potencies [45]. Further analyses established that *TRAC*-CAR T cells were far less exhausted than conventional T cells after 17 days. The latter persisted in tumor-bearing mice but were unable to reject the tumor. In vitro studies analyzing CAR expression following contact with antigen revealed a new aspect of CAR biology. *TRAC*-encoded CARs are internalized and degraded in the hours following exposure to antigen, in contrast to retrovirally encoded CAR, which in a large fraction of T cells was expressed at a higher level than *TRAC*-CAR and only marginally internalized. The most striking difference in CAR expression, however, occurred in the next 24 hours in the kinetics of CAR re-expression, which was faster and greater when the CAR was transcribed off a powerful enhancer/promoter such as EF1α or the Moloney murine leukemia virus long terminal repeat. These findings support a model of CAR T cell exhaustion whereby the *TRAC*-encoded CAR is expressed at an optimal level, affording efficient antigen recognition and subsequent CAR internalization; CAR re-expression, depending on de novo translation and hence on CAR transcription, replenishes cell surface CAR expression with optimal kinetics. Stronger promoters that yield higher baseline expression or faster (premature) CAR re-expression, however, accelerate T cell exhaustion by not providing the T cells a rest before re-engaging target. This model suggests that uninterrupted serial killing, which is favored by high CAR expression, is detrimental to the overall anti-tumor response by accelerating T cell exhaustion over time.

These findings have several clinical implications. The first concerns T cell dosing. These findings on the regulation of CAR expression imply that a large fraction of conventionally generated CAR T cells are at high risk of accelerated exhaustion and thus ineffective and dispensable. The engineering of more functional T cells will reduce the T cell dose and scale down manufacturing parameters. The second concerns CAR toxicity. Since CRS and neurotoxicity are associated with high T cell levels and their peak expansion, one may predict that *TRAC*-CAR T cells administered in lower dose will generate milder toxicities. This hypothesis remains to be demonstrated. We are presently scaling up the cGMP manufacture of TRAC-CAR T cells to test their safety and efficacy in a clinical trial where responses and toxicities could be compared to those encountered with current CAR therapies. The third is that *TRAC*-CAR T cells, which lack TCR expression as a consequence of TCR disruption, are a potential source for off-the-shelf T cells endowed with superior functional properties.

Conclusions

CD19-specific CAR T-cell immunotherapy has shown significant anti-tumor activity in the treatment of high-risk relapsed and refractory leukemias and lymphomas, and has revolutionized the treatment landscape for patients with advanced lymphoid malignancies.

This cellular therapy has remarkable efficacy, even in highly chemotherapy-resistant tumors, with response rates up to 90% for patients with relapsed B-cell ALL and greater than 60% for patients with relapsed NHL. However manipulating the immune system is also associated with unique and potentially life-threatening toxicities. With the recent approval by the US FDA of CD19 CAR T cells for ALL and NHL, there will be expanded use of these therapies in many clinical centers, including several without prior experience with CAR T cells. Successful implementation of CAR T therapy in the clinical setting will require an understanding of potential complications such as CRS and neurotoxicity, as well as clear guidelines for the management of these toxicities. The field of engineered T cells is rapidly evolving with current and future clinical trials exploring new targets, as well as novel approaches to increase potency and safety, including the use of suicide genes and gene editing.

Acknowledgments

FINANCIAL SUPPORT

This research was supported in part by NIH/NCI Cancer Center Support Grant P30 CA008748 (MAP, MS). The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health.

References

1. Brentjens RJ, Davila ML, Riviere I, et al. CD19-targeted T cells rapidly induce molecular remissions in adults with chemotherapy-refractory acute lymphoblastic leukemia. Sci Transl Med. 2013; 5:177ra138.
2. Brentjens RJ, Riviere I, Park JH, et al. Safety and persistence of adoptively transferred autologous CD19-targeted T cells in patients with relapsed or chemotherapy refractory B-cell leukemias. Blood. 2011; 118:4817–4828. [PubMed: 21849486]
3. Grupp SA, Kalos M, Barrett D, et al. Chimeric antigen receptor-modified T cells for acute lymphoid leukemia. N Engl J Med. 2013; 368:1509–1518. [PubMed: 23527958]
4. Curran KJ, Brentjens RJ. Chimeric antigen receptor T cells for cancer immunotherapy. J Clin Oncol. 2015; 33:1703–1706. [PubMed: 25897155]
5. Davila ML, Bouhassira DC, Park JH, et al. Chimeric antigen receptors for the adoptive T cell therapy of hematologic malignancies. Int J Hematol. 2014; 99:361–371. [PubMed: 24311149]
6. Kershaw MH, Westwood JA, Darcy PK. Gene-engineered T cells for cancer therapy. Nat Rev Cancer. 2013; 13:525–541. [PubMed: 23880905]
7. Park JH, Geyer MB, Brentjens RJ. CD19-targeted CAR T-cell therapeutics for hematologic malignancies: interpreting clinical outcomes to date. Blood. 2016; 127:3312–3320. [PubMed: 27207800]
8. Maude SL, Teachey DT, Porter DL, Grupp SA. CD19-targeted chimeric antigen receptor T-cell therapy for acute lymphoblastic leukemia. Blood. 2015; 125:4017–4023. [PubMed: 25999455]
9. Tasian SK, Gardner RA. CD19-redirected chimeric antigen receptor-modified T cells: a promising immunotherapy for children and adults with B-cell acute lymphoblastic leukemia (ALL). Therapeutic advances in hematology. 2015; 6:228–241. [PubMed: 26425336]
10. Maude SL, Frey N, Shaw PA, et al. Chimeric antigen receptor T cells for sustained remissions in leukemia. N Engl J Med. 2014; 371:1507–1517. [PubMed: 25317870]
11. Davila ML, Riviere I, Wang X, et al. Efficacy and toxicity management of 19-28z CAR T cell therapy in B cell acute lymphoblastic leukemia. Sci Transl Med. 2014; 6:224ra225.
12. 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; 385:517–528. [PubMed: 25319501]

13. Gardner RA, Finney O, Annesley C, et al. Intent-to-treat leukemia remission by CD19 CAR T cells of defined formulation and dose in children and young adults. Blood. 2017; 129:3322–3331. [PubMed: 28408462]
14. Kochenderfer JN, Dudley ME, Kassim SH, et al. Chemotherapy-refractory diffuse large B-cell lymphoma and indolent B-cell malignancies can be effectively treated with autologous T cells expressing an anti-CD19 chimeric antigen receptor. J Clin Oncol. 2015; 33:540–549. [PubMed: 25154820]
15. Turtle CJ, Hanafi LA, Berger C, et al. Immunotherapy of non-Hodgkin's lymphoma with a defined ratio of CD8+ and CD4+ CD19-specific chimeric antigen receptor-modified T cells. Sci Transl Med. 2016; 8:355ra116.
16. Gardner R, Finney O, Smithers H, et al. CD19CAR T Cell Products of Defined CD4:CD8 Composition and Transgene Expression Show Prolonged Persistence and Durable MRD-Negative Remission in Pediatric and Young Adult B-Cell ALL. Blood. 2016; 128:219–219.
17. Sadelain M, Riviere I, Riddell S. Therapeutic T cell engineering. Nature. 2017; 545:423–431. [PubMed: 28541315]
18. Turtle CJ, Hay KA, Hanafi LA, et al. Durable Molecular Remissions in Chronic Lymphocytic Leukemia Treated With CD19-Specific Chimeric Antigen Receptor-Modified T Cells After Failure of Ibrutinib. J Clin Oncol. 2017; 35:3010–3020. [PubMed: 28715249]
19. Ghosh A, Politikos I, Perales MA. Stop and go: hematopoietic cell transplantation in the era of chimeric antigen receptor T cells and checkpoint inhibitors. Curr Opin Oncol. 2017
20. Lee DW, Gardner R, Porter DL, et al. Current concepts in the diagnosis and management of cytokine release syndrome. Blood. 2014; 124:188–195. [PubMed: 24876563]
21. Riddell SR, Jensen MC, June CH. Chimeric antigen receptor–modified T cells: clinical translation in stem cell transplantation and beyond. Biol Blood Marrow Transplant. 2013; 19:S2–5. [PubMed: 23085599]
22. Perales MA, Sauter CS, Armand P. Fast Cars and No Brakes: Autologous Stem Cell Transplantation as a Platform for Novel Immunotherapies. Biol Blood Marrow Transplant. 2016; 22:17–22. [PubMed: 26485445]
23. Kenderian SS, Porter DL, Gill S. Chimeric Antigen Receptor T Cells and Hematopoietic Cell Transplantation: How Not to Put the CART Before the Horse. Biol Blood Marrow Transplant. 2017; 23:235–246. [PubMed: 27638367]
24. Porter DL, Levine BL, Kalos M, Bagg A, June CH. Chimeric antigen receptor-modified T cells in chronic lymphoid leukemia. N Engl J Med. 2011; 365:725–733. [PubMed: 21830940]
25. Hay KA, Hanafi LA, Li D, et al. Kinetics and Biomarkers of Severe Cytokine Release Syndrome after CD19 Chimeric Antigen Receptor-modified T Cell Therapy. Blood. 2017
26. Orlowski RJ, Porter DL, Frey NV. The promise of chimeric antigen receptor T cells (CARTs) in leukaemia. Br J Haematol. 2017; 177:13–26. [PubMed: 27977050]
27. Teachey DT, Lacey SF, Shaw PA, et al. Identification of Predictive Biomarkers for Cytokine Release Syndrome after Chimeric Antigen Receptor T-cell Therapy for Acute Lymphoblastic Leukemia. Cancer discovery. 2016; 6:664–679. [PubMed: 27076371]
28. Bhoj VG, Arhontoulis D, Wertheim G, et al. Persistence of long-lived plasma cells and humoral immunity in individuals responding to CD19-directed CAR T-cell therapy. Blood. 2016; 128:360–370. [PubMed: 27166358]
29. Neelapu SS, Tummala S, Kebriaei P, et al. Chimeric antigen receptor T-cell therapy – assessment and management of toxicities. Nature reviews Clinical oncology. 2017
30. Maus MV, Nikiforow S. The Why, what, and How of the New FACT standards for immune effector cells. Journal for immunotherapy of cancer. 2017; 5:36. [PubMed: 28428885]
31. Zhou X, Brenner MK. Improving the safety of T-Cell therapies using an inducible caspase-9 gene. Exp Hematol. 2016; 44:1013–1019. [PubMed: 27473568]
32. Wang X, Chang WC, Wong CW, et al. A transgene-encoded cell surface polypeptide for selection, in vivo tracking, and ablation of engineered cells. Blood. 2011; 118:1255–1263. [PubMed: 21653320]
33. Zhou X, Dotti G, Krance RA, et al. Inducible caspase-9 suicide gene controls adverse effects from alloreplete T cells after haploidentical stem cell transplantation. Blood. 2015; 125:41034113.

34. Brudno JN, Kochenderfer JN. Toxicities of chimeric antigen receptor T cells: recognition and management. Blood. 2016; 127:3321–3330. [PubMed: 27207799]
35. Taraseviciute A, Kean L, Jensen MC. Creation of the First Non-Human Primate Model That Faithfully Recapitulates Chimeric Antigen Receptor (CAR) T Cell-Mediated Cytokine Release Syndrome (CRS) and Neurologic Toxicity Following B Cell-Directed CAR-T Cell Therapy. Blood. 2016; 128:651–651.
36. Uchida N, Hargrove PW, Lap CJ, et al. High-efficiency transduction of rhesus hematopoietic repopulating cells by a modified HIV1-based lentiviral vector. Mol Ther. 2012; 20:1882–1892. [PubMed: 22871664]
37. Hanawa H, Hematti P, Keyvanfar K, et al. Efficient gene transfer into rhesus repopulating hematopoietic stem cells using a simian immunodeficiency virus-based lentiviral vector system. Blood. 2004; 103:4062–4069. [PubMed: 14976042]
38. Riviere I, Sadelain M. Chimeric Antigen Receptors: A Cell and Gene Therapy Perspective. Mol Ther. 2017; 25:1117–1124. [PubMed: 28456379]
39. Urnov FD, Rebar EJ, Holmes MC, Zhang HS, Gregory PD. Genome editing with engineered zinc finger nucleases. Nat Rev Genet. 2010; 11:636–646. [PubMed: 20717154]
40. Joung JK, Sander JD. TALENs: a widely applicable technology for targeted genome editing. Nat Rev Mol Cell Biol. 2013; 14:49–55. [PubMed: 23169466]
41. Carroll D. Genome engineering with targetable nucleases. Annu Rev Biochem. 2014; 83:409–439. [PubMed: 24606144]
42. Sander JD, Joung JK. CRISPR-Cas systems for editing, regulating and targeting genomes. Nat Biotechnol. 2014; 32:347–355. [PubMed: 24584096]
43. Doudna JA, Charpentier E. Genome editing. The new frontier of genome engineering with CRISPR-Cas9. Science. 2014; 346:1258096. [PubMed: 25430774]
44. Eyquem J, Mansilla-Soto J, Giavridis T, et al. Targeting a CAR to the TRAC locus with CRISPR/Cas9 enhances tumour rejection. Nature. 2017; 543:113–117. [PubMed: 28225754]
45. Zhao Z, Condomines M, van der Stegen SJ, et al. Structural Design of Engineered Costimulation Determines Tumor Rejection Kinetics and Persistence of CAR T Cells. Cancer Cell. 2015; 28:415–428. [PubMed: 26461090]