Review article

CAR-T Immunotherapy Limitations and Advancements for Relapse and Refractory Pediatric B-Cell Malignancies

Diana Woller[1,2*], Brenda Barnes[1,3], Joel E. Mortensen[1,4]

Medical Laboratory Sciences Program, College of Allied Health Science, University of Cincinnati, Cincinnati, Ohio[1]

Cell Therapy and Regenerative Medicine, University of Utah, Salt Lake City, Utah[2]

Allen College-UnityPoint Health, Waterloo, Iowa[3]

Cincinnati Children's Hospital Medical Center, Cincinnati,Ohio[4]

Chimeric antigen receptors (CARs) are genetically engineered, T-lymphocyte receptors that target hematological cancer cells and solid tumors. CAR-T cell immunotherapies for B-Cell malignancies target CD19 positive cells and have shown dramatic results in treating pediatric patients since becoming licensed as tisagenlecleucel by the US Food and Drug Administration in 2018. Tisagenlecleucel is used to treat relapsed or refractory B-cell acute lymphoblastic leukemia (ALL) and large diffuse B-cell lymphomas (DLBCL) in children and young adults up to age 25. While CAR-T immunotherapies have shown continued promise, crucial limitations are being investigated to improve anti-CD19 CAR-T toxicity, off-target events, bridging and dual treatment requirements, and manufacturing capabilities. This review examines the current limitations and advancements of anti-CD19 CAR-T immunotherapy for pediatric B-cell malignancies focusing on relevant improvement strategies for engineering, efficacy, and patient safety.

Key words: Anti-CD19 CAR-T cells; pediatric acute lymphoblastic leukemia; pediatric diffuse large B-cell lymphoma; replapsed/refractory B-cell malignancies; limitations of Anti-CD19CAR-T cell therapy; anti-CD19 CAR-T manufacturing advancements; cytokine release syndrome; neurotoxicity; immunotherapy

Introduction

Chimeric antigen receptor T-cell (CAR-T) immunotherapies have been a revolutionary advancement in treating various cancers, specifically in hematological malignancies. There are currently over 500 active CAR-T clinical trials worldwide and 6 Food and Drug Administration (FDA) approved commercial products since 2017.[1] Anti-CD19 CAR-T cell therapy in the treatment of B-cell malignancies has been significantly successful in providing long-term remission for patients up to age 25.[2] Tisagenlecleucel was the first FDA approved CD19- CAR-T therapy and is the new standard of care for relapsed and pediatric refractory to young adult B-cell acute lymphoblastic leukemia (B-Cell ALL).[3] Since FDA approval in 2018, tisagenlecleucel has treated over 5,400 pediatric patients.[2] As CAR-T approvals expand and production technology advances, increasingly more patients are anticipated to receive targeted immunotherapies.

Prior to CAR-T advancements, patients with B-cell malignancies received multiple lines of intensive myeloablative chemotherapy regimens. While complete remission (CR) is observed in nearly 80% of cases with standard

Accepted: August 17, 2022

*Corresponding author: Diana Woller, Email: diana.woller@hsc.utah.edu

treatment, many patients end up with relapsed and refractory disease within 5 years.[4] Intensive chemotherapy requires the destruction of cancer and healthy cells, which is highly taxing on patients. Historically, allogeneic hemopoietic stem cell transplantation (allo-HSCT) was the only curative option to overcome relapse.[5] However, the risk of graft-versus-host disease (GVHD) and a high relapse rate has remained a continued challenge in treating residual disease. Ultimately this standard of care requires continued, life-long treatment.

Within the last 5 years, however, CAR-T immunotherapy has shown promise in the field of oncology with novel technology to target cancer-antigens. Pediatric patients with relapsed and refractory B-cell malignancies who have undergone CD19-targeted CAR-T immunotherapy show 70-90% CR.[6] These results vary between institution due to the dual therapies administered to prevent toxicity and infection following treatment.[6] CR has been most effectively reported in pediatric B-cell ALL compared juvenile cases and diffuse large B-cell lymphoma.[4] Cytotoxic effects, such as cytokine release syndrome (CRS) have been observed in 77% of patients treated with tisagenlecleucel.[7] CRS is graded by severity and can be potentially life-threatening.

While promising, these novel targeted immunotherapies have critical limitations that are currently being investigated. These limitations include severe cytotoxicity such as CRS and neurotoxicity, off-target CAR-T functionality, and manufacturing constraints. This review article discusses the current limitations and advancement strategies for engineering safe and effective CD19 CAR-T immunotherapy for pediatric relapsed and refractory B-cell malignancies.

Background

Pediatric B-cell malignancies include B-cell ALL and DLBCL. B-cell ALL is the result of rapid growing lymphoblasts developing from B-cell precursors which target the immune system.[4] Eighty percent of B-cell ALL patients are infants and juveniles.[4] DLBCL is the most common form of non-Hodgkin lymphoma (NHL) and represents 10-20% of pediatric NHL cases.[4] DLBCL arises from the malignant development of B-cells typically occurring in lymph nodes.[4] In both forms of these B-cell malignancies, the CD19 molecule, which is expressed on all B-cell lymphocytes during each stage of differentiation, acts as a key target for CAR-T targeting agents. The generation of the synthetic T-cell receptors attempt to optimize patient outcomes by enriching naïve, memory, and stem-cell like phenotypes.[8]

CARs are synthetic receptors made with four components including the antigen binding domain, the hinge region, a transmembrane domain, and intracellular signaling domains.[8] The antigen binding domain targets the extracellular cancer surface antigens. Anti-CD19 CAR-T cells are manufactured by isolating a patient's T-cells *ex-vivo* from autologous peripheral blood mononuclear cells (PBMNCs). The CAR gene is inserted via lentiviral transduction, along with the anti-CD19 protein. The cells are then expanded, tested for safety, purity, and potency, and then cryopreserved for later transplantation.[9,10,11] Within a few weeks following transplantation, CAR-T cells will multiply in the body, detect and target CD19+ B-cells for destruction.[3]

Following transplantation, patients are at increased risk of CRS, infection, and neurotoxicity. As CD19 CAR-T cells activate and proliferate, other cell types are activated resulting in high serum concentrations of pro-inflammatory cytokines.[12] Multiple grades of CRS have been reported in 56-100% of pediatric B-cell ALL treatment.[13] Serious adverse reactions can occur within hours of transplantation. Due to these immune reactions, dual treatments are often necessary to limit side-effects, including the use of interleukin-6 (IL-6) corticosteroids.[9] However, due to institution-specific guidelines on treating CRS, present data shows heterogeneous results, thus standard guidelines have not been

established. Current research suggests CAR-T dose compared to bone marrow tumor burden have a positive correlation to CRS severity.[12] Higher doses may not be more effective, as previously thought.[12,13,15,16] However, ongoing research is working to improve CRS symptoms.

Along with the risk of toxicity and infection, the manufacturing process for CD19 CAR-T cells presents limitations. CAR-T cell manufacturing is costly and is limited to sponsor specific manufacturing sites, which significantly reduces patient accessibility. Many patients must travel to prominent cancer facilities to receive treatment. The manufacturing process is complex and requires a pre-determined number of cells for expansion. Pediatric patients with B-cell malignancies already have limited T-cell production, thus manufacturing reproducibility varies by each individual patient. [16,17] The quality in starting material is hard to determine until transduction and expansion occur.[17]

Anti-CD-19 CAR-T cell engineering and manufacturing strategies

Since the advent of CAR-T immunotherapies, there have been four generations of implemented CAR-T technology. The first generation was limited to signaling capacity and inability for sustained cytokine release.[18] The second generation was modified to have additional costimulatory signaling domains which enhanced survival and expansion of activated T-cells. The third generation expanded by having more effective costimulatory domains.[18] The fourth generation has seen further genetic modification to redirect T-cells for universal cytokine-mediated killing (TRUCKs).[18] Finally, the fifth and current generation contains one extra intracellular domain.[19] The evolution of CAR-T generations has focused on improving T-cell activation and costimulatory domains following antigen recognition.[18] While rapid new developments are being investigated, increasing patient numbers versus engineering and manufacturing capabilities poses limitations, specifically for pediatric patient populations.

The starting material for anti-CD19 CAR-T cells is collected via autologous PBMNC apheresis from the patient. For standard hematopoietic stem cell collections, patients receive a mobilization regimen prior to collection to optimize the available stem cells. However, CAR-T cell collections do not require mobilization drugs, therefore the number of CD3+ lymphocytes collected is dependent on the individual patient's disease state and prior chemotherapy bridged with CAR-T therapy.[20] Patient's receiving CAR-T therapy must first undergo chemo-lymphodepletion, which supresses the immune system, allowing for increased CAR-T cell persistence. Most importantly, lymphodepletion requires a favorable microenvironment for CAR-T cells by eliminating homeostatic cytokines.[20]

Genetic factors, medical history, age, and demographics vary from patient to patient; thus, pre-determining treatment efficacy is difficult. Data collected from pediatric T-cell collections shows 77% of patients reaching the collection requirement for starting materials, while 97% of patients only reach the minimum cell count requirement.[20] Factors such as poor collection efficiency or granulocyte and monocyte contamination contribute to these figures.[20] Granulocytes and monocytes pose risk of inhibiting the T-cell enrichment process. The starting leukapheresis material affects downstream anti-CD19 CAR-T production, thus it is critical for the manufacturing facilities to set cell count starting requirements to optimize downstream T-cell isolation processes. However, these collection requirements may be under- or over-estimated between patients, resulting in insufficient collections or excess material which is discarded.

Following cell collection, products are shipped to the manufacturing facility. The cells must maintain stable temperature as deviations will affect the cell's viability. To mitigate facility limitations, cells shipped for tisagenlecleucel manufacturing are cryopreserved and thawed for production, which has been shown to significantly reduce cellular viability.[20] Currently, there is only one facility

in the United States that manufactures tisagenlecleucel, located in Morris Plains, New Jersey, but other international facilities are being developed.[17] CAR-T cells are considered phase 1 investigational drugs; therefore, the FDA sets regulations for good manufacturing practices (GMP).[21] These regulations include guidance for personnel, quality control (QC), facilities and equipment, manufacturing and record keeping, laboratory controls, packaging, labeling, distribution, as well as cell and gene specific requirements.[21] These regulations ensure purity, potency, and safety of the final product. Due to the stringent nature of FDA and other regulatory adherence, expanding facilities is a complex, costly process.

The manufacturing process requires the use of unidirectional and multidirectional flow of materials and staff to limit risk of contamination or cross-contamination.[17] Advanced instrumentation is required for each facility manufacturing anti-CD19 CAR-T cells. The starting material is first washed and fractionated; CD3+ T-cells are selected using an antibody-conjugated magnetic bead process, followed by T-cell activation with the 4-1BB costimulatory domain and anti-CD19 CAR complimentary DNA (cDNA) transduction with retroviral vectors and mRNA electroporation.[22] The domain, 4-1BB, is expressed on activated T-cells and natural killer (NK) cells and acts as an inducible costimulatory receptor. In the malignant B-cell environment, 4-1BB restores effector functions of dysfunctional T-cells, therefore its use in CAR-T manufacturing has been promising.[22]

Antigen-presenting cells, such as dendritic cells and artificial antigen-presenting cells, are used to stimulate the expansion of CAR-T cells.[17] *Ex vivo* expansion of T-cells is only accomplished with sustained activation.[22] This step is the area of research focused on producing more effective CAR-T generations. Following activation and expansion, the anti-CD19 CAR-T cells are then tested and ensured for purity, potency, and safety prior to shipment back to the treating facility.

Critical quality control points are essential for maintaining and producing quality product. It is important that the starting material is properly enriched to eliminate unwanted cells for selection. CAR expression indicates efficacy of stable expression post-transplantation. The ratio of CD4+ and CD8+ T-cells must be maintained to prevent downstream CRS toxicity.[23] Each step in the engineering and manufacturing process must be evaluated with checkpoints.

Cytokine release syndrome and neurotoxicity

CRS and neurotoxicity are noted as the most prominent and severe reactions following anti-CD19 CAR-T immunotherapy, caused by generalized immune system activation. The introduction of anti-CD19 CAR-T cells results in immune cell cross activation and systemic cytokine release.[24] Severe symptoms of CRS include, fever, rigors, hypotension, kidney failure, coagulation, hypoxia, and respiratory distress.[25] Neurological toxicities are observed as a separate symptom; however, cytokines have been implicated in causing neurotoxic symptoms.[25] Neurotoxic symptoms include headache, encephalopathy, delirium, tremors, peripheral neuropathy, seizures, and aphasia.[26]

The onset of CRS is observed by elevated levels of inflammatory cytokines such as interferon-gamma (IFN- γ), interleukin-6 (IL-6), interleukin-8 (IL-8), interleukin-10 (IL-10), and interleukin-12 (IL-12).[26] CRS is graded by severity on a scale of 1 to 4. CRS has historically been graded with variability among institutions, thus the data has been difficult to compare due to this unclear consensus. Various grading systems have been found to have overlapping symptomatology and described using differing terms.

In 2018, the American Society for Transplantation and Cellular Therapy (ASTCT) developed consensus recommendations to establish a grading system based on symptomatic algorithms.[25] The current, established grading system determined by ASTCT is as follows: CRS

grade 1 is noted as a mild reaction such as fever; grade 2 is noted with hypotension and mild hypoxia which is quickly resolved with non-steroid anti-inflammatory drugs (NSAIDs), and prophylactic medications; grade 3 is indicative of hypotension requiring vasopressors or clinical sequelae requiring prolonged treatments; and grade 4 indicates life-threatening symptoms requiring urgent intervention, organ failure, or ventilatory support.[25]

CRS symptoms are observed within the first 14 days following immunotherapy initiation. Data from patients treated with tisagenlecleucel shows 77% of patients experience some grade of CRS within the first 3 days of treatment, and 48% of patients experience CRS graded ≥ 3 within the first 8 days of treatment.[27] Due to this, patients are closely monitored for 4 weeks following CAR-T transplantation. Tocilizumab, which blocks the inflammatory IL-6 protein, is the gold standard for treatment of CRS. [26,28] Tocilizumab is a human IL-6 receptor monoclonal antibody originally approved to treat rheumatologic diseases. In 2017, the drug was approved by the FDA to treat CRS in patients 2 years of age and older.[29]

Tocilizumab has been shown to be effective in treating COVID-19 symptoms, which has resulted in severe shortages of the drug.[30] To mitigate these unexpected drug shortages, current studies are exploring the use of anakinra, a recombinant IL-1 receptor antagonist, which is also an FDA approved drug used for rheumatologic disease in treating pediatric CRS and immune effector cell-associated neurotoxicity syndrome (ICANS).[31]

ICANS represents a more diverse symptomatic profile than CRS. Early signs of ICANS are indicated by expressive speech patterns, tremors, attention impairment and lethargy.[25] Most notably, pediatric patients with symptoms of expressive aphasia have shown to be a hallmark of ICANS diagnoses.[25] Like CRS grading, ICANS is graded on a severity scale from 1 to 4. Neurological and encephalopathy assessments are evaluated twice daily to monitor neurotoxicological events following anti-CD19 CAR-T transplantation. Pediatric ICANS is reported in conjunction with CRS symptoms, at a median of 2 days following CRS onset.[32] While ICANS symptoms are generally reversible, 47% of pediatric patients impacted with ICANS following anti-CD19 CAR-T therapy required admission to the intensive care unit (ICU).[33] Recovery from severe ICANS ranges from 27-75 days.[34] Risk of developing ICANS has been correlated with early CAR-T expansion *in vivo,* however more research is needed.[35]

Anti-CD19 CAR-T cell efficiacy

Despite the success of anti-CD19 CAR-T immunotherapy for pediatric patients, poor clearance of malignant cells and prominent adverse events complicate the therapy's efficacy and safety. There are currently approximately 200 clinical trials studying pediatric anti-CD19 CAR-T immunotherapies.[35] Many of these studies focus on improving the pharmacokinetics and safety of anti-CD19 immunotherapies and optimizing chemotherapy and bridging therapies.

The efficacy of 5th generation anti-CD19 CAR-T therapy requires pre-treatment chemotherapy and bridging therapy, however there are few patients who receive treatment without bridging. Bridging therapy, which is determined by the treating facility, greatly increases the efficacy of anti-CD19 CAR-T activity. Bridging therapy involves high-dose chemotherapy, followed by low dose chemotherapy, radiation therapy and anti-CD19 CAR-T-transplantation coupled with symptomatic treating agents.[15] As previously discussed, this process eliminates circulating lymphocytes and cellular contaminants and reduces the risk of disease progression prior to transplantation. Due to the complexity of bridging therapy, current studies aim to optimize treatment approaches, as continued chemotherapy and radiation can be taxing on the patient. The lymphodepleting bridging therapy occurs for 4 weeks prior to transplantation.[36] Diagnostic analysis of minimal residual disease (MRD) is

currently not a required measurement prior to T-cell collection or CAR-T manufacturing; however, evidence supports MRD measurements correlate with the probability of CAR-T efficacy.[36]

Since original licensing, the five-year tisagenlecleucel treatment data shows a 55% survival rate of 79 pediatric and young adult B-cell ALL patients.[27] Relapse-free survival data indicates that 44% of patients remained in remission for a median of 43 months.[27] Prior to anti-CD19 CAR-T immunotherapy, the five-year survival rate for pediatric and young adult patients was 10%.[27] Reported efficacy analysis was based on determination of the rate and duration of response. Dual treatment tocilizumab indicates 298% higher area under the curve (AUC) from day 0 to day 28 compared to patients who only received corticosteroids. [27] Prior HSCT was noted in 47% of treated pediatric patients.[27]

CAR engineering is specifically important in understanding efficacy. As anti-CD19 CAR-T cell generation evolves, strategies to engineer enhanced antigen recognition with minimal immune activation remain the focus. Current research is evaluating the efficacy of bispecific ligand-binding domains in which two antigens activate the specific CAR.[37] This design shows promising evidence of eliminating antigen escape *in vivo*. However, off-target toxicity is still observed. Alternatively, molecular CAR expression and stability has been shown to correlate with alteration of the hinge and transmembrane domains.[37] Manipulation of the length of the hinge and transmembrane domains not only correlate with potency, but also significantly reduces neurotoxicity as observed in a phase I clinical trial.[38]

Conclusion

New strategies for anti-CD19 CAR-T development are rapidly expanding, as researchers and clinicians gain new insights on the efficacy and safety of design and administration. CAR-T cell therapy has significantly increased survival rates in relapsed and refractory pediatric B-cell malignancy as compared to conventional chemotherapy interventions.[4,8,27] Anti-CD19 CAR-T immunotherapy has greatly improved over the span of 10 years in clinical trials and the 5 years since FDA approval. Targeting more specific molecular and cellular pathways while limiting off-target toxicity remains the most prominent improvement strategy. As anti-CD19 CAR engineering improves, manufacturing efforts must also expand to extend patient access. Examining MRD diagnostic data to develop patient specific CARs has shown promise in the development of more individuals CAR-T immunotherapies.[36]

Future pediatric treatment strategies aim to develop anti-CD19 CAR-T cells from healthy donors to eliminate risk of disease burden cells affecting efficacy.[39] Patients prior treatments limit the effectiveness of the CAR-T cell product due to T-cell dysfunction. Allogeneic anti-CD19 CAR-T cell therapy has been shown to be effective in adult DLBCL, Chronic Lymphocytic Leukemia (CLL), and Mantle Cell Lymphoma (MCL).[40] Developing allo-CAR-T cells come with its own set of complications, including donor recruitment strategies, human leukocyte antigen (HLA) matching, and limiting the risk for GVHD in the recipient.

Ultimately, current anti-CD19 CAR-T cell immunotherapies has greatly reduced relapse and refractory disease in pediatric B-cell cancers. As the understanding of malignant-burdened molecular pathways expands, improved generations of CARs will advance. Future directions aim to suppress cytotoxic effects and reduce required bridging therapies and dual treatments.

References

1. Albinger N, Hartmann J, Ullrich E. Current status and perspective of CAR-T and CAR-NK cell therapy trials in Germany. Gene Ther. 2021 Sep;28(9):513-527. doi: 10.1038/s41434-021-00246-w. Epub 2021 Mar 22. PMID: 33753909; PMCID: PMC8455322.
2. Maude SL, Laetsch TW, Buechner J, Rives S, Boyer M, Bittencourt H, Bader P, Verneris MR, Stefanski HE, Myers GD, Qayed M, De Moerloose B, Hiramatsu H, Schlis K, Davis KL, Martin PL, Nemecek ER, Yanik GA, Peters C, Baruchel A, Boissel N, Mechinaud F, Balduzzi A, Krueger J, June CH, Levine BL, Wood P, Taran T, Leung M, Mueller KT, Zhang Y, Sen K, Lebwohl D, Pulsipher MA, Grupp SA. Tisagenlecleucel in children and young adults with B-cell lymphoblastic leukemia. N Engl J Med. 2018 Feb 1;378(5):439-448. doi: 10.1056/NEJMoa1709866. PMID: 29385370; PMCID: PMC5996391.
3. Pasquini MC, Hu ZH, Curran K, Laetsch T, Locke F, Rouce R, Pulsipher MA, Phillips CL, Keating A, Frigault MJ, Salzberg D, Jaglowski S, Sasine JP, Rosenthal J, Ghosh M, Landsburg D, Margossian S, Martin PL, Kamdar MK, Hematti P, Nikiforow S, Turtle C, Perales MA, Steinert P, Horowitz MM, Moskop A, Pacaud L, Yi L, Chawla R, Bleickardt E, Grupp S. Real-world evidence of tisagenlecleucel for pediatric acute lymphoblastic leukemia and non-Hodgkin lymphoma. Blood Adv.2020 Nov 10;4(21):5414-5424. doi: 10.1182/bloodadvances.2020003092. Erratum in: Blood Adv. 2021 Feb 23;5(4):1136. Erratum in: Blood Adv. 2022 Mar 22;6(6):1731. PMID: 33147337; PMCID: PMC7656920.
4. Buechner J, Caruana I, Künkele A, Rives S, Vettenranta K, Bader P, Peters C, Baruchel A, Calkoen FG. Chimeric antigen receptor T-cell therapy in paediatric B-cell precursor acute lymphoblastic leukaemia: curative treatment option or bridge to transplant? Front Pediatr. 2022 Jan 25;9:784024. doi: 10.3389/fped.2021.784024. PMID: 35145941; PMCID: PMC8823293.
5. Sermer D, Batlevi C, Palomba ML, Shah G, Lin RJ, Perales MA, Scordo M, Dahi P, Pennisi M, Afuye A, Silverberg ML, Ho C, Flynn J, Devlin S, Caron P, Hamilton A, Hamlin P, Horwitz S, Joffe E, Kumar A, Matasar M, Noy A, Owens C, Moskowitz A, Straus D, von Keudell G, Rodriguez-Rivera I, Falchi L, Zelenetz A, Yahalom J, Younes A, Sauter C. Outcomes in patients with DLBCL treated with commercial CAR T cells compared with alternate therapies. Blood Adv. 2020 Oct 13;4(19):4669-4678. doi: 10.1182/bloodadvances.2020002118. PMID: 33002134; PMCID: PMC7556134.
6. Wei G, Hu Y, Pu C, Yu J, Luo Y, Shi J, Cui Q, Wu W, Wang J, Xiao L, Wu Z, Huang H. CD19 targeted CAR-T therapy versus chemotherapy in re-induction treatment of refractory/relapsed acute lymphoblastic leukemia: results of a case-controlled study. Ann Hematol. 2018 May;97(5):781-789. doi: 10.1007/s00277-018-3246-4. Epub 2018 Feb 7. PMID: 29417201.
7. Terwilliger T, Abdul-Hay M. Acute lymphoblastic leukemia: a comprehensive review and 2017 update. Blood Cancer J. 2017 Jun 30;7(6):e577. doi: 10.1038/bcj.2017.53. PMID: 28665419; PMCID: PMC5520400.
8. Haen SP, Groh C, Schumm M, Backert L, Löffler MW, Federmann B, Faul C, Dörfel D, Vogel W, Handgretinger R, Kanz L, Bethge WA. Haploidentical hematopoietic cell transplantation using in vitro T cell depleted grafts as salvage therapy in patients with disease relapse after prior allogeneic transplantation. Ann Hematol. 2017 May;96(5):817-827. doi: 10.1007/s00277-017-2941-x. Epub 2017 Feb 28. PMID: 28247058.
9. Pulsipher MA. Are CAR T cells better than antibody or HCT therapy in B-ALL? Hematology Am Soc Hematol Educ Program. 2018 Nov 30;2018(1):16-24. doi: 10.1182/asheducation-2018.1.16. PMID: 30504287; PMCID: PMC6246000.
10. Murthy H, Iqbal M, Chavez JC, Kharfan-Dabaja MA. Cytokine release syndrome: current perspectives. Immunotargets Ther.

2019 Oct 29;8:43-52. doi: 10.2147/ITT. S202 015. PMID: 31754614; PMCID: PMC6825470.
11. Fujiwara K, Tsunei A, Kusabuka H, Ogaki E, Tachibana M, Okada N. Hinge and transmembrane domains of chimeric antigen receptor regulate receptor expression and signaling threshold. Cells. 2020 May 9;9 (5):1182. doi: 10.3390/cells9051182. PMID: 32397414; PMCID: PMC7291079.
12. Rossi J, Paczkowski P, Shen YW, Morse K, Flynn B, Kaiser A, Ng C, Gallatin K, Cain T, Fan R, Mackay S, Heath JR, Rosenberg SA, Kochenderfer JN, Zhou J, Bot A. Preinfusion polyfunctional anti-CD19 chimeric antigen receptor T cells are associated with clinical outcomes in NHL. Blood. 2018 Aug 23;132(8):804-814. doi: 10.1182/blood-2018-01-828343. Epub 2018 Jun 12. PMID: 29895668; PMCID: PMC6107882.
13. Nickolay L, Mock U, Phillip B, Cheung GWK, Zhan H, Peggs K, Johnston I, Kaiser A, Pule M, Thrasher A, & Qasim W. Automated lentiviral transduction of T cells with CARS using the CliniMACS prodigy. Molecular Therapy: J of the Am Soc of Gene Therapy. 2016 (24) S180–S181. https://doi.org/10.1016/s1525-0016(16)33264-6
14. Jackson Z, Roe A, Sharma AA, Lopes FBTP, Talla A, Kleinsorge-Block S, Zamborsky K, Schiavone J, Manjappa S, Schauner R, Lee G, Liu R, Caimi PF, Xiong Y, Krueger W, Worden A, Kadan M, Schneider D, Orentas R, Dropulic B, Sekaly RP, de Lima M, Wald DN, Reese JS. Automated manufacture of autologous CD19 CAR-T cells for treatment of non-hodgkin lymphoma. Front Immunol. 2020 Aug 7;11: 1941. doi: 10.3389/fimmu.2020.01941. PMID: 32849651; PMCID: PMC7427107.
15. Sheth VS, Gauthier J. Taming the beast: CRS and ICANS after CAR T-cell therapy for ALL. Bone Marrow Transplant. 2021 Mar; 56(3):552-566. doi: 10.1038/s41409-020-01134-4. Epub 2020 Nov 24. PMID: 33230186; PMCID: PMC8592274.
16. Frey NV, Shaw PA, Hexner EO, Pequignot E, Gill S, Luger SM, Mangan JK, Loren AW, Perl AE, Maude SL, Grupp SA, Shah NN, Gilmore J, Lacey SF, Melenhorst JJ, Levine BL, June CH, Porter DL. Optimizing chimeric antigen receptor T-Cell therapy for adults with acute lymphoblastic leukemia. J Clin Oncol. 2020 Feb 10;38(5):415-422. doi: 10.1200/JCO.19.01892. Epub 2019 Dec 9. PMID: 31815579; PMCID: PMC8312030.
17. Abou-El-Enein M, Elsallab M, Feldman SA, Fesnak AD, Heslop HE, Marks P, Till BG, Bauer G, Savoldo B. Scalable manufacturing of CAR T cells for cancer immunotherapy. Blood Cancer Discov. 2021 Sep;2(5):408-422. doi: 10.1158/2643-3230.BCD-21-0084. Epub 2021 Aug 3. PMID: 34568831; PMCID: PMC8462122.
18. Subklewe M, von Bergwelt-Baildon M, Humpe A. Chimeric antigen receptor T Cells: A race to revolutionize cancer therapy. Transfus Med Hemother. 2019 Feb;46(1):15-24. doi: 10.1159/000496870. Epub 2019 Feb 5. PMID: 31244578; PMCID: PMC6558337.
19. Mehrabadi AZ, Ranjbar R, Farzanehpour M, Shahriary A, Dorostkar R, Hamidinejad MA, Ghaleh HEG. Therapeutic potential of CAR T cell in malignancies: A scoping review. Biomed Pharmacother. 2022 Feb;146:112512. doi: 10.1016/j.biopha. 2021. 112512. Epub 2021 Dec 9. PMID:34894519.
20. Gee AP. GMP CAR-T cell production. Best Pract Res Clin Haematol. 2018 Jun;31 (2):126-134. doi: 10.1016/j.beha.2018.01.002. Epub 2018 Feb 2. PMID: 29909913.
21. Guidance for industry: CGMP for phase 1 investigational drugs, U.S. Department of Health and Human Services Food and Drug Administration Center for Drug Evaluation and Research (CDER) Center for Biologics Evaluation and Research (CBER) Office of Regulatory Affairs (ORA) July 2008.
22. Wang X, Rivière I. Clinical manufacturing of CAR T cells: foundation of a promising therapy. Mol Ther Oncolytics. 2016 Jun 15;3:16015. doi: 10.1038/mto.2016.15. PMID: 27347557; PMCID: PMC4909095.
23. Fesnak AD . The challenge of variability in chimeric antigen receptor T cell manufacturing. Regen Eng Transl Med 2020;6:322–9.
24. Sterner RC, Sterner RM. CAR-T cell therapy: current limitations and potential strategies. Blood Cancer J. 2021 Apr 6;11

(4):69. doi: 10.1038/s41408-021-00459-7. PMID: 33824268; PMCID: PMC8024391.
25. Lee D, Santomasso B, Locke F, Ghobadi A, Turtle C, Brudno J, Maus M, Park J, Mead E, Pavletic S, Go W, Eldjerou L, Gardner RA, Frey N, Curran KJ, Peggs K, Pasquini M, DiPersio JF, van den Brink MRM, Komanduri KV, Grupp SA, Neelapu SS. ASTCT consensus grading for cytokine release syndrome and neurologic toxicity associated with immune effector cells. Biol Blood Marrow Transplant. 2019 Apr;25(4):625-638. doi: 10.1016/j.bbmt.2018.12.758. Epub 2018 Dec 25. PMID: 30592986.
26. Ceschi A, Noseda R, Palin K, Verhamme K. Immune checkpoint inhibitor-related cytokine release syndrome: analysis of WHO global pharmacovigilance database. Front Pharmacol. 2020 May 4;11:557. doi: 10.3389/fphar.2020.00557. PMID: 32425791; PMCID: PMC7212758.
27. KYMRIAH® (tisagenlecleucel) [package insert]. East Hanover, New Jersey: Novartis Pharmaceuticals Corporation; 2022.
28. Sesques P, Ferrant E, Safar V, Wallet F, Tordo J, Dhomps A, Karlin L, Brisou G, Vercasson M, Hospital-Gustem C, Schwiertz V, Ranchon F, Rioufol C, Choquet M, Sujobert P, Ghergus D, Bouafia F, Golfier C, Lequeu H, Lazareth A, Novelli S, Devic P, Traverse Glehen A, Viel S, Venet F, Mialou V, Hequet O, Chauchet A, Arkam Y, Nicolas-Virelizier E, Peyrade F, Cavalieri D, Ader F, Ghesquières H, Salles G, Bachy E. Commercial anti-CD19 CAR T cell therapy for patients with relapsed/refractory aggressive B cell lymphoma in a European center. Am J Hematol. 2020 Nov;95(11):1324-1333. doi: 10.1002/ajh.25951. Epub 2020 Aug 25. PMID: 32744738.
29. Si S, Teachey DT. Spotlight on tocilizumab in the treatment of CAR-T-Cell-Induced cytokine release syndrome: clinical evidence to date. Ther Clin Risk Manag. 2020 Aug 4;16:705-714. doi: 10.2147/TCRM.S223468. PMID: 32801727; PMCID: PMC7414980.
30. Verma AA, Pai M, Saha S, et al. Managing drug shortages during a pandemic: Tocilizumab and COVID-19. CMAJ. 2021; 193(21):E771-E776
31. Diorio C, Vatsayan A, Talleur AC, Annesley C, Jaroscak JJ, Shalabi H, Ombrello AK, Hudspeth M, Maude SL, Gardner RA, Shah NN. Anakinra utilization in refractory pediatric CAR T-cell associated toxicities. Blood Adv. 2022 Jun 14;6(11):3398-3403. doi: 10.1182/bloodadvances.2022006983. PMID: 35395068; PMCID: PMC9198909.
32. Brown BD, Tambaro FP, Kohorst M, Chi L, Mahadeo KM, Tewari P, Petropoulos D, Slopis JM, Sadighi Z, Khazal S. Immune effector cell associated neurotoxicity (ICANS) in pediatric and young adult patients following chimeric antigen receptor (CAR) T-Cell therapy: can we optimize early diagnosis? Front Oncol. 2021 Mar 8;11:634445. doi: 10.3389/fonc.2021.634445. PMID: 33763368; PMCID: PMC7982581.
33. Gutierrez C, Brown ART, Herr MM, Kadri SS, Hill B, Rajendram P, Duggal A, Turtle CJ, Patel K, Lin Y, May HP, Gallo de Moraes A, Maus MV, Frigault MJ, Brudno JN, Athale J, Shah NN, Kochenderfer JN, Dharshan A, Beitinjaneh A, Arias AS, McEvoy C, Mead E, Stephens RS, Nates JL, Neelapu SS, Pastores SM. The chimeric antigen receptor-intensive care unit (CAR-ICU) initiative: Surveying intensive care unit practices in the management of CAR T-cell associated toxicities. J Crit Care. 2020 Aug;58:58-64. doi: 10.1016/j.jcrc.2020.04.008. Epub 2020 Apr 15. PMID: 32361219; PMCID: PMC7321897.
34. Yassine F, Iqbal M, Murthy H, Kharfan-Dabaja MA, Chavez JC. Real world experience of approved chimeric antigen receptor T-cell therapies outside of clinical trials. Curr Res Transl Med. 2020 Nov;68(4):159-170. doi: 10.1016/j.retram.2020.05.005. Epub 2020 Aug 15. PMID: 32811793.
35. Hayden PJ, Roddie C, Bader P, Basak GW, Bonig H, Bonini C, Chabannon C, Ciceri F, Corbacioglu S, Ellard R, Sanchez-Guijo F, Jäger U, Hildebrandt M, Hudecek M, Kersten MJ, Köhl U, Kuball J, Mielke S, Mohty M, Murray J, Nagler A, Rees J, Rioufol C, Saccardi R, Snowden JA, Styczynski J, Subklewe M, Thieblemont C, Topp M, Ispizua ÁU, Chen D, Vrhovac R, Gribben JG, Kröger N, Einsele H,

Yakoub-Agha I. Management of adults and children receiving CAR T-cell therapy: 2021 best practice recommendations of the European Society for Blood and Marrow Transplantation (EBMT) and the Joint Accreditation Committee of ISCT and EBMT (JACIE) and the European Haematology Association (EHA). Ann Oncol. 2022 Mar;33(3):259-275. doi: 10.1016/j.annonc. 2021.12.003. Epub 2021 Dec 16. PMID: 34923107.

36. Qi Y, Zhao M, Hu Y, Wang Y, Li P, Cao J, Shi M, Tan J, Zhang M, Xiao X, Xia J, Ma S, Qiao J, Yan Z, Li H, Pan B, Sang W, Li D, Li Z, Zhou J, Huang H, Liang A, Zheng J, Xu K. Efficacy and safety of CD19-specific CAR T cell-based therapy in B-cell acute lymphoblastic leukemia patients with CNSL. Blood. 2022 Jun 9;139(23):3376-3386. doi: 10.1182/blood. 2021013733. PMID: 35338773.

37. Rafiq, S, Hackett, C & Brentjens RJ. Engineering strategies to overcome the current roadblocks in CAR T cell therapy. Nat Rev Clin Oncol. 2020. 17;147–167. https://doi.org/ 10.1038/s41571-019-0297-y

38. Yang Y, McCloskey JE, Yang H, Puc J, Alcaina Y, Vedvyas Y, Gomez Gallegos AA, Ortiz-Sánchez E, de Stanchina E, Min IM, von Hofe E, Jin MM. Bispecific CAR T cells against EpCAM and Inducible ICAM-1 overcome antigen heterogeneity and generate superior antitumor responses. Cancer Immunol Res. 2021 Oct;9(10):1158-1174. doi: 10.1158/2326-6066. CIR-21-0062. Epub 2021 Aug 2. PMID: 34341 066; PMCID: PMC8492509.

39. Ying Z, Huang XF, Xiang X, Liu Y, Kang X, Song Y, Guo X, Liu H, Ding N, Zhang T, Duan P, Lin Y, Zheng W, Wang X, Lin N, Tu M, Xie Y, Zhang C, Liu W, Deng L, Gao S, Ping L, Wang X, Zhou N, Zhang J, Wang Y, Lin S, Mamuti M, Yu X, Fang L, Wang S, Song H, Wang G, Jones L, Zhu J, Chen SY. A safe and potent anti-CD19 CAR T cell therapy. Nat Med. 2019 Jun;25(6):947-953. doi: 10.1038/s41591-019-0421-7. Epub 2019 Apr 22. PMID: 31011207; PMCID: PMC7518381.

40. Chavez JC, Yassine F, Sandoval-Sus J, Kharfan-Dabaja MA. Anti-CD19 chimeric antigen receptor T-cell therapy in B-cell lymphomas: current status and future directions. Int J Hematol Oncol. 2021 Aug 3;10(2): IJH33. doi: 10.2217/ijh-2020-0021. PMID: 34540198; PMCID: PMC8445151.