CAR T-Cell Therapy in Hematological Malignancies: Staying Ahead of a Rapidly Evolving Landscape

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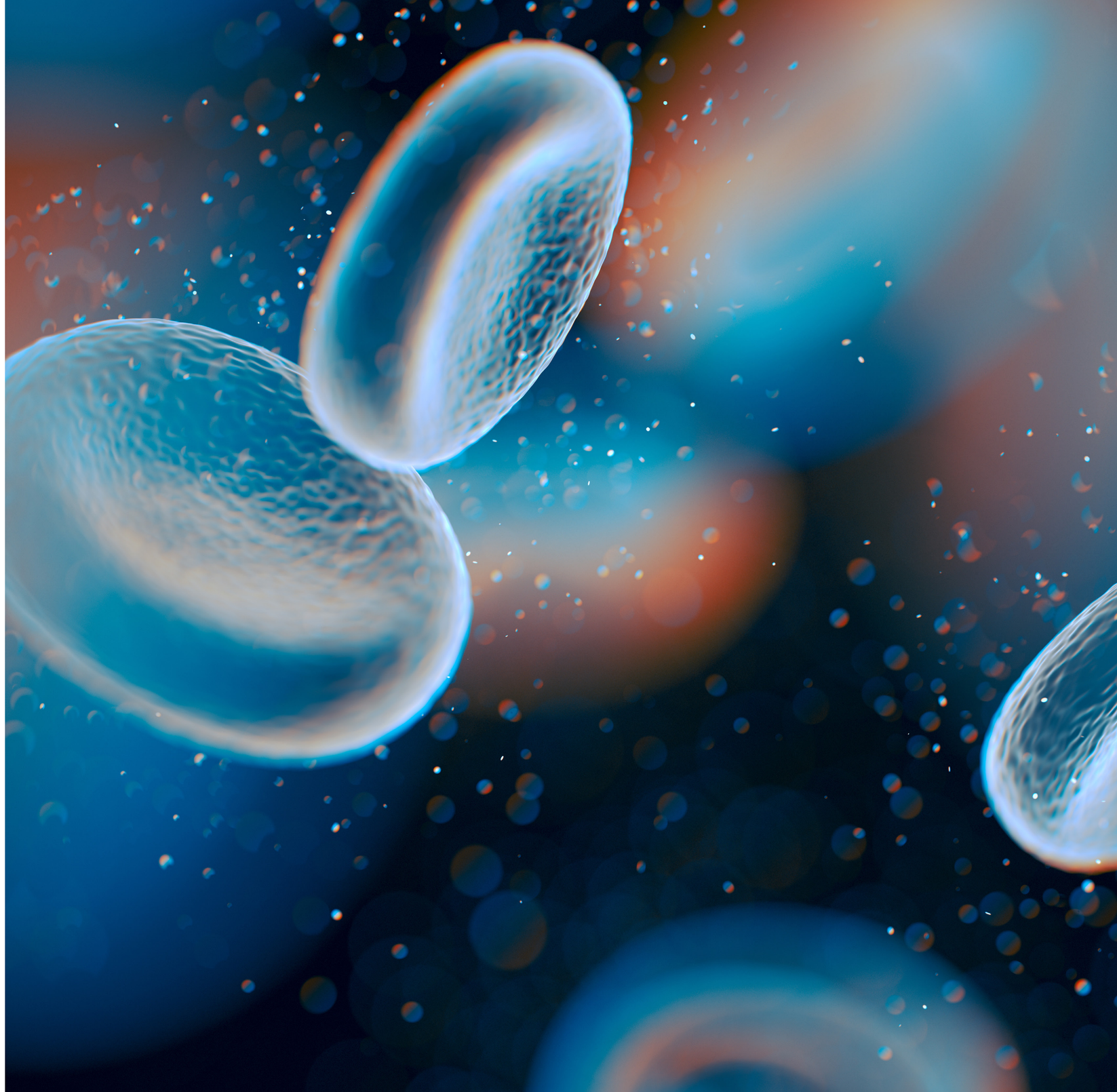


Introduction

Over the past few years, the approval of two CD19-directed chimeric antigen receptor (CAR) T-cell therapies for B-cell cancers has ignited enthusiasm among researchers and patients for translating this treatment approach to other hematological malignances. Research in the field of CAR T-cell therapies has intensified, as scientists explore ways to increase the impact of these treatments or expand the addressable patient population. Progress is being made in our collective efforts to unlock the full potential of CAR T-cell therapies—whether it is evaluating CAR T-cell therapy as first- or second-line treatment; optimizing CAR T-cell therapy for other tumors, both liquid and solid; or innovating in the development or manufacturing of next-generation or off-the-shelf therapies that are safer, more effective, or more accessible for patients, providers, and payers.

In this eBook, we explore the current landscape of CAR T-cell therapies, from real-world data on approved CD19-targeted products to imminent or emerging treatments for other hematological malignancies. We review the progress, opportunities, and challenges in this space, highlighting key clinical trial data and insights, and mapping out future directions.

About Precision for Medicine

Precision for Medicine is an accelerator of innovation, devoted to supporting the development of new medical treatments and breakthroughs. As a precision medicine clinical research services organization, Precision has brought together new technologies, expertise, and operational scale to help the life sciences industry improve the speed, cost, and success rate of bringing life-changing therapies to patients.

Precision has a long history of successful work in hematology trials, conducting multiple studies in 2500 sites with 11,500 patients. We combine extensive experience in cell therapy with a deep understanding of how to leverage both science and technology to advance hematology studies. Our Adoptive Cell Therapy Working Group brings together experts from across our global organization to stay at the leading edge of cell therapy study management—from data considerations to the regulatory landscape to patient safety.

Approved CAR T-cell Therapies: How Real-World Experience Compares With Clinical Trial Data

Chimeric antigen receptor (CAR) T-cell therapy is a cell-based gene therapy that works to harness the power of the immune system to recognize and eliminate cancer cells. The development of CAR T-cell therapies involves genetic modification of T cells to transform them into robust, tumor-specific T cells. Importantly, CAR T cells are living drugs, with the capability of cloning themselves.

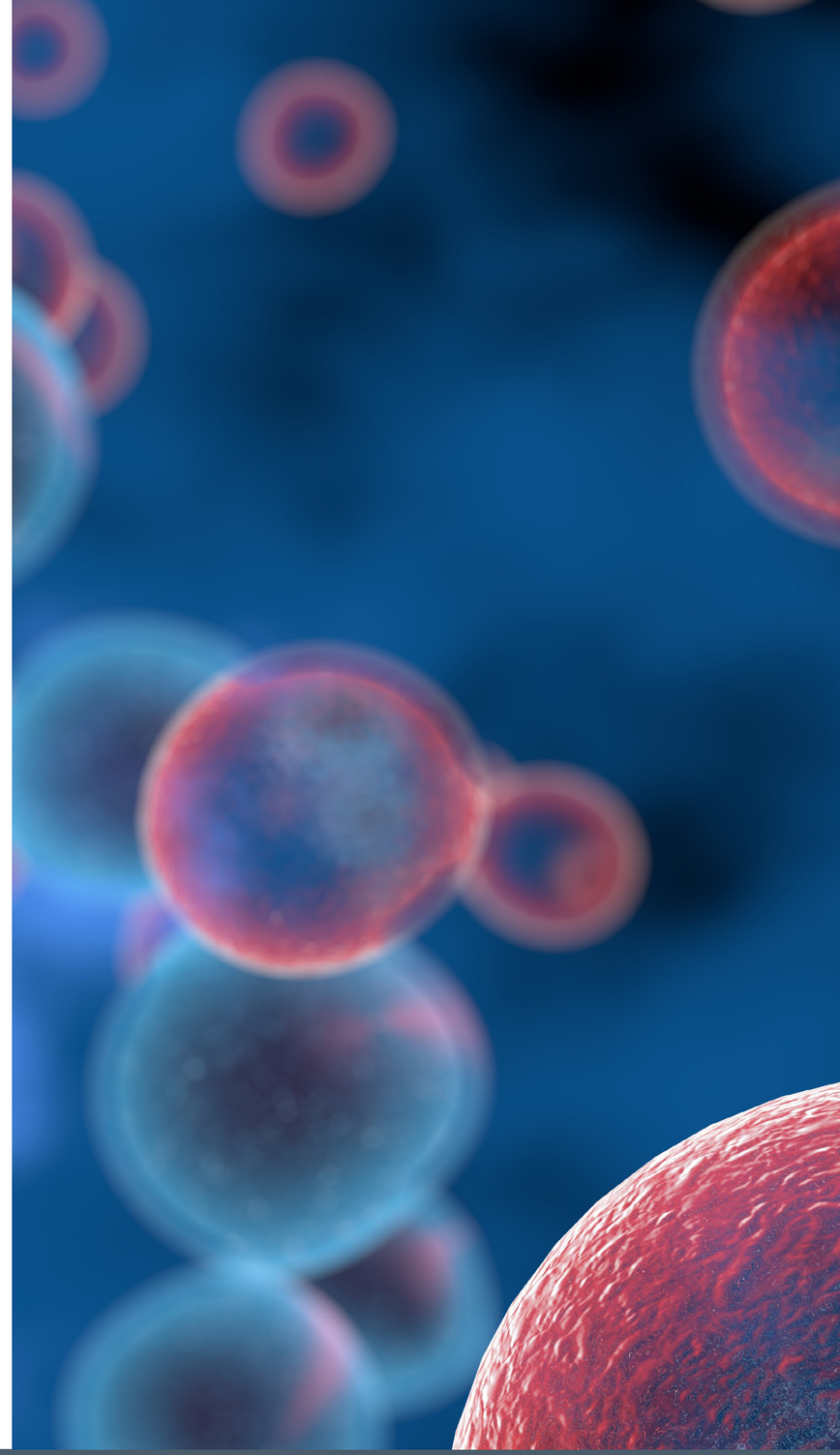
Approved CAR T-Cell Therapies

Two anti-CD19 CAR T-cell products, tisagenlecleucel (tisa-cel) and axicabtagene ciloleucel (axi-cel), have been approved by the U.S. Food and Drug Administration (FDA) and the European Medicines Agency (EMA) for the treatment of acute lymphoblastic leukemia (ALL) and diffuse large B cell lymphoma (DLBCL). More recently, in July 2020, the FDA approved brexucabtagene autoleucel, another CD19-directed CAR T-cell therapy, for patients with relapsed or refractory mantle cell lymphoma.[1]

Real-World Experience

In Children and Young Adults With R/R ALL

The tisa-cel approval for ALL in August 2017 was based on the results of the pivotal ELIANA trial, in which 79 patients were infused with autologous CAR T-cells. Complete remission (CR) was observed in 82% of patients and among these, 98% had minimal residual disease negative (MRD-) disease. With a median follow-up of 24 months, 45% of patients had ongoing response and relapse-free survival at 18 months was 66%. Only 8 patients with complete remission went on to allogeneic stem cell transplant while the remainder sought no further treatment. In patients who relapsed after CAR T-cell therapy, 75% were found to have CD19-negative disease.[2]



1. National Cancer Institute. CAR T-Cell Therapy Approved by FDA for Mantle Cell Lymphoma, August 24, 2020.

2. Maude SL et al. Tisagenlecleucel in children and young adults with B-Cell lymphoblastic leukemia. *N Engl J Med*. 2018;378:439-448.

3. Grupp S et al. Tisagenlecleucel Chimeric Antigen Receptor (CAR) T-Cell Therapy for Relapsed/Refractory Children and Young Adults with Acute Lymphoblastic Leukemia (ALL): Real World Experience from the Center for International Blood & Marrow Transplant Research (CIBMTR) Cellular Therapy (CT) Registry [abstract]. In: The 61st ASH Annual Meeting.; December 7-10; Orlando, Florida.

Figure 1. **Tisa-Cel in ALL: Clinical Trial and Real-World Experience**[3]

	ELIANA	Real World
Patients infused	N = 75	N = 159 (103 ≧ 3 mo)
Best CR rate at 3 mo	81% (100% MRD-)	88% (100% MRD-)
6 month DOR	NR	77%
EFS	50% (13.6 mo)	68% (6 mo)
OS	76% (13.6 mo)	94% (6 mo)
Grade ≧ 3 CRS	49% (Penn scale)	13.3% (ASTCT)
Grade ≧ 3 NT	13% (MedDRA SMQ)	8.6% (ICANS)

Recently, Grupp et al. summarized the real-world experience with tisa-cel using the Center for International Blood and Marrow Transplant Research (CIBMTR) Cellular Therapy Registry (see Figure 1). Overall, efficacy outcomes were similar and safety outcomes seemed to be more favorable in the real-world setting compared with the pivotal clinical trial, though the median follow-up in the real-world analysis was only 6 months. At 3 months post infusion, 88% of patients achieved CR in the real-world setting compared with 81% in the ELIANA trial. The rates of grade 3 cytokine release syndrome (CRS) and neurological events in the real-world setting were 13.3% and 8.6%, respectively, compared with 49% and 13% in the clinical trial.[3]

Real World Experience: In Diffuse Large B-Cell Lymphoma

Axi-cel was approved for DLBCL in October 2017 based on the results of the ZUMA-1 registrational study. Tisa-cel was approved for a similar patient population in May 2018 based on data from the JULIET trial.

Analysis of ZUMA-1 with a median follow-up of 15.4 months revealed that 82% of 108 patients treated with axi-cel achieved an objective response and 58% achieved a complete response. Two-year follow-up data from ZUMA-1 with a medical follow-up of 27.1 months demonstrated that 83% of patients had an objective response and 58% had a complete response. The median duration of response was 11.1 months and the median progression-free survival was 5.9 months. Grade 3 or worse CRS and neurological events occurred in 11% and 32% of patients, respectively.[4]

4. Locke FL et al. Long-term safety and activity of axicabtagene ciloleucel in refractory large B-cell lymphoma (ZUMA-1): a single-arm, multicentre, phase 1-2 trial. Lancet Oncol. 2019;20(1):31-42.

5. ASH 2018.

Figure 2. **Axi-cel in DLBCL: Clinical Trial and Real-World Experience**[5]

	ZUMA-1 (N = 111)	6 center experience (N = 136)	17 center experience (N = 295)
Median age, yrs	58 (23-76)	61 (21-79)	58 (64-77)
Prior ASCT %	25	30	31
Bridging therapy %	0	57	55
T cell not infused	10 (9%) 1 PD 2 CR 1 product failure 6 AEs (1 death)	13 (9.6%) 6 PD 1 CR 3 product failure 2 infection; 1 others	21 (7.1%) 12 PD 1 CR 7 product failure 1 infection
Not Meet ZUMA-1 Criteria %	0%	62%	43%
Best ORR % day 30	78	74	80
Best CR %	52 (ITT 47)	49 (ITT 44)	47 (ITT 44)
CR % at day 90	58	n/a (53 at month 6)	57

Two evaluations of real-world data were presented at ASH 2018 (see Figure 2). Notably, a significant number of patients who were infused with axi-cel in the real-world setting would not have met the inclusion/exclusion criteria for ZUMA-1 and would have been considered less fit than the original study participants. And yet, the response rates in the real-world setting are comparable.[5]

In the JULIET trial, with a median follow-up of 14 months, 40% of patients achieved complete responses and 12% had partial responses. At 12 months after initial response, rate of relapse-free survival was estimated to be 65%. Grade 3 or worse CRS and neurological events occurred in 22% and 12% of patients, respectively.[6]

In the real-world setting, efficacy outcomes for patients who were infused with tisa-cel were similar to those demonstrated in JULIET. In an analysis of 80 patients with R/R DLBCL, the overall response rate was 58%, with 40% of patients achieving a complete response. Grade 3 or worse CRS and neurological events occurred in 4% and 5% of patients, respectively.[7]

CAR T-Cell Therapies in Routine Clinical Practice

To date, the use of tisa-cel and axi-cel in the real-world setting has largely demonstrated efficacy and safety outcomes consistent with the pivotal trials supporting their approvals. Ongoing analysis of real-world experience will continue to add to our understanding of how these therapies can—and should—be incorporated into clinical practice.

6. Schuster SJ et al. Tisagenlecleucel in adult relapsed or refractory diffuse large B-cell lymphoma. *N Engl J Med*. 2019;380:45-56.

7. Jaglowski S et al. Tisagenlecleucel Chimeric Antigen Receptor (CAR) T-Cell Therapy for Adults with Diffuse Large B-Cell Lymphoma (DLBCL): Real World Experience from the Center for International Blood & Marrow Transplant Research (CIBMTR) Cellular Therapy (CT) Registry [abstract]. In: The 61st ASH Annual Meeting.; December 7-10; Orlando, Florida.

On the Horizon: CAR T-Cell Therapy for Multiple Myeloma

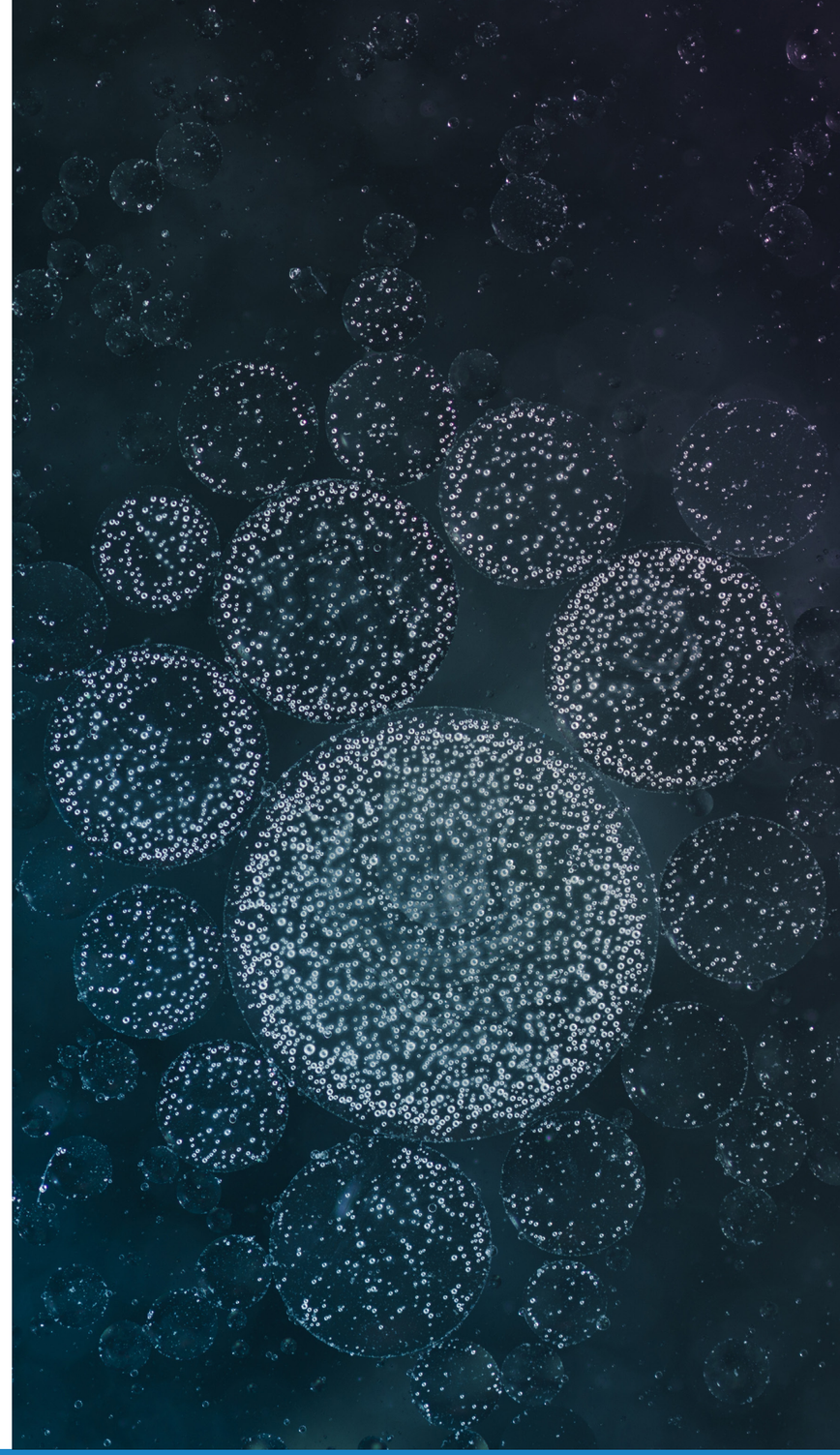
Multiple myeloma (MM) is an hematological malignancy caused by clonal proliferation of malignant plasma cells. In Western countries, MM accounts for approximately 10% of hematological malignancies and 1% of all cancers.[8]

Multiple myeloma is an incurable disease that accounts for ~10% of hematological malignancies[8]

A number of therapeutic agents, including immunomodulatory drugs, proteasome inhibitors, and monoclonal antibodies, have led to significant advances in the treatment of MM. However, the disease remains incurable and the vast majority of patients ultimately become refractory to—or unable to tolerate—treatment.

Targeting BCMA with CAR T-Cell Therapy

CAR T-cell therapy targeting B-cell maturation antigen (BCMA) has shown encouraging results in MM. BCMA has been the target of choice due to its high expression on the surface of malignant plasma cells and restricted expression in most normal cells other than mature B-cells where expression is low.[9] Currently, there are more than 50 clinical trials worldwide studying BCMA-directed CAR T-cell therapies.

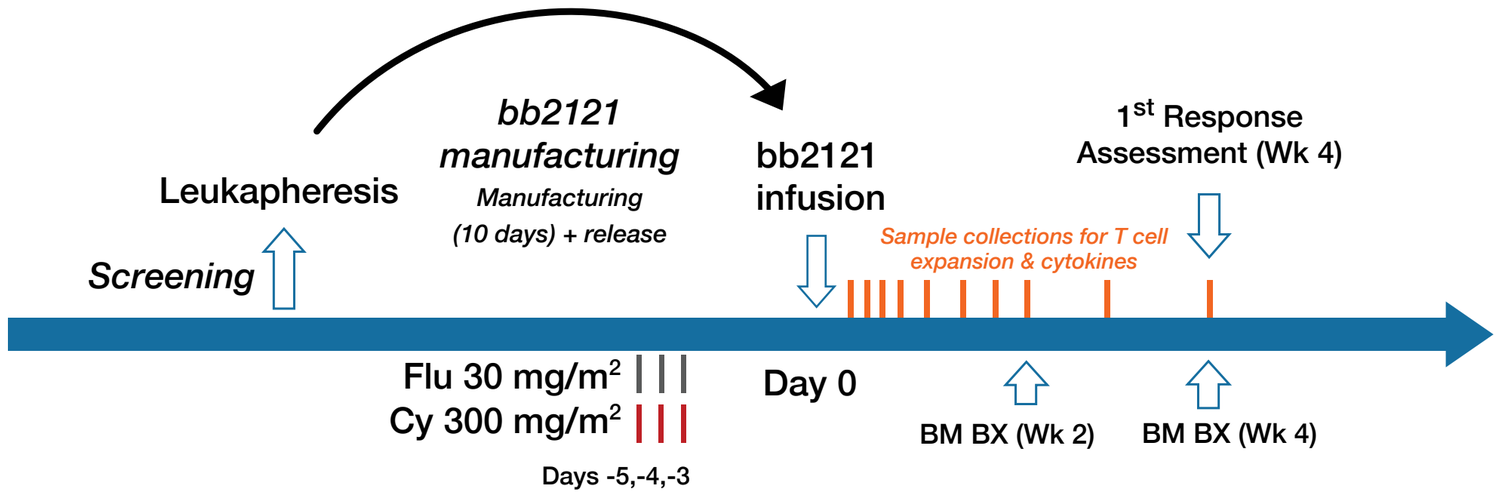


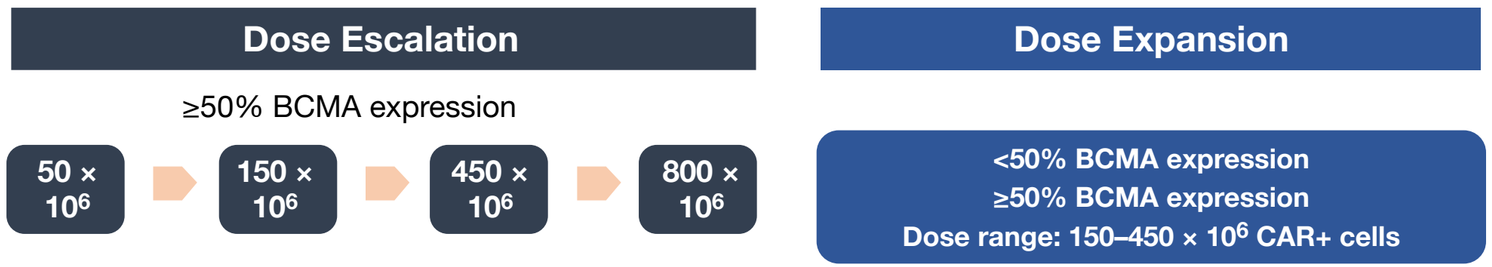
8. Timmers M et al. Chimeric antigen receptor-modified T cell therapy in multiple myeloma: beyond B cell maturation antigen. *Front Immunol*. 2019;10:1-12.

9. Garcia-Guerrero E, Sierro-Martinez B, Perez-Simon JA. Overcoming chimeric antigen receptor (CAR) modified T-cell therapy limitations in multiple myeloma. *Front Immunol*. 2020;11:1128.

10. Friedman KM et al. Effective targeting of multiple B-cell maturation antigen-expressing hematological malignances by anti-B-cell maturation antigen chimeric antigen receptor T cells. *Hum Gene Ther*. 2018;29:585-601.

Figure 3. **Phase 1 Anti-BCMA CAR T-Cell Trial for Multiple Myeloma: Study Design**





BCMA, B-cell maturation antigen; BM BX, Bone marrow biopsy; Cy, cyclophosphamide: Flu, fludarabine.

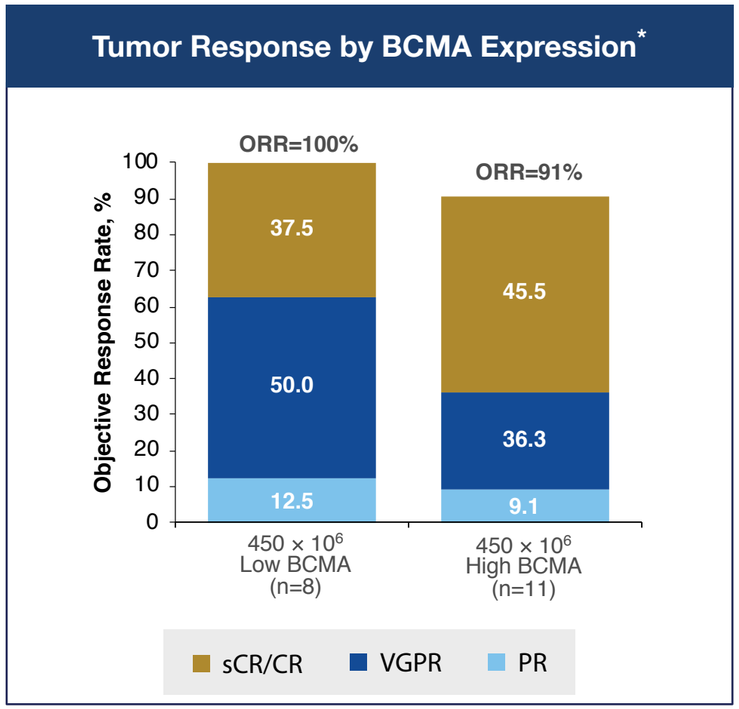
Idecaptagene cicleucel (ide-cel, previously bb2121) is a CAR T-cell therapy targeting BCMA. Ide-cel is produced by transducing autologous T cells with a lentiviral vector encoding a second-generation CAR with an anti-BCMA single-chain variable fragment, a CD137 (4-1BBB) costimulatory motif, and a CD3-zeta signaling domain.[10] An open-label, phase 1 study (CRB-401) involving patients with relapsed or refractory MM who had received at least 3 previous lines of therapy was conducted with a primary endpoint of safety. The study consisted of 2 parts: a dose escalation phase starting at 50 million cells and going up to 800 million cells, and a dose-expansion phase (See Figure 3).

Initial results of this phase 1 study showed that, among 33 patients who received ide-cel, 85% had a clinical response lasting a median of 10.9 months without any ongoing myeloma therapy. Complete responses (CRs) were achieved in 45% of patients and were observed across all doses from 150–800 million CAR+ T cells. Notably, response appeared to be independent of tumor BCMA expression (see Figure 4). High response rates were also observed in patients with high-risk cytogenetic profiles, progressive disease during their last line of therapy, or extramedullary disease at baseline. Median progression-free survival (PFS) was 11.8 months, with 40% of study participants free of progression at 12 months.[11]

Precision's Cell Therapy Working Group stays on the leading edge of advances in the industry, delivering insights on unique regulatory environment, study operations, data considerations, logistics, and patient safety management of cell therapy studies. Learn more >>

11. Raje N, et al. Anti-BCMA CAR T-cell therapy bb2121 in relapsed or refractory multiple myeloma. *N Engl J Med*. 2019;380:1726-1737..

Figure 4. **Tumor Response Seems to Be Independent of BCMS Expression[11]**



***All responses were confirmed and assessed according to the International Myeloma Working Group Uniform Response Criteria for Multiple Myeloma.**

Data are for BCMA expression on bone marrow plasma cells at screening. Low BCMA is <50% bone marrow plasma cells expression of BCMA; high BCMA is defined as ≥50%. CR, complete response; ORR, objective response rate; PR, partial response; sCR, stringent CR; VGPR, very good partial response. ORR is defined as attaining sCR, CR, VGPR, or PR, including confirmed and unconfirmed responses.

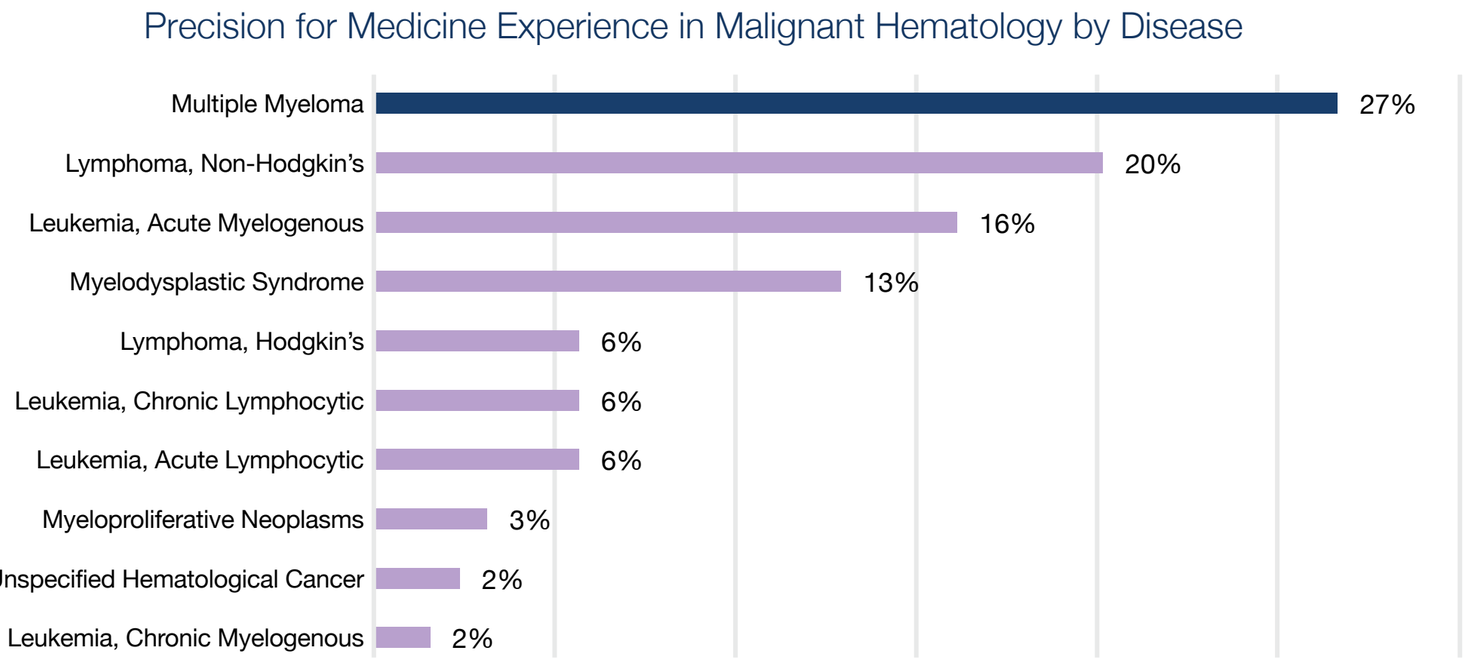
Based on these promising data, ide-cel advanced to the pivotal phase 2 KarMMa study evaluating its efficacy and safety. KarMMA enrolled 140 patients, of whom 128 were treated with ide-cel across the target dose levels of 150–450 million CAR+ T cells. The primary endpoint of the study was overall response rate as assessed by an independent review committee according to the International Myeloma Working Group criteria. Secondary endpoints included complete response rate, time to response, duration of response, progression-free survival, overall survival, minimal residual disease evaluated by next-generation sequencing, and safety.[12]

Results from KarMMA were presented at ASCO in May 2020. These data showed an objective response rate (ORR) of 73% and a median PFS of 8.6 months. The most common any-grade toxicities were cytopenias (97%) and cytokine release syndrome (CRS; 84%). CRS was mainly grade 1/2, though 5 patients had grade 3, one had grade 4, and one had grade 5. Neurotoxicity was observed in 23 patients (18%). Median peak CAR+ T-cell expansion occurred at 11 days post infusion and persistence was durable, with CAR+ T cells detected in 59% of patients at 6 months and 36% of patients at 12 months.[13]

In September 2020, Bristol Myers Squibb and Bluebird Bio, Inc., announced that the FDA had accepted their biologics' license application (BLA) for ide-cel for priority review, making ide-cel the first CAR T-cell therapy for MM accepted for regulatory review. The proposed indication is for treatment of adult patients with MM who have received at least 3 prior therapies, including an immunomodulatory agent, a proteasome inhibitor, and an anti-CD38 antibody.

12. Bristol Myers Squibb. U.S. Food and Drug Administration (FDA) Accepts for Priority Review Bristol Myers Squibb and bluebird bio Application for Anti-BCMA CAR T Cell Therapy Idecabtagene Vicleucel (Ide-cel, bb2121), September 22, 2020. Available at https://news.bms.com/news/details/2020/U.S.-Food-and-Drug-Administration-FDA-Accepts-for-Priority-Review-Bristol-Myers-Squibb-and-bluebird-bio-Application-for-Anti-BCMA-CAR-T-Cell-Therapy-Idecabtagene-Vicleucel-Ide-cel-bb2121/default.aspx.

Precision for Medicine has deep expertise across a broad range of hematological malignancies, including multiple myeloma.



Other Multiple Myeloma CAR T-Cell Therapies in the Pipeline

Ciltacabtagene autoleucel (cilta-cel, LCAR-B38M, or JNJ-4528) is another anti-BCMA CAR T-cell therapy being investigated for relapsed or refractory MM. To date, cilta-cel has been studied in three phase 1 and phase 2 trials—LEGEND-2, CARTITUDE-1, and CARTIFAN-1. Data from LEGEND-2, a phase 1/2 trial in China, demonstrated an ORR of 88%, with 74% of patients achieving CR. Among complete responders, over 90% reached minimal residual disease (MRD). Median PFS was 20 months overall and 28 months among those achieving MRD negativity.[14] CARTITUDE-1 is an ongoing phase 1b/2 study and interim top-line data shows an ORR of 97%.[14] CARTIFAN-1 is an ongoing, confirmatory phase 2 study with no results officially released to date. In September 2020, cilta-cel was given breakthrough therapy designation in China.[15]

Other potential targets being explored include CD38, CD138, CD19, and immunoglobulin kappa light chain, either alone or as multitargeted CAR T-cell products. Studies of these targets have generally shown high response rates, but have yet to achieve durable responses.

Hope on the Horizon

Despite advancements in treatment, nearly all patients with MM eventually have a relapse and new options are needed. CAR T-cell therapies targeting BCMA have shown promise in patients with heavily pretreated relapsed or refractory MM and approval of the first CAR T-cell therapy for MM is expected in 2021.

13. Munshi NC et al. Idecabtagene vicleucel (ide-cel; bb2121), a BCMA-targeted CAR T-cell therapy, in patients with relapsed and refractory multiple myeloma (RRMM): Initial KarMMa results. *J Clin Oncol*. 2020;38:15 Suppl,8503.

14. Myeloma Research News. Cilta-cel, CAR T-cell Treatment, Named Breakthrough Therapy in China. September 1, 2020.

15. Myeloma Research News. Advanced Multiple Myeloma Patients Responding to Janssen's CAR T-cell Therapy, Trial Data Show. December 10, 2019.

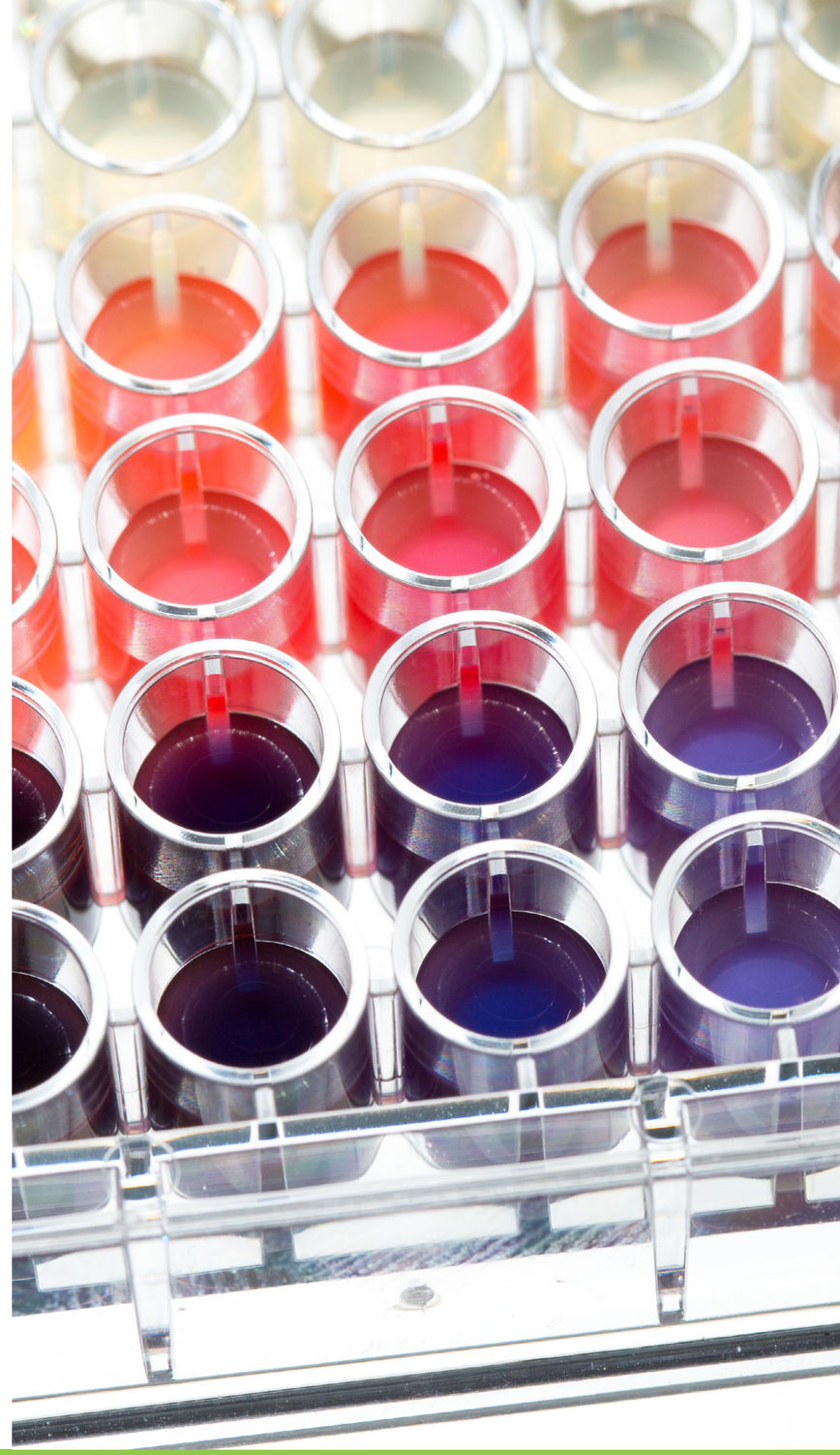
Update on CAR T-Cell Therapy for Acute Myeloid Leukemia

For patients with acute myeloid leukemia (AML), relapse after conventional chemotherapy remains a significant problem and a major cause of death.The only potentially curative treatment available for AML is allogeneic hematopoietic stem cell transplantation (allo-HSCT); however, relapse following allo-HSCT is not uncommon and is associated with poor prognosis.[16] Currently, there is substantial research efforts aimed at studying CAR T cells for the treatment of AML and other myeloid malignancies.

Challenges in Developing CAR T-Cell Therapies for AML

One of the major challenges for developing a CAR T-cell (or other engineered cell) therapy for AML is lack of a target as pristine as CD19 for acute lymphoblastic leukemia and B-cell lymphoma or B-cell maturation antigen (BCMA) for multiple myeloma. The ideal cell therapy target for AML is yet to be identified, as myeloid antigens are often co-expressed on a wide range of normal cells including hematopoietic stem/progenitor cells.[16] An ideal target would be expressed on both myeloid blood and myeloid stem cells, but not on any normal cells or tissues.

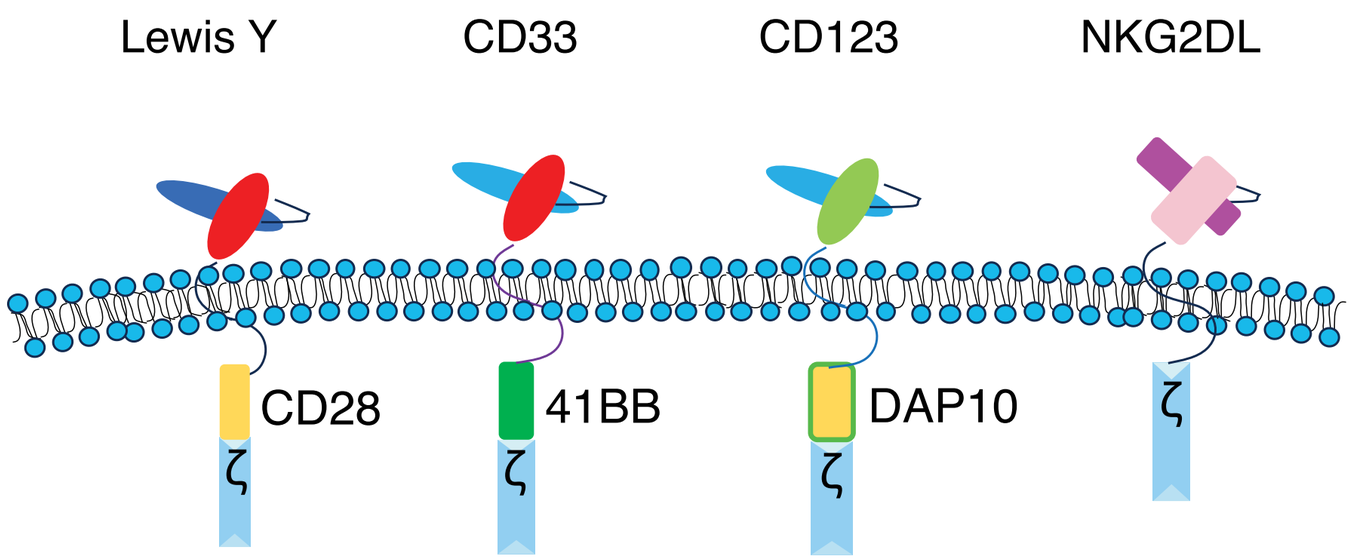
Another challenge may be the manufacture of CAR T-cells in patients with AML, as AML cells may secrete soluble factors that inhibit T cell proliferation.[17] In addition, patients who are candidates for CAR T-cell therapy will likely have undergone treatments that make it difficult to obtain quality autologous T cells for CAR T-cell manufacture.



16. Mardiana S, Gill S. CAR T cells for acute myeloid leukemia: State of the art and future directions.

17. Kenderian SS, June CH, Gill S. Generating and Expanding Autologous Chimeric Antigen Receptor T Cells from Patients with Acute Myeloid Leukemia, in Fortina P, Londin E, Park JY, Kricka LJ. editors. Acute Myeloid Leukemia: Methods and Protocols. New York, NY: Springer New York; 2017, p. 267–276.

Figure 5. **AML Targets in the Clinic**



Targets Being Studied for AML

Despite the lack of an ideal target for AML, there are a variety of targets being tested in both the preclinical and clinical settings (see Figure 5).

The first reported clinical trial demonstrating biological activity of CAR T cells in AML utilized a second-generation CAR directed against the Lewis Y antigen. While only one of the patients treated had a transient response, this was nevertheless an important study as it demonstrated CAR T-cell biological activity in AML without hematopoietic toxicity.[18]

There are currently approximately 40 CAR T-cell clinical trials enrolling patients with AML. Most of these investigational products are targeting C-type lectin-like molecule-1 (CLL-1), CD33, or CD123.[19]

- **CLL-**1 is an attractive target due to its high expression in AML and reported absence in healthy hematopoietic stem and progenitor cells (HSPCs)[16]
- **CD33** is almost ubiquitously expressed on AML blasts.[16] In addition, studies have shown that more than 60% of AML samples with CD33 expression also express CLL-1, raising the possibility of creating a dual-specific CAR T cell[20]

  A preliminary report of a first-in-human trial of a dual CD33-CLL-1 CAR T-cell therapy indicated that 2 patients with refractory/relapsed AML (R/R AML) experienced complete remission accompanied by pancytopenia within 3 weeks of CAR T-cell infusion. Both patients experienced hematopoietic recovery after undergoing anti-thymocyte globulin (ATG)-based HSCT

18. Ritchie DS et al. Persistence and efficacy of second generation CAR T cell against the LeY antigen in acute myeloid leukemia. *Mol Ther*. 2013;21:2122-2129.
19. US National Library of Science ClinicalTrials.gov. (2020). Available online at: https://clinicaltrials.gov/ct2/results?term=CARandcond=AMLandSearch=Applyandrecrs=aandage_v=andgndr=andtype=andrslt=~.
20. Bakker ABH et al. C-type lectin-like molecule-1. *Cancer Res*. 2004;64:8443.
21. Liu F et al. First-in-human CLL1-CD33 compound CAR T cell therapy induces complete remission in patients with refractory acute myeloid leukemia: update on phase 1 clinical trial. *Blood*. 2018;132:901.

Figure 6. **CD123 CAR T-Cell Trials for R/R AML in North America**

	costim	Gene delivery	T cell source	dose	Status
NCT02159495 City of Hope	CD28	lentivirus	allo or auto	50-500M	Active
NCT02623582 Penn	4-1BB	mRNA electroporation	auto	4M/kg x 4 4M/kg x 6	Terminated 10.2017
NCT03190278 Cellectis	4-1BB	lentivirus	universal donor (UCART)	6.25 x 10^4/kg to 6.25 x 10^6/kg	Active
NCT03766126 Penn	4-1BB	lentivirus	auto	2x10^6/kg 10%/30%/60%	Opened 2.2019

22. Dana-Farber Cancer Institute. Blastic Plasmacytoid Dendritic Cell Neoplasm (BPDCN) Center.

23. Budde et al. SOHO 2019.

24. BusinessWire. First Patient Dosed with Cellectis' New Allogeneic UCART123 Product Candidate for Relapsed/Refractory Acute Myeloid Leukemia, January 15, 2020. Available at https://www.businesswire.com/news/home/20200115005813/en/First-Patient-Dosed-with-Cellectis%E2%80%99-New-Allogeneic-UCART123-Product-Candidate-for-RelapsedRefractory-Acute-Myeloid-Leukemia.

- **CD123** is also almost universally expressed on AML blasts. There are 4 main CD123 CAR T-cell therapy clinical trials in North America (see Figure 6)

City of Hope is currently conducting a clinical trial of a CD123-directed allogeneic or autologous CAR T-cell product. This clinical trial has 2 arms—one for patients with R/R AML and the other for patients with blastic plasmacytoid dendritic cell neoplasm (BPDCN), a rare and aggressive malignancy of the bone marrow and blood.[22] The study utilizes second-generation CD28-ζ CAR T cells targeting CD123 manufactured by lentiviral transduction. Early results revealed that one patient treated with 50 million CAR+ T cells experienced a transient morphologic leukemia-free state and then achieved a significant blast reduction after receiving a subsequent CAR T-cell infusion 3 months later. Three of 6 patients treated with 200 million CAR+ T cells experienced complete remission. Notably, no dose-limiting toxicity including cytopenia has been reported.[23]

In 2016, the University of Pennsylvania initiated a clinical trial of CD123-directed CAR T cells manufactured via mRNA electroporation with the goal of preventing long-term CAR T-cell persistence and mitigating the risk of severe myeloablation. While no measurable antitumor response was observed, this study did demonstrate a favorable safety profile—no obvious vascular, hematological, or neurological toxicity was reported despite expression of CD123 on healthy hematopoietic tissues. Based on this, the University of Pennsylvania initiated a new clinical trial using a lentiviral transduction system in February 2019, which is still underway.

Cellectis is using a universal CAR T-cell (UCART) program and lentiviral-based system to deliver CARs into off-the-shelf health donor T cells. Their UCART123 phase 1 studies were put on hold in September 2017 due to grade 5 toxicities, but after adjustments in both the lymphodepletion regimen and the CAR+ T-cell dose, the trial has resumed and the first patient was dosed in January 2020.[24]

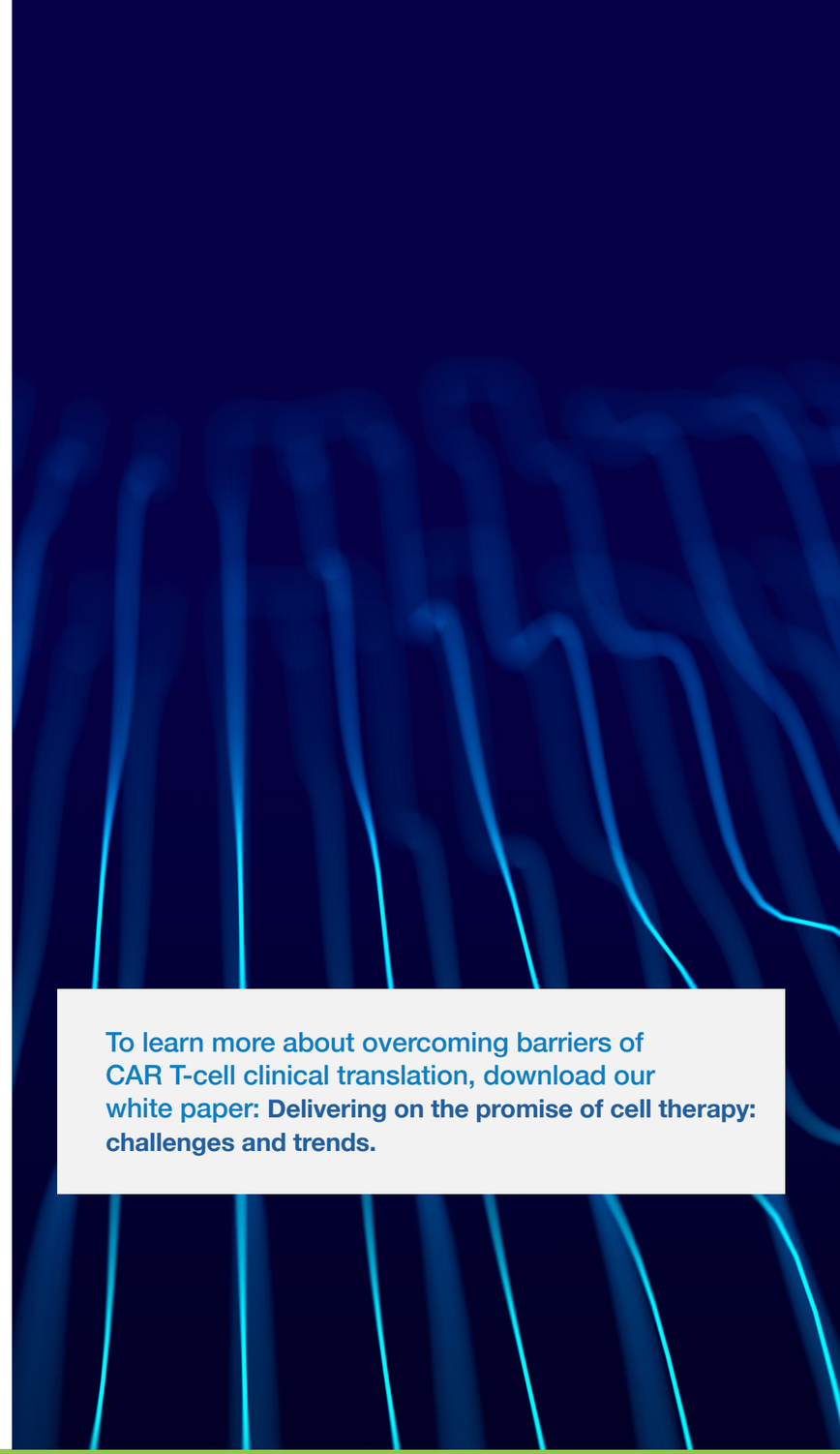
Approaches for Overcoming Barriers to Clinical Translation of CAR T-cells in AML

The principal barrier limiting the clinical translation of CAR T-cell therapy in AML is the absence of an AML-specific antigen. Whereas the complete ablation of CD19-expressing B cells is clinically tolerated, prolonged myeloablation resulting from CAR T-cells targeting myeloid antigens that are also expressed on normal myeloid progenitors can lead to fatal infections and bleeding complications.[16] Strategies that are being investigated to address this barrier include:[16]

- **Limiting CAR T-cell persistence** through alternative CAR constructs or safety switches to prevent prolonged myeloablation
- **Creating an artificial AML-specific antigen** using a gene-editing technology to edit a donor allograft. The strategy behind this approach is to edit out a CAR target antigen from a donor allograft and then transplant those edited HSPCs into a patient. Once engrafted, CAR T cells directed at the target antigen are manufactured from the same donor and infused into the patient, with the objective of allowing for normal hematopoiesis by the transplanted HSPCs. A clinical trial involving the use of allogeneic CD33-/- HSCT prior to CD33-directed CAR T-cell infusion is being developed at the University of Pennsylvania for patients with R/R AML
- **Identifying AML-specific neoantigens** such as mutations contributing to leukemogenesis or splice variants

Next Steps for CAR T-Cell Therapy in AML

While early clinical trial results show that CAR T-cell therapy may be a feasible option for AML, the full therapeutic potential of CAR T cells in this hematological malignancy remains limited by the lack of an AML-specific cell surface antigen. Intense research is underway, however, to overcome this and other barriers to clinical translation of CAR T cells in AML. Rationally designed clinical trials based on a comprehensive understanding of AML will be critical for advancing CAR T-cell development for this aggressive disease.



To learn more about overcoming barriers of CAR T-cell clinical translation, download our white paper: **Delivering on the promise of cell therapy: challenges and trends.**

Current Landscape of Allogeneic CAR T-Cells

While autologous CAR T-cell therapy has proven effective in certain hematological malignancies, it has its challenges:

- **Lymphopenic patients.** Some patients are lymphopenic and may find it difficult to generate a CAR T-cell product
- **Manufacturing logistics.** Manufacturing is complex and may be difficult to scale
- **Kinetics of disease.** Malignancies can progress rapidly, leaving little time for generating the CAR T-cell product and infusing it back into the patient
- **Inter-patient variation in quality of product.** By its very nature, autologous products will vary in quality from one patient to the next, which may ultimately impact efficacy
- **Cost and access.** The high cost of autologous CAR T-cell therapy makes it unaffordable for many patients and healthcare systems

Allogeneic, off-the-shelf CAR T cells are actively being explored as an alternative to autologous therapies. These CAR T cells are generated from healthy donors and would consist of standardized, validated batches that could potentially reduce production costs and allow multiple infusions from a single donor. It is hypothesized that since these T cells are derived from healthy donors, they might have more potent antitumor effects than patient-derived T cells, though this is yet to be proven.

Strategies for Generating Allogeneic CAR T-Cells

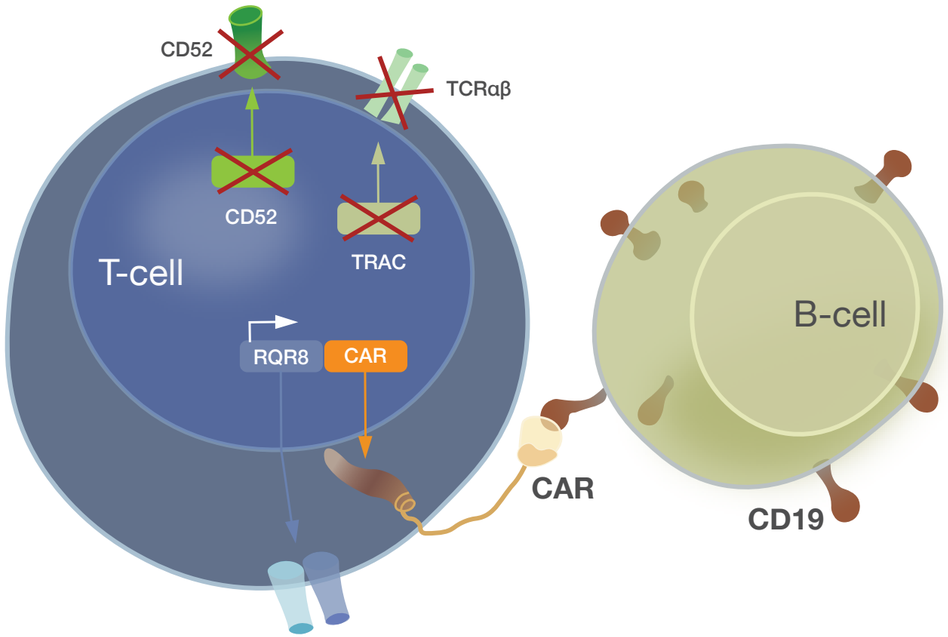
A key challenge in generating allogeneic CAR-T cells is preventing host immune rejection of the infused allogeneic product. One strategy for preventing rejection is to immunosuppress the patient, not just with standard fludarabine and cyclophosphamide lymphodepletion, but also with a drug such as alemtuzumab, an anti-CD52 monoclonal antibody that eliminates host T cells. One approach for preventing the infused CAR T cells from also being eliminated by alemtuzumab is to edit CD52 out of the donor T cells. In order to prevent the graft-versus-host disease that would inevitably occur with an intact endogenous T-cell receptor (TCR), gene-editing technology is also needed to remove the TCR from the donor T cells.

Challenges Associated With Allogeneic CAR T-Cells

In addition to graft-versus-host disease, there are a number of other challenges associated with allogeneic CAR T cells:

- **Rejection** of the third-party CAR
- **Intense lymphodepletion required,** leading to a higher risk of infections
- **Lack of clarity on donor characteristics** and how to define an optimal donor
- **Complex manufacturing** involving technically challenging gene-editing steps
- **Unknown risks** of combined viral transduction and gene editing

Figure 7. **Schematic of UCART19**



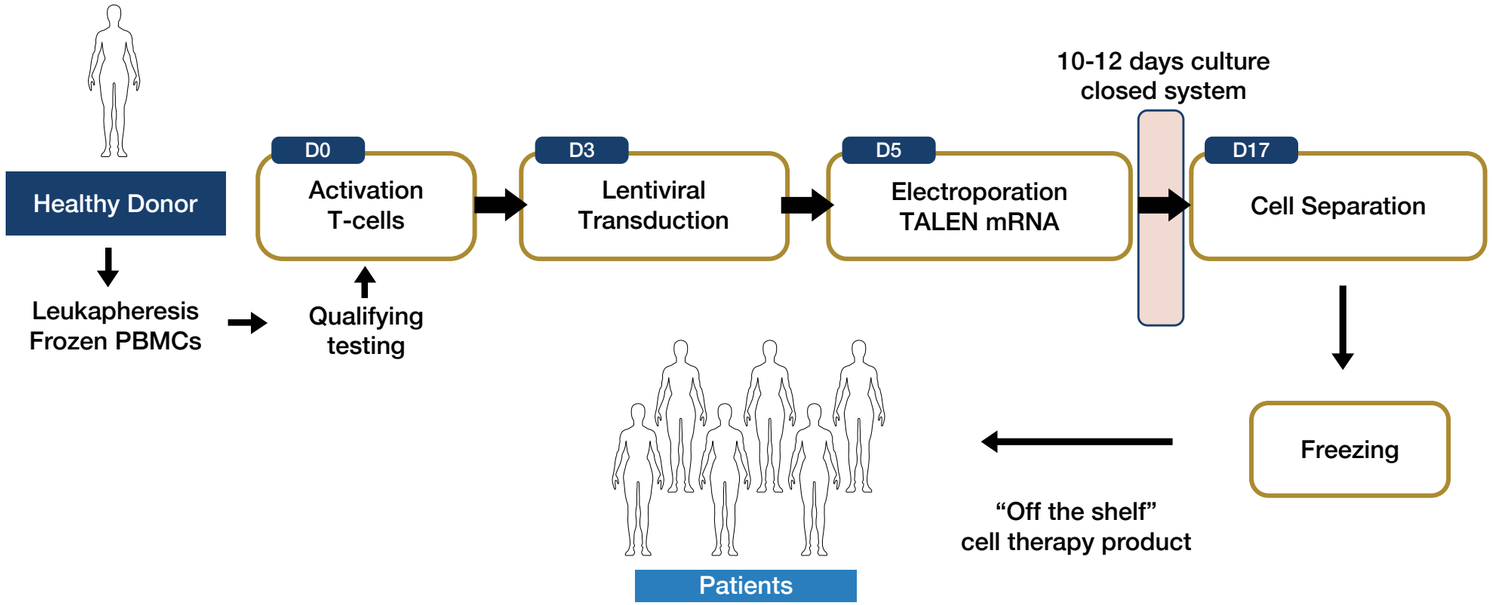
Allogeneic CAR T-Cells in the Clinic

UCART19 was the first allogeneic, off-the-shelf CAR T-cell product studied in clinical trials. A schematic diagram of UCART19 is shown in Figure 7.

The CD19 CAR is introduced in the standard way and transcription activator-like effector nucleases (TALEN® technology) are used to knock out both CD52 and TCR alpha through genome editing. In addition, the T cells are engineered to co-express the RQR8 gene as a safety switch, with the objective of rendering the T cells sensitive to the monoclonal antibody rituximab.[25]

25. Smith J et al. UCART19, an allogeneic "off-the-shelf" adoptive T-cell immunotherapy against CD19+ B-cell leukemias. *J Clin Oncol*. 2015;33:15(Suppl)3069.

Figure 8. **UCART19 Manufacturing Process**



The manufacturing process for UCART19 (see Figure 8) requires 19 days to run and up to 9 weeks for quality control.

There are 2 ongoing clinical trials for UCART19:

1. **CALM trial**, a phase 1 dose-escalation trial in patients with relapsed/refractory (R/R) adult B-cell acute lymphoblastic leukemia (B-ALL)
2. **PALL trial**, a phase 1 single-dose study in R/R pediatric B-ALL

Inclusion criteria for both trials are morphological (>5%) or minimal residual disease positive (MRD+) disease and exhaustion of available treatment options. Exclusion criteria are clinically suspected extramedullary disease, evidence of active infection, and presence of UCART19 donor-specific anti-HLA antibodies.

26. Benjamin et al. ASH 2018.

Figure 9. **UCART19 Safety Profile**

N=21	G1 n(%)	G2 n(%)	G3 n(%)	G4 n(%)	G5 n(%)	All grades n(%)
AEs related to UCART19						
Cytokine release syndrome	4 (19.0)	12 (57.1)	2 (9.5)	1 (4.8)	-	19 (90.5)
Neurotoxicity events	7 (33.3)	1 (4.8)	-	-	-	8 (38.1)
Acute skin graft-versus-host disease	2 (9.5)	-	-	-	-	2 (9.5)
AEs related to lymphodepletion and/or UCART19						
Prolonged cytopenia	-	-	-	6 (28.5)	-	6 (28.5)
Viral infections	1 (4.8)	2 (9.5)	4 (19.0)	1 (4.8)	-	8 (38.1)
Neutropenic sepsis				1 (4.8)	1 (4.8)	2 (9.5)
Febrile neutropenia/septic shock					1 (4.8)	1 (4.8)
Pulmonary hemorrhage					1 (4.8)	1 (4.8)

As first-in-human studies, CALM and PALL are focused primarily on safety. A pooled analysis of the 2 trials presented at ASH 2018 showed that the adverse events with UCART19 were similar to those with autologous CD19-directed CAR T-cell therapies (see Figure 9) and were manageable. Graft-versus-host disease was seen in 2 of the 21 patients and was limited to grade 1.[26]

The pooled analysis demonstrated that 82% of patients who received fludarabine, cyclophosphamide, and alemtuzumab prior to infusion of UCART19 achieved a complete response. Of the 21 patients treated, 4 did not receive alemtuzumab. In these patients, there was no expansion of the CAR T cells and no disease response. The early response rate is comparable to what is seen with autologous CAR T cells. In the setting of allogeneic CAR T cells, however, long-term persistence of the engineered cells is not expected and therefore most patients ultimately went on to a subsequent consolidation allogeneic stem cell transplant.

Figure 10. **Universal CAR T Cells in Development**

Developer	CAR T cell product	Target antigen	Allogeneic technology	Tools andvectorization	Development phase and trial reference
Allogene Therapeutics	ALLO-715	BCMA	TRAC and CD52 KO	TALEN mRNA (KO)	Preclinical
Allogene Therapeutics and Servier	UCART19	CD19	TRAC KO with orwithout CD52 KO	TALEN mRNA (KO)	Phase I in relapsed/refractory B cell ALL (NCT02746952, CALM study); phase I in relapsed/refractory B cell ALL (NCT02808442); phase I in lymphoid malignancies (NCT02735083)
Atara Biotherapeutics	Anti-CD19 EBV CTL therapy	CD19	Use of EBV-specificcell lines	Retroviral vector	Preclinical
Cellectis	UCART-123	CD123 (also known as IL-3RA)	TRAC KO	TALEN mRNA (KO)	Phase I in AML (NCT03190278); phase I in BPDCN (NCT03203369)
	UCART-22	CD22	TRAC and CD52 KO	TALEN mRNA (KO)	IND
	UCART-CS1	CS1 (also known as SLAMF7)	TRAC and CS1 KO	TALEN mRNA (KO)	Preclinical
	UCART-CLL1	CLL1	TRAC and B2M KO;CAR at the TRAC locus	TALEN mRNA (KO); AAV6 (TI)	Preclinical
Celyad	CYAD-101	NKG2D	Expression of aTRAC-inhibitor-ymolecule peptide consisting of a truncated form of CD3ζ	Retroviral vector (co-ex-pression of TRAC-inhibitory molecule with CAR)	Phase I in CRC (NCT03692429), alloSHRINK)
Chinese People's Liberation Army General Hospital	UCART019	CD19	TRAC and B2M KO	CRISPR gRNA and Cas9 mRNA(KO)	Phase I in B cell leukemia and phase II inB cell lymphoma (NCT03166878)
	Mesothelin CAR T cells	Mesothelin	TRAC and PD1 KO	CRISPR gRNA and Cas9 mRNA(KO)	Phase I in solid tumors (NCT03545815)
	Universal dual-specificity CD19 and CD20 or CD19 and CD22 CAR T cells	CD19 and CD22 or CD19 and CD20	TRAC KO	CRISPR gRNA and Cas9 mRNA(KO)	Phase I in B cell leukemia and phase II in B cell lymphoma (NCT03398967)
CRISPR Therapeutics	CTX-101	CD19, BCMA or CD70	TRAC and B2M KO	CRISPR gRNA and Cas9 mRNA(KO)	Preclinical (B cell malignancies (CD19),MM (BCMA) or solid tumors (CD70))
Fate Therapeutics	FT-819	CD19	TRAC KO in iPSC-derived T cells	CRISPR gRNA and Cas9 mRNA(KO)	Preclinical
Memorial Sloan Kettering Cancer Center	CD19 CAR T cells	CD19	TRAC KO; CAR at the TRAC locus	CRISPR gRNA and Cas9 mRNA (KO)	Preclinical
Poseida Therapeutics	P-BCMA-ALL01	BCMA	TRAC and MHC class I KO	CRISPR gRNA and dead Cas9 fused to Clo51 nuclease (Cas-CLOVERTM) (KO)	Preclinical
Precision Biosciences and Servier	PBCAR-0191	CD19	TRAC KO; CAR at the TRAC locus	Meganuclease mRNA (KO); AAV6	Phase I in NHL and phase II in B cell ALL (NCT0366000)
Sangamo Therapeutics	CD19 CAR T cells	CD19	TRAC and B2M with or without CISH KO; CAR at the TRAC locus	ZFN mRNA (KO); AAV6 (TI)	Preclinical
Shanghai Bioray Laboratory	CD19 UCART	CD19	TRAC and MHC class I KO	CRISPR gRNA and Cas9 mRNA (KO)	Phase I in B cell ALL and B cell NHL (NCT03229876)
Tessa Therapeutics	CAR-transduced Vy9Vδ2 cells	Undisclosed	αβ T-cell depletion	Cell sorting	Preclinical
University College London	CD19 CAR T cells	CD19	TRAC KO	CRISPR gRNA and Cas9 mRNA (KO)	Preclinical
	CD3 CAR T cells	CD3	TRAC and CD3 KO	TALEN mRNA (KO)	Preclinical
University of Ghent	Haematopoietic progenitor- derived CAR T cells	CEA	Monospecific TCR-transgenic cells lack-ing endogenous rearrangements	Differentiation of haematopoietic progenitors to T cells	Preclinical
University of Minnesota	CD19 CAR T cells	CD19	TRAC KO	TALEN mRNA, megaTAL mRNA or CRISPR gRNA and Cas9 mRNA (KO)	Preclinical
University of Pennsylvania	CD19 CAR T cells	CD19	TRAC and B2M KO; TRAC, B2M and FAS KO; TRAC, B2M, PDC1 and CTLA4 KO	CRISPR shRNA in lentiviral vector and Cas9 mRNA (KO)	Preclinical
University of Singapore	CD19 CAR T cells	CD19	TRAC inhibition via anti-CD3ε PEBLs	PEBLs (inhibition)	Preclinical

Currently, there are a number of different allogeneic CAR T cells in development (see Figure 10).

Improving the Safety of Allogeneic CAR T-cell Therapies

There are a number of potential strategies for improving the safety of allogeneic CAR T-cell products. One is to reduce the need for the alemtuzumab lymphodepleting regimen. This could be done by knocking out beta-r macroglobulin to eliminate MHC class I-mediated rejection. Even if this is done, NK cell-mediated rejection could still occur. One avenue for addressing this would be to introduce inhibitory molecules like HNAG that would prevent NK cell-mediated rejection of the allogeneic CAR T-cell.

Looking to the Future

Allogeneic CAR T-cells represent an opportunity to industrialize and standardize CAR T-cell products with consistent pharmaceutical release criteria, lowering cost and increasing access to transformative cellular therapies. Challenges remain, however, in increasing the safety and persistence of allogeneic CAR T-cells and this will continue to be an active area of research.

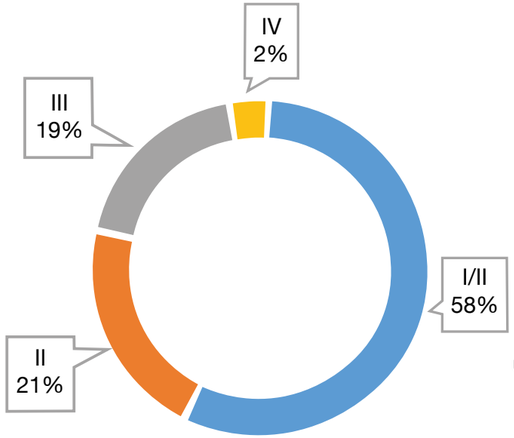
Conclusion

CAR T-cell therapy is one of the most promising approaches in anticancer therapy for hematological malignancies. The success of CD-19 targeted CAR T cells has paved the way for a new wave of CAR T-cells targeting other tumor antigens, with the aim of broadening the treatment scope beyond B-cell hematological malignances. Increasing focus on allogeneic, off-the-shelf CAR T-cell products that address many of the challenges associated with autologous CAR T cells offers a tantalizing glimpse into a future where these innovative therapies can be standardized, immediately available, redosed, or combined to deliver truly personalized cancer treatment.

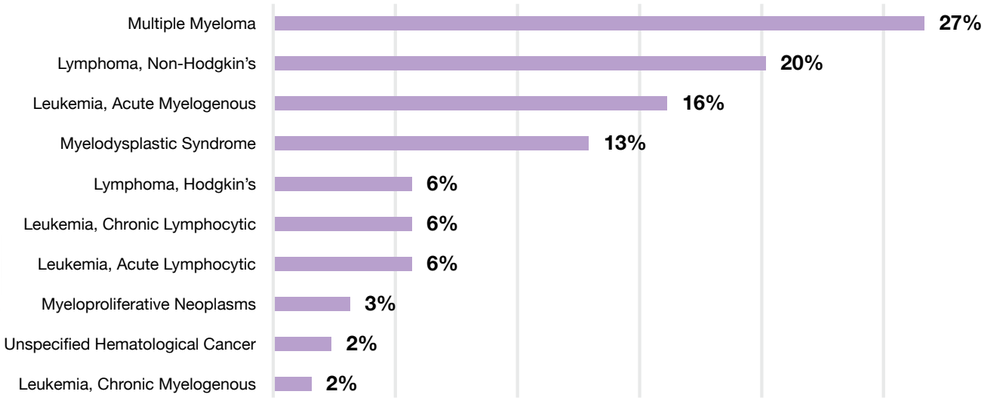
Clinical trials are the key to bringing these life-changing therapies to patients as safely and quickly as possible. At Precision for Medicine, we have deep experience across both the entire clinical development cycle and the spectrum of hematological malignancies.

Precision's Deep Expertise in Hematology Programs

Hematology by Phase

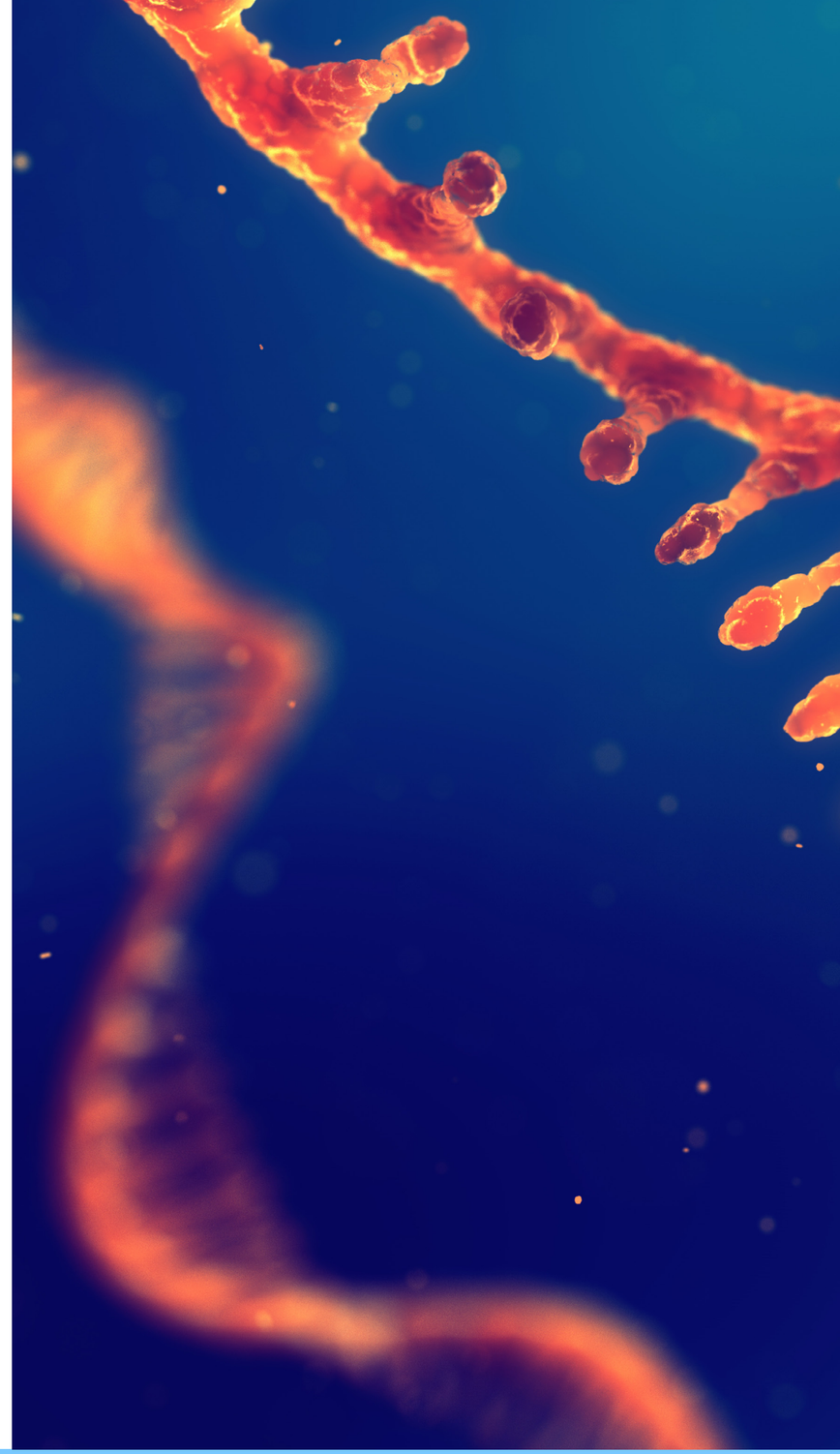


Malignant Hematology by Disease



Helping You Advance Your Cell Therapy Research

As a global clinical research organization with more than 20 years of industry experience, we integrate clinical trial execution with deep scientific knowledge, laboratory expertise, and advanced data sciences to maximize insights into patient biology and accelerate clinical development.



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