

Facts About Chimeric Antigen Receptor (CAR) T-Cell Therapy

Introduction

Autologous chimeric antigen receptor (CAR) T-cell therapy for the treatment of hematologic malignancies has generated intense interest from patients, families and healthcare providers. Clinical trials are underway for multiple B-cell malignancies, including B-cell acute lymphoblastic leukemia (B-ALL), non-Hodgkin lymphoma (NHL, including mantle cell lymphoma [MCL], follicular lymphoma [FL] and marginal zone lymphoma [MZL]), as well as other hematologic malignancies such as Hodgkin lymphoma (HL), chronic lymphocytic leukemia (CLL), acute myeloid leukemia (AML) and multiple myeloma (MM).

The first 2 autologous CAR T-cell therapies, tisagenlecleucel (Kymriah®) and axicabtagene ciloleucel (Yescarta®), were approved by the US Food and Drug Administration in 2017. In 2020, the FDA approved brexucabtagene autoleucel (Tecartus™). Lisocabtagene maraleucel (Breyanzi®) and idecabtagene vicleucel (Abecma®) were approved in 2021. Ciltacabtagene autoleucel (Carvykti™) was approved in March 2022. The movement of these and other products currently in development into clinical practice will require sufficient understanding by all parties of the technology and medical management surrounding the use of these personalized, “living” biologics in patients with cancer. This publication will explain the rationale behind CAR T-cell therapy, describe approved therapies, summarize efficacy results to date, detail significant risks that have emerged, provide practical medical management information, and highlight some unique challenges involved in the anticipated integration of this therapy into clinical practice.

Highlights

- CAR T-cell therapy equips a patient's T-cells with the ability to detect and destroy malignant cells by combining the specificity of a monoclonal antibody with the cytotoxic and memory capabilities of endogenous T-cells.
- CAR T-cell therapy involves isolating a patient's T-cells and then transfecting them with the CAR gene, expanding the cells, and re-infusing them into the patient.
- Tisagenlecleucel (Kymriah®) is approved for the treatment of patients up to 25 years of age with B-ALL (refractory or in second or later relapse). It is also approved for adult patients with relapsed or refractory (r/r) large B-cell lymphoma after two or more lines of systemic therapy and for adult patients with relapsed or refractory (r/r) follicular lymphoma (FL) after two or more lines of systemic therapy.
- Axicabtagene ciloleucel (Yescarta®) is approved for the treatment of adults with large B-cell lymphoma (LBCL) who either did not respond to first-line standard chemotherapy or relapsed within 12 months of chemotherapy and adult patients with relapsed or refractory follicular lymphoma (FL) after two or more lines of systemic therapy.
- Brexucabtagene autoleucel (Tecartus™) is approved for the treatment of adults with r/r MCL and adult patients with relapsed or refractory B-cell precursor acute lymphoblastic leukemia (ALL).
- Lisocabtagene maraleucel (Breyanzi®) is approved for the treatment of adult patients with relapsed or refractory large B-cell lymphoma after one prior therapy.
- Idecabtagene vicleucel (Abecma®) is approved for the treatment of adult patients with relapsed or refractory multiple myeloma after four or more prior lines of therapy.
- Ciltacabtagene autoleucel (Carvykti™) is approved for the treatment of adult patients with relapsed or refractory multiple myeloma who have already tried four or more prior lines of therapy
- CARs used in four of the approved products and in the most advanced clinical trials are directed against CD19, which is expressed on the surface of the vast majority of B cells, including B-cell malignancies.
- In the majority of cases, the time from T-cell harvest to infusion of CAR T-cells is several weeks.
- After infusion, CAR T-cells reach peak levels in the majority of patients between 1 and 2 weeks after infusion, though this may vary.
- The degree of expansion and persistence of CAR T-cells is one indicator of efficacy.
- Clinical trial data have demonstrated high efficacy for r/r B-cell acute lymphoblastic leukemia, and lasting remissions have been reported.
- Clinical trial data have demonstrated encouraging results for r/r chronic lymphocytic leukemia and non-Hodgkin lymphoma, with durable remissions reported.
- CAR T-cell therapy is associated with serious side effects, including cytokine release syndrome, neurotoxicity, macrophage activation syndrome and B-cell aplasia. Patient deaths have been reported. Management algorithms have been developed to mitigate these risks.
- Additional CAR targets are under investigation for the treatment of other hematologic malignancies, as well as solid tumors.
- Ongoing studies of CAR T-cells in hematologic malignancies are exploring new target antigens, streamlining the manufacture process, and long-term efficacy.

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What is a chimeric antigen receptor (CAR)?

Manufacturing a targeted T-cell

CAR T-cell therapy essentially leverages the natural ability of the human immune system to detect and destroy cancer cells. In order to effectively eliminate cancer cells, cytotoxic T-cells must first recognize them as dangerous. The T-cells must then be activated, proliferate, and effectively kill the target cell.

CARs are genetically engineered cell surface receptors designed to equip a patient's own T-cells with the ability to recognize and bind to antigens (cell surface proteins) found on tumor cells.

CARs consist of an extracellular domain capable of binding tightly to a tumor antigen, fused to a signaling domain partly derived from the T-cell receptor (TCR) (**Figure 1**).

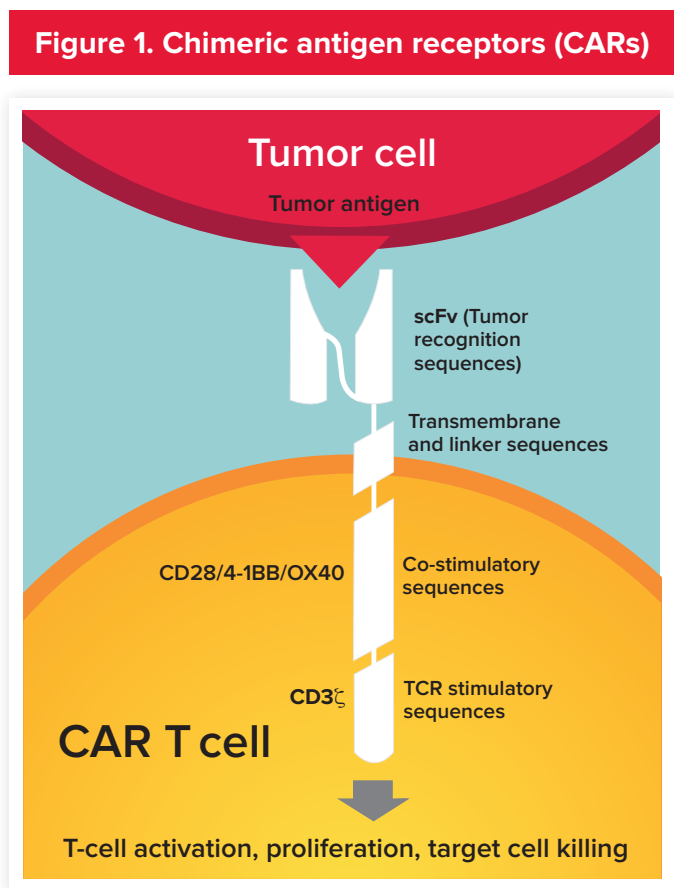


Figure 1. The structure of a CAR

The extracellular, tumor-antigen recognition domain (scFv) consists of a fragment of a monoclonal antibody specific for the desired target. The intracellular signaling domain consists of a portion of the endogenous T-cell receptor (TCR) signaling domain (CD3 ζ) and a co-stimulatory domain (CD28, 4-1BB, and OX40 have all been used as co-stimulatory domains in CARs in clinical trials). Binding of tumor target antigen results in T-cell activation, proliferation, and target cell elimination.

The CAR is activated when the extracellular domain binds to a tumor antigen, resulting in the activation of the T-cell cytotoxic response and tumor cell destruction.

The CAR extracellular domain consists of a fragment of a tumor-specific monoclonal antibody (a single-chain variable fragment, or scFv). The scFv used in four of the approved products (Kymriah[®], Yescarta[®], Tecartus[™], Breyanzi[®]) and in the most advanced clinical trials for B-cell malignancies is one directed against the cell surface protein CD19, which is expressed on the surface of the vast majority of B-cell malignancies, in addition to normal B cells. Abecma[®] and Carvykti[™] target B-cell maturation antigen (BCMA). Other potential CAR tumor target antigens are under active investigation.

The intracellular portion of the CAR consists of the signaling portion of the receptor, which when activated by tumor antigen binding to the scFv causes T-cell activation, proliferation and cytokine secretion to eliminate the tumor cell. Full activation of endogenous T-cells requires 2 signals, and CARs are designed to replicate both. For CAR T-cells, the first signal is provided by the intracellular signaling portion of the T-cell receptor (TCR) (the CD3 zeta [ζ] subunit, **Figure 1**). The second signal is provided by a “co-stimulatory” domain typically consisting of CD28, 4-1BB, or OX40. The external antibody portion of the CAR is anchored to the membrane by linker and transmembrane sequences.

The chimeric CAR molecule thus combines the specificity of a monoclonal antibody with the cytotoxic and memory capabilities of endogenous T-cells.

Autologous CAR T-cell therapy involves genetically transferring the CAR gene directly into a patient's own T-cells, which have been collected by leukapheresis. The CAR equips the T-cells to recognize and destroy cancer cells when infused back into the patient.

CARs differ in design, the vector used for gene transfer, and manufacturing processes. CAR designs may differ in the scFv region, intracellular co-stimulatory domains, and linker and transmembrane sequences (**See Figure 1**).

How does CAR T-cell therapy work?

CAR T-cell therapies require a high degree of coordination between the primary oncology team, the certified prescribing oncologist/center, and the manufacturing facility in which the CAR T-cells are generated. The process often begins with a referral from a primary oncologist who has determined their patient is eligible for CAR T-cell therapy. Suitability for treatment is confirmed by the certified treatment center, where the patient will have continued care throughout the entire process, from pre-screening to long-term follow up. While specific protocols vary for each product and clinical trial, CAR T-cell therapy generally involves the following steps:

1. Patients are evaluated to determine if CAR T-cell therapy is safe and appropriate. The patient's cells are then collected by leukapheresis.

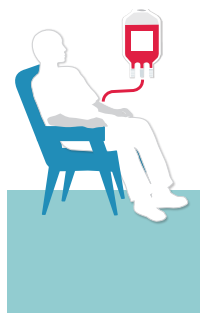


In general, patients eligible for CAR T-cell therapy must:

- Have tumors that are positive for the CAR target (CD19, for example).
- Have an adequate number of T-cells for collection. The threshold for a patient's required absolute lymphocyte count varies by protocol.
- Not have an active, uncontrolled infection.
- Have adequate performance status and organ function.
- Not have certain relevant comorbidities, such as certain cardiovascular, neurologic, or immune disorders.

It is important to note that prescreening tests and precise criteria for eligibility vary by treatment regimen or protocol, malignancy and CAR T-cell product.

2. T-cells are harvested from the patient by leukapheresis.

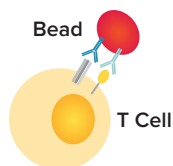


The patient's treatment regimen is frequently altered to increase the likelihood that a sufficient number of functional T-cells can be collected. This may include the avoidance of:

- Corticosteroids within a certain time frame prior to leukapheresis.
- Salvage chemotherapy within a certain time frame prior to leukapheresis.

Depending on the product or clinical trial, the collected cells may be frozen and shipped to a Good Manufacturing Practice (GMP) facility for further processing. CAR T-cell manufacturing facilities are highly specialized, requiring complex infrastructure and highly skilled, knowledgeable staff. There, lymphocytes are enriched, and cell subsets (such as CD4+ and CD8+ T-cells) may be selected.

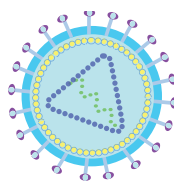
3. T-cells are activated.



The isolated T-cells are placed in culture and are activated, typically by exposing them to antibody coated beads.

The capacity of T-cells to grow *in vitro* varies from patient to patient. A significant number of patients have been excluded from selected clinical trials due to inadequate *in vitro* T-cell expansion capacity. The development of "off-the-shelf" (allogeneic) CAR T-cells, described below, is an area of active investigation as a means to broaden CAR T-cell therapy.

4. The CAR gene is introduced into activated T-cells *in vitro*.



Means of introducing the CAR gene into T-cells include the use of several viral vectors, which results in permanent genome modification and persistent CAR expression. These vectors have been shown to have low oncogenic potential and limited immunogenicity. Other expression systems, including plasmid-based and transient expression systems, are currently in development.

5. The CAR T-cells are expanded *in vitro*.

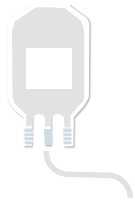


In order to generate sufficient numbers of CAR T-cells for therapy, the cells are expanded using a variety of culture systems.

Following expansion, the cells are washed and concentrated, and samples are removed for quality testing. Finally, the CAR T-cells may be frozen for shipment to the infusion site.

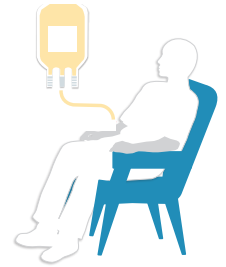
While the CAR T-cells are being manufactured, patients may require "bridging chemotherapy" to control their cancer. This may be at the discretion of the primary oncologist.

6. Prior to CAR T-cell infusion, the patient undergoes “preconditioning” chemotherapy.



In the days prior to infusion of CAR T-cells, the patient undergoes chemotherapy to deplete endogenous lymphocytes, which allows for the engraftment and expansion of CAR T-cells. Lymphodepletion “makes room” for the CAR T-cells and reduces immunosuppressive cells that may threaten CAR T-cell expansion. Lymphodepletion also releases endogenous intracellular inflammatory cytokines, which promote CAR T-cell activity once the cells are infused. Preconditioning regimens vary by protocol and by individual patient.

7. The CAR T-cells are infused back into the patient.



The cells, which are typically delivered to the infusion site and kept in a frozen state until just before the infusion, are thawed and infused into the patient. In some studies, cells are given by fresh infusion. Depending on the product or clinical trial, cells may be infused in the inpatient setting, or in the outpatient setting with careful monitoring.

The dose of CAR T-cells varies by protocol; finding the optimal cell doses, number of doses, and infusion timing that will provide maximal efficacy with minimal toxicity are areas of active investigation.

CAR T-cells generally reach peak levels between 1 and 2 weeks after infusion. The degree of expansion and persistence of CAR T-cells may be indicators of CAR T-cell efficacy. The capacity for CAR T-cells to develop into memory cells has been demonstrated – instances of sustained persistence of CD19-directed CAR T-cells out several years have been reported.

Figure 2. CAR T-cell manufacturing process

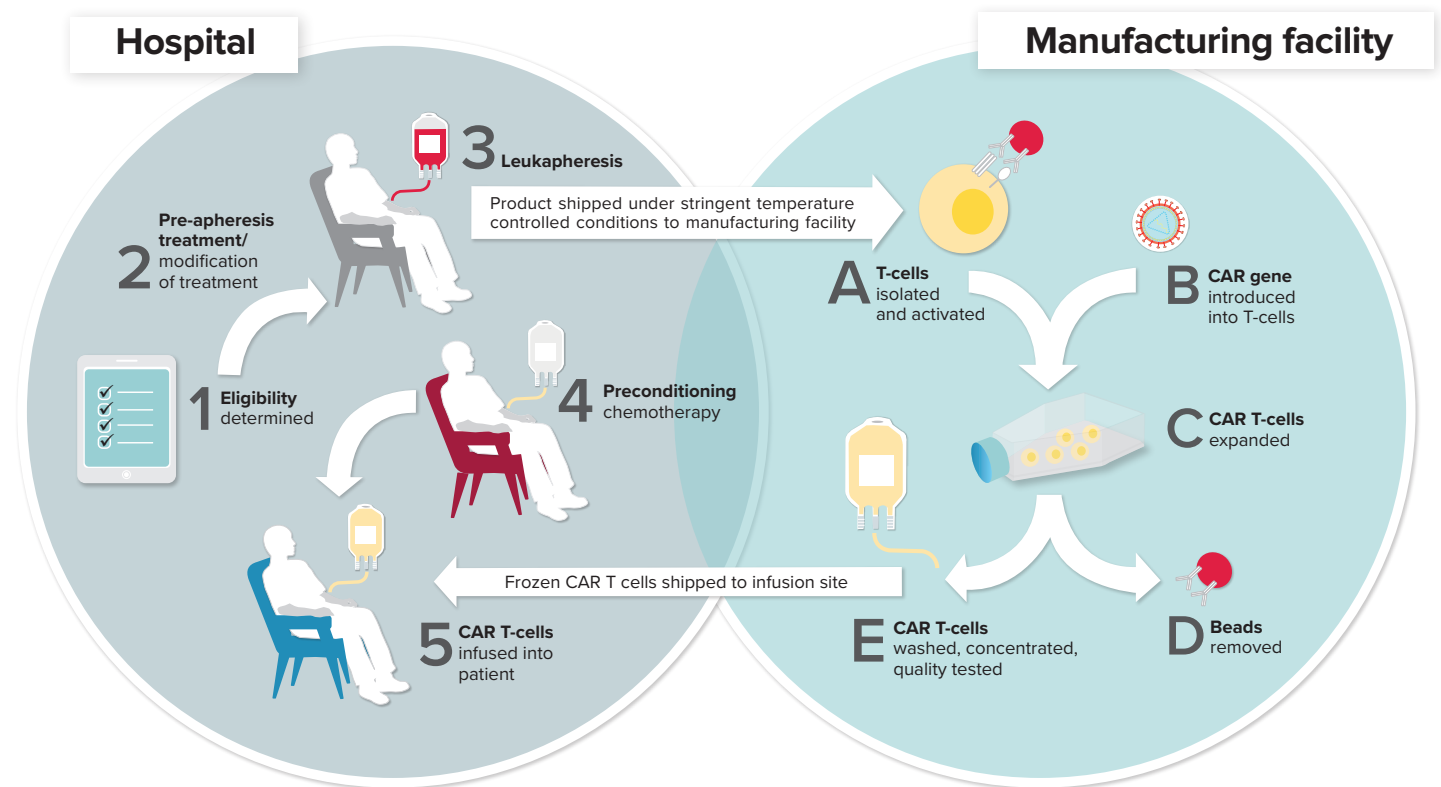


Figure 2. The time from endogenous T-cell collection to CAR T-cell infusion varies, but typically takes several weeks. The entire process, from prescreening to CAR T-cell infusion, is summarized.

Clinical trial results

Efficacy

The most advanced clinical trials, including those completed for approved therapies, have evaluated CD19- directed CAR T-cell therapy for the treatment of relapsed and refractory B-cell acute lymphoblastic leukemia (r/r B-ALL). While various studies differed in patient selection criteria, CAR design, method of gene transfer, cell culture techniques, preconditioning regimens, and the timing and dose of cell infusions, high efficacy has been observed at multiple centers. While clinical trial data are continually being updated, complete response (CR) rates have ranged from 70% to 90%, and lasting remissions have been reported. In the phase 2 pivotal trial for tisagenlecleucel, the overall remission rate (ORR, defined as CR/CRi within 3 months) for patients with r/r B-ALL was 82% (n = 50). All infused patients with a best overall response of CR/CRi were minimal residual disease (MRD) negative; 95% of these patients were negative by day 28.

Relapse occurs and may, in some cases, be related to tumor cells losing expression of CD19 (CD19 “escape variants”) or limited persistence of CAR T-cells. Relapse data are continually being updated as patients proceed further out from treatment.

Encouraging clinical responses have also been reported in trials of CD19-directed CAR T-cells for the treatment of r/r chronic lymphocytic leukemia (r/r CLL) and r/r non- Hodgkin lymphoma (r/r B-NHL). While encouraging, response rates for CAR T-cell therapy for the treatment of r/r CLL and r/r B-NHL have not approached those seen in B-ALL.

In the phase 2 trial for tisagenlecleucel in adult r/r diffuse large B-cell lymphoma (DLBCL), the overall response rate was 52% and the CR rate was 40%. The approval of tisagenlecleucel for adult patients with relapsed or refractory follicular lymphoma (FL) was based on the ELARA trial, a multicenter, single-arm, open-label trial evaluating tisagenlecleucel. Among 90 patients in the primary efficacy analysis, the ORR was 86% with a complete response (CR) rate of 68%.

In the phase 1 and 2 pivotal trials for axicabtagene ciloleucel in patients with refractory large B-cell lymphoma, the overall response rate was 83% and the CR rate was 58%. On March 5, 2021, axicabtagene ciloleucel was approved for adult patients with relapsed or refractory follicular lymphoma (FL) after two or more lines of systemic therapy. Approval was based on ZUMA-5, a single-arm, open-label, multicenter trial. Among 81 patients in the primary efficacy analysis, the ORR was 91% with a complete remission (CR) rate of 60% and a median time-to-response of 1 month. Clinical trials are underway in FL, MCL and MZL.

In April 2022, axicabtagene ciloleucel was approved for patients with large B-cell lymphoma (LBCL) who either did not respond to first-line standard chemotherapy or relapsed within 12 months. This approval was based on results from the ZUMA-7 study, which showed clear benefits of earlier CAR T-treatment. One-time axi-cel infusion led to significant improvement in event-free survival compared to the current standard of care, which includes

chemotherapy and a stem cell transplant. Axi-cel treated patients were 2.5 times more likely to be alive two years later and have no signs of disease progression despite having no additional treatment.

In the phase 2 trial for brexucabtagene autoleucel in patients with r/r MCL, the complete remission rate was 62% and the objective response rate was 87%.* In October, 2021 the FDA approved brexucabtagene autoleucel to add a new indication for adult patients with relapsed or refractory (r/r) B-cell precursor acute lymphoblastic leukemia.

Idecabtagene vicleucel and ciltacabtagene autoleucel use CAR T-cells that target the B-cell maturation antigen (BCMA) have been approved for the treatment of relapsed and refractory multiple myeloma. The CARTITUDE-1 study of ciltacabtagene autoleucel resulted in a 98% response rate to treatment and 78% stringent complete response rate. The trials of idecabtagene vicleucel found an overall response rate of 72%, very good partial response of 54% and a stringent complete response of 29%.

While many patients with B-ALL that have undergone successful CAR T-cell therapy proceed to allogeneic hematopoietic stem cell transplant (HSCT), other patients have not. Precisely how CAR T-cell therapy will fit into current treatment paradigms – and whether it will best serve as a replacement for transplant or a bridge to transplant – are still open questions.

Relapses may not respond to a second infusion of the same CAR T-cells. This may be due to multiple factors:

1. The relapse may be due to escape variants, in which the malignancy no longer expresses the target antigen.
2. The CARs may be immunogenic, such that the patient’s immune system eliminates a second infusion of CAR T-cells due to an immune response.

Approaches to treating relapse due to escape variants, including using CAR T-cells directed against a second target, are under investigation.

CD19 has proven to be an effective CAR target for any B-cell malignancies and is the basis for four of the approved therapies. Two additional therapies were approved for multiple myeloma targeting CD38. Other CARs in clinical trials target additional markers found on a wide variety of hematologic malignancies. “Ideal” target antigens for CAR T-cell therapy would be restricted to tumor cells. However, most CAR T-cell targets are also expressed on normal cells. The tissue distribution of the marker must be taken into consideration in order to avoid the destruction of non-malignant or essential cells.

Identification of additional tumor markers that can be leveraged for CAR T-cell therapy across a wide range of hematologic malignancies is an area of active investigation.

* Brexucabtagene autoleucel was granted accelerated approval by the FDA for r/r MCL. Continued approval may depend on verification of response rates and description of clinical benefit in a confirmatory trial.

Safety

While CAR T-cell therapy has shown tremendous promise, data from clinical trials and the use of commercial products reveal a similar pattern of serious toxicities. Consideration of CAR T-cell therapy should take into account the potential for circulating CAR T-cells to persist for years. The American Society for Transplantation and Cellular Therapy (ASTCT) has published consensus guidelines for grading the severity of the 2 toxicities most commonly seen in patients who have received CAR T-cell therapy: cytokine release syndrome (CRS) and neurotoxicity. Toxicity prevention and management are primary focuses of current investigation.

Risks and Adverse Effects

Cytokine release syndrome (CRS)

CRS is a systemic inflammatory response due to high circulating levels of inflammatory cytokines, resulting from CAR T-cell expansion and activation in response to tumor antigen binding. Some degree of CRS has been observed in most CD19 and BCMA directed CAR T-cell clinical trials, ranging from mild flu-like symptoms (malaise, fatigue, myalgia, nausea, anorexia) to high fever, tachycardia, hypotension, hypoxia and organ failure requiring vasopressors, ventilatory support and supportive care in the intensive care unit (ICU).

CRS typically occurs within the first 1 to 3 weeks after cell infusion. CRS severity appears to correlate with disease burden in B-ALL. CRS grading systems and treatment algorithms have been established as part of each treatment protocol and may be center-specific.

Severe CRS can be mitigated by the use of anti-IL-6 receptor monoclonal antibody (e.g., tocilizumab) and corticosteroids; however, higher dose corticosteroids have the disadvantage of potentially dampening the anti-tumor response, though more recent analyses have shown that this is not as much a concern for some CD19 products as initially thought.. Tocilizumab is frequently used as a front-line treatment for severe CRS, though assessment of its effect on CAR T-cell proliferation and activity is ongoing. The reported incidence of CRS of any severity following CD19- targeted CAR T-cell therapy varies among clinical trials.

In the majority of cases, CRS is reversible, and depending on management strategies, typically resolves in patients by 2 to 3 weeks. However, patient deaths have occurred as a result of severe CRS following CAR T-cell therapy.

All approved products are available only through a restricted program under a Risk Evaluation and Mitigation Strategy (REMS), and all have specific instructions in their prescribing information for managing CRS and neurotoxicity.

Neurotoxicity

Neurotoxicity (also called Immune Effector Cell-Associated Neurotoxicity Syndrome) has often been observed in clinical trials and in commercial use of CD19 and BCMA directed CAR T-cell therapy. The incidence of neurotoxicity (of any severity) reported in different clinical trials has varied. Symptoms include confusion, delirium, dysphasia, global encephalopathy and seizures. Symptoms of neurotoxicity may not occur at the same time as CRS and may not be controlled by IL-6 receptor blocking with tocilizumab. Effective management strategies are under active investigation. Treatment approaches include corticosteroids and more recently the use of IL1-R blockade (anakinra) and supportive care, which may include anti-epileptic medication.

Patients experiencing neurotoxicity may require ICU support, depending on the severity of symptoms. Patients with mild symptoms can sometimes be observed without receiving steroids. A neurologist is often consulted. The mechanism of neurotoxicity is unknown, though thought to be related to endothelial activation. CAR T-cells have been found in the spinal fluid of affected patients. While reversible in the majority of cases, cerebral edema associated with neurotoxicity is a rare but potentially fatal complication of CAR T-cell therapy, and deaths have occurred as a result.

Macrophage activation syndrome (MAS)/ hemophagocytic lymphohistiocytosis (HLH)

MAS typically occurs concurrently with CRS, with many clinical manifestations overlapping. Laboratory findings associated with the activation of macrophages include high levels of ferritin, C-reactive protein (CRP) and d-dimer. Transaminitis and elevated triglycerides have been observed, with hypofibrinogenemia and bleeding occurring in a small number of patients. IL-6 receptor blocking therapy with tocilizumab and corticosteroids has been effective in some cases of MAS and CRS. In some cases of HLH, etoposide chemotherapy can be used.

B-cell aplasia (“on-target, off-tumor” toxicity)

Normal B cells and B-cell precursors express CD19, and B-cell aplasia that can last for months or years is an expected consequence of successful CD19 and BCMA directed CAR T-cell therapy. Patients may receive immunoglobulin replacement therapy and appropriate antimicrobial prophylaxis.

Prolonged cytopenia

Prolonged cytopenias occur with all CAR T-cell therapies with neutropenia the most common, followed by thrombocytopenia and occasionally anemias. Prolonged cytopenias with bleeding and infection requiring stem cell

transplantation for hematopoietic recovery resulting in death have occurred following treatment with Abecma® and Carvykti™.

Parkinsonism and Guillain-Barre syndrome (GBS)

Parkinsonism and its associated complications resulting in fatal or life-threatening reactions have occurred following

treatment with Carvykti. A single, fatal case of GBS occurred following Carvykti therapy despite treatment with intravenous immunoglobulins.

A summary of the toxicities associated with CD19-directed CAR T-cell therapy, in addition to timing and management strategies, is shown in **Table 1**.

Table 1. Toxicities associated with CAR T-cell therapy*

	Associated Therapy	Signs and Symptoms	Timing	Management
Cytokine release syndrome (CRS)	All approved CAR T-cell therapies	Fever, myalgia, hypotension, hypoxia, potential organ failure	Usually within 1-3 weeks post infusion	<ul style="list-style-type: none"> • Tocilizumab • Corticosteroids • Severe CRS may require vasopressors, ventilatory support and supportive care in the ICU
Neurotoxicity (Immune Effector Cell-Associated Neurotoxicity Syndrome [ICANS])	All approved CAR T-cell therapies	Confusion, delirium, hallucinations, encephalopathy, aphasia, facial paresis, mutism, myoclonus, tremors, somnolence, seizures	Median time to onset varies but generally within 1 week. May or may not be concurrent with CRS	<ul style="list-style-type: none"> • Corticosteroids • Supportive care, which may include anti-epileptic medication
Macrophage activation syndrome (MAS)	<ul style="list-style-type: none"> • ABECMA® (idecabtagene vicleuce)l • CARVYKTI™ (ciltacabtagene autoleuce)l • TECARTUS® (brexucabtagene autoleuce)l 	High levels of ferritin, CRP, d-dimer; hypofibrinogenemia associated with bleeding, transaminitis and elevated triglycerides	Concurrently or shortly after CRS	<ul style="list-style-type: none"> • Tocilizumab • Corticosteroids • Etoposide (in some cases of hemophagocytic lymphohistiocytosis [HLH])
B-cell aplasia	All approved CAR T-cell therapies	Hypogammaglobulinemia	Within first few weeks post infusion, may last indefinitely	<ul style="list-style-type: none"> • Immunoglobulin replacement therapy • Prophylactic antibiotics in some cases
Prolonged Cytopenias	All approved CAR T-cell therapies	Neutropenias and thrombocytopenias, anemias	Up to two months (occasionally longer) after treatment	<ul style="list-style-type: none"> • Monitor blood counts prior to and after treatment • Myeloid growth factor and blood product transfusion
Parkinsonism	CARVYKTI™ (ciltacabtagene autoleuce)l	Occurs with neurotoxicity but with Parkinsonian symptoms distinct from ICANS: tremor, bradykinesia, involuntary movements, stereotypy, loss of spontaneous movements, masked facies, apathy, flat affect, fatigue, rigidity, psychomotor retardation, micrographia, dysgraphia, apraxia, lethargy, confusion, somnolence, loss of consciousness, delayed reflexes, hyperreflexia, memory loss, difficulty swallowing, bowel incontinence, falls, stooped posture, shuffling gait, muscle weakness and wasting, motor dysfunction, motor and sensory loss	Median onset 43 days with ongoing symptoms at 530 days post infusion.	<ul style="list-style-type: none"> • Monitor patient for symptoms that may be delayed in onset • Manage with supportive care • There is limited efficacy information with medications used for the treatment of Parkinson's disease for the improvement or resolution of parkinsonism symptoms following Carvykti treatment

CRP = C-reactive protein

*Specific instructions for managing toxicities associated with tisagenlecleucel (Kymriah®), axicabtagene ciloleucel (Yescarta®), brexucabtagene autoleucel (Tecartus™), lisocabtagene maraleucel (Breyanzi®), idecabtagene vicleuce (Abecma®), and ciltacabtagene autoleucel (Carvykti™) are provided in the prescribing information.

The future

Strategies to maximize outcomes of CAR T-cell therapies, minimize toxicities, broaden targets beyond CD19, and target solid tumors are all areas of active investigation. In addition, standards establishing how CAR T-cell therapy might best fit within current treatment paradigms have not yet been determined.

Improving CAR T-cell safety

Efforts to manage the risk of serious toxicity include:

1. Developing methods to predict individual patient risk of CRS and neurotoxicity so that the dose and timing of CAR T-cell infusion can be adjusted accordingly (“risk-adapted dosing”).
2. Modulation of CAR T-cell activity and persistence, including:
 - **Development of CARs** that are activated only in the presence of small molecule drugs that can be administered according to patient tolerability (known as “switchable” or “multi-chain” CARs).
 - **“Suicide” systems**, allowing for the destruction of CAR T-cells should life-threatening toxicity occur. Numerous methods are in development, including co-expressing pro-apoptotic genes under the control of inducible promoters along with the CAR gene.
 - **Transient expression systems** that provide CAR expression only for approximately 7 days. Repeated CAR T-cell infusions would theoretically be required for effective disease control.
3. Improving the specificity of CARs. Efforts have included the development of:
 - **“Affinity-tuned” CARs**, designed with a lower affinity for the target antigen, theoretically narrowing CAR targeting only to tumor cells that greatly overexpress the antigen.
 - **“Tandem” CARs**, in which the 2 CAR cytoplasmic signaling domains are separated onto 2 different CAR molecules having different tumor target specificities. CAR T-cell activation results only in the presence of both target antigens, increasing specificity.

Improving CAR T-cell efficacy

Numerous efforts to improve the efficacy and persistence of CAR T-cells are underway and include:

1. Combining CAR T-cells with other therapies, including PD-1 blocking antibodies or kinase inhibitors.
2. Development of “armored CARs” that co-express pro-inflammatory cytokines such as IL-12 or IL-15 to allow for increased CAR T-cell proliferation and persistence in the face of tumor-mediated immunosuppression.
3. Co-expression of 2 CARs that target different antigens (CD19 and CD22, for example) with the goal of reducing remissions due to escape variants.

Improving and streamlining the manufacturing process

Efforts to streamline and standardize the manufacture of CAR T-cells in a cost-effective and time-efficient manner are underway. These include the development of “universal” or “off-the-shelf” CARs, which lack endogenous T-cell markers, allowing for allogeneic CAR T-cell therapy. This would streamline production while broadening CAR T-cell therapy to those patients who lack sufficient numbers of endogenous T-cells for processing.

One “off the shelf” approach is the use of CAR-armed natural killer (NK) cells, which can target tumors without antigen presentation or HLA matching. Anti-CD19 CAR-NK cells have been used with some success in early clinical trials targeting lymphoid tumors.

While CAR T-cell therapy holds great promise, emerging data from clinical trials and post-marketing surveillance of approved therapies will continue to inform its appropriate place in the clinical management of hematologic malignancies.

Frequently asked questions

1. Which patients are considered good candidates for CAR T-cell therapy?

Patients receiving Yescarta®, Kymriah®, Tecartus™, Breyanzi®, Abecma®, or Carvykti™ should be deemed by their physician to have adequate organ function and performance status to tolerate the therapy.

Currently, patients enrolled in CAR T-cell clinical trials are those with advanced relapsed or refractory malignancy. The suitability of CAR T-cell therapy as a first line treatment in hematologic malignancies has not been studied, though earlier use in high-risk B-ALL and LBCL patients is being explored. While protocols vary for each trial, in general, patients eligible for CAR T-cell therapy must:

- Have tumors that are positive for the CAR target (CD19, for example)
- Have an adequate number of T-cells for collection. The threshold for a patient's required absolute lymphocyte count varies among clinical trials.
- Not have an active, uncontrolled infection, including hepatitis B, hepatitis C, or HIV.
- Have adequate performance status and organ function.
- Not have certain relevant comorbidities, such as certain cardiovascular, neurologic, or immune disorders. These may vary among clinical trials.

2. Where does the infusion of CAR T-cells take place?

Depending on the protocol, cells may be infused in the inpatient setting, or with careful monitoring in the outpatient setting.

3. Does successful CAR T-cell therapy eliminate the need for transplant?

The standard of care for patients who achieve complete remission with conventional treatment for B-ALL is to proceed with allogeneic HSCT; however, whether this is warranted in all cases after successful CAR T-cell therapy is an open question – proceeding to transplant is not standard of care for NHL post-CAR T-cell therapy.

4. How do we help connect patients to the appropriate clinical trials?

Information Specialists at The Leukemia & Lymphoma Society engage patients in conversations about the role of clinical trials in their treatment, help them develop a list of questions to ask their doctor about participating in a clinical trial, provide personalized clinical trial navigation when appropriate, and assist in trying to overcome the obstacles to enrollment. Contact: 800.955.4572.

References:

- Abate-Daga D, Davila ML. CAR models: next generation CAR modifications for enhanced T-cell function. *Mol Ther Oncolytics*. 2016;3:16014. doi:10.1038/mto.2016.14
- Abecma® [prescribing information]. Summit, NJ: Celgene Corporation, a Bristol-Myers Squibb Company; 2021.
- Abecma: response rate. Bristol-Myers Squibb. <https://www.abecmahcp.com/efficacy/>
- Almasbak H, Aarvak T, Vemuri MC. CAR T cell therapy: a game changer in cancer treatment. *J Immunol Res*. 2016; 2016:5474602. doi:10.1155/2016/547602
- Batlevi CL, Matsuki E, Brentjens RJ, et al. Novel immunotherapies in lymphoid malignancies. *Nat Rev Clin Oncol*. 2016;13(1):25-40.
- Bonifant CL, Jackson HJ, Brentjens RJ, Curran KJ. Toxicity and management in CAR T-cell therapy. *Mol Ther Oncolytics*. 2016;3:16011. doi:10.1038/mto.2016.11
- CARVYKTI™ [prescribing information]. Horsham, PA: Janssen Biotech, Inc.
- Dai H, Wang Y, Lu X, Han W. Chimeric antigen receptors modified T-cells for cancer therapy. *J Natl Cancer Inst*. 2016;108(7). pii:djv439. doi:10.1093/jnci/djv439
- Breyanzi® [prescribing information]. Bothell, WA: Juno Therapeutics, a Bristol-Myers Squibb Company; 2021.
- Fesnak AD, June CH, Levine BL. Engineered T cells: the promise and challenges of cancer immunotherapy. *Nat Rev Cancer*. 2016;16:567-581.
- Frey NV, Porter DL. CAR T-cells merge into the fast lane of cancer care. *Am J Hematol*. 2016;91(1):146-150.
- Gandhi, U., Cornell, R., Lakshman A., et al. Outcomes of patients with multiple myeloma refractory to CD38-targeted monoclonal antibody therapy. *Leukemia*. 2019;33(9). <http://www.ncbi.nlm.nih.gov/pubmed/30858549>.
- Jackson HJ, Rafiq S, Brentjens RJ. Driving CAR T-cells forward. *Nat Rev Clin Oncol*. 2016;13(6):370-383.
- Kymriah® [prescribing information]. East Hanover, NJ: Novartis Pharmaceuticals Corporation; 2018.
- Lee DW, Gardner R, Porter DL, et al. Current concepts in the diagnosis and management of cytokine release syndrome. *Blood*. 2014;124(2):188-95.
- Lee DW, Santomaso BD, Locke FL, et al. ASTCT consensus grading for cytokine release syndrome and neurologic toxicity associated with immune effector cells. *Biol Blood Marrow Transplant*. 2019;25:625-38.
- Liu E, Marin D, Banerjee P, et al. Use of CAR-transduced natural killer cells in CD19-positive lymphoid tumors. *N Engl J Med*. 2020;382:545-53.
- Locke FL, Ghobadi A, Jacobson CA. 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;20:31-42.
- Maude S, Barrett DM. Current status of chimeric antigen receptor therapy for haematological malignancies. *Br J Haematol*. 2016;172(1):11-22.
- Maude SL, Laetsch TW, Buechner J, et al. Tisagenlecleucel in children and young adults with B-cell lymphoblastic leukemia. *N Engl J Med*. 2018;378:439-48.
- Maude SL, Teachey DT, Porter DL, Grupp SA. CD19-targeted chimeric antigen receptor T-cell therapy for acute lymphoblastic leukemia. *Blood*. 2015;125(26):4017-4023.
- Maus MV, Levine BL. Chimeric antigen receptor T-cell therapy for the community oncologist. *Oncologist*. 2016;21(5):608-617.
- Neelapu SS, Tummala S, Kebriaei P, et al. Chimeric antigen receptor T-cell therapy - assessment and management of toxicities. *Nat Rev Clin Oncol*. 2018;15:47-62.
- Onea AS, Jazirehi AR. CD19 chimeric antigen receptor (CD19 CAR)-redirected adoptive T-cell immunotherapy for the treatment of relapsed or refractory B-cell non-Hodgkin's lymphomas. *Am J Cancer Res*. 2016;6(2):403-424.
- Schuster SJ, Bishop MR, Tam CS, et al. Tisagenlecleucel in adult relapsed or refractory diffuse large B-cell lymphoma. *N Engl J Med*. 2019;380:45-56.
- Singh N, Perazzelli J, Grupp SA, Barrett DM. Early memory phenotypes drive T cell proliferation in patients with pediatric malignancies. *Sci Transl Med*. 2016;8(320):320ra3. doi:10.1126/scitranslmed.aad5222
- Tecartus™ [prescribing information]. Santa Monica, CA: Kite Pharma, Inc; 2020.
- Turtle CJ, Riddell SR, Maloney DG. CD19-targeted chimeric antigen receptor- modified T-cell immunotherapy for B-cell malignancies. *Clin Pharm Ther*. 2016;100(3):252-258.
- US Food and Drug Administration. FDA approves brexucabtagene autoleucel for relapsed or refractory mantle cell lymphoma. 7/27/2020.
- Wang X, Riviere I. Clinical manufacturing of CAR T cells: foundation of a promising therapy. *Mol Ther Oncolytics*. 2016;3:16015. doi:10.1038/mto.2016.15
- Yescarta® [prescribing information]. Santa Monica, CA: Kite Pharma, Inc.; 2020.

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We're Here to Help

LLS is the world's largest voluntary health organization dedicated to funding blood cancer research, education and patient services. LLS has regions throughout the country and in Canada. To find the region nearest to you, visit our website at www.LLS.org/chapterfind or contact

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LLS offers free information and services for patients and families touched by blood cancers as well as for healthcare professionals. The resources listed below are available to you and your patients.

Consult with an Information Specialist. Information Specialists are highly trained social workers, nurses, and health educators who assist through treatment, financial, and social challenges. They offer up-to-date disease and treatment information. Language services are available.

For more information, please

- Call: (800) 955-4572 (M-F, 9 a.m. to 9 p.m. ET)
- Visit: www.LLS.org/IRC
- Email: infocenter@LLS.org
- Live chat: www.LLS.org/informationsspecialists

Clinical Trials Support Center (CTSC). Work one-on-one with an LLS clinical trial nurse navigator who will personally assist throughout the entire clinical trial process. A nurse navigator will help identify potential clinical trials and overcome the barriers to enrollment (navigators help HCPs

and patients). For more information about this free service, please

- Call an Information Specialist (800) 955-4572 to be referred to the CTSC
- Visit: www.LLS.org/CTSC
- Complete a referral form at: www.LLS.org/CTSCreferral

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- **CAR T-Cell Therapy Facts** for patients. www.LLS.org/booklets and select treatment.

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Additional Resource

The National Cancer Institute (NCI)

www.cancer.gov

(800) 422-6237

The National Cancer Institute, part of the National Institutes of Health, is a national resource center for information and education about all forms of cancer, including CAR T-cell therapy. The NCI also provides a clinical trial search feature, the PDQ® Cancer Clinical Trials Registry, at www.cancer.gov/clinicaltrials, where healthcare professionals and patients can look for clinical trials.