Chimeric Antigen Receptor-T Cell Therapy

Practical Considerations for Implementation in Europe

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Abstract

Chimeric antigen receptor (CAR)-T cell therapy is a new class of cellular immunotherapies that involves ex vivo genetic modification of T cells to incorporate an engineered CAR. After infusion into the patient, the CAR-expressing T cells recognize specific tumor targets and induce an immune response against them. The technology utilized is fundamentally different from previously available cancer treatments. Currently, most CAR-T cell therapies use autologous T cells. Tisagenlecleucel (formerly CTL019) is an anti-CD19 CAR-T cell therapy that was recently approved in the United States for the treatment of pediatric and young adult patients with relapsed/refractory B-cell acute lymphoblastic leukemia (B-ALL). Tisagenlecleucel has shown robust in vivo expansion and long-term persistence, clinically meaningful durable response and remission rates, and overall survival benefit in pediatric and young adult patients with relapsed/refractory B-ALL and in relapsed/refractory diffuse large B-cell lymphoma. Common adverse events (AEs) include cytokine release syndrome, which may require hospitalization and admission to an intensive care unit, neurological toxicities, and B-cell aplasia. These AEs are manageable when treated by an appropriately trained team. Additional research is required to further develop AE management protocols. In this review, we describe regulatory requirements, clinical considerations, and site-level requirements for clinical study implementation of CAR-T cell therapy in Europe. We also provide a case study of the European experience from the first global clinical trial for tisagenlecleucel, which may serve as a useful starting point for investigators and clinicians looking to implement CAR-T cell therapy at their institutions.

Introduction

Chimeric antigen receptor (CAR)-T cell therapy is a new class of adoptive cellular immunotherapy for cancer treatment involving ex vivo genetic manipulation of T cells, using either lentiviral or retroviral vectors or nonviral gene transfer systems to express engineered CARs specific for particular tumor targets.[1,2] These reprogrammed CAR-T cells are then infused into the patient, where they initiate targeted immune responses against cells expressing the corresponding antigen. Currently, most CAR-T cell therapies in development utilize autologous T cells. The technology and strategy of using autologous, genetically modified, and adoptively transferred T cells for individualized cancer treatment differs fundamentally from available small-molecule or biologic therapies, and as with all new therapeutic modalities, introduces new regulatory and logistic considerations for medical practice. Recently, tisagenlecleucel (CTL019), directed against the B-lymphocyte antigen CD19, became the first CAR-T cell therapy to be approved in the United States (US) for the treatment of patients up to 25 years of age with B-cell precursor acute lymphoblastic leukemia (B-ALL) that is refractory or in second or later relapse. Axicabtagene ciloleucel

(KTE-C19) was the second CAR-T cell therapy approved in the US, and is indicated for the treatment of adult patients with certain types of large B-cell lymphoma who have not responded to or who have relapsed after at least 2 other systemic therapies. In this review, we provide an overview of the regulatory, clinical, and site-level requirements for clinical implementation of CAR-T cell therapy in the European setting, using experience gained from clinical trials with tisagenlecleucel.

Currently, CAR-T cell therapies that have progressed most in clinical development are primarily used for the treatment of several relapsed/refractory B-cell malignancies, particularly pediatric and young adult B-ALL, adult chronic lymphocytic leukemia (CLL), and adult diffuse large B-cell lymphoma (DLBCL).[3–20] Several structural features differentiate between the CAR constructs in clinical development. A select list of CAR-T cell therapies in development, along with their respective target antigens, viruses used for transduction, costimulatory molecules, and indications are provided in Table 1.[3–23] Most of these CARs target CD19, a marker present on the surface of B cells. There is a significant unmet clinical need in B-ALL and DLBCL for patients with relapsed/refractory disease, and investigation of novel, improved treatment options is warranted. Newly diagnosed B-ALL is usually treated with combination chemotherapy, which yields high complete remission (CR) rates and 5-year survival rates of over 90% in pediatric patients.[24–28] Cure rates are much lower for the 15% to 20% of children who relapse, and have not significantly improved over the past 2 decades.[27–31] Cure rates are also lower for adult patients and patients with refractory disease.[26,32] Higher-risk patients with B-ALL may benefit from immunotherapies, including the anti-CD19/CD3 bispecific T-cell engager antibody blinatumomab and the anti-CD22 antibody-drug conjugate inotuzumab ozogamicin.[29,32,33] These targeted therapies have demonstrated improved remission rates and median durations of remission compared with standard of care chemotherapy among adult patients with relapsed/refractory B-ALL[34,35] and are associated with high rates of remission and long relapse-free survival among pediatric patients in early phase studies.[36,37] Despite these promising results, these therapies are not curative.[35]

The 5-year relative survival rate for adult DLBCL in 2006–2008 was 55.4%.[38] Treatment involves the use of chemotherapy, rituximab, and radiotherapy, depending on age and risk.[39] Although outcomes are often good for DLBCL patients who receive combined-modality therapy,[40] more than 30% of patients will relapse.[39] Therapy for relapsed or refractory DLBCL consists of salvage chemotherapy with rituximab followed by high-dose chemotherapy and autologous stem-cell transplantation (SCT).[39] Survival rates for these patients are very poor, with 20% remaining alive at 2 years and a median overall survival (OS) between 4 and 13 months, especially for patients refractory to second-line or later therapy or who relapse within 1 year following initial treatment.[41,42]

Molecular biology and mechanism of action

Tisagenlecleucel uses a self-inactivating minimal lentiviral vector containing the anti-CD19 CAR transgene that was selected and designed to avoid potential induction of secondary cancers or formation of replication-competent pathogenic viruses.[1] This CAR comprises a murine single-chain antibody fragment that recognizes CD19 and is fused to the CD8-alpha hinge and transmembrane region, followed by the intracellular signaling domains from the costimulatory molecule 4–1BB (CD137) and the CD3-zeta chain (Fig. 1). When the CAR binds to CD19 in vivo, the CD3-zeta component transmits a signal to initiate T-cell activation and target cell elimination, leading to the release of cytokines.[43] The 4–1BB component acts as the required second signal for T cell activation and enhances the expansion, persistence, and anti-tumor activity of tisagenlecleucel.[44] In comparison with other CARs that utilize CD28 costimulation, use of 4-1BB has resulted in preferential expansion of memory T cells and enhanced CAR-T cell persistence.[45] In addition, the

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Table 1

Select List of CAR-T Cell Therapies in Clinical Development

CAR-T Cell Therapy	Target Antigen/Virus/Costimulatory Molecule	Target Indication(s)
Tisagenlecleucel (CTL019)[4–8] Novartis Pharmaceuticals	CD19/Lentivirus/4–1BB	Pediatric/young adult relapsed/refractory B-cell ALL Adult relapsed/refractory DLBCL

Axicabtagene ciloleucel (KTE-C19)[9,10,21–23]
   Kite Pharma
	CD19/Gammaretrovirus/CD28	Adult relapsed/refractory NHL, including:
		   DLBCL
		   Primary mediastinal B-cell lymphoma
		   Transformed follicular lymphoma
		   Mantle cell lymphoma
		   ALL

CD19 CAR-T cells[11,12] Fred Hutchinson Cancer Research Center	CD19/Lentivirus/4-1BB	Adult relapsed/refractory B-cell malignancies (including ALL and CLL)
JCAR017[13] Juno Therapeutics	CD19/Lentivirus/4-1BB	Adult relapsed/refractory DLBCL Primary mediastinal B-cell lymphoma Follicular lymphoma Mantle cell lymphoma
bb2121[14–16] bluebird bio	B cell maturation antigen/Lentivirus/4-1BB	Adult relapsed/refractory multiple myeloma
19–28z CAR-T cells[17,18] Memorial Sloan-Kettering Cancer Center	CD19/Gammaretrovirus/CD28	Adult relapsed/refractory B-cell ALL
Anti-CD22 CAR-T cells[19] National Cancer Institute	CD22/Lentivirus/Not stated	Pediatric/young adult relapsed/refractory B-cell malignancies
UCART19 (allogeneic CAR-T cells)[20] Cellectis	CD19/Lentivirus/4-1BB	Relapsed/refractory ALL

ALL = acute lymphoblastic leukemia, CAR-T = chimeric antigen receptor T cell, CLL = chronic lymphocytic leukemia, DLBCL = diffuse large B-cell lymphoma, NHL = non-Hodgkin lymphomas.
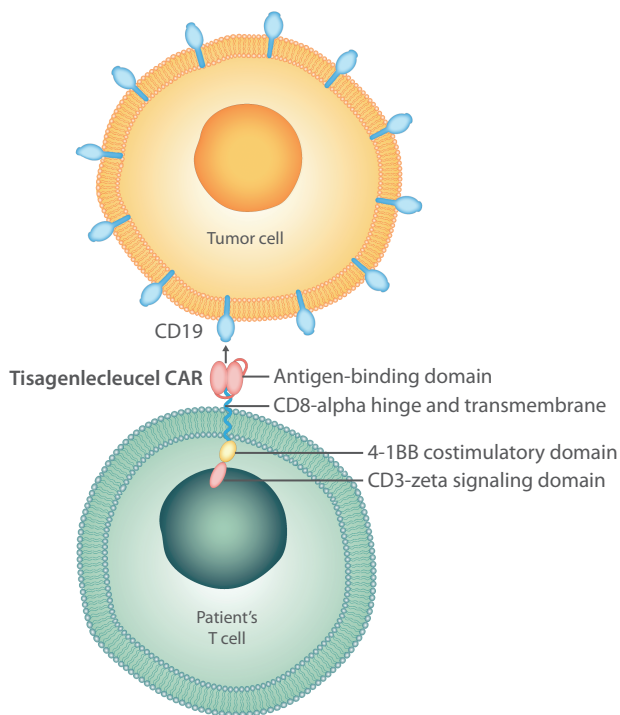
Figure 1. Structure and mechanism of action of tisagenlecleucel.

integration of a CD8-alpha hinge and transmembrane domain into a CAR containing the CD28 signaling domain has been shown to reduce the level of production of the cytokines interferon-gamma and tumor necrosis factor-alpha and to reduce

activation-induced cell death, thereby increasing cell survival, compared with CARs containing a CD28 hinge and transmembrane domain.[46] These differences result in genetically and phenotypically unique CAR-T cells and may explain differences in emerging clinical data for CAR-T cell therapies. However, additional research is needed to fully define the impact of the various domains on the short- and long-term clinical effects.

Early clinical studies confirmed the proposed mechanism of action for tisagenlecleucel, demonstrating in vitro reactivity against CD19-positive cells with high cytokine production, expansion of tisagenlecleucel in vivo, elimination of detectable CD19-positive leukemic cells, and tisagenlecleucel expansion and persistence that were correlated to response.[3,47,48] Persistence of tisagenlecleucel was detectable 14 to 49 months after infusion in adult CLL patients who achieved CR, and the cells retained activity against CD19 up to 3 years after infusion.[3] Persistence of tisagenlecleucel in pediatric and young adult B-ALL patients was detectable for up to 2 years.[48] Tisagenlecleucel expansion was associated with the development of cytokine release syndrome (CRS).[3,48]

Manufacturing process

Manufacturing of tisagenlecleucel occurs at a central facility and must be coordinated closely with the treatment center to ensure timely management of each patient leading up to infusion (Fig. 2).[49] An overview of the manufacturing process has recently been published[1]; briefly, it begins with collecting leukocytes by nonmobilized leukapheresis. When the targeted number of CD3-positive cells and total nucleated cells are collected, the cells are cryopreserved and shipped to the manufacturing facility. After thawing, the cells are processed to enrich in T cells and remove
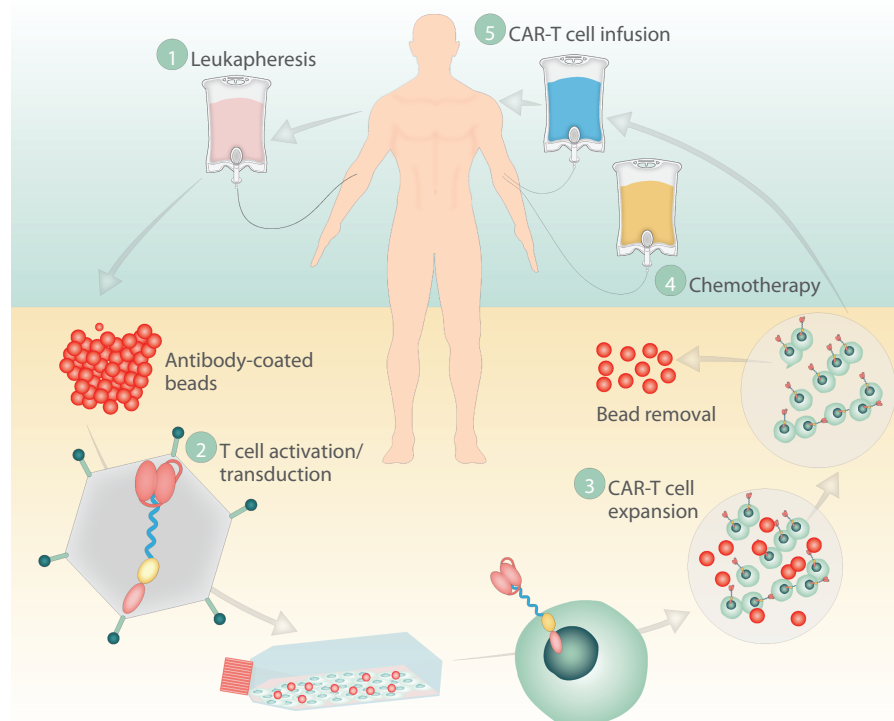

Figure 2. Manufacturing of tisagenlecleucel. Reprinted with permission from reference 49.

other cell types such as monocytes, blasts, and other B-lineage cells. The T cells are activated by binding to anti-CD3/CD28 antibody-coated paramagnetic beads and transduced with the lentiviral vector containing the anti-CD19 CAR transgene. The T cells are cultured for several days to reach the required minimum number of CAR-positive T cells. The cells are then separated from the beads, harvested, washed, and once again cryopreserved. Full release testing, including for purity, identity (to ensure the presence of the CAR transgene), potency (to ensure appropriate CAR expression and cytokine secretion), viability, and sterility, is completed before release and shipment of the final product to the clinical site.[1]

Regulatory requirements

CAR-T cell therapies in Europe are overseen by the European Medicines Agency's Committee for Advanced Therapies.[50] They fall under the category of advanced therapy medicinal products (ATMPs), are more specifically classified as genetically modified cells and thus considered gene therapy medicinal products, and therefore must undergo the central marketing authorization procedure. The clinical trial approval process is governed under Directive 2001/20/EC, but not yet fully harmonized between European Union (EU) member states.[50] This may in part change in 2019 when the new EU Clinical Trials Regulation (Regulation EU No 536/2014) is set to go into effect, providing a single application point for clinical trials and a harmonized approval procedure across the EU.[51] Since genetically modified autologous T cells are considered to be genetically modified organisms (GMOs), clinical trials for CAR-T cell therapies will also require approval for the use and release of a GMO, which is based on a risk assessment for the environment and for the healthcare professionals handling the product. However, GMOs are classified and regulated differently among EU member states, leading to different application processes with varying timelines. This specific authorization comes in addition to approval from ethics committees and national competent authorities (Table 2).[50–54]

The environmental risk assessment (ERA) must be performed according to the requirements of the EU member state in which the trial will be performed. While there is detailed guidance on the ERA requirements for a marketing authorization application, the national requirements and application documentation for clinical studies vary depending on whether the member state regards use of the GMO-containing medicinal product in the clinical trial to be "contained use" or "deliberate release."[55] Legislation

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Table 2

Typical Regulatory Requirements for Tisagenlecleucel Therapy in the European Setting[50–54]

Advanced Therapy Medicinal Products classification and central marketing authorization (Regulation [EC] No 1394/2007 and Directive 2001/83/EC)

Genetically modified organism environmental risk assessment (Directive 2001/18/EC and Commission Decision 2002/623/EC)

Country/site-specific requirements

  Manufacturing authorization according to good manufacturing practice standards (Commission Directive 2003/94/EC)

  Clinical trial authorization (Directive 2001/20/EC; to be replaced in 2019 by Clinical Trials Regulation EU No 536/2014)

  Ethics and biosafety committee approvals

  "Chain of identity" requirements

  Unique rules for product storage

  Importation and customs requirements

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concerned with GMOs was drafted primarily with plant GMOs in mind, with a goal to protect consumers and crops from contamination. Consequently, information requests are not always relevant to clinical trials, and application forms are generally not designed for medicinal products. In addition, the various agencies tasked with the evaluation of GMO use applications may also be responsible for the evaluation of transgenic plants, genetically modified foods and feeds, or environmental biosafety, depending on the member state, and therefore do not necessarily review the application with a focus on clinical studies in the context of a hospital. In a number of member states, GMO applications for clinical trials are managed by a different government agency than the competent health authority. This often leads to delays in authorizations as these bodies do not necessarily operate according to the same timetables. Furthermore, in some countries, the evaluation involves a public consultation, adding to the time needed for evaluation, and bearing the risk for the industry of releasing confidential data to the public. It is unclear how the EU Clinical Trials Regulation will specifically affect a harmonized approach to CAR-T cell therapy trials, as the new Regulation does not take into account the GMO aspects of these therapies. Additionally, submission of relevant documentation is not foreseen in the upcoming clinical trial applications portal, which would render the submission of clinical trial applications with GMOs virtually impossible in the future, and therefore needs to be addressed.

Other important aspects are the technical and logistical requirements for shipping products between countries, including country-specific "chain of identity" requirements. Indeed, the collection, storage, shipping, and labeling of cells follow a unique set of rules for importation and customs clearance. Several varying regulations apply, depending on whether the EU member state regards white blood cells as blood-derived products (Directive 2002/98/EC) or as tissues and cells for further manufacturing (Directive 2004/23/EC). Depending on this classification, various labeling and cell donor testing requirements may apply across the various EU countries. Due to the lack of harmonization concerning donor testing requirements, as pertinent to the development of tissue and cell-based products versus blood-derived products, source material procured within 1 jurisdiction may not meet the donor eligibility requirements within another jurisdiction. Therefore, before first-in-human clinical trials, all country/regional-specific donor testing requirements must be taken into account, and should be met to enable clinical use of these products. A list of useful government and society websites is provided in Table 3.

Before applying for a clinical trial authorization, the manufacturer must request manufacturing authorization from the appropriate competent authority, which may involve inspection to confirm good manufacturing practice (GMP) compliance (Commission Directive 2003/94/EC). Manufacturing licenses from 1 EU member state are mutually recognized by all other member states. A guideline on specific GMP requirements applying to ATMPs is currently available that gives useful additional information.[56] If the manufacturer is outside the EU, the importer is required to ensure that the product is manufactured in accordance with EU standards and must obtain an import license.[52]

Clinical considerations

The procedures and criteria for patient selection and enrollment, treatment, toxicity management, and follow-up were thoroughly defined in the clinical research protocols for tisagenlecleucel

Useful Government and Society Websites

Government/Society	Website
EMA	http://www.ema.europa.eu/ema/
EMA: Advanced Therapy Medicinal Products	http://www.ema.europa.eu/ema/index.jsp?curl=pages/regulation/general/general_content_000294.jsp
EMA: Genetically Modified Organisms (Directive 2001/18/EC)	http://eur-lex.europa.eu/legal-content/EN/TXT/?qid=1407852294664&uri=CELEX:32001L0018
EMA: List of National Competent Authorities in the European Economic Area	http://www.ema.europa.eu/ema/index.jsp?curl=pages/medicines/general/general_content_000155.jsp&mid=WC0b01ac0580036d63
Foundation for the Accreditation of Cellular Therapy	http://www.factwebsite.org/
Joint Accreditation Committee—International Society for Cellular Therapy (ISCT) and European Society for Blood and Marrow Transplantation (EBMT)	http://www.jacie.org/
Austrian Network for Gene Therapy	http://angt.austrianova.com/angt/
British Society of Gene Therapy	https://www.bsgct.org/
European Society of Gene and Cell Therapy	https://www.esgct.eu//Home.aspx
Finnish Gene Therapy Society	http://fsgt.fi/
French Society of Cellular and Gene Therapy	https://www.sftcg.fr/
German Gene Therapy Society	http://www.dg-gt.de/
Netherlands Society of Gene and Cell Therapy	http://www.nvgct.nl/
Spanish Society of Gene and Cell Therapy	https://www.setgyc.es/
Swedish Society for Gene and Cell Therapy	http://www.ssgct.org/

EMA = European Medicines Agency, ICH = International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use.

(Fig. 3). Briefly, in the phase 2 tisagenlecleucel clinical trials in pediatric and young adult patients with B-ALL and adult patients with DLBCL, patients had relapsed or refractory disease measurable at screening, had no available standard treatment options, an expected survival of >12 weeks, and adequate organ function (to ensure they would be capable of tolerating CRS).[48,57,58] A complete overview of inclusion and exclusion criteria is published on ClinicalTrials.gov.[57,58] At the screening visit, patients and their families or parents were informed about the clinical trial concept, procedures and timelines, and on what to expect from CAR-T cell therapy based on the limited number of clinical trials, both in terms of efficacy and possible CAR-T cell therapy-specific adverse events (AEs). They were also informed that manufacturing failures can occur. After informed study consent was obtained, patients underwent trial-specific screening investigations, including confirmation of measurable CD19-positive disease and a nonmobilized leukapheresis to harvest T cells. Protocol-associated leukapheresis guidelines provided specific washout periods for certain antineoplastic drugs and immune modulating substances prior to tisagenlecleucel infusion to avoid impairment of CAR-T cell function/expansion. In order to avoid transfer of alloreactive lymphocytes from previous SCT, leukapheresis had to take place at least 3 months after transplantation, and tisagenlecleucel infusion at least 6 months after transplantation.[49]

During the manufacturing process, most patients received individualized bridging chemotherapy to control their disease until tisagenlecleucel infusion.[48,49] While it only takes approximately 2 weeks to complete the manufacturing process for tisagenlecleucel in most instances, the time from leukapheresis to infusion of the final product may be 4 weeks or more due to the requirement for quality assessment and release controls, and/or due to logistical reasons (eg., shipment of cell products). The time period between leukapheresis and infusion, in which the disease has to be kept stable, can be clinically challenging in patients with aggressive disease. The goal of the bridging chemotherapy is to maximize disease control without compromising organ function or causing toxicity (including infections due to cytopenias) that might render the patient ineligible for tisagenlecleucel infusion, or intolerant for the toxicity of a potential CRS.[49] Importantly, in contrast to hematopoietic SCT, it is not necessary to achieve CR prior to tisagenlecleucel infusion. Bridging therapy regimens in the current phase 2 tisagenlecleucel trials varied and were individualized to the patient, with intensity and composition adapted to their disease,[49] previous response to treatment, time frame until infusion, and pre-existing toxicity. Lymphodepleting chemotherapy is employed to enhance engraftment and persistence of tisagenlecleucel.[49,59,60] In the tisagenlecleucel clinical trials, lymphodepletion was performed in the vast majority of patients, except those already experiencing profound
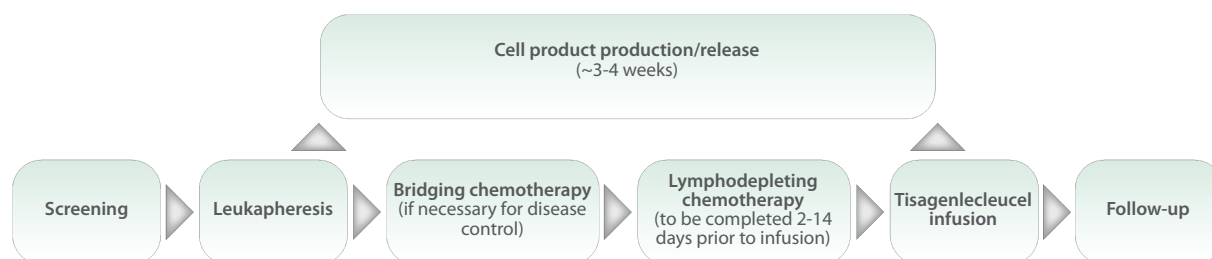
Figure 3. **Clinical process flow of tisagenlecleucel therapy.**

lymphopenia (white blood cell count <1000/μL).[48] Standard lymphodepletion per protocol consisted of fludarabine (30 mg/m^2 IV daily for 4 doses) and cyclophosphamide (500 mg/m^2 IV daily for 2 doses) and was timed so that tisagenlecleucel was infused 2 to 14 days after lymphodepletion was completed.

Toxicity management

Several clinical trials have established the safety profile of tisagenlecleucel following infusion. The nature of AEs was generally consistent across clinical studies with some variability in frequency and severity between indications. CRS, a significant toxicity often associated with CAR-T cell therapy, has been reported in patients with relapsed/refractory B-ALL at rates ranging from 78% to 90% of patients. Severe CRS has been reported in 27% to 38% of patients, correlating with high disease burden and greater tisagenlecleucel expansion.[4–6,61,62] CRS is a systemic inflammatory response syndrome related to activated T-cell proliferation and the release of inflammatory cytokines, and can result in life-threatening shock and multiple organ dysfunction syndrome.[63–66] A specific CRS grading system and CRS management algorithm have been established for the clinical studies with tisagenlecleucel[3]; attempts are ongoing to establish a uniform CRS grading and management system across CAR-T cell therapies.[66] Symptoms of CRS typically include a prodromal syndrome comprising high fever, tachycardia, and myalgias; higher-grade CRS can require intensive care unit (ICU) management for hypotension, respiratory failure, and organ dysfunction (most commonly hepatic and renal dysfunction).[63,65,66] There may also be a biphasic presentation for CRS. Another major toxicity is CAR-T cell-related encephalopathy syndrome (CRES), which can be mild and self-limiting when occurring during or shortly after CRS resolution. Encephalopathy can manifest with confusion, delirium, hallucinations, aphasia, and/or seizures, and usually resolves within days.[63–66] Early signs include impaired attention, language, and handwriting, and should be monitored carefully with writing tests and mini-mental status examinations (MMSEs). Recently, a simpler CAR-T cell therapy-specific version of the MMSE has been developed.[66] A more severe encephalopathy can occur, associated with symptoms that may vary based on the specific CAR-T cell therapy and disease states involved, including seizures, motor weakness, incontinence, reduced alertness, and cerebral edema. Cerebral edema has been observed with certain CAR-T cell therapies, but not in the current phase 2, global tisagenlecleucel trials. Presently, the pathogenesis of CRES is incompletely understood, but endothelial activation and disruption of the blood-brain barrier may play a role.[66]

In the tisagenlecleucel trials, a detailed algorithm defined the clinical management of CRS.[66] The anti-interleukin-6 (anti-IL-6) receptor antibody tocilizumab can alleviate CRS symptoms[63–65] without affecting tisagenlecleucel efficacy, but also without impacting the occurrence of encephalopathy.[63,64,67] Anti-IL-6 therapy can be used to treat early symptoms of CRES; corticosteroids are advised in severe cases.[66] In the current phase 2 tisagenlecleucel trials, corticosteroids were only used as second-line therapy, in case of refractory hypotension or other severe CRS symptoms not responsive to the first dose of tocilizumab.[63–66] Third- and fourth-line management included repeated doses of tocilizumab and use of the anti-IL-6 antibody siltuximab. In particularly severe situations, other T cell-directed approaches (eg, anti-thymocyte globulin, alemtuzumab, or cyclophosphamide) were potential options; however, use of these agents was not required in the current phase 2 tisagenlecleucel trials. Current research is evaluating the predictive value of a variety of biomarkers for severe CRS[68] and early use of tocilizumab,[67,69] which may help clinicians better manage CRS in the future.

CD19-directed CAR-T cell therapy does not differentiate between malignant and nonmalignant cells, which can lead to depletion of normal B cells and hypogammaglobulinemia, often continuing as long as tisagenlecleucel persists.[64,65] Intravenous or subcutaneous immunoglobulin can be used to manage hypogammaglobulinemia on a regular basis in children (often necessary every fourth week) and in adults (mainly in the context of recurrent infections).[64,65]

Components of macrophage-activation syndrome (MAS) have been seen concurrently with CRS, including liver dysfunction, cytopenias, and coagulopathy.[47,48] In addition to anti-cytokine treatment of MAS according to the CRS algorithm, MAS-associated coagulopathy requires frequent monitoring of fibrinogen levels[66] and proactive substitution either with cryoprecipitate or fibrinogen concentrate to avoid bleeding complications.

Other AEs associated with tisagenlecleucel therapy in pediatric and young adult patients with B-ALL include neutropenia with high fever (61%), infections (44%), cytopenias not resolved after 28 days (35%), and tumor lysis syndrome (3%).[61]

Unit and staff preparation

When implementing CAR-T cell therapy, unit and staff preparation should include all members of the multidisciplinary team (including nursing, hematology staff and residents, neurology, emergency department and ICU, pharmacy, and social workers; Table 4).[49,54,63] Ideally, the treatment center should be accredited by the Foundation for the Accreditation of Cellular Therapy[70] in the US or the Joint Accreditation Committee—International Society for Cellular Therapy (ISCT) and European Society for Blood and Marrow Transplantation (EBMT; JACIE) in Europe.[71] If leukapheresis is conducted at a separate facility, communication between the CAR-T cell therapy treatment center and the leukapheresis center should be established in order to coordinate the timing of any mandatory treatment washout periods.[49] Staff should be provided with education on CAR-T cell therapy technology and training on proper procedures for product handling in order to collect, cryopreserve, store, and transport the leukapheresis product promptly to the manufacturing site. Nonmedical staff training can also help with the coordination of tisagenlecleucel therapy, including training for couriers on proper product handling, for social workers on addressing patient needs, and for patients and their families on recognizing and reporting AEs.[49]

Once the leukapheresis material is collected and shipped to the manufacturing center, the patient should undergo bridging and lymphodepleting chemotherapy. Treating physicians should be experienced in designing individualized, risk- and toxicity-adapted bridging approaches. Staff should be capable of managing chemotherapy administration and AEs, and adjusting the timing of chemotherapy according to when the tisagenlecleucel product is ready for infusion. Therefore, communication and cooperation should be established between the clinical team, manufacturing team, and courier services, and any issues or questions regarding product quality and timing of shipment should be proactively addressed.[49]

To ensure that every patient receives the correct product and that the product has been handled properly, a "chain of custody" or "chain of identity" process should be established to link

Table 4

Recommended Site Capabilities and Education for Tisagenlecleucel Therapy Implementation

Site Capabilities	Education
FACT- or JACIE-accredited, transplant-capable treatment center	Medical/treatment center staff
Management of bridging chemotherapy administration and AEs	CAR-T cell therapy technology
Management of lymphodepleting chemotherapy administration and AEs	Product handling procedures for collection (leukapheresis), cryopreservation, storage,
Communication with manufacturing team and courier services;	transport, receipt of finished product, thawing, and infusion
adjustment of chemotherapy schedule as needed	Monitoring and management of AEs (recognizing CRS symptoms, admitting severe
Establishment of "chain of custody" or "chain of identity"	cases to ICU, prescribing anti-IL-6 therapy (tocilizumab) and appropriate supportive care[63]
process to track product for each patient[54]	
Biosafety officer	Non-medical staff education[49]
	Couriers: temperature and timing requirements for product storage and transport
	Social workers: transportation and lodging needs as dictated by treatment schedule;
	insurance reimbursement and other support as needed
	Patient and families: recognition and reporting of CAR-T cell therapy AEs (including CRS,
	neurological toxicities, and fever)

AE = adverse event, CAR-T = chimeric antigen receptor T cell, CRS = cytokine release syndrome, FACT = the Foundation for the Accreditation of Cellular Therapy, ICU = intensive care unit, IL-6 = interleukin-6, JACIE = Joint Accreditation Committee—International Society for Cellular Therapy (ISCT) and European Society for Blood and Marrow Transplantation (EBMT).

patient identifiers (eg, name, date of birth, and unique patient identification numbers) with the product batch identification number (Fig. 4).[49,54] To further ensure this chain of custody is maintained in the current phase 2 tisagenlecleucel trials, a dedicated manufacturing team is assigned to work on a single product at a time, with no transfer between teams.[49,54]

Treatment center staff must follow appropriate protocols for product handling to receive, thaw, and infuse the finished tisagenlecleucel product. Personal protective equipment must be used to prevent medical personnel from being exposed to the product during administration, and specific measures should be in place to handle accidental spills, first aid, decontamination, and waste disposal, with appropriate training documented. Treatment staff, including nurses, should be able to properly monitor and manage AEs, including recognizing CRS when it occurs and admitting patients with severe cases of CRS to an ICU.[49,63] The ICU staff should be familiar with CAR-T cell therapy and be aware of appropriate therapy needs, such as avoiding corticosteroids and the possibility of using anti-IL-6 therapy.[49,63] An ICU with experience in pediatric intensive care (PICU) is recommended when treating pediatric patients. The

pharmacy should have an adequate supply of anti-IL-6 medication and appropriate supportive care as needed.[49]

Tisagenlecleucel clinical trial data

Tisagenlecleucel was initially evaluated in studies with small population sizes,[4,5,7,48] including in B-ALL[47] and multiple myeloma,[72] and continues to be studied in larger multicenter trials in B-ALL[6,73] and DLBCL.[8] In a phase 1/2a trial (N = 30; 29 with B-ALL)[48] in pediatric and adult patients with CD19-positive leukemia and lymphoma, tisagenlecleucel therapy led to CR in 27 patients (90%) at 1 month. Subsequent relapse occurred between 6 weeks and 8.5 months in 7 patients, and 19 patients remained in remission after a median follow-up of 7 months. A total of 7 patients died after disease progression; there were no deaths due to tisagenlecleucel. At 6 months, OS was 78% (95% confidence interval [CI], 65–95). This study also assessed tisagenlecleucel expansion and persistence. By flow cytometry, a high median peak of tisagenlecleucel cells was observed in responders with 39.8% of CD3-positive cells (range, 4.4–69.3) being tisagenlecleucel cells. The 3 patients who did not respond had median peak
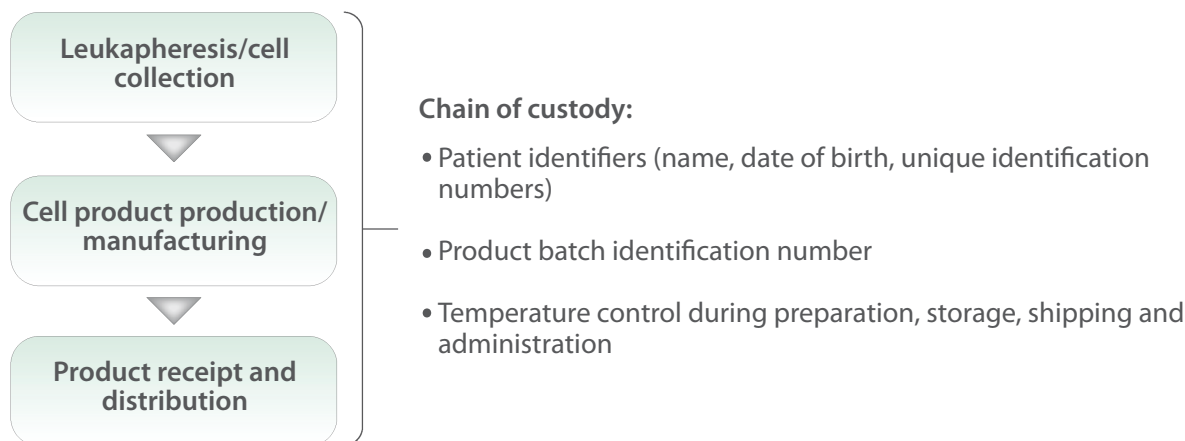

Figure 4. Chain of custody requirements for tisagenlecleucel therapy.[49,54]

cells between 0.2% and 8.2% of CD3-positive cells. The cells were detectable by flow cytometry ≤11 months. By qPCR, tisagenlecleucel was detectable in patients with sustained remissions until 2 years. All patients had peak levels >5000 copies/µg, and 26 patients had peak levels >15,000 copies/µg. Mild-to-moderate CRS developed in 22 of the 30 patients and required hospitalization for febrile neutropenia. Severe CRS developed in 8 patients (27%) and required intensive care admission. Tocilizumab was administered to 9 patients, and rapidly reversed symptoms over 1 to 3 days; 4 patients received a second dose. Glucocorticoids were provided to 6 patients. Neurological toxicities occurred in 13 patients and resolved over 2 to 3 days without intervention. B-cell aplasia occurred in all patients who responded to therapy, was managed with immunoglobulin supplementation, and continued for 1 year after tisagenlecleucel cells were no longer detectable by flow cytometry.[48]

Tisagenlecleucel has been further studied in children and young adults with relapsed/refractory B-ALL in a phase 1/2a trial,[4] a phase 2 trial,[5] and in the first global, multicenter, pivotal trial for tisagenlecleucel, ELIANA (ClinicalTrials.gov, NCT02435849).[6,73] These studies demonstrated high remission and OS rates.[4–6] In ELIANA, the overall response rate (rate of CR plus CR with incomplete blood count recovery) within 3 months after tisagenlecleucel infusion was 83%, with negative minimal residual disease by flow cytometry in all responding patients; the median duration of remission was not reached at a median follow-up of 6 months.[6] High rates of CRS were observed.[4–6] Severe cases of CRS were associated with high disease burden, usually required hemodynamic or respiratory support, and were reversible with tocilizumab. Grade 3 or 4 AEs included hypotension, hypoxia, increased aspartate aminotransferase, neurological toxicities, and neutropenia with high fever.[4–6] Pooled safety data from the 2 phase 2 studies ELIANA and ENSIGN (ClinicalTrials.gov, NCT02228096), both evaluating tisagenlecleucel in pediatric/young adult patients with relapsed/refractory B-ALL, found no new safety signals.[61] Overall, data from the global multisite studies are consistent with data from the earlier, single-site studies.

A phase 2a study (NCT02030834)[7] and the global, multicenter, pivotal JULIET trial (NCT02445248)[8] evaluated tisagenlecleucel therapy in relapsed/refractory DLBCL. Both trials reported high response rates (best overall response rate at 3 months follow-up in JULIET was 59%[8]) and evidence of durable responses (median duration of response not reached at 3 months follow-up in JULIET[8]). The types of toxicities observed were similar to those reported in previous tisagenlecleucel studies.[7,8]

Axicabtagene ciloleucel clinical trial data

Axicabtagene ciloleucel, a CD19-directed CAR-T cell therapy with a CD28 costimulatory domain,[23] has also been evaluated in adult patients with relapsed/refractory DLBCL (ZUMA-1, NCT02348216)[9] and in pediatric/young adult patients with ALL (ZUMA-4, NCT02625480).[10] The ZUMA-1 study in DLBCL provided evidence of durable responses, and the 4 treated ALL patients in the ZUMA-4 study experienced minimal residual disease–negative remission. In both studies axicabtagene ciloleucel had a toxicity profile consistent with earlier CAR-T cell therapy studies.[9,10]

Experience from clinical implementation of tisagenlecleucel in Europe

The global ELIANA trial was conducted at 25 sites in 11 countries. The experience gained from implementation of the ELIANA trial at the Oslo University Hospital in Norway can be used to illustrate successful implementation of CAR-T cell therapy.[74] Oslo University Hospital is a tertiary care center with maximal services, including a JACIE-accredited, experienced transplant center and experienced PICU in very close proximity to the pediatric tisagenlecleucel infusion site.

The regulatory approval process was initiated by a face-to-face, joint scientific advice meeting between representatives from the commercial sponsor (Novartis Phama AG, Basel, Switzerland), the Norwegian national coordinator for the tisagenlecleucel program, and the Norwegian Medicines Agency (NoMA), the Norwegian Directorate of Health, and the Norwegian Environment Agency. The purpose of this meeting was to give insight into the clinical program, clarify any uncertainties, and agree on the submission process as the CAR-T cell therapy represented a new treatment paradigm. The outcome was that a standard Clinical Trial Application was to be submitted to NoMA. A notification of contained use and a Gene Therapy Application was submitted to the Norwegian Directorate of Health. The Norwegian Environment Agency declared the use of tisagenlecleucel in the protocol to not be deliberate release; hence, approval from them was not required. All tisagenlecleucel products were to be manufactured in the US and shipped to Europe via Germany, which required prior German manufacturing and import authorization (MIA) for gene product shipment.

Hospital staff in Oslo were familiarized with the complex trial-specific logistics and, because Oslo served as an international site for the Nordic area, with recruitment of patients across borders. Trial implementation and treatment required a multidisciplinary approach involving clinical departments (pediatrics/adults, PICU/ICU, and cell therapy), and preclinical/laboratory services (hematology, pathology, immune flow cytometry, genetics, and clinical immunology). Extensive training on expected AEs and recognizing early symptoms of CRS was required for nurses, physicians on all levels, including those who were on call, and PICU/ICU staff. Treatment staff were specifically trained on how to "read" the patient, as the clinical situation with tisagenlecleucel is different from that of all other types of therapies and can change rapidly, especially when severe CRS develops. Clear criteria and procedures were established for patient referral to the PICU/ICU, either for better observation and monitoring, or treatment. Nurses and physicians were familiarized with CRS management algorithms, including protocol criteria for initiation of anti-cytokine treatment, and the potential need for aggressive fibrinogen substitution for severe CRS/MAS. Case reports, simulations, and educational material for the setup of CAR-T cell therapy were also provided by the commercial sponsor. Laboratory personnel were proactively primed to immediately collect and analyze samples if necessary. It proved valuable and necessary to have a dedicated CAR-T person in charge, and access to advice from sites with extensive CAR-T cell therapy experience. Weekly calls were organized with global Novartis team members, principal investigators in the EU, and experienced colleagues from US sites to discuss patient updates, logistics, clinical advice, and toxicity management.

Specific aspects of CAR-T cell therapy and differences from other oncologic approaches (eg, characteristics and rationale of bridging therapy, which differs from the common leukemia treatment approach to achieve rapid, deep remission) were repeatedly discussed during national and Nordic network meetings. Referring centers were explicitly instructed to contact the study site as early as possible in order to perform the leukapheresis procedure immediately, prior to any therapy that might impair T cell function.

Referred patients were screened for study inclusion within 4 days, including informed consent, bone marrow and central nervous system assessment, evaluation of clinical eligibility, and leukapheresis. Standby slots in the local cell therapy department were reserved to ensure that leukapheresis could be performed immediately and that timelines were maintained, especially with regard to the often aggressive course of the disease. After successful screening and leukapheresis, patients received individualized bridging therapy at their local oncologic treatment center. Any delays in manufacturing or shipping timelines were communicated back to the local site and to the patient in order to modify bridging therapy. The leukapheresis cell products were cryopreserved and were shipped to the Novartis manufacturing facility in Morris Plains, NJ. After the cell product passed quality control and was accepted for manufacturing, patients were enrolled in the trial. Patients were transferred to Oslo 8 days before tisagenlecleucel infusion for lymphodepleting chemotherapy and were required to stay in close proximity to the hospital until day 21 after infusion. The first efficacy assessment, including bone marrow examination, was performed on day 28.

Patients were encouraged to return to the study site for all follow-up visits, which was feasible even for international patients. Response and safety assessments for ELIANA patients will continue for a total of 5 years, with visits every month for the first 6 months, every third month for months 6 to 24, and every 12 months thereafter. Long-term safety follow-up will be continued for a total of 15 years under a separate protocol, which will monitor for potential delayed AEs associated with gene therapies consistent with regulatory requirements.[75]

The future of CAR-T cell therapy

CAR-T cell therapies, and in particular tisagenlecleucel, have demonstrated improved therapy outcomes for patients with relapsed/refractory B-ALL and DLBCL, and provide a new option for patients who may otherwise have no adequate treatment alternatives. Although serious toxicities such as CRS and neurological toxicities are common, clinical trials have shown that these can be managed with appropriate preparation and training. Research is also currently ongoing on potential biomarkers and early intervention for these toxicities in order to better identify and manage patients with severe toxicity. As more experience is gained with CAR-T cell therapies and as they become more widely understood and available, they can be further developed for use across a broad range of malignancies, potentially including solid tumors. Predictive markers of response will have to be developed, particularly in disease states with lower long-term disease-free survival rates (such as DLBCL). In addition, the role of CAR-T cell therapies as substitutes for autologous or allogeneic SCT should be explored. The European Hematology Association predicts that treatment of leukemias will evolve from disease models to target models and will become more individualized for each patient. This will lead to improved prognosis for high-risk patients as well as reduced morbidity overall.[76]

Conclusions

CAR-T cell therapies are a new modality for cancer treatment. CD19-directed CAR-T cell therapies are in development for B-ALL, DLBCL, and other B-cell malignancies. The recent US approval of tisagenlecleucel for the treatment of patients up to 25 years of age with B-cell precursor ALL that is refractory or in second or later relapse marks a milestone for this new approach to cancer treatment. Tisagenlecleucel has demonstrated significant clinical benefit in all studies published thus far. Although AEs such as CRS, neurological toxicities, and B-cell aplasia are common, the majority of events are self-limiting or manageable when treated by an appropriately trained multidisciplinary team. ICU care may be necessary for management of severe CRS. Further investigation into this therapy and refinement of treatment and implementation protocols are important in facilitating broader clinical use. Logistical and regulatory requirements are still being refined globally and are expected to become more streamlined with time. Experience gained from successful implementation of tisagenlecleucel therapy in the ELIANA and JULIET trials may serve as a useful starting point for investigators and clinicians looking to implement CAR-T cell therapy in Europe within their own institutions.

Acknowledgments

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