CTL019 (tisagenlecleucel): CAR-T therapy for relapsed and refractory B-cell acute lymphoblastic leukemia

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Abstract: Over the past decades, survival of patients with acute lymphoblastic leukemia (ALL) has dramatically improved, but the subgroup of patients with relapsed/refractory ALL still continues to have dismal prognosis. As an emerging therapeutic approach, chimeric antigen receptor-modified T-cells (CAR-T) represent one of the few practice-changing therapies for this subgroup of patients. Originally conceived and built in Philadelphia (University of Pennsylvania), CTL019 or tisagenlecleucel, the first CAR-T approved by the US Food and Drug Administration, showed impressive results in refractory/relapsed ALL since the publication on two pediatric patients in 2013. It is in this context that we provide a review of this product in terms of manufacturing, pharmacology, toxicity, and efficacy studies. Evaluation and management of toxicities, particularly cytokine release syndrome and neurotoxicity, is recognized as an essential part of the patient treatment with broader use of IL-6 receptor inhibitor. An under-assessed aspect, the quality of life of patients entering CAR-T cells treatment, will also be reviewed. By their unique nature, CAR-T cells such as tisagenlecleucel operate in a different way than typical drugs, but also provide unique hope for B-cell malignancies.

Keywords: CTL019, tisagenlecleucel, B-cell acute lymphoblastic leukemia

Pediatric and adult acute lymphoblastic leukemia (ALL): the unmet needs

ALL represents the most common cancer among children with 25% of cancer diagnoses in people under age 15.1 Dramatic improvement in survival has been achieved over the past decades for this subgroup, leading to a 5-year survival rate of 90% for all subtypes combined among children and adolescents.2 Therefore, most recent pediatric trials now aim to reduce long-term toxicity and focus on refractory/relapsed (r/r) ALL that has a much worse prognosis. Current overall survival (OS) for this population is approximately 20% at 5 years.3,4 In adults, ALL is much less frequent and represents only 0.2% of all cancers.1 Prognosis is also less encouraging, with an expected 5-year OS between 20% and 40% despite complete remission (CR) rates of 85%–90%.5-7 This is partly explained by the reduced tolerance to chemotherapy and the different genetic profiles: a large proportion of patients with Philadelphia t(9;22) positive and Ph-like profile,8 a greater number of patients with MLL gene rearrangement t(4;11), monosomy 7, or trisomy 8.9 Among adult patients with Philadelphia-negative ALL, outcome after relapse remained extremely poor, with 5-year OS under 15%.5

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These specific challenges in both the pediatric and adult population led to the emergence of innovative therapies, such as targeted therapy with monoclonal antibodies or bispecific T-cell engagers, personalized vaccines, and immunocellular therapy.

Immunocellular therapy aims to harness the power of a patient’s own immune system to fight malignancy. One of those therapeutic approaches involves the use of engineered and activated cytotoxic T cells. Chimeric antigen receptor-modified T-cells (CAR-T cells) with B-cell antigen specificity are a promising therapy for B-cell malignancies and demonstrated impressive clinical efficacy to date.

The idea of adoptive immunotherapy using lymphocytes to attack leukemia was developed in the early 1990s. After cloning the zeta-chain of T cell antigen receptor, the first chimeric antigen receptor was conceived by Eshhar et al.10,11 Many molecular and configurational modifications have been attempted with this product in order to optimize its antitumor efficacy.12

Many North American groups have developed CAR-T products and started clinical trials with anti-CD19 therapies for B-cell malignancies such as non-Hodgkin lymphoma (NHL), chronic lymphoid leukemia (CLL), and ALL. These groups include, among others, Memorial Sloan Kettering Cancer Center (MSKCC), University of Pennsylvania (UPenn) and the Children’s Hospital of Philadelphia (CHOP), Fred Hutchinson Cancer Research Center (FHCRC), and the National Cancer Institute (NCI). In 2010, Kochenderfer et al published the first case report of a patient with refractory and relapsed stage IVB follicular lymphoma showing an impressive response to anti-CD19 CAR-T cells.13 Later, in 2011, results in CLL were published in heavily treated patients showing an overall response rate (ORR) of 57%–100% with 29%–66% complete remission (CR) rate.14,15

In 2012, the University of Pennsylvania was the first to create a research alliance with a pharmaceutical company, Novartis, aiming to develop CAR-T cells for commercialization after its initial clinical success. The product from this alliance, CTL019, later known as tisagenlecleucel, was the first CAR-T treatment approved by the US Food and Drug Administration (FDA). The initial results of CTL019 in ALL were published in 2013 and will be reviewed in this paper.16 Since then, many trials are ongoing with various CAR-T products for different indications, and with promising results. In this article, we will focus on the manufacturing and pharmacology aspects of CTL019, as well as side effects management and efficacy studies for r/r ALL.

Pharmacology of CAR-T cells – CTL019

CD19 CAR-T design

CARs for hematological malignancies have been first designed to recognize CD19 antigen on the surface of B-cells, including normal lymphocytes and leukemic cells. The choice of CD19 for target in immunotherapy comes from its appealing characteristics: being uniformly expressed in B-cell leukemia/lymphomas and healthy B-cells but not on other normal tissues.17,18 Furthermore, targeting normal B-cell lymphocytes is an acceptable on target/off tumor toxicity, as B-cell aplasia can be managed in the clinic with intravenous or subcutaneous immunoglobulins, which will be detailed later.

As mentioned previously, the idea of adoptive cell therapy was developed more than 30 years ago. Before the first CAR-T cells, the concept of lymphokine-activated killer was developed,19 followed by tumor-infiltrating lymphocytes (TILs).20 One major advantage of the CAR-T cells over the TIL is their ability to be human leucocytes antigen (HLA)/T cell receptor (TCR) independent. By obviating the need for presentation of antigen in HLA, it makes this technology a more accessible and universal one.

Basic CAR structure includes an intracellular T-cell activation domain, an extracellular hinge region, a transmembrane domain, and an extracellular antigen-recognition moiety that is usually derived from an antibody (single-chain variable fragment [scFv]).21 The hinge, or spacer element, is conceived to optimize the accessibility of the epitope. Second-generation CAR-T cells encode for a co-stimulatory domain, such as CD2822 or members of the tumor-necrosis factor receptor family such as CD137 (4-1BB).23 This addition of co-stimulation domain provides better cytokine production and proliferation, and enhances persistence of CAR-T cells.22,24 Third-generation CAR-T includes a second co-stimulatory domain using the abovementioned co-stimulatory domains and/or others such as CD27, ICOS, or CD134 (OX40).25

Regarding CTL019, the structure is composed of an anti-CD19 scFv (FMC63) for antigen recognition, a CD8–α hinge region, 4-1BB co-stimulatory domains, and CD3ζ as a signaling domain, with an activation through intracellular immunoreceptor tyrosine-based activation motifs (ITAM; Figure 1).21,26 As described in Table 1, CTL019 from CHOP/UPenn and CAR-T cells built by FHCRC in Seattle have a very similar structure, with 4-1BB costimulatory domain, and the use of a lentivirus vector. The hinge is different,
with CTL019 using CD8 and FHCRC an IgG4. Of note, the latter team also uses a manipulated composition ratio of CD4:CD8. Other teams reported in Table 1 use mostly CD28 as co-stimulatory domain and gamma-retrovirus as vector. Clinical results among different products will be discussed further.

**Manufacturing CTL019**

The process begins with the collection of a sufficient number of effector T cells from the patient’s peripheral blood through leukapheresis. The process is similar to peripheral blood stem cell (PBSC) collection, but aiming for mononuclear cells with the goal of obtaining a maximum of CD3⁺T cells. A minimum of absolute lymphocyte count (ALC) of 500 cells/µL or a CD3⁺ cell count of 150 cells/µL (if ALC is under 500 cells/µL) is required to start apheresis. The time to harvest the required number of cells is usually between 2 and 3 hours.²⁷,²⁸ Considerations enter into account regarding pediatric vs the adult leukapheresis technique. The smaller blood volume in children needs to have a slower rate of leukapheresis and priming the apheresis volume, in order to reduce the perfusion-related adverse effects such as hypovolemia, hypothermia, and hypocalcemia.²⁹,³⁰ For both age populations, cytotoxic and immunomodulator therapies must have been stopped for at least 2 weeks before collection (except for asparaginase and donor lymphocyte infusions that should be stopped at least 4 weeks, intrathecal methotrexate for 1 week, and steroids for 72 hours).³⁰ Moreover, patients should be at least 3 months after an allogeneic stem cell transplant (allo-SCT) and 2 months from a T-cell lytic agent such as clofarabine.²⁸ These requirements imply that the disease be stable during this time-lapse, and therefore can
Table 1 Comparison of second-generation CD19 CARs in North America for relapsed B-ALL

| Institution; industry and collaborators | Antigen-recognition moiety/signaling domain/co-stimulatory domain | Dose (CAR-T cells/kg) | Lymphodepletion regimen | Vector | Results |
|----------------------------------------|---------------------------------------------------------------|-----------------------|--------------------------|--------|---------|
| CHOP/UPenn; Novartis                   | FMC63–CD8α                                                  | CD3+                | Investigator's choice (mostly Cy + Flu) | LV     | Ped and adults: CR: 90% (MRD neg 22/25), 6 mo OS 78%, EFS 67%.
|                                        |                                                              |                      |                          | Ped: CR: 81% (95% MRD neg), 12 mo OS 76%, 50% EFS. |
| FHCRC; Juno                            | FMC63–IgG4                                                  | CD3+                | Cy ± Flu, or CE          | LV     | Adults: CR: 93% (MRD neg 86%) and 50% OS 82%. |
|                                        |                                                              |                      |                          | Ped: MRD – CR: 93% (89% ITT), estimated 12 mo OS 69.5% and EFS 50.8%. |
| NCI, NIH                               | FMC63–28z                                                   | CD3+                | Cy + Flu                 | γRV    | Ped: CR: 70% (66.7% ITT), MRD neg 85% or 60% ITT. |
| MSKCC; Juno                            | FMSJ25C1–28z                                               | CD3+                | 1 or 3 × 10⁸             | γRV    | Ped: CR: 83% (6.7% MRD neg), median EFS 6.1 mo, median OS 12.9 mo. |
| MDACC, NCI; ZioPharm; Intrexon          | FMC63–IgG4                                                  | CD3+                | Cy ± Flu or physician's choice | SBT    | Adults: CR: 69%, 1y PFS: 53% and OS 63%.
| Baylor; Bluebird; Celgene               | FMC63–CD8α                                                  | CD28                | –                       | γRV    | No clinical results yet. |
|                                        |                                                              |                      |                          |        |         |

Notes: *Fixed ratio ~1:1 of CD4+ and CD8+ purified TcD and TcN, and with expressing EGFR ( truncated human epidermal growth factor receptor). *Fixed CD4:CD8 ratio of ~1:1 with expressing EGFRs.

Abbreviations: Baylor, Baylor College of Medicine; CARs, chimeric antigen receptors; CE, cyclophosphamide and etoposide; CHOP, Children’s Hospital of Philadelphia; Cy, cyclophosphamide; Flu, fludarabine; FHCRC, Fred Hutchinson Cancer Research Center; ITT, intention to treat; Juno, Juno Therapeutics; LV, lentivirus; MDACC, University of Texas MD Anderson Cancer Center; MSKCC, Memorial Sloan Kettering Cancer Center; NCI, National Cancer Institute; NIH, National Institutes of Health; UPenn, University of Pennsylvania Health system; Ped, pediatric and young adults; γRV, gamma-retrovirus; SBT, sleeping beauty transposon; TCM, Central memory T cell; Tn, Naive T cell; MRD, Minimal residual disease; EFS, event-free survival; CR, complete remission.

lead to exclusion of highly aggressive leukemia and/or active graft-versus-host disease. This represents a fragile balance between good quality and quantity of T effector cells and the urge to treat a refractory patient. The analogous material from leukapheresis is then shipped to a manufacturing facility. Novartis has currently two facilities to produce CTL019, one in Morris Plains, NJ, and the other in Lupke, Germany. UPenn produces CTLO19 for its clinical trials at the Clinical Cell and Vaccine Production Facility (CVPF) in Philadelphia, USA and the other in Leipzig, Germany. Intrexon produces CTLO19 for its clinical trials at the Clinical Cell and Vaccine Production Facility (CVPF) in Philadelphia, USA. The former material is then used to produce CTL019, one in Morris Plains, NJ, and the other in Lupke, Germany.
manufacturing and lacks, until now, data arguing for clinical superiority. Heterogeneity in T lymphocytes populations can contribute to the differences in efficacy and toxicity profiles of the different CAR-T cells. In CTL019 manufacturing, there is no selection of subpopulation on the T-cell content. The FHCRC group, however, has added the step of selection of T-cells subset in order to have a defined composition for its CAR-T product. In their study in adults with r/r B-ALL, they aim to obtain a 1:1 ratio of CD4+ and CD8+ T cells and enrich the CD8+ lymphocytes population to target CD8+ T CM.

The latter is achieved through a two-step method with depletion of CD4+, CD14+, and CD45RA+ followed by selection of CD62L+.

In the pediatric counterpart study, they also fixed the ratio at 1:1 for CD4+ and CD8+ T cells, but without the enrichment part.

On their side, City of Hope group has led two trials with CAR-T cells enriched in T CM from either CD8+ alone or CD4/8+ combined, in patients with relapsed NHL. Infusion of CAR-T cells occurred 2–3 days following autologus hematopoietic stem cell infusion. Furthermore, Baylor College of Medicine (BCM) group also conceived an allogeneic CD19 CAR-T cells virus specific for patients with B-cell malignancies relapsing after allo-hematopoietic stem cell transplant (allo-HSCT). Mononuclear cells from transplant donors are used to generate EBV-transformed lymphoblastoid B-cell to become APCs for subsequent viral stimulations. After this process, there is a transduction in CD19 CAR-T cells. This approach aimed to provide both antiviral and antitumor effects on the recipient.

Lentiviruses deliver RNA that is reversed-transcribed into DNA in the target cell. This DNA coding the CAR then integrates into the host genome through a process catalyzed by the vector integrase enzyme and several key sequences. Other methods of nonviral gene transfer such as Sleeping Beauty transposon are being investigated. The integration of vector DNA near an oncogene of the host cells is a concern of vector DNA near an oncogene of the host cells is a concern for all vectors. However, it seems that lentivirus carries a lower risk of mutagenesis compared with retrovirus because of its sites of integration away from the cellular promoter.

Previous experience of the last decade reported from Scholler et al has shown no cases of oncogenicity even with retroviral vector in the context of CAR-T manufacturing.

The production of CAR-T-cell therapies has largely increased over the past few years, to serve many patients distributed among treatment sites, internationally. It has a highly controlled process to ensure the quality of this product. The vector producer company is also subjected to these quality control tests and has been described by Levine et al in a recent review. The viral vector encoding the CAR can be frozen at −80°C and stay stable for 4 years, and up to 9 years in some reports. During the manufacturing time, bridging chemotherapy is often required to control the leukemia until the CTL019 manufacturing process is complete.

Once the CAR-T cells product is ready, it is shipped back to the treatment center. At the same time, lymphodepleting regimen is administered to the patient to optimize the therapy. The latter has been shown to facilitate the T-cell expansion in vivo and reduce the disease burden and, therefore, the risk of cytokine release syndrome (CRS). The combination of cyclophosphamide (CY) and fludarabine (Flu) vs CY alone for lymphodepletion regimen seems to provide better results in terms of CAR-T cells persistence and disease-free survival, although no randomized study has been designed for this purpose.

Mechanism of action

The first-generation CAR had a powerful cytotoxicity, but a poor tumor control and limited persistence, due to premature exhaustion. Next, investigators tried to overcome this problem by mimicking physiologic T-cell activation. They therefore discovered the 2-signal rule in order to have complete activation, persistence, and anergy prevention. This was achieved by adding a co-stimulator such as 4-1BB, CD28, CD27, OX40, or ICOS to the TCR CD3ζ chain. In CTL019, this sustained activation is achieved through the TNF receptor superfamily member 9: 4-1BB, also known as CD137. The latter is a type II transmembrane protein that binds to the TNF-receptor-associated factor 2 (TRAF2), essential to activate NF-kB via p38 mitogen-activated protein kinase (MAPK) and Jun N-terminal kinase (JNK) (Figure 1).

Downstream to this activation of NF-kB, phosphoinositide 3 kinase (PI3K) and protein kinase B (PKB), 4-1BB then upregulates anti-apoptotic factors such as Bcl-2, Bcl-xl, and Bfl-1. It also acts through interaction with endogenous TCR signaling. This leads to potent T-cell proliferation, cell-cycle progression via extracellular signal-regulated kinase (ERK) and phosphoinositide 3 kinase (PI3K), cytokine secretion such as Interleukin-2 (IL-2), IL-4, IL-5, and IFN-gamma, increased cytolytic potential, prevention of clonal deletion and activation-induced cell death, and decreased sensitivity to transforming-growth-factor beta (TGF-β) inhibition. All these mechanisms are extrapolated from physiologic roles, and so the exact function into CAR-T cells remains to be elucidated.

Upon CARs multi-homodimerization with CD19 epitope, CD3ζ containing ITAMs serve as substrate for the Src-family kinase lymphocyte-specific protein tyrosine kinase
(Lck). Intracellular signaling leads to phosphorylation of the endogenous CD3 complex, interaction with TCR, and activation of MAPK cascade and nuclear factor of activated T-cells (NFAT). In fact, a recent study in xenografted syngeneic murine models demonstrates that TCR signaling impairs the ability of CD8 CAR-T cells to expand, leading to their exhaustion and clearance. This effect was not observed in CD4 CAR-T cells, underlining a significant difference for future CAR models.

Pharmacokinetics

“By its peculiar immunocellular mechanism”, standard pharmacokinetic components such as biodistribution, excretion, and metabolism are difficult to apply.

Once injected into the patient, there is an expansion of CAR-T cells, notably CTL019, from 1,000 to more than 10,000-fold. The peak is achieved between 10 and 14 days and coincides or follows the onset of clinical symptoms and CRS, between 1 and 21 days after the first infusion. In fact, for CTL019, this expansion often correlates with the severity of the CRS and the preinfection tumor burden in children. A small decline is observed after peak expansion, probably due to redistribution in tissues, bone marrow, and peripheral blood before the maximal expansion.

Efficient migration is a key factor for antitumor efficacy, as malignant cells are known to remodel their microenvironment. The CAR-T cells were proved to be trafficked in the bone marrow, and decreased to 60%–70% of peripheral blood levels 3–6 months later in pediatric B-ALL. CTL019 was also found in the cerebrospinal fluid, maybe explaining the absence of CNS relapses among children with ALL. Interestingly, no relationship between neurological events and CAR-T cells load into CNS was established.

Different CAR-T cells persistence has been observed among studies. Of note, longer persistence was correlated with longer event-free survival (EFS) in the studies of CTL019 and other CARs. Recent studies have confirmed CTL019 levels measurable in up to 780 days among patients with ALL. On their side, MSKCC demonstrated that their CAR-T-cell (19-28z) had persistence between 3 and 8 weeks in adults with r/r B-ALL, although their long-term study was limited by bridging to allo-HSCT after CAR-T cells for a majority of patients. Finally, Fred Hutchinson’s team has observed persistence of CAR-T cells in a defined CD4:CD8 ratio beyond 6 months, especially in patients receiving lymphodepleting regimen containing CY/Flu.

Many explanations can be hypothesized from the results above. First, lymphodepletion clearly enhances CARs efficacy as demonstrated in murine and clinical studies, principally by eradication of regulatory T cells. Therefore, CY/Flu compared with CY alone was demonstrated superior by Turtle et al from FRCHC. In the CTL019 pivotal trial recently published by Maude et al, almost all patients received a lymphodepletion with the combination of CY/Flu.

Then, the long-term persistence of CAR-T cells seems to correlate with $T_{CM}$ and $T_{SCM}$ concentration in the infused products. Some authors have demonstrated that CD8 $T_{CM}$ have a superior long-term persistence in primate models. As stated above, recent work on murine model demonstrates that TCR activation in CARs leads to premature exhaustion and clearance of CD8 CARs.

Regarding co-stimulatory domain, 41BB vs CD28 is clearly a component to explore as many confounding factors between clinical studies complicate their interpretation. In vivo, CD28 drives more rapid expansion with early tumor control, whereas 41BB drives more memory T-cell functions with a slower kinetic. Moreover, the question of third-generation CAR-T cells in order to combine this efficacy and persistence is currently under study.

The infused dose is another aspect that should be clarified as wide range of doses has been employed in studies (Table 1). Either a dose correlated to the burden of the disease or a fixed dose remains to be explored, taking into account toxicity. Recent data on CTL019 demonstrate no correlation between dose and expansion.

To measure the persistence of CAR-T, flow cytometry and qPCR are available. There is a strong correlation between the circulating levels of CD3 CAR-T cells measured by flow cytometry and qPCR. However, flow cytometry measures CAR-T cells by their expressed surface protein and qPCR measures the integration of the transgene into T-cell. Therefore, qPCR may not necessarily reflect functional CAR-T cells. Of note, qPCR was used for CTL019 studies. Duration of B-cell aplasia is indeed a surrogate to the CAR-T cells persistence and functionality.

Efficacy studies of CTL019 in r/r ALL

Since the initial clinical report by Grupp et al in 2013, CTL019 continue to show promising results in patients with r/r ALL. The latter reported the use of CAR-T cells in two children with r/r ALL, including one after hematopoietic stem cell transplantation and blinatumomab administration. They received 1.4×10^6 and 1.2×10^6 CTL019 cells/kg with etoposide and CY as...
lymphodepletion for one of the patients. The other patient had no lymphodepletion before as a highly cytotoxic therapy was administered 6 weeks before. Both experienced CR at 1 month post-infusion. One patient had sustained remission 11 months later at the time of publication and the other relapsed 2 months later with a CD19 negative leukemia escape.16

In 2014, Maude et al published the clinical results of their pilot study of CTL019 at CHOP for r/r ALL.49 The pediatric cohort (22 years old and younger) included 25 patients, and 5 adult patients from the population studied. Among them, two were blinatumomab-resistant and 15 had a previous HSCT. Lymphodepleting regimen was variable, including 50% Flu/CY. Some patients received no lymphodepletion. One month after infusion, 90% (27/30) of them were in complete morphologic remission and 22/27 were MRD negative by flow cytometry. At 6 months, EFS was 67% and OS rate was 78%, with a 68% probability of CTL019 persistence. There was no death related to the product.

The recent CTL019 study published (ELIANA trial) has confirmed the previous results. This was a phase II multicenter trial in pediatric and young adults that enrolled 92 patients. Of note, among those 92 patients enrolled, 17 were not infused: seven because of manufacturer issues, seven died before infusion, and three had severe adverse events. They received between 0.2 and 5.4x10^6 CTL019 cells/kg (median: 3.1x10^6 CTL019 cells/kg) and 96% had lymphodepleting chemotherapy before infusion, mostly CY/Flu. At 12 months, EFS rate was 50% and OS was 76%. Moreover, as stated before, CTL019 was noted to persist for 20+ months, with a median duration of 168 days. All responding patients had B-cell aplasia.57

Given those impressive results, CAR-T cell studies are currently running internationally. At the time of publication, three clinical trials are ongoing for CTL019. A phase II open-label multicenter trial for pediatric and young adults with r/r B-ALL is recruiting patients. This trial is available in centers in Canada and Europe and is sponsored by Novartis (NCT03123939). There is also a phase 1 with a humanized CTL019 (huCTL019) for pediatric and young adults with ALL or Diffuse large B-cell lymphoma (DLBCL) open in CHOP at Pennsylvania (NCT02374333). Finally, a phase II adult trial of CTL019 is actually recruiting ALL patients with MRD positive disease for use of CAR-T cells in upfront therapy (NCT02935543).

Safety and tolerability

Besides their remarkable results, CAR-T cells can cause toxicity by several mechanisms such as damage in normal tissues if the tumor-associated antigen is expressed in those tissues; acute anaphylaxis56 and tumor lysis syndrome (TLS).48 Two common and potential severe toxicities have been described with CAR-T therapies: CRS, an entity caused by cytokines released from activated CAR-T cells, and neurologic toxicity that can occur, associated or not with CRS.77 Both toxicities will be detailed in this section. Other most common adverse effects affecting more than 20% of patients receiving tisagenlecleucel are: hypogammaglobulinemia, infections–pathogen unspecified, fever, viral infectious disorders, loss of appetite, nausea, diarrhea, vomiting, headache, encephalopathy and delirium, hypotension, bleeding episodes, tachycardia, hypoxia, fatigue, and acute kidney injury. Of note, grade 3 and 4 events were experienced by 88% of patients.57

In the CTL019 pilot study, no death was linked to the treatment and seven patients died after 30 days, all from relapse or progression of B-ALL.48 In ELIANA trial, 19 deaths occurred, mostly from ALL relapse but one from CRS complication and two from infection, within 30 days of infusion.57 Because of those serious adverse reactions, including some fatal complications in patients receiving tisagenlecleucel, the FDA approved this product with a Risk Evaluation and Mitigation plan.78

For its effective application, clinicians must learn how to manage these associated toxicities. The underlying goal is a fair balance between preventing life-threatening toxicities and keeping the best chance for a beneficial antitumor effect. The CARTOX (CAR-T-cell-therapy-associated TOXicities) Working Group was created with the goal of providing general guidelines to manage those patients. The group has recently published a framework for a proactive management strategy of different and frequent complications after CAR-T treatment for adult patients.79 The final proposition is a three-step approach to assessment and management of acute toxicities associated with CAR-T-cell therapy.

Step 1: Systematic monitoring of patient’s clinical and biological parameters to determine the nature of the CAR-T cell-related toxicity, in order to diagnose CRS, CAR-T cell-related encephalopathy syndrome (CRES), and hemophagocytic lymphohistiocytosis/macrophage activation syndrome (HLH/MAS).

Step 2: Grading the severity of CRS, CRES, and HLH/MAS using the criteria adapted from Lee et al and the Common Terminology Criteria for Adverse Events, version 4.0.77,80

Step 3: Treating the toxicities according to the suggested management algorithms for CRS, CRES, and HLH/MAS.

The use of the CTCAE to grade CRS is debatable as it was developed before the emergence of cellular therapy and can underestimate this phenomenon.81 The University of
Cytokine release syndrome (CRS)

The CRS is a systemic inflammation response that is produced by elevated levels of cytokines, associated with T-cell in vivo activation and proliferation. CRS is not unique to anti-CD19 therapies because it is related to T-cell engagement with a tumor antigen and symptoms may be delayed, depending on the kinetics of T-cell activation, the main difference between cytokine storm (eg, after transplant) and CRS. The clinical features include high fever, malaise, fatigue, myalgia, nausea, anorexia, tachycardia/hypotension, capillary leak, cardiac dysfunction, renal impairment, hepatic failure, and disseminated intravascular coagulation. Most patients experience CRS 1–14 days after CAR-T cell infusion. The median time was 4 days in all recent trials, with more severe presentation if symptoms appear after 24 hours of CAR-T infusion. It is quite unusual for CRS to start after 2 weeks of CAR-T infusion. Accurate predictors for severe CRS have not yet a defined place in clinical practice, because of technical difficulties to monitor serum cytokines and its cost. Currently, under investigation is the use of well-known C-reactive protein, which is made by hepatocytes in response to IL-6, as a laboratory marker of CRS onset and severity. As stated above, a high leukemia burden seems to be associated with a higher risk of CRS. One of the key mediators of CRS after CAR-T-cell therapy is IL-6, but a dramatic increase in other cytokines, such as interferon-gamma, granulocyte macrophage-colony stimulation factor (GM-CSF), IL-10, and IL-5, is also present.

In the ELIANA trial, CRS occurred in 77% of cases. Notably, 35/75 patients were admitted to the intensive care unit for CRS management with 19 of them needing vasoressor support, 10 needing mechanical ventilation, 7 dialysis, and 28 received tocilizumab.

Tocilizumab (Atlastum [Chugai Pharmaceutical Co., Tokyo, Japan], Actemra [Hoffmann-La Roche, Basel, Switzerland]) is a humanized monoclonal antibody against IL-6 receptor (IL-6R). It blocks IL-6-induced signal transduction pathways through competitive inhibition of IL-6 binding to its receptors and has been currently used to treat patients with rheumatoid arthritis. Tocilizumab has become a mainstay in the control of complications from T-cell-engaging therapies for several groups after being reported by CHOP that CRS can be successfully ameliorated with this IL-6R inhibitor. Concomitant to the approval of tisagenlecleucel, the FDA has approved it as a treatment for CRS after CAR-T-cell therapy. The recommended dose of tocilizumab for CRS is 12 mg/kg for children under 30 kg and 8 mg/kg for patients more than 30 kg (pediatric and adults).

Siltuximab (CNTO 328, Sylvant; Janssen Pharmaceutica, Beerse, Belgium) is a human murine chimeric monoclonal antibody against IL-6. It is FDA-approved since 2014 for the treatment of multicentric Castleman’s Disease. It is used off-label for management of CRS and, as tocilizumab, can induce rapid reversal of symptoms in most patients. Even if tocilizumab is the only approved drug to treat CRS, its advantage, compared with siltuximab, remains to be proved. A theoretical disadvantage of tocilizumab is the fact that its administration is associated with an increase of the IL-6 level in the blood (as it binds the IL-6 receptor) and possible passive diffusion of IL-6 into the central nervous system, which could increase the risk of neurotoxicity with tocilizumab.

Corticosteroids can also control inflammatory responses and are effective in the management of CRS, CRES, and HLH/MAS associated with cellular therapies. However, as corticosteroids suppress T-cell function and can induce T-cell apoptosis, their use can impair the function and the durability of the cells infused. So, corticosteroids should be reserved for CRS that is refractory to anti IL-6 therapy.

No unified grading system has been validated prospectively for CRS, but it is a first step to managing complications. The guidelines will evolve with our better knowledge of cellular therapies. However, since CAR-T-cell therapy is going beyond the research context, it is imperative that a grading system that can be applied globally be developed in the near future. In Table 2, we summarize the two grading systems available and guidelines for management of CRS published so far.

CAR-T-cell-related encephalopathy syndrome (CRES)

The pathophysiology of neurological toxicity is still unclear and the neurological symptoms do not follow the same time course as systemic CRS. Two explanations are considered: first, passive diffusion of cytokines into the brain; and second, trafficking of T-cells to the brain. Both hypotheses are supported by findings in the serum and in the CSF, respectively. The clinical presentation is a toxic encephalopathy, presenting as decreased attention, dysphasia, and impaired handwriting. Patients can experience other symptoms including confusion, disorientation, agitation, aphasia, somnolence, and tremors. In severe cases (grade 2 or more), seizures, increased intracranial pressure, papilledema, and cerebral edema also occur.
Table 2 CRS assessment, grade, and management

| Grade | Toxicity | Management |
|-------|----------|------------|
| 1 | Mild reaction | Fever ≥38°C, nausea, fatigue, headache, myalgia, malaise | Symptomatic treatment only  
  • Antipyretic, antiemetics if needed  
  • Keep patient well hydrated, with special attention in water balance  
  • Empiric broad-spectrum antibiotic and filgrastim if neutropenic |
| 2 | Moderate reaction: Symptoms require and respond to moderate intervention | Hospitalization needed for management of symptoms:  
  • Hypoxia with O₂ requirement <40%  
  • Hypotension responding to fluid or low-dose vasopressors  
  • Grade 2 organ toxicity or grade 3 LFT | • Supplemental oxygen  
  • IV fluid bolus, if no response, starting vasopressors  
  • Tocilizumab (or siltuximab)  
  • Patients at high risk or refractory hypotension; steroids to consider, and transfer to ICU |
| 3 | Severe reaction: Symptoms require and respond to aggressive intervention | Hypoxia with O₂ requirement ≥40%  
  Hypotension requiring multiple fluid boluses and/or vasopressors  
  Grade 3 organ toxicity or grade 4 LFT  
  Coagulopathy requiring cryoprecipitate or plasma transfusion | • Transfer to ICU  
  • High-flow supplemental oxygen or noninvasive support  
  • Management of organ toxicities by standard guidelines, including transfusion for coagulopathy  
  • Tocilizumab (or siltuximab)  
  • Corticosteroids |
| 4 | Life-threatening symptoms | Ventilator support  
  Grade 4 organ toxicity (excluding LFT) | • Mechanical ventilation  
  • IV fluids, vasopressors  
  • Hemodynamic and respiratory monitoring  
  • Tocilizumab/siltuximab and corticosteroid  
  • Management of organ toxicities by standard guidelines |

Notes: Adapted from the Penn Grading Scale (From Porter DL, Hwang WT, Frey NV, et al. Chimeric antigen receptor T cells persist and induce sustained remissions in relapsed refractory chronic lymphocytic leukemia. Sci Transl Med. 2015;7(303):ra139.14 Reprinted with permission from AAAS and Lee et al (with permission of Blood, Current concepts in the diagnosis and management of cytokine release syndrome, Lee DW, Gardner R, Porter DL, et al, 124(2), copyright 2014).15 Regarding vasopressor: different definitions are used by Penn Grading Scale and Lee et al scale. In the Penn Grading Scale, low-dose vasopressors define a grade 3 CRS and high-dose or multiple vasopressors are included in the grade 3 CRS of Lee scale. Grading of organ toxicities is performed according to CTCAE version 4.04.03.46

Abbreviations: CRS, cytokine release syndrome; iCU, intensive care unit; IV, intravenous; LFT, liver function test.

In the ELIANA trial, neurologic effects were present in 40% of patients within 8 weeks after infusion and 13% were grade 3.67 In the pilot study, 13/25 patients had cerebral toxicity with self-limiting symptoms and complete resolution without apparent sequelae.68 No cerebral edema or grade 4 toxicity was reported in those two studies.

CRES has been reported as a biphasic manifestation: the first phase occurs with high fever and other CRS symptoms in the first 5 days after infusion and a second phase that occurs after CRS subsides, usually after 5 days of infusion. In about 10% of patients, however, a delayed neurotoxicity can occur 3–4 weeks after CAR-T-cell therapy.79

For its treatment, anti IL-6 can reverse the CRES concomitant with CRS, but it is not effective when CRS is not present. In this situation, corticosteroids remain the treatment of choice. CRES usually lasts for 2–4 days, but it can vary from a few hours to weeks. CRES occurring post-CRS is usually more severe.79,82 It is generally reversible; however, some rare fatal cases have been reported.37,82,97

HLH/MAS

HLH is a rare condition characterized by inappropriate immune activation and cytokine release that typically presents with fever and splenomegaly in association with hyperferritinemia, coagulopathy, hypertriglyceridemia, and cytopenias.98 These traditional criteria are not specific and are frequently present even in patients with low-grade CRS, which can complicate the diagnosis of HLH/MAS in the context of CAR-T-cell therapy. In CTL019 trials, this condition was not described thus far. However, severe CRS can evolve into HLH and clinicians must be aware about this complication in order to avoid fatal outcomes.

CARTOX has proposed a definition for HLH/MAS after CAR-T-cell therapy: a peak serum ferritin level of >10,000 ng/mL with any two of the following: grade ≥3 increase in serum bilirubin, aspartate aminotransferase, or alanine aminotransferase levels; grade ≥3 oliguria or increase in serum creatinine levels; grade ≥3 pulmonary edema; presence of hemophagocytosis in bone marrow or organs based on histopathological assessment of cell morphology and/or CD68 immunohistochemistry.79

These patients should be treated with anti-IL6 therapy as for CRS (and corticosteroids if clinically indicated), which has been able to reverse this clinical situation. If the patient does not improve in 48 hours, additional therapy with etoposide should be considered and intrathecal cytarabine
as well as for patients with neurotoxicity-associated HLH. Even if there is no evidence to support the latter practice in patients with CAR-T-cell-associated HLH, this recommendation is supported by the preference for those drugs in refractory HLH.79

It is critical that the community continue to investigate the pathophysiology and patterns of these unique toxicities and find ways to modify its administration or the CAR-T constructs to increase safety.79 In addition to medications, preparation of clinical staff to recognize toxicity and effectively manage these patients is key to improving overall safety.

B-cell aplasia
Therapies targeting B-cells such as anti-CD20 antibodies like rituximab, kinase inhibitors such as ibrutinib, and CAR-T cells can lead to absolute and functional hypogammaglobulinemia and/or the inability to respond to vaccines.100

CAR-T cells can probably be associated with “on target/off tumor” because of their direct attack on normal tissues that have the shared expression of the targeted antigen. This situation is well illustrated by the extended B-cell aplasia and hypogammaglobulinemia presented after anti-CD19 CAR-T-cell therapies.75,93,95 In the ELIANA trial, the probability of B cell aplasia at 6 months was 83%, and was present in all patients responding to CAR-T cells.79 Fortunately, for CTL019 and other anti-CD19 CAR-T therapies, this situation can be easily managed with intravenous or subcutaneous immunoglobulin replacement therapy without compromising the patient’s quality of life (QoL). Otherwise, if the target is present in the vital organs, the on target/off tumor effect can result in severe or fatal toxicity, perhaps one of the greatest obstacles for CAR-T-cell therapy in other diseases such as solid tumors.101,102

The immunoglobulin replacement therapy remains controversial and needs to be individualized, considering the disease, therapy, and serum antibody titers. The recommendation resulting from the last review by Ueda et al is a starting dose of 500 mg/kg/monthly intravenously or 200 mg/kg/weekly subcutaneously with a target serum IgG level over 400–500 mg/dL.106 Serum IgG level and/or specific antibody titers and infections should be monitored and used to individualize therapy.

Patient-focused perspective
Since more than 85% of children will survive 5 years or longer after a diagnosis of ALL in developed countries, the diminished QoL of the survivors obviously becomes a concern.2,103 On the other hand, it means that approximately 15% of children diagnosed with childhood ALL will experience a relapse.104 Indeed, for adults with ALL, it is only 20%–40% who will achieve long-term remission.6,7

For most relapsed patients, the treatment of choice remains an allo-HSCT,105 with a success rate of about 40%.106,107 However, morbidity after HSCT remains elevated for long-term survivors and includes endocrinology complications such as impairment growth, hormone deficiency, thyroid and sex hormone deficiencies, infertility, and second malignancies.103,108–110 In this context, CAR-T-cell therapy has shown remission rates and OS that seem to considerably exceed other therapies. However, some crucial points should also be taken into account with this innovative therapy, such as its unique toxicity profile, and its development and costs. Indeed, the cost of $475,000 announced by Novartis for a single infusion, compared with the estimate of allo-HSCT of about $200,000, has been viewed with a lot of criticism.111,112 Furthermore, this number does not include collateral costs during therapy, such as apheresis, cell infusion, and management of complications, including intensive care unit stays and use of tocilizumab.78

Eligible patients for this new therapy need to face more obstacles: geographic barriers, with less than 40 centers in the US authorized to administer the therapy and the time required to manufacture these cells, which can prevent patients with a more aggressive disease from receiving the therapy. In the ELIANA trial, 18% of patients were unable to receive the infusion, leading to the death of six patients, while seven patients failed to pass the manufacturing stage.67 All these points can overestimate the potential of cure of anti-CD19 CAR-T therapies. Of note, Novartis foresees that the actual time of manufacture for tisagenlecleucel will be shorter compared with the manufacturing time required during the last clinical trials.

Quality of life (QoL)
A French prospective study published in 2017 by Michel et al showed the results of a late-effect program (the LEA program) comparing transplanted vs non-transplanted children in a long-term follow-up.113 Despite major physical complications in the HSCT group, clinical consequences on the QoL were relatively mild. Those findings demonstrate acceptance and adaptation of a handicap, allowing him/her to have a better QoL. Also, an important point highlighted in this study was that some events adversely affecting QoL of transplant patients have been detected in adults but not in children, suggesting impairment in the adulthood of these younger survivors.
If we look at the specific population of patients who have received an infusion of CTL019, we see such an important difference in instantaneous QoL, starting from day 28, and continued improvement after 3 and 6 months as shown in ELIANA’s QoL study presented at ASH Meeting in 2017.\textsuperscript{114} Studies described above used different QoL scales, limiting the comparison between them. Moreover, it is essential to analyze this QoL in two different ways: short-term follow-up comparing with relapsed and refractory leukemia and long-term follow-up comparing with a normal population and children after HSCT, for example.

The timing of the CAR-T-cell treatment should also be considered to assess QoL. If CAR-T is given after two or three lines of treatment, patients might present less cumulative toxicity vs if CAR-T is given after being refractory to several lines of treatment or after a first transplant. It is likely that there would be significant differences regarding morbidities burden between these two groups of patients.

Although it is early to show the superiority of CAR-T-cell therapy to deliver a better QoL, the first results are promising once the adverse effects period ends without permanent morbidities. As stated above, those results need to be compared in a prospective long-term follow-up scenario with other patients who received chemotherapy with or without allo-HSCT instead of CAR-T cells, and with normal population control.

Conclusion

CTL019 and other CAR-T-cell therapies represent an impressive new approach of anti-cancer therapy and have a strong potential to transform lives of patients with fatal diseases. Optimal structure, T-cells selection and enrichment, doses, and lymphodepleting regimen remain relevant and hot topics in the field of cellular therapy. In spite of those questions, tisagenlecleucel has proven its efficacy leading to its approval by the FDA. However, its peculiar and severe toxicities must be improved in future trials.

Its recent approval has opened singular discussion about the need for a new policy and improved infrastructure to achieve the best clinical benefit, and, moreover, raised the concern about better access for patients worldwide. The price of a single infusion is undoubtedly an obstacle to this commercialization.\textsuperscript{112} Actually, tisagenlecleucel has the promise of meaningful benefits but at a considerable social cost and with toxicities.

Disclosure

Henrique Bittencourt and Pierre Teira are consulting for Novartis Oncology. The other authors report no conflicts of interest in this work.

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