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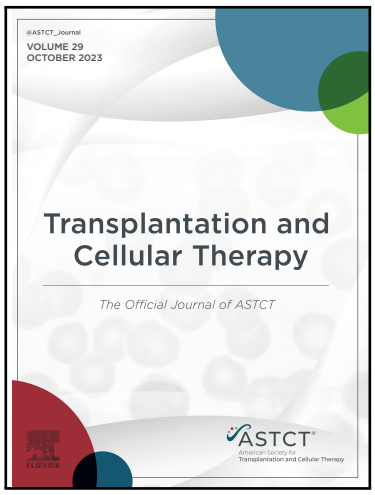

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Opportunities and Challenges with CAR T-cell Treatment of Children and Young Adults with B-Cell Acute Lymphoblastic Leukemia: Review and Recommendations from the Westhafen Intercontinental Group*

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Highlights:

- Chimeric antigen receptor (CAR) improves outcomes in young relapsed B-ALL patients
- CAR T-cell challenges include toxicity, poor persistence, and antigen escape
- Large studies determined relapse risk allowing personalized approaches to HCT post-CAR

Abstract

Chimeric Antigen-Receptor T-cells (CAR T-cells) targeted at pediatric B-cell precursor acute lymphoblastic leukemia (B-ALL) have changed the paradigm for treatment of relapsed and refractory B-ALL. We present a comprehensive review and recommendations approaching this topic from the Westhafen Intercontinental Group, which is comprised of leaders from the International Berlin Frankfurt, Muenster (iBFM) Stem Cell Transplantation Committee, the Center for International Blood and Marrow Transplant Research (CIBMTR) Pediatric Cancer Working Committee, the Children's Oncology Group (COG) Cellular Therapy Committee, the Pediatric Diseases Working Party (PDWP) of the European Society for Bone and Marrow Transplantation (EBMT) and the Pediatric Transplantation and Cellular Therapy Consortium (PTCTC). In this paper we examine the current state of CAR T-cell therapy in pediatric B-ALL, assess current and emerging integration of CAR T-cells into treatment algorithms, and discuss emerging strategies to overcome existing challenges.

Introduction

Chimeric Antigen-Receptor T-cells (CAR T-cells) for pediatric B-cell precursor acute lymphoblastic leukemia (B-ALL) have significantly improved outcomes and hence redefined expectations and treatment approaches for B-ALL. The Food and Drug Administration (FDA) and the European Medicines Agency's (EMA) approval of tisagenlecleucel (Kymriah) marked a watershed moment in the field of cellular immunotherapy, offering hope to patients who had exhausted conventional treatment options, many of whom had failed multiple lines of therapy including allogeneic hematopoietic stem cell transplantation (HCT).

Despite advances, challenges persist. The complexity of manufacturing, high production costs, and limited accessibility continue to impact widespread adoption. In addition, the field grapples with clinical challenges including CAR T-cell associated toxicities, lack of CAR T-cell persistence evidenced by early loss of B-cell aplasia (BCA), and antigen escape. In addition, although CAR T-cells can lead to responses in patients with extramedullary disease (EMD), relapses can sometimes occur in sanctuary sites such as the central nervous system (CNS) despite CAR T-cell persistence, and optimal uses in these patients are unknown. There is a critical need to understand and address these limitations.

This paper is a comprehensive review that includes recommendations from the Westhafen Intercontinental Group, which is comprised of leaders from the International Berlin Frankfurt, Muenster (iBFM) Stem Cell Transplantation Committee, the Center for International Blood and Marrow Transplant Research (CIBMTR) Pediatric Cancer Working Committee, the Children's Oncology Group (COG) Cellular Therapy Committee, the Pediatric Diseases Working Party (PDWP) of the European Society for Bone and Marrow Transplantation (EBMT) and the Pediatric Transplantation and Cellular Therapy Consortium (PTCTC). Consensus was achieved

through representatives from each of the groups that were tasked with paper planning, writing, and editing. In this paper, we will examine the current state of CAR T-cell therapy in pediatric and young adult B-ALL, assess current and emerging integration of CAR T-cells into treatment algorithms, and discuss emerging strategies to overcome existing challenges. We will also explore recent technological advances in CAR design, novel approaches to toxicity management, and innovative solutions to enhance manufacturing efficiency and accessibility. Additionally, we will discuss ongoing clinical trials and future directions that may further expand the role of this transformative therapy in pediatric leukemia treatment.

Current uses and outcomes

Trials leading to the approval of tisagenlecleucel (tisa-cel) were performed without randomization in pediatric and young adults patients with either refractory or multiply relapsed B-ALL.(1,2). Since the FDA and the EMA approval of tisa-cel in children and young adults in 2017, there has been a steady stream of approvals of other cellular and gene therapy products in adults. As of May of 2025, however, tisa-cel remains the only CAR T-cell therapy approved in children by the FDA and the EMA, with indications limited to multiply relapsed or refractory (r/r) B-ALL. Brexucabtagene autoleucel (brexu-cel) and obecabtagene autoleucel (obe-cel) are approved for adults with relapsed B-ALL.

Relapsed/Refractory Disease

CAR T-cells have led to impressive complete remission (CR) rates (70%–90%) in children and adults with r/r B-ALL, (3–7). These high response rates (8) have been observed regardless of white blood cell count, cytogenetics, number of prior therapies, chemotherapy responsiveness, or other factors associated with chemotherapy responsiveness. Despite these early results, a substantial number of patients eventually experience relapse due to CAR T-cell failure. The two primary mechanisms of failure are 1) loss of functional CD19 CAR T-cells before disease eradication, and 2) leukemia relapse due to CD19 target antigen loss on B-ALL blasts.

Risk factors for CAR T-cell failure include (Table 1):

1. **High disease burden** (9–11) Several studies have demonstrated that high disease burden prior to infusion (>5% blasts) is associated not only with lower relapse free survival but also with a higher likelihood of CD19-negative relapses.
2. **Failure of prior treatment with blinatumomab** (10,12,13) Rather than any exposure to CD19-directed therapy with blinatumomab, only non-response to blinatumomab has been associated with inferior event-free survival (EFS).
3. **Early Loss of B-cell aplasia** (BCA) (7,14): Defined variably in different reports as <1% to <3% CD19+ cells among total lymphocytes (or an absolute count ≥10 to 50/µl), BCA can be used as a measure of in vivo CD19 CAR T-cell functional activity. Loss of BCA implies the absence of functional CAR T-cells, and if it occurs within 6 months post-infusion, it is associated with a high risk of CD19-positive relapse.

4. **Target antigen loss** (14–16): CD19 loss/downmodulation can result due to truncated proteins, genetic mutations, epigenetic changes or lymphoid-to-myeloid lineage switch. Subsets of patients, including those with high disease burden and/or KMT2A rearrangements, have been identified as being at a higher risk for antigen loss.
5. **Lower CAR T-cell dose**(17): Higher doses of tisa-cel have been associated with better long-term outcomes.
6. **Use of autologous CAR T-cells early after relapse post-HCT**(18): Patients who relapsed within six months following allogeneic HCT and received tisa-cel manufactured from their cells collected post-HCT experienced significantly poorer disease free survival (DFS) compared to those who relapsed beyond six months after transplant. This compelling observation requires further study to validate and understand possible mechanisms contributing to failure.

7. **Suboptimal fludarabine dosing** (19,20) Suboptimal fludarabine exposure (area under the curve [AUC] <13.8 h/L) has been associated with shorter CAR T-cell persistence and an increased risk of relapse.

Relapse after HCT

Historically, outcomes for patients with B-ALL experiencing relapse after HCT have been particularly poor. Second HCT attempts in this cohort of patients have significant limitations due to high treatment-related mortality (TRM) and contraindications for further total body irradiation (TBI) (21–23). For patients who have already undergone allogeneic HCT, CAR T-cell therapy can serve as an effective rescue therapy (23,24). One study showed that the outcome was associated with the time elapsed between HCT and relapse, with an EFS of 55.5% for patients relapsing beyond 6 months and 18.5% for patients relapsing prior to 6 months after HCT (18). These dismal outcomes in patients with very early relapses after HCT (<6 months) may be explained both by the refractoriness of the disease, and T-cells collected shortly after immunosuppression discontinuation may be dysfunctional and have impaired in vivo expansion (25–27). One potential way to address this issue would be to manufacture T-cells directly from the transplant donor. This approach has been explored in several clinical trials, with promising results in small studies (28–31), but is not currently FDA/EMA approved.

Extra-Medullary Disease (EMD)

Only 10-20% of newly diagnosed B-ALL patients present with EMD. However, at recurrence, a higher proportion of patients (15%-25%) relapse with some combination of medullary/extramedullary involvement (21% with isolated CNS disease and less than 1% with isolated testicular relapse)(32). Non-CNS EM disease is likely underdiagnosed, as full body imaging is not a standard part of evaluation at most centers (33).

Recent evidence has shown that patients with CNS disease at diagnosis or relapse who undergo CAR T-cell therapy have similar outcomes to those without CNS disease, with no increase in severe ICANS (≥grade 3) (34–36). However, in a retrospective report, patients treated with tisa-cel for an isolated CNS relapse had a high incidence of a subsequent CNS relapse (36). There are conflicting data with non-CNS EMD. While some groups noted no

difference in outcomes when compared to CNS-EMD or isolated marrow disease(35) a large retrospective trial found that active EMD at infusion was independently associated with worse EFS(10). Localized transient toxicities have occurred at sites of EMD following CAR T-cells including erythema, swelling, and pain, as well as a report of bilateral retinal detachment with temporary vision loss in a patient with ocular involvement. (37,38)

CAR-T cells in special populations

The current FDA/EMA indication does not include treatment with CAR T-cell products for patients with first relapse of B-ALL. CAR T-cell therapy should be considered at first relapse for very selected categories of patients, such as those affected with genetic conditions associated with poor outcomes due to excessive toxicity with conventional treatment.

Patients with Down Syndrome

Patients with Down syndrome-associated acute lymphoblastic leukemia (DS-ALL) are at risk of chemotherapy-associated toxicities and poor outcomes. CAR T-cell therapy offers potential cure in refractory patients with toxicity profiles comparable to non-Down syndrome patients (39–41).

Other special populations

Patients with chromosomal instability syndromes, such as Nijmegen Breakage Syndrome, who develop B-ALL and have an indication for HCT may do better with reduced-intensity conditioning (RIC) regimens compared to myeloablative protocols involving TBI(42). As a result, these patients are likely at a higher risk of developing mixed chimerism following HCT, which increases their susceptibility to relapse. In such cases, it may be reasonable to consider consolidation with CAR T-cell therapy when mixed chimerism is detected post-transplant to reduce the risk of disease recurrence. This approach has been reported to be successful in a single patient(43).

Additional consideration should be given to two subsets of patients who have been reported to experience decreased outcomes due to adverse cytogenetic traits. The first includes patients with KMT2A rearrangements (KMT2Ar). While these patients can achieve long-term DFS similar to other cytogenetic subsets treated with CAR T-cells(44), if they relapse, they are at increased risk for lineage switch, and salvage for those relapsing with lineage switch is very poor (10,45). The second subset includes patients with Li-Fraumeni Syndrome (TP53 germline mutations) and somatic TP53 mutated leukemia. Studies from China have shown lower DFS, significant risk of failure in these patients (46,47). While other studies have not identified TP53 as a poor risk factor, TP53 characterization of high-risk patients with B-ALL patients have not been uniformly performed by many centers and further study is warranted(10,44).

Challenges for the field

The primary biologic challenges can be summarized as toxicities associated with CAR T-cell therapies and leukemia relapse due to CD19 target loss (antigen escape), and loss of functional CAR T-cells (lack of T-cell persistence), while the socio-economic challenges related to CAR T-cell therapy are complex and multifaceted (Figure 1).

Toxicity associated with CAR T-cell therapy

Although hypogammaglobulinemia, cytokine release syndrome (CRS) and Immune effector cell–associated neurotoxicity syndrome (ICANS) are the most common and well described toxicities associated with CAR T-cell therapy, recently Immune-effector cell associated HLH-like syndrome (IEC-HS), and Immune effector cell-associated hematotoxicity (ICAHT), have been described. (48,49)

Hypogammaglobulinemia and risk of infections

BCA and hypogammaglobulinemia are expected on target adverse events of successful CAR T-cell therapy but increase the risk of life-threatening infections. Long-term immunoglobulin replacement therapy is routinely performed in pediatrics, with response and persistence varying by patient. Most institutions use immunoglobulin G (IgG) levels below 400 mg/dL as the threshold for supplementation, higher levels may be needed for those with recurrent infections despite-IgG replacement(50–52)

Cytokine Release Syndrome (CRS)

Cytokine Release Syndrome (CRS) is caused by the significant release of inflammatory cytokines, a self-limited process that initially presents with fever and flu-like symptoms (headaches, myalgias) in mild cases and can progress to a sepsis-like constellation with hypotension and hypoxia, leading to organ dysfunction, capillary leak, and coagulopathy. CRS can be successfully treated with anti–interleukin-6 receptor (IL-6R) therapies (e.g., tocilizumab), often in combination with steroids (44,45). The severity of CRS is measured by staging. High tumor burden prior to lymphodepletion is the strongest predictive factor for severe CRS. Both the American Society for Transplantation and Cellular Therapy (ASTCT) and the EBMT/EHA consensus guidelines for CRS have been broadly adopted (45–48).

Currently, due to the lack of evidence supporting effective prophylactic strategies for CRS in patients receiving CAR T-cells, no formal recommendations exist for prophylaxis. However, there is evidence supporting the early use of tocilizumab at Grade I CRS in patients presenting risk factors for severe CRS, with the aim of preventing progression to severe CRS (8). The impact of tocilizumab on CAR T-cell expansion and persistence appears negligible (49), this approach should be considered for a selected group of patients, including:

- Patients with high disease burden (e.g., >5% to >25% blasts) before CAR T-cell infusion
- Patients with pre-existing cardiac or pulmonary comorbidities
- Patients with CRS onset within 24 hours of CAR T-cell infusion

Immune effector cell–associated neurotoxicity syndrome (ICANS)

Neurological manifestations associated with CAR T-cell–induced immune effector cell–associated neurotoxicity syndrome (ICANS) range from language dysfunction or aphasia, handwriting difficulties, and cognitive impairment to altered mental status or delirium, seizures, coma, and fatal cerebral edema. Neurological toxicity has been reported less frequently in pediatric patients and tends to be short-lived. Although rare, fatal cerebral edema has been documented (50)

The pathophysiology is likely related to disruption of the blood-brain barrier (BBB) secondary to systemic cytokine release, high levels of cytokines in the cerebrospinal fluid, and/or direct CAR T-cell attack of CD19-positive mural cells in CNS tissues (53,54). Unlike CRS, CNS symptoms have not responded well to tocilizumab, as it does not cross the BBB. ICANS has generally been treated with high-dose steroids, anakinra or other approaches. The timing of treatment for ICANS is controversial, but concerns about its rare, fatal form have led to near-uniform recommendations for the treatment of patients with grade 3 or higher ICANS (54–56). Rapid peak expansion, severe CRS and higher dose of CAR T-cells have been highlighted as risk factors for severe ICANS, although, interestingly, pre-CAR T-cell CNS disease has not been clearly associated with the severity of neurological manifestations (57–60)

Of note, neurocognitive impairment and neuropsychiatric disorders are emerging as long-term side effects associated with ICANS in adults, but the incidence of these late manifestations in children is unknown (61,62). Given the lack of sufficient evidence, anti-seizure prophylaxis is generally not universally recommended. However, seizure prophylaxis with levetiracetam—a medication generally well tolerated in children, with rare and minor side effects—should be considered for high-risk patients, including those with:

- History of neurological disorders (e.g., seizures, posterior reversible encephalopathy syndrome)
- Evidence of neurological abnormalities on imaging

Immune-effector cell associated Hemophagocytic Lymphohistiocytosis (HLH)-like syndrome (IEC-HS)

Immune-effector cell associated HLH-like syndrome (IEC-HS) has been described as life-threatening immune activation. Onset is usually after CRS is resolving, or after an initial improvement with CRS directed treatment. IEC-HS is associated with high fever, hyperferritinemia, prolonged cytopenia, and can lead to multiorgan failure(48). There may be overlap with CRS in some patients; the later onset disease occurs more frequently with certain approaches to CD22-targeted CARs. Given the lack of prospective trials in this area, published ASTCT working group treatment recommendations include a patient-tailored stepwise approach with anakinra with or without glucocorticoids, followed by ruxolitinib, emapalumab or low-dose etoposide.(48,63)

Immune effector cell-associated hematotoxicity (ICAHT)

Prolonged cytopenias (30-90 days), particularly neutropenia (<500/mm3) occur in a subset of patients (approximately 10% of patients experience persistent cytopenia one year after treatment) (64,65). Cytopenias in combination with hypogammaglobulinemia can predispose patients to serious infectious complications(66), and patients with B-ALL seem to be more likely to be affected than other B-cell targeted diseases. The use of B-ALL specific tools to risk stratify patient's susceptibility to develop hematoxicity is important for post-CAR T-cell care(67). The Pediatric Real World CAR Consortium *(PRWCC)* has also published a score for predicting risk of severe, prolonged neutropenia(68). Management of cytopenias is mostly supportive with transfusions. Some patients may respond to G-CSF or thrombopoietin receptor

agonists (69,70); in patients that have severe persistent cytopenias and a history of prior HCT, CD34+ selected hematopoietic cell boosts have been beneficial (71,72).

Antigen escape

Combined data from the ELIANA and ENSIGN trials showed rates of CD19-positive and CD19-negative disease recurrence were 36% and 64%, respectively (73,74).

There are different proposed mechanisms for the emergence of antigen escape:

1. Pre-existing target-negative tumor clones.
2. Antigen gene mutations, alternative splicing or methylation. (15,75)
3. Deficiencies in antigen processing and presentation to the T-cells unrelated to CD19. (76,77).
4. Lineage switch (commonly observed in patients with KMT2A rearrangements) leading to loss of the target antigen(16,78).
5. Epitope masking(79)
6. Trogocytosis and Antigen redistribution. While antigen redistribution usually refers to the movement of antigens from membrane to an intracellular location (80,81), trogocytosis refers to the exchange of plasma membrane fragments. (82–84).

To address these challenges, researchers have pursued multiple approaches, including testing combinations with other therapies, such as enhanced or armored CAR T-cells with IL18 (85) or radiation therapy prior to CAR T-cell infusion (86), or concomitant use with chidamide (a histone deacetylase inhibitor) to upregulate tumor antigens (87). In addition to searching for novel targets, which has proven difficult due to challenges in finding candidates with acceptable on-target, off-tumor toxicity profiles.

Multi-antigen approach: Multi-targeted CAR T-cell, sequential cell and immune therapies

Cell and immune therapy approaches have been devised to target a second lymphoid antigen to overcome CD19 antigen escape. CD22 has been the most extensively studied and is considered an attractive target, both in CAR constructs and with inotuzumab ozogamicin (88). CD22 can be downregulated; therefore, this approach is often combined with HCT, as an increased risk of relapse is expected (55,89,90).

There is concern that administering CD22-targeted therapy before CD19 CAR T-cell treatment may impair T-cell expansion, potentially reducing therapeutic efficacy (91). Although not yet commercially available, sequential or simultaneous CD19 and CD22 CAR infusions are being studied. There have been studies published with promising results (92,93)

Multitargeted CAR T-cells offer a promising strategy to combat antigen escape; however, early experience has revealed significant limitations. Several approaches have been tested clinically, including co-administration of two CAR T-cell products targeting different antigens, co-transduction of T-cells with two separate vectors encoding different CARs, the use of a bicistronic vector encoding two CARs, and tandem CARs (94,95). To date, the major limitation of these studies (89–92) has been the limited persistence of CAR T-cells, which

precludes assessment of the impact of multi-antigen targeting on CD19-negative relapse. Addressing this challenge will likely be necessary to fully realize the potential of this approach.

T-cell persistence

A decade has passed since Maude et al. (7) first identified the correlation between CAR T-cell persistence in peripheral blood and BCA. Subsequent data from the ELIANA and ENSIGN trials have refined our understanding (18,73) revealing that B-cell recovery alone does not necessarily imply relapse.

CAR T-cell persistence involves multiple factors, with T-cell functionality proving more important than CAR detection (7,96), as evidenced by the practice of using BCA as a key indicator of T-cell persistence. T-cell phenotype—including memory versus effector status and activation versus exhaustion markers—influences persistence. (97,98) (99) The persistent antigenic stimulation of T cells can lead to dysfunction (100–102), where exhausted T-cells exhibiting a characteristic pattern of inhibitory receptors, and transcription factors display altered metabolism, low proliferative capacity, and a reduced cytotoxicity and secretion of effector cytokines (103)

Potential T-cell Persistence Improvement Strategies

Architecture

The sequential generations of CAR T-cells have not only improved the cytotoxic ability of the T-cells but also aided in persistence. The addition of the 4-1BB (CD137) domain to CAR constructs promoted the induction of CD8+ T-cells with increased oxidative metabolism and heightened mitochondrial biogenesis, two characteristics of the least differentiated memory T-cells. The structure of the single chain fragment variable (scFv) can modify persistence, as seen in obecabtagene autoleucel (Obe-cel), a CD19 CAR T-cell (FAST OFF CAR), with a lower affinity than FMC63 (the scFv in tisa-cel), which has led to higher in vitro proliferation and cytotoxicity and greater in vivo proliferative and antitumor activity compared with FMC63 CAR T-cells(104). There are numerous newer constructs that integrate systems with modulated CAR expression and intermittent activation(105). Oxygen sensitive CAR expression is also being studied by utilizing the subdomain of HIF1α to modulate CAR expression according to oxygen availability in the tumor microenvironment(106).

Another approach to enhance persistence such as incorporating vaccination with tumor antigens (107,108), or incorporating oncolytic virus into treatment(109–112). Recent studies have also shown that CAR T-cells engineered to express and deliver non-coding RNA can promote expansion and effector memory differentiation of CAR T-cells leading to higher persistence and less exhaustion(113)

Cell Culture Optimization

Modifications in manufacturing techniques have led to significant changes in functionality and phenotype. Both the type of culture medium used for ex vivo expansion and the duration of expansion(101,114) influence cellular behavior in vivo, including their phenotypic differentiation, proliferation, and efficacy. The use of fetal bovine serum (FBS) versus human serum or human platelet lysate have all shown differences in outcomes. The use of RetroNection for lentiviral

transductions(115–117), specific CD4/CD8 ratios, and agents like dasatinib have been used to increase transduction efficiencies and have demonstrable influence on T-cell performance (118).

Cytokines Used to Yield Undifferentiated CAR-T Cells

The most studied is Interleukin (IL)-2 (115), has played an essential role in the manufacturing process, as it stimulates cell proliferation and maintains cell viability during the expansion phase. IL-2 can lead to shorter lived phenotypes. Some studies have shown that, during the expansion phase of CD28-based CD19 CAR T-cells, a mixture of IL-7 and IL-15 increased the number and proportion of a T-cell subpopulation with T-cell memory stem cell and central memory-like phenotypes (101). Some newer generation of CARs include inducible gene expression cassette encoding a transgenic cytokine, to enhance T-cell activity within tumor microenvironment (85,119,120)

Patient Access and Regulatory Considerations

The regulatory landscape presents additional complexities. Current European Union legislation requires pharmaceutical licensing for CAR T-cell therapy. The EMA supports academic investigators in licensing CAR T-cells and other advanced therapy medicinal products (ATMPs) (93). Single-center approaches prove inefficient and time-consuming (121)

The current development pathway mirrors traditional drug development, requiring FDA biological license application (BLA) submission and approval after demonstrating efficacy and safety. Academic institutions typically lack the infrastructure for conducting pivotal trials necessary for commercial approvals, though orphan drug designation provides some incentives.

Regarding cost recovery, the Code of Federal Regulations (CFR) Title 21 Part 312, subpart A section 312.8 allows academic institutions to recover specified costs under an investigational new drug (IND) application if they meet certain criteria:

- Evidence of potential clinical benefit
- Possibility of advantages over existing treatments
- Essential safety and efficacy data collection
- Financial necessity for trial continuation

The FDA's authority does not extend to determining reimbursement mechanisms. Even with approved INDs, patients rely heavily on insurance coverage, and product pricing remains constrained by allowable production cost calculations.

Potential solutions have emerged, although these may vary across different continents. For example:

- A hybrid model, where academic centers continue production with expanded distribution capabilities.
- Automation to address production challenges.
- Novel reimbursement strategies, such as limiting pharmaceutical licensing to specific vectors or CAR constructs rather than to individual patient cell products.

Industry experts advocate for innovative solutions, such as establishing new entities like the Pediatric Advanced Medicines Biotech (PAMB) to advance late-stage development and commercialization of pediatric cell and gene therapies outside traditional biopharmaceutical models in the United States(122). Developing consensus on these solutions is crucial (123).

Controversial Role of HCT as consolidation after CD19 CAR T-cells: HCT for all vs. selective use

Prior to CAR T-cells, the universal standard of care (SOC) for patients with high-risk, r/r B-ALL was to proceed to HCT following the achievement of a CR. Today the role of consolidative HCT post tisa-cel is being debated, with notable regional and institutional practice differences.

An estimated 35-40% patients (124) are cured by tisa-cel as a stand-alone therapy, a central question in the field is whether CAR T-cell therapy should be used to avoid the need for HCT in this group or if all patients should be consolidated with HCT. Proceeding with HCT after CAR T-cell therapy can potentially reduce the risk of recurrence in some categories of patients. Studies from the National Institutes of Health (NIH) showed that therapy with CAR T-cells using the CD28 co-stimulatory domain in pediatrics and young adults had improved survival when HCT was given 4 to 8 weeks after the CAR T-cell infusion(125). The short half-life of CARs with a CD28 co-stimulatory signal, almost invariably require a consolidative HCT to avoid relapse for patients with B-ALL, as a survival advantage has been demonstrated in children and young adults consolidated with HCT(126).

For patients receiving CD19 targeted CAR T-cells using 4-1BB costimulatory domains (tisa-cel and obe-cel) the decision to proceed to transplant is nuanced. A subset of patients will have sustained remission without further therapy. To date, the available data rely on nonrandomized, retrospective analyses, and are potentially subject to biases (127–129). In the ELIANA update, 11/79 or 14% of patients in a tisa-cel mediated remission went to HCT. Of the 8 patients from these 11 who had follow-up data available, none had relapsed(73). Reason to proceed to HCT was not described.

Over the last three decades, TRM after HCT has decreased, due to increasing precision in donor matching, better graft-versus-host disease (GVHD) prevention and management, and overall improvements in supportive care. As conditioning for B-ALL in pediatrics and AYA has traditionally included high-dose TBI, pediatric HCT survivors are at increased risk of early development of chronic health conditions, with over 60% of HCT survivors reporting at least one chronic condition, which in turn can lead to late TRM (130). In one study, consolidative transplant after CAR T-cell therapy improved leukemia-free survival in patients who were not previously transplanted, but this benefit was not observed in those who had previously been transplanted(128). A European retrospective study highlighted a survival benefit of consolidative HCT in patients without evidence of disease recurrence, when compared to those who had disease relapse or MRD positivity after CAR T-cells. In this study, no difference was noted in OS, LFS, and NRM between outcomes of consolidative HCT of patients undergoing a first or a second HCT after CAR T-cell treatment. (131) More study is required of patients undergoing a

second HCT after CAR T-cell therapy, but current literature supports recommendations for two patient types.(131)

In patients who are eligible to proceed to HCT and do not have a history of prior HCT, there is reasonable evidence to recommend consolidative HCT in patients who:

a) receive a CAR T-cell therapy using CD28-costimulatory domains, OR
b) experience loss of BCA within 6 months of CAR T-cell infusion, OR
c) present with MRD positivity at any level after CAR T-cell infusion.

In patients who are eligible to proceed to HCT and do not have a history of prior HCT, patients that may be considered for consolidative HCT are those who:

a) have an appropriate donor available and desire to proceed with HCT, AND/OR
b) have a high disease burden (prior studies have defined >5% blasts up to >25%) pre-lymphodepletion, AND/OR
c) have a history of prior treatment failure with blinatumomab, AND/OR
d) another relapse is unlikely to be treatable, whether due to history of refractoriness or adverse cytogenetics.

The optimal approach for patients who have previously been transplanted and have early loss of BCA has yet to be determined and requires special consideration. Our proposed treatment algorithm is included in Figures 2 and 3. Patient specific features should be considered to balance pros and cons of consolidative HCT. Including time elapsed since first transplant and characteristics of the previous HCT (conditioning regimen and donor type). The presence of CD19 negative clone before CAR T-cell infusion, donor availability and co-morbidities, and previous toxicities should be accounted for in the decision-making process. Potential alternative approaches other than HCT are discussed below.

Reinfusion and maintenance therapy

In cases where patients achieve initial remission following a CD19 CAR with a 4-1BB co-stimulatory domain and do not proceed to HCT, is there a role for CAR T-cell reinfusion to overcome short persistence (loss of BCA)? Investigators from Children's Hospital of Philadelphia (CHOP) recently published a retrospective review of children and young adults with r/r B-ALL treated on three CD19 CAR clinical trials or with commercial tisa-cel between 2012 and 2020 who received at least one reinfusion of the same product (132). While some patients re-established BCA and demonstrated improved persistence following reinfusion, this was observed mostly in those who were given reinfusions because of emergence of CD19-positive hematogones in the bone marrow versus those with robust peripheral B-cell recovery. Other studies addressing whether reinfusion is beneficial are ongoing and have generally focused on infusions for loss of BCA or relapse.

An alternative approach is to treat patients with early loss of BCA with maintenance therapy. In a small UK retrospective study, 5 out of 8 patients treated with this approach

remained in molecular remission at last follow up (median follow-up time from loss of BCA was 21.5 months) and 3 relapsed with CD19-positive disease (133). Further larger studies of this approach are ongoing.

Similarly, maintenance with tyrosine kinase inhibitors (TKI) in small cohorts of Ph+ B-ALL patients have been explored as an approach, derived from post-transplant management of these patients, to reduce the risk of disease relapse. However, data are limited to small cohorts of patients, and the benefit of TKI in children post-CART still warrants further study (134).

Discussion

CAR T-cell therapy has transformed the treatment landscape for patients with relapsed or refractory B-ALL, not only improving the chances of sustained remissions but has also facilitated the eligibility of some patients for HCT who would otherwise have been deemed ineligible due to the severity of their disease, other underlying conditions (e.g. active infections) or treatment failures. The efficacy of CAR T-cell therapy, particularly targeting CD19, has been well documented, with studies reporting high remission rates and durable responses(135) and a favorable toxicity profile. While adverse events such as CRS and ICANS remain a concern, many of these effects are manageable with supportive care and timely interventions.(136,137). Furthermore, the safety of CAR T-cell therapy has been underscored by studies demonstrating that most adverse reactions occur within the initial weeks post-infusion and are controllable (70,138).

Despite these advances, challenges persist, such as antigen escape that leads to CD19 negative relapses, or poor T-cell persistence. Multifaceted approaches are required to overcome these challenges, including multi-antigen targeting strategies to mitigate escape, enhanced CAR designs, and accurate patient risk stratification to identify which patients may require consolidative therapies.

Among the most pressing issues are cost and production scalability. Equally concerning is the reality that CAR T-cell therapies that show promise in clinical trials remain challenging to produce commercially, particularly for rare pediatric indications. The term "valley of death" aptly describes the substantial gap between basic science achievements and their clinical implementation (139) This gap is primarily driven by limited commercial interest, resulting in restricted access to products from academic centers and significant regulatory and financial barriers to conducting prospective investigational trials.

The effectiveness of any therapy depends on its accessibility. Currently, patients outside academic center catchment areas or those facing financial constraints often cannot access these potentially life-saving treatments. (140,141) To address these challenges, the ASTCT established the ACT to Sustain (Adoptive Cell Therapy to Sustain) task force (142). This initiative focuses on scenarios where the current model fails patients, including cases involving effective CAR T-cells without commercial partners, off-label indications, and rare diseases that would benefit from gene or cellular therapy. (143,144)

Despite challenges CAR T-cell therapy represents a paradigm shift in the management of relapsed or refractory B-ALL, offering hope for cure and improved quality of life for patients. While significant obstacles remain, the potential benefits make these challenges worth addressing through continued research and clinical development. The favorable toxicity profile and potential to facilitate HCT eligibility secures CAR T-cell therapy's spot as a cornerstone of treatment for r/r B-ALL.

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Manuscript was conceived and written by C.D.R, K.K and M.A.P. All other authors participated in the consensus development process through discussion, manuscript review and editing.

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Figures:

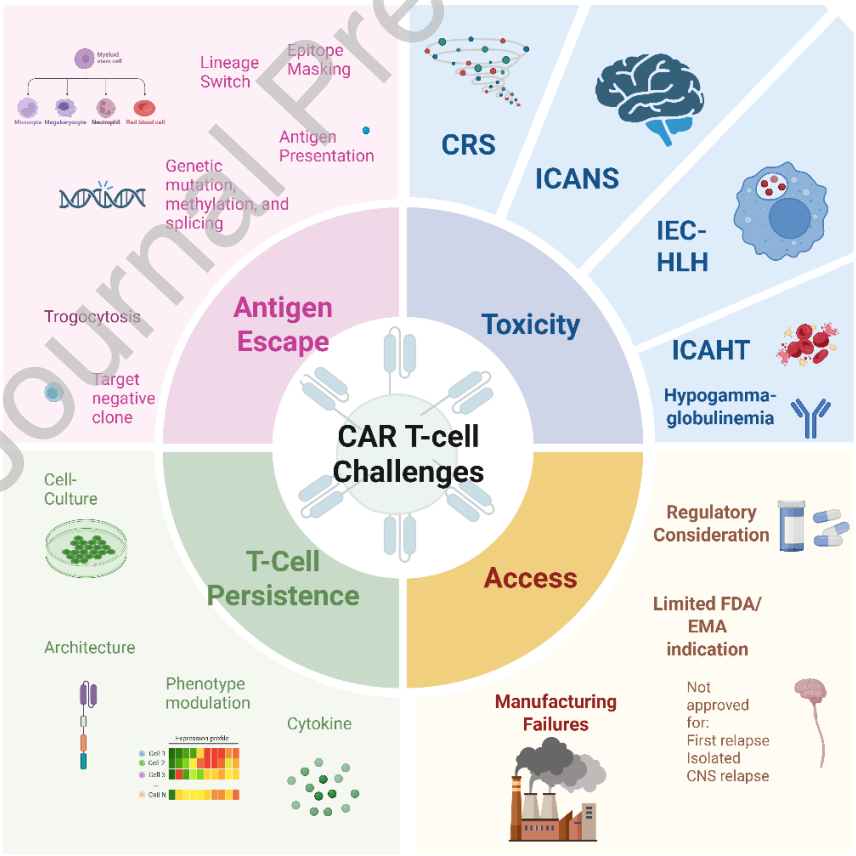

Figure 1 Challenges in Chimeric Antigen Receptor (CAR) Therapies - Food and Drug Administration (FDA), European Medicines Agency's (EMA), extramedullary disease (EMD), cytokine release syndrome (CRS) Immune effector cell–associated neurotoxicity syndrome (ICANS) Immune-effector cell associated HLH-like syndrome (IEC-HS), and Immune effector cell-associated hematotoxicity (ICAHT) Created in BioRender. Deimundo Roura, C. (2025) https://BioRender.com/t90006r

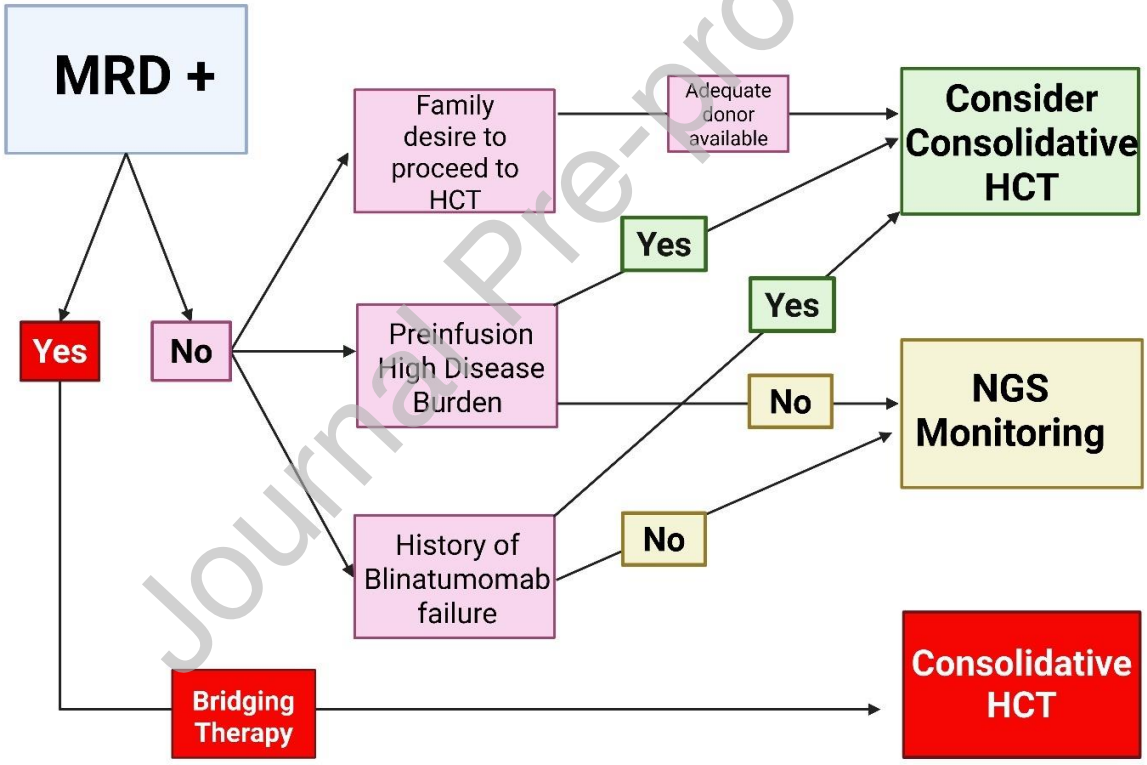

Figure 2 Algorithm based on Minimal Residual Disease (MRD), at either quantitative PCR (qPCR) or flow cytometry level. Hematopoietic Cell Transplant (HCT), Next Generation Sequencing (NGS) Created in BioRender. Deimundo Roura, C. (2025) https://BioRender.com/lo22o1z

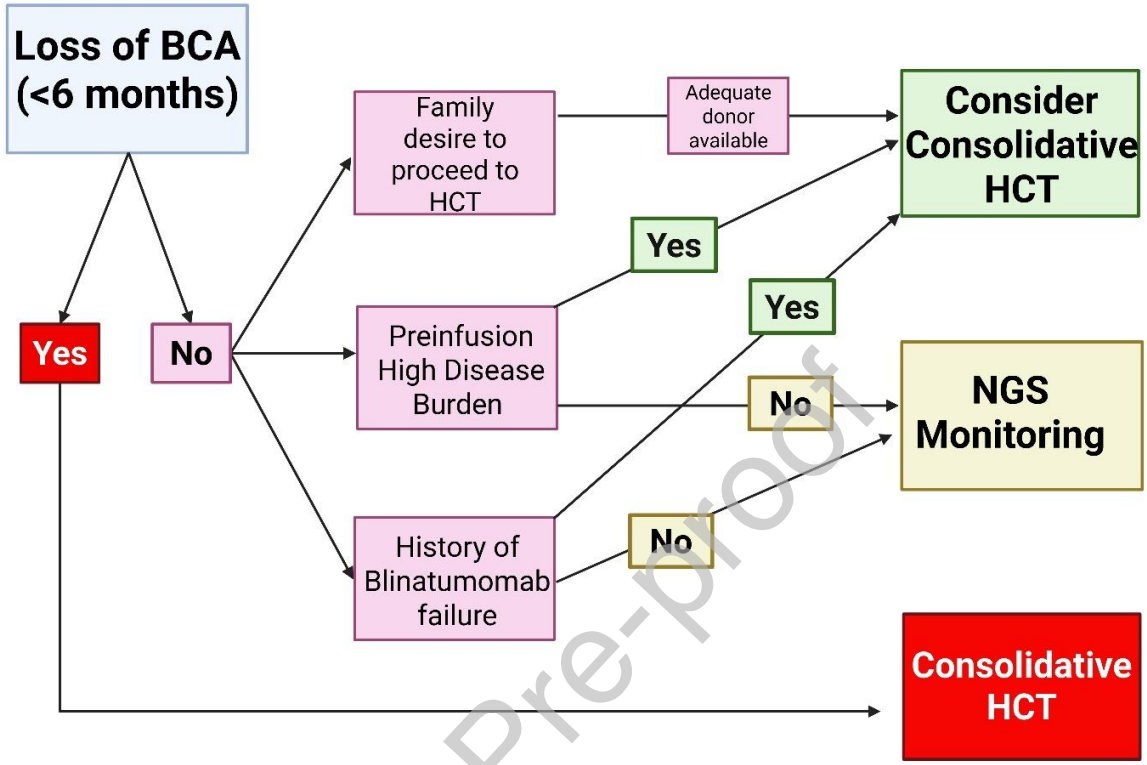

Figure 3 Algorithm based on loss of B-cell aplasia (BCA) within 6 months of infusion. Hematopoietic Cell Transplant (HCT), Next Generation Sequencing (NGS) Created in BioRender. Deimundo Roura, C. (2025) https://BioRender.com/lo22o1z

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1126 Table:

Risk Factors for CAR T-cell Therapy Failure	
High disease burden (≥5% bone marrow blasts)	CAR-MA studies (N =420)(10,45): HD burden (≥5% bone marrow blasts) was associated with inferior EFS, RFS, and OS. HD burden was independently associated with worse EFS (HR 2.5, P < .001) by multivariable analysis, and specifically associated with a higher cumulative incidence of CD19− relapse (HR 5.2, P < .001). CHOP clinical trials: in a trial of tisa-cel (N = 70)(9), patients with HD burden (>40% blasts) had inferior 24-mo EFS (34% vs 78%) and OS (60% vs 92%) compared with LD burden. In a trial of humanized CD19 CAR (N = 74), HD burden was associated with inferior RFS(145). PRWCC study(146) (N = 185): patients with HD burden (≥5% bone marrow blasts) had lower 12-mo EFS (31% vs 70%, P < .0001) and OS (58% vs 85%, P <.0001) compared with LD burden. HD burden was independently associated with OS by multivariable analysis (HR 5.1, P = .002). St Jude and JHU study(147) (N = 30): HD burden (≥5% bone marrow blasts) was independently associated with inferior EFS (HR 6.0, P = .038) and OS (HR 4.2,P = .015). Robert Debre and Saint Louis University Hospitals study (148)(N = 51): HD burden (≥1% bone marrow blasts) was associated with a higher cumulative incidence of CD19− relapse (SHR 10.4, P = .03) in a competing risks analysis.
Non-response to blinatumomab	CAR-MA study (N = 420): blinatumomab non-responders had lower CR rates to CD19 CAR T cells and worse 6-mo EFS (CR, 65%; EFS, 27%) than blinatumomab responders (CR, 93%; EFS, 67%) or blinatumomab-naïve patients (CR, 94%; EFS, 73%). (10) CHOP study (N = 166): composite outcome of NR, CD19− MRD/relapse was more frequent in blinatumomab-exposed patients.(149)

	Robert Debre and Saint Louis University Hospitals study(148) (N = 51): prior blinatumomab was associated with early CAR failure (P = .01), increased CIR (HR 2.6), and shorter EFS (HR 3.0) and OS (HR 5.5).
Short CAR persistence (loss of BCA)	Pooled ELIANA/ENSIGN analysis (N = 143): loss of BCA within 1 y was associated with increased relapse risk (HR 4.5, P < .001). Patients with loss of BCA within 6 mo had a 24-mo EFS of 14%.(14) Seattle PLAT-02 trial32 (N = 45): loss of BCA was associated with increase relapse risk (HR 3.5, P = .04).(6) CHOP humanized CD19 CAR T-cell trial (N = 74): when treated as a time varying covariate, B-cell recovery was associated with worse RFS (P = .011).(145)
Cell dose	PRWCC (n=185) OS, EFS, and RFS were improved in patients who received higher doses of tisa-cel (P = .031, .0079, and .0045, respectively) without increasing toxicity profile (17)
Timing post HCT	Real world data form Germany (N=81): relapsing within 6 months of allo-HCT pEFS of 18.4% (pOS = 16.0%); the pEFS for those relapsing later was 55.5% (pOS = 74.8%) (18)
Inadequate dose of fludarabine	PRWCC study (N = 152): suboptimal fludarabine exposure, defined as AUC <13.8 mg × h/L and estimated by a validated population pharmacokinetic model, was associated with a higher CIR (HR 2.5, P = .005) and higher risk of a composite end point of relapse or loss of BCA (HR 2.0, P = .01) compared with optimal fludarabine exposure.(20) Princess Maxima study (N = 26): a cumulative fludarabine AUC <14 mg × h/L was associated with a higher frequency of CD19+ relapse within 1 y (100% vs 27%, P = .0001) and probability of losing BCA within 6 mo (77% vs 37%, P =.009) than AUC >14 mg × h/L.(150)

Table 1 Risk for CAR T-cell Therapy Failure. Area under the curve (AUC), Children's Hospital of Philadelphia (CHOP), John Hopkins University (JHU), detectable minimal residual disease by next generation sequencing, (NGS-MRD), St Jude Children's Research Hospital (St Jude), Pediatric Real World CAR Consortium (PRWCC)

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