haematologica
Journal of the Ferrata Storti Foundation

Approaches for bridging therapy prior to chimeric antigen receptor T cells for relapsed/refractory acute lymphoblastic B-lineage leukaemia in children and young adults

by Tobias Feuchtinger, Peter Bader, Marion Subklewe, Maike Breidenbach, Semjon Willier, Markus Metzler, Nicola Gökbuget, Julia Hauer, Fabian Müller, Paul-Gerhard Schlegel, Michael Frühwald, Christoph Schmid, Anja Troeger, Claudia Baldus, Roland Meisel, Annette Künkele, Max Topp, Jean-Pierre Bourquin, Gunnar Cario, Arend von Stackelberg, and Christina Peters

Received: June 20, 2023.
Accepted: February 9, 2024.

Citation: Tobias Feuchtinger, Peter Bader, Marion Subklewe, Maike Breidenbach, Semjon Willier, Markus Metzler, Nicola Gökbuget, Julia Hauer, Fabian Müller, Paul-Gerhard Schlegel, Michael Frühwald, Christoph Schmid, Anja Troeger, Claudia Baldus, Roland Meisel, Annette Künkele, Max Topp, Jean-Pierre Bourquin, Gunnar Cario, Arend von Stackelberg, and Christina Peters. Approaches for bridging therapy prior to chimeric antigen receptor T cells for relapsed/refractory acute lymphoblastic B-lineage leukaemia in children and young adults.
Haematologica. 2024 Feb 15. doi: 10.3324/haematol.2023.283780 [Epub ahead of print]

Approaches for bridging therapy prior to chimeric antigen receptor T cells for relapsed/refractory acute lymphoblastic B-lineage leukaemia in children and young adults

Authors: Tobias Feuchtinger,[1,2,3] Peter Bader,[4] Marion Subklewe,[2,5] Maike Breidenbach,[1,2] Semjon Willier,[1,3] Markus Metzler,[2,6] Nicola Gökbuget,[7] Julia Hauer,[2,8] Fabian Müller,[2,9] Paul-Gerhardt Schlegel,[2,10] Michael Frühwald,[2,11] Christoph Schmid,[2,12] Anja Troeger,[2,13] Claudia Baldus,[14] Roland Meisel,[15] Annette Künkele,[16] Max Topp,[2,17] Jean-Pierre Bourquin,[18] Gunnar Cario,[19]Arend von Stackelberg,[16] and Christina Peters[20]

Affiliations: [1] Department of Paediatric Haematology, Oncology, Hemostaseology and Stem Cell Transplantation, Dr. von Hauner Children's Hospital, University Hospital, LMU Munich, Germany; [2] Bavarian Cancer Research Center (BZKF), R/R ALL Study Group; [3] Department of Pediatrics and Adolescent Medicine, Division of Pediatric Hematology and Oncology, Medical Center, Faculty of Medicine, University of Freiburg, Freiburg, Germany; [4] Goethe University, University Hospital, Department for Children and Adolescents, Division for Stern Cell Transplantation, Immunology and Intensive Care, Frankfurt, Germany; [5] Department of Medicine III, University Hospital, LMU Munich, Munich, Germany; [6] Department of Pediatrics and Adolescent Medicine, University Hospital Erlangen, Erlangen, Germany; [7] Department of Medicine II, Haematology/Oncology, Goethe University, Frankfurt, Germany; [8] Department of Pediatrics and Children's Cancer Research Centre, TUM School of Medicine, Children's Hospital Munich Schwabing, Technical University of Munich, Munich, Germany; [9] Department of Internal Medicine 5, Haematology and Oncology, University Hospital of Erlangen, Friedrich- Alexander University of Erlangen-Nuremberg (FAU), Erlangen, Germany; [10] University Children's Hospital Wuerzburg, Wuerzburg, Germany; [11] Pediatrics and Adolescent Medicine, Swabian Children's Cancer Center, University Medical Center Augsburg, Augsburg, Germany; [12] Department of Internal Medicine, University Medical Centre Augsburg, Augsburg, Germany; [13] Department of Paediatric Haematology, Oncology and Stem Cell Transplantation, University of Regensburg, Regensburg, Germany; [14] Department of Internal Medicine II, University Hospital Schleswig-Holstein, Kiel, Germany; [15] Division of Paediatric Stern Cell Therapy, Department of Paediatric Oncology, Haematology and Clinical Immunology, Medical Faculty, Heinrich-Heine- University, Duesseldorf, Germany; [16] Charite-Universitätsmedizin Berlin, corporate member of Freie Universität Berlin and Humboldt Universität zu Berlin, Department of Paediatric Oncology and Haematology, Berlin, Germany; [17] Department of Medicine II, University Hospital of Wuerzburg, Wuerzburg, Germany; [18] Department of Oncology, University Children's Hospital Zürich, Zürich, Switzerland; [19] Department of Pediatrics, University Medical Center Schleswig-Holstein, Campus Kiel, Germany, Kiel, Schleswig-Holstein, Germany;[20] St. Anna Children's Hospital, St. Anna Children's Research Institute, Medical University Vienna, Vienna, Austria

Keywords: B-ALL, CAR T cells, bridging therapy

Running title: Guidance pre-CAR T bridging chemotherapy R/R ALL

Acknowledgements: This work was supported by the Bavarian Cancer Research Centre.

Author Contributions: All authors participated in the consensus process. The manuscript was written by TF and reviewed by all authors.

Declaration of Interests: TF received Research Support from Miltenyi Biotec and Servier. The remaining authors declare no competing interests. This manuscript contains description with off-label use of drugs.

Data-sharing statement: The authors make the original data and protocols of the guidance process available to other investigators without unreasonable restrictions and can be requested from the corresponding author.

Word count: Abstract n= 173 and the main text n=5000. Two figures and two tables.

Current address for correspondence:
Tobias Feuchtinger MD, Department of Pediatrics and Adolescent Medicine, Division of Pediatric Hematology and Oncology, Medical Center, Faculty of Medicine, University of Freiburg, Freiburg, Germany
Mathildenstrasse 1, 79106 Freiburg
Phone: +49 89 4400 57945 Fax: +49 89 4400 54719
Email: tobias.feuchtinger(at)uniklinik-freiburg.de

Abstract

The ongoing development of immunotherapies, including chimeric antigen receptor (CAR) T cells, has revolutionized cancer treatment. In paediatric relapsed/refractory B-lineage acute leukaemia antiCD19-CARs induced impressive initial response rates, with event-free survival plateauing at 30-50% in long-term follow-up data. During the interval between diagnosis of relapse or refractoriness and CAR T cell infusion, patients require a bridging therapy. To date, this therapy has consisted of highly variable approaches based on local experience. Here, in an European collaborative effort of paediatric and adult haematologists, we summarise current knowledge with the aim of establishing a guidance for bridging therapy. This includes treatment strategies for different patient subgroups, the advantages and disadvantages of low- and high-intensity regimens, and the potential impact of bridging therapy on outcome after CAR T cell infusion. This guidance is a step towards a cross-institutional harmonization of bridging therapy, including personalized approaches. This will allow better comparability of clinical data and increase the level of evidence for the treatment of children and young adults with relapsed/refractory B-lineage ALL until CAR T cell infusion.

Introduction

Treatment of children and young adults with relapsed/refractory (r/r) B-lineage acute lymphoblastic leukaemia (B-ALL) remains a challenge (1). Despite significant progress, relapse remains the leading cause of treatment failure while primary resistant ALL is relatively rare in children and young adults. The development of gene engineered T cells with a chimeric antigen receptor (CAR) has revolutionized cancer immunotherapy (2). In recent years, treatment regimens for patients with r/r B-ALL have improved significantly, particularly with the approval of antiCD19-CAR T cell therapy by the FDA and EMA. Initial response rates to antiCD19-CAR T cell therapy are high (3, 4). However, the majority of patients relapse following CAR T cell treatment with a 5-year event-free survival (EFS) of 30-50 % in paediatric cohorts and approximately 20% in adult cohorts (5-8).

CAR T cell manufacturing may take several weeks before start of lymphodepleting chemotherapy and the subsequent infusion of CAR T cells. Anti-leukemic treatment during this period can be subdivided into a first phase until leukapheresis and a second phase ("bridging therapy") until start of lymphodepleting therapy (Figure 1). Especially prior to leukapheresis, treatment should not reduce T cell fitness. The optimal bridging therapy has not yet been defined and varies between centres and clinical protocols. To date, it has mainly been based on centre-specific experiences and often every single patient received an individualized protocol. Harmonization is urgently needed to further improve evidence of these treatments. However, personalized approaches based on prior therapy, pre-existing disease burden and responsiveness to single agents, are important considerations in the management of this patient cohort. Potentially high rates of prior organ toxicities in this heavily pre-treated and often refractory

patient population have further diversified bridging therapy (9). In this report, we will balance the need for harmonization with the requirement of personalized approaches.

A growing number of reports emphasize the importance of bridging therapy across different age groups (10). Recent data have highlighted that a lower disease burden at time of CAR T cell infusion correlates with improved long-term survival (11, 12). The beneficial effect of low disease burden may be a result of disease biology or a result of effective bridging therapy. In addition, high disease burden is associated with increased treatment-related complications, such as cytokine release syndrome (CRS) and immune effector cell-associated neurotoxicity syndrome (ICANS) (13, 14). Patients may therefore benefit from an effective reduction in disease burden prior to CAR T cell therapy. However, recent studies in adults and smaller paediatric cohorts have shown that high intensity bridging chemotherapy is associated with an increased incidence of infectious complications without improving long-term outcomes (9, 15, 16). Compared to bridging prior to allogeneic hematopoietic stem cell transplantation (HSCT), where studies on prognostic parameters for outcome have been conducted over decades (17, 18), the evidence for bridging before to CAR T cell therapy is sparse and inconsistent. Future prospective trials will need to demonstrate the relevance of MRD and other prognostic parameters for outcome after CAR T cell therapy.

In this report, we review the bridging treatment strategies and clinical outcomes in children and young adults with r/r B-ALL, treated with CD19-specific CAR T cells and define consensus statements to harmonize approaches in the future. There will be no “one size fits all” in bridging therapy, but steps towards harmonization will strengthen the analysis of registry data, improve evidence and enable consecutive prospective clinical trials.

Methods

In a collaborative effort of paediatric and adult haematologists, we summarize current knowledge with the aim of establishing a guidance for bridging therapy, as a step towards cross-institutional harmonization of bridging therapy. The guidance text is written with the aim to allow better comparability of clinical data and increase the level of evidence for the treatment of children and young adults with relapsed/refractory B-lineage ALL until CAR T cell infusion.

The methodological approach used to set up this guidance and to reach a consensus, included several steps: First, approved centres for CAR T cell therapies in Austria, Switzerland and Germany were contacted and asked to participate in a process leading to a guidance. Second, a consensus workshop was organized to discuss the topics of treatment strategies for different patient subgroups, the advantages and disadvantages of low-intensity and high-intensity regimens, personalized, vs uniform strategies, timing of approaches and the potential impact of bridging therapy on outcome after CAR T cell infusion. A systematic literature search was performed before and after the workshop using PubMed. The results of the workshop were shared in writing among the co-authors to define a consensus wording.

Aims of bridging therapy

Bridging therapy aims to control leukaemia burden until pre-CART lymphodepletion. To date, there are not enough conclusive results from prospective clinical trials to clearly define the aim of bridging therapy regarding long-term outcome. However, an increasing number of non-randomised controlled clinical trials and large real-world registry studies have provided data to make recommendations. High tumour burden has been shown to be associated with increased

risk of cytokine-release-syndrome (13, 14, 19). In addition, several phase II trials and recent real-world data have shown that morphological remission (< 5% blasts in the bone marrow) is associated with a significantly improved outcome in terms of event-free survival (EFS) and overall survival (OS) (12). In comparison to allogeneic hematopoietic stem cell transplantation (HSCT) (17, 18), negative minimal residual disease (MRD) status prior to CAR T cell therapy has not yet been shown to improve long-term outcome compared to patients starting with morphological remission alone (12). For patients that start bridging therapy with a low disease burden, the aim of therapy is rather prevention of disease progression or maintenance of disease at low-burden.

Bridging therapy primarily aims at achieving a low leukaemia burden (BM < 5% blasts) before starting lymphodepletion without severe toxicity (B4 Evidence).

Timing and duration of bridging therapy

Start of bridging therapy is guided by the label of the CAR products, that usually require cytomorphological evidence of relapse/nonremission and consecutive scheduling of an apheresis. Starting bridging therapy in a situation of increasing MRD appears to be reasonable, but evidence is currently lacking. The duration of bridging therapy has historically depended on the duration of CAR T cell manufacturing. Manufacturing that included cryopreservation and shipping, required a duration of roughly 6 weeks up to several months, whereas fresh manufacturing required 10-14 days. Recent improvement in manufacturing protocols have shortened these durations (20, 21). A short bridging therapy has been preferred based on data comparing one or two cycles of bridging, each approx. 4-6 weeks (9). However, the counterbalance between achieving remission (see above) and reducing the number of treatment

cycles has not been clarified. Furthermore, the published preference for one cycle contains a potential bias, due to the selection of chemosensitive patients. Based on the limited data in the literature and expert recommendations, the authors suggest one cycle of bridging therapy, if possible, a preferred duration of no more than two cycles and an earlier discontinuation of bridging therapy if a remission is achieved.

Start of bridging is defined by the label of the CAR product (cytomorphological relapse/nonremission). Duration of bridging therapy as short as remission is achieved (preferentially one cycle) and up to 12 weeks (D Evidence).

Treatment prior to apheresis

Treatment for control of leukaemia prior to leukapheresis is usually not considered "bridging therapy", although it is part of the window between the decision to plan a CAR T cell treatment and the start of the lymphodepleting therapy. Treatment prior to apheresis is particularly challenging, as lymphotoxic agents should not harm T cells that will be used to generate of the CAR T cells product. The number and functionality of lymphocytes in peripheral blood may be low due to leukemic growth that crowds out physiological haematopoiesis, as well as from the therapy itself. Ideally, any anti-leukemic therapy should be preceded by the collection of T cells to generate a CAR product with optimal T cell expansion capacities (22, 23). This may be feasible even in the presence of overt relapse, although inadvertent B-cell transduction has been described as a rare event (24).

Acceptance criteria for apheresis are defined in most protocols as a threshold of peripheral blood T cell counts >150-200/µL. In an analysis of more than one hundred procedures (age 1-25 years),

the median reported absolute lymphocyte count ranged from 142-6944 cells/µL (25). Although 21.6% had absolute lymphocyte counts less than 500 cells/µL, the mononuclear cell target was obtained in 100% of all apheresis harvests and T-lymphocyte collection efficiency was 83.4%, with only 9.8% minor adverse events (25). Elevated blast counts in peripheral blood have been described to interfere with successful apheresis and CAR production (26, 27), although the association between high blasts counts and lower $CD3^+$ cell collection yields, may be due to lower numbers of circulating T cells. The threshold of peripheral blasts, below which apheresis is acceptable, has yet to be defined. In such patients, apheresis can be planned after an initial cycle of cytoreductive treatments. If the T cell count is too low or T cell collection is not feasible, chemotherapy should be administered to debulk leukaemia burden, with the aim of performing apheresis in an interval during bridging therapy (28). Timing of therapy prior to apheresis has to consider the wash-out calculation of the applied regimen. Most protocols define a threshold of absolute lymphocyte counts (ALC) >300-500/µL to achieve a successful collection by processing 3-4x total blood volume. In some protocols an ALC of 200/µL is also accepted. CD3 counts should be >150-200/µL, but >100/µL CD3 is also accepted. However, these pragmatic recommendations are mostly derived from unpublished local feasibility studies.

In case that a sufficient lymphocyte number could not be achieved for apheresis, or relapse very early after HSCT, an allogeneic CAR product from a healthy donor is an emerging approach. Recent reports have described the CAR production from the stem cell donor (29). In case of a sufficient chimerism after HSCT, the CAR product would be infused into a syngeneic immune system. In a meta-analysis, 16 studies with 220 patients have been identified with a low GvHD rate (30). However, no commercial product has been authorized yet. Preclinical research also included the development of universal of-the-shelf allogeneic CAR T cells using advanced

engineering approaches (31). Initial clinical data however also showed that challenges of this approach still remain (32). In all allogeneic CAR approaches, bridging therapy would start up front from the day of eligibility to CAR T cell therapy.

Apheresis should be performed as early as possible before or during bridging therapy. Wash-out of lymphotoxic agents must be considered. Currently used thresholds are either CD3 >150-200/µL or ALC of >300-500/µL (Evidence level based on expert opinion).

Patient subgroups

Currently anti-CD19 CAR T cells are approved for r/r ALL. This includes a heterogeneous patient population with primary refractory disease, ≥ 2nd relapse and relapse after HSCT, patients from different 1st-line protocols and different numbers of prior lines of therapies. This heterogeneity raises the question of whether one single approach can be applied to all subgroups or if each of these different subgroups require a separate bridging regimen. Based on the available data, a harmonized approach could be recommended for most relapse patients (D Evidence), as the available reports do not support a strategy of different approaches in subgroups (6, 33). Furthermore, patients under 18 years of age (including infant leukaemia) and those over 18 years of age could be treated with the same bridging therapy and do not require separate approaches. In Europe most younger adults with ALL receive a BFM-like intensive initial treatment protocols, comparable to standard paediatric protocols in adolescents. In the US, many centres use hyperCVAD as a backbone (34). In elderly patients dosing is usually adjusted and choice of bridging therapies may differ due to different pre-treatment toxicity-profiles. Despite molecular heterogeneity in the disease cohort in terms of aggressiveness (e.g. TCF3-PBX1,

ETV6-RUNX1), we have not been able to provide evidence that specific molecular subgroups require a different bridging approach.

Exemptions to a harmonized bridging approach arise from several specific considerations: First, in patients that show progressive disease under first-line therapy, a bridging regimen with these same elements of standard induction, is unlikely to achieve a remission. In these rare patients, a higher-intensity regimen or 2nd-line therapy with agents different from the previous line is reasonable. Second, patients who relapse within 100 days of HSCT are particularly vulnerable to haematological toxicity and may require even more reduced low-intensity regimens (35). Third, radiotherapy of extramedullary manifestation of relapsed ALL may be used for disease control.

Patients who relapse after stem cell transplantation or with second or later relapse can receive the same bridging regimen. Early relapse after HSCT may require dose-reduction. Primary-refractory disease requires a change of treatment line with potentially required escalation of intensity to control disease (C3 Evidence).

Low-intensity versus high-intensity regimens

Many patients with r/r ALL are heavily pre-treated and prevention of toxicity is a high priority. Therefore, low-intensity regimens have been preferred over high-intensity regimens as bridging therapy (9). Patients receiving high-intensity regimens have a higher incidence of grade 3 - 4 infections and experience more toxicities than patients treated with low-intensity regimens. Furthermore, recent data have shown no superior results in MRD-negative patients compared to MRD-positive patients analysed prior to start of lymphodepletion (12).

The distinction between low-intensity and high-intensity regimens has not been properly defined. The term “low-intensity” refers to the combination of agents, not the dose and should not be mixed up with lower dosing of single agents. Although this definition may be arbitrary and lacks sound evidence, we consider it a valuable pragmatic approach. Nevertheless, for most regimens, there will be no controversy between high and low-intensity, but the boundary between “high” and “low” remains difficult to define. Even with an identical regimen, patients with different sensitivities to chemotherapy may experience very different durations of cytopenia. Alternative definitions such as “number of drugs per cycle or per week” or other systems could be proposed, but they also lack validation in clinical studies. A low-intensity regimen contains cycles of steroids, vincristine and asparaginase. Other options include low-dose cytarabine (75-100 mg/m^2 on four consecutive days) and agents used as maintenance therapy (e.g. oral 6-Mercaptopurine, Methotrexate). Anthracyclines as part of a bridging therapy are not considered a first choice due to their high toxic potential. The number of lines of prior therapy should be reviewed as part of the history when considering which is the appropriate bridging therapy to initiate. In some primary refractory patients, leukaemia has already shown to be refractory against the above mentioned low-intensity regimens and requires more personalized approaches.

Asparaginase lacks cumulative organ toxicity of conventional chemotherapy. This advantage is offset by its long in vivo activity against lymphocytes. As patients with r/r ALL are usually heavily pre-treated, asparaginase is an option for bridging therapy. According to the recommendation for e.g. tisagenlecleucel, pegylated asparaginase (peg-asparaginase) should be stopped at least 4 weeks before leukapheresis and also before CAR T cell infusion (36). Asparaginase is considered a potential component of low-intensity regimens, due to the definition of low/high-intensity mentioned above. However, it can also be associated with

serious complications of coagulopathy, thrombosis, pancreatitis, allergy and liver toxicity which may pose a risk for CAR therapy. Recommendations on asparaginase are based on the rational expert opinion only, as no controlled clinical trials are available yet.

If possible, one cycle of chemotherapy until CAR T cell administration should be preferred instead of two or more cycles to reduce the risk of serious infections. Morphologic remission is pursued, but complete regeneration of healthy haematopoiesis is not mandatory. CAR T cells should be administered as soon as possible after disease control (D Evidence).

Whenever possible, a low-intensity regimen should be preferred, such as a backbone with vincristine, dexamethasone or low-dose cytarabine. Recommendations for time intervals between different agents and leukapheresis/CAR infusion should be considered (D Evidence). Administration of peg-asparaginase during bridging therapy with at least 4 weeks wash-out, prior to CAR T cell therapy, could be considered due to its low cumulative organ toxicity (C3 Evidence).

Considerations on personalized approaches to individual patients in the context of prior lines of therapy, prior toxicities and disease burden

Responsiveness to prior lines of therapy is important for the choice of bridging regimen, especially in primary refractory disease. In patients with multiple relapses or relapse after HSCT, the number of prior lines of therapy has not yet been identified as a prognostic marker. Although there is no evidence available from clinical studies, it appears obvious that patients with progressive disease under a certain regimen, require a change of drug substances to achieve a remission.

Previous toxicities must be taken into account when choosing a bridging regimen (e.g. avoidance of VCR in patients with severe neuropathy). Especially uncontrolled viral and fungal infections serve as contraindications for high-dose and prolonged steroid treatment. Prevention of prolonged neutropenia is already addressed by the recommendation of a low-intensity bridging regimen (see above). Severe organ dysfunction (kidney, liver and heart) requires an individualized regimen, but case reports suggest, that even these are not insurmountable challenges for a therapy aimed at disease control (37).

Disease burden has been thought to be a relevant marker for prognosis in the past but not been proven in recent reports (9). However, "responsiveness to prior lines of therapy" appears to be more important, e.g. in case of a steroid responsive disease, high blast counts are usually manageable. Disease burden at the end of bridging therapy has been associated with a significantly improved outcome (see above) (12), whereas disease burden a start of bridging therapy has not yet been defined as a significant risk factor. Several reports have hypothesized, that starting bridging already at an MRD level, may lead to a beneficial outcome (38, 39) but sound evidence is still missing and bridging data are sparsely available.

A rationale for a personalized approach in bridging therapy is guided by responsiveness to prior lines of therapy and by severe prior toxicities (D Evidence).

Immunotherapy prior to CAR T cell therapy

Antibody-based immunotherapy in B-lineage has been a tremendous success, but in bridging therapy it carries a potential risk that administration prior to CAR T-cell infusion may jeopardize the expansion of CAR T cells in the absence of the target antigen. Blinatumomab is a bispecific

T cell engager (BiTE) that links CD3 expressed on T cells with CD19 on B cells/B-ALL cells, leading to direct targeting and destruction of $CD19^+$ leukaemia cells. In initial studies investigating anti-CD19 CAR T cell therapies, a prior anti-CD19 immunotherapy like blinatumomab was considered to be a risk factor for non-response to CAR T cells (11). Meanwhile, recent registry data have demonstrated similar rates of EFS in patients with prior blinatumomab treatment and those without (11). In contrast in another study among 51 patients with r/r BCP-ALL (median age 17 years) infused with tisagenlecleucel after lymphodepletion, prior blinatumomab was associated with an increased CIR, and a shorter EFS and OS (19). Therefore, the role of blinatumomab prior to CAR T cell therapy require further investigation.

Furthermore, patients with a history of CAR T cell treatment showed still relevant (but lower) response rates to bridging, second CAR T cell infusions and/or HSCT in small case series, even when the same CAR product was used (40). However, blinatumomab nonresponse and high-disease burden were independently associated with worse RFS and EFS following CD19-CAR therapy. Furthermore, patients with a diminished CD19 expression ($CD19^{dim}$), have a risk of immune escape and consecutive relapse rate is high (11). In case of high CD19 expression, blinatumomab can be given up to 4-6 weeks prior to CAR T cell infusion. For a strong functional CAR T cell response, recovery of $CD19^+$ cells should be present for in vivo CAR T cell activation and expansion. Systematic data on blinatumomab immediately prior to apheresis are not available. However, bi-specific T cell engagers such as blinatumomab can cause T cell exhaustion and an impact on functionality of the CAR T cell product remains to be investigated.

Inotuzumab ozogamicin (InO) is an anti-CD22 antibody conjugated to the cytotoxic antibiotic calicheamicin. InO is approved for first or subsequent relapse of CD22 expressing B-ALL in adults. The landmark phase III randomized study demonstrated superiority of InO in r/r B-ALL

over standard chemotherapy (41). In children and adolescents Phase I and II studies have proven the value of InO, with impressive responses reported for r/r leukaemia (42-44). Several reports on the sequential use of InO and CAR T cell infusion from Europe and the US show similar outcomes compared to r/r patients who did not receive InO. Since the number of reported cases is still low and outcome often reflects severity of disease, the use of InO for bridging therapy appears feasible but its role remains to be clarified. Of note, the severe depletion of B-lineage lymphocytes through the administration of InO did not prevent later CAR expansion in vivo (45-47).

Prior use of anti-B-cell-immunotherapy (e.g. blinatumomab) is not a contraindication for CAR T cell therapy. CD19 expression should be measured prior to CAR T cell administration (B4 Evidence). Sequential therapy with inotuzumab-ozogamicin and CD19 CARs appears feasible, but controlled trials are needed to clarify the evidence for long-term efficacy (C2 Evidence).

Intrathecal therapy

CAR T cells have been shown to migrate into the cerebrospinal fluid (CSF) (13). However, active central nervous system (CNS) disease has been a contraindication for CD19 CAR T cell therapy in clinical trials. In recent retrospective studies, CAR T-cells were found to be initially effective even in cohorts with CNS disease, though, high incidence of a subsequent CNS relapse has been reported,(48, 49). Therefore, most approaches included intrathecal (i.th.) chemotherapy until resolution of CNS disease. “Triple” therapy (ITT) with a combination of methotrexate, cytarabine and prednisolone was considered first choice in relapsed disease. Monotherapy or a combination of 2 agents has also been used in r/r patients. A combination of steroids with

cytarabine has been discussed, either because of prior irradiation of the CNS or because of a rational that MTX prior to apheresis may damage T cells. At initial diagnosis of ALL, post-induction CNS prophylaxis with ITT did not improve 5-year DFS for children with HR B-ALL compared to intrathecal monotherapy with MTX (50). In the absence of systematic data for r/r ALL, ITT remains the standard of care for CNS prophylaxis in r/r ALL.

Intrathecal therapy against active disease should be continued until CNS remission, preferentially 2x/week. CNS remission is achieved when 2 consecutive CSF samples are negative for leukemic blasts. In cases without active CNS disease, a prophylactic treatment with intrathecal chemotherapy is recommended at least every 2-4 weeks.

All patients with r/r-ALL should receive intrathecal (i.th.) chemotherapy prior to CAR T cell therapy. In case of active CNS disease, i.th. therapy should be continued (1-2x/week) until CNS remission (2 consecutive negative CSF samples). “Triple” therapy is first choice with methotrexate, cytarabine and prednisolone (B4 Evidence).

Molecular targeted therapy

Screening for druggable molecular targets in all patients with relapsed and refractory malignancies has become routine in most centres. Therefore, combining conventional chemotherapy agents with molecularly targeted therapies is an obvious rationale. However, patient-specific druggable molecular targets beyond ABL-class mutations or Philadelphia-like ALL are rare. In a multicentre phase I study, the combination of chemotherapy with the BCL-2 inhibitor venetoclax and the BCL-XL/BCL-2 inhibitor navitoclax was well-tolerated and showed promising results in r/r B-ALL (51). In relapsed Ph+ B-ALL, leukaemia cells should be first

analysed for the presence of ABL1 kinase domain mutations. Treatment decisions and choice of a tyrosine kinase inhibitor (TKI) could then be based on these results. Dasatinib and nilotinib are both second-generation TKIs that have shown activity against r/r Ph+ ALL in early-phase clinical trials (52, 53). In addition, ponatinib is a third-generation TKI that also inhibits the T315I mutation seen in the Abl kinase domain (54). Of note, the combination of chemotherapy with small molecules requires special awareness due to the increased risk for bone marrow toxicity with prolonged cytopenia and subsequent serious infections. As kinase inhibitors may have potential impact on CAR T cell activation and expansion, they should be washed out and caution is appropriate to use it continuously before and after CAR T infusion (55).

Targeted therapy could be used in addition to bridging after apheresis based on specific mutations in the individual patient but can lead to prolonged cytopenia (C3 Evidence).

Wash out before CAR T cell infusion

Almost all drugs against ALL have a relevant toxicity against healthy lymphocytes (on-target-off-tumour effect). In order to achieve production of sufficient and high-quality CAR T cells which show optimal in vivo expansion, wash-out periods must be considered during bridging therapy. Based on the pharmacokinetics of the agents and small case series, an expert recommendation could be as follows: therapeutic systemic doses of steroids should be stopped > 72 hours prior to CAR T cell infusion (physiological replacement of hydrocortisone is continued if necessary). Medications with 6-thioguanine or intrathecal methotrexate should be stopped at least 1 week prior to CAR T cell infusion. Anthracyclines, cytarabine and methotrexate should be stopped at least 2 weeks prior to CAR infusion. Longer cessation is required for asparaginase (see below), antibodies used as serotherapy in HSCT such as antithymocyte globulin (ATG) or

alemtuzumab and all chemotherapy that is particularly toxic to T cells. These T-cell lytic agents include clofarabine and others and should also be used with caution due to long wash-out times before apheresis and CAR infusion as well. Manuals for commercial CAR products recommend 8 weeks wash-out. However, these recommendations are mainly based on rationales or expert opinion and not on evidence from clinical trials.

Clinical trials

Patients with multiple relapses or those who have failed multiple prior lines of therapies represent a need for novel experimental therapies. In recent years several new agents have been described that are typically tested in early clinical trials targeting refractory disease. However, early clinical trials are often limited to the use of single-agent therapies. Due to the extensive pre-treatment with combinatorial multi-drug regimens, monotherapy carries a relevant risk of non-response. The level of evidence for bridging therapy is generally limited due to the rarity of the disease and the consecutive small patient cohorts. For this reason, enrolment in early clinical trials is strongly recommended. A careful balance between patient safety and innovation is therefore essential.

Inclusion of patients into phase-I/II-studies is encouraged. Single-agent regimens for bridging chemotherapy are not recommended.

Conclusion

Currently, there are no evidence-based treatment recommendations available for bridging therapy in r/r ALL prior to CAR T cell therapy. The level of evidence is not yet strong enough to

define a single first-line bridging therapy. Here, we summarize the available data in order to harmonize the inconsistent approaches across centres with rationales at the level of expert recommendation and some B and C evidence. Although harmonization is needed, a single treatment regimen will not be applicable because individual patients present with a wide range of pre-treatments and different history of responses to therapy. Therefore, this guidance does not define a "gold standard", but will help centres to improve the evidence for clinical decisions.

First choice is one cycle of low-intensity regimen to induce morphological remission, i.e. < 5% blasts in bone marrow. A backbone treatment may include vincristine, dexamethasone, asparaginase plus targeted therapy if available (Figure 2). In case of high CD19 expression, blinatumomab can be given up to 4-6 weeks prior to CAR T cell infusion, to allow a recovery of $CD19^+$ cells that play a role for CAR T cell activation and expansion. If relapse occurs within 100 days after HSCT, a significant dose reduction of chemotherapy is recommended. Inclusion into clinical studies is encouraged but single-agent bridging is not recommended.

Overall, bridging therapy is an area with an urgent need for better evidence from clinical trials. Beyond conventional generation of evidence, novel approaches such as drug response profiling using functional precision oncology, may further improve the safety and efficacy profile for r/r patients. This includes testing of patient-derived ALL blasts with drugs to identify vulnerabilities and novel combinations (56).

This guidance may help to streamline the diversity of approaches to a small number of rational regimens, enable improved analysis of registry data and support the set-up of prospective clinical trials. Increased evidence from clinical studies is needed in the future.

Themes

Definitions

- Bridging therapy is defined as anti-leukemic treatment between leukapheresis and start of lymphodepleting therapy prior to CAR T cell infusion. Treatment prior to leukapheresis is considered separately in this report.
- Assessment of evidence level relies on the NCI levels of evidence for adult and paediatric cancer treatment studies and is described in detail in Table 1.
- The definition of relapse in relapsed/refractory diseases is used in accordance with the labelling of CAR T cell therapies: Second or higher relapse and first relapse after HSCT are considered r/rALL. First relapse without prior HSCT is currently not an in-label indication for CARs and is therefore not included into the definition of r/r ALL.
- Primary refractory disease among r/r diseases is defined as non-remission after end of consolidation therapy, as applied and defined in the current paediatric protocols of AIEOP-BFM, ALLtogether, COG and others. In protocols designed primarily for adults, end of first consolidation is defined as primary refractory disease, such as in GMALL protocols.
- The term “disease burden” is often used in the field of CAR T cell therapy. However, there is no common definition in ALL. It usually refers to the percentage of blast cells in the bone marrow. The definitions for “high disease burden” range from ≥5% blasts (12), or ≥40% (57) to ≥50% blasts (58) and “low disease burden” respectively. Some reports, also consider the percentage of blasts in peripheral blood as “disease burden”. For extramedullary disease, the term is not specified.

- Remission of ALL is defined as <5% blast on cytomorphological analysis of bone marrow aspirate smears.

- Minimal residual disease (MRD) in bone marrow is detected by either IG/TCR real-time PCR, fusion gene transcript assessment, next-generation sequencing, or flowcytometry as described (59). A detection threshold of at least 10^{-4} is currently required to define MRD negativity.

- Toxicity grading of chemotherapy and immunotherapy is performed according to the Common Terminology Criteria for Adverse Events (CTCAE; Version 5.0; published: November 27, 2017; https://ctep.cancer.gov/protocoldevelopment/electronic_applications/docs/ctcae_v5_ quick _reference_5x7.pdf).

- Cytokine release syndrome (CRS) is a potentially life-threatening toxicity that has been observed following immunotherapies for cancer. CRS is associated with elevated circulating levels of inflammatory cytokines. The definition of CRS is based on widely used grading systems (60, 61).

- Immune effector cell-associated neurotoxicity syndrome (ICANS) may manifest as encephalopathy, tremor, seizures and headache. The definition of ICANS is based on published criteria (62). The understanding of clinical risk factors and pathophysiology is rapidly improving (63, 64).

- This consensus statement discusses high-intensity and low-intensity bridging therapy. The intensity of bridging therapy is as variable as the number of different regimens that have been used. High-intensity has recently been defined as myelosuppression > 7 days (9).

References:

1. Sun W, Malvar J, Sposto R, et al. Outcome of children with multiply relapsed B-cell acute lymphoblastic leukemia: a therapeutic advances in childhood leukemia & lymphoma study. Leukemia. 2018;32(11):2316-2325.

2. Sadelain M. CD19 CAR T Cells. Cell. 2017;171(7):1471.

3. Maude SL, Frey N, Shaw PA, et al. Chimeric antigen receptor T cells for sustained remissions in leukemia. N Engl J Med. 2014;371(16):1507-1517.

4. Brentjens RJ, Davila ML, Riviere I, et al. CD19-targeted T cells rapidly induce molecular remissions in adults with chemotherapy-refractory acute lymphoblastic leukemia. Sci Transl Med. 2013;5(177):177ra138.

5. Laetsch TW, Maude SL, Rives S, et al. Three-Year Update of Tisagenlecleucel in Pediatric and Young Adult Patients With Relapsed/Refractory Acute Lymphoblastic Leukemia in the ELIANA Trial. J Clin Oncol. 2023;41(9):1664-1669.

6. Maude SL, Laetsch TW, Buechner J, et al. Tisagenlecleucel in Children and Young Adults with B-Cell Lymphoblastic Leukemia. N Engl J Med. 2018;378(5):439-448.

7. Park JH, Riviere I, Gonen M, et al. Long-Term Follow-up of CD19 CAR Therapy in Acute Lymphoblastic Leukemia. N Engl J Med. 2018;378(5):449-459.

8. Shah BD, Ghobadi A, Oluwole OO, et al. KTE-X19 for relapsed or refractory adult B-cell acute lymphoblastic leukaemia: phase 2 results of the single-arm, open-label, multicentre ZUMA-3 study. Lancet. 2021;398(10299):491-502.

9. Shahid S, Ramaswamy K, Flynn J, et al. Impact of Bridging Chemotherapy on Clinical Outcomes of CD19-Specific CAR T Cell Therapy in Children/Young Adults with Relapsed/Refractory B Cell Acute Lymphoblastic Leukemia. Transplant Cell Ther. 2022;28(2):72.

10. Ghorashian S, Jacoby E, De Moerloose B, et al. Tisagenlecleucel therapy for relapsed or refractory B-cell acute lymphoblastic leukaemia in infants and children younger than 3 years of age at screening: an international, multicentre, retrospective cohort study. Lancet Haematol. 2022;9(10):e766-e775.

11. Myers RM, Taraseviciute A, Steinberg SM, et al. Blinatumomab Nonresponse and High-Disease Burden Are Associated With Inferior Outcomes After CD19-CAR for B-ALL. J Clin Oncol. 2022;40(9):932-944.

12. Schultz LM, Baggott C, Prabhu S, et al. Disease Burden Affects Outcomes in Pediatric and Young Adult B-Cell Lymphoblastic Leukemia After Commercial Tisagenlecleucel: A Pediatric Real-World Chimeric Antigen Receptor Consortium Report. J Clin Oncol. 2022;40(9):945-955.

13. Lee DW, Kochenderfer JN, Stetler-Stevenson M, et al. T cells expressing CD19 chimeric antigen receptors for acute lymphoblastic leukaemia in children and young adults: a phase 1 dose-escalation trial. Lancet. 2015;385(9967):517-528.

14. Turtle CJ, Hanafi LA, Berger C, et al. CD19 CAR-T cells of defined CD4+:CD8+ composition in adult B cell ALL patients. J Clin Invest. 2016;126(6):2123-2138.

15. Perica K, Flynn J, Curran KJ, et al. Impact of bridging chemotherapy on clinical outcome of CD19 CAR T therapy in adult acute lymphoblastic leukemia. Leukemia. 2021;35(11):3268-3271.

16. Pinnix CC, Gunther JR, Dabaja BS, et al. Bridging therapy prior to axicabtagene ciloleucel for relapsed/refractory large B-cell lymphoma. Blood Adv. 2020;4(13):2871-2883.

17. Bader P, Kreyenberg H, Henze GH, et al. Prognostic value of minimal residual disease quantification before allogeneic stem-cell transplantation in relapsed childhood acute lymphoblastic leukemia: the ALL-REZ BFM Study Group. J Clin Oncol. 2009;27(3):377-384.

18. Peters C, Dalle JH, Locatelli F, et al. Total Body Irradiation or Chemotherapy Conditioning in Childhood ALL: A Multinational, Randomized, Noninferiority Phase III Study. J Clin Oncol. 2021;39(4):295-307.

19. Dourthe ME, Rabian F, Yakouben K, et al. Determinants of CD19-positive vs CD19-negative relapse after tisagenlecleucel for B-cell acute lymphoblastic leukemia. Leukemia. 2021;35(12):3383-3393.

20. Ghassemi S, Durgin JS, Nunez-Cruz S, et al. Rapid manufacturing of non-activated potent CAR T cells. Nat Biomed Eng. 2022;6(2):118-128.

21. Blache U, Popp G, Dunkel A, Koehl U, Fricke S. Potential solutions for manufacture of CAR T cells in cancer immunotherapy. Nat Commun. 2022;13(1):5225.

22. Das RK, Vernau L, Grupp SA, Barrett DM. Naive T-cell Deficits at Diagnosis and after Chemotherapy Impair Cell Therapy Potential in Pediatric Cancers. Cancer Discov. 2019;9(4):492-499.

23. Singh N, Perazzelli J, Grupp SA, Barrett DM. Early memory phenotypes drive T cell proliferation in patients with pediatric malignancies. Sci Transl Med. 2016;8(320):320ra3.

24. Ruella M, Xu J, Barrett DM, et al. Induction of resistance to chimeric antigen receptor T cell therapy by transduction of a single leukemic B cell. Nat Med. 2018;24(10):1499-1503.

25. Ceppi F, Rivers J, Annesley C, et al. Lymphocyte apheresis for chimeric antigen receptor T-cell manufacturing in children and young adults with leukemia and neuroblastoma. Transfusion. 2018;58(6):1414-1420.

26. Allen ES, Stroncek DF, Ren J, et al. Autologous lymphapheresis for the production of chimeric antigen receptor T cells. Transfusion. 2017;57(5):1133-1141.

27. Myers RM, Shah NN, Pulsipher MA. How I use risk factors for success or failure of CD19 CAR T cells to guide management of children and AYA with B-cell ALL. Blood. 2023;141(11):1251-1264.

28. Kansagra AJ, Frey NV, Bar M, et al. Clinical utilization of Chimeric Antigen Receptor T-cells (CAR-T) in B-cell acute lymphoblastic leukemia (ALL)-an expert opinion from the European Society for Blood and Marrow Transplantation (EBMT) and the American Society for Blood and Marrow Transplantation (ASBMT). Bone Marrow Transplant. 2019;54(11):1868-1880.

29. Del Bufalo F, Becilli M, Rosignoli C, et al. Allogeneic, donor-derived, second-generation, CD19-directed CAR-T cells for the treatment of pediatric relapsed/refractory BCP-ALL. Blood. 2023;142(2):146-157.

30. Chen S, Zhang Y, Fang C, et al. Donor-derived and off-the-shelf allogeneic anti-CD19 CAR T-cell therapy for R/R ALL and NHL: A systematic review and meta-analysis. Crit Rev Oncol Hematol. 2022;179:103807.

31. Bonini C, Chapuis AG, Hudecek M, et al. Genome Editing in Engineered T Cells for Cancer Immunotherapy. Hum Gene Ther. 2023;34(17-18):853-869.

32. Benjamin R, Graham C, Yallop D, et al. Genome-edited, donor-derived allogeneic anti-CD19 chimeric antigen receptor T cells in paediatric and adult B-cell acute lymphoblastic leukaemia: results of two phase 1 studies. Lancet. 2020;396(10266):1885-1894.

33. Rives Sea. Tisagenlecleucel in pediatric and young adult patients with relapsed/refractory (r/r) B-cell acute lymphoblastic leukemia (B-ALL): Final analyses from the ELIANA study. Oral presentation at: 2022 Hybrid Congress of the European Hematology Association (EHA). 2022;Abstract #S112.

34. Jabbour E, Short NJ, Jain N, et al. The evolution of acute lymphoblastic leukemia research and therapy at MD Anderson over four decades. J Hematol Oncol. 2023;16(1):22.

35. Bader P, Rossig C, Hutter M, et al. CD19-CAR-T cells are an effective therapy of post-transplant relapse in B- ALL patients: Real-World Data from Germany. Blood Adv. 2023;7(11):2436-2448.

36. Mahadeo KM, Khazal SJ, Abdel-Azim H, et al. Management guidelines for paediatric patients receiving chimeric antigen receptor T cell therapy. Nat Rev Clin Oncol. 2019;16(1):45-63.

37. Khan I, Khan N, Wolfson N, et al. Safety of CAR-T Cell Therapy in Patients With Renal Failure/Acute Kidney Injury: Focused Review. Clin Hematol Int. 2023;5(2-3):122-129.

38. Niu J, Qiu H, Xiang F, et al. CD19/CD22 bispecific CAR-T cells for MRD-positive adult B cell acute lymphoblastic leukemia: a phase I clinical study. Blood Cancer J. 2023;13(1):44.

39. Hu GH, Cheng YF, Zuo YX, et al. Chimeric Antigens Receptor T Cell Therapy Improve the Prognosis of Pediatric Acute Lymphoblastic Leukemia With Persistent/Recurrent Minimal Residual Disease in First Complete Remission. Front Immunol. 2021;12:731435.

40. Schultz LM, Eaton A, Baggott C, et al. Outcomes After Nonresponse and Relapse Post-Tisagenlecleucel in Children, Adolescents, and Young Adults With B-Cell Acute Lymphoblastic Leukemia. J Clin Oncol. 2023;41(2):354-363.

41. Kantarjian HM, DeAngelo DJ, Stelljes M, et al. Inotuzumab Ozogamicin versus Standard Therapy for Acute Lymphoblastic Leukemia. N Engl J Med. 2016;375(8):740-753.

42. Bhojwani D, Sposto R, Shah NN, et al. Inotuzumab ozogamicin in pediatric patients with relapsed/refractory acute lymphoblastic leukemia. Leukemia. 2019;33(4):884-892.

43. Brivio E, Locatelli F, Lopez-Yurda M, et al. A phase 1 study of inotuzumab ozogamicin in pediatric relapsed/refractory acute lymphoblastic leukemia (ITCC-059 study). Blood. 2021;137(12):1582-1590.

44. O'Brien MM, Ji L, Shah NN, et al. Phase II Trial of Inotuzumab Ozogamicin in Children and Adolescents With Relapsed or Refractory B-Cell Acute Lymphoblastic Leukemia: Children's Oncology Group Protocol AALL1621. J Clin Oncol. 2022;40(9):956-967.

45. Ceolin V, Brivio E, van Tinteren H, et al. Outcome of chimeric antigen receptor T-cell therapy following treatment with inotuzumab ozogamicin in children with relapsed or refractory acute lymphoblastic leukemia. Leukemia. 2023;37(1):53-60.

46. Wullenkord R, Reicherts C, Mikesch JH, et al. Sequential therapy with inotuzumab ozogamicin, CD19 CAR T cells, and blinatumomab in an elderly patient with relapsed acute lymphoblastic leukemia. Ann Hematol. 2021;100(2):587-589.

47. Rubinstein JD, Breese EH, Krupski MC, et al. The Choice of Either Conventional Chemotherapy or Inotuzumab Ozogamicin as Bridging Regimen Does Not Appear To Impact Clinical Response to CD19-Directed CAR-T Therapy in Pediatric B-ALL. Transplant Cell Ther. 2023;29(5):311.

48. Jacoby E, Ghorashian S, Vormoor B, et al. CD19 CAR T-cells for pediatric relapsed acute lymphoblastic leukemia with active CNS involvement: a retrospective international study. Leukemia. 2022;36(6):1525-1532.

49. Leahy AB, Newman H, Li Y, et al. CD19-targeted chimeric antigen receptor T-cell therapy for CNS relapsed or refractory acute lymphocytic leukaemia: a post-hoc analysis of pooled data from five clinical trials. Lancet Haematol. 2021;8(10):e711-e722.

50. Salzer WL, Burke MJ, Devidas M, et al. Impact of Intrathecal Triple Therapy Versus Intrathecal Methotrexate on Disease-Free Survival for High-Risk B-Lymphoblastic Leukemia: Children's Oncology Group Study AALL1131. J Clin Oncol. 2020;38(23):2628-2638.

51. Pullarkat VA, Lacayo NJ, Jabbour E, et al. Venetoclax and Navitoclax in Combination with Chemotherapy in Patients with Relapsed or Refractory Acute Lymphoblastic Leukemia and Lymphoblastic Lymphoma. Cancer Discov. 2021;11(6):1440-1453.

52. Ottmann O, Dombret H, Martinelli G, et al. Dasatinib induces rapid hematologic and cytogenetic responses in adult patients with Philadelphia chromosome positive acute lymphoblastic leukemia with resistance or intolerance to imatinib: interim results of a phase 2 study. Blood. 2007;110(7):2309-2315.

53. Ottmann OG, Larson RA, Kantarjian HM, et al. Phase II study of nilotinib in patients with relapsed or refractory Philadelphia chromosome--positive acute lymphoblastic leukemia. Leukemia. 2013;27(6):1411-1413.

54. Cortes JE, Kim DW, Pinilla-Ibarz J, et al. A phase 2 trial of ponatinib in Philadelphia chromosome-positive leukemias. N Engl J Med. 2013;369(19):1783-1796.

55. Leclercq G, Haegel H, Schneider A, et al. Src/lck inhibitor dasatinib reversibly switches off cytokine release and T cell cytotoxicity following stimulation with T cell bispecific antibodies. J Immunother Cancer. 2021;9(7):e002582.

56. Letai A, Bhola P, Welm AL. Functional precision oncology: Testing tumors with drugs to identify vulnerabilities and novel combinations. Cancer Cell. 2022;40(1):26-35.

57. Talleur AC, Qudeimat A, Metais JY, et al. Preferential expansion of CD8+ CD19-CAR T cells postinfusion and the role of disease burden on outcome in pediatric B-ALL. Blood Adv. 2022;6(21):5737-5749.

58. DeAngelo DJ, Advani AS, Marks DI, et al. Inotuzumab ozogamicin for relapsed/refractory acute lymphoblastic leukemia: outcomes by disease burden. Blood Cancer J. 2020;10(8):81.

59. Bader P, Kreyenberg H, Ossenkoppele G. Monitoring Minimal Residual Disease in ALL and AML. In: Carreras E, Dufour C, Mohty M, Kroger N, editors. The EBMT Handbook: Hematopoietic Stem Cell Transplantation and Cellular Therapies. 7th ed. Cham (CH): 2019. p. 431-436.

60. Lee DW, Gardner R, Porter DL, et al. Current concepts in the diagnosis and management of cytokine release syndrome. Blood. 2014;124(2):188-195.

61. Brudno JN, Kochenderfer JN. Toxicities of chimeric antigen receptor T cells: recognition and management. Blood. 2016;127(26):3321-3330.

62. Lee DW, Santomasso BD, Locke FL, et al. ASTCT Consensus Grading for Cytokine Release Syndrome and Neurologic Toxicity Associated with Immune Effector Cells. Biol Blood Marrow Transplant. 2019;25(4):625-638.

63. Brown BD, Tambaro FP, Kohorst M, et al. Immune Effector Cell Associated Neurotoxicity (ICANS) in Pediatric and Young Adult Patients Following Chimeric Antigen Receptor (CAR) T-Cell Therapy: Can We Optimize Early Diagnosis? Front Oncol. 2021;11:634445.

64. Mahdi J, Dietrich J, Straathof K, et al. Tumor inflammation-associated neurotoxicity. Nat Med. 2023;29(4):803-810.

Table 1: NCI levels of evidence for adult and paediatric cancer treatment studies *

Level of evidence	Definition
A1 Evidence	Randomized controlled clinical trial (RCT) (double-blinded or nonblinded) with an end point of overall survival (OS) from a defined time, total mortality, or cause-specific mortality.
A2 Evidence	Meta-analysis of RCTs with an end point of OS from a defined time, total mortality, or cause-specific mortality.
A3 Evidence	RCT (double-blinded or nonblinded) with an end point of quality of life that is well-collected, clinically meaningful, and carefully assessed.
B1 Evidence	RCT (double-blinded or nonblinded) with an end point of event-free survival (EFS), disease-free survival (DFS), or progression-free survival (PFS) differences.
B2 Evidence	Meta-analysis of RCTs with an end point of EFS, DFS, PFS, or carefully assessed quality of life.
B3 Evidence	RCT (double-blinded or nonblinded) with an end point of tumour response rate or quality-of-life measurement that does not reach the level described in A3.
B4 Evidence	Nonrandomized, multicentre, prospective, controlled clinical trial with a planned comparison of efficacy including an end point of OS from a defined time, total mortality, cause-specific mortality, carefully assessed quality of life, EFS, DFS, PFS, or tumour response differences.
C1 Evidence	Case series or other observational study design, including trials with non-consecutive cases, with an end point of OS from a defined time, total mortality, cause-specific mortality, or carefully assessed quality of life.
C2 Evidence	Case series or other observational study design, including trials with non-consecutive cases, with an end point of EFS, DFS, or PFS differences.
C3 Evidence	Case series or other observational study design, including trials with non-consecutive cases, with an end point of tumour response rate or quality-of-life measurement that does not reach the level described in A3.
D Evidence	Anecdotal experience or expert opinion.

* NCI = National Cancer Institute; https://www.cancer.gov/publications/pdq/levels-evidence/treatment#_70

Table 2: Chemotherapy regimens

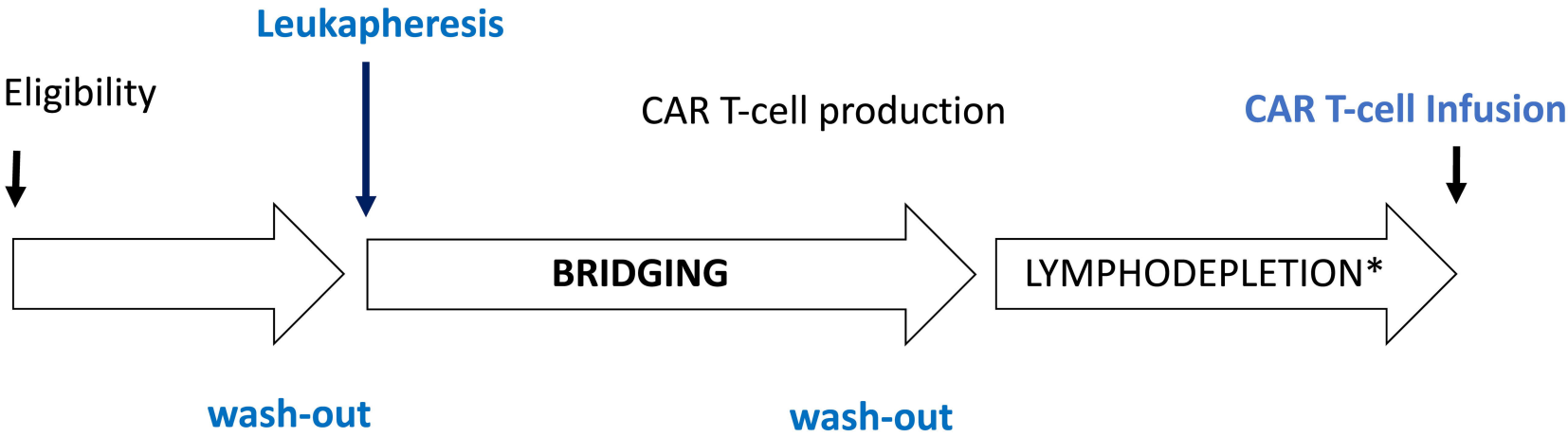
Low intensity regimen	Medication
VDA	Vincristine, Dexamethasone (or Prednisone), PEG-Asparaginase
Maintenance	6-Mercaptopurine (daily), Methotrexate (weekly, p.o.), ± Hydroxyurea
VDC	Vincristine, Dexamethasone (or Prednisone), Cytarabine 75mg/m^2/d on four consecutive days
High intensity regimen	
VDA plus …	VDA plus (one of the following) ± Anthracyclines (Daunorubicin or Idarubicin or others) or ± Cyclophosphamide or ± Etoposide or ± i.v. Methotrexate or ± i.v. high-dose Cytarabine
TT	Dexamethasone (or Prednisone) plus Thiotepa
Clof	Dexamethasone (or Prednisone) plus Clofarabine
Immunotherapy	
Blinatumomab	Dosing according to the approved label is recommended. Dosing recommendations for adults may be considered in adolescents.
Inotuzumab	Increasing evidence of efficacy is counterbalanced by B-cell aplasia.
others	Rituximab in case of CD20 expression. Several other lineage-specific antibody treatments are in development.
Targeted therapy	
BCL-2	Venetoclax, Navitoclax
Based on individual genotype of blasts	Tyrosine kinase inhibitors in case of Ph1 ALL (Dasatinib, Nilotinib, Ponatinib)

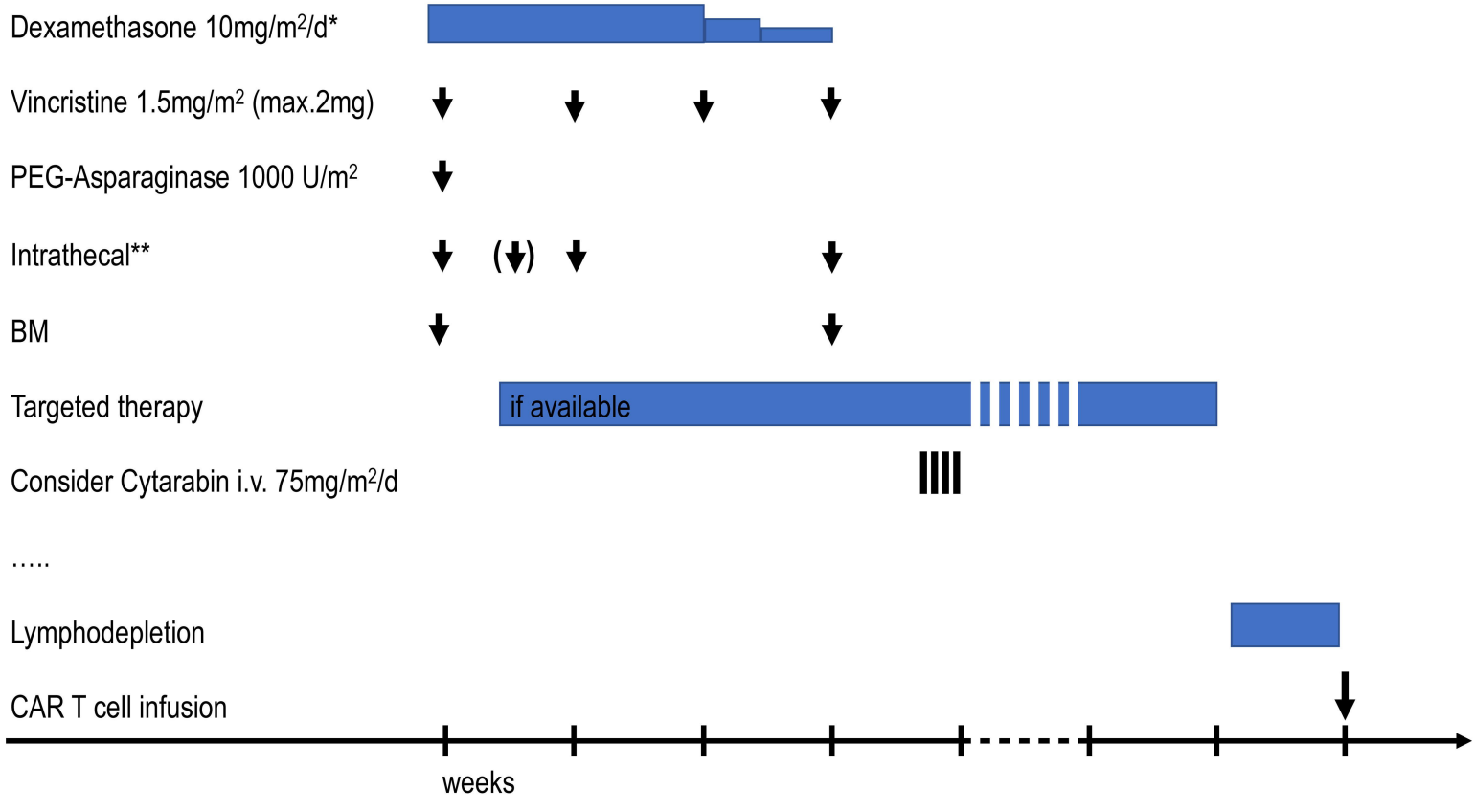
TT = Thiotepa; Clof = Clofarabin; BCL-2 = B cell lymphoma 2 protein family

Legend Figure 1: Bridging therapy is usually considered as the phase between leukapheresis and start of lymphodepleting therapy. However, control of leukaemia starts earlier already prior to leukapheresis. Therefore, treatment is subdivided into a first phase until leukapheresis and a bridging therapy phase until start of lymphodepleting therapy and consecutive infusion of CAR T cells.

* Lymphodepleting therapy has been variable within and between different study protocols. A typical example of lymphodepletion contains fludarabin and cyclophosphamide.

Legend Figure 2: Backbone of a low-intensity bridging therapy before infusion of CAR T cells. BM = Bone marrow aspiration.


Leukapheresis
Eligibility
CAR T-cell production
CAR T-cell Infusion
BRIDGING
LYMPHODEPLETION*
wash-out
wash-out



* Consider Dexa every other week if possible; **Methotrexate, Cytarabin, Prednisolone