ALL-IC REL 2024

Childhood ALL 1st Relapse Guidance, ALL-IC Study Group

Current Best Practice Guidance for
1st Relapse of Childhood Acute Lymphoblastic Leukemia
Optimized for the ALL-IC Group

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For further data, contact details, information on our patient-registry, see
https://semmelweis.hu/tuzoltoklinika/en/researches/all-ic/

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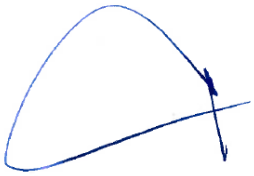
SYNOPSIS AND SIGNATURES

Title	**Childhood ALL 1st Relapse Guidance, ALL-IC Study Group "ALL-IC REL 2024"**
Steering committee	Dániel Erdélyi, Hungary – chair Juan Tordecilla, Chile Marko Kavčič, Slovenia Mirella Ampatzidou, Greece Monica Makiya, Argentina Sophia Polychronopoulou, Greece Volkan Hazar, Turkey
Background	The Acute Lymphoblastic Leukemia Inter Continental Relapse (ALL-IC REL) Group decided to update its earlier guideline for pediatric ALL 1st relapse. By standardizing therapy and involving flow cytometry based MRD in treatment stratification, patient outcomes greatly improved due to that guideline. Because of the new PdL relapse definitions, new targeted therapies and changing risk stratification, the guideline has been revised.
Objectives	• To improve the outcome of ALL 1st relapse • To set up an international treatment platform with homogenized treatment approach among the ALL-IC community also involving non-ALL-IC countries • To prepare the way for future clinical studies within the ALL-IC or the IntReALL study group
Design	This is not a formal clinical trial, but a "current best practice guidance" optimized for the resources available in the ALL-IC countries at present. No randomizations are involved.
Inclusion & exclusion criteria	Inclusion criteria: • Confirmed diagnosis of 1st relapse of precursor lymphoblastic leukemia • Age 0-18 years at the time of relapse • Written informed consent • The participating center has access to flow cytometry MRD measurement as per standards of the ALL-IC study group Exclusion criteria: • Mature B-ALL • Pregnant or breastfeeding patient
Risk group stratification	**I. Non-T cell ALL 1st relapses** Patients are grouped for standard risk (SR), high risk (HR), very high risk (VHR) and isolated extramedullary central nervous system (IEM-CNS) relapse treatment approaches and according to eligibility for stem cell transplantation. Criteria for SR: late isolated/combined bone marrow (BM) relapse Criteria for HR: early isolated/combined BM relapse Criteria for VHR: 1) very early isolated/combined BM relapse; 2) All relapses, regardless relapse time and location, with very high-risk genetic features [*KMT2A::AFF1* fusion, *TCF3::PBX1* fusion, *TCF3::HLF* fusion, *TP53* alterations, hypodiploidy (<44 chromosomes)] Criteria for IEM-CNS: All CNS relapses with no bone marrow involvement (at 5% cut-off)

	II. T-cell ALL 1st relapses Criteria for IEM-CNS: All CNS relapses with no bone marrow involvement (at 5% cut-off) Criteria for HR: All other relapses. **III. Eligibility for SCT** 1) All T-cell relapses; 2) All very early and early relapses; 3) All patients with poor initial treatment response defined as bone marrow MRD ≥ 0.1% by flow cytometry or residual extramedullary leukemia on day 29 or MRD reappearance ≥ 0.1% any time later.

Agreed on 13th May 2024:

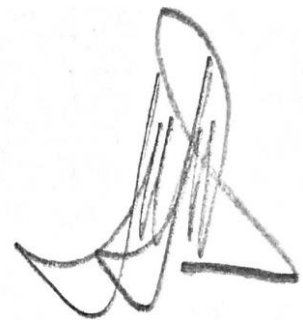
Dániel Erdélyi, Hungary



Juan Tordecilla, Chile

Marko Kavčič, Slovenia

Mirella Ampatzidou, Greece

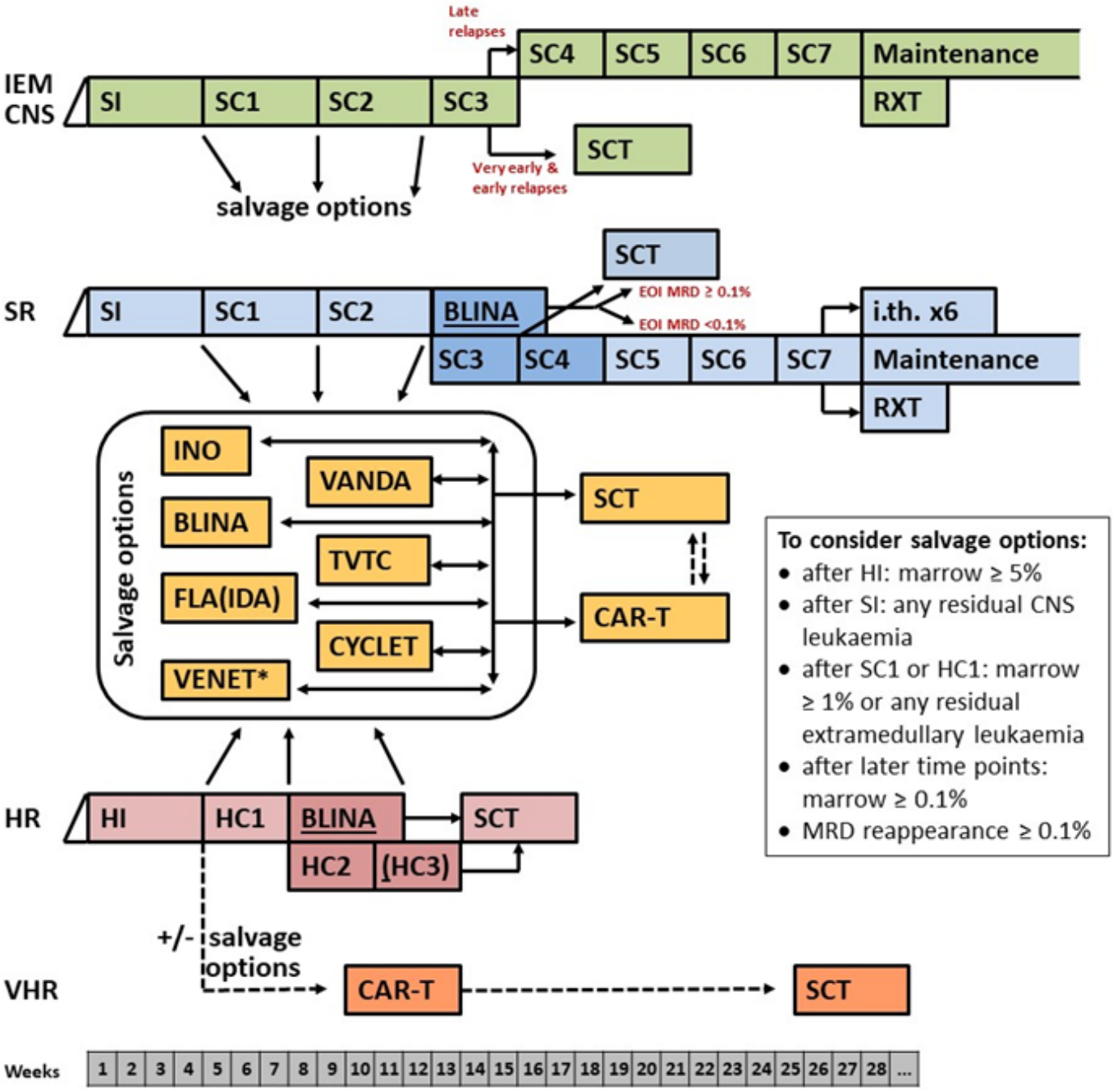


Monica Makiya, Argentina

Sophia Polychronopoulou, Greece

Volkan Hazar, Turkey

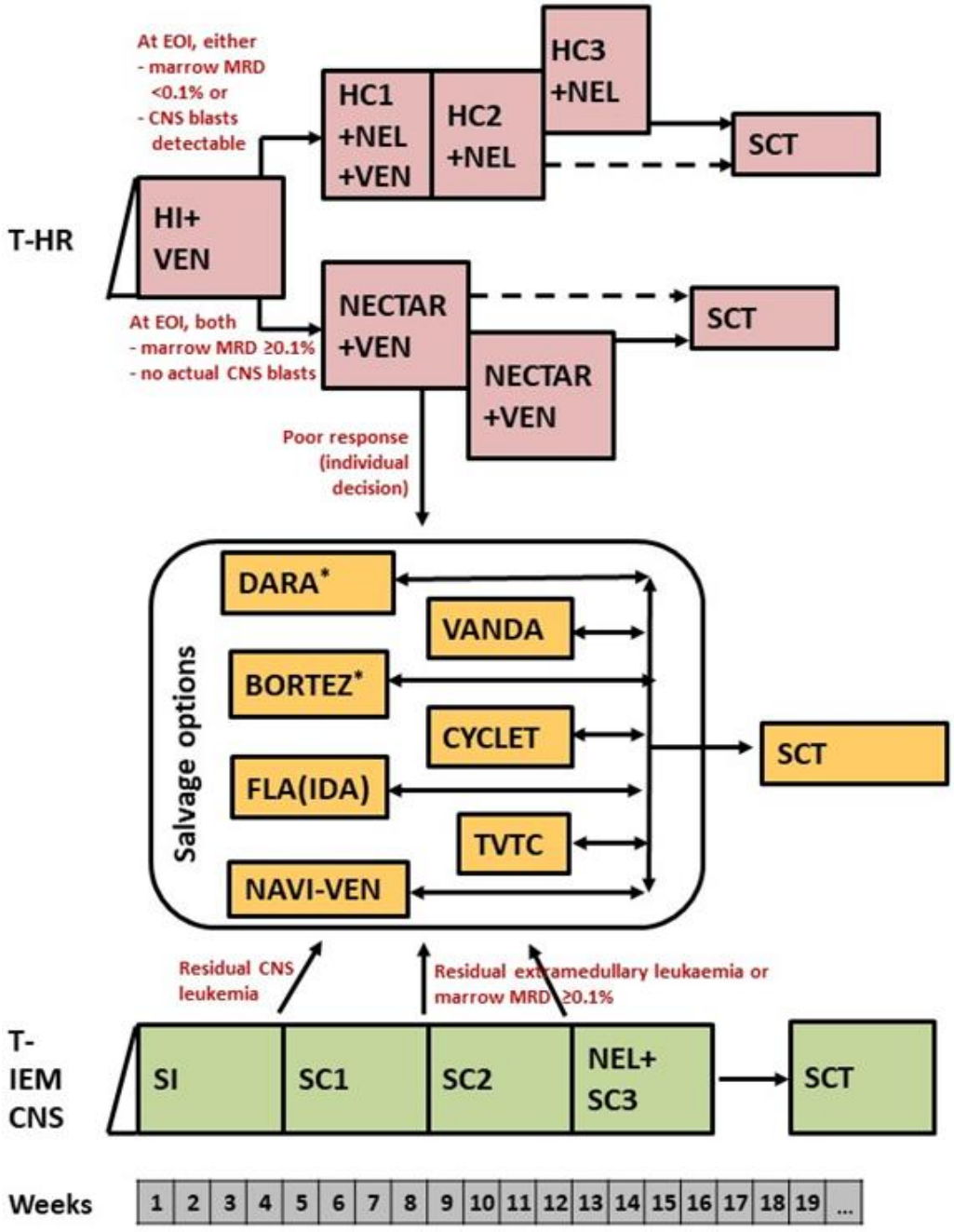
OVERVIEW: ALL-IC REL 2024 NON-T-CELL TREATMENT STRATEGY



EOI: end of induction. MRD: minimal residual disease

* Venetoclax is always given in a combination with chemotherapy (of multiple choices)

OVERVIEW: ALL-IC REL 2024 T-CELL TREATMENT STRATEGY



EOI: end of induction. MRD: minimal residual disease

* Daratumumab and bortezomib are always given in a combination with chemotherapy (of multiple choices)

LIST OF ABBREVIATIONS

ADC	Antibody drug conjugate
ALL	Acute lymphoblastic leukemia
ALL-IC	The Acute Lymphoblastic Leukemia Intercontinental Study Group
AML	Acute myeloid leukemia
AML1	Acute myeloid leukemia 1 (gene)
ARA-C	Cytarabine
BCP	B-cell precursor
BFM	Berlin-Frankfurt-Münster Study Group
BM	Bone marrow
BMP	Bone marrow puncture
BSA	Body surface area
CAR	Chimeric antigen receptor
CNS	Central nervous system
COG	Children's Oncology Group
CR	Complete remission
CSF	Cerebrospinal fluid
CT	Computer tomography
CTCAE	Common Terminology Criteria for Adverse Events
DI	Delayed intensification
DMSO	Dimethylsulfoxide
EFS	Event free survival
EM	Extramedullary
EOI	End of induction
F1-2	ALL Rez BFM 2002 protocol "F" cycles
FAB	French-American-British morphological classification
FC	Flow cytometry
FD	Family donor
FEDRRAL	Federated Relapsed Refractory Acute Leukemia
FISH	Fluorescein in situ hybridization
GI	Gastrointestinal
GR	Good response
HC	High-risk arm consolidation
HD	High-dose
HI	High-risk arm induction
HLA	Human leucocyte antigen
HR	High risk
iAMP21	Intrachromosomal amplification of chromosome 21
IEM	Isolated extramedullary
InO	Inotuzumab ozogamycin
IntReALL	International Study for Treatment of Childhood Relapsed ALL
IT	Intrathecal treatment
IV	Intravenous treatment
linEF	Linear ejection fraction
M1/2/3	Cytological bone marrow status 1-3
mAb	Monoclonal antibody
MD	HLA matched donor
MDS	Myelodysplastic syndrome
MFD	Matched family donor

MMD	HLA Mismatched donor
MMFD	Mismatched family donor
MMUD	Mismatched unrelated donor
MPAL	Mixed (immuno)phenotype acute leukemia
MRD	Minimal residual disease
MRI	Magnetic resonance imaging
MSD	Matched sibling donor
MUD	Matched unrelated donor
NGS	New generation sequencing
NHL	Non-Hodgkin lymphoma
NT5C2	5'-nucleotidase, cytosolic II (gene)
PBX1	PBX homeobox 1 (gene)
PCR	Polymerase chain reaction
PdL	Ponte-di-Legno criteria
PEG	Polyethylene glycol
PET	Positron emission tomography
PR	Poor response
PRES	Posterior reversible encephalopathy syndrome
qPCR	Quantitative polymerase chain reaction
R blocks	ALL relapse chemotherapy courses of the BFM group (SC4-7 cycles)
RBC	Red blood cell count
REL	Relapse
SC	Standard risk arm consolidation
SCT	Stem-cell transplantation
SG	Study group
SI	Standard risk arm remission induction
SOC	Standard of care
SOP	Standard operational procedure
SOS	Sinusoidal obstruction syndrome
SR	Standard risk
TBI	Total body irradiation
TCF3	Transcription factor 3 (gene, alias of E2A)
TCR	T-cell receptor
TKI	Tyrosine kinase inhibitor
TP53	Tumour protein p53 (gene)
TPN	Total parenteral nutrition
UD	Unrelated donor
ULN	Upper limit of normal range
VHR	Very high risk
WBC	White blood cell count

1. INTRODUCTION

1.1. Introduction from the founder and first chair of the ALL-IC REL Project

The "ALL-IC REL 2024" guidelines for the first relapse of pediatric Acute Lymphoblastic Leukemia (ALL) mark a significant milestone in the journey towards refining the management of this challenging condition. Grounded in the comprehensive findings from the observational registry trial, the ALL-IC REL 2016 protocol, which meticulously documented the treatment and outcomes of a diverse cohort of 500 children within the ALL-IC network, this update synthesizes a wealth of clinical research with real-world patient experiences.

This foreword introduces the guidelines as a culmination of concerted efforts to distill insights from the vast data collected through the 2016 protocol. By closely examining the responses and outcomes of patients stratified into standard-risk and high-risk categories, and leveraging advancements in minimal residual disease (MRD) assessment and immunotherapy, these guidelines propose a nuanced, evidence-based approach to treatment. They particularly focus on addressing the pressing needs of high-risk patients, who have historically faced lower response rates to induction therapy and higher relapse rates post-transplant.

Acknowledging the diverse healthcare landscapes across the consortium's participating countries, the "ALL-IC REL 2024" guidelines emphasize a balance between standardization and flexibility. This approach ensures that the protocols are not only rooted in the latest clinical research but also adaptable to the varied settings in which they will be implemented, aiming to provide equitable access to effective treatment for all children with relapsed ALL.

As a testament to the collaborative spirit of the ALL-IC Study Group, the "ALL-IC REL 2024" guidelines embody the drive towards continuous improvement in pediatric oncology care. By marrying the rigor of clinical research with the practicalities of real-world application, these guidelines aim to set a new benchmark in the treatment of pediatric ALL relapses, paving the way for better survival rates and quality of life for affected children worldwide. This forward-looking approach highlights the group's commitment to not just addressing the current challenges in treating relapsed ALL but also to innovating and refining strategies to meet the evolving needs of this patient population on a global scale.

Prof. Janez Jazbec

1.2. Introduction from the current chair of the ALL-IC REL Project

The ALL-IC REL project has not organized clinical studies so far. The main focus has been to create and repeatedly update a best practice treatment guidance on ALL 1st relapse in children, optimized for high-middle income countries. The protocol is based mostly on the studies of the International Relapse Refractory Acute Lymphoblastic Leukemia (IntReALL) group for their information and advice. A common ALL-IC REL registry was also established in the REDCap platform. Pseudonymized patient data are entered here with disease features, follow up MRD values, toxicity grading and outcome. With homogenized treatment strategies and a large cohort from an international registry, we can learn from our experience.

As per the knowledge of the steering committee, this guidance is applied in the following countries, either in all or just some of the centers: Argentina (GATLA), Armenia, Bulgaria, Chile, Croatia, Georgia, Greece, Hungary, Lebanon, North Macedonia, Romania, Russia, Serbia, Slovenia, Turkey, Ukraine. We presume it is being used in various middle-income countries worldwide without our knowledge.

In 2022, we distributed a short update of our treatment protocol to incorporate changes triggered by new evidence: optimal treatment strategy for isolated CNS relapses; benefit of blinatumomab; major changes to the risk group stratification. We also provided colleagues with salvage options and recommendations when and how to choose from them. At that time, the plan was to create a fully written detailed protocol.

Finally, we changed our mind and decided to write a totally new protocol version due to further relevant additions and amendments: incorporation of the new Ponte-di-Legno definitions of relapse and remission; describing T-cell relapse treatment backbones different from our previous protocol versions; changes in CNS diagnostics; updating salvage options to mention only the most important points.

We are quite convinced that these changes will result in obvious improvement in the long term cure rate of children with ALL relapse, together with reduction of the treatment's toxicity for a large proportion of patients.

Daniel Erdelyi, MD PhD habil

2. BACKGROUND AND RATIONALE

Though survival of children with ALL has considerably improved over the past few decades, relapsed ALL remains a leading cause of mortality in children with cancer. Prognostic factors for treatment failure are duration of first remission, immunophenotype of the malignant clone, site of relapse, early treatment response and genetic features. The prognosis of patients with relapsed ALL was substantially improved with intensive multidrug chemotherapy and allogeneic stem cell transplantation (allo-SCT) in the majority of patients at the cost of acute and long-term toxicity including early and late treatment-related deaths.

Preliminary analysis of patients treated as per the ALL-IC REL 2016 protocol is encouraging. Patients classified as standard risk (SR) with late bone marrow (BM) and/or extramedullary (EM) relapses and without high-risk genetic features achieved high remission rates (>95%) and favorable long-term event-free survival rates (>65%) with conventional consolidation and maintenance chemotherapy if, after induction therapy, the marrow leukemia burden could be reduced below the MRD level of 0.1% by flow cytometry.

About 50% of SR patients experience poor MRD response after conventional chemotherapy induction and require intensified consolidation through allo-SCT to achieve EFS rates comparable to that of SR patients with good MRD response.

Patients classified as high risk (HR) with early BM/EM relapse still demonstrate poor response rates (at the end of induction) of around 65% being treated on standard induction therapy, and high rates of subsequent relapse. Consequently, these patients all require allo-SCT for consolidation of 2nd remission.

Patients with isolated extramedullary relapse (IEM) had a better outcome with more CNS directed treatment in induction / consolidation chemotherapy as provided by the ALL-REZ BFM backbone compared to conventional induction as provided with the ALL-R3 induction (IntReALL SR 2010, unpublished results).

Patients with very high risk (VHR) genetic features [*KMT2A::AFF1* t(4;11), *E2A3::PBX1 (also called TCF3::PBX1)* t(1;19), *TCF3::HLF* t(17;19) rearrangements, hypodiploidy (<44 chromosomes), *TP53* alteration] and/or very early relapses (i.e. those occurring within 18 months after initial diagnosis) have a limited benefit from conventional chemotherapy and allo-SCT since their event-free survival (EFS) rate has been reported to be below 20%.

There is a need for new therapeutic means to further increase survival, and to replace chemotherapy and radiotherapy with targeted less toxic drugs. For the B-cell precursor (BCP) immunophenotype group, the CD3/19 directed bi-specific monoclonal antibody blinatumomab has shown better efficacy and less toxicity compared to conventional consolidation chemotherapy in children with relapsed B-cell precursor (BCP) ALL with and without extramedullary (EM) involvement in randomized trials. The superiority of blinatumomab has been shown for HR patients in a randomized trial in Europe[(1)] and for HR and SR patients in a randomized trial conducted by the Children's Oncology Group (COG) (AALL1331 trial) [(2,3)]. Thus, blinatumomab is considered as the best standard of care (SOC) for early consolidation in this indication.

Inotuzumab ozogamicin (InO) is a CD22 directed humanized monoclonal antibody linked to the toxin calicheamicin, belonging to the class of antibody-drug conjugates (ADC). CD22 is expressed on nearly all BCP-ALLs. After antigen binding, InO is internalized, and the toxin released causing DNA

damage and inducing apoptosis. InO has shown high MRD negative remission rates in adult and pediatric patients with relapsed/refractory BCP ALL and a safe and effective dose has been identified in pediatric phase I/II trials. It is generally accepted as being a good treatment choice for patients not responding to chemotherapy [4-6].

No similarly well proven immunotherapy options have been developed for T-cell relapses. So far, venetoclax and nelarabine were proven to be effective drugs in this patient group. We recommend their routine use due to the poor survival achieved with the ALL-IC REL 2016 as well as other protocols containing traditional chemotherapy agents and allo-SCT.

In light of these developments and the accessibility of these treatments in the majority of the ALL-IC countries, a need for revision has arisen in the ALL-IC REL group.

Cellular immunotherapies, namely chimeric antigen receptor (CAR)-T cell therapy is not available for most ALL-IC patients yet. Hence this guidance only includes this treatmen as an option.

3. OBJECTIVES

Main goals of this treatment protocol are:

- To set up a large international platform allowing for optimization of standard treatment strategies.
- To improve the outcome of children and adolescents with first relapse of acute lymphoblastic leukemia,
- To prepare the way for future clinical studies on ALL relapse within the ALL-IC or the IntReALL study groups.

On the one hand, resources and availability of novel therapeutic agents are somewhat heterogenous within the ALL-IC group and in further countries interested in using this guidance. Rightly, the best available treatment is pursued among every set of circumstances. On the other hand, conclusions can only be drawn from large, rather homogenously treated cohorts, if patients' data are filled into registries. Hence, this protocol aims to find the balance to fulfil both above considerations. Novel drugs that are more expensive or difficult to access are therefor put down as optional in this guidance.

There is no legal framework around this protocol. It has been written and agreed by delegates of national groups from the ALL-IC community, as a joint recommendation. Decision and responsibility lie upon the treating physician if and how (with what modifications) this protocol is applied.

4. PROTOCOL DESIGN AND DESCRIPTION

4.1. Design

This is not a research study, but a current best practice guidance optimized for the present resources available at the ALL-IC countries. It is designed as prospective open label international multicenter multiple arm observational study with a registry, without randomization.

Inclusion criteria:

- Confirmed diagnosis of 1st relapse of acute lymphoblastic leukemia;
- Age 0-18 years at the time of relapse;
- Written informed consent.

Exclusion criteria:

- Mature B-ALL;
- Pregnant patient;
- Lactation.

During the course of treatment, individual patients are excluded in case of one of the following situations:

- Withdrawal of consent;
- Pregnancy;
- Retroactive failure to fulfill inclusion/exclusion criteria;
- Significant noncompliance;
- New medical conditions not allowing for continuation of the protocol conform treatment.

Patients excluded from the study are further observed and considered for intention to treat analyses unless they withdraw their consent for registration within the study.

4.2. Requirements for centers

Requirements for treatment centers applying this guidance:

- A physician fully trained in pediatric hematology/oncology should direct the treatment at the site;
- Access to hematology/pathology laboratory facilities for cytological diagnosis;
- Access to flow cytometry MRD measurement as per standards of the ALL IC-BFM 2009 study as a minimum;
- Access to radiotherapy facilities;
- Access to intensive care;
- Fulfilling the national criteria for pediatric hematology/oncology centers.

4.3. Treatment strategy

Fundamentally, this guidance mostly adapts the strategies of standard (non-investigational) arms of IntReALL trials to ALL-IC countries. The main deviation from IntReALL 2020 is that MRD will be measured by flow cytometry instead of the PCR based approach at IntReALL, BFM backbone chemotherapy will be used instead of UK R3 backbone chemotherapy in the SR arm, blinatumomab is not used in the IEM-CNS arm, new recommendations were made for T-ALL relapses to utilize novel agents routinely (nelarabine, venetoclax if available at the treating center).

When establishing the indication of SCT in the SR group, the same MRD time point and cut off will be used as that found effective in the ALL REZ BFM 2002 trial and the IntReALL 2010 SR trial by qPCR. Various study groups found that MRD by flow cytometry and MRD by IgH or TCR based qPCR correlate very well in general, as well as in the specific situation of childhood ALL relapse end-of-induction time point.

5. DEFINITIONS

5.1 Definition of relapse and of organ involvement

Regarding relapse, remission and treatment failure definitions, the ALL-IC REL group adheres to the new Ponte-di-Legno (PdL) consensus[7]. According to this, even 1% of bone marrow blasts are enough to define relapse, though only if confirmed by more testing methods and/or times of testing.

The diagnostic procedures before the initiation of induction are essential to establish the correct diagnosis and to define the site of relapse and organ ALL infiltration. Assessment of the bone marrow and all extramedullary sites should take place before starting any anti-leukemic therapy, apart from emergency situations (e.g. hyperleukocytosis-hyperviscosity syndrome, tracheobronchial compression, superior vena cava syndrome, increased intracranial pressure, DIC).

5.1.1. Bone marrow relapse

Bone-marrow involvement is assessed by light microscopy applying the French-American-British (FAB) criteria and by flow cytometry at default. BM aspirations from at least one site (usually posterior iliac spine) is mandatory. Routine performance of bilateral aspiration and/or biopsy and histopathology evaluation are not study requirements but are optional based on local standards. If the aspiration is insufficient (punctio sicca), a bone marrow biopsy should be taken instead and referred to pathology. In this case, the aspiration is repeated after a few days of dexamethasone pre-phase, which usually leads to mobilization of the blast cells. Bone marrow involvement quantified by cytomorphology is supplemented by immunophenotyping and genetic techniques. Consensus criteria for BM relapse definition are described in Table 1.[7]

Table 1. Consensus criteria for defining relapse in pediatric ALL based on bone marrow (Ponte-di-Legno criteria)[7]. If all criteria within any one of the rows of the table are met, bone marrow relapse is established. A second bone marrow examination is only needed if the diagnosis cannot be established from the first sample.

BM 1		**BM 2***
Cytomorphology	**MRD test(s)****	**(if needed)**
M3	(any/not necessary)	(any/not necessary)
(any/not necessary)	One test ≥ 25%	(any/not necessary)
M2	One test ≥ 1%	(any/not necessary)
M2	(any/not necessary)	Cytomorphology M2
(any/not necessary)	One test ≥ 5% plus another test ≥ 1%	(any/not necessary)
(any/not necessary)	Three test ≥ 1%	(any/not necessary)
(any/not necessary)	One test ≥ 1%	two MRD tests with ≥ 1%***

* Second bone marrow evaluation ≥ 1 week later

** Flow cytometry or FISH or karyotype or PCR-MRD or NGS-MRD demonstrating leukemia-specific markers are meant by MRD test(s).

*** One of these tests can be the same method as at 1st type point

See the new Ponte-di-Legno criteria of bone marrow relapse outlined in Table 1. This allows for defining relapse already at ≥ 1% leukaemic involvement. However, note that in case of overt

extramedullary relapse with detectable bone marrow involvement below 5%, the patient will be categorized and stratified to treatment arms as isolated extramedullary relapse in the ALL-IC REL 2024 protocol. Patients with overt CNS or testicular relapse plus bone marrow involvement of ≥ 5% are regarded as combined medullary-extramedullary relapses.

5.1.2. Central nervous system relapse

Methods of CSF testing are:

- manual cell counting in Fuchs-Rosenthal's or Nageotte's chamber (counting should be performed on ≥ 3 µL of CSF and cell numbers per 1 µL should be calculated);
- automated cell counting with cell analyzers – with minimum validated sensitivity threshold of 5 WBCs/µL;
- cytospin: cytomorphology analysis from stained preparations centrifuged from ideally 400 µL of CSF; identifying ≥ 2 blasts are considered as positive finding;
- flow cytometry performed from ideally 2 mL of CSF, positivity accepted if ≥ 10 blasts are identified (irrespective of initial CSF volume).

Regarding CNS staging categories and definitions, those of the ALL-IC Rel 2024 study protocol are applied (stages 1a, 1b, 2a, 2b, 2c, 3a, 3b, 3c), but with altered role of CSF flow cytometry. We accept CSF flow positivity despite a negative cytospin as true positivity, to stratify patients as CNS2. However, in case of positive cytomorphology, a good quality flow analysis can rule out relapse or CNS involvement because its specificity exceeds that of the cytomorphology analysis.

If the CSF is contaminated with blood, the following procedure is recommended after discussion with the national study center. If blasts are present in the CSF and the peripheral blood shows no blasts, a CNS relapse is assumed. If blasts are seen both in the peripheral blood and the CSF, then the Steinherz/Bleyer algorithm is applied: if the ratio of WBC/RBC in the CSF exceeds the double of the same ratio in the peripheral blood, a CNS involvement is assumed, otherwise a contamination is assumed.

A CNS relapse is classically diagnosed when morphologically leukemic lymphoblasts are detected in the CSF and there is a pleocytosis of >5/µL nucleated cells by either manual or automated cell counting. The presence of blasts among CSF WBCs should be shown by cytospin and/or flow cytometry analyses.

Following the new Ponte-di-Legno consensus criteria[7], CNS relapse can also be established without CSF pleocytosis, based on two CSF samples obtained at least 1 week apart, with leukemic cells identified by cytomorphology at both time points, plus one unequivocally positive MRD method (e.g. flow cytometry, FISH from CSF) confirming the leukemic origin of the CSF cells in at least one of the two time points. This is especially important for diagnosing and defining isolated CNS relapses. However, in case there is overt bone marrow involvement (>5%), if CNS blasts are detected only by cytomorphology and flow but without CSF pleocytosis, then the case is regarded as an isolated marrow relapse and stratified to therapy arms of the ALL-IC REL 2024 protocol accordingly.

In unclear situations a case-by-case decision may be necessary.

If clinical signs of CNS involvement are present such as visual disturbances, polyphagia, cranial nerve palsies in the absence of CSF pleocytosis, the presence of meningeal infiltration or CNS masses should be assessed by radiological scans, preferably MRI. However, focal neurological signs (e.g. facial palsy presenting at the same time as marrow relapse) define CNS3 stage even without positive CSF findings or abnormal imaging findings.

CNS infiltrates seen on imaging can also define CNS3 stage even without positive CSF findings. However, histopathology or cytology is warranted to establish the fact of relapse. The histopathology or cytology sample can be originated from any site of the body (e.g., bone marrow, testicle, pleural effusion) or, in the absence of these, they should be obtained from the meninges or the intracranial mass.

5.1.3. Testicular relapse

A testicular relapse is diagnosed in case of a uni- or bilateral painless testicular enlargement with infiltration of leukemic lymphoblasts confirmed by biopsy. The extent of enlargement has to be documented using an orchidometer. Ultrasound scan is recommended to detect leukemic infiltration. In case of a clinically normal or inconclusive contralateral testis, a subclinical involvement should be assessed by biopsy.

If leukemia relapse is established by histopathological or cytological tests from other sites (e.g. blood, bone marrow or CSF) and both testicles are obviously abnormal physically or by ultrasound findings with the history of their recent appearance, then testicular biopsies may not be needed for confirmation.

5.1.4. Other extramedullary sites of relapse

EM tumor burden should be confirmed by imaging and/or biopsy. Any site, organ or tissue may be infiltrated by leukemia. To detect clinically not overt organ involvement, a chest X-ray and an explorative ultrasound examination of the abdomen and other lymph node regions must be performed in all patients. Additionally, other radiological measures such as MRI or CT or PET-CT may be used to determine the extent of organ involvement.

Usually, no specific local treatment is necessary for these sites since systemic chemotherapy is not impaired by blood barriers. However, involvement of bradytrophic sites such as the anterior chamber of the eye may require specific diagnostics and local therapy.

Imaging could be diagnostic if combined with other sites, but a positive biopsy is needed to define relapse if it is the only potential relapse site.

5.1.5. Combined bone marrow relapse

Bone marrow leukemic involvement ≥ 5% and any non-BM relapse. Regarding CNS involvement, only CNS3 (not CNS2) categories are considered to define a combined relapse.

5.1.6. Isolated bone marrow, isolated central nervous system, isolated extramedullary relapse

Any sole BM, CNS or EM relapses, respectively, as defined above, without presence of leukemic infiltration in other sites.

A BM relapse with CNS2 is regarded as isolated BM relapse. A CNS3 relapse with <5% blasts in the marrow is regarded as isolated CNS relapse.

5.2. Definitions of treatment response

5.2.1. Definition of complete remission

CR is to be assessed no earlier than the end of induction (EOI).

For CR, the following is required:

- BM: Cytomorphology M1 and flow (and optional extra MRD methods) yield < 1% residual leukemia (the cut-off for CR is different from the SCT indication).
- CNS: CSF WBC ≤ 5/μL and < 2 blasts are identified by cytomorphology in the centrifuged preparation and no masses or leukemic infiltration are seen radiologically (if seen on presentation of relapse). Ideally 400 μL of CSF should be centrifuged for cytomorphology analysis.
- Testes: Normalization of physical alterations and initial ultrasound abnormalities, or a negative biopsy if clinical examination is not considered normal.
- Other EM: No evidence of leukemic infiltration as evaluated clinically and by imaging; a preexisting leukemic mass (mediastinal mass included) must have decreased at least to one-third of the initial tumor volume; to be confirmed by biopsy in case of doubt.

If an extramedullary, local leukemic lesion seems to be persistent at the end of induction or later during consolidation, a PET-CT imaging is recommended. In case of a positive PET-CT scan result, the lesion or at least one lesion must be biopsied. Lymphoblastic infiltration seen by histopathology or cytology or flow cytometry from the sample is needed so that complete remission can be disproven, the fact of poor response established.

5.2.2. Cytomorphologic bone marrow assessment

Aplastic bone marrow (M0)

Representative bone marrow aspirate with only few nucleated cells (mostly lymphocytes, cellularity resembles normal blood count in cytological analysis) without signs of regenerating normal hematopoiesis and with residual leukemic cells < 5%.

Morphological bone marrow remission (M1)

Representative bone marrow aspirate with less than 5% lymphoblasts assessed, satisfactory cellularity and signs of regenerating normal hematopoiesis.

Partial morphological response / marrow involvement (M2)

Bone marrow with ≥ 5% and < 25% of lymphoblasts irrespective of the cellular content.

Metaplastic marrow (M3)

Bone marrow with ≥ 25% of lymphoblasts irrespective of the cellular content.

Non-representative bone marrow

Markedly reduced cellularity despite signs of regeneration in the peripheral blood; differential count of nucleated cells in the marrow largely corresponding to that in the peripheral blood. Such a bone

marrow aspirate should be repeated, particularly when therapeutic decisions are taken based on the result.

5.2.3. Minimal residual disease recurrence

A reconversion after MRD negativity to reproducible MRD positivity is called MRD reappearance. A reconfirmation is strongly recommended. This finding does not fulfill the conditions for the definition of subsequent relapse.

5.2.4. Subsequent relapse

A subsequent relapse is defined as evidence of ALL after achievement of 2nd CR. The same definitions apply to establishing a 2nd relapse as the definitions described above for the first ALL relapse.

6. RISK GROUP STRATIFICATION

6.1. Definition of risk groups

Treatment stratification based on time, site of relapse, genetics and immunophenotype is shown in Table 2.

NON-T IMMUNOPHENOTYPE RELAPSES

Isolated Extramedullary CNS (IEM-CNS): Relapses with CNS-3 stage CNS involvement and < 5% blasts in the marrow, regardless of time to relapse. Involvement of other EM sites besides the CNS doesn't exclude the patient from this category.

Standard Risk (SR): Late relapses excluding IEM-CNS patients and those with very high-risk genetic features.

High Risk (HR): Early BCP-ALL relapses excluding IEM-CNS patients and those with very high-risk genetic features.

Very High Risk (VHR): Very early relapses and/or very high risk genetic features: *KMT2A::AFF1* t(4;11), *E2A3::PBX1* (also called *TCF3::PBX1*) t(1;19), *TCF3::HLF* t(17;19) rearrangements, hypodiploidy (< 44 chromosomes), *TP53* alteration

T-IMMUNOPHENOTYPE RELAPSES

T Isolated Extramedullary CNS (T-IEM-CNS): Relapses with CNS-3 stage CNS involvement and <5% blasts in the marrow, regardless of time to relapse. Involvement of other EM sites besides the CNS don't exclude the patient from this category.

T High Risk (T-HR): All other T-ALL relapses

Table 2. Treatment stratification based on time, site of relapse, genetics and immunophenotype

	NON-T-IMMUNOPHENOTYPE			
	Non-high risk genetics		**Very high risk genetics**	
	IEM-CNS	**All others**	**IEM-CNS**	All others
Very early	IEM-CNS arm	VHR arm	IEM-CNS arm	VHR arm
Early	IEM-CNS arm	HR arm	IEM-CNS arm	VHR arm
Late	IEM-CNS arm	SR arm	IEM-CNS arm	VHR arm

	T-IMMUNOPHENOTYPE			
	IEM-CNS	**All others**	**IEM-CNS**	**All others**
Very early	T-IEM-CNS arm	T-HR arm	T-IEM-CNS arm	T-HR arm
Early	T-IEM-CNS arm	T-HR arm	T-IEM-CNS arm	T-HR arm
Late	T-IEM-CNS arm	T-HR arm	T-IEM-CNS arm	T-HR arm

Abbreviations: HR, high risk; IEM-CNS, isolated extramedullary relapses with CNS involvement; SR, standard risk; T, T-immunophenotype; VHR, very high risk

The time-point of relapse is defined in relation to the date of primary diagnosis and the date of completion of primary therapy (Table 3).

Table 3. Definitions of categories based on time of relapse

Time-point	After primary diagnosis		After completion of primary therapy
Very early	< 18 months	and	< 6 months
Early	≥ 18 months	and	< 6 months
Late			≥ 6 months

Completion of primary therapy is defined as the end of the antileukemic therapy of the frontline protocol. This is in most cases the end of the maintenance therapy, but may also be the last treatment after interruption of the intensive treatment, or of an inadequately short primary therapy. Data from the ALL-REZ BFM Study Group clearly show that the end of frontline therapy is more important for the prognosis than the absolute duration of 1st CR (unpublished). "Good risk" i.e. late relapses can be withheld by a prolongation of maintenance therapy, and ALL relapses have a good prognosis if they occur more than 6 months after the end of an inadequately short primary therapy.

6.2. Genetic risk groups

Very high-risk genetic features include:

- *KMT2A::AFF1* fusion, t(4;11) (q21;q23)
- *TCF3::PBX1* (also called *E2A3::PBX1*) fusion, t(1;19) (q23;p13)
- *TCF3::HLF* fusion, t(17;19) (q22;p13)
- *TP53* alterations
- hypodiploidy (< 44 chromosomes)

Treatment of Philadelphia positive relapses and those with ABL-class fusion are suggested to be risk-stratified as per their non-genetic features (immunophenotype, location and time of relapse) but with the addition of tyrosine kinase inhibitors to chemotherapy. This can be dasatinib or imatinib. If available, then we recommend dasatinib. Even more importantly dasatinib over imatinib for patients with CNS involvement and for those who relapsed after imatinib use in frontline therapy.

7. MINIMAL RESIDUAL DISEASE RESPONSE

7.1. Quantification of MRD

Numerous research evaluating MRD have demonstrated that there is a high concordance between flow cytometry (FC)-MRD and PCR-based MRD. In the ALL-IC REL protocol, MRD detection is evaluated by flow cytometry (FC) in bone marrow samples, based on Amendment to ALL IC-BFM 2009 Standard Operating Procedure ≥ 6 color FLOW-MRD detection in ALL (8). Centers are encouraged to perform FCM-MRD analyses according to the already mentioned accredited standardized flow procedure, the published guidelines of the I-BFM Flow Network and the standardized EuroFlow ALL MRD strategy [(9-14)].

7.1.1. MRD quantification by flow cytometry

In order to assess treatment response, bone marrow MRD is quantified at defined time points by flow cytometry (FC). Consensus diagnostic panels have been defined and there are published detailed guidelines for standardized staining, analysis and reporting (9). One can use any validated panel as long as it includes the markers listed below. It is mandatory to analyze the diagnostic sample with the respective standard combinations for MRD analysis of BCP using the same antibody clones and conjugates.

Quality control

Standardization and appropriate quality controls should be applied to instrument, procedure and reagents. Each instruments have a proper quality controls (QC) FacsCanto/Lyric (BD Biosciences) – CS&T beads, Navios/Dx Flex (Beckman Coulter)–Flow Check-Flow set Beads) or using Rainbow beads and target channels of 7th peak as outlined by Euroflow (www.euroflow.org).

FC MRD laboratories are suggested to participate in the following QC programs: for example Euroflow, UKNEQAS, ALLICBFM flow.

Markers and panels

Mandatory markers for ALL bone marrow samples (at least 6 colours, a CD45/CD34 or lineage marker backbone is advisable):

For BCP-ALL: CD19, CD10, CD20, CD34, CD38, CD45
Advisable markers: CD58, CD73, CD81, CD304, CD22, CD24, CD123.

The combination of monoclonal antibodies (mAbs) per tube is based on a backbone of mAbs: in BCP-ALL CD19 (pan B cell marker), CD10/CD34 (as immature/blast cell marker), CD20 (for discrimination of mature B-Cells) and CD45 (as pan leukocyte marker and quality control of BM composition). It is recommended to use the same fluorochrome conjugate for CD19 throughout all tubes, since the initial gate is set on CD19 vs SSC. If blinatumomab is used for treatment, then CD22 vs SSC or CD24 vs SSC is better to use as the initial gate.

Syto X (nucleated cell dye: DNA>RNA dye that stains nucleated cells without a need for permeabilization) should be used in order to determinate evaluability of the sample. A gate including all Syto+ events allow calculation of the % of erythroblasts in the CD45-/Syto+ region (taking into

account that there could be CD19+CD45- blasts that should be checked for in order to avoid including them in the calculation of erythroblasts). There are many SYTO dies with different emission spectra, and they can be measured in different channels (SYTO16 in FL1 (blue laser – 525nm), SYTO 40 – violet laser - 480nm)

For T-ALL: CD7, sCD3, CD5, CD99, CD34, CD8, CD48, CD4.
Advisable markers: CD56, cyCD3, TdT, CD2

The backbone markers for T-ALL: CD7 (pan T- and NK-cell marker), CD34/CD99 (blast cell marker), sCD3 (mature T-cells) and CD45 (as pan leukocyte marker and quality control of BM composition). It is recommended to use the same fluorochrome conjugate for CD7 throughout all tubes, since the initial gate is set on CD7 vs SSC. The best marker combinations for determine T cell blast are CD48 vs CD99 or sCD3 vs CD5.

This guideline for flow MRD determination is based on ALLIC-BFM 2009 FC MRD SOP Amendment, June 2014, Dworzak M. & Kappelmayer J.

Analysis and report

The end-induction MRD measurement is crucial for further treatment stratification and for the estimation of prognosis. The timing of this marrow sample is strictly the 29th day of induction. Marrow aspiration should take place on d29 irrespective of the patient's blood count or other clinical issues (e.g. ongoing infection) unless it is un-safe to perform the sampling.

A group of ≥30 leukemic cells on flow cytometry are unequivocal MRD. Hence, for a 0.1% cut-off, at least 30,000 viable cells (SYTO+) have to be counted, however, for a precise determination of MRD and MRD negativity it is necessary to acquire enough events in order to be sensitive and specific at the 0.01% level. The minimal recommended number of nucleated events to be acquired 300 000.

Most children would be pancytopenic at this time point (d29), so large volume of bone marrow should be taken for flow cytometry. Hence, we have to consider that the bone marrow sample is not diluted by blood. A sample is counted as bone marrow sample if the proportion of erythroid population is higher than 2% otherwise the sample is diluted by blood and MRD negativity is not reliable.

In case less than 30 000 viable (SYTO+) cells can be collected by FC measurements but more than 30 leukemic events are detected: the samples are MRD positive over the 0.1% level.

If less than 30 000 viable (SYTO+) cells can be collected and less than 30 leukemic events are detected: the test is not informative, not evaluable. Sampling should be repeated within a week, when the peripheral white cell count is rising.

If two reliable MRD results are obtained and are conflicting, the higher value should be used for the relevant clinical decisions.

The standard operational procedure (SOP) of day 29 flow cytometry MRD measurement is available for ALL-IC certified laboratories on request.

In case of T-ALL only the results of tubes with surface markers should be used for MRD determination.

Report

Number of total acquired events,
Number of SYTO+ events,
% of SYTO+ cells,
% of blasts among SYTO+ cells,
% of erythroblasts among SYTO+ cells.

CNS involvement

The number of blasts in CNS is also an important feature of the disease. The measurement of the number of blasts is CNS by flow cytometry is one of the best choices. At least 1.5 mL but rather 2 mL CSF should be used for the flow cytometric measurements. Because the cells are very vulnerable and can be destroyed easily in CSF, using Transfix is advisable to preserve the surface markers for flow cytometric measurements. Since the cell number in CSF is usually very low, only one tube can be run. For this tube the best panel, which proved to be the most useful to detect blasts in bone marrow, should be used for assessing the CSF.

Analysis and report

Sometimes the SYTO staining of blasts are lower in the CNS than normal cells therefore using a wider SYTO gate and FSC-SSC gating to exclude debris is necessary.
Ten blasts in CSF (rather than 30 as in marrow) are regarded as true positivity, irrespective of the volume of the CSF. The absolute number of blasts should be reported instead of the % of blasts.

7.2. Time points of residual disease evaluation

Rationale

MRD is quantified at defined time points during treatment of all patients. These measurements are paramount for indicating or avoiding SCT, or guiding salvage options.

Bone marrow MRD testing

At standard risk arm:

- SI day 15 - optional
- SI day 29 – mandatory
- Before SC2 – mandatory if any residual leukemia was detected at previous assessment (may also be important for deciding on need for salvage)
- Before Blinatumomab or SC3 – mandatory
- During consolidation: optional. However, mandatory after each cycle only if any detectable leukemia was present at the time point before the given cycle at any site (marrow MRD or any EM site positive)
- Before SCT – mandatory
- End of maintenance therapy – optional

At non-T high risk arm:

- HI day 15 – optional
- HI day 29 – mandatory
- Before Blinatumomab or HC2 – mandatory

- After each cycle optional; but mandatory if
    - any detectable leukemia was present just before the given cycle at any site (marrow MRD or any EM site positive)
    - marrow ≥ 0.1% MRD or any extramedullary leukemia was detected at the end of induction
- Before SCT – mandatory

At IEM-CNS arms:

- Day 29: perform if any marrow involvement was identified at the time of relapse
- During consolidation: after each further cycle only if, before the given cycle, any residual leukemia was detected at any site (marrow or CNS or testicle or other EM site)
- Before SCT (if SCT is indicated) – mandatory
- End of maintenance therapy – optional

At non-T very high risk arm:

- After each cycle of therapy.

At T high risk arm, for late relapse:

- HI day 15 – optional
- HI day 29 – mandatory
- After consolidation cycles – optional. But mandatory if
    - any detectable leukemia was present just before the given cycle at any site (marrow MRD or any EM site positive)
    - marrow ≥ 0.1% MRD or any extramedullary leukemia was detected at the end of induction
- Before SCT – mandatory

At T high risk arm, for early and very early relapse:

- HI day 15 – optional
- HI day 29 – mandatory
- After consolidation cycles – mandatory
- Before SCT – mandatory

Monitoring EM sites

- End of induction: mandatory to check all sites that were involved at the time of relapse.
- Later time points for patients with good response: optional.
- Later time points for patients with suboptimal response: mandatory; assess all sites (even those that were previously not affected) before each cycle of treatment.
- Before SCT: mandatory.
- End of maintenance: optional.

Marrow sampling for flow (or qPCR) MRD evaluation after any treatment cycle is ideal just before exiting aplasia. If needed, sampling and MRD measurement can be repeated after marrow recovery.

Only a baseline schedule of assessments is required for good responders to relapse-therapy. Patients who didn't reach remission at the end of induction or those who had persisting MRD afterwards may benefit from more intensive monitoring. In these patients, we recommend testing for MRD reappearance or further relapse at all possible sites after each cycle. This is because many of these patients will progress while on treatment. Leukemia may well present at sites different from original, e.g. in the CNS or the testicles while on treatment for bone marrow disease or vice versa.

7.3. Poor response, indication of salvage therapies

Unfortunately, more than 20% of SR and more than 50% of HR patients don't reach remission by the end of the induction cycle. The subsequent, 'consolidation' cycles may still induce remission. We suggest considering a shift to 'Salvage options' from the standard treatment backbones in the following situations:

A) Non-T-cell relapses:

- after HI: marrow residual leukemia ≥ 5%
- after SI; any physically or radiologically detectable residual extramedullary leukemic mass confirmed by biopsy, or in case of any detectable CSF blasts;
- after SC1 or HC1: ≥ 1% marrow MRD or any residual extramedullary leukemia, any CSF blasts
- after later time points: ≥ 0.1% marrow MRD
- MRD reappearance ≥ 0.1% after previous flow/molecular remission, any new or reappearing extramedullary leukemic involvement.

B) T-cell relapses:

- At T-IEM CNS arm:
    - if extramedullary leukemia is detected at d29 of induction
    - marrow MRD ≥ 0.1% any time after SC1
- At T-HR arm:
    - persisting or emerging CNS disease after induction
    - poor response to the first NECTAR-VEN cycle
    - in case of MRD reappearance later the course

Although BM MRD ≥ 5% and/or M2/M3 cytomorphology after SI are considered as treatment failure, we do not recommend switching to salvage options after SI, since the SC1 block that follows is practically another induction cycle. Therefore, the recommended time point for switching to salvage options for SR patients is after SC1, if marrow MRD is ≥ 1%.

These cut off values are arbitrary and only stand as approximate recommendations. The decision for switching to salvage options should be carefully assessed by the treating physician-team. E.g. for an early isolated marrow BCP-relapse and 3% marrow MRD post HC1, a blinatumomab cycle as next step in the protocol backbone is probably the best decision anyway. However, with an MRD of 30% at the same time point and CD22pos BCP immunophenotype, salvage with inotuzumab would be the recommended choice.

We recommend that these cases be discussed at some international expert forum, e.g. at a FEDRRAL meeting or via the email-list of the ALL-IC REL tumor board.

It is very important to have convincing evidence of the residual leukemia before proclaiming treatment failure and diverging from the standard treatment backbones. Hence it is strongly advised to confirm findings with additional methods. E.g., for both marrow and CSF, a combination of morphology, flow cytometry, and ideally additional FISH, qPCR, or NGS MRD methods can be used, or alternatively the sampling and the only available test repeated. Regarding extramedullary masses, biopsy and histopathology conformation is required.

Arranging funds and permissions to administer new targeted agents may be timely. In many countries, the time of a full chemotherapy cycle is needed for this, so the drug can only be given a cycle later than the decision made to mandate its use. If so, it's worth considering the initiation of these arrangements to prepare for later administration of novel expensive therapies if:

- after SI or HI, BM MRD > 1% or any residual extramedullary disease is present [These cut-offs are for initiating arrangements to prepare for later immunotherapy administration (since it might be time-consuming), and not for immediate initiation of salvage options, as stated in the text. Patients can continue to SC1 or HC1 during the time waiting for funding / permissions for immunotherapy.]
- after SC1 or HC1, any BM MRD. [Any BM MRD after SC1 or HC1 is enough to start immunotherapy orders and procedures. This is a warning sign and not an indication for immediate immunotherapy. After SC1 MRD > 1% is the cut-off for setting the indication for immediate salvage options, as stated before].
- Any MRD reappearance during the course of therapy.

Bridging to immunotherapy with a less toxic cycle may be better than proceeding with intensive chemotherapy. E.g. an SC2 or a HD-MTX or a Capizzi-MTX cycle can be used while waiting for arrangements re. inotuzumab or CAR-T therapy.

8. INDICATIONS FOR ALLOGENEIC STEM CELL TRANSPLANTATION

The SCT procedure is not part of this protocol. It is recommended to include patients with SCT indication into the national SCT studies and protocols, as far as available, to warrant a quality controlled and homogeneous treatment.

SCT is indicated for all **T-cell immunophenotype relapses** regardless of site and time to relapse. Standard indications of allogeneic hematopoietic stem cell transplantation for **non-T-cell relapses** are summarized in Table 4.

Regarding the IEM-CNS group, all such very early and early BCP-ALL relapses should be directed towards SCT. (For very early BCP-ALL relapses, CAR-T cell therapy is the preferred option where this treatment is available). In the very unusual situation of an EOI marrow MRD ≥ 0.1% detected in a late IEM-CNS BCP-ALL relapse case, such a patient should also receive SCT.

Regarding the SR group, patients are eligible for allogeneic SCT if the bone marrow MRD quantified by flow cytometry at the end of induction is ≥ 0.1%, that is 10^{-3}. If MRD cannot be quantified at this point, then even SR patients with late BM relapse are eligible for SCT.

If SCT is indicated in an SR patient but it cannot be performed (lack of resources, non-compliance, etc.), few-ten percent of chance for long term survival can still be expected with prolonged intravenous chemotherapy plus maintenance. Probably addition of immunotherapies can increase the chances somewhat further.

Resistance to therapy can also indicate SCT in the SR or the IEM-CNS arm. Bone marrow MRD reappearance at a level ≥0.1% by flow after any time post SC1 indicates SCT. Also, any detectable extramedullary leukemia at the end of induction or later should prompt SCT. For these indications, it is paramount to clearly prove the leukemic character of the cells or masses detected. For this, an extra method (e.g. morphology, FISH, QPCR MRD, NGS MRD) regarding bone marrow or CSF; histopathology re. other extramedullary sites or repeated sampling and testing with the same method should ideally confirm the fact of treatment failure. We recommend that these cases get discussed at some international expert forum, e.g. at a FEDRRAL meeting or via the email-list of the ALL-IC REL tumor board.

All **HR relapses** are eligible for allogeneic SCT. If CAR-T therapy is not available, **VHR BCP relapses** should also be treated with SCT just like for VHR T-ALL relapses (until CAR-T for T-ALL becomes available and proves to be effective).

Just like with T-cell relapses, the outlook of non-T HR/VHR patients is very poor (<10% long-term survival) without SCT, either with chemotherapy or with added humoral immunotherapies. This situation may arise when not reaching remission, or lack of donors or severe toxicities hindering SCT, or due to no consent to SCT from the patient/family. CAR-T cell therapy is the only alternative with reasonable chance for long-term cure. If this is not available either, we leave the decision of further treatment to the responsible physician, who may consider A) switching to experimental treatments (Phase I or II studies if available), or B) providing palliative therapy. There is little point in fighting with toxic chemotherapy and humoral immunotherapies for a miniscule chance for cure.

If SCT is indicated, marrow MRD of < 0.1% and clearance of all extramedullary sites are prerequisites of starting the conditioning. The chance of a post-SCT relapse is very high if these conditions are not fulfilled. Therefore, extra treatment to reduce the leukemic burden before the SCT should be attempted, or alternatively CAR-T cell therapy given for such patients. SCT with ≥ 0.1% marrow MRD or detectable residual extramedullary disease can only be considered as a desperate last resource, while informing the family about the gravely low chance for cure despite unproportionately high chance and grade of toxicities associated with SCT. It is probably wiser to turn to palliation and provide the patient with months of good quality life at home on oral chemotherapy instead of the difficulties of the SCT process and its complications.

In general, CAR-T therapy may be an alternative solution and potentially replace SCT. It is expected that the toolkit and the indications of CAR-T therapies will expand in the coming years, plus CAR-T clinical studies might take in patients with classic SCT indications. The treating physician should seek expert advice and should be allowed to decide which treatment to prefer.

Table 4. Indication for allo-SCT for patients with non-T cell immunophenotype

SCT donor	**SR**			**HR / VHR**	**IEM-CNS**	
	MRD GR	**MRD PR**	**MRD ND**	**All**	**Late***	**Early or very early**
MD**	No	Yes	Yes	Yes	No	Yes
MMD***	No	Yes	Yes	Yes	No	Yes

*Note that all T-ALL relapses require SCT. ** Matched donor is defined as at least 9 out of 10 HLA allele identical with high-resolution typing of HLA A, B, C, and Dq, Dr. *** Mismatched donor is defined as less than 9 out of 10 HLA allele identical (= more than 1 antigen mismatch). Abbreviations: GR, good response as defined by induction d29 bone marrow MRD <0.1%; HR, high risk; SCT, hematopoietic stem-cell transplantation; IEM, isolated extramedullary; MD, matched donor; MMD, mismatched donor; MRD, minimal residual disease after induction; ND, not done; PR, poor response defined as induction d29 bone marrow MRD ≥0.1%; SR, standard risk; VHR, very high risk.

9. TREATMENT

9.1. General recommendations on chemotherapy

The routine use of G-CSF is recommended after each myelotoxic consolidation cycle, also adjusting it to clinical need.

9.1.1. Dose reduction

Dose reductions may be considered in specific situations where there is unacceptable toxicity or significant treatment delays due to impaired tolerance to the treatment. It is important to reassess the need for dose reduction before each treatment element to ensure it is necessary and appropriate for the individual patient. In general, one or several cytotoxic drugs may be reduced to two-thirds (2/3) of the scheduled protocol dosage. This reduction in dose aims to minimize toxicity while still maintaining the effectiveness of the treatment. An alternative option is to replace toxic chemotherapy cycles by immunotherapy. E.g. we recommend replacing any two SC cycles by one cycle of blinatumomab for CD19+ patients with unacceptably severe toxicities from chemotherapy.

When patients experience a relapse after stem cell transplant (SCT), the treatment approach will depend on several factors, including the timing of the relapse and the patient's overall health. Here are some general considerations:

- Timing of relapse: If a patient relapses at least half a year after SCT, full-dose high-risk (HR)-arm chemotherapy may be attempted. However, if the relapse occurs earlier, the treatment intensity may need to be reduced based on individual circumstances.
- Patient health condition: The patient's overall health and ability to tolerate aggressive treatment will also be considered. If the patient has significant comorbidities or adverse effects from prior treatments, the treatment approach may need to be adjusted.
- Curative or palliative intent: Note the very poor outlook, usual chemoresistance and increased risk of severe toxicities in patients who relapse within 6 months of SCT. If the goal of the therapy is curative, a more aggressive approach may be taken, preferring targeted treatment options. However, if the goal is palliative or symptom management, a less intense treatment may be more appropriate.
- Individual basis: The decision to reduce treatment intensity should be made on an individual basis.

Ideally, immunotherapies and dose-reduced chemotherapy followed by CAR-T cell therapy should be offered to patients who relapse after SCT. If this is not available, then a second SCT may offer the chance for cure. However, in general, one should not attempt unreduced chemotherapy as per this protocol for relapses that occur within half a year after SCT, due to its expectable severe toxicity. For these cases, one can try immunotherapy and reduced chemotherapy at individual basis. We recommend that difficult cases be discussed at some international expert forum, e.g. at a FEDRRAL meeting or via the email-list of the ALL-IC REL tumor board.

9.1.1.1. Infants or those with body weight below 10 kg

We recommend following the instructions of the Interfant-21 trial protocol in this regard. In the rare case when an infant or a patient below 10 kg body weight relapses, calculate the chemotherapy doses based on body surface and the reduction described in table 5. However, don't reduce doses of intrathecal drugs, glucocorticoids, asparaginase, vincristine, folinic acid and blinatumomab.

Table 5. Dose reduction according to age

Age	Dose modification
0 – 2 months:	2/3 of the calculated dose based on BSA
2 – 6 months:	3/4 of the calculated dose based on BSA
> 6 months	>6 months Full dose

9.1.1.2. Obesity

There are no general recommendations for dose reduction in obese patients receiving chemotherapy. Dosing should be individualized based on several factors, including body weight, BSA, and renal and hepatic function. We recommend the equation below.

To calculate a corrected BSA for children who have BMI higher than 98 centiles, use

- dosing weight: use the weight corresponding to the BMI at 98 centiles (kg/m^2) at the patient's height;
- patient's actual height.

In general, the doses should be capped calculations with 2 m^2 body surface for very tall and/or obese patients.

9.1.1.3. Down syndrome patients

Patients with Down syndrome who experience a relapse of acute lymphoblastic leukemia (ALL) are known to have a poorer tolerance to treatment, with a higher induction death rate and mortality rate in CR2 compared to other patients. This population may also have poorer tolerance to high-dose methotrexate (HD-MTX) specifically. To manage the risk of treatment-related toxicities in this population, it is recommended to give the first application of HD-MTX at shortened infusion duration of 24 hours. Additionally, the dose may be reduced to 500 mg/m^2 if necessary, based on the patient's individual circumstances and overall health status. If the initial application of high-dose methotrexate (HD-MTX) given at a reduced dose and infusion duration of 24 hours is well tolerated by the patient, subsequent HD-MTX treatments can be adjusted according to the protocol's scheduled doses and durations. Regarding other drugs used in the treatment protocol, there are no general recommendations for dose reduction in patients with Down syndrome. However, due to their potentially poorer tolerance to treatment, the option of dose reduction may be applied more generously in patients with Down syndrome compared to other patients and the antimicrobial prophylaxis options be maximized. Whenever possible, risky intensive chemotherapy should be replaced by blinatumomab or other less toxic immunotherapy.

9.1.2. Treatment intensity

The analysis of previous studies indicates that treatment intensity plays a crucial role in the success of relapse therapy. Therefore, it is important to carefully consider the timing and duration of treatment-free intervals, especially during the first two cycles of therapy. Prolonging these intervals should only be considered in cases of life-threatening complications where the patient is at immediate risk. The entry criteria for subsequent treatment cycles, including blood count criteria, are set to prevent the early initiation of the next block and to ensure that the patient has recovered sufficiently to tolerate further treatment.

If there is prior experience with individual patients that indicates a high likelihood of delays or significant risks associated with the timely delivery of therapy due to intolerance, then the treatment protocol may allow for dose reduction. Dose reduction can help mitigate the adverse effects of treatment and improve the patient's ability to tolerate therapy without compromising its overall effectiveness. Indeed, delaying therapy may be necessary if the patient experiences severe toxicity and the decision to delay treatment should be reassessed regularly, at least every other day. It is essential for the treating physician to closely monitor the patient's response to treatment and adjust the protocol as needed to accommodate their individual treatment tolerance and needs.

9.1.3. Novel drugs that are expensive or difficult to access

Some new agents or therapeutic options are not available in certain ALL-IC / ALL-IC REL countries, or they are not funded. At the time of writing this protocol, we are aware of such problems regarding blinatumomab, inotuzumab, CAR-T therapy (tisagenlecleucel), nelarabine, daratumumab. To less extent, access to venetoclax and bortezomib may also be problematic. For patient populations at some rural areas, access to allogeneic SCT may be limited by long-range travel and displacement of (a part of) the family.

This protocol was created in a way to accommodate these difficulties. On the one hand, the authors aimed to point towards the best treatment providing the highest chance of long-term cure, by the lowest risk of severe toxicities. On the other hand, if resources are limited, then the option is available to omit the agents that are not available or chose treatment arms that are possible to provide among the given circumstances. The treating physicians should decide on the optimal treatment adapted to their situation.

Even if none of the above-mentioned novel treatment options are available, centers can follow this guidance. It is absolutely foreseeable that many treatment pathways will be modified, either due to non-response, or toxicities – and to be realistic, also due to drug-access issues. These patient stories are also very useful for analysis and learning. So, we strongly request colleagues to include these cases when they fill their anonymized patient data into the ALL-IC REL registry.

9.2. Systemic treatment

9.2.1. Non-T cell SR arm

Induction consists of the optional prephase followed by the standard induction cycle (SI) according to the ALL-IC REL 2016 treatment guidance. On day 29, marrow cytology and MRD status are examined, and extramedullary sites are assessed. Patients with MRD ≥ 0.1% (at least 1 leukemic cell out of 1000 mononuclear cells equal to MRD ≥ 10^{-3}) and/or those with residual extramedullary disease at the EOI are allocated to allogeneic SCT after the blinatumomab (or alternatively the SC3) cycle. The other patients continue with the consolidation cycles and maintenance therapy of 24 months duration and a total treatment duration of 131 weeks.

According to COG's AALL 1331 study (3), replacing chemotherapy with blinatumomab in a cohort of low-risk 1st relapses comes with survival benefit. Therefore, it has been decided that blinatumomab is preferred to two SC cycles (SC3 and SC4 type, one each) from the ALL-IC REL 2016 protocol to increase long term cure rate and to reduce toxicity. It is an option to adhere to the ALL-IC REL 2016 protocol without giving blinatumomab but giving all SC cycles (as ALL-IC REL 2016 protocol) in the following situations:

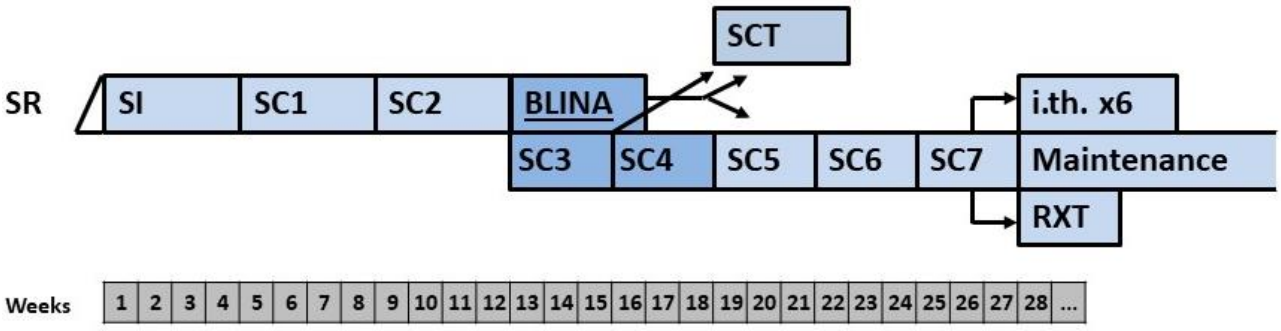
- Any contraindication of blinatumomab, e.g. CSF WBC > 5/uL or CNS infiltration seen radiologically at the time when blinatumomab would be administered,
- in case of severe blinatumomab toxicity,
- CD19neg blasts,
- if blinatumomab is not available.

At the beginning of maintenance therapy, all patients without CNS irradiation receive triple intrathecal chemotherapy once every 4 weeks for a total of 6 times. Patients with CNS-3 involvement receive cranial irradiation at a dose of 18 Grays (Gy) and have no intrathecal therapy during maintenance treatment. Boys with testicular relapse or possibly those with other extramedullary relapses receive local treatment as described in section 9.3. Local therapy.

SR definition

See chapter 6.1. to identify patients to be stratified to this treatment backbone.

Overview of SR treatment arm:



9.2.1.1. SI block

(SR arm remission induction, week 1-4) (from ALL-IC REL 2016)

Agent	Dosage	Application	Week 1							Week 2							Week 3							Week 4						
Dexamethasone	20 mg/m²/d	PO																												
Vincristine	1,5 mg/m²	IV																												
Methotrexate	1 g/m²	IV 36hr																												
Cytarabine	2x3 g/m²/d	IV 3hr																												
PEG-Asparaginase	1000 U/m²	IV 2hr/IM																												
Methotrexate	Age dep.	IT																												
Cytarabine	Age dep.	IT																												
Prednisolone	Age dep.	IT																												
		Days	1	2	3	4	5	6	7	1	2	3	4	5	6	7	1	2	3	4	5	6	7	1	2	3	4	5	6	7

Criteria to start and guide the course

No specific blood count or clinical condition is mandatory to start week 1 and 3. In case of renal insufficiency (elevated creatinine) or major liver insult (but isolated transaminase elevation of any grade without relevant abnormality of the synthetic and metabolic functions of the liver are not regarded as major liver insult) MTX 1g/m² should be avoided and induction should be started with week 3. If possible, administer Week 1 treatment (formerly cycle F1) afterwards from day 15, so that the day 29 assessment of treatment response can be clear-cut.

Diagnostic measures

Bone marrow aspirate at day 1 of week 3 is optional. Results might help to guide treatment in case of clinical complications.

Bone marrow assessment on day 29 is compulsory; it is of key importance for further treatment stratification.

- A decisive unequivocal flow report is expected with MRD either ≥ 0.1% to indicate SCT or <0.1% to indicate chemotherapy without SCT.
- A nonrepresentative marrow at this time point warrants repeated sampling and the start of the next cycle should be postponed accordingly.
- If aplastic marrow is gained, blasts ≥ 0.1% cannot be proven and the sensitivity of flow cytometry MRD 0.1% is not reached, then the aspiration should be repeated within one week.
- Patients with no reliable d29 MRD assessment have established indication to receive SCT, see Table 4 in Chapter 8.
- In case of uncertainty, we recommend that these cases be discussed at some international expert forum, e.g. at a FEDRRAL meeting or via the ALL-IC REL tumor board email-list.

Note: if, after the SI cycle, there is evidence of residual CNS leukemia, consider a shift to ‘Salvage options’ from the standard treatment backbones (see Chapter 9.4).

Dexamethasone

20 mg/m² (max. 40 mg/day) orally divided in two daily doses on day 1-5 of week 1 and 3. A dexamethasone prophase can be started from day -5 until day 0 at a dose of 6 mg/m² in case of high tumor burden or to bridge the time until start of the study.

Vincristine

1.5 mg/m² (maximum single dose 2 mg) as 15 min short infusion (or as IV bolus not on the same day as IT therapy) on day 1 and 6 of week 1 and on day 1 of week 3.

Methotrexate
1 g/m² IV over 36 hours starting on day 1 of week 1. The first 10% is given as a 30 min bolus and the remaining 90% as a continuous infusion for 35.5 hours. Concomitant alkaline hydration with 3000 ml/m²/24 hours is given on day 1 and 2. Rescue with folinic acid of 15 mg/m² is given at 48 and 54 hours after start of MTX. Serum methotrexate levels can be measured at 36h and 48h after the start of the MTX infusion, and the dose of folinic acid adapted to elevated MTX serum levels. See further details at "Impaired elimination of methotrexate" in Chapter 11.2.

PEG-asparaginase
1000 units/m² IM or as 2-hour IV infusion on day 4 of week 1 and 3. The infusion of PEG-asparaginase should be started at a reduced rate and increased stepwise, if applicable. See further notes on alternative agents "Asparaginase preparations" in Chapter 9.5.1.

Cytarabine
3 g/m² as 3-hour infusion every 12 hours on day 1 and 2 (a total of 4 doses) of week 3. A prophylaxis of conjunctivitis with eye drops and of neuropathy with vitamin B6 at a dose of 100 mg/m² IV is recommended prior to each cytarabine dose.

Intrathecal chemotherapy
Age adapted doses of methotrexate, cytarabine and prednisolone (see Table 6 in section 9.3.3.) are administered on day 2 of week 1 and on day 5 of week 3. This can be administered after the methotrexate infusion. Patients with CNS-2/3 status should receive additional intrathecal injections on day 5 of week 1 and 2 plus weekly doses until two totally blast-negative CSF samples are obtained (to be checked ideally by both cytomorphology and flow cytometry of CSF).

9.2.1.2. SC1 block
(SR arm, 1st cycle of consolidation, week 5-8) (from ALL-IC REL 2016)

Agent	Dosage	Application	Week 5							Week 6							Week 7							Week 8						
Dexamethasone	6 mg/m²/d	PO																												
Vincristine	1,5 mg/m²	IV																												
Idarubicin	6 mg/m²	IV 2hr																												
PEG-Asparaginase	1000 U/m²	IV 2hr / IM																												
Methotrexate	Age dep.	IT																												
Cytarabine	Age dep.	IT																												
Prednisolone	Age dep.	IT																												
		Days	1	2	3	4	5	6	7	1	2	3	4	5	6	7	1	2	3	4	5	6	7	1	2	3	4	5	6	7

Diagnostic measures
Bone marrow aspirate at induction day 1 of week 5 is obligatory to assess cytological remission and MRD response (see chapter 7.2).
Note: If after the SI cycle, residual CNS leukemia is detected, consider a shift to 'Salvage options' from the standard treatment backbones (see Chapter 9.4) instead of giving an SC1 cycle. We recommend that these cases get discussed at some international expert forum, e.g. at a FEDRRAL meeting or via the email-list of the ALL-IC REL tumor board.

Criteria to start and guide the SC1 course

Start of week 5 and application of vincristine / idarubicin at weeks 6-8 requires recovering blood counts (neutrophils ≥ 0.5 x 10^9/L, platelets ≥ 50 x 10^9/L), mucositis less than grade 3, and no essential organ dysfunction. Dexamethasone and asparaginase should be given without discontinuation.

Dexamethasone

6 mg/m² orally divided in two daily doses on day 1-7 of week 5 and 6, tapering the dose to 3 mg/m² on day 1-3 of week 7, 1.5 mg/m² on day 4-6 of week 7, and 0.75 mg/m² on day 7 of week 7 and day 1-2 of week 8.

Vincristine

1.5 mg/m² (maximum single dose 2 mg) as 15 min short infusion (or as IV bolus not on the same day as IT therapy) on day 1 of week 5-8.

Idarubicin

6 mg/m² as infusion over 2 hours on day 1 of week 5-8.

PEG-asparaginase

1000 units/m² as 2-hour infusion on day 1 of week 5 and day 4 of week 6. The infusion of PEG-asparaginase is started at a reduced rate and increased stepwise, if applicable. See further notes on alternative agents "Asparaginase preparations" in Chapter 9.5.1.

Intrathecal chemotherapy

Age adapted doses of methotrexate, cytarabine and prednisolone (see Table 6 in section 9.3.3.) are administered on day 1 of week 5 and 7. CNS-3 patients receive an additional triple intrathecal therapy on day 1 of week 6.

9.2.1.3. SC2 block

(SR arm, 2nd cycle of consolidation, week 9-12) (ALL-IC REL 2016)

Agent	Dosage	Application	Week 9							Week 10							Week 11							Week 12						
Cyclophosphamide	1 g/m²/d	IV 1 hr	▮																											
Cytarabine	75 mg/m²	IV			▮	▮	▮	▮				▮	▮	▮	▮															
Thioguanine	60 mg/m²	PO	▬	▬	▬	▬	▬	▬	▬	▬	▬	▬	▬	▬	▬	▬														
Methotrexate	Age dep.	IT			▮							▮																		
Cytarabine	Age dep.	IT			▮							▮																		
Prednisolone	Age dep.	IT			▮							▮																		
		Days	1	2	3	4	5	6	7	1	2	3	4	5	6	7	1	2	3	4	5	6	7	1	2	3	4	5	6	7

Diagnostic measures

Bone marrow aspirate at day 1 of week 9 is optional to assess cytological remission and MRD response. However, for patients with detectable residual leukemia at the end of SI, this bone marrow test is mandatory.

Note: If after SC2 cycle or later time points ≥ 0.1% marrow MRD is detected or there is evidence of residual extramedullary disease or marrow MRD reappearance ≥ 0.1% after previous flow/molecular remission, consider a shift to 'Salvage options' from the standard treatment backbone (see Chapter

9.4). We recommend that these cases get discussed at some international expert forum, e.g. at a FEDRRAL meeting or via the email-list of the ALL-IC REL tumor board.

Criteria to start and guide the course
Start of week 9 requires recovering blood counts (neutrophils $\geq 0.5 \times 10^9$/L, platelets $\geq 50 \times 10^9$/L), mucositis less than grade 3, and no essential organ dysfunction. Cytarabine courses on day 3 of weeks 9 and 10 are given irrespectively of the blood count as long as the clinical status allows for treatment continuation.

Cyclophosphamide
1 g/m² as 1-hour infusion on day 1 of week 9. Mesna is administered at a dose of 400 mg/m² IV prior to as well as 4 and 8 hours after the cyclophosphamide. Hydration with 3000 ml/m² is administered for 24 hours from start of cyclophosphamide.

Cytarabine
75 mg/m² as IV bolus on day 3-6 of week 9 and 10.

Thioguanine
60 mg/m² orally on day 1-7 of week 9 and 10.

Intrathecal chemotherapy
Age adapted doses of methotrexate, cytarabine and prednisolone (see Table 6 in section 9.3.3.) are administered on day 3 of week 9 and 10.

9.2.1.4. Blinatumomab block

(SR arm, 3rd cycle of consolidation, week 13-16) (Preferred to be given instead of SC3 and SC4 cycle of the ALL-IC REL 2016 protocol)

For detailed information about the Blina cycle that will be given instead of SC3 and SC4 cycles, see the Blinatumomab section in Chapter 9.4.3.

Diagnostic measures
Bone marrow aspiration and MRD assessment before the blinatumomab cycle is obligatory.

Criteria to start and guide the course
Start of week 12 requires recovering blood counts (neutrophils $\geq 0.5 \times 10^9$/L, platelets $\geq 50 \times 10^9$/L), and no essential liver dysfunction.

Blinatumomab
A single course of blinatumomab consists of 28 days of continuous intravenous infusion, followed by 7-14 treatment free days (35-42 days in total).

Intrathecal chemotherapy
Age adapted doses of methotrexate, cytarabine and prednisolone (see Table 6 in section 9.3.3.) are administered on days 15 and 28 of blinatumomab infusion.

9.2.1.5. SC3, SC5, SC7 blocks

(SR arm, 3rd, 5th, 7th cycle of consolidation, week 13-15, 19-21, 25-27) (from ALL-IC-REL 2016)

Agent	Dosage	Application	Week 13, 19, 27							Week 14, 20, 26							Week 15, 21, 27						
Dexamethasone	20 mg/m²/d	PO																					
Mercaptopurine	100 mg/m²/d	PO																					
Vincristine	1,5 mg/m²	IV																					
Methotrexate	1 g/m²	IV 36hr																					
Cytarabine	2x2 g/m²/d	IV 3hr																					
PEG-Asparaginase	1000 U/m²	IV 2hr / IM																					
Methotrexate	Age dep.	IT																					
Cytarabine	Age dep.	IT																					
Prednisolone	Age dep.	IT																					
		Days	1	2	3	4	5	6	7	1	2	3	4	5	6	7	1	2	3	4	5	6	7

Diagnostic measures

If an SC3 cycle is given, bone marrow aspiration at day 1 of week 13 is obligatory to confirm cytological remission and MRD response. Before SC5 and SC7, bone marrow aspiration and CSF tests are indicated if the patient had any detectable leukemia just before the previous treatment block. If allogeneic SCT is planned for week 17 (or 19 if blinatumomab was administered), BM aspirate is done in context with the SCT preparation and can be postponed accordingly.

Criteria to start and guide the course

Start of the course requires recovering blood counts (neutrophils $\geq 0.5 \times 10^9$/L, platelets $\geq 50 \times 10^9$/L), mucositis less than grade 3, and no essential organ dysfunction.

In case of prolonged treatment delays, dose reductions have to be considered according to those described at chapter 9.1.1 have to be considered. SC3 cycle is indicated if blinatumomab can´t be administered. After the SC3 course, at week 17, allogeneic SCT is scheduled, if indicated.

Dexamethasone

20 mg/m² (maximum 40 mg/d) orally, divided in two daily doses on day 1-5, and 10 mg/m² on day 6 of these blocks.

6-Mercaptopurine

100 mg/m² orally on day 1-5 of these blocks.

Vincristine

1.5 mg/m² (maximum single dose 2 mg) as 15 min short infusion (or as IV bolus not on the same day as IT therapy) on day 1 and 6 of these blocks.

Methotrexate IV

1 g/m² IV over 36 hours starting on day 1 of each course. Out of the total, 10% is given as a 30 min bolus and the remaining 90% as a continuous infusion for 35.5 hours. Concomitant alkaline hydration with 3000 ml/m²/24 hours is given on day 1 and 2. Rescue with folinic acid of 15 mg/m² is given at 48 and 54 hours after start of MTX. Serum methotrexate levels can be measured at 36h and 48h after

the start of the MTX infusion, and the dose of folinic acid adapted to elevated MTX serum levels. The management is guided according to recommendations "Impaired elimination of methotrexate" in Chapter 11.2.

Cytarabine IV
2 g/m² as 3-hour-infusion every 12 hours on day 5 (a total of 2 doses) of SC3, SC5, SC7 cycles. A prophylaxis of conjunctivitis with eye drops and of neuropathy with vitamin B6 at a dose of 100 mg/m² IV is recommended prior to each cytarabine dose.

PEG-asparaginase
1000 units/m² as 2-hour infusion on day 6 of each of these cycles. The infusion of L-asparaginase should be started at a reduced rate and increased stepwise, if applicable. See further notes on alternative agents "Asparaginase preparations" in Chapter 9.5.1.

Intrathecal chemotherapy
Age adapted doses of methotrexate, cytarabine and prednisolone (see Table 6 in section 9.3.3.) are administered on day 2 of each course after the methotrexate infusion.

9.2.1.6. SC4, SC6 blocks

(SR arm, 4th and 6th cycle of consolidation, week 16-18, 22-24) (ALL-IC-REL 2016)

Agent	Dosage	Application	Week 13, 19, 27							Week 14, 20, 26							Week 15, 21, 27						
Dexamethasone	20 mg/m²/d	PO																					
Thioguanine	100 mg/m²/d	PO																					
Vindesine	3 mg/m²	IV																					
Methotrexate	1 g/m²	IV 36hr																					
Ifosfamide	400 mg/m²	IV 1hr																					
Daunorubicine	35 mg/m²	IV 24hr																					
PEG-Asparaginase	1000 U/m²	IV 2hr / IM																					
Methotrexate	Age dep.	IT																					
Cytarabine	Age dep.	IT																					
Prednisolone	Age dep.	IT																					
		Days	1	2	3	4	5	6	7	1	2	3	4	5	6	7	1	2	3	4	5	6	7

Diagnostic measures
Before SC4 and SC6, bone marrow aspiration and CSF tests are indicated if the patient had any detectable leukemia just before the previous treatment block. Otherwise assessment at this time point is optional.

Criteria to start and guide the course
Start of the course requires recovering blood counts (neutrophils ≥ 0.5 x 10^9/L, platelets ≥ 50 x 10^9/L), mucositis less than grade 3, and no essential organ dysfunction. In case of prolonged treatment delays, dose reductions according to the specific guidelines outlined in chapter 9.1.1 have to be considered. SC4 cycle is indicated after SC3 cycle if blinatumomab can´t be administered.

Dexamethasone
20 mg/m² (maximum 40 mg/d) orally divided in two daily doses on day 1-5, and 10 mg/m² on day 6 of each SC4, or SC6 course, week 16 and 22.

Thioguanine
100 mg/m² orally on day 1-5 of each SC4 or 6 course, week 16 and 22.

Vindesine
3 mg/m² as 15 min short infusion (or as IV bolus not on the same day as IT therapy) on day 1 of each SC4 or 6 course, week 16 and 22. In case vindesine is not available, it can be substituted with vincristine 1.5 mg/m² (maximum single dose 2 mg) as 15 min short infusion (or as IV bolus not on the same day as IT therapy).

Methotrexate IV
1 g/m² IV over 36 hours starting on day 1 of each SC4, or SC6 course, week 16 and 22. 10% of the dose is given as a 30 min bolus and the remaining 90% as a continuous infusion for 35.5 hours. Concomitant alkaline hydration with 3000 ml/m²/24 hours is given on day 1 and 2. Rescue with folinic acid of 15 mg/m² is given at 48 and 54 hours after start of MTX. Serum methotrexate levels can be measured at 36h and 48h after the start of the MTX infusion, and the dose of folinic acid adapted to elevated MTX serum levels. The management is guided according to recommendations "Impaired elimination of methotrexate" in Chapter 11.2.

Ifosfamide
400 mg/m² as a 1-hour infusion day 1-5 of each SC4 or SC6 course, week 16 and 22. Mesna is administered at a dose of 200 mg/m² IV prior to as well as 4 and 8 hours after Ifosfamide. Hydration with 1500 ml/m²/day is administered from start of Ifosfamide until day 5.

Daunorubicin
35 mg/m² as a 24-hour infusion on day 5 of each SC4 or SC6 course, week 16 and 22.

PEG-asparaginase
1000 units/m² as 2-hour infusion on day 6 of each SC4 or SC6 course, week 16 and 22. The infusion of L-asparaginase should be started at a reduced rate and increased stepwise. See further notes on alternative agents "Asparaginase preparations" in Chapter 9.5.1.

Intrathecal chemotherapy
Age adapted doses (see Table 6 in section 9.3.3.) of methotrexate, cytarabine, and prednisolone are administered on day 2 of each SC4, SC6 course, week 16 and 22, after the methotrexate infusion. Patients with CNS-3 stage at relapse receive an additional triple IT therapy on day 6.

9.2.1.7. Maintenance chemotherapy for patients with no CNS3 involvement at time of relapse

(SR arm, week 28-131) (ALL-IC-REL 2016 protocol SR arm)

Agent	**Dosage**	**Application**	**week 28-49**																					**week 49-131**						
Mercaptopurine	50 mg/m²/d	PO																												
Methotrexate	20 mg/m²/wk	PO																												
Methotrexate	Age dep.	IT																												
Cytarabine	Age dep.	IT																												
Prednisolone	Age dep.	IT																												
		Weeks	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	.	.	.	.	.	131

General structure

Maintenance therapy is given throughout from week 28 for a total of 104 weeks (=2 years). During the first 24 weeks, a total of 6 applications of intrathecal triple chemotherapy are given only to those patients with no indication for radiotherapy.

Diagnostic measures

Bone marrow aspirate at day 1 of week 28 at start of maintenance therapy and at the end of maintenance therapy (after week 131) are not obligatory and can be done at the discretion of the treating investigator to confirm cytological remission and continuous MRD response.

Criteria to start and guide the course

Start of maintenance (week 28) requires leukocytes >2.0 x 10^9/L, neutrophils > 0.5 x 10^9/L, platelets > 80 x 10^9/L, and a clinical status allowing for treatment continuation.

The dosing of mercaptopurine and methotrexate is adapted to the leukocyte count which should range between 2.0 and 3.0 x 10^9/L (150% of 6MP/MTX dose if WBC > 3.0 x 10^9/L, 100% if WBC > 2.0 and < 3.0 x 10^9/L, 50% if WBC > 1.0 and < 2.0 x 10^9/L or if the lymphocyte count drops below 0.3 x 10^9/L, 0% if WBC < 1.0 x 10^9/L).

Both drugs are started with reduced doses and weekly increased. In case of drop of leukocytes < 1.0 x 10^9/L, neutrophils < 0.5 x 10^9/L, and/or platelets < 80 x 10^9/L or in case of febrile episodes, the treatment is interrupted.

Mercaptopurine

50 mg/m² (adapted individually to leukocyte counts and toxicity) orally every day (day 1-7 of week 28 - 131). Doses should be taken at least one hour after the evening meal without milk products.

Methotrexate

20 mg/m² (adapted individually to leukocyte counts and toxicity) orally once per week as a single dose.

Radiotherapy

If indicated, testicular irradiation can be applied in parallel with maintenance chemotherapy (also mercaptopurine) at the beginning of the maintenance phase.

9.2.1.8. Maintenance chemotherapy for those with CNS3 stage at time of relapse

(SR arm, week 28-131) (ALL-IC-REL 2016 protocol SR arm)

Agent	Dosage	Application	Weeks 28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	.	.	.	.	.	131
Mercaptopurine*	50 mg/m^2/d	PO					■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■
Methotrexate	20 mg/m^2	PO	▮	▮	▮	▮	▮	▮	▮	▮	▮	▮	▮	▮	▮	▮	▮	▮	▮	▮	▮	▮	▮	▮	▮	▮	▮	▮	▮	▮
Radiotherapy			■	■	■	■																								

General structure
Maintenance therapy is given throughout from week 28 for a total of 104 weeks (= 2 years).

Diagnostic measures
Bone marrow aspirate at day 1 of week 28 at start of maintenance therapy and at the end of maintenance therapy (after week 131) are not obligatory and can be done at the discretion of the treating investigator to confirm cytological remission and continuous MRD response.

Criteria to start and guide the course
Start of maintenance (week 28) requires leukocytes > 2.0 x 10^9/L, neutrophils > 0.5 x 10^9/L, platelets > 80 x 10^9/L, and a clinical status allowing for treatment continuation.

The dosing of mercaptopurine and methotrexate is adapted to the leukocyte count which should range between 2.0 and 3.0 x 10^9/L (150% of 6MP/MTX dose if WBC > 3.0 x 10^9/L, 100% if WBC > 2.0 and < 3.0 x 10^9/L, 50% if WBC > 1.0 and < 2.0 x 10^9/L or if the lymphocyte count drops below 0.3 x 10^9/L, 0% if WBC < 1.0 x 10^9/L).

Both drugs are started with reduced doses and weekly increased. In case of drop of leukocytes < 1.0 x 10^9/L, neutrophils < 0.5 x 10^9/L, and/or platelets < 80 x 10^9/L or in case of febrile episodes, the treatment is interrupted.

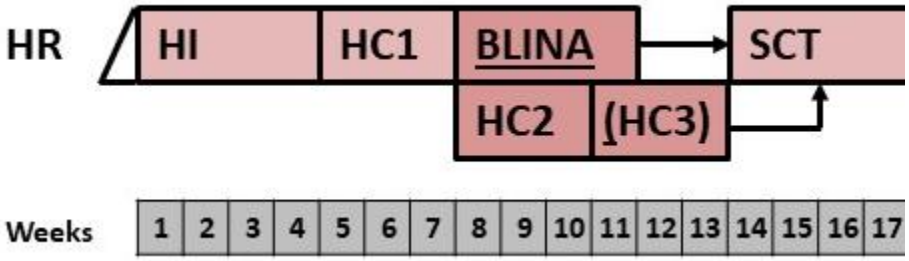
Mercaptopurine
50 mg/m² (adapted individually to leukocyte counts and toxicity) orally every day (day 1-7 of week 28 - 131). Doses should be taken at least one hour after the evening meal without milk products. *It is started after completion of radiotherapy. It is not used along with radiotherapy because of increased risk of CNS tumors.*

Methotrexate
20 mg/m² (adapted individually to leukocyte counts and toxicity) orally once per week as a single dose.

9.2.2. Non-T cell HR arm

See chapter 6.1. to identify patients to be stratified to this treatment backbone.

Overview of the HR treatment arm:



HR patients will receive induction (HI) with a modified ALL R3 schema.
Studies demonstrated the benefit of blinatumomab over consolidation chemotherapy in high risk relapses. For this reason, blinatumomab is preferred over HC2 and HC3 in the previous protocol (2, 3). Exceptions, when HC2 and HC3 should be chosen instead of blinatumomab:

- for T-ALL or any other CD19neg cases;
- in case of severe blinatumomab toxicity;
- any contraindication of blinatumomab, e.g. CSF WBC > 5/µl or CNS infiltration seen radiologically at the time when blinatumomab would be administered;
- if blinatumomab is not available.

Consolidation consists of an HC1 course at week 5 and followed by one cycle of blinatumomab at week 8, then allogeneic SCT.

In case of not using blinatumomab, consolidation consists of an HC1 course at week 5, HC2 course at week 8 and HC3 course at week 11, then transplantation.

9.2.2.1. Non-T cell HI block

(HR arm, induction cycle, week 1-4) (originally R3 induction)

Agent	Dosage	Application	Week 1							Week 2							Week 3							Week 4						
Dexamethasone	20 mg/m^2/d	PO	■	■	■	■	■	■	■								■	■	■	■	■	■	■							
Vincristine	1,5 mg/m^2	IV			■							■							■							■				
Mitoxantrone	10 mg/m^2	IV 1hr	■	■																										
PEG-Asparaginase	1000 U/m^2	IV 2hr / IM			■														■											
Methotrexate	Age dep.	IT	■							■							□													
Cytarabine	Age dep.	IT	■							■							□													
Prednisolone	Age dep.	IT	■							■							□													
		Days	1	2	3	4	5	6	7	1	2	3	4	5	6	7	1	2	3	4	5	6	7	1	2	3	4	5	6	7

Criteria to start and guide the course

No specific blood count or clinical condition is mandatory to start the HI cycle. In case of some major comorbidity, complication or critical situation, the treatment for these may precede the start of HI

cycle. A steroid prephase may be applied on the discretion of the responsible physician. This may also be the choice if the patient is at high risk of tumor lysis syndrome.

Diagnostic measures
Bone marrow aspirate MRD testing is mandatory on day 1 of week 5.

Dexamethasone
20 mg/m² (maximum 40 mg/d) orally divided into two daily doses on days 1-5 of weeks 1 and 3. A dexamethasone prephase can be started from day -5 until day 0 at a dose of 6 mg/m² in case of high tumor burden or to bridge the time until start of the study.

Vincristine
1.5 mg/m² (maximum single dose 2 mg) as 15 min short infusion or as IV bolus (not on the same day as IT therapy) on day 3 of weeks 1-4.

Mitoxantrone
10 mg/m^2 as 1-hour infusion on days 1 and 2 of week 1.

PEG-asparaginase
1,000 units/m² as a 2-hour infusion or intramuscularly on day 3 of weeks 1 and 3. The infusion of asparaginase should be started at a reduced rate and increased stepwise, if applicable. See further notes on alternative asparaginase agents at "Asparaginase preparations" in Chapter 9.5.1.

Intrathecal chemotherapy
Age adapted dose (see Table 6 in section 9.3.3.) of triple intrathecal chemotherapy is administered on day 1 of weeks 1 and 2. Patients with CNS involvement receive additional intrathecal injections weekly thereafter, until two leukemia free CSF samples are obtained.

9.2.2.2. Non-T cell HC1 block
(HR arm, 1st cycle of consolidation, weeks 5-7) (Modified from ALL AIEOP-BFM HR1 course)

Agent	Dosage	Application	Week 5							Week 6							Week 7						
Dexamethasone	10 mg/m^2/d	PO																					
Vincristine	1.5 mg/m^2/d	IV																					
Cytarabine	2 gr/m^2	IV 3 hr																					
Methotraxate	1 gr/m^2	IV 36 hr																					
Cyclophosphamide	200 mg/m^2	IV 1 hr																					
PEG-Asparaginase	1000 IU/m^2	IV 2 hr / IM																					
Methotrexate	Age dep.	IT																					
Cytarabine	Age dep.	IT																					
Prednisolone	Age dep.	IT																					
		Days	1	2	3	4	5	6	7	1	2	3	4	5	6	7	1	2	3	4	5	6	7

Diagnostic measures

Bone marrow aspirate on day 1 of week 5 of HI induction is mandatory to assess cytological remission and MRD response. Results should reveal a representative marrow. In case of a non-representative or aplastic marrow, the analysis should be repeated, and the start of cycle HC1 postponed accordingly. If ≥ 5% blasts are identified, the team should consider shifting to rescue options.

Criteria to start and guide the course

Start of the course requires recovering blood counts (neutrophils ≥ 0.5 x 10^9/L, platelets ≥ 50 x 10^9/L), mucositis less than grade 3, and no essential organ dysfunction. In case of prolonged treatment delays, dose reductions may be considered according to the specific guidelines outlined in section 9.1 "General recommendations on chemotherapy".

Dexamethasone

10 mg/m² orally divided into two daily doses on days 1-5 of week 5.

Vincristine

1.5 mg/m² (maximum single dose 2 mg) as 15 min short infusion or as IV bolus (not on the same day as IT therapy) on days 1 and 6 of week 5.

HD-Cytarabine

2 g/m^2/dose as 3-hour-long infusion every 12 hours (total of 2 doses) on day 5 of week 5. Prophylaxis of conjunctivitis with eye drops every 6 hours during administration and of neuropathy with vitamin B6 at a dose of 100 mg/m^2 IV prior to each cytarabine dose is recommended.

Methotrexate IV

1 g/m^2 IV over 36 hours starting on day 1 of week 5. 10% is given as a 30 min bolus and the remaining 90% as a continuous infusion over 35.5 hours. Concomitant alkaline hydration with 3000 ml/m^2/24 hours is given on day 1 and 2. Serum methotrexate levels can be measured at 36 hours and 48 hours after start of MTX infusion. Rescue with folinic acid at 15 mg/m^2 is given at 48 and 54 hours after start of MTX. The dose can be adapted to elevated MTX serum levels. See further details under "Impaired elimination of methotrexate" in Chapter 11.2.

Cyclophosphamide

200 mg/m^2/dose as a 1-hour-long infusion every 12 hours on days 2-4 (total of 5 doses). Mesna is administered at 70 mg/m^2/dose before and at 4 and 8 hours after start of each cyclophosphamide infusion. Hydration with 3000 ml/m^2 is administered from start of cyclophosphamide until day 5.

PEG-asparaginase

1,000 units/m² as a 2-hour-long infusion or intramuscularly on day 6 of week 5. The infusion of asparaginase should be started at a reduced rate and increased stepwise, if applicable. See further notes on alternative asparaginase agents at "Asparaginase preparations" in Chapter 9.5.1.

Intrathecal chemotherapy

Age adapted doses (see Table 6 in section 9.3.3.) of methotrexate, cytarabine and prednisolone are administered on day 2 of week 5. Patients with CNS involvement receive an additional intrathecal injection on day 7 of week 5.

9.2.2.3. Blinatumomab block

(HR arm, 2nd cycle of consolidation) (Instead of HC2 and HC3 cycle ALL-IC-REL 2016)

For detailed information about the Blina cycle that will be given instead of HC2 and HC3 cycles, see the Blinatumomab section in Chapter 9.4.3.

Only patients with > 10% CD19 positive leukemic cells at the latest marrow time point, and no overt CNS leukemia can start this course. Blinatumomab is not an ideal course for those, either, who have any CSF blasts after HC1, as blinatumomab doesn't pass the blood-brain-barrier. Patients with CD19-disease, those with residual CNS involvement, and those who have no access to blinatumomab should proceed to course HC2.

Diagnostic measures

Bone marrow aspiration and assessment of extramedullary sites before the blinatumomab cycle is obligatory.

Patients can proceed to SCT after Blina depending on MRD negativity and donor availability.

9.2.2.4. Non-T cell HC2 block

This will be given after HC1 in cases when blinatumomab is contraindicated or isn't available.

(HR arm, 2nd cycle of consolidation, weeks 8-10) (Modified ALL AIEOP-BFM HR3 course)

Agent	Dosage	Application	Week 8							Week 9							Week 10						
Dexamethasone	10 mg/m^2/d	PO	■	■	■	■	■	■	■														
Cytarabine	2 gr/m^2	IV 12 hr	■	■	■	■																	
Etoposide	100 mg/m^2	IV 2 hr			■	■	■	■	■														
PEG-Asparaginase	1000 IU/m^2	IV 2 hr / IM						■															
Methotrexate	Age dep.	IT					■																
Cytarabine	Age dep.	IT					■																
Prednisolone	Age dep.	IT					■																
		Days	1	2	3	4	5	6	7	1	2	3	4	5	6	7	1	2	3	4	5	6	7

Diagnostic measures

Bone marrow aspirate on day 1 of week 8 is obligatory.

Criteria to start and guide the course

Start of the course requires recovering blood counts (neutrophils ≥ 0.5 x 10^9/L, platelets ≥ 50 x 10^9/L), mucositis less than grade 3, and no essential organ dysfunction. In case of prolonged treatment delays, dose reductions may be considered according to the specific guidelines outlined in section 9.1 "General recommendations on chemotherapy".

Instead of HC2, consider salvage options if ≥1% blasts are identified in the marrow after cycle HC1 or any residual extramedullary leukemia, e.g. any CSF blasts are detected.

Dexamethasone

10 mg/m²/d (maximum 40 mg/d) orally divided into two daily doses on days 1-6 of week 8.

Cytarabine IV
2 g/m^2/dose as 3-hour-long infusion every 12 hours (total of 4 doses) on days 1-3 of week 8. Prophylaxis of conjunctivitis with eye drops every 6 hours during administration and of neuropathy with vitamin B6 at a dose of 100 mg/m^2 IV prior to each cytarabine dose is recommended.

Etoposide
100 mg/m^2/dose as 2-hour-long infusion every 12 hours on days 3-5 (total of 5 doses) of week 8. It should not be infused over < 1 hour, since it can lead to hypotension.

PEG-asparaginase
1,000 units/m² as a 2-hour infusion or intramuscularly on day 6 of week 8. The infusion of asparaginase should be started at a reduced rate and increased stepwise, if applicable. See further notes on alternative asparaginase agents at "Asparaginase preparations" in Chapter 9.5.1.

Intrathecal chemotherapy
Age adapted doses (see Table 6 in section 9.3.3.) of methotrexate, cytarabine, and prednisolone are administered on day 5 of week 8.

9.2.2.5. Non-T cell HC3 block

This will be given after HC2 in cases when blinatumomab is contraindicated or isn't available.
HR arm, 3rd cycle of consolidation, weeks 11-13) (Modified ALL AIEOP-BFM HR2 course)

Agent	Dosage	Application	Week 11							Week 12							Week 13						
Dexamethasone	10 mg/m^2/d	PO																					
Vincristine	1.5 mg/m^2	IV																					
Daunorubicin	30 mg/m^2	IV 24 h																					
Methotrexate	1 g/m^2	IV 36 h																					
Ifosfamide	800 mg/m^2	IV 1 h																					
PEG-Asparaginase	1000 U/m^2	IV 2hr / IM																					
Methotrexate	Age dep.	IT																					
Cytarabine	Age dep.	IT																					
Prednisolone	Age dep.	IT																					
		Days	1	2	3	4	5	6	7	1	2	3	4	5	6	7	1	2	3	4	5	6	7

Diagnostic measures
Bone marrow aspirate at day 1 of week 11 is optional. It is recommended to assess cytological remission and MRD response in patients who were MRD positive at the previous time point.

Criteria to start and guide the course
Start of the course requires granulocyte ≥ 0.5 x1 0^9/L, thrombocytes ≥ 50 x 10^9/L and a clinical status allowing for treatment continuation and no essential organ dysfunction. In case of prolonged treatment delays, dose reductions may be considered according to the specific guidelines outlined in section 9.1 "General recommendations on chemotherapy".

Dexamethasone
10 mg/m²/d orally divided into two daily doses on days 1-5 of week 11.

Vincristine
1.5 mg/m² (maximum single dose 2 mg) as 15 min short infusion or as IV bolus (not on the same day as IT therapy) on days 1 and 6 of week 11.

Daunorubicin
30 mg/m^2 as 24-hour infusion on day 5 of week 11.

Methotrexate IV
1 g/m^2 IV over 36 hours starting on day 1 of week 5. 10% is given as a 30 min bolus and the remaining 90% as a continuous infusion over 35.5 hours. Concomitant alkaline hydration with 3000 ml/m^2/24 hours is given on day 1 and 2. Serum methotrexate levels can be measured at 36 hours and 48 hours after start of MTX infusion. Rescue with folinic acid at 15 mg/m^2 is given at 48 and 54 hours after start of MTX. The dose can be adapted to elevated MTX serum levels. See further details under "Impaired elimination of methotrexate" in Chapter 11.2.

Ifosfamide
800 mg/m^2/dose as a 1-hour infusion every 12 hours on days 2-4 (total of 5 doses). Mesna at a dose of 300 mg/m^2 is given before start of infusion and 4 and 8 hours after start of infusion. Hydration with 3000 ml/m^2/d on days 2-5.

PEG-asparaginase
1,000 units/m² as a 2-hour infusion or intramuscularly on day 6 of week 11. The infusion of asparaginase should be started at a reduced rate and increased stepwise, if applicable. See further notes on alternative asparaginase agents at "Asparaginase preparations" in Chapter 9.5.1.

Intrathecal chemotherapy
Age adapted doses (see Table 6 in section 9.3.3.) of methotrexate, cytarabine and prednisolone are administered on day 2 of week 11.

After HC3, patients can proceed to SCT depending on MRD negativity and donor availability.

9.2.3. Non-T cell IEM-CNS arm

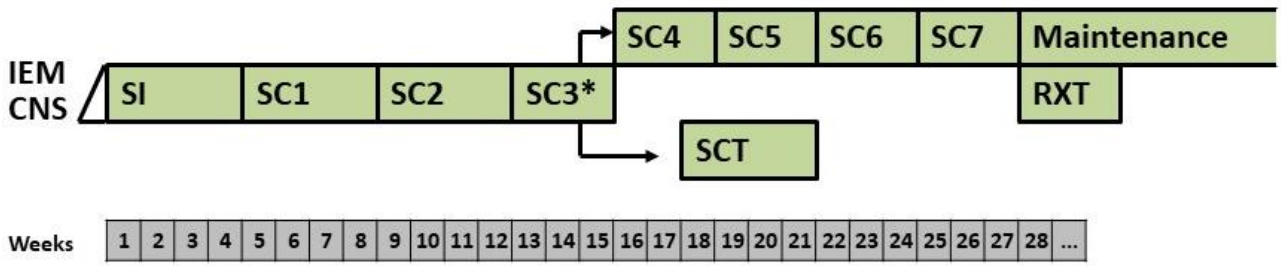
A COG study (AALL 1331) compared blinatumomab vs chemotherapy according to UK ALL R3 backbone in BCP IEM relapses and they showed non-superiority of blinatumomab in this group. When they compared those with isolated or combined marrow relapse with IEM relapses, they also revealed the excess of second isolated CNS relapses in patients with first IEM relapses [(3)]. On the other hand, the multinational, multicenter trial IntReALL 2010 study compared the use of chemotherapy according to ALL REZ BFM 2002 vs UK ALL R3 backbones and among patients with IEM-CNS relapses, they found significantly better outcomes among those who received treatment according to the ALL REZ BFM arm.

Therefore, we recommend that IEM-CNS patients receive therapy according to the ALL-IC REL 2016 standard risk backbone that is based on the ALL REZ BFM 2002 study protocol, irrespective of time to relapse.

IEM-CNS definition

See chapter 6.1. to identify patients to be stratified to this treatment backbone.

Overview of IEM-CNS treatment backbone:



* The IntReALL group plans to replace the SC3 block with a cycle of blinatumomab in the IEM-CNS arm. It is not a violation of the ALL-IC REL 2024 guidance to follow that practice.

SCT is recommended after cycle SC3 in the following patients:

- all very early and all early non-T immunophenotype IEM-CNS relapses;
- among late non-T immunophenotype IEM-CNS relapses for those with poor response to induction (any extramedullary leukemia detected e.g. CNS2 equivalent CSF blastosis, or marrow MRD ≥ 0.1%)

Late non-T immunophenotype IEM-CNS relapses with good response to induction should proceed to further consolidation cycles up to SC7 and then maintenance therapy with cranial irradiation of 18 Grays (Gy). They receive no intrathecal chemotherapy during maintenance treatment.

For detailed information about the chemotherapy cycles SI to SC7, see chapter 9.2. Note the extra IT chemotherapy doses in most chemotherapy cycles that are indicated for CNS3 relapse cases.

9.2.3.1. Non-T cell late IEM-CNS relapse maintenance chemotherapy

Maintenance therapy is given throughout from week 28 for a total of 104 weeks (= 2 years). Patients will receive therapeutic cranial radiotherapy in the beginning of the maintenance period, and triple IT treatments will not be given. For detailed information about maintenance therapy, see chapter 9.2.1.7

Agent	Dosage	Application	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	.	.	.	.	.	131
Mercaptopurine*	50 mg/m^2/d	PO					■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■
Methotrexate	20 mg/m^2	PO	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■
Radiotherapy			■	■	■	■																								
		Weeks																												

* There is evidence to suggest that the use of thiopurines during cranial irradiation may predispose to secondary brain tumors. Therefore, thiopurines are omitted during CNS irradiation.

Very rare patients who can't be treated with cranial radiotherapy (e.g. due to very young age, or family not consenting to irradiation) should instead receive extra IT triple chemotherapy doses during i.e. a total of 12 IT triple chemotherapy doses during maintenance. This issue may be worth discussing at some international expert forum, e.g. at a FEDRRAL meeting or via the email-list of the ALL-IC REL tumor board.

9.2.3.2. Non-T cell IEM relapses other than CNS

Patients with BCP-ALL IEM relapses with no overt CNS involvement (no CNS3 status) should be treated as per the SR, HR or VHR arm depending on time to relapse and genetics, just like combined relapses.

9.2.4. Non-T cell VHR arm

Very high risk (VHR) ALL relapses follow a dismal clinical course. These patients are eligible for experimental therapies due to the poor results historically obtained using conventional treatments. The IntReALL study group recommends individualized treatment based on in vitro or xenograft drug response profiling and CAR-T cell therapy which are not available in most ALL-IC countries.

If there is no option for experimental therapies, the ALL-IC REL Group recommends that these patients follow the HR arm. In case of an induction failure, the salvage options could be applied according to the physician's choice.

9.2.5. T cell HR arm

Relapsed T-ALL represents a unique entity with therapeutic challenges. In this frame, distinct treatment options and algorithm are recommended in this protocol to maximize the chance to achieve CR2 and subject patients to SCT, the only present curative consolidation for this patient group.

Given the disappointing EFS rates (< 30%), an efficient common induction is needed in order to identify chemo-sensitive patients who may proceed to SCT rapidly and also in order to set up a new standard of care for T-ALL relapse.

Venetoclax, a small compound targeting BCL2 and enhancing cell apoptosis, is a promising agent in T-ALL relapse. In a recent US study, venetoclax combined with chemotherapy resulted in a CR rate of 52.6% and MRD negativity was achieved in at least 31% of the patients [(15)], with other studies verifying the initial promising results, although the numbers of patients are still limited [(16)]. In our protocol, venetoclax, if available, is recommended for all patients with relapsed T-ALL, concurrently with HI and HC1 cycle.

Nelarabine is recommended for any T-ALL relapse patient, although its cost may limit its use. Funding for nelarabine should be requested immediately at the time of relapse and in any case prior to the end of induction, in order to solve time availability issues. The combination of nelarabine with HC blocks is the recommended and preferable choice for CNS positive patients, as nelarabine penetrates the blood-brain-barrier, and the HC cycles contain other CNS-active drugs like dexamethasone, HD-ARA-C and HD-MTX. Potential severe neurotoxicity should be considered. Combination of nelarabine with other neurotoxic agents should be avoided or limited. Intrathecal chemotherapy must not be administered on days when nelarabine is given or on the previous/subsequent day. Nelarabine treatment should be interrupted in case of any neurotoxicity CTC grade 3 or over. There are other alternative ways of implementing Nelarabine into the therapy schedule, based on certain indications and algorithm.

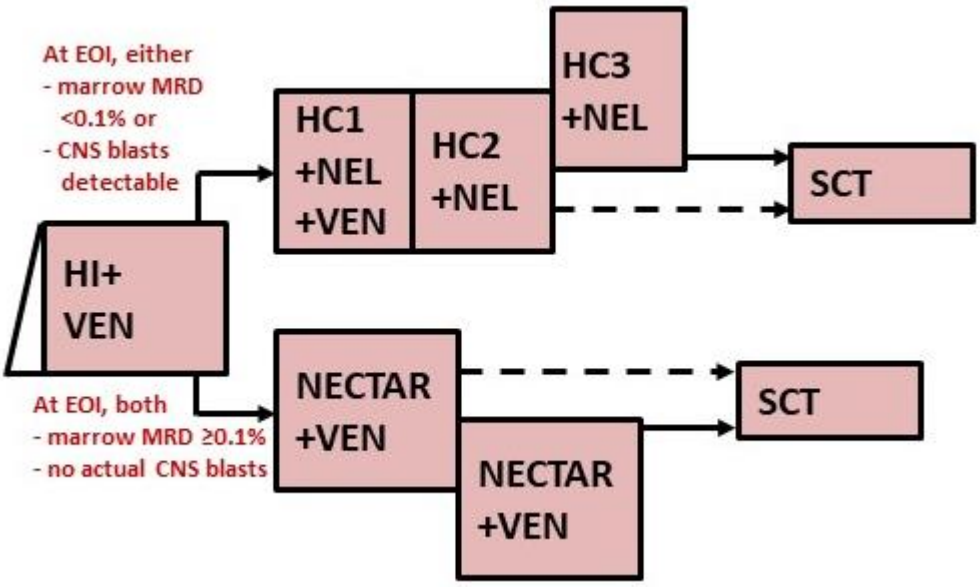
Considerations [(17-19)]

- Depending on availability and toxicity, nelarabine can be added to all 3 HC cycles;
- This way of administration of nelarabine may be a good choice especially for treatment resistant CNS disease as nelarabine penetrates the blood-brain-barrier, and the HC cycles contain other CNS-active drugs like dexamethasone, HD-ARA-C, HD-MTX.
- Consider its potentially severe neurotoxicity. Limit combining nelarabine with other neurotoxic agents. Do not administer intrathecal chemotherapy on days when nelarabine is given or on the previous/subsequent day! Nelarabine treatment should be interrupted in case of any neurotoxicity CTC grade 3 or over.
- There are other alternative ways how nelarabine can be inserted into the therapy, e.g. the NECTAR cycle.

HR definition

See chapter 6.1. to identify patients to be stratified to this treatment backbone.

Overview of HR treatment arm:



Patients with insufficient response to induction (EOI marrow MRD ≥ 0.1%) but no detectable CNS involvement at this time point should be shifted to the NECTAR+VEN arm. For all other patients, we recommend the consolidation cycles used in the HR arm of the ALL-IC REL 2016 protocol, with added venetoclax and nelarabine.

It is very difficult to write clearcut criteria to abandon this scheme and turn to salvage options. The decision has to be made on an individual basis. We recommend that these cases be discussed at some international expert forum, e.g. at a FEDRRAL meeting or via the email-list of the ALL-IC REL tumor board.

9.2.5.1. HI + VEN block

(HR arm, induction cycle, week 1-4) (originally R3 induction + venetoclax)

Agent	Dosage	Application	Week 1							Week 2							Week 3							Week 4						
Venetoclax	Age/Wt dep.																													
Dexamethasone	20 mg/m^2/d	PO																												
Vincristine	1.5 mg/m^2	IV																												
Mitoxantrone	10 mg/m^2	IV 1hr																												
PEG-Asparaginase	1000 U/m^2	IV 2hr / IM																												
Methotrexate	Age dep.	IT																												
Cytarabine	Age dep.	IT																												
Prednisolone	Age dep.	IT																												
		Days	1	2	3	4	5	6	7	1	2	3	4	5	6	7	1	2	3	4	5	6	7	1	2	3	4	5	6	7

Criteria to start and guide the course

No specific blood count or clinical condition is mandatory to start the HI cycle. In case of some major comorbidity, complication or critical situation, the treatment for these may precede the start of HI cycle. A steroid prephase may be applied on the discretion of the responsible physician. This may also be the choice if the patient is at high risk of tumor lysis syndrome.

Diagnostic measures

Bone marrow aspirate MRD testing is mandatory on day 1 of week 5.

Dexamethasone

20 mg/m² (maximum 40 mg/d) orally divided into two daily doses on days 1-5 of weeks 1 and 3. A dexamethasone prephase can be started from day -5 until day 0 at a dose of 6 mg/m² in case of high tumor burden or to bridge the time until start of the study.

Mitoxantrone

10 mg/m^2 as 1-hour-long infusion on days 1 and 2 of week 1.

Vincristine

1.5 mg/m² (maximum single dose 2 mg) as 15 min short infusion or as IV bolus (not on the same day as IT therapy) on day 3 of weeks 1-4.

Table 7. Pediatric Venetoclax doses weight adaption to adult 400 mg/day equivalent

For patients < 2 years of age	**Venetoclax dose (mg)**
Newborn- < 1 month	10
1 - < 3 months	20
3 - < 6 months	50
6 months- < 1 year	125
1 - < 2 years	200
For patients ≥ 2 years of age	**Venetoclax dose (mg)**
10 - < 20 kg	250
20 - < 30 kg	300
30 - < 45 kg	400
≥ 45 kg	600

Venetoclax

Weight adjusted dosage to achieve the equivalent of adult 400 mg/day, on days 1-21 of weeks 1-3, or for 21 consecutive days since the drug can be obtained and initiated (weight adaptation to adult 400 mg/day equivalent is shown in Table 7 below).

Caution: Due to interaction, best to avoid concomitant azol prophylaxis/therapy besides venetoclax, opt for amphotericin-B or echinocandins. Also note increased absorption with crushed venetoclax tablets. In case there is no other option and tablets are crushed, monitor drug levels or reduce the daily total dose.

PEG-asparaginase

1000 units/m² as 2-hour-long infusion or intramuscularly on day 3 of weeks 1 and 3. The infusion of asparaginase should be started at a reduced rate and increased stepwise, if applicable. See further notes on alternative asparaginase agents at “Asparaginase preparations” in Chapter 9.5.1.

Intrathecal chemotherapy

Age adapted dose (see Table 6 in section 9.3.3.) of triple intrathecal chemotherapy is administered on day 1 of weeks 1 and 2. Patients with CNS involvement receive additional intrathecal injections weekly thereafter, until two leukemia free CSF samples are obtained.

9.2.5.2. HC1 + VEN + NEL block

Agent	Dosage	Application	Week 6							Week 5							Week 6							Week 7						
Nelarabin	650 mg/m^2/d	IV 2 hr																												
Dexamethasone	10 mg/m^2/d	PO																												
Vincristine	1.5 mg/m^2/d	IV																												
Cytarabine	2 g/m^2	IV 3 hr																												
Methotraxate	1 g/m^2	IV 36 hr																												
Cyclophosphamide	200 mg/m^2	IV 1 hr																												
PEG-Asparaginase	1000 IU/m^2	IV 2 hr / IM																												
Methotrexate	Age dep.	IT																												
Cytarabine	Age dep.	IT																												
Prednisolone	Age dep.	IT																												
Venetoclax	Age/Wt dep.	PO																												
		Days	1	2	3	4	5	6	7	1	2	3	4	5	6	7	1	2	3	4	5	6	7	1	2	3	4	5	6	7

Considerations[15,16,18,19]

In case of MRD good response (< 0.1%), and no detectable CNS disease at EOI, the ALL-IC Rel Study Group strongly recommends the use of nelarabine to be given before every consolidation block (HC1, HC2 and HC3), if available, with the addition of venetoclax also in HC1.

Diagnostic measures

Bone marrow aspiration and assessment of other body compartments for residual leukemia before or at day 1 of Nelarabine is the same as that described under the HC cycle it precedes. Bone marrow aspirate on day 1 of week 5 of HI induction is mandatory to assess cytological remission and MRD response. Results should reveal a representative marrow. In case of a non-representative or aplastic marrow, the analysis should be repeated, and the start of cycle HC1 postponed accordingly. If ≥5% blasts are identified, the team should consider shifting to rescue options.

Criteria to start and guide the course

Start of nelarabine requires recovering blood counts (neutrophils ≥0.5x10^9/L, platelets ≥ 50 x 10^9/L), mucositis less than grade 3, and no essential organ dysfunction as the start of the HC cycle it is combined with. When Nelarabine is given on days 1-5, HC cycle should be administered starting on day 8. No blood count criteria should be considered on day 8 to continue with the HC cycle.

Nelarabine

650 mg/m^2/dose as a 2-hour-long infusion on days 1-5 of week 5 (total of 5 doses). No hyperhydration is required.

- NOTE: Intrathecal chemotherapy must not be administered between day -1 and day 6 of Nelarabine course. It is thought to increase the risk of severe, even life threatening central nervous system toxicity.
- Up to three nelarabine courses can be inserted into the high-risk arm. These 5-day-long courses should be given before HC1, HC2, HC3.
- Adding Nelarabine to these cycles are optional, as it may not be available at many centers.

Dexamethasone
10 mg/m² orally divided into two daily doses on days 1-5 of week 6.

Vincristine
1.5 mg/m² (maximum single dose 2 mg) as 15 min short infusion or as IV bolus (not on the same day as IT therapy) on days 1 and 6 of week 6.

HD-Cytarabine
2 g/m^2/dose as 3-hour-long infusion every 12 hours (total of 2 doses) on day 5 of week 6. Prophylaxis of conjunctivitis with eye drops every 6 hours during administration and of neuropathy with vitamin B6 at a dose of 100 mg/m^2 IV prior to each cytarabine dose is recommended.

Methotrexate IV
1 g/m^2 IV over 36 hours starting on day 1 of week 6. 10% is given as a 30 min bolus and the remaining 90% as a continuous infusion over 35.5 hours. Concomitant alkaline hydration with 3000 ml/m^2/24 hours is given on day 1 and 2. Serum methotrexate levels can be measured at 36 hours and 48 hours after start of MTX infusion. Rescue with folinic acid at 15 mg/m^2 is given at 48 and 54 hours after start of MTX. The dose can be adapted to elevated MTX serum levels. See further details under "Impaired elimination of methotrexate" in Chapter 11.2.

Cyclophosphamide
200 mg/m^2/dose as a 1-hour infusion every 12 hours on days 2-4 (total of 5 doses) of week 6. Mesna is administered at 70 mg/m^2/dose before and at 4 and 8 hours after start of each cyclophosphamide infusion. Hydration with 3000 ml/m^2 is administered from start of cyclophosphamide until day 5.

PEG-asparaginase
1,000 units/m² as a 2-hour-long infusion or intramuscularly on day 6 of week 6. The infusion of asparaginase should be started at a reduced rate and increased stepwise, if applicable. See further notes on alternative asparaginase agents at "Asparaginase preparations" in Chapter 9.5.1.

Venetoclax
See Chapter 9.3.1. By starting on day 1 of week 6, 21 days consecutively.

Intrathecal chemotherapy
Age adapted doses (see Table 6 in section 9.3.3.) of methotrexate, cytarabine and prednisolone are administered on day 2 of week 6. Patients with CNS involvement receive an additional intrathecal injection on day 7 of week 6.
Caution: To avoid increasing neurotoxicity, there should be an interval of at least 2 days between triple IT and nelarabine treatment.

9.2.5.3. HC2 + NEL block

Agent	Dosage	Application	Week 10							Week 11							Week 12							Week 13						
Nelarabine	650 mg/m^2/d	IV 2 hr	▮	▮	▮	▮	▮																							
Dexamethasone	10 mg/m^2/d	PO								▬	▬	▬	▬	▬	▬	▬														
Cytarabine	2 g/m^2	IV 3hr								▮	▮	▮	▮																	
Etoposide	100 mg/m^2	IV 2hr										▮	▮	▮	▮	▮														
PEG-Asparaginase	1000 U/m^2	IV 2hr / IM														▮														
Methotrexate	Age dep.	IT									▮																			
Cytarabine	Age dep.	IT									▮																			
Prednisolone	Age dep.	IT									▮																			
		Days	1	2	3	4	5	6	7	1	2	3	4	5	6	7	1	2	3	4	5	6	7	1	2	3	4	5	6	7

Diagnostic measures

Bone marrow aspirate on day 1 of week 10 is obligatory. Instead of HC2 + NEL, consider salvage options if ≥ 1% blasts are identified in the marrow after cycle HC1 + VEN + NEL or any residual extramedullary leukemia, e.g. any CSF blasts are detected.

Criteria to start and guide the course

Start of nelarabine cycle requires recovering blood counts (neutrophils ≥0.5x10^9/L, platelets ≥ 50 x 10^9/L), mucositis less than grade 3, and no essential organ dysfunction as the start of the HC cycle it is combined with. When nelarabine is given on days 1-5, HC cycle should be administered starting on day 8. No blood count criteria should be considered on day 8.

Nelarabine

650 mg/m^2/dose as a 2-hour long infusion by starting on day 1 of week 10, 5 days consecutively. No hyperhydration is required.

Dexamethasone

10 mg/m²/d (maximum 40 mg/d) orally divided into two daily doses on days 1-6 of week 11.

Cytarabine IV

2 g/m^2/dose as 3-hour-long infusion every 12 hours (total of 4 doses) on days 1-3 of week 11. Prophylaxis of conjunctivitis with eye drops every 6 hours during administration and of neuropathy with vitamin B6 at a dose of 100 mg/m^2 IV prior to each cytarabine dose is recommended.

Etoposide

100 mg/m^2/dose as 2-hour-long infusion every 12 hours on days 3-5 (total of 5 doses) of week 11. It should not be infused over < 1 hour, since it can lead to hypotension.

PEG-asparaginase

1,000 units/m² as a 2-hour-long infusion or intramuscularly on day 6 of week 11. The infusion of asparaginase should be started at a reduced rate and increased stepwise, if applicable. See further notes on alternative asparaginase agents at “Asparaginase preparations” in Chapter 9.5.1.

Intrathecal chemotherapy

Age adapted doses (see Table 6 in section 9.3.3.) of methotrexate, cytarabine and prednisolone are administered on day 2 of week 11. Patients with CNS involvement receive an additional intrathecal injection on day 7 of week 11.

Caution: To avoid increasing neurotoxicity, there should be an interval of at least 2 days between triple IT and nelarabine treatment.

Patients can proceed to SCT after HC2+NEL depending on MRD negativity and donor availability. If no donor is available, treatment can proceed with a HC3+ NEL cycle. If no MRD negativity is reached, then salvage options should be considered.

9.2.5.4. HC3 + NEL block

Agent	Dosage	Application	Week 14							Week 15							Week 16							Week 17						
Nelarabine	650 mg/m^2/d	IV 2 hr																												
Dexamethasone	10 mg/m^2/d	PO																												
Vincristine	1,5 mg/m^2	IV																												
Daunorubicin	30 mg/m^2	IV 24 h																												
Methotrexate	1 g/m^2	IV 36 h																												
Ifosfamide	800 mg/m^2	IV 1 h																												
PEG-Asparaginase	1000 U/m^2	IV 2hr / IM																												
Methotrexate	Age dep.	IT																												
Cytarabine	Age dep.	IT																												
Prednisolone	Age dep.	IT																												
		Days	1	2	3	4	5	6	7	1	2	3	4	5	6	7	1	2	3	4	5	6	7	1	2	3	4	5	6	7

Diagnostic measures

Bone marrow aspirate at day 1 of week 14 is optional. It is recommended to assess cytological remission and MRD response in patients who were MRD positive at the previous time point.

Criteria to start and guide the course

Start of nelarabine cycle requires recovering blood counts (neutrophils ≥0.5x10^9/L, platelets ≥ 50 x 10^9/L), mucositis less than grade 3, and no essential organ dysfunction as the start of the HC cycle it is combined with. When nelarabine is given on days 1-5, HC cycle should be administered starting on day 8. No blood count criteria should be considered on day 8 to continue with the HC cycle.

Nelarabine

650 mg/m^2/dose as a 2-hour long infusion by starting on day 1 of week 10, 5 days consecutively. No hyperhydration is required.

Dexamethasone

10 mg/m²/d orally divided into two daily doses on days 1-6 of week 15.

Vincristine

1.5 mg/m² (maximum single dose 2 mg) as 15 min short infusion or as IV bolus (not on the same day as IT therapy) on days 1 and 6 of week 15.

Daunorubicin

30 mg/m^2 as a 24-hour-long infusion on day 5 of week 15.

Methotrexate IV
1 g/m^2 IV over 36 hours starting on day 1 of week 15. 10% is given as a 30 min bolus and the remaining 90% as a continuous infusion over 35.5 hours. Concomitant alkaline hydration with 3000 ml/m^2/24 hours is given on day 1 and 2. Serum methotrexate levels can be measured at 36 hour and 48 hour after start of MTX infusion. Rescue with folinic acid at 15 mg/m^2 is given at 48 and 54 hours after start of MTX. The dose can be adapted to elevated MTX serum levels. See further details under "Impaired elimination of methotrexate" in Chapter 11.2.

Ifosfamide
800 mg/m^2/dose as a 1-hour-long infusion every 12 hours on days 2-4 (total of 5 doses). Mesna at a dose of 300 mg/m^2 is given before start of infusion and 4 and 8 hours after start of infusion. Hydration with 3000 ml/m^2/d on days 2-5.

PEG-asparaginase
1,000 units/m² as a 2-hour-long infusion or intramuscularly on day 6 of week 15. The infusion of asparaginase should be started at a reduced rate and increased stepwise, if applicable. See further notes on alternative asparaginase agents at "Asparaginase preparations" in Chapter 9.5.1.

Intrathecal chemotherapy
Age adapted doses (see Table 6 in section 9.3.3.) of methotrexate, cytarabine and prednisolone are administered on day 2 of week 15.
Caution: To avoid increasing neurotoxicity, there should be an interval of at least 2 days between triple IT and nelarabine treatment.
Patients can proceed to SCT after HC3+NEL depending on MRD negativity. If no MRD negativity is reached, then salvage options should be considered.

9.2.5.5. NECTAR + VEN block

Agent	Dosage	Application	Week 5							Week 6							Week 7							Week 8						
Nelerabine	650 mg/m^2/d	IV 2 hr	▮	▮	▮	▮	▮																								
Etoposide	100 mg/m^2/d	IV 2 hr	▮	▮	▮	▮	▮																								
Cyclophosphamide	400 mg/m^2/d	IV 1 hr	▮	▮	▮	▮	▮																								
Methotrexate	Age dep.	IT															▮							▮						
Cytarabine	Age dep.	IT															▮							▮						
Prednisolone	Age dep.	IT															▮							▮						
Venetoclax	Age/Weight dep.	PO								▬	▬	▬	▬	▬	▬	▬	▬	▬	▬	▬	▬	▬	▬							
		Days	1	2	3	4	5	6	7	1	2	3	4	5	6	7	1	2	3	4	5	6	7	1	2	3	4	5	6	7

Criteria to start and guide the course
Start of the cycle requires recovering blood counts (neutrophils ≥0.5x10^9/L, platelets ≥ 50 x 10^9/L), mucositis less than grade 3, and no essential organ dysfunction.

Nelarabine:
650 mg/m^2/doseas a 2-hour-long infusion daily on five consecutive days.

Etoposide:
100 mg/m^2/dose as a 2-hour-long infusion daily on five consecutive days. It should not be infused over < 1 hour, since it can lead to hypotension.

Cyclophosphamide:
400 mg/m^2/dose as a 1-hour-long infusion daily on five consecutive days. Hydration and MESNA to be administered according to institution standard of care procedures.

Venetoclax
See Chapter 9.3.1. By starting on day 1 of week 6, 14 days consecutively.

Intrathecal therapy:
Age adapted doses (see Table 6 in section 9.3.3.) of intrathecal cytarabine, methotrexate and prednisolone on days 15 and 22.

9.2.5.5.1. MRD good response (MRD < 0.1%) after 1 cycle of NECTAR + VEN

In case of good response is detected after the 1st NECTAR+VEN cycle, patients can either proceed to SCT or receive one more cycle of NECTAR + VEN and then proceed to allo-SCT.

9.2.5.5.2. MRD sufficient response after 1 cycle of NECTAR + VEN

In case marrow residual leukemia was reduced by more than one order by the 1st cycle of NECTAR + VEN, but MRD is still around 0.1% or higher, then it makes sense to proceed with another similar cycle before proceeding to allo-SCT.

9.2.5.5.3. MRD sufficient response after 1 cycle of NECTAR + VEN

If the response to the first NECTAR + VEN cycle is poor, then a shift to salvage options is recommended.

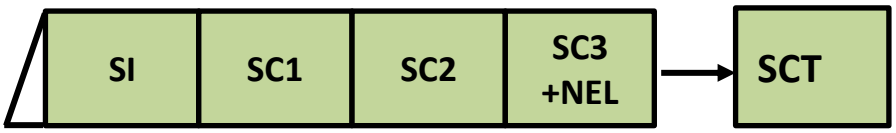
9.2.6. T cell IEM-CNS arm

Regardless of the time of relapse, all T cell IEM-CNS relapses will be considered as HR and treatment approaches will be terminated with allo-SCT. The ALLIC group strongly recommends the use of nelarabine in patients with T-cell IEM-CNS before transplantation, in conjunction with SC3, to treat possible minimal residual CNS disease due to its good CNS penetration.

IEM-CNS definition

See chapter 6.1. to identify patients to be stratified to this treatment backbone.

Overview of IEM-CNS treatment backbone:



9.2.7.1. SI, SC1, SC2 blocks

See Chapter 9.2.1.1., 9.2.1.2. and 9.2.1.3.

9.2.7.2. SC3 + NEL block

Agent	Dosage	Application	Week 13							Week 14							Week 15							Week 16						
Nelarabine	650 mg/m^2/d	IV 2 hr																												
Dexamethasone	20 mg/m^2/d	PO																												
Mercaptopurine	100 mg/m^2/d	PO																												
Vincristine	1.5 mg/m^2	IV																												
Methotrexate	1 g/m^2	IV 36 hr																												
Cytarabine	2x2 g/m^2/d	IV 3 hr																												
PEG-Asparaginase	1000 U/m^2	IV 2 hr / IM																												
Methotrexate	Age dep.	IT																												
Cytarabine	Age dep.	IT																												
Prednisolone	Age dep.	IT																												
		Days	1	2	3	4	5	6	7	1	2	3	4	5	6	7	1	2	3	4	5	6	7	1	2	3	4	5	6	7

Criteria to start and guide the course

Start of the course requires recovering blood counts (neutrophils ≥ 0.5 x 10^9/L, platelets ≥ 50 x 10^9/L), mucositis less than grade 3, and no essential organ dysfunction. In case of prolonged treatment delays, dose reductions have to be considered according to those described at chapter 9.1.1.

Nelarabine

650 mg/m^2/dose as a 2-hour long infusion daily on five consecutive days on days 1-5 of week 13. No hyperhydration is required.

Dexamethasone
20 mg/m² (maximum 40 mg/d) orally, divided in two daily doses on day 1-5, and 10 mg/m² on day 6 of these blocks.

6-Mercaptopurine
100 mg/m² orally on day 1-5 of these blocks.

Vincristine
1.5 mg/m² (maximum single dose 2 mg) as 15 min short infusion (or as IV bolus not on the same day as IT therapy) on day 1 and 6 of week 14.

Methotrexate IV
1 g/m² IV over 36 hours starting on day 1 of week 14. Out of the total, 10% is given as a 30 min bolus and the remaining 90% as a continuous infusion for 35.5 hours. Concomitant alkaline hydration with 3000 ml/m²/24 hours is given on day 1 and 2. Rescue with folinic acid of 15 mg/m² is given at 48 and 54 hours after start of MTX. Serum methotrexate levels can be measured at 36h and 48h after the start of the MTX infusion, and the dose of folinic acid adapted to elevated MTX serum levels. The management is guided according to recommendations “Impaired elimination of methotrexate” in Chapter 11.2.

Cytarabine
2 g/m² as 3-hour-infusion every 12 hours on day 5 (a total of 2 doses) of SC3 cycle. A prophylaxis of conjunctivitis with eye drops and of neuropathy with vitamin B6 at a dose of 100mg/m² IV is recommended prior to each cytarabine dose.

PEG-asparaginase
1,000 units/m² as 2-hour infusion on day 6 of each of this cycle. The infusion of L-asparaginase should be started at a reduced rate and increased stepwise, if applicable. See further notes on alternative agents “Asparaginase preparations” in Chapter 9.5.1.

Intrathecal chemotherapy
Age adapted doses of methotrexate, cytarabine and prednisolone (see Table 6 in section 9.3.3.) are administered on day 2 of each course after the methotrexate infusion.

9.3. Local therapy

9.3.1. General information

Local therapy of extramedullary relapses of ALL in children is very important part of their treatment. Involvement of extramedullary sites in children with ALL relapse has been registered in about 35-40% of patients depending on the frontline protocol. However, in the last decade due to more effective frontline protocols, the rate of all but also of extramedullary relapses has been decreased. Since the disease is protected from chemotherapy by biologic blood barriers in extramedullary sanctuary sites such as the CNS and the testes, specific local therapy in addition to systemic chemotherapy is generally recommended.

9.3.2. Radiation therapy

Radiation therapy is given to control disease in compartments protected from effective systemic chemotherapy by biologic blood barriers. This concerns CNS and testicular relapses. Furthermore, in case of persistence of leukemia in extramedullary regions such as CNS mass, mediastinum, lymph nodes, bone, skin, orbit, or other organs, local radiotherapy might add to the efficacy of combined immuno-chemotherapy. This concept is generally accepted only 1) in the form of a local boost to total body irradiation as part of conditioning for SCT, or 2) as palliative therapy. Local radiotherapy in other indications (e.g. as a separate treatment element not related to SCT) is a non-evidence-based addition to the treatment - this should be discussed individually at national or international forums.

Whether protective CNS irradiation is necessary in patients with isolated bone marrow relapse remains controversial. In a retrospective analysis the BFM group has clearly shown, that the rate of subsequent medullary and CNS relapses can be significantly reduced by preventive CNS irradiation at 12 Gy (16). With the ALLR3 trial, outstanding results in IR patients with BM relapse and MRD good response have been achieved without preventive cranial irradiation. Omitting cranial irradiation in childhood ALL has been propagated by various groups due to the documented radiation associated late effects such as neuro-behavioral changes and secondary malignancies. The ALL-IC REL group has decided to omit preventive cranial irradiation for all patients with isolated bone marrow relapse and to replace it with 6 triple intrathecal chemotherapy applications during maintenance therapy.

9.3.2.1. Central nervous system relapse

In CNS relapse, the accepted local treatment is intensified intrathecal therapy and cranial irradiation. The adequate dose (18 versus 24 Grays [Gy]) and mode of CNS irradiation (cranial versus craniospinal) remains controversial [(20, 21)]. In some protocols the administration of 24 Gy to cranium and 15 Gy to spine are recommended [(22)]. The ALL-IC Rel Group, parallel to the IntReALL Consortium, has decided to apply 18 Gy to the cranium with daily fractions of 1.5 to maximum 2.0 Gy, with the following exceptions. If radiation therapy was given as part of the frontline therapy (1st presentation of ALL), and the interval from the first course of radiation therapy is shorter than 24 months, or the previous radiation dose exceeded 15 Gy, then the radiation dose should be reduced to 15 Gy. For patients with CNS mass (leukaemic tumour on imaging) 24 Gy irradiation dose is recommended.

Patients with CNS relapse and indication for allogeneic SCT will receive TBI at 12 Gy supplemented by a cranial boost of 6 Gy. Radiation therapy is principally performed using high-voltage technique (telecobalt or linear accelerator). The exact reproducibility of the daily positioning has to be ensured for example using masks for immobilization. During irradiation of the CNS, individual attenuators have to be made to protect the visceral cranium and the anterior cervical soft tissues. The retroorbital spaces and the skull base have to be well included in the radiation field. If the entire neuroaxis is irradiated, dosage gaps and overlaps of adjacent fields have to be avoided using divergence compensation. Emphasis is placed on a homogeneous distribution of the radiation dose. Principally, all fields are irradiated in each session. Single fractions should be administered 5 times per week. During follow-up, regular ophthalmologic assessments are required to detect and treat radiation cataracts in a timely manner.

Very young children suffer from severe impairment of neurocognitive development post cranial irradiation. Therefor it is contraindicated to subject children under 2 years of age to cranial irradiation. Protection of the eye lenses is also not possible due to the low lying frontal skull base. Between age 2 and 4 years, the contraindication is relative, the treating physician should weigh up the benefit and the toxicity and decide. Patients who can't receive radiotherapy can either be treated with CAR-T-cells in case of allogeneic SCT indication, or receive 12 doses if IT triple chemotherapy in a 4-weekly pace during maintenance.

There is evidence to suggest that the use of thiopurines during cranial irradiation may predispose to the occurrence of brain tumors. Therefore, thiopurines are omitted during CNS irradiation.

9.3.2.2. Testicular relapse

The testes are the second most frequent sites of extramedullary involvement. Local therapy is not uniform in different study groups. ALL REZ BFM relapse strategies have recommended to remove a clinically involved testis and to irradiate a contralateral clinically not involved and biopsy negative testis with 15 Gy, or with 18 Gy if biopsy positive. It has been shown that this strategy keeps the possibility of spontaneous puberty in a substantial part of patients.

ALL-IC Rel Group recommendations for patients with testicular relapse:

Option 1: Orchiectomy and reduced irradiation of the contralateral testicle

In case of a unilateral clinical involvement, the contralateral testis should be biopsied during the orchiectomy procedure. If the biopsy shows no involvement, local irradiation with 15 Gy is given. After this dose sufficient residual endocrine function is expected to allow the spontaneous onset of puberty. If the biopsy is positive or not performed, the clinically not involved testis should be irradiated with 18 Gy.

Testicular involvement documented by ultrasound alone without clinical enlargement has to be confirmed by biopsy and will be treated like a clinically non-involved testis based exclusively on the result of the biopsy.

During orchiectomy, a testicular prosthesis could be implanted. The cosmetic result may be better compared to the testicular atrophy following local irradiation with 24 Gy, but the hormonal dysfunction is identical.

Option 2: No orchiectomy but full dose irradiation of both testicles.

Boys with testicular infiltration at presentation receive local irradiation of both testicles (irrespectively of the side and extent of involvement) with 24 Gy divided in 12 daily fractions.

In case allogeneic SCT is planned, the orchiectomy should be performed at diagnosis or during early consolidation. In context of total body irradiation, the radiation dose given to the testes should be increased to 18 Gy giving a 6 Gy boost if orchiectomy was not performed initially.

If no SCT is planned, the testicular radiotherapy should be performed at the very beginning of maintenance therapy, timed similarly to CNS radiotherapy as described in chapter 9.2.3.1. However, mercaptopurine doesn't have to be omitted during testicular radiotherapy.

There is no lower age limit for testicular irradiation like that described for cranial radiotherapy.

9.3.3. Intrathecal chemotherapy

All patients in BCP-ALL SR group who have no indication for SCT should receive CNS radiotherapy or 6 intrathecal chemotherapy applications during maintenance therapy after SC7 block depending on CNS involvement. Patients with CNS1 or CNS2 relapses receive triple intrathecal therapy (Table 6) once every 4 weeks for a total of 6 times on day 1 of weeks 28, 32, 36, 40, 44, 48 and they are not subject to cranial radiotherapy. Patients with CNS3 relapses receive cranial irradiation at a dose of 18 Gy just before the start of the maintenance therapy without intrathecal therapy during maintenance.

The intrathecal treatment during the induction and consolidation phase of the therapy and the days of application are indicated in the relevant chemo cycles' figures (SI, SC1-SC7, HI, HC1-HC3). In all cases the intrathecal therapy is triple. Age adapted doses of methotrexate, cytarabine and prednisolone are described in Table 6.

Table 6. Age-dependent intrathecal chemotherapy doses

Age (years)	Methotrexate (mg)	Cytarabine (mg)	Prednisolone (mg)*
<1	6	16	4
≥1 - <2	8	20	6
≥2 - <3	10	26	8
≥3	12	30	10

*In case of unavailability of Prednisolone, dexamethasone doses according to age: < 1years, 1 mg; ≥ 1 year to < 2 years, 2 mg; ≥ 2 years to < 3 years, 3 mg; ≥ 3 years 4 mg.

In case a CNS3 patient cannot be irradiated, e.g. due to very young age or lack of parental consent, such a child should receive 12 IT triple chemotherapy doses during maintenance, with prolonged 4-weekly pace (based on no evidence but expert advice).

9.4. Salvage options

9.4.1. General remarks

See the concept and the rough indications of salvage therapies in chapter 7.3.

Below, we give a set of possible salvage treatments and provide some points to support the choice among these options. Alternatively, these patients are encouraged to participate in early phase clinical trials.

Patients needing salvage therapy should have marrow, CSF and potentially further extramedullary site assessment after each treatment cycle and previous or suspicious other extramedullary sites should also be examined. This is because patients who are resistant to relapse treatment are unpredictable, they frequently develop leukemic infiltration at new or previously cleared sites.

Late relapses tend to respond to chemotherapy, even if slowly. There is considerable chance to get them into a suitable state for SCT by ongoing intensive chemotherapy, even if targeted therapies are not available. Contrary to this, early and especially very early relapses with no good initial treatment response will hardly get into deep remission with any chemotherapy. Novel targeted treatment options, especially inotuzumab, blinatumomab or venetoclax are more likely to bridge these patients to SCT, providing hope for long term cure.

In very early relapses, or in any patients who don't reach remission at the end of relapse induction therapy, it would be reasonable to perform drug response profiling (by in vitro cell culture or xenograft models) and take these in consideration re. treatment individualization - if such testing is available. The physician may try to get in touch with the organizers of the HEM-iSMART study.

Typical 1st choice salvage treatments for residual leukemia in the CNS:

- FLA-(IDA)-(VEN) for any immunophenotype
- CAR-T therapy for BCP-ALL

Typical 1st choice salvage treatments for residual leukemia at other sites, for BCP-ALL:

- Residual leukemia > 25%: inotuzumab ozogamicin (if blasts are CD22+),
- Residual leukemia between 5-25%: inotuzumab ozogamicin or blinatumomab (consider the greater efficacy but more toxicity, especially SOS caused by inotuzumab ozogamicin),
- Residual leukemia < 5%: blinatumomab,
- If no novel therapies are available, then FLA-(IDA)-(VEN) may be the 1st choice.

Typical 1st choice salvage treatments for residual leukemia at other sites, for T-ALL:

- Cycles combining daratumumab and chemotherapy are first choice,
- Cycles combining bortezomib and chemotherapy are second choice,
- If no novel therapies are available, then FLA-(IDA) may be the 1st choice.

See more details and further options in this chapter below.

In general, it makes sense to attempt immunotherapy for the patients who prove to be chemotherapy-resistant. However, if no immunotherapies are available, probably there is little difference among the expectable effect of various chemotherapy cycles. It is also an option not to switch to salvage cycles but to proceed as per the protocol (e.g. with cycle HC1 after non-remission to HI).

The salvage cycles may be given after each other in various sequences as indicated if the patient has ongoing residual leukemia. However, if the marrow MRD is <0.1% and no extramedullary leukemia remains, it is advised to proceed to SCT as soon as possible. MRD reappearance and on-treatment further relapse are frequent in patients with resistant disease after relapse.

Chemotherapy, monoclonal antibodies, the above salvage options used even in combinations are insufficient themselves to provide long term cure for poorly responding relapse patients, or even for well responding high risk relapses (apart from very rare examples). Cellular immunotherapies, that are SCT and/or CAR-T, are needed to offer a chance for long-term cure.

9.4.2. Inotuzumab ozogamicin block

Inotuzumab ozogamicin (InO) is a CD22-directed humanized monoclonal antibody conjugated to the potent cytotoxin calicheamicin. CD22 is widely expressed on B-ALL blasts and is rapidly internalized upon antibody binding, making it an excellent target for immune-targeted chemotherapy in BCP-ALL if CD22 expression is demonstrated on blasts (even only partial expression of CD22 is sufficient).

At present, InO is mostly recommended for patients who couldn't reach remission by chemotherapy. In adults, its higher remission rate and lower toxicity was demonstrated over alternative chemotherapy options in a prospective clinical trial [(23)]. Less pediatric evidence is available, but the perception is similar.

Considerations [(24, 25)]

- Only for B-cell precursor CD22pos immunophenotype cases (subclonal or partial CD22-expression are not contraindications);
- 1st choice among salvage options if marrow blasts ≥ 25%;
- Can be considered if marrow blasts ≥ 5% (InO may be more likely to be effective but is also more toxic than blinatumomab, especially in view of later SOS – sinusoidal obstruction syndrome, alias VOD, veno-occlusive disease of the liver);
- May repeat InO cycles if response is observed;
- Avoid in relevant liver toxicity;
- Avoid or minimize the number of InO cycles if SCT is planned. If no other good enough option is available, best to insert InO earlier in the sequence of cycles, further away from SCT in order to reduce the risk of SOS;
- Do not repeat InO if MRD negativity is achieved, not to increase SOS risk during SCT;
- InO doesn't exert sufficient effect in the CNS; it is not the appropriate choice if CSF WBC > 5/uL or CNS infiltration is seen radiologically.
- Monthly IgG level monitoring is recommended after InO therapy due to the resulting B-cell aplasia that can last for months or years, and immunoglobulin substitution as required.

Overview of the cycle:

Agent	Dosage	Application	Week 1							Week 2							Week 3						
Inotuzumab	0.8 mg/m^2/d	IV 1hr	▮																				
Inotuzumab	0.5 mg/m^2/d	IV 1hr								▮							▮						
Methotrexate	Age dep.	IT	▮																				
Cytarabine	Age dep.	IT	▮																				
Prednisolone	Age dep.	IT	▮																				
		Days	1	2	3	4	5	6	7	1	2	3	4	5	6	7	1	2	3	4	5	6	7

Criteria to start and guide the course

Start of InO cycle requires recovering blood counts (neutrophils ≥ 0.5 x 10^9/L, platelets ≥ 50 x 10^9/L), mucositis less than grade 3, and no essential organ dysfunction, total bilirubin ≤ 3 x upper limit of normal (ULN) unless the patient has documented Gilbert syndrome, AST/ALAT ≤ 10x ULN, absence of uncontrolled infection, no history of prior sinusoidal obstruction syndrome (SOS/VOD).

Inotuzumab ozogamicin

One cycle consists of 3 doses: 0.8 mg/m^2 on day 1, 0.5 mg/m^2 on days 8 and 15 as a 1-hour-long IV infusion. May repeat InO cycles each 28 days at 0.5 mg/m^2 dose for 3 doses if response is observed.

Supportive medications:

- Dexamethasone 5 mg/m^2/dose 30-60 minutes before the start of each inotuzumab infusion,
- Paracetamol 15 mg/kg/dose 30-60 minutes before the start of each inotuzumab infusion
- Chlorphenamine 0.15 mg/kg/dose 30-60 minutes before the start of each inotuzumab infusion

Intrathecal chemotherapy

Age adapted doses (see Table 6 in section 9.3.3.) of methotrexate, cytarabine, and prednisolone are administered on day 1.

9.4.3. Blinatumomab (BLINA) block

Overview of the cycle: Blinatumomab cycle 1 for patients under 45 kg
(Patients 45 kg or over receive fix doses, see them described in the text):

Agent	Dosage	Application	Week 1							Week 2							Week 3							Week 4							5
Blinatumomab	5 µg/m^2/d (max 9 µg/d)	IV continuous	▬	▬	▬	▬	▬	▬	▬	▬	▬	▬	▬	▬	▬	▬															
	15 µg/m^2/d (max 28 µg/d)	IV continuous								▬	▬	▬	▬	▬	▬	▬	▬	▬	▬	▬	▬	▬	▬	▬	▬	▬	▬	▬	▬	▬	▬	
Methotrexate	Age dep.	IT	▮														▮														▮
Cytarabine	Age dep.	IT	▮														▮														▮
Prednisolone	Age dep.	IT	▮														▮														▮
		Days	1	2	3	4	5	6	7	1	2	3	4	5	6	7	1	2	3	4	5	6	7	1	2	3	4	5	6	7	1

Considerations [26-28]

Blinatumomab (BLINA) can be used as a salvage option in poor extracranial response to therapy, only if blasts are CD19 positive:

- Only for CD19pos BCP immunophenotype cases;

- Can be considered as 1st choice if marrow blasts are detectable but < 25%, especially if < 5%. It may also be tried in case of heavier disease burden but it is less likely to be effective then, so if possible, BLINA should be administered after debulking;
- 1st choice if severe infectious adverse events contraindicate myelotoxic chemotherapy (e.g. invasive fungal infection);
- BLINA doesn't exert sufficient effect in the CNS, it is contraindicated if CSF WBC > 5/uL or CNS infiltration is seen radiologically;
- May repeat BLINA cycles, and if so, it might make sense to alternate it with other treatment blocks instead of immediate repeats, this is to avoid resistance (no evidence, only expert opinion);
- Ideally, do not repeat BLINA cycles if MRD negativity is achieved and SCT is readily available as MRD reappearance is possible after any subsequent BLINA cycle;
- ideally, avoid BLINA if you plan to use CAR-T later (both are anti-CD19 immunotherapies) and you have similarly good treatment choices.
- Monthly IgG level monitoring is recommended after BLINA therapy due to the resulting B-cell aplasia that can last for months or years, and immunoglobulin substitution as required.

Criteria to start and guide the course

Start of BLINA cycle requires recovering blood counts (neutrophils $\geq$ 0.5 x 10^9/L, platelets $\geq$ 50 x 10^9/L), mucositis less than grade 3, and no essential organ dysfunction. Total bilirubin $\leq$ 3x ULN unless the patient has documented Gilbert syndrome. No clinically relevant CNS pathology requiring treatment (e.g. unstable epilepsy) and ideally no acute or uncontrolled chronic infection or other concurrent disease or medical condition that could be exacerbated by the treatment.

Diagnostic measures

Physical and neurological examination	Physical and neurological examination daily during the first days of infusion. Most neurologic events start with a prodromal phase of kinetic tremor. Daily testing is recommended.
Full blood count and chemistry (liver function, urea, kreatinin, electrolytes)	Hemoglobin and platelets can fall significantly during the first few days of infusion. Transient rise in transaminases during first few days of infusion. Recommendation: hematology and chemistry 3 times on the first week, and weekly after that.
BM aspirate on day 1	For all patients.
BM aspirate on day 15	Only if MRD $\geq$ 5% at the beginning of the cycle.
BM aspirate on day 29	Mandatory only if residual leukemia was found in any body compartment at the start of blinatumomab cycle. Otherwise, optional.
CSF analysis with each IT chemotherapy administration	Triple intrathecal therapy as per protocol according to age. CSF cell count, cytospin-morphology, flow cytometry confirmatory test according to institutional/national guidelines.

BM: Bone marrow; CSF: cerebrospinal fluid

Blinatumomab
Blinatumomab is administered as a continuous, 28-day-long intravenous infusion.

Patients under 45 kg:
For patients under 45 kg the dose is 15 μg/m^2/day (maximum 28 μg/day). Blinatumomab should be commenced at 5 μg/m^2/day (Max 9 μg/day) for the first 7 days and then increased to 15 μ/m^2/day (max 28 μg/day) for days 8-28.

Patients over 45 kg:
For patients above 45 kg, the dosage is 28 μg/day. Blinatumomab should be commenced at 9 μ/day for the first 7 days and then increased to 28 μ/day for days 8-28.

Local guidelines may allow other dosing variations. We strongly suggest starting with the smaller dose in order to minimize neurotoxicity and cytokine release syndrome in case of the following patient groups:
- MRD ≥ 5% at the beginning of the cycle
- Patients with combined CNS relapse
- Clinically relevant CNS pathology, CNS comorbidity
- Any other cases when the recognition of neurological symptoms can be difficult (e.g.: intellectual disability, infants/very young children, altered state of consciousness)
If further cycle(s) of blinatumomab are administered, the starting dose in these subsequent cycles can be 15 μg/m^2/day (maximum 28 μg/m2/day), to give this dose throughout the 28 days.

NOTE: Do not flush the blinatumomab infusion line! When administering via a multi-lumen venous catheter, blinatumomab should be infused through a dedicated lumen. See further notes about adverse effects and management in Chapter 10.

Supportive medications
- Dexamethasone 5 mg/m^2 IV 30-60 minutes before the start of the blinatumomab infusion.
- Paracetamol 60 mg/kg/day divided into 4 doses orally or IV during the first 48 hours to reduce pyrexia.

Intrathecal chemotherapy
Age adapted doses (see Table 6 in section 9.3.3.) of methotrexate, cytarabine, and prednisolone are administered on day 1 of week 3 and 5. For CNS3 relapses, also on da1 of week 1.

We recommend starting the next cycle 1 or 2 weeks after the end of the blinatumomab infusion, depending on the patient's blood count and liver function tests.

9.4.4. FLA (IDA) block

Agent	Dosage	Application	Week 1						
Fludarabine	30 mg/m^2/d	IV 30 minutes	▮	▮	▮	▮	▮		
Cytarabine	2 g/m^2/d	IV 3hr	▮	▮	▮	▮	▮		
(Idarubicin)	8 mg/m^2/d	IV 1hr			▯	▯	▯		
(Peg-Asparaginase)	1000 U/m^2	IV 2hr/IM						▯	
Methotrexate	Age dep.	IT						▮	
Cytarabine	Age dep.	IT						▮	
Prednisolone	Age dep.	IT						▮	
		Days	1	2	3	4	5	6	7

Considerations [29-31]

- For any immunophenotype.
- First choice if the above immunotherapies can't be given (e.g. due to contraindications or no target CD markers or lack of funding/availability);
- First choice for patients with overt or persistent CNS disease (both fludarabine and HD-cytarabine penetrate the blood-brain-barrier)
- This toxic cycle may be repeated maximum twice more.
- Idarubicin is an optional part of this cycle. It is recommended in marrow non-remission cases but may be withheld in marrow MRD negative patients or in patients with higher risk of cardiac or myelotoxicity or in cases when this cycle is given at 2nd or 3rd time. The physician should also take the patient's cumulative anthracycline dose into consideration.
- Asparaginase can also be optionally added to this cycle, to patients with resistant extracranial disease.

Criteria to start and guide the course

Starting FLA (IDA) cycle requires neutrophils > 1.0 x 10^9/L, platelets > 50 x 10^9/L, and a clinical and cardiological status allowing for treatment continuation. If a second FLA course or any other subsequent myelotoxic cycle is planned, it should be started not earlier than 28 days after the start of the first course.

Diagnostic measures

Bone marrow aspiration at day 1 of FLA (IDA) cycle and on bone marrow recovery after the cycle, and assessment of other body compartments are mandatory for patients on salvage therapy.

Fludarabine

30 mg/m² as a 30 minutes infusion on days 1-5.

Cytarabine (IV)

2 g/m²/dose, as 3-hour infusion on days 1-5. The infusion should start 4 hours after the end of the Fludarabine infusion. A prophylaxis of conjunctivitis with eye drops and of neuropathy with vitamin B6 at a dose of 100 mg/m² IV is recommended prior to each cytarabine dose, and at least until 48 hours after the last (fifth) infusion.

Idarubicin (optional part of the cycle)
8 mg/m² as a 1 hr infusion on days 3, 4 and 5. The infusion is started just after the Ara-C infusion has been stopped.

PEG-asparaginase (optional part of the cycle)
1,000 units/m² as a 2-hour infusion or intramuscularly on day 6. The infusion of asparaginase should be started at a reduced rate and increased stepwise, if applicable. See further notes on alternative asparaginase agents at "Asparaginase preparations" in Chapter 9.5.1.

Intrathecal chemotherapy
Age adapted doses (see Table 6 in section 9.3.3.) of methotrexate, cytarabine, and prednisolone are administered on day 6.

Venetoclax can optionally be added to FLA-(IDA) cycles.

9.4.5. VANDA block

Agent	Dosage	Application	Week1							Week2							Week3							Week4						
Dexamethasone	20 mg/m²/d	PO/IV																												
Cytarabine	2 g/m²	IV 3 hr																												
Mitoxatrone	8 mg/m²/d	IV 1 hr																												
Etoposide	150 mg/m²/d	IV 2 hr																												
Peg-Asparaginase	1000 U/m²	IV 2 hr / IM																												
Methotrexate	Age dep.	IT																												
Cytarabine	Age dep.	IT																												
Prednisolone	Age dep.	IT																												
		Days	1	2	3	4	5	6	7	1	2	3	4	5	6	7	1	2	3	4	5	6	7	1	2	3	4	5	6	7

Considerations [32, 33]

- This stands mostly as an option for SR arm patients who didn't receive mitoxantrone and didn't receive HC2 (containing similar drugs)
- Very toxic cycle, one of the last resorts
-

Criteria to start and guide the course

Start VANDA cycle requires recovering blood counts (neutrophils ≥ 0.5 x 10^9/L, platelets ≥ 50 x 10^9/L), mucositis less than grade 3, and no essential organ dysfunction and a clinical status allowing for treatment continuation.

Dexamethasone

20 mg/m² (max. 40 mg/day) orally/IV divided in two daily doses on day 1-5.

Cytarabine (IV)

2 g/m²/doses, as 3-hour-long infusion every 12 hours on day 1 and 2 (a total of 4 doses). A prophylaxis of conjunctivitis with eye drops and of neuropathy with vitamin B6 at a dose of 100 mg/m² IV is recommended prior to each cytarabine dose.

Mitoxantrone

8 mg/m² as 1-hour-long infusion on days 3 and 4.

Etoposide

150 mg/m²/dose as a 2-hour-long infusion on days 3, 4 and 5 (total of 3 doses). It should not be infused over < 1 hour since it can lead to hypotension.

PEG-asparaginase

1,000 U/m² as a 2-hour-long infusion or intramuscularly on day 6. The infusion of asparaginase should be started at a reduced rate and increased stepwise, if applicable. See further notes on alternative asparaginase agents at chapter "Asparaginase preparations" in Section 9.5.1.

Intrathecal chemotherapy

Age adapted doses (see Table 6 in section 9.3.3.) of methotrexate, cytarabine, and prednisolone are administered on day 5.

9.4.6. CYCLET block

(Cylophosphamide, clofarabine, etoposide)

Agent	Dosage	Application	Week1							Week2							Week3							Week4						
Clofarabine	40 mg/m^2	IV 2 hr	▮	▮	▮	▮	▮																							
Etoposide	100 mg/m^2	IV 2 hr	▮	▮	▮	▮	▮																							
Cyclophosphamide	400 mg/m^2	IV 1 hr	▮	▮	▮	▮	▮																							
Methotrexate	Age dep.	IT	▮																											
Cytarabine	Age dep.	IT	▮																											
Prednisolone	Age dep.	IT	▮																											
		Days	1	2	3	4	5	6	7	1	2	3	4	5	6	7	1	2	3	4	5	6	7	1	2	3	4	5	6	7

Considerations[42, 43]

- Clofarabine containing cycles were found to be non-superior to other salvage chemotherapy strategies in published trials. There may be individual patients who respond well to it, though, irrespective of immunophenotype.
- This is a very toxic cycle, one of the last resorts.
- This cycle can be repeated if the patient responds well to it.

Criteria to start and guide the course

Starting this cycle requires recovering blood counts (neutrophils $\geq 0.5 \times 10^9$/L, platelets $\geq 50 \times 10^9$/L), mucositis less than grade 3, and no essential organ dysfunction and a clinical status allowing for treatment continuation.

Clofarabine

40 mg/m^2 as 2-hour-long infusion daily on five consecutive days.

Etoposide

100 mg/m^2/dose as a 2-hour infusion daily on five consecutive days. It should not be infused over < 1 hour since it can lead to hypotension.

Cyclophosphamide

400 mg/m^2/dose as a 1-hour infusion daily on five consecutive days.

Intrathecal therapy:

Age adapted doses (see Table 6 in section 9.3.3.) of intrathecal cytarabine, methotrexate and prednisolone on day 1.

9.4.7. TVTC block

(Topotecan, vinorelbine, thiotepa, clofarabine)

Agent	Dosage	Application		Week 1							Week 2							Week 3						
Topotecan	1 mg/m^2/d	IV contin. inf.																						
Vinorelbine	20 mg/m^2	IV 10 min																						
Thiotepa	15 mg/m^2	IV 4 hr																						
Clofarabine	40 mg/m^2	IV 2 hr / IM																						
Dexamethasone	45 mg/m^2	PO																						
Cytarabine	Age dep.	IT																						
Prednisolone	Age dep.	IT																						
		Days	0	1	2	3	4	5	6	7	1	2	3	4	5	6	7	1	2	3	4	5	6	7

Considerations [34]

- Clofarabine containing cycles were found to be non-superior to other salvage chemotherapy strategies in published trials. There may be individual patients who respond well to it, though, irrespective of immunophenotype.
- This is a very toxic cycle, one of the last resorts.

Criteria to start and guide the course

Starting this cycle requires recovering blood counts (neutrophils ≥ 0.5 x 10^9/L, platelets ≥ 50 x 10^9/L), mucositis less than grade 3, and no essential organ dysfunction and a clinical status allowing for treatment continuation.

Topotecan

1 mg/m^2/day IV as continuous infusion on days 1-4 (96 hours).

Vinorelbine

20 mg/m^2 IV over 6-10 minutes, once a week, as intravenous push, over 6–10 minutes, on day 1 of weeks 1, 2 and 3.

Thiotepa

15 mg/m^2 IV over 4 hours, once a week, as intravenous push, over 6–10 minutes, on day 1 of weeks 1, 2 and 3.

Clofarabine

40 mg/m^2 IV, over 2 hours, on 5 consecutive days, from day 4 onwards. This cycle might be repeated once, in that case the dose of clofarabine should be reduced to 30 mg/m^2 in the 2nd cycle.

Dexamethasone

15 mg/m^2/dose every 8 hours on day 4 as prophylaxis against capillary leak syndrome.

Intrathecal therapy

Age adapted doses (see Table 6 in section 9.3.3.) of IT cytarabine and prednisolone on day 0.

G-CSF may be started on day 2 of week 2 as supportive care measure.

9.4.8. Daratumumab-chemotherapy combinations

Daratumumab (DARA) is an anti-CD38 antibody that is being explored for patients with ALL, whose blasts highly express CD38. It has been used as monotherapy or in combination with chemotherapy showing potential benefits, but still few data.

Considerations [34, 35]

- DARA may be tried for CD38pos disease, mostly T-ALL relapses that don't respond to chemotherapy
- It should be administered in combination with chemotherapy

9.4.8.1. Delphinus Cycle 1 (VPLD-DARA)

Agent	Dosage	Application	Week 1							Week 2							Week 3							Week 4							Week 5						
Daratumumab	16 mg/kg/d	IV 1 hr																																			
Prednisolone	40 mg/m^2/d	PO																																			
Vincristine	1.5 mg/m^2	IV																																			
Doxorubicin	60 mg/m^2	IV 1hr																																			
PEG-Asparaginase	2 500 U/m^2	IV 2hr/IM																																			
		Days	1	2	3	4	5	6	7	1	2	3	4	5	6	7	1	2	3	4	5	6	7	1	2	3	4	5	6	7	1	2	3	4	5	6	7

Criteria to start and guide the course

Start of the course requires recovering blood counts (neutrophils ≥ 0.5 x 10^9/L, platelets ≥ 50 x 10^9/L), mucositis less than grade 3, and no essential organ dysfunction.

Daratumumab

Daratumumab 16 mg/kg IV once a day on Days 1, 8, 15 and 22 given in Cycles 1–2. Infusion rate should be increased slowly and gradually over at least 8 hours.

Supportive care measures besides daratumumab administration:

Dexamethasone 5 mg/m^2/dose PO/IV 60 minutes before the start of each daratumumab infusion.

Paracetamol 15 mg/kg/dose PO/IV 60 minutes before the start of each daratumumab infusion.

Chlorphenamine 0.15 mg/kg/dose IV 60 minutes before the start of each daratumumab infusion.

Prednisone

40 mg/m^2 PO divided twice a day on days 1–28, then tapered.

Vincristine

1.5 mg/m² (maximum single dose 2 mg) as 15 min short IV infusion on days 1, 8, 15 and 22.

Doxorubicin

60 mg/m^2 IV as 60 min infusion once on day 1.

PEG-asparaginase

2500 units/m² as 2-hour-long infusion on days 2 and 16. The infusion should be started at a reduced rate and increased stepwise. See further notes on "Asparaginase preparations" in Chapter 9.5.1.

9.4.8.2. Delphinus Cycle 2

Agent	Dosage	Application	Week 6							Week 7							Week 8							Week 9						
Daratumumab	16 mg/kg/d	IV	▮							▮							▮							▮						
Methotrexate	5 g/m²/d	IV 24hr		▮																										
Cyclophosphamide	1 g/m²/d	IV 1hr															▮													
Cytarabine	75 mg/m²	IV/SC	▮															▮	▮	▮	▮				▮	▮	▮	▮			
Mercaptopurine	60 mg/m²/g	PO															▮	▮	▮	▮	▮	▮	▮	▮	▮	▮	▮	▮	▮	▮	▮
		Days	1	2	3	4	5	6	7	1	2	3	4	5	6	7	1	2	3	4	5	6	7	1	2	3	4	5	6	7

Cycle 2 combination with daratumumab could be administered instead of nelarabine-based consolidation therapy, in case nelarabine is not available.

Criteria to start and guide the course

Start of the course requires recovering blood counts (neutrophils ≥ 0.5 x 10^9/L, platelets ≥ 50 x 10^9/L), mucositis less than grade 3, and no essential organ dysfunction.

Daratumumab

Daratumumab 16 mg/kg IV once a day on Days 1, 8, 15 and 22 given in Cycles 1–2. Infusion rate should be increased slowly and gradually over at least 8 hours.

Supportive care measures besides daratumumab administration:

Dexamethasone 5 mg/m²/dose PO/IV 60 minutes before the start of each daratumumab infusion.

Paracetamol 15 mg/kg/dose PO/IV 60 minutes before the start of each daratumumab infusion.

Chlorphenamine 0.15 mg/kg/dose IV 60 minutes before the start of each daratumumab infusion.

Methylprednisolone 0.25 mg/kg (max 20 mg) PO or an equivalent dose of an intermediate- or long-acting corticosteroid orally for 2 days starting after the administration of daratumumab.

Methotrexate IV

5 g/m² IV over 24 hours on day 2. Out of the total, 10% is given as a 30 min bolus and the remaining 90% as a continuous infusion for 23.5 hours. Concomitant alkaline hydration with 3000 ml/m²/24 hours is given on day 1 and 2. Rescue with folinic acid and methotrexate level monitoring is different for this cycle from those of the 36-hour-long infusion regimen's. For detailed instructions, see the frontline ALL protocol ALL IC-BFM 2022.

Cyclophosphamide

1,000 mg/m²/dose as a 1-hour-long infusion on day 15. Mesna is administered at 70 mg/m²/dose before and at 4 and 8 hours after start of each cyclophosphamide infusion. Hydration with 3000 ml/m² is administered from start of cyclophosphamide for a total of 24 hours.

Cytarabine

75 mg/m² as a 1-hour-long infusion or SC once a day on days 16–19 and days 23–26.

6-mercaptopurine

60 mg/m² PO once a day on days 15–28.

9.4.9. Bortezomib combined with chemotherapy

Considerations

Although some previous studies suggest that the use of bortezomib provides a survival benefit only for T-LL and not for T-ALL patients[46], the COG results provide strong evidence that bortezomib reinduction could be a salvage option for T-ALL patients in first relapse. The study enrolled 32 patients with T-cell disease (T-ALL=22 and T-LL=10) and demonstrated a CR2 rate of 68% ± 10% for T-ALL patients. Outcome for patients with T-ALL achieving MRD negative status following reinduction was also favorable (75% ± 37% 3-year EFS and 67% ± 8% 3- year OS)[44]. In this frame, we can recommend bortezomib-based chemotherapy approach as a bridge to SCT for patients who failed to achieve remission with other recommended treatment regimens.

Most published data in ALL are on bortezomib combined either with R3-induction based or with classic 3-drug or 4-drug induction chemotherapy +/- cyclophosphamide. In these studies, the traditional bortezomib dosing was applied: a total of 4 doses 1.3 mg/m^2 each given twice weekly. However, massive amount of evidence in adult hematological malignancies support the fact that bortezomib is less toxic and equally effective if given once weekly. It is therefore reasonable to give the bortezomib doses weekly for heavily pretreated children.[46] Other data suggest that the neurotoxicity of bortezomib is lower if given SC compared to IV.

Some, but less data is available on another proteosome inhibitor, the carfilzomib in the context of ALL.

9.4.10. Further options

Further salvage options may be:

- Venetoclax in combination with various chemotherapy cycles (others than listed above) are good salvage options for treatment-resistant BCP-ALL relapses, not only T-ALL relapses.
- CAR-T cell therapy may be an option for patients whose chemotherapy resistance makes SCT futile. CAR-T cell therapy may be performed in non-remission state and be either curative itself or bridge to SCT.
- Targeted biological therapies may arise as options based on molecular genetic studies of the given leukemic clone.
- HD-MTX as per osteosarcoma or B-NHL protocols (8-12 g/m^2 in 3-6-hour-long infusion). Higher dose, shorter duration infusions of HD-MTX might work e.g. in persistent CNS disease.
- HD-ARAC as per AML-BFM HAM cycle (3 doses of 3 g/m^2 over 3 hours, given every 12 hours) with or without mitoxantrone especially in persistent CNS disease.
- Dexamethasone combined to the above drugs can be given in higher doses (20 mg/m^2/day) or over longer time then described in various cycles especially in persistent CNS disease.
- IT triple chemotherapy can be administered as frequently as 3-times weekly in persistent CNS disease.
- IV and/or IT rituximab (if blasts are CD20 positive) or IT etoposide or IT thiotepa (as per brain tumor protocols).
- Alemtuzumab.

It is difficult to give a solid recommendation when SCT is indicated after CAR-T therapy. The practice of various working groups differs. Any, even presently firm recommendation may change very soon as this is a topic only recently debated and studied. We recommend that such questions get discussed at some international expert forum, e.g. at a FEDRRAL meeting or via the email-list of the ALL-IC REL tumor board.

9.5. Alternative agents

9.5.1. Asparaginase preparations

This guidance advises for the use of PEG-asparaginase at first line.
In case of overt allergic reaction, one dose of PEG-asparaginase will be replaced by Erwinia-asparaginase at a dose of 20 000 units/m² IV or IM (capped at 30 000 units at many centers) every 2nd day for a total of 7 doses if available in the given country.
Monitoring of asparaginase activity and antibodies may be performed according to local or national agreements, or few published international recommendations.
In countries where PEG-asparaginase as first-line preparation is not available, native E. coli-asparaginase may be given as first-line as long as it has been tolerated in previous treatments (4 doses given every 3rd day replacing 1 dose of PEG-asparaginase) at a dose of 10 000 units/m^2 on days 3 and 6 of week 1 and days 2 and 5 of week 2, calculated from the date PEG-asparaginase would be due.

9.5.2. Tyrosine kinase inhibitors (TKIs)

TKIs should be used in all cases where blast genetic features support their use (Philadelphia chromosome, other ABL class fusions typically) or if in vitro or xenograft drug response profiling suggests its efficacy. Combining chemotherapy with imatinib seems to be less toxic than dasatinib, however some data suggest dasatinib is more effective than imatinib. We suggest using dasatinib but leave the decision to the treating physician. There are a few scenarios where dasatinib is of major advantage: in case of isolated or combined CNS relapses because of its penetration to the CNS; also in relapses after frontline imatinib.

10. SIDE EFFECTS OF CHEMOTHERAPEUTIC DRUGS

See a summary of adverse effects below.[36]

Dexamethasone (DEXA)
Mechanism of action: Dexamethasone is an important component of ALL treatment, responsible for glucocorticoid receptor mediated lympholysis.
Metabolism: Dexamethasone is metabolized via CYP450 enzymes (mainly CYP3A4) and glutathione S-transferases and is transported by ABCB1.
Elimination: Hepatic.
Short-term side effects: increased appetite, weight gain (especially facial and abdominal), hyperglycemia, hypopotassemia, hyponatremia, headache, changes in personality or mood (sleeping problems, fatigue, muscle weakness), hypertension, acne, hirsutism, increased risk of infection.
Long-term side effects: decreased muscle mass and muscle weakness, impaired wound healing, osteoporosis, avascular necrosis, cataracts and impaired growth.

Vincristine (VCR)
Mechanism of action: Vincristine is a vinca alkaloid derived from the plant Catharanthus roseus, a tubulin-interactive agent inhibiting the microtubule polarization, that affects the M-phase of the cell cycle. Another mechanism includes interfering with nucleic acid and protein synthesis by blocking glutamic acid utilization.
Metabolism: Hepatic.
Elimination: Hepatic, rapid biliary excretion
Contraindication: Charcot-Marie-Tooth syndrome.
Side effects: Neurotoxicity: a) sensory neuropathy: paresthesia, numbness, impaired touch sensitivity/temperature recognition/vibration, neuropathic pain, jaw pain; b) motor neuropathy: extremity weakness, walking difficulties, deteriorated reflexes and fine motor abilities, impaired balance, muscle cramps; c) autonomic neuropathy: constipation, paralytic ileus, incontinence, urinary retention, orthostatic hypotension, d) seizures (rarely).
Usually no nausea/vomiting, myelosuppression, alopecia (usually minimal hair loss), inappropriate ADH secretion, hepatic sinusoidal obstruction syndrome.
Warning! Intrathecal administration of Vincristine is lethal.
Warning! Extravasation of the drug causes severe tissue necrosis – topical hot pads should be applied, a dilution of Hyaluronidase and saline solution should be administered subcutaneously.

Vindesine (VDS)
Mechanism of action: Vindesine is a vinca alkaloid that affects the M-phase of the cell cycle, a mitosis blocker that interferes with the formation and growth of microtubules by inhibiting tubulin polymerization into microtubules.
Metabolism: Hepatic.
Elimination: Biliary, renal.
Contraindication: Charcot-Marie-Tooth syndrome.
Caution: Incompatible with solution pf pH>6.0.
Interaction: Vindesine leads to Methotrexate loss of efficacy.

Side effects: Usually no nausea/vomiting, myelosuppression, alopecia (usually minimal hair loss), inappropriate ADH secretion. Neurotoxicity is less frequently reported compared to vincristine.
Warning! Extravasation of the drug causes severe tissue necrosis – topical hot pads should be applied, a dilution of Hyaluronidase and saline solution should be administered subcutaneously.

Methotrexate (MTX)
Mechanism of action: Methotrexate is an antimetabolite that interferes with folate metabolism (specific reversible inhibition of dihydrofolate reductase).
Metabolism, enzymes and transporters: ABCB1, ABCC1-4, ABCG2, FPGS, GGH, OAT1-4, OATP1A1/B1/B3, OATP4C1, RFC, PCFT.
Elimination: Primary renal, hepatic.
Caution: Drugs interfering with protein-binding or renal clearance (ex. Cotrimoxazole, non-steroidal anti-inflammatory drugs) should be avoided before and during high-dose methotrexate courses)
Side effects:
- Methotrexate low oral dose: mouth ulcers, skin rashes.
- Methotrexate intermediate/high IV dose: myelosuppression, severe mucositis, skin rashes, hepatotoxicity, rarely: acute renal failure, neurotoxicity; no/mild nausea and vomiting, no hair loss. Systemic over-exposure secondary to impaired clearance is associated with skin rash, severe enteritis, and myelosuppression.
- Methotrexate low intrathecal dose: neurotoxicity – headache, vertigo, ataxia, convulsions, abnormal vision.

Warning! Increased sensitivity to Methotrexate in trisomy 21 and disorders of homocysteine metabolism.

Cytarabine (ARA-C)
Mechanism of action: Cytarabine is a deoxynucleoside analogue that after incorporation into DNA inhibits DNA polymerase and terminates DNA chain elongation. It is converted into the triphosphate form within the cell and competes with cytidine to incorporate itself into the DNA. The process of DNA replication ceases, specifically during the S phase of the cell cycle, making it a specific drug for rapidly dividing cells.
Metabolism: Hepatic.
Elimination: Cytarabine is a pro-drug which is activated to triphosphate form intracellularly. Hepatic metabolism and renal excretion of inactive metabolites.
Side effects: Myelosuppression, nausea & vomiting, mucositis, diarrhoea, gastro-intestinal inflammation and ulceration, abnormal liver function, fever, myalgia, and arthralgia (flu-like syndrome), peripheral edema, sepsis, abdominal pain, urticaria and skin ulcers, abnormal renal function, neuritis and CNS toxicity (cerebellar ataxia/encephalopathy), headaches, pneumonitis, conjunctivitis, alopecia.

6-Mercaptopurine (MP)
Mechanism of action: Mercaptopurine is a purine analog that affects the G1 and S-phase of the cell cycle. It is transformed by the enzyme hypoxanthine phosphoribosyl transferase into triphosphate nucleotides that work as antagonists to endogenous purines required for DNA replication, RNA and protein synthesis. There is also a secondary pathway via thiopurine methyltransferase (TPMT), which produces inactive methylated bases that inhibit purine de novo synthesis.

Metabolism: Hepatic
Elimination: Renal
Side effects: Myelosuppression (dose-limiting, most common in homozygote thiopurine methyltransferase carriers), hepatic toxicity (elevated transaminase levels), pancreatic toxicity, nephropathy, nausea, vomiting, mucositis, diarrhea, hyperuricemia, crystaluria, skin rash, fever.
Warning! Macrophage activation syndrome (MAS) is a rare but life-threatening side effect of 6-Mercaptopurine administration.

6-Thioguanine (TG)
Mechanism of action: Thioguanine is an antimetabolite (purine antagonist) that affects the S-phase of the cell cycle. Thioguanine competes with hypoxanthine and guanine for the enzyme hypoxanthine-guanine phosphoribosyltransferase (HGPRTase) and is converted to 6-thioguanilyic acid (TGMP). It inhibits the biosynthesis of glutamine-5-phosphoribosylpyrophosphate amidotransferase, the first enzyme unique to the de novo pathway of purine ribonucleotide synthesis, and the conversion of inosinic acid (IMP) to xanthylic acid (XMP) by competition for the enzyme IMP dehydrogenase. Thioguanine nucleotides are incorporated into both the DNA and the RNA by phosphodiester linkages, the overall result of its action is a sequential blockade of the utilization and synthesis of the purine nucleotides.
Metabolism: Hepatic.
Side effects: Myelosuppression, nausea and vomiting, hepatic toxicity (veno-occlusive disease), mucositis, diarrhea, hyperuricemia, crystalluria, neurotoxicity (peripheral neuropathy).

Peg-Asparaginase (ASP)
Mechanism of action: Pegaspargase is a conjugate of monomethoxypolyethylene glycol (mPEG) and L-asparaginase (L-asparagine amidohydrolase), an asparagine-specific enzyme that converts L-asparagine into aspartic acid and ammonia, depleting plasma asparagine. Low levels of asparagine inhibit DNA and RNA synthesis and cell division, especially in leukemia cells (dependent on exogenous sources of asparagine), causing apoptosis as the final result.
Metabolism: As a pegylated form of L-asparaginase, pegaspargase is expected to be metabolized by ubiquitously distributed proteolytic enzymes throughout the body.
Elimination: Due to its high molecular weight, pegaspargase is not excreted renally.
Contraindication: acute or chronic pancreatitis
Most important side effects: hypersensitivity, anaphylaxis, coagulopathy, thrombosis, hypercholesterolemia, lowered insulin secretion, pancreatitis, hepatotoxicity, encephalopathy, kidney damage, transient secondary hypothyroidism, hypoparathyroidism.
Warning! Asparaginase enhances the effect of Vincristine, Vindesine and Etoposide.

Cyclophosphamide (CPM)
Mechanism of action: Cyclophosphamide is an alkylating agent, oxazophosphorine, that has a cytotoxic effect in the S phase of the cell cycle, inhibiting protein synthesis through DNA and RNA crosslinking. It is metabolized in the liver to phosphoramide mustard that forms cross-linkages within and between adjacent DNA strands and acrolein, the principal-agent responsible for the manifestation of hemorrhagic cystitis. In addition to antimitotic and antineoplastic effects, cyclophosphamide has immunosuppressive effects and selectivity for T cells.

Metabolism: Hepatic. 75% of the drug is activated by cytochrome P450 isoforms, CYP2A6, 2B6, 3A4, 3A5, 2C9, 2C18, and 2C19.
Elimination: Cyclophosphamide is eliminated primarily in the form of metabolites. 10-20% is excreted unchanged in the urine and 4% is excreted in the bile following IV administration.
Side effects: alopecia, myelosuppression, nausea and vomiting, hemorrhagic cystitis caused by acrolein (toxic metabolite), inappropriate ADH secretion, cardiotoxicity, bronchospasm, renal tubular and glomerular damage, stomatitis, dermatitis, skin rash, facial flushing during injection, anphylaxis, eosinophilia, alveolitis, neurotoxicity, hepatotoxicity, taste alterations, Steven-Johnson syndrome, gonadal toxicity, secondary malignancies.
Interactions: Cyclophosphamide increases insulin and anesthetics effect. Its effect is increased by allopurinol, paracetamol, barbiturates.
Warning! Hemorrhagic cystitis to be prevented by hyper-hydration and concomitant administration of MESNA.

Ifosfamide (IFO)
Mechanism of action: Ifosfamide is an oxazaphosphorine alkylating agent with cytotoxic effect in the G2 phase of the cell cycle. It is metabolized in the liver by CYP450 enzymes to active metabolites – phosphoramide mustard derivatives and acrolein, that bind to DNA and inhibit DNA synthesis. They cause cell damage by forming interstrand or intrastrand crosslinks and by upregulating the reactive oxygen species (ROS), resulting in irreparable DNA damage and the cessation of protein formation.
Metabolism: Primarily hepatic.
Elimination: Renal.
Interactions: Ifosfamide increases the effect of anticoagulants and dexamethasone. N-acetylcysteine causes loss of efficacy of Ifosfamide.
Side effects: alopecia, myelosuppression, hemorrhagic cystitis (more severe than with cyclophosphamide), nephrotoxicity (renal tubular and glomerular damage), metabolic acidosis, nausea and vomiting, stomatitis, dermatitis, skin rash, cardiotoxicity, neurotoxicity (encephalopathy), hepatotoxicity, inappropriate ADH secretion, secondary malignancies.
Warning! More nephrotoxic and urotoxic than Cyclophosphamide. Hemorrhagic cystitis to be prevented by hyper-hydration and concomitant administration of MESNA.

Idarubicin (IDA)
Mechanism of action: Idarubicin is an anthracycline with no phase-specific action, that forms DNA complexes by intercalation between base pairs. It inhibits topoisomerase II activity by stabilizing the DNA-topoisomerase II complex, preventing the religation portion of the ligation-religation reaction that topoisomerase II catalyzes.
Metabolism: Hepatic.
Elimination: Predominately biliary and to a lesser extent by renal excretion, mostly in the form of idarubicinol.
Side effects: Myelosuppression, stomatitis, nausea, vomiting, diarrhea, alopecia, cardiotoxicity, rash, elevated bilirubin and transaminases, peripheral neuropathy, seizure, headache, inflammatory disease of mucous membrane, red discoloration of urine.
Warning! Extravasation of the drug causes severe tissue necrosis – DMSO 99% and topical cooling should be applied.

Daunorubicin (DNR)

Mechanism of action: Daunorubicin is an anthracycline aminoglycoside that acts by intercalating between the DNA base pairs, causing the DNA double helix to uncoil and inhibit the topoisomerase II enzyme, resulting in single and double-strand breaks, thus inhibiting DNA and RNA synthesis. It also inhibits polymerase enzyme activity, dysregulating the gene expression and resulting in free radical damage to the DNA and ultimately resulting in apoptosis, mitochondrial injury, and programmed cell death.

Elimination: Mostly hepatically. 40% of daunorubicin is excreted in the bile while 25% is excreted in an active form (daunorubicin or daunorubicinol) in the urine. In the liposomal formulation, only 9% of active molecules are excreted in the urine.

Caution: Incompatible with solutions of pH>8.0, light protection necessary.

Dose-limiting side effects: Myelosuppression, cardiac toxicity, hepatotoxicity.

Non-limiting side effects: alopecia, mucositis, nausea, vomiting, diarrhoea, skin abnormalities (rash, dermatitis, hyperpigmentation skin/nails), secondary malignancies.

Warning! Extravasation of the drug causes severe tissue necrosis – DMSO 99% and topical cooling should be applied; intravenous Dexamethasone might be considered in case of a high dose of Daunorubicin.

Mitoxantrone

Mechanism of action: Mitoxantrone is an anthracenedione that inhibits the action of DNA topoisomerase II. Its action appears not to be phase-specific and it inhibits DNA and RNA synthesis by intercalating into DNA, causing template disorder, steric obstruction.

Metabolism: Hepatic.

Elimination: The parent drug and metabolites are excreted primarily via hepatobiliary excretion with small amounts excreted in the urine.

Side effects: Myelosuppression, nausea, vomiting, cardiac toxicity, alopecia, diarrhoea, headache, fever, and stomatitis, blue to green discoloration of urine, elevated liver function tests, allergic reactions, seizures, jaundice, and renal failure.

Etoposide (VP 16)

Mechanism of action: Etoposide (epipodophyllotoxin) is a topoisomerase II inhibitor with phase-specific action, primarily in the late S and G2 phases of the cell cycle. It uses the topoisomerase II cleavage complexes (topoisomerase II creates and reseals double-stranded DNA breaks during the replication process) and inhibits the second step of the reaction, DNA re-ligation. The etoposide-topoisomerase II complex triggers a mutagenic and cell death pathway, working best in tumor cells with higher levels of topoisomerase II enzymes.

Metabolism: Primarily hepatic (through O-demethylation via the CYP450 3A4 isoenzyme pathway) with 40% excreted unchanged in the urine.

Elimination: Renal and biliary excretion. Glucuronide and/or sulfate conjugates of etoposide are excreted in the urine.

Caution: It should not be diluted with glucose or buffered solutions with pH>8 (precipitation). Grapefruit products should be avoided (inhibition of CYP3A4 metabolism, which may increase the serum concentration of etoposide).

Dose-limiting side effects: Myelosuppression

Non-limiting side effects: Nausea, diarrhea, mucositis, anorexia, alopecia, hypertension following rapid intravenous infusion; transient liver function abnormalities; anaphylactic-like reaction with fever, chills, bronchospasm, dyspnea and tachycardia (readministartion is safe with premedication – corticosteroids and antihistamines); peripheral neuropathy; etoposide induced secondary AML.
Warning! Fast intravenous infusion causes hypotension.

Blinatumomab (Blina)

A) Infusion reaction:

Infusion reactions are very common within the first few hours of the blinatumomab infusion. It is usually in the form of fever, flu-like symptoms. It can be indistinguishable from cytokine release syndrome. If convinced this is an infusion reaction (and not cytokine release syndrome), it can be managed symptomatically using paracetamol, and hydrocortisone. Ibuprofen can be given if fever is unresponsive to paracetamol. There is no need to stop the infusion, unless there are other signs of cytokine release syndrome. Blood cultures and CRP should be taken and consider the use of antibiotics as a supportive care, if the patient is neutropenic.

B) Cytokine Release Syndrome (CRS):

Cytokine release syndrome is a constellation of inflammatory symptoms resulting from T-cell engagement and proliferation. In many patients, symptoms are mild and flu-like with fevers and myalgia. However, some patients experience a severe inflammatory response syndrome including vascular/capillary leak, hypotension, pulmonary oedema, coagulopathy (DIC), leading to multiorgan failure. There have been reported cases of HLH/Macrophage Activation Syndrome (MAS) during treatment with blinatumomab.

The Common Terminology Criteria for Adverse Events (CTCAE) version 5.0 will be used for the definition of CRS.[(24)]

CRS is graded according to the revised CRS grading system:

CRS parameter	Grade I.	Grade II.	Grade III.	Grade IV.
Fever	≥ 38 °C	≥ 38 °C	≥ 38 °C	≥ 38 °C
With				
Hypotension	None	Not requiring vasopressors	Requiring vasopressors with or without vasopressin	Requiring multiple vasopressors (excluding vasopressin)
and/or				
Hypoxia	None	Requiring low-flow nasal cannula or blow-by	Requiring high-flow nasal cannula, face-mask, nonrebreather mask, or Venturi mask	Requiring positive pressure (CPAP, BiPAP, intubation and mechanical ventilation

Treatment of CRS:

Grade	Management
Grade I.	• Continue Blinatumomab infusion at current rate • Give paracetamol, chlorphenamine +/- hydrocortisone

Grade II.	• If hypotension occurs, give one 20 ml/kg fluid bolus. • If hypotension is unresponsive to one fluid bolus, or if the child has multiple/extensive comorbidities: - pause blinatumomab infusion - give dexamethasone 0.4 mg/kg/day (maximum 24 mg/day) preferably IV, in 3 divided doses until symptoms resolve. The dose should be weaned by at least 25% over 4 days. - consider tocilizumab (see below) • Restart infusion 48 hours after resolution of CRS
Grade III. and Grade IV.	• Stop Blinatumomab infusion. • Give dexamethasone 0.4 mg/kg/day (maximum 24 mg/day) preferably IV, in 3 divided doses until symptoms resolve. The dose should be weaned by at least 25% over up to 4 days. • Give tocilizumab (see below) • Do not restart Blinatumomab infusion unless discussed and agreed with a hematology consultant. A decision may be taken to discontinue blinatumomab treatment.

Tocilizumab is a recombinant humanised monoclonal antibody to IL-6. IL-10, IL-6, and INF-γ are the most highly elevated cytokines in patients who develop cytokine release syndrome CRS after receiving blinatumomab. Use tocilizumab in children who develop Grade 2 CRS that is refractory to 20ml/kg fluid bolus, and those who develop grade 3 or 4 CRS.

Dose: Weight ≥ 30 kg is 8 mg/kg IV administered over one hour.
Weight < 30 kg is 12 mg/kg IV over one hour.

C) Neurological toxicity:

Neurological symptoms caused by blinatumomab can include: cranial nerve disorders, deliria (including confusion), demyelinating disorders, dementia and amnestic conditions, movement disorders (including parkinsonism), disturbances in thinking and perception, neurological disorders, psychiatric disorders, seizures, schizophrenia and other psychotic disorders, cognitive and attention disorders and disturbances, communication disorders and disturbances.

Due to the great variety of symptoms, it is essential to assess and document the child's neurological status regularly.

For grading and assessment, we suggest using the Common Terminology Criteria for Adverse Events (CTCAE) version 5.0.

- Management of CTCAE Grade I. neurotoxicity:
  - Blinatumomab infusion may be temporarily stopped, but do NOT disconnect and discard blinatumomab.
  - NO dexamethasone is required.
  - Reassess patient after 10-12 hours (or overnight) and consider restarting the infusion.
- Management of CTCAE Grade > II. neurotoxicity:
  - Blinatumomab infusion should be interrupted and intravenous dexamethasone should be administered 0.4mg/kg/day (maximum 24mg/day) three times a day until symptoms subside. Dexamethasone should be weaned by at least 25% per day over at least 4 days.
  - Treatment should be interrupted for at least 48 hours.

- If the symptoms resolved, the cycle can be resumed at the reduced dose of 5 µg/m^2/day (or 3.75 µg/m^2/day if patient was receiving 5 µg/m^2/day when event occurred).
- If the neurologic event was a seizure, appropriate prophylactic anticonvulsant treatment should be administered for the rest of the cycle.
- In case of Grade III/IV. neurotoxicity, blinatumomab should be discontinued permanently.

Further drugs

For adverse effects of further chemotherapy and targeted immunological or biological agents, please look up the drug SPCs and current publication.

11. EMERGENCIES

The main problem of intensive multi-agent chemotherapy is the combination of direct organ and mucosal toxicity and marked immunosuppression which may result in serious infections. A number of protective and supportive measures are urgently required to prevent potentially serious harm associated with therapy.

11.1. Acute tumor lysis syndrome

The acute tumor lysis syndrome (TLS) is rare in children with relapsed ALL, since this type of leukemia in general is relatively resistant to therapy. Acute TLS can occur when large numbers of leukemic cells are rapidly destroyed, leading to the release of various substances, including purine degradation products (xanthine, hypoxanthine, and uric acid), potassium, and phosphate. This can result in potentially life-threatening complications such as renal damage, hyperkalemia, hypocalcemia, arrhythmia, seizures.

To prevent acute tumor lysis syndrome and its associated complications, several interventions are commonly used:

- Forced diuresis: This involves maintaining a high urine output (usually 3-5 L/m^2/day) through the administration of intravenous fluids. Adequate hydration helps promote the excretion of uric acid, also phosphate and prevent its precipitation in the renal tubules. Close monitoring of fluid balance is important, and the diuretic furosemide may be used as needed.
- Allopurinol: allopurinol is a medication that inhibits the production of uric acid. It is typically administered at a dose of 10 mg/kg/day (300 mg/m^2/day, max. 800 mg PO) to prevent hyperuricemia. By reducing uric acid levels, the risk of uric acid crystal formation and subsequent renal damage is mitigated.
- Rasburicase: rasburicase is a recombinant urate oxidase enzyme that rapidly breaks down uric acid. It is used in cases of significant hyperuricemia, renal insufficiency, or marked hyperleukocytosis. Rasburicase can quickly lower uric acid levels and prevent or treat TLS-related complications. We recommend using rasburicase for patients with WBC > 100 G/l, or those with serum uric acid > 1000 µmol/L once or > 600 µmol/L measured repeatedly (if measured so, to be repeated in 4-6 hrs).
- Hemodialysis: In severe cases of hyperkalemia, hyperphosphatemia, hyperuricemia, or renal insufficiency that do not respond to conservative measures, hemodialysis may be necessary. Hemodialysis helps remove waste products and correct electrolyte imbalances.
- Careful antileukemic therapy; application of steroid prephase or delaying treatment until TLS resolves.

It is crucial to closely monitor laboratory parameters, including uric acid levels, electrolytes, and renal function, to promptly identify and address any TLS-related complications.

11.2. Impaired elimination of methotrexate

It is advisable to measure methotrexate levels after the MHD IV MTX infusions. The serum methotrexate level 48 hours after the start of methotrexate infusion is generally below 0.5 µmol/L. Otherwise, folinic acid rescue is extended at six hourly intervals beyond the scheduled doses at 48

and 54 hours, until the methotrexate level falls below 0.25 µmol/L. The dose of folinic acid depends on the methotrexate level and is calculated as 15 mg/m^2 antagonizing up to 1 µmol/L serum methotrexate. If the methotrexate level at 48 hours is > 2.0 µmol/L, alkaline diuresis with 3 to 4.5 L/m^2 is used in addition. If the methotrexate level at 48 hour is > 5 µmol/L or in cases of marked intolerance with severe vomiting, diarrhea and neurological symptoms, the use of carboxy peptidase should be considered. Carboxy peptidase results in enzymatic cleavage of methotrexate. In this case, contact the national or international study coordinator. If a decreased elimination of methotrexate is apparent at 36 hours (MTX level > 10 µmol/L), a methotrexate serum level at 42 hours is recommended. In this case the administration of leucovorin should be adjusted to a dose equivalent to that recommended by the rescue scheme at 42 hours (15 mg/m^2 antagonizing up to 1 µmol/L serum methotrexate). If the value is > 5 µmol/L, the dose of folinic acid is calculated using the following formula:

leucovorin (mg) = MTX at 42h (µmol/L) x body weight (kg).

11.3. Extravasation of anthracyclines or vinca alkaloids

In case of extravasation of an anthracycline, the extravasate as well as the tissue fluid and blood should first be aspirated, using the existing venous access and, if possible, diluted by instilling normal saline before removing the vascular line. Topical application of dimethylsulfoxide (DMSO 99%) four drops per 10 cm^2 skin three times a day for several days may ameliorate the course. The local area of skin should be kept cool for several days.
In case of extravasation of a vinca alkaloid, the extravasate as well as tissue fluid and blood should be aspirated, using the existing venous access. Then hyaluronidase (150 units/mL NaCl 0.9%) should be injected into that area using the existing venous access before removing it. Subsequently, the affected tissue can be infiltrated subcutaneously with several small injections of hyaluronidase. The local area should be kept warm (in contrast to the cooling recommended for anthracycline extravasations). If necrosis develops despite these local measures, early surgical revision should be considered.

12. SUPPORTIVE CARE

Supportive care is performed according to national and local guidelines. A regular pneumocystis carinii prophylaxis is recommended as well as an effective antimycotic prophylaxis.

12.1. Anti-infectious prophylaxis

The routine use of G-CSF is recommended after each myelotoxic consolidation cycle.

From the start of treatment until the end of maintenance or until SCT the following measures may optionally be applied (for supportive care during SCT see SCT protocols):
Pneumocystis carinii prophylaxis: Cotrimoxazole, 2-3 mg/kg trimethoprime (10-15 mg/kg sulfamethoxazole) BID on 2 days per week (e.g. Saturday and Sunday). The drug should not be given the same day as high dose methotrexate. Cotrimoxazole may cause prolonged cytopenias. If this is suspected, the drug should be interrupted or discontinued. As an alternative inhalation with pentamidine 300 mg once a month or Dapsone 4 mg/kg weekly needs to be considered.
Local antifungal prophylaxis: Amphotericin B suspension for local (oral mucosa and upper GI tract) candida prophylaxis: The amphotericin suspension is carefully spread over the entire oral mucosa and then swallowed. If prophylaxis with amphotericin suspension is not feasible or if thrush becomes apparent despite prophylaxis, fluconazole (8-12 mg/kg/d) is recommended. Hepatic toxicity and possible drug resistance have to be considered.
Systemic antifungal prophylaxis: In case of prolonged aplasia, systemic antifungal prophylaxis according to preferences at the different centers may be applied. Schemes such as daily oral voriconazole, posaconazole or intermittent liposomal amphotericin-B 3x/week have been applied. Note the interaction between azol antifungals and vinca alkaloids.

12.2. Anti-emetic treatment

Ondansetron (two doses of 5 mg/m^2/day) may be used for highly emetogenic treatment elements such as high-dose Ara-C, ifosfamide and cyclophosphamide. Additional treatment with aprepitant, metoclopramide or dimenhydrinate may be required, if that agent is insufficient particularly in adolescents. Many treatment elements already include the administration of dexamethasone, so that no further anti-emetic effect can be expected from this agent.

12.3. Febrile neutropenia

In case of a neutrophil count below 0.5 x 10^9/L and fever greater than 38.3°C or greater than or equal to 38ºC for at least an hour, systematic antibiotic and possibly anti-fungal treatment has to be administered. Particularly patients with a high therapeutic risk (e.g. patients with very early relapse during initial treatment or fever at the beginning of cytopenia) require rapid escalation of antibiotics to be able to control severe infections until the regeneration of cells.

12.4. Transfusion associated graft versus host disease

To avoid TA-GVHD, we recommend using irradiated blood products to patients who received fludarabine or clofarabine, and also to SCT patients from the start of conditioning.

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