STANDARD CLINICAL PRACTICE RECOMMENDATIONS
ACUTE LYMPHOBLASTIC LEUKEMIA (ALL)

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Table of contents Page

CHAPTER 1

Philadelphia-chromosome Negative (Ph-) Acute Lymphoblastic Leukemia (ALL)

1. BACKGROUND AND RATIONALE

1.1 Background

The cure rates and survival outcomes for pediatric patients with Acute lymphoblastic leukemia (ALL) have improved dramatically over the past several decades. Improvements are largely due to advances in the understanding of the molecular genetics, underlying biology and pathogenesis of the disease, the incorporation of risk-adapted therapy, the advent of new targeted agents and the use of allogeneic hematopoietic stem cell transplantation (HSCT). Acute lymphoblastic leukemia (ALL) in children and adolescents is now considered a disease comprising very heterogeneous subentities which carry the same “look” - the leukemic phenotype - but harbor widely different activated pathways. Cytogenetics and molecular genetics reveal the wide variety in this disease (Moorman*, et al* 2010, Pui*, et al* 2011) and high resolution sequencing has also disclosed the enormous clonal heterogeneity of the disease (Fischer*, et al* 2015, Koren*, et al* 2014, Notta*, et al* 2011, Roberts*, et al* 2014a). It has previously also been noticed that the individual response to treatment (as assessed by minimal residual disease, MRD) is a very reliable parameter of outcome in nearly all of ALL subgroups (Borowitz*, et al* 2008, Cave*, et al* 1998, Schrappe*, et al* 2012, van Dongen*, et al* 1998). Recurrence of disease is most likely due to intrinsic resistance of disease subclones, and may emerge after short or extended periods of remission (Eckert*, et al* 2011, Ford*, et al* 2015, Ma*, et al* 2015), or through escape mechanisms (Alsadeq*, et al* 2015, Krause*, et al* 2015).

Within the ALL strategy committee of the **International BFM Study Group (I-BFM-SG)** a number of highly successful clinical trials for childhood ALL have been developed over the last decades, which were derived from the original "BFM backbone". Progress in treatment outcome has been made mainly through refinement of risk stratification and a more personalized risk-adaptation of treatment. Modifications of essentially all elements of therapy have been evaluated, and the results debated at the annual I-BFM-SG meetings. This large inter-group effort helped new I-BFM-SG members to decide on treatment protocols best adapted to the local situation with regard to infrastructure, resources, supportive-care potential and laboratory capacities.

One of the most important achievements made first by the ALL-BFM group 30 years ago was the recognition that early treatment response, in particular the response to the prednisone prephase (absolute blast count on day 8, after 7 days of prednisone and x1 dose of IT MTX) is the strongest prognostic factor. Due to the highly reproducible results when analyzing the reduction of blasts in the peripheral blood (PB) most groups have introduced the prednisone response into their stratification system. Later on, several groups also evaluated the early response in the bone marrow, mostly on day 15 of treatment (BM d15), adding prognostic information.

In 1991, some groups (AIEOP, BFM, DCLSG, EORTC) started evaluating the response as measured by a more sensitive technique, namely the molecular detection of leukemia-specific gene rearrangements, i.e. of the T-cell receptor (TCR) gene and the immunoglobulin heavy chain (IgH) gene. The results have revealed that the level of minimal residual disease (MRD) at defined time points could provide very specific prognostic information. The major disadvantage of this approach was and still is the enormous logistic and technologic burden when used on a large number of patients. The AIEOP and BFM study groups nevertheless decided in 1998 to develop a protocol that would try to skip basically all risk group definitions derived from factors at diagnosis (age, WBC, immunophenotype) and base the new strategy also exclusively on the prednisone pre-phase response and MRD level in week 5 and 12 of therapy (Riehm, *et al* 1987, Conter, *et al* 1998, Schrappe, *et al* 2000b, Conter, *et al* 2000).

Since then, several clinical trials have demonstrated that it is feasible to monitor MRD in very large cohorts of ALL. These trials have succeeded to demonstrate the strong prognostic impact of quantified treatment response (Brüggemann*,et al* 2006, Flohr,*et al* 2010, Conter *et al* 2010, Möricke,*et al* 2010, Schrappe*,et al* 2011, Vora*,et al* 2014).

The AIEOP-BFM ALL 2000 trial started in the summer 2000 with a central study question: Can such a new response-based stratification improve the overall outcome and also reduce toxicity by adjusting treatment intensity more specifically to the patient's individual relapse risk? In all risk groups, treatment modifications were introduced on a controlled basis (Conter,*et al* 2010). The main randomized study referred to the use of different steroids (dexamethasone vs prednisone) in Protocol IA.

Other I-BFM-SG members developed a trial, **ALL-IC- BFM 2002** *(ClinicalTrials.gov Identifier: NCT00764907),* with a similar stratification system and risk assignment by response, but without the need to use the extensive and expensive techniques for MRD detection. It became clear that the combination of response evaluation in PB on day 8, in BM on day 15 and in BM on day 33 (end of induction) might provide the tool to adjust treatment intensity accordingly, allowing the combination of forces together with the AIEOP/BFM 2000 trial. In addition, prospective studies on MRD by using various techniques, were developed.

Following ALL-IC- BFM 2002, **ALL-IC- BFM 2009** trial was developed *(ALL IC 2009 Trial of the I-BFM Study Group, 2009)*, with a similar stratification pattern and focusing on three study design questions: 1) the outcome of SR patients, defined by SR according to ALLIC 2002 criteria and a FC-MRD load <0.1% on day 15, 2) the addition of an early intensification, protocol IB (Augmented BFM) and its impact on survival, 3) the optimal dose of MTX randomly evaluated in consolidation. Results of randomized study design questions are anticipated and ready to be published.

Regarding frontline therapy in ALL, treatment relies mainly on combinations of hormone treatment (glucocorticoids), amino acid depletion (Asparaginase) but also on alkylating agents and antimetabolites, in addition to classical metaphase blockers and anthracyclines (Möricke*, et al* 2010). So-called novel agents which may target activated driver pathways have so far been of limited value, with the exception of tyrosine-kinase inhibitors in the rare subgroup with Philadelphia-chromosome positive ALL (Biondi*, et al* 2012, Schultz*, et al* 2009). Recently, novel risk groups of precursor-B-cell ALL called Philadelphia-like (or *BCR-ABL*-like) ALL have been identified (Den Boer*, et al* 2009, Mullighan*, et al* 2009). Some of the genetic lesions found are potentially targetable by existing agents which has raised some hope for more effective treatment in patients at increased risk of relapse (Loh*, et al* 2013, Weston*, et al* 2013). Trial **AIEOP-BFM ALL 2000** has been the platform to evaluate and validate new signatures for high-risk subpopulations of ALL (Boer*, et al* 2016, Cario*, et al* 2010, Dörge*, et al* 2013, Fischer*, et al* 2015, Palmi*, et al* 2012, Zaliova*, et al* 2014). For instance, the presence of the *CRLF2-P2RY8* fusion and of *IKZF1* deletions have been detected as high-risk features of ALL. However, it was also shown that the prognostic effect is different dependent on the degree of measurable treatment resistance. The comprehensive molecular characterization of the fatal t(17;19) positive ALL with the *TCF3-HLF* fusion gene was the key to identify novel disease mechanisms and treatment targets. In this trial two randomized studies on intensified use of Peg-asparaginase were implemented.

Trial **AIEOP-BFM ALL 2017** is currently active *(ClinicalTrials.gov Identifier: NCT03643276),* aiming to take all of the above novel circumstances into account for the treatment of patients at increased risk of failure. Risk of recurrence depends both on intrinsic genetic features as well as on individual treatment response, thus, all patients need risk-adapted genetic screening for prognostically relevant lesions, and response-oriented treatment adaptation. Thus, trial AIEOP-BFM ALL 2017 is aiming to contribute to better understanding of disease mechanisms, to future improvement of diagnostics, to better definition of response assessment as well as to individual risk adaptation of therapy. Of note in this trial different randomized studies have been implemented, with two of them including the use of immunotherapy agent Blinatumomab.

The current ESCP or PH Negative ALL has been mainly derived from the ALLIC-2002 protocol whose solid results in terms of efficacy, toxicity and follow-up have been published (Stary, *et al* 2014). However, modifications have been introduced in some parts for the ESCP for Ph Neg ALL (e.g. biological indicators of higher risk, BMT indications, CRT strategy) based on consolidated evidence from more recent AIEOP-BFM ALL experiences/protocols.

1.2 Aim

The aim of the current project, which is a joint initiative of SIOP Europe and the ERN PaedCan called the **European Standard Clinical Practice (ESCP) Project,** is to provide clear, homogenous and widely accepted standard clinical practice recommendations for newly diagnosed ALL patients among European widening countries. This Project has emerged as a major strategic objective from the recent SIOPE and ERN PaedCan meetings and the survey conducted by the SIOPE CRC Chairs among the ECTGs and National Associations of Pediatric Hematology and Oncology (NAPHOs) Chairs.

This project aims to develop and approve clinical recommendations reflecting current best practice for childhood acute lymphoblastic leukemia (ALL). We strongly believe that widely available standard clinical practice protocols reflecting the standard clinical practice, as defined by the collective European experts in each field (i.e. the ECTGs) will provide a benchmark for all European Widening Countries.

2. PATIENT GROUP

2.1 Patient Group

The current ESCP recommendations apply to:

- ✓ Newly diagnosed ALL patients
- ✓ Age≤18 years
- ✓ Diagnosis of ALL ensured by all the diagnostic criteria defined according to ESCP Guidelines
- ✓ Admission, diagnosis and therapy performed by experienced centers
- ✓ Any responsibilities linked to the application of the present ESCP solely lie with the attending physician of the center where the patient is actually being treated.

3. DEFINITIONS

3.1 Definitions of Response and Remission Status

3.1.1 Prednisone response

The Prednisone response is evaluated at the end of the cytoreductive prephase with 7 days Prednisone and one dose intrathecal Methotrexate. The absolute blast count in the peripheral blood on day 8 is decisive for categorization.

- **Prednisone Good Response (PGR):** absolute blast count in peripheral blood < 1000/µl
- **Prednisone Poor Response (PPR):** absolute blast count in peripheral blood ≥ 1000/µl

The Prednisone response is relevant for risk stratification in T-ALL and for alloHSCT indication in infants with BCP-ALL and *KMT2A* rearrangement. In other patients with BCP-ALL (or with unknown immunophenotype) it is no longer used for risk stratification as long as MRD results are available.

3.1.2 Bone Marrow (BM) status

The cut-off limits for the definition of bone marrow (BM) status are shown in **Table 1** below.

Table 1: Definition of BM Status

BM Status	M1	M2	M3
% blasts in BM	<5%	≥5-<25%	≥25%

3.1.3 Complete remission

Complete remission can *per definitionem* not be stated before day 33 of Protocol I.

Complete remission (CR) has been achieved when the following criteria are fulfilled:

• <5% blast cells (M1) in representative bone marrow with sufficient cellularity and signs of regeneration of normal myelopoiesis. In case <5% blast cells are documented in aplastic bone marrow, a new bone marrow aspiration needs to be performed after one week to confirm early response and CR in a representative regenerative sample. Those patients are still classified as early responders despite delay in regeneration.

• ≤ 5 nucleated cells/µl in CSF, or > 5 nucleated cells/µl and no evidence of blasts in cytospin.

• no evidence of leukemic infiltrates as evaluated clinically and by imaging; a preexisting mediastinal mass must have decreased at least to 1/3 of the initial tumor volume.

Identification of residual blast cells by PCR or flow cytometry is *not* decisive for assessment of complete remission.

3.1.4 Late-Response

A patient, who is not in CR on day 33 but achieves CR in the regenerating bone marrow with Protocol IB or any of the three HR blocks is characterized as having Late-Response.

3.1.5 Morphological Non-Response (resistance to protocol treatment)

A patient, who has not achieved CR in the regenerating bone marrow after the 3rd HR block, is classified as having morphological Non-Response (resistance to protocol).

3.1.6 Molecular Non-Response (resistance to protocol treatment)

A patient who has an MRD load of ≥ 5x10-4 after having received the high-risk treatment is classified as having molecular Non-Response (resistance to protocol).

3.1.7 Relapse

The diagnosis of relapse can only be made if complete remission has been achieved before. If a relapse is diagnosed, the patient should be promptly enrolled on the relapse trial with which the participating center has the best experience. **Table 2** summarizes the definitions for various kinds of relapse seen in ALL.

Table 2: Definition of Relapse by Site

Isolated BM	✓ ≥ 25% lymphoblasts in bone marrow without extramedullary involvement. ✓ ≥ 5% and <25% lymphoblasts in bone marrow and confirmation of the prior clonal abnormality by flow cytometry and/or cytogenetics/FISH and/or PCR.
Isolated CNS	✓ >5/µl nucleated cells in CSF and morphologically unequivocal evidence of lymphoblasts ✓ If an intracranial mass is detected by imaging without evidence of blasts in CSF and this tumor is the only site of the suspected relapse, a biopsy is required.
Isolated testicular	✓ Unilateral or bilateral painless hard testicular tumor ✓ If the testis is the only site of the suspected relapse, a biopsy of the tumor is mandatory.

Isolated infiltrates at other sites	✓ Diagnosis needs imaging and/or biopsy
Combined	✓ ≥ 5% lymphoblasts in bone marrow and at least one extramedullary site localization

3.2 Definitions of stratification-relevant genetic aberrations

3.2.1 IKZF1plus

The *IKZF1*plus type is defined as *IKZF1* deletion co-occurring with deletion in *CDKN2A or CDKN2B* (only homozygous deletions) or *PAX5* or *PAR1 (P2RY8-CRLF2*) in the absence of *ERG* deletion. Taking into consideration the potential co-occurrence of *IKZF1*plus with other genetic aberrations, a hierarchy is defined for predefined recurrent genetic aberrations with well-defined prognosis. Furthermore, *IKZF1*plus will have no consequence for risk stratification in patients with negative PCR-MRD or FC-MRD at the end of induction phase (MRD TP1).

In summary, *IKZF1*plus will qualify patients for HR treatment if they are *not* MRD-negative at TP1 *and not* detected positive for *ETV6-RUNX1* (*TEL-AML1*), *TCF3-PBX1* (*E2A-PBX1*) or *KMT2A* (*MLL*) rearrangement other than *KMT2A-AFF1* (*MLL-AF4*).

3.2.2 Hypodiploidy

Hypodiploidy as high-risk factor is defined by a modal chromosome number of less than 45 chromosomes. Patients with less than 44 chromosomes will in addition have an indication for alloHSCT except those FC/PCR-MRD-negative at TP1.

Genetic findings in conventional karyotyping showing a high-hypodiploid modal chromosome number (e.g. 44 chromosomes) with a complex aberrant karyotype need further diagnostic work-up to decide if hypodiploidy is applicable for stratification to HR.

3.2.3 Other genetic aberrations

Identification of the fusion genes *ETV6-RUNX1, KMT2A-AFF1*, other *KMT2A* rearrangements (only infants) and *TCF3-HLF* (previous nomenclature *TEL-AML1, MLL* rearrangements, *MLL-AF4, E2A-HLF*) is also essential for risk group allocation.

3.3 Definitions of organ involvement

3.3.1 CNS status and CNS disease

Symptomatic central nervous system (CNS) disease at the onset of ALL is rare and the diagnosis of CNS involvement is thus usually made in asymptomatic patients. Although fewer than 5% of children with ALL have CNS disease at the time of diagnosis, it remains the most common site of extramedullary leukemia. Some children, such as those with T-cell ALL, have a higher incidence of CNS leukemia which may be explained by the propensity of T-cells to migrate to extramedullary sites. Leukemic blasts in CSF are identified in about 15 to 30% of patients at diagnosis (Bürger*, et al* 2003, Gajjar*, et al* 2000, Mahmoud*, et al* 1993), the majority of whom lacks neurological symptoms.

A comprehensive definition of CNS disease regards several aspects, such as definition of CNS status including blood contamination of the cerebrospinal fluid (CSF), presence of bulk tumor and CNS symptoms.

3.3.1.1 CNS status

The CNS status is determined by the number of nucleated cells in CSF, the presence of blasts in initial CSF before start of chemotherapy, the finding of CSF contamination with blood and the presence of clinical and imaging findings of CNS disease.

Bloody CSF may obscure the diagnosis of CNS leukemia at the presentation of ALL.

The CNS status is defined as follows:

- **CNS 1:** no clinical or imaging findings of CNS disease and absence of blasts on cytospin preparation in CSF, regardless of the number of white blood cells (WBCs) and regardless of red blood cells (RBCs) or bloody contamination.

- **CNS 2:** no clinical or imaging findings of CNS disease ***and***:
  - ✓ **CNS 2a:** <10/µl RBCs and no macroscopical contamination with blood; ≤ 5/µl WBCs and cytospin positive for blasts
  - ✓ **CNS 2b:** Macroscopical contamination with blood and/or ≥ 10/µl RBCs; ≤ 5/µl WBCs and cytospin positive for blasts
  - ✓ **CNS 2c:** Macroscopical contamination with blood and/or ≥ 10/µl RBCs; > 5/µl WBCs and cytospin positive for blasts but negative by algorithm as specified below (i.e. WBC/RBC in CSF < 2x WBC/RBC in blood).

- **CNS 3**: CNS Disease
  - ✓ **CNS 3a:** <10/µl RBCs and no macroscopical contamination with blood; > 5/µl WBCs and cytospin positive for blasts
  - ✓ **CNS 3b:** ≥ 10/µl RBCs and/or macroscopical contamination with blood, > 5/µl WBCs and cytospin positive for blasts and positive by algorithm as specified below (i.e. WBC/RBC in CSF > 2x WBC/RBC in blood).
  - ✓ **CNS 3c:** Clinical or imaging findings of CNS disease:
    - CNS masses or clear leptomeningeal infiltration on Magnetic Resonance Imaging (MRI) and/or Computed Tomography (CT)
    - Cranial nerve palsies if not caused by extracerebral manifestations

Algorithm for classification of CNS positivity (CNS 2c or CNS 3b) in case of initial traumatic lumbar puncture:

CSF WBC / CSF RBC > 2x Blood WBC / Blood RBC

In the case of a lumbar puncture with high blood contamination (e.g. > 100 RBC/µl), the above-mentioned algorithm may not be applicable.

If the CSF is taken after start of glucocorticoid treatment or chemotherapy, the CSF is considered evaluable until the third day of Prednisone treatment if no other systemic or intrathecal chemotherapy has been given. Beyond day 3, the CNS status cannot be defined and the patient has nevertheless to be treated according to the actual findings.

3.3.1.2 CNS disease

The initial CNS status does not influence the risk group allocation, yet determines the presence of CNS disease and the CNS-directed treatment administered:

- ✓ Patients with CNS status **CNS 1 and CNS 2** receive the regular intrathecal injections.
- ✓ Patients with CNS status **CNS 3** are considered having CNS disease (CNS positive) and receive additional intrathecal injections in Protocol IA and during reintensification treatment.

3.3.2 Testicular involvement

Testicular involvement is diagnosed clinically and is defined as the presence of painless enlargement of one or both testes. For patients with initial testicular involvement, no modifications in risk group allocation or therapeutic management are planned.

Overt testicular involvement at diagnosis is rare. Occult testicular leukemic infiltration at diagnosis, however, may be quite common in the presence of a high tumor burden. The leukemic infiltration is mainly in the interstitial spaces, but it has also been observed to invade and accumulate beneath the Sertoli cell layer. Destruction of the tubules by the infiltrate occurs in advanced cases, and bilateral microscopic testicular infiltration is common despite unilateral clinical testicular enlargement.

3.3.3 Mediastinal mass

Mediastinal enlargement is more commonly associated with T-immunophenotype ALL and is not a stratification criterion.

3.3.4 Other organ involvement

Involvement of other organs at the onset of ALL does not have an impact on stratification. Obligatory initial diagnostic imaging includes chest X-ray only. Further tests (i.e. further skeletal X-rays, soft tissues or abdomen ultrasonography) should be based on clinical indications (e.g. persistent bone pain or skeletal instability, abdominal pain, palpable abdominal mass). The same strategy should be applied in presence of signs suspicious for leukemic involvement of any other organ (such as kidney, spinal cord, ovary, etc).

4. DIAGNOSTICS

4.1 Initial Diagnostics

4.1.1 Initial Diagnostic workup of ALL

The initial diagnostic workup for patients with ALL should include a thorough medical history and physical examination, along with laboratory and imaging studies, where applicable. Laboratory studies include a complete blood count (CBC) with platelets and differential, a blood chemistry profile, liver function tests, and disseminated intravascular coagulation panel. The blood chemistry panel should include a tumor lysis syndrome panel (including measurements for serum lactate dehydrogenase, uric acid, potassium, phosphates, and calcium). Appropriate imaging studies should also be performed to detect extramedullary involvement. All patients should be evaluated for opportunistic infections as appropriate. In addition, an echocardiogram or cardiac scan should be considered for all patients due to the use of anthracyclines as the backbone of nearly all treatment regimens. The initial clinical and laboratory diagnostic workup for all newly diagnosed ALL patients is presented in **Table 3.**

Table 3: Initial clinical and laboratory diagnostic workup for all newly diagnosed ALL patients

INITIAL DIAGNOSTIC WORKUP	MANDATORY	OPTIONAL
History and physical exam	X	
Complete Blood Count (CBC)/Differential	X	
Chemistry Profile/Liver function tests/Tumor Lysis Syndrome Panel (LDH, uric acid, K, Ca, Phos)	X	
Disseminated Intravascular Coagulation (DIC) Panel (D-dimer, fibrinogen, prothrombin time, partial thromboplastin time)	X	
CT/MRI of head with contrast, in case of neurologic symptoms	X	
Chest X-Ray, to rule out mediastinal mass	X	
Whole Body PET/CT, if lymphoblastic lymphoma suspected		X
Lumbar Puncture (LP), with intrathecal chemotherapy (IT)	X	
Ultrasonography (US) of neck and abdomen	X	
Testicular exam, scrotal ultrasound as indicated	X	
Infection evaluation, screening for opportunistic infections	X	
ECG & Echocardiography (Echo-CG)	X	
Fundoscopy		X
Central venous access of choice	X	
Pharmacogenomic testing for TPMT, NUTD15		X
Cancer predisposition syndromes (i.e. germline TP53 mutations in hypodiploidy)		X

4.1.2 Initial biological characterization of ALL

The basic diagnostic program regarding diagnosis and biological characterization of ALL, which is essential for the choice of proper therapy that will in turn affect the ultimate prognosis, is outlined in **Table 4.**

Table 4 Mandatory initial diagnostics for the biological characterization of ALL

Diagnostic evaluation method	
Cytomorphology *[from native material without additives (e.g. EDTA)]*	➢ Bone marrow: Myelogram (MGG) ➢ Peripheral blood: Complete blood count Differential hemogram ➢ CSF: Cell count (counting chamber) MGG stained cytospin preparation
Flow Cytometry	➢ Bone marrow (and/or peripheral blood): Immunophenotyping DNA index (optional) Identification of suitable FCM-MRD targets
Cytogenetics	➢ Bone marrow (and/or peripheral blood): High-resolution G-banding (Numerical & structural aberrations)
Molecular genetics	➢ Bone marrow (and/or peripheral blood): Identification of suitable PCR-MRD targets Comprehensive genetic leukemia characterization (Tab.6,7, Fig.2)

4.1.2.1 Cytomorphology

The diagnosis of ALL is made based primarily on the cytologic examination of the bone marrow (BM), peripheral blood (PB) and cerebrospinal fluid (CSF). Diagnosis of ALL can be made if ≥ 25% of the nucleated cells in the BM are lymphoblasts. PB count including differential hemogram is obligatory and has preferably to be determined before possible red cell or platelet transfusion. BM should preferably be used for establishing the diagnosis, but PB may be sufficient if cytomorphological diagnosis can unambiguously be made from the PB cells. Well spread BM smears should be stained with Romanowsky dyes, preferably May-Grünwald-Giemsa (MGG). The myelogram and FAB score should be done on 500 nucleated cells from a good BM smear stained with MGG. Conventional cytochemistry (PAS, AcP, MPO, SBB, NACE, ANAE/ANBE ± NaF) is not routinely required but may be helpful in otherwise uncertain cases. However, examination of myeloperoxidase is mandatory by cytochemistry or by immunophenotyping. Usually, panoptic staining, again preferably with MGG, is enough for the cytologic evaluation of PB films and CSF cytospin preparations. For this purpose, it is preferable to prepare native PB smears and CSF cytospins, i.e. without EDTA, heparin, albumin etc, so that the cytologic detail could be better appreciated.

4.1.2.2 Immunophenotyping

Immunophenotyping by flow cytometry is an integral part of the initial work-up of every ALL patient due to its direct consequences regarding stratification and therapy. Flow cytometric analysis and interpretation/reporting of the findings should be done according to the AIEOP-BFM ALL Immunophenotyping Consensus Guidelines. The immunologic classification of ALL and criteria for its separation from AML & B-ALL/NHL are given in **Appendix I.**

4.1.2.3 Genetic classification

The emphasis of the diagnostic evaluation of the genetic make-up of leukemia cases is put on obtaining the relevant genetic information that is essential for risk stratification and treatment rather than on the respective ascertainment technology. It aims to identify and delineate all pertinent lesions in the most comprehensive as well as cost- and time-efficient way. The diagnostic workflow and applied technology should be understood as a highly recommended suggestion and can either be partially replaced or supplemented according to the special local requirements. Depending on the available infrastructure and logistic set-up in the various countries and laboratories, the necessary ascertainment procedures may therefore be adapted and vary accordingly.

The revised World Health Organization (WHO) 2016 ALL genetic classification is outlined in **Table 5.**

Table 5: The revised World Health Organization (WHO) 2016 ALL genetic classification.

New provisional entities have been included (BCR-ABL1-like ALL and intrachromosomal AML1amplification (iAMP21).

GENETIC ABERRATION	FREQUENCY IN CHILDHOOD ALL	FREQUENCY UN ADULT ALL
High hyperdiploidy (51-67 chr.)	25%	7%
t(12;21)(p13;q22)-ETV6/RUNX1	22%	2%
11q23/KMT2A rearrangements	8%	10%
t(1;19)(q23;p13.3)-TCF3/PBX1	5%	3%
t(9;22)(q34;q11.2)-BCR/ABL1	3%	25%
Hypodiplpoidy(<45 chr.)	1%	2%
t(5;14)(q31.1;q32.3)-IL3/IGH	<1%	<1%
2016 PROVISIONAL ENTITIES		
"BCR-ABL1-like" ALL		
Intrachromosomal AML1 amplification (iAMP21)		

4.1.2.3.1. Favourable Genetic Risk Features

The most common chromosomal abnormality in pediatric ALL is **hyperdiploidy,** defined as >50 chromosomes or DNA Index≥1.16 and accounting for approximately 20% of pediatric ALL *(Moorman et al, 2016, Pui et al, 2019).* The ***ETV6-RUNX1*** subtype resulting from chromosomal translocation t(12;21)(p13;q22) is also among the most frequent subtypes in childhood ALL (20-25%). Both hyperdiploidy and *ETV6-RUNX1* subtypes are associated with favorable prognosis, although latest research is indicating that the presence of subclones and additional aberrations may impair disease clearance and eventually moderate favourable prognosis *(Ampatzidou et al, 2019).* Additionally, emerging data suggests that the presence of intragenic ***ERG* deletion** is associated with favorable outcomes in pediatric BCP-ALL *(Clappier E et al, 2014).*

4.1.2.3.2. Unfavourable Genetic Risk Features

Several genetic aberrations and chromosomal abnormalities are well-recognized prognostic biomarkers of high-risk disease, including hypodiploidy, *KMT2A* translocations, *TCF3-HLF* fusion and *BCR-ABL1* ALL *(Moorman et al,2016).*

Hypodiploidy is associated with inferior outcomes and is observed in 1% of pediatric patients while low hypodiploidy is associated with a high frequency of *TP53* alterations, which are germline in 50% of cases *(Moorman et al,2016).*

***KMT2A* gene rearrangements**, previously referred to as the human mixed lineage leukemia (*MLL*) gene, occur in approximately 5% of pediatric ALL cases, with a higher incidence in infants and have

long been associated with high risk disease and poor prognosis *(Pui et al, 2019, Moorman et al, 2016).*

The fusion gene ***TCF3-HLF***, resulting from the translocation t(17;19)(q22;p13), defines a rare subtype of pediatric ALL (1%) and has recently been associated with poor outcomes *(Fischer U et al, 2015).* On the other hand, the fusion gene *TCF3-PBX1* occurs in approximately 5% of pediatric ALL cases and is associated with intermediate outcomes *(Felice M et al, 2011).*

iAMP21 occurs in 2% of pediatric ALL and is characterized by amplification of a portion of chromosome 21, detected by fluorescence in situ hybridization (FISH) with a probe for the *RUNX1* gene. BCP-ALL with iAMP21 is more frequent in adolescents and young adults (AYAs) and is associated with adverse prognosis when treated with low-intensity regimens *(Moorman et al, 2013).*

BCR-ABL1 **or Ph-positive ALL** is not so frequent in childhood ALL (2%) compared to the AYAs population and has long been associated with poor prognosis *(Arico M et al, 2000).*

In BCP-ALL, mutations in the **Ikaros gene (*IKZF1*)** are seen in approximately 15%–20% of patients with pediatric BCP-ALL and at a higher frequency in patients who are also *BCR-ABL1* positive. In several studies, *IKZF1* mutations are associated with poor prognosis and a greater incidence of relapse *(Boer JM et al, 2016, Mullighan CG et al, 2009).* An analysis of the MRD-dependent prognostic impact of *IKZF1* deletions with co-occurring deletions in *CDKN2A*, *CDKN2B*, *PAX5*, or *PAR1* in the absence of *ERG* deletion conferred poor outcomes in pediatric patients with BCP-ALL. In this frame, a new pediatric ALL subtype has been introduced, the ***IKZF1*****plus subgroup,** defined as *IKZF1* deletion co-occurring with deletion in *CDKN2A or CDKN2B* (only homozygous deletions) or *PAX5* or *PAR1 (P2RY8-CRLF2*) in the absence of *ERG* deletion. The ***IKZF1*****plus subgroup** is associated with delayed disease clearance and adverse prognosis and is currently incorporated in modern risk group stratification. *(Stanula M et al, 2018).*

BCR-ABL1–like **or Ph-like ALL** is a subgroup of BCP-ALL associated with unfavorable prognosis that occurs in approximately 15% of childhood ALL. Despite the fact that this subgroup is Ph-negative, it is characterized by an otherwise similar gene expression profile to the Ph-positive ALL subgroup, including an IKZF1 mutation that accounts for approximately 40% of Ph-like ALL cases. **It is noteworthy that the expression profile of the *BCR/ABL*-like BCP ALL is only partly the same as in Ph-like ALL.** The presence of the *BCR-ABL1-like* signature is indicative of adverse prognosis and is is typically associated with gene fusions and mutations that activate tyrosine kinase pathways. These gene fusions and mutations include ABL-class rearrangements (ie, *ABL1*, *ABL2*, *PDGFRA*, *PDGFRb*, *FGFR*), *JAK-STAT* rearrangements and/or mutations (ie, *CRLF2*, *EPOR*, *JAK1*, *JAK2*, *JAK3*, *TYK2*, *SH2B3*, *IL7R*) and other rearrangements in *FLT3*, *NTRK3*, *LYN*, and, *PTK2B* genes. Genomic profiling studies indicate that the majority of Ph-like ALL cases have cytokine receptor- or kinase-activating alterations, posing unique challenges and suggesting potential role for ABL-class tyrosine kinase inhibitors (TKIs) or JAK small molecule inhibitors in effort to improve disparate and inferior outcomes otherwise driven from the underlying biology *(van der Veer A et al, 2013, Roberts KG, 2017).*

4.1.2.3.3. Genetic Abnormalities Associated With T-ALL

T-ALL is characterized by activating *NOTCH1* mutations and rearrangements of transcription factors *TLX1* (*HOX11*), *TLX3* (*HOX11L2*), *LYL1*, *TAL1* and *KMT2A*. More than 50% of T-ALL cases have activating *NOTCH1* mutations, and approximately 10%–15% of T-ALL cases have mutations in the *NOTCH1*-targeting E3 ligase *FBXW7*, which leads to prolonged *NOTCH1* activation. In patients with T-ALL, *NOTCH1* and *FBXW7* mutations have generally been associated with favorable prognosis and lower MRD levels. Nevertheless, it is unclear if these mutations are independent predictors of outcome and may serve as surrogate markers, or if concurrent absence of *RAS* or *PTEN* mutations is required. In this context, genetic abnormalities characterizing T-ALL have not yet been incorporated in current risk stratification algorithms *(Breit S, 2006, Clappier E et al, 2010, Jenkinson S, 2016).*

4.1.2.4 Methodology for genetic evaluation and molecular characterization

In addition to classic morphology, **conventional cytogenetics** of the whole karyotype, with **high-resolution G-banding** being the gold standard, and **molecular genetics (at least FISH)** to investigate prognostically important fusion genes (*BCR/ABL1*, *KMT2A/AFF1* and *ETV6/RUNX1*) are indispensable. If available, techniques of molecular cytogenetics **[MLPA** (**Multiplex Ligation-**

dependent Probe Amplification), RT-PCR, CGH] are encouraged, as they may reveal changes that would otherwise escape detection *(Jarosova M et al. 2000)*. The presence of recurrent genetic abnormalities should be evaluated using karyotyping of **G-banded metaphase chromosomes (conventional cytogenetics),** interphase **FISH** assays, and **reverse transcription-polymerase chain reaction (RT-PCR) testing.** FISH probes and RT-PCR primers should include those capable of detecting major recurrent genetic abnormalities. RT-PCR should measure transcript sizes (ie, p190 vs p210) of *BCR-ABL1* in BCP-ALL. Generally, the preferred method for identifying all relevant categories of gene fusions is the hierarchical FISH screening with specific double-color split-apart probe sets to ascertain whether or not a particular (hub) gene is involved. The first set covers the most common fusions and the second the rare ones. Screening for rare fusion genes can be restricted to cases in which the presence of all other primary lesions (*ETV6, ABL1, KMT2A* or *TCF3* fusions and hyper-/haplo-/hypodiploidy) have been excluded. The respective fusion partners should be identified subsequently in all hub gene-positive cases with either translocation-specific dual-color/dual-fusion FISH probe sets or, alternatively, with any other specific single or multiplex RT-PCR or any form of RNA- or DNA sequencing approach. The determination of the nature of the fusion partner and more specifically, eventually also the fusion breakpoint sequence is optional but highly desirable because it can eventually be used for treatment decisions and MRD surveillance. Additional FISH probes that may be useful to consider but remain optional include centromeric probes for chromosomes 4, 10, and 17 to detect hyperdiploidy, *CDKN2A* at 9p21.3 to detect deletions, probes to detect cryptic t(X;14)(p22;q32)/t(Y;14)(p11;q32) *IGH-CRLF2* rearrangements and probes to detect cryptic *JAK2* and *FGFR1* rearrangements. Additionally, the combined FISH and array approach can be viewed as an optional, extended and refined form of standard cytogenetics. Metaphase spreads may still be required to resolve the topologic composition of the genome, to decipher potential rearrangements and to map particular lesions with FISH on chromosomes.

If samples are *ETV6-RUNX1*– and *BCR-ABL1*–negative, testing for other gene fusions and mutations associated with Ph-like ALL is optional but encouraged in selected patients, and may aid in risk stratification. Low-density arrays, next-generation sequencing (NGS)–based assays, and multiplex RT-PCR are typically used to detect signature or cryptic rearrangements and mutations characteristic of Ph-like ALL.

Generally, all relevant quantitative changes can be defined by DNA-index, cytogenetics, FISH, MLPA and specific DNA-based PCR analyses. However, none of these methods can either alone or even in combination deliver the overall necessary information with a nearly complete and comparable standardized precision as DNA array analysis can. Therefore, although it remains optional, the preferred method for capturing all relevant small- and large-scale quantitative changes simultaneously in a comprehensive manner is DNA array analysis. The study-wide use of standardized high-density arrays that should be composed of SNP and oligo probes allows the joint collection, exchange and evaluation of the derived data on a sequence-based level. It thereby facilitates not only data validation and quality control programs but makes the obtained data also future-proof because they can easily be merged with all other sequence-derived (RNA, DNA and methylation) data sets. Finally, any appropriately sized copy number aberrations can in principle serve as a template for custom-made FISH probes and therefore in principle be utilized as a FISH-traceable marker for monitoring treatment response or disease progression on a single cell level, which may be especially pertinent for the analyses of particular tissues (e.g. cerebral fluid, testes).

Overall, the diagnostic workflow and applied technology should be understood as a highly recommended suggestion and can either be partially replaced or supplemented according to the special local requirements. As mentioned in Section 4.1.2.3 and depending on the available infrastructure and logistic set-up in the various countries and laboratories, the necessary ascertainment procedures may be adapted accordingly.

Proposed guidelines for basic genomic screening methodology are outlined in **Table 6**. Mandatory and optional genetic diagnostics of stratification-relevant and other important prognostic aberrations are presented in **Table 7**.

Table 6: Proposed guidelines for basic genomic screening methodology (Moorman AV. SIOPE Meeting, Prague 2019)

Genetic aberrations/ karyotyping abnormality patterns	**Methods**					
	Array (optional)	**FISH**	**RT-PCR**	**MLPA**	**G/R Banding**	**NGS (optional)**
High hyperdiploidy (HeH; 51-65/67 chromosomes)	X				X	X
Low Hypodiploidy (HoL; 30-39 chromosomes)	X				X	X
Near haploidy (NH; 25-29 chromosomes)	X				X	X
iAMP21	X	X				X
KMT2A rearrangements		X			X	X
BCR-ABL1, ETV6/RUNX1, TCF3-HLF, TCF3-PBX1		X	X		X	X
ABL-class fusions and other (i.e. *ABL1, ABL2, CSF1R, PDGFRB*) (optional)		X				X
Copy Number Alterations *BTG1, CDKN2A/2B, EBF1, ETV6, IKZF1, PAR1, PAX5, RB1*	X			X		X

Table 7: Mandatory and optional genetic diagnostics of stratification-relevant and other important prognostic aberrations

GENETIC ABERRATION	RELEVANT FOR STRATIFICATION	MANDATORY/ OPTIONAL	TARGET PATIENT GROUP
BCR-ABL1	Yes	Mandatory	All patients
KMT2A rearrangement	Yes (only in infant ALL)	Mandatory	Infant ALL
KMT2A/AFF1	Yes	Mandatory	All patients
Identification of other *KMT2A* partner gene	No	Optional	
ETV6/RUNX1	Yes	Mandatory	All Bcp patients
TCF3/PBX1	No	Mandatory	All Bcp patients
TCF3/HLF	Yes	Mandatory	All Bcp patients
Hypodiploidy	Yes	Mandatory	All Bcp patients
Hyperdiploidy	No	Mandatory	All Bcp patients
IKZF1 deletion	Yes	Mandatory	All Bcp patients, except pts with *BCR-ABL1*, *ETV6/RUNX1*, *KMT2A* rearrangement, *TCF3* rearrangement, or hypodiploidy
PAX5 deletion	Yes (only in case of *IKZF1plus* subgroup)	Mandatory (only in case of IKZF1plus subgroup)	
CDKN2A/2B deletion			
P2RY8/CRLF2 (*PAR1* del)			
ERG deletion	Yes (only in case of *IKZF1plus* subgroup)	Mandatory (only in case of IKZF1plus subgroup)	All patients meeting criteria for *IKZF1plus* definition
ABL1 rearrangement	No	Optional	All Bcp patients with positive MRD on day 33, except pts with *BCR-ABL1*, *ETV6/RUNX1*, *KMT2A* rearrangement, *TCF3* rearrangement, or hypodiploidy
ABL2 rearrangement	No	Optional	
CSF1R rearrangement	No	Optional	
PDGFRB rearrangement	No	Optional	
IGH rearrangement	No	Optional	
CRLF2 rearrangement	No	Optional	
EPOR rearrangement	No	Optional	
ETV6 rearrangement	No	Optional	
NTRK3 rearrangement	No	Optional	
JAK2 mutations	No	Optional	
NOTCH1 mutations	No	Optional	T-ALL pts
TLX1(*HOX11*),*TLX3*(*HOX11L2*), *LYL1*, *TAL1* rearrangements	No	Optional	T-ALL pts

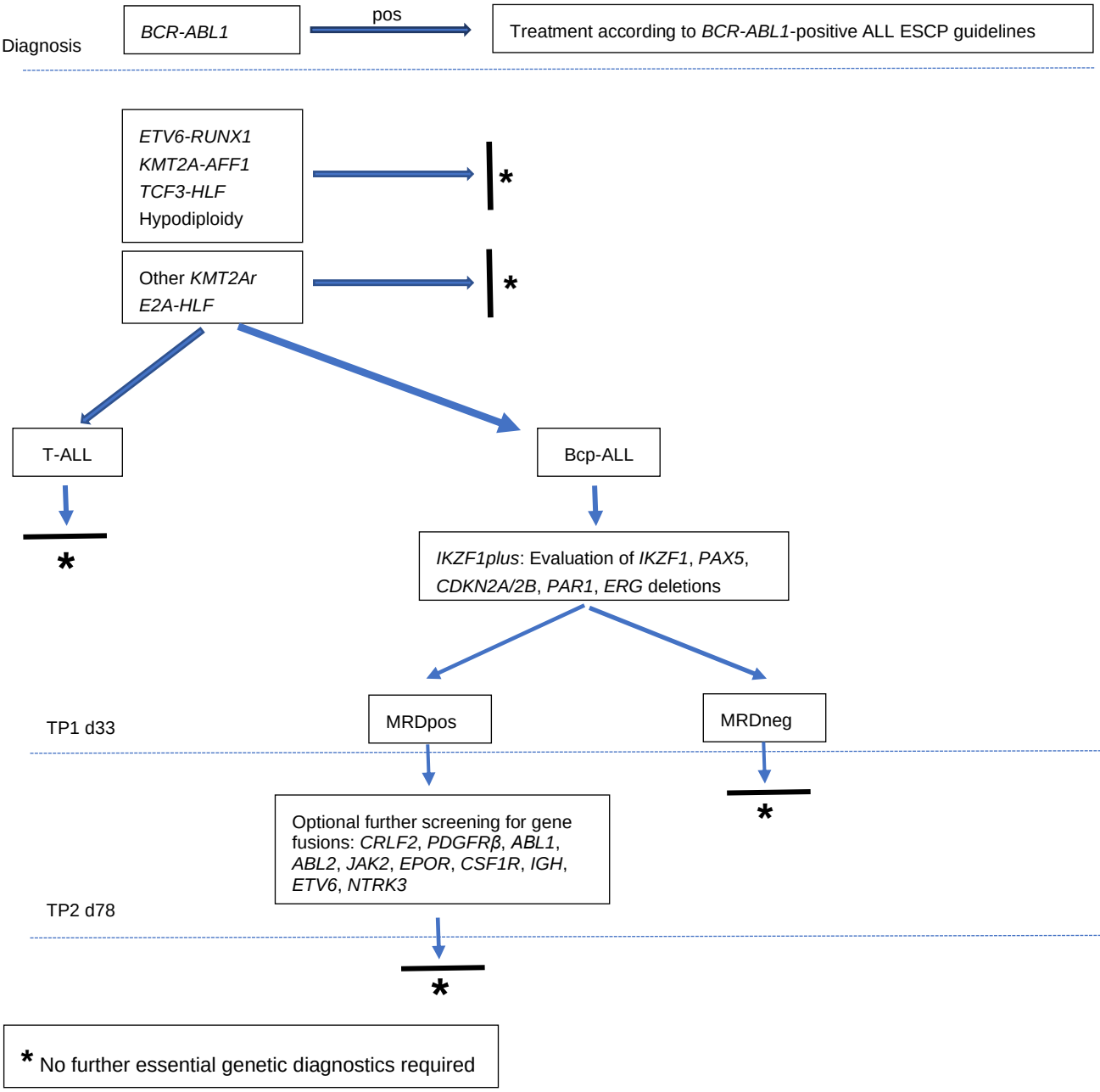
4.1.2.5 Proposed genetic algorithm, basic genetic diagnostic requirements and workflow

All patients must be investigated for the presence of *BCR-ABL1* upon diagnosis. Additionally, all patients (BCP-ALL and T-ALL) should further be screened for *KMT2A* (*MLL*) rearrangements, and the fusion partner should be identified in *KMT2A*-positive cases. The absence or presence of *TCF3-HLF* (or *E2A-HLF*), *TCF3-PBX1* (or *E2A-PBX1)*, *ETV6-RUNX1* (or *TEL-AML1*) and hypodiploidy should be ascertained in all patients with BCP-ALL. Depending on the individual workflow in the lab, it is not necessarily required to investigate all BCP-ALL for all these aberrations if a hierarchical screening

approach is followed, assuming that these genetic aberrations usually are mutually exclusive. In order to identify patients with an *IKZF1*plus pattern (for definition see 3.2.1), BCP-ALL patients not presenting one of the specific aberrations *ETV6-RUNX1*, rearranged *KMT2A,* rearranged *TCF3,* or hypodiploidy are investigated for deletions of *IKZF1*, *PAX5*, *CDKN2A*, *CDKN2B*, *CRLF2* or *ERG*. The information on the stratification-relevant aberrations *KMT2A-AFF1* (or *MLL-AF4*), *TCF3-HLF, ETV6-RUNX1,* hypodiploidy and *IKZF1*plus has to be available by the end of induction (d33, TP1) at the latest.

To allow the identification of kinase-activating genetic alterations potentially targetable by tyrosine kinase inhibitors, translocations of *PDGFRB, ABL1, ABL2, CSF1R* as well as *CRLF2, IGH, EPOR* and *NTRK3* are optionally analyzed in all BCP-ALL with positive MRD result at TP1 (d33) and not positive for *ETV6-RUNX1*, rearranged *KMT2A,* rearranged *TCF3,* or hypodiploidy. Patients with *CRLF2* translocations frequently carry *JAK2* mutations. Therefore, sequence analysis can optionally be performed to detect *JAK2* mutations. The final results of these analyses will be reported until TP2 (d78/d92). Algorithm of basic genetic diagnostics and genetic workflow is presented in **Figure 1**.

Figure 1: Algorithm of basic genetic diagnostics



4.1.2.6 Biobanking

Apart from establishing harmonized diagnostic procedures, a comprehensive harmonized strategy for tissue banking and biological material storage at a national level is essential, enabling biological studies to improve knowledge on the disease, discover new risk factors and potential targets for new drugs. Depending on the available infrastructure and logistic set-up in the various countries and laboratories, local and national tumour banks may be linked to centralised tissue banks and report on availability and quantity of tumour material with a specific set of clinical and biological information. This allows for searching of material for research projects in rare subgroups. Furthermore, the storage and documentation needs to be standardized, warranting high quality and reliability of any requested material.

4.1.2.7 DNA index- Evaluation of hypodiploidy by flow-cytometric analysis

Ploidy can be determined by genetic methods such as metaphase karyotyping or DNA array analyses or by measuring the DNA content by flow cytometry. Flow cytometry expresses the DNA content as DNA index (DI), a ratio between the normal amount of fluorescence seen in a diploid cell and the fluorescent content of the bone marrow blasts (in G0/G1) at diagnosis. However, hypodiploidy cannot be definitely excluded by a normal DI. On the contrary, in case of a clear “hypodiploid” DI, this finding can be considered reliable. The analysis of DI is not mandatory but can be helpful in cases with inconclusive genetic findings.

The following diagnostic procedure for the evaluation of hypodiploidy is suggested:

- If DI is < 0.8 or the conventional cytogenetics clearly shows less than 45 chromosomes no further confirmation by another method is required.
- If DI is ≥ 0.8 and < 1.00, confirmation by another method is mandatory, e.g.: Metaphase cytogenetics: a normal karyotype is only reliable, if at least 20 metaphases could be analyzed. Otherwise, a third method is required (DNA array, MLPA, centromerFISH).

4.1.2.8 PCR-MRD marker establishment

In case this is feasible, PCR-MRD diagnostics (establishment and testing of suitable PCR targets, standard dilution series) is proposed and encouraged. The DNA amount (and the corresponding number of mononucleated cells) required for a successful MRD analysis depends on the number of established markers, DNA quality and the need for repetition of experiments. As a general guideline 1x 10^7 cells (corresponding to about 20 µg DNA) are the minimally required amount.

4.1.3 General diagnostics and diagnostics of extramedullary disease

A careful clinical work-up documentating the patient’s baseline status and potential extramedullary manifestations of the disease, is required including history and physical examination of the patient as well as extensive laboratory and instrumental diagnostic procedures **(Section 4.1.1, Table 3).** Additional investigations may be indicated in specific situations that should be determined by the treating center.

For definition of organ involvement including CNS involvement see section 3.3.

4.1.3.1 Diagnostics of CNS status

Pretherapeutic lumbar puncture (LP) and examination of the CSF at diagnosis is an essential part of the initial staging and is necessary for the assessment of initial CNS status. Only in exceptional cases, e.g. a large mediastinal tumor with considerable respiratory impairment, the first LP may be postponed. Hyperleukocytosis >100 000/µl is *per se not a* contraindication for LP under conditions of a clinical effective hemostasis. Patients with high hyperleukocytosis are prone to be CNS-positive, careful diagnostics of the CNS status is therefore of particular importance in those patients. In addition to chemistry (protein, glucose), the cell count of nucleated cells *and* erythrocytes is to be determined in a counting chamber (e.g. Fuchs-Rosenthal chamber), and the cell morphology must be assessed on a high-quality cytospin preparation as described below.

Additional findings with consequences for classification of CNS involvement are diagnosed clinically and by CNS imaging. Careful neurological physical examination should be accomplished in all patients. Cranial imaging (MRI or CT) (in BFM by MRI preferably) should be accomplished only in patients with neurological symptoms or CNS3a.

For definition of the CNS status see section 3.3.1.

Technique of cytospin preparation:

Mix the CSF specimen thoroughly but gently. Depending on the CSF cell count, centrifuge 100-500 µl of the specimen (1000 rpm for 5 min) onto a dry and uncoated slide.

Traumatic lumbar puncture:

In any case, a visible blood contamination of the CSF has to be well documented, since it may have impact on CNS status and treatment.

In case of traumatic LP with macroscopically visible blood contamination, ensure that the CSF is clearing, and then administer intrathecal Methotrexate.

If the punctured fluid appears to be mainly blood and is not clearing, it remains unclear whether the needle is in the intrathecal space

- do not administer intrathecal Methotrexate,

- perform LP at a different site, immediately. If the immediate re-puncture is not feasible, delay LP to the following day. In this case, postpone glucocorticoid administration to the following day as well, except for patients, whose clinical condition requires the immediate start of treatment.

4.1.3.2 Diagnosis of initial testicular involvement

If typical signs are present, such as the recent emergence of a painless swelling of the testicle(s) without symptoms/signs of inflammation or infection, then sonographic examination of both testes is mandatory. A biopsy is not routinely necessary. Nevertheless, other diseases such as infection (orchitis, epididymitis, cellulitis), vascular abnormalities (hydrocele/varicocele), or scrotal hernia should be reliably ruled out. If there is uncertainty in this regard, then a biopsy must be performed. Primary orchiectomy is not indicated, however. Initial testicular involvement is not primarily included in the risk stratification process. Nevertheless, biopsy-proven testicular leukemia persisting beyond induction therapy should be managed along the HR strategy, and may eventually need local radiotherapy (18 Gy) as well (see Treatment Section).

4.2 Diagnostics during the course of therapy-Response evaluation

4.2.1 Cytomorphological response and remission evaluation in BM and PB

Cytomorphological response evaluation should be reviewed by each treating center.

4.2.1.1 Peripheral blood on treatment day 8 (Prednisone response)

The Prednisone response is evaluated on day 8 (before application of Vincristine/Daunorubicin) after 7 days of Prednisone and one intrathecal application of MTX. Day 1 is the day of the first Prednisone application. Prednisone poor-response qualifies for high-risk treatment. Only for T-ALL this represents a stratification criterion.

Prednisone response is defined by the absolute blast cell count in peripheral blood on day 8 regardless of the absolute blast cell count at diagnosis. For definition of Prednisone good- response and Prednisone poor-response see section 3.1.1.

The Prednisone response is determined by cytomorphology on a blood smear which should be prepared with native peripheral blood without any additives (i.e. without EDTA).

4.2.1.2 Bone marrow on treatment day 15 and day 33

Investigation of cytomorphological response in bone marrow on protocol days 15 and 33 of Protocol IA is mandatory in all patients. Protocol days 15 and 33 do not necessarily take place 15 or 33 days after start of therapy, but are defined by the treatment schedule, i.e. protocol day 15 is the day of the

second VCR/DNR dose and protocol day 33 is the fourth day after the 4th VCR/DNR. The bone marrow puncture at protocol day 15 has to be performed before application of the 2nd VCR/DNR.

Morphologic and minimal residual disease (MRD) based investigation of BM day 33 is crucial for further risk-adapted stratification. In any case, a puncture, if clinically possible, should be performed on protocol day 33 (i.e. 4 days after 4th VCR/DNR). A re-puncture must be performed before start of Consolidation/Protocol IB in the case of insufficient cellularity or DNA content. The MRD finding will be taken into consideration for further risk stratification.

4.2.1.3 Later time points

For all patients, cytomorphological evaluation of remission in bone marrow is recommended at the start of the subsequent treatment elements (Protocol M, Protocol II, all HR blocks, before HSCT, at the beginning and end of maintenace treatment), for confirming retaining complete remission (CR) as defined in Section 3.1.3.

4.2.2 Response evaluation of extramedullary manifestations

Definitions of remission of extramedullary manifestations are described in section 3.3.

4.2.2.1 CSF

In the case of detectable blasts in CSF at diagnosis, the CSF should be carefully controlled at subsequent therapeutic LPs until it is free of blasts. In general, determination of cell count of the CSF and cytomorphology (cytospins) in case of a positive count is recommended at every therapeutic lumbar puncture (LP), even in patients without initial CNS involvement.

4.2.2.2 Mediastinal tumor

Regression of an initial mediastinal mass should be reevaluated on day 33 of Protocol IA. In the case of incomplete regression of the tumor, imaging should be repeated after completion of Consolidation/Protocol IB. If CT examinations were performed at diagnosis *and* on day 33, the tumor volume for calculation of the tumor regression should be used. If only chest X-ray imaging is available, the tumor regression should be calculated using the product of the largest transversal and sagittal diameters of the mediastinal mass.

4.2.2.3 Other manifestations

In general, initial findings of extramedullary leukemic infiltrations should be reevaluated on day 33. In the case of incomplete regression, evaluation should be repeated after completion of Consolidation/Protocol IB.

4.2.3 Minimal Residual Disease (MRD)

4.2.3.1 MRD by PCR of clone-specific TCR and Ig gene rearrangements

To assess the treatment response in the bone marrow, the presence and dynamics of minimal residual disease (MRD) is evaluated by molecular genetic analysis of clone-specific T-cell receptor and immunoglobulin gene rearrangements by real-time quantitative PCR. To ensure a high quality of the PCR-MRD results, it is required that the laboratories follow the guidelines for EuroMRD membership and that the MRD analyses and the interpretation of the results are performed according to the published guidelines of the EuroMRDconsortium (*van der Velden, et al* 2007).

4.2.3.1.1. PCR-MRD time points

The PCR-MRD response at distinct protocol time points is essential for risk group and treatment stratification.

Bone marrow puncture for MRD (and cytomorphological) evaluation is mandatory at the following time points:

All patients:

- **TP 1: at end of Protocol IA (d33).** This time point is decisive for stratification to HR Group. In the absence of HR criteria and PCR-MRD negativity at TP1 with quantitative range of $<10^{-4}$, MRD-TP1 is sufficient for stratification to the SR group.
- **TP 2 (d78/d92, after Protocol IB, i.e. at start of Protocol M or HR-1'):** This time point is decisive for final stratification in patients without TP1 HR criteria. For patients with HR criteria by TP1, TP2 is decisive for SCT indication (see Treatment Section).

HR patients:

- **TP HR1:** After the 1st HR-Block (HR-1), when hematopoiesis has recovered (only for pts with MRD d78$\geq 10^{-4}$)
- **TP HR2:** After the 2nd HR-Block (HR-2), when hematopoiesis has recovered (only for pts with MRD d78$\geq 10^{-4}$)
- **TP HR3:** After the 3rd HR-Block (HR-3), when hematopoiesis has recovered.
- **TP HSCT:** Before HSCT, for HR patients fulfilling criteria for HSCT, in the case of long delay after TP HR3 or if another treatment element was given in between.

Other time points:

- Subsequent measurements in any case of MRD load increase.

4.2.3.1.2. Logistical aspects of PCR-MRD analyses

The DNA amount (and the corresponding number of mononucleated cells) required for a successful MRD analysis depends on the number of established markers, DNA quality and the need for repetition of experiments. As a general guideline, 5 x 10^6 cells (corresponding to about 10 µg DNA) are the minimally required amount at the follow-up time points.

At **TP1** (end of induction) and **TP2,** the MRD load is essential for risk stratification.

4.2.3.2 MRD by flow cytometry

4.2.3.2.1. FCM-MRD on day 15

MRD by flow cytometry (FCM) on day 15 is relevant for risk stratification. Therefore, investigation of this time point is obligatory for all patients:

- Patients with detection of ≥10% blast cells by FCM in bone marrow on day 15 are eligible for treatment in the HR group regardless of PCR-MRD status on TP1 and TP2.
- Patients with missing or inconclusive PCR-MRD results, are eligible for treatment in SR if <0.1% blast cells are detectable by FCM in bone marrow on day 15.

4.2.3.2.2. FCM-MRD on TP1 and TP2 if no PCR-MRD is available

Risk stratification using FCM-MRD results on TP1 and TP2 is accepted and highly recommended in patients either without initial material to perform PCR-MRD or without PCR-MRD marker with a quantitative range of ≤ 10^{-3} or in any other case that PCR-MRD is not feasible, depending on the available infrastructure and logistic set-up in the various countries and laboratories. In that case, PCR-MRD substitution by FCM-MRD (10^{-5} sensitivity) is obligatory.

It is required that the laboratories follow the required recommendations and that the MRD analyses and the interpretation of the results are performed according to the published guidelines of the AIEOP-BFM Flow Network and the fully standardized EuroFlow ALL MRD strategy (*Dworzak M, Cytometry B Clin Cytom 2008, Bruggemann M et al, Leukemia 2010, Theunissen P et al, Blood 2017, Dworzak M, Cytometry B Clin Cytom 2018*).

The schematic outline of recommended time-points for MRD evaluation (obligatory and optional) are summarized in **Tab.8.** The detailed flow chart for MRD-based risk stratification is outlined in **Section 5.**

Table 8: Recommended time-points for follow-up BMPs and MRD evaluation

Pt Population			BMP/MRD (FC-MRD or PCR-MRD)				
	Day15	Day 33 (End IA)	Day 78 (End IB)	After HR-1	After HR-2	After HR-3	Before HSCT
SR and IR pts	X	X	X				
HR pts	X	X	X	X (only for pts with MRDd78≥10^{-4})	X (only for pts with MRDd78≥10^{-4})	X	
HR pts qualifying for HSCT	X	X	X	X (only for pts with MRDd78≥10^{-4})	X (only for pts with MRDd78≥10^{-4})	X	X

4.3 Monitoring of toxicity

4.3.1 Toxicity during treatment

Close and careful monitoring of the patients is indispensable throughout the treatment. Supportive care and treatment of toxic effects of the chemotherapy and other complications should follow the common clinical practice. Non-binding guidelines regarding specific chemotherapy toxicities and recommendations for their management are specified in the Supportive Care Section.

4.3.2 Follow-up, late side effects

Recommendations for follow-up diagnostics and close monitoring and handling of late side-effects are specified in the Supportive Care Section.

5. PROGNOSTIC RISK STRATIFICATION

5.1 Risk Group Assignment

5.1.1 Standard Risk Group (SR)

Age ≥ 1 yr – < 6 yr (only when MRD data is not available or inconclusive)
and Initial WBC < 20,000/µL (only when MRD data is not available or inconclusive)
and FC-MRD d15<0.1% and M1 BM on day 15
and MRD d33<$5x10^{-4}$ and M1 BM on day 33
and MRD d78<$5x10^{-4}$ and M1 BM on day 78
and ETV6/RUNX1 or hyperdiploidy patients with FC-MRDd15<0.1% and MRDd33/d78<$5x10^{-4}$
and non-T ALL
and no IKZF1del, iAMP21 or CRLF2overexpression

All criteria must be fulfilled.

5.1.2 Intermediate Risk Group (IR)

All patients not included in SR or HR Groups

PB day 8: < 1,000 blasts/µL (Good Prednisone Response-GPR)-Only for T-ALL
and Age < 1 yr* or ≥ 6 yr (only when MRD data is not available or inconclusive)

or WBC ≥ 20,000/µL (only when MRD data is not available or inconclusive)

or FC-MRDd15 >0.1% and <10% and M1/M2 BM on day 15,
and MRDd33<$5x10^{-4}$ and M1 BM on day 33
and MRDd78<$5x10^{-4}$ and M1 BM on day 78

or CRLF2 or IKZF1del or iAMP21 with MRDd15<10% and MRDd33/d78<$5x10^{-4}$

and no HR genetics as defined in the HR-Group

***Infants <1 year should be stratified and treated according to ESCP Guidelines for Infant ALL described in following section.

5.1.3 High Risk Group (HR)

PB on day 8: ≥ 1,000 blasts/µL (Poor Prednisone Response-PPR)-Only for T-ALL
or FC MRD d15≥10% or M3 BM on day 15 (if FC MRDd15 inconclusive)
or no CRd33

or MRD d33≥$5x10^{-4}$ or M2/M3 marrow on day 33 (if FC MRDd33 inconclusive)
and/or MRD d78≥$5x10^{-4}$ or M2/M3 marrow on day 78 (if FC MRDd78 inconclusive)
or BCR/ABL1* or KMT2A/AFF1 rearrangement
or Age<1 year and any KMT2A rearrangement#
or Hypodiploidy ≤ 44
or t(17;19)(q23;p13) (TCF3/HLF)
or IKZF1plus with FC-MRD d15≥0.1% or MRDd 33/d78≥$5x10^{-4}$

* BCR/ABL1+ patients should be stratified and treated according to ESCP Guidelines for Ph+ ALL described in following section.

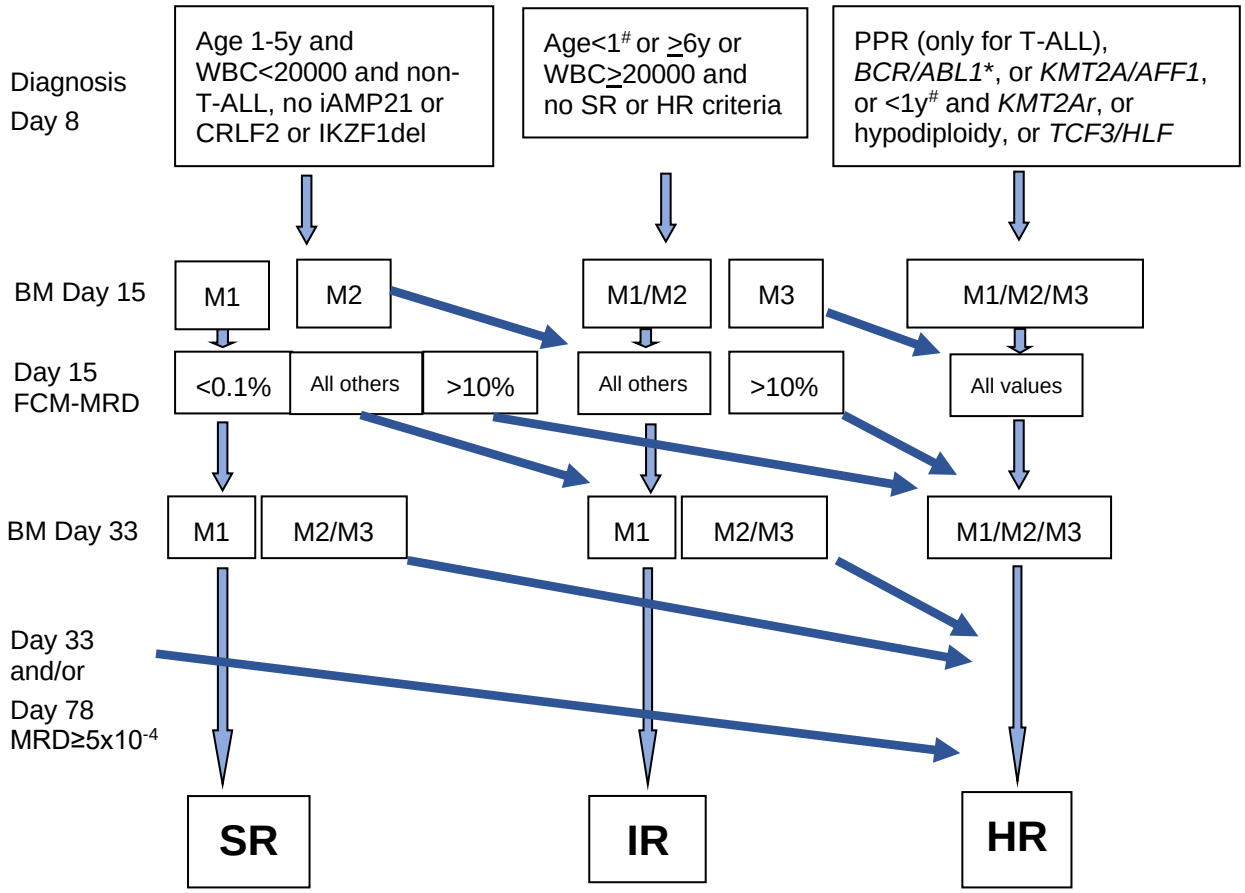
Infants <1 year should be stratified and treated according to ESCP Guidelines for Infant ALL described in following section.

5.1.4 Final risk group allocation in all patients

Risk Group stratification based on WBC, age, genetics and treatment response on days 15, 33 and 78 is outlined in **Figure 2.**

Final risk group assignment by MRD is outlined in **Table 9.**

Figure 2: Risk Group stratification based on WBC, age, genetics and treatment response on days 15, 33 and 78.



***** *BCR/ABL1*+ patients should be stratified and treated according to ESCP Guidelines for Ph+ ALL described in following section.

#Infants <1 year should be stratified and treated according to ESCP Guidelines for Infant ALL described in following section.

Table 9: Final risk group assignment by MRD values in ALL patients

	MRD TP2 (MRDd78)	
	$<5x10^{-4}$	$\geq 5x10^{-4}$
FCM-MRD_{d15} <0.1%	SR	HR
FCM-MRD_{d15} 0.1-10%	IR	HR
FCM-MRD_{d15}>10%	HR	HR
Any HR criteria at TP1 (day 33)	HR	HR
No HR criteria at TP1 (day 33) and MRD at TP1 (MRDd33)		
$<5x10^{-4}$ and SR criteria fulfilled	SR	HR
$<5x10^{-4}$ and SR criteria not fulfilled	IR	HR
$\geq 5x10^{-4}$	HR	HR

6. TREATMENT

6.1 Introduction and General Remarks

6.1.1 Basis for dosage

The IV and PO dosage of cytostatic drugs is in principle determined based on the body surface area (BSA) of the patient. The BSA should be calculated always at the start of each phase of treatment. In addition, the BSA must be calculated before each MTX infusion in Protocol mM/M (x4) and every 4 weeks during the maintenance therapy. Dosage should be updated at each of these time points.

Specific situations:

- Dosage for infants ≥ 1 year of age, whose body weight is less than 10 kg:

  In this case, BSA-based dosage should be converted to mg/kg (U/kg) according to the following formula

  $$\text{Dose in mg/kg (U/kg)} = 1/30 \times \text{dose in mg/m}^2\ (\text{U/m}^2)$$

- Dosing for obese children with a body weight > 2 SD or > 125% of the ideal body weight (IBW) should be made on the basis of the BSA corresponding to the adjusted IBW, which is calculated according to the following formula:

  $$\text{Adjusted IBW} = \text{IBW} + 0.25\ (\text{Actual BW} - \text{IBW})$$

  The IBW is calculated using appropriate age and sex standards available in the form of tables or nomograms.

  For patients who have gained weight on steroids, the pre-steroid weight is more appropriate for drug dosing. If this exceeds 125% IBW, then the adjusted IBW is calculated using the pre-steroid actual weight.

Pharmacokinetic, pharmacodynamic, and clinical data have established that dosing of intrathecal medications (MTX, ARA-C, prednisone, 0.9% NaCl) should be adapted to age in lieu of BSA at the time of treatment delivery. The age-adjusted dosage of the drugs used IT in this trial is shown in **Tab. 10**.

Table 10: Dosage of IT Medications by Age Attained at Time of Therapy

Age (yr)	MTX (mg)
≥ 1 < 2	8
≥ 2 < 3	10
≥ 3	12

In addition to age-adjusted dosage, the following remarks are pertinent to LP and IT medication:

- Each CSF obtained by a diagnostic or therapeutic puncture should be examined for:
  - Chemistry (total protein, G, lactate)
  - Cell count in Fuchs-Rosenthal's or Nageotte's chamber
  - Cytomorphology and differential count on a cytospin preparation made by a standard technique

- The findings should be always carefully documented and sent along with 2 unstained cytospin preparations at the specified time points as well as in case of a positive finding and any suspicion or uncertainty to the national reference laboratory

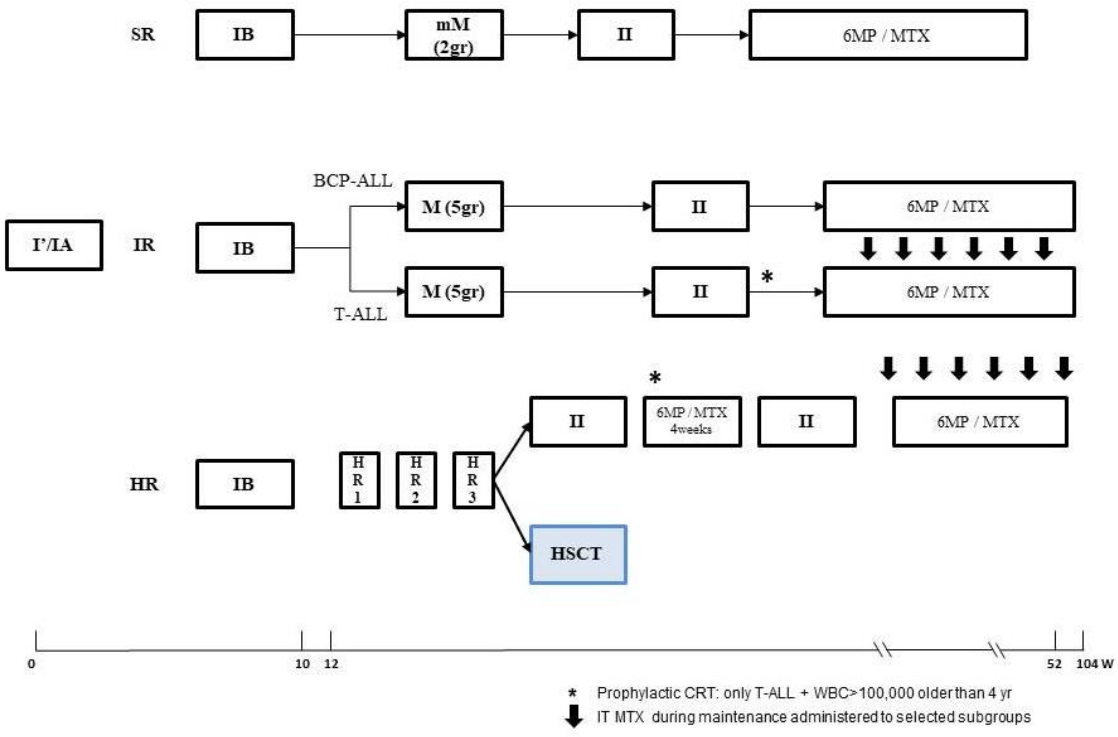
6.1.2 Timetable

Each patient should be registered at the national data management office whether available once the diagnosis of ALL has been made, i.e. within 24 – 72 h. The first complete blood count and differential available upon admission prior to hydration, transfusion, and antineoplastic treatment including prednisone should be considered as the initial one for the purpose of documentation and further evaluation.

The first day of administering cytostatic drug(s) of a given treatment element will be labeled day 1 of that element.

The duration of treatment in all risk groups is 104 weeks overall. The global treatment plan of the individual strategic groups is outlined in **Fig. 3**. Induction is followed by a consolidation/intensification phase, after which the patients are directed to reinduction therapy or transplant. In addition to specific situations, radiotherapy is included in the treatment of specific subgroups of patients during or subsequent to the late reinduction phase of treatment. After intensive chemotherapy with or without radiotherapy all patients not undergoing SCT will receive maintenance therapy for the remainder of 24 months of overall treatment.

Figure 3. Overview ESCP ALL Treatment Plan



6.2 SR & IR Therapy Branch

6.2.1 Introduction

The SR patients with BCP-ALL only receive 2 doses of DNR in induction (Protocol I'), as all available data suggest that this reduction is safe, i.e. without any risk for the rate of relapse. All IR (as well as HR) patients should receive Protocol I with 4 doses of the drug. Two weeks after induction and consolidation (IA+IB), the “extracompartment” phase of therapy is strated and diversified. IR BCP ALL patients and patients with T-ALL should receive Protocol M, i.e. HD MTX (5g/m^2/24h x4 q 2 weeks), whereas SR patients with BCP-ALL receive Protocol mM, i.e. MD MTX (2g/m^2/24h x4 q 2 weeks). Two weeks following extracompartment treatment, all

patients of each risk group are directed to late intensification therapy with Protocol II. Finally, 2 weeks after the last intensive therapy element, all patients are put on oral maintenance therapy with daily 6-MP and weekly MTX for the remainder of 24 months (104 weeks) of overall treatment.

Results from different study groups demonstrated that it is feasible and safe sparing T-cell patients from prophylactic cranial irradiation, which is now restricted to some small subgroups of patients. Patients with initial CNS involvement (CNS3) who are at least 4 year-old will receive therapeutic cranial irradiation (tCRT), whereas those younger than 4 year-old will receive CNS directed therapy with IT MTX during maintenance (see chapter 6.5.2). Moreover, the administration of intrathecal MTX injections during maintenance is limited to specific groups of patients, as described in chapter 6.5.1.

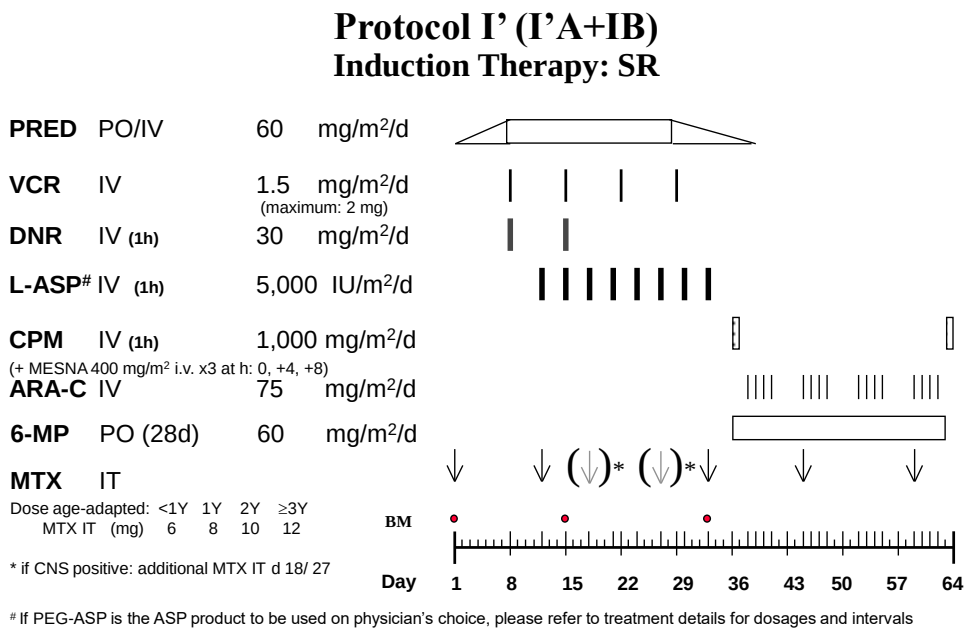
6.2.2 Induction and Consolidation Therapy (SR/IR/HR ALL)

6.2.2.1 Protocol I'A/IA

Protocol I'A is designed for the induction therapy of SR patients, whereas Protocol IA should be used for induction in all the other. The former prescribes only 2 doses, and the latter 4 doses of DNR q 30 mg/m^2.

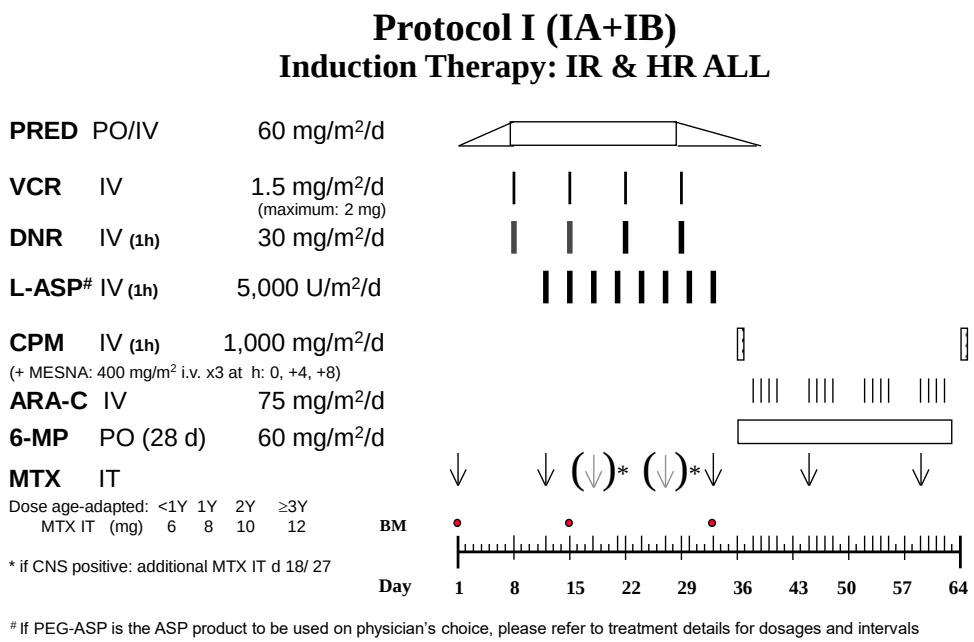
Dosage should be based on the BSA calculated at the beginning of each phase, i.e. on day 1 and 36. Protocol I'A' & Protocol IA are illustrated in **Fig. 4** and **Fig. 5**, respectively.

Fig. 4: Protocol I' (I'A+IB) for Induction Therapy and Consolidation Therapy in SR



If PEG-ASP is the ASP product to be used on physician's choice, please refer to treatment details for dosages and intervals

Fig. 5: Protocol I (IA+IB) for Induction Therapy and Consolidation Therapy in IR and HR



If PEG-ASP is the ASP product to be used on physician's choice, please refer to treatment details for dosages and intervals

Phase I'A and Phase IA (Induction)

Precautions and therapy regulation

Close monitoring of the patient is essential, and measures to prevent or treat tumor lysis syndrome (TLS), metabolic derangements, infections and other complications should be undertaken. For detailed recommendations regarding careful monitoring and supportive care, it is referred to the protocol appendix. Protocol I'/I, being the induction element of therapy, should be adhered to as far as possible, especially during the first phase. Treatment may be delayed only in specific conditions. Severe granulocytopenia in the absence of infection is not per se a reason for treatment delay or dose reduction in this treatment phase

Treatment schedule

IT MTX **Intrathecal methotrexate** at age-adjusted dosage on day 1, 12 and 33.

- In case of initial CNS involvement (CNS 3), see chapter 3.3.1.1, additional MTX IT is administered on days 18 and 27.
- LP is urgently necessary for the assessment of initial CNS status. Therefore, the first LP may be postponed only in exceptional situations.

Age (yr)	≥ 1 < 2	≥ 2 < 3	≥ 3
Dosage (mg)	8	10	12

Tilt head-down position for at least 2 h after LP.

PRED **Prednisone/Prednisolone** 60 mg/m²/d, PO/IV, in 3 single doses per day.

Day 1 – 7: Begin with ca 25% of the calculated dose and increase rapidly depending on clinical response (reduction of the blast count in PB and of organ size), laboratory findings (urea, creatinine, uric acid, electrolytes, phosphate) and diuresis to a final dosage of

60 mg/m^2/d (e.g. daily increments to 50- 75- 100% of the final dosage). Full dose should be reached as fast as possible, by day 4 at the latest. The cumulative dose of prednisone in the first 7 days of therapy must be greater than 210 mg/m^2. For patients with a great tumor burden (high leukocytosis, significant organomegaly) a lower starting dosage (0.2 – 0.5 mg/kg/d) must be chosen in order to prevent acute tumor lysis syndrome.

Day 8 – 28: Prednisone 60 mg/m^2/d, in 3 single doses PO.

From day 29: Tapering to withdrawal of prednisone over 9 days by halving the dosage every third day, with the highest dose given in the morning.

VCR **Vincristine** 1.5 mg/m^2/d, IV (maximal single dose 2 mg), on day: 8, 15, 22, 29 (4 doses).

DNR **Daunorubicin** 30 mg/m^2/d, PI, over 1 h

- x2 on day: 8, 15 (Protocol I')- in SR ALL only.
- x4 on day: 8, 15, 22, 29 (Protocol I)- in IR & HR ALL.

ASP **E. coli L-asparaginase** at 5,000 I.U./m^2/d, PI, over 1 h, on day: 12, 15, 18, 21, 24, 27, 30, 33 (8 doses).

Alternatively, PEG-L-Asparaginase could be used at 2,500 I.U./m^2, PI, over 1 h, on day 12 and 26 (2 doses) (maximal single dose 3,750 I.U.).

In case of a hypersensitivity reaction to the native E. coli ASP formulation, which leads to discontinuation of treatment, two options are offered:

- Peg Asparaginase to be used at 2,500 I.U./m^2, PI, over 1 h. One dose of PEG-ASP 2,500 I.U./m^2 substitutes 4 doses of the native E. coli ASP.
- Erwinia Asparaginase could be given at a dosage of 20,000 I.U./m^2/dose p.i. (1 h) or i.m. every other day (6 doses)

Phase I/B (Consolidation)

Requirements for beginning phase I/B

- Adequate clinical condition and no serious infection according to the assessment of the investigator
- Normal renal function
- Blood counts
  - Granulocytes ≥ 500/µl
  - Platelets ≥ 50 000/µl

Therapy regulation in phase I/B

The minimum requirements to begin a cytarabine (ARA-C) block are:

- WBC ≥ 500/µL
- Platelets ≥ 30,000/µL

As far as possible, an ARA-C block should not be interrupted. However, should an ARA-C block be postponed or interrupted, then 6-mercaptopurine (MP) also must be withheld for the same period of time. The missing MP doses should be subsequently delivered to make up the planned cumulative total dose of 1,680 mg/m^2 (28 x 60 mg/m^2).

For the second cyclophosphamide (CPM) dose to be given, the minimum requirements are:

- WBC ≥ 1,000/µL
- Granulocytes ≥ 300/µL

- Platelets ≥ 50,000/µL
- Creatinine level within normal range for age

Treatment schedule

CPM **Cyclophosphamide** 1,000 mg/m^2/d, PI, over 1 h, on day: 36, 64.

- Hyperhydration 3,000 ml/m^2 over 24 hours: G5% + NaCl 0.45% + 90 mEq/m^2 KCl
- Give Mesna (Uromitexan®) 400 mg/m^2/dose, IV, x3, before and at 4 and 8 hours from the start of the CPM infusion.
- Furosemide 0.5-1 mg/kg i.v. if input > output + 400 ml/m^2/12h.

MP **6-Mercaptopurine** 60 mg/m^2/d, PO, day: 36 – 63 (=28 days), to be taken in the evening on a fasting stomach without milk.

ARA-C **Cytarabine** 75 mg/m^2/d, IV, in 4 blocks, over 4 days each, on day: 38-41, 45-48, 52-55, 59-62.

IT MTX **Intrathecal methotrexate** at age-adjusted dosage on the same day as the first dose of ARA-C in block 2 (day 45) and block 4 (day 59):

Age (yr)	≥ 1 < 2	≥ 2 < 3	≥ 3
Dosage (mg)	8	10	12

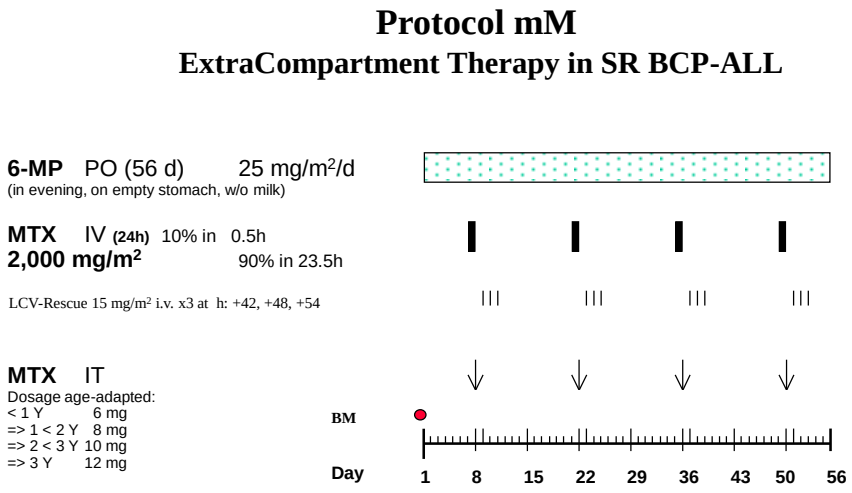
Tilt head-down position for at least 2 h after IT MTX.

6.2.3 ExtraCompartment Therapy (SR ALL)

6.2.3.1 Protocol mM (SR BCP-ALL)

Protocol mM is given to SR BCP-ALL patients and begins 2 weeks following the end of Protocol I'/I. Dosage is to be adjusted to the BSA determined at the start of the protocol as well as prior to each MTX infusion. Protocol mM is shown in **Fig. 6** below.

Fig. 6: Protocol mM for ExtraCompartment Therapy in SR BCP-ALL



Requirements for beginning Protocol mM

- Adequate clinical condition and no serious infection in complete cytomorphologic remission.
- Normal renal function. Dose adjustments of MD-MTX are recommended in the case of reduced creatinine clearance.
- No urinary obstruction
- GOT/GPT < 10 x upper normal limit
- Bilirubin < 3 x upper normal limit with normal direct bilirubin
- Recovering (increasing) blood count
    - Granulocytes ≥ 500/µL
    - Platelets ≥ 50,000/µL

Drug interactions

Avoid the administration of cotrimoxazol, nonsteroidal anti-inflammatory medications and penicillins simultaneously to MD-MTX and as long as the MTX level is not less than 0.25 µmol/l.

Avoid sun exposure (also solarium) during MD-MTX-containing treatment elements.

Treatment schedule

MP **6-Mercaptopurine** 25 mg/m^2/d, PO, day: 1 – 56, in the evening on a fasting stomach without milk.

MD MTX: **Medium-dose methotrexate** 2,000 mg/m^2/d, PI, over 24 h, q 14 days (x4) on day: 8, 22, 36, 50. 1/10 of the total dose (200 mg/m^2) should be administered PI over 30 minutes as a loading dose, immediately followed by the remaining 9/10 of the total dose (1,800 mg/m^2) given PI over 23.5 h.

- Good urine output should be established at least from –4 h to +72 h from the start of the MTX infusion by adequate IV hydration.
- Urine pH > 7 must be maintained at least from –4 h to +72 h from the start of the MTX infusion by adequate IV alkalinization.
- Fluid balancing q 12 h. If intake > output by +400 ml/m2 /12h, then furosemide 0.5 mg/kg body weight (maximum: 20 mg) IV should be administered.

- Routine assessment of the serum MTX levels is not necessary. However, careful clinical monitoring of the patient is mandatory. Specifically, oliguria/anuria, hypertension, edema, weight gain, emesis, confusion, blurred vision, significant elevation of serum creatinine, etc may be manifestations of slow elimination of MTX. In these cases, MTX levels must be assessed statim, and appropriate measures undertaken promptly (forced diuresis/alkalinization, more stringent monitoring of fluid balance, vital functions and blood chemistry, adequate LCV rescue, and possibly CPD G2).

LCV **Leucovorin rescue** is given at hours 42, 48, and 54 (15 mg of the racemic form or 7,5 mg/m2 each of the levo-form).

IT MTX **Intrathecal methotrexate** dosage adjusted to age on days 8, 22, 36, 50, during MD-MTX infusion.

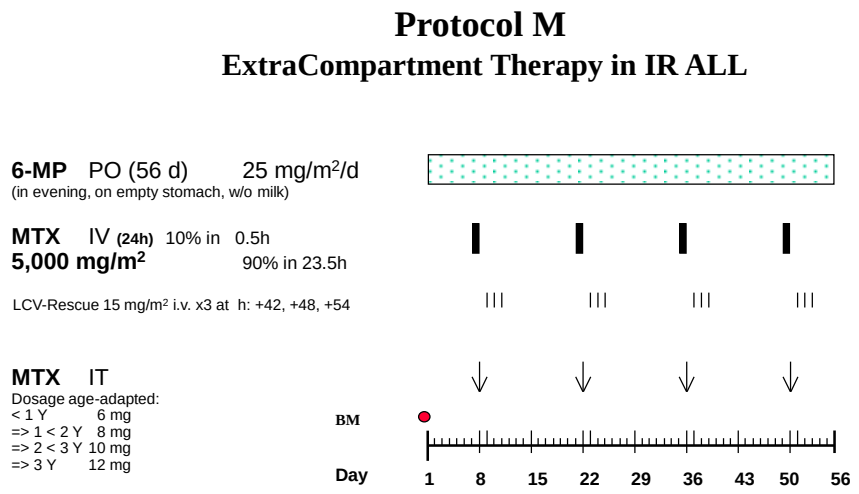
Age (yr)	≥ 1 < 2	≥ 2 < 3	≥ 3
Dosage (mg)	8	10	12

Tilt head-down position for at least 2 h after IT MTX.

6.2.3.2 Protocol M (IR T-ALL, IR BCP-ALL)

T-ALL patients as well as BCP-ALL patients classified as IR should be consolidated with HD MTX, i.e. 5 g/m^2/24h x4 q 14 days, two weeks following the end of Protocol I. Dosage is to be adjusted to the BSA determined at the start of the protocol as well as prior to each MTX infusion. Protocol M is shown in **Fig. 7** below.

Fig. 7: Protocol M for ExtraCompartment Therapy in IR BCP-ALL and IR T-ALL



Requirements for beginning Protocol M

- Adequate clinical condition and no serious infection in complete cytomorphologic remission.
- Normal renal function. Dose adjustments of HD-MTX are recommended in the case of reduced creatinine clearance.
- No urinary obstruction

- GOT/GPT < 10 x upper normal limit
- Bilirubin < 3 x upper normal limit with normal direct bilirubin
- Recovering (increasing) blood count
  - Granulocytes ≥ 500/µL
  - Platelets ≥ 50,000/µL

Drug interactions

Avoid the administration of cotrimoxazol, nonsteroidal anti-inflammatory medications and penicillins simultaneously to HD-MTX and as long as the MTX level is not less than 0.25 µmol/l. Avoid sun exposure (also solarium) during HD-MTX-containing treatment elements.

Treatment schedule

MP **6-Mercaptopurine** 25 mg/m^2/d, PO, day: 1 – 56, in the evening on a fasting stomach without milk.

HD MTX **High-dose methotrexate** 5,000 mg/m^2/d, PI, over 24 h, q 14 days (x4) on day: 8, 22, 36, 50. 1/10 of the total dose (500 mg/m^2) should be administered PI over 30 minutes as a loading dose, immediately followed by the remaining 9/10 of the total dose (4,500 mg/m^2) given PI over 23.5 h.

- Alkalinization aiming at and maintaining a urine pH ≥ 7 from –4 h through +72 h as of the start of the MTX infusion, checking every urine portion by dipstick.
- Intense IV hydration with crystalloids (5% G/0.45% NaCl/7.45% KCl) from –4 h through +72 h as of the start of the MTX infusion, checking fluid balance q 12 h, and administering furosemide 0.5 mg/kg (maximum: 20 mg) IV if input > output by > 400 ml/m2 /12h.
- Leucovorin rescue: 15 mg/m^2 i.v. of the racemic product or 7.5 mg/m^2 of the levo-product 42, 48 and 54 hrs after the start of the Methotrexate infusion. The leucovorin dose depends on the Methotrexate plasma level. For details of Methotrexate level monitoring and regulation of leucovorin rescue see Appendix II.
- For detailed guidelines of the management of impaired Methotrexate excretion see Appendix II.

IT MTX **Intrathecal methotrexate** dosage adjusted to age on days 8, 22, 36, 50, during HD-MTX infusion.

Age (yr)	≥ 1 < 2	≥ 2 < 3	≥ 3
Dosage (mg)	8	10	12

Tilt head-down position for at least 2 h after IT MTX.

6.2.4 Reinduction Therapy (SR/IR ALL)

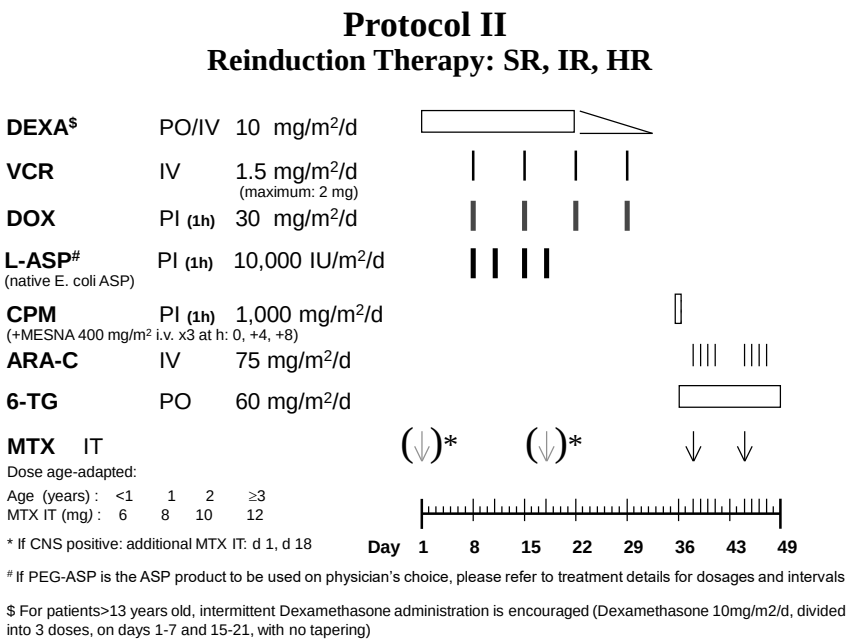
6.2.4.1 Protocol II

Protocol II is given as reintensification phase for all SR and IR patients. It consists in two phases: Protocol IIA (day 1-35) and Protocol IIB (day 36-50)

It is used x1 for late reinduction therapy in SR and IR patients, beginning 2 weeks after Protocol mM/M. In addition, it is also destined as part of the reinduction therapy in HR patients who do not undergo transplant. The reinduction strategy for HR patients is discussed below (chapter 6.3.4).

Dosage is a function of BSA as determined at 2 key time points: on day 1 and 36, i.e. at the start of phase II/1 and phase II/2, respectively.

Fig. 8: Protocol II for Reinduction Therapy in SR and IR patients



If PEG-ASP is the ASP product to be used on physician's choice, please refer to treatment details for dosages and intervals

$ For patients>13 years old, intermittent Dexamethasone administration is encouraged (Dexamethasone 10mg/m2/d, divided into 3 doses, on days 1-7 and 15-21, with no tapering)

Requirements for beginning Protocol II

- Adequate clinical condition and no serious infection
- Recovering (increasing) blood counts
  - Granulocytes ≥ 500/µl
  - Platelets ≥ 50 000/µl

Phase II/A

Regulation of therapy in Phase II/A

- In the case of severe neuropathy, VCR may be deleted.
- In the case of insufficient WBC recovery (WBC < 500/µL or granulocytes < 200/µL), doxorubicin/vincristine doses can be postponed until blood count recovery.

Treatment schedule

DEXA **Dexamethasone** 10 mg/m²/d, PO/IV, divided into 3 doses per day, on day: 1-21 (21 days). By day 22 taper down stepwise to withdrawal over 9 days by halving the dose on every third day.

For adolescent patients≥13 years old, intermittent Dexamethasone administration is encouraged (Dexamethasone 10 mg/m²/d, PO/IV, divided into 3 doses per day, on days: 1-7 and 15-21, with no subsequent tapering), in attempt to decrease the risk of osteonecrosis.

VCR **Vincristine** 1.5 mg/m^2/d, IV, (maximal single dose 2 mg), on day: 8, 15, 22 and 29 (4 doses).

DOX **Doxorubicin** 30 mg/m^2/d, PI, over 1 h, on day: 8, 15, 22 and 29 (4 doses).

ASP **E. coli L-asparaginase** 10,000 U/m^2/d, PI, over 1 h, on day: 8, 11, 15, 18.

Alternatively, PEG-L-Asparaginase should be used at 2,500 I.U./m^2, PI, over 1 h, on day 8 (1 dose) (maximal single dose 3,750 IU).

- If the patient has not yet displayed an overt hypersensitivity reaction (HSR) to the drug, it is preferable to administer the same formulation as in Protocol I'/I (native E. coli L-asparaginase or PEG-L-asparaginase).

  In case of a HSR to the native E. coli ASP formulation, which leads to discontinuation of treatment, two options are offered:
  - A PEG-L-asparaginase, to be used at 2,500 U/m^2, PI, over 1 h, on day 8 or when the HSR has occurred;
  - Erwinia chrysanthemi ASP should be given at a dosage of 20,000 U/m^2/dose PI (1 h) every second day (days: 8, 10, 12, 14, 16, 18) for 2 weeks (6 doses).

IT MTX **Intrathecal methotrexate** at age-adjusted dosage on day 1 and 18 only if **initial CNS** involvement.

Age (yr)	≥ 1 < 2	≥ 2 < 3	≥ 3
Dosage (mg)	8	10	12

Tilt head-down position for at least 2 h after IT MTX.

Phase II/B

Requirements for starting phase II/B

- Adequate clinical condition and no serious infection
- Creatinine within normal limits
- Recovering (increasing) blood counts
  - Granulocytes ≥ 500/µl
  - Platelets ≥ 50 000/µl

Therapy regulation in phase II/B

The minimum requirements to begin a cytarabine (ARA-C) block are:

- WBC ≥ 500/µL
- Platelets ≥ 30,000/µL

As far as possible, a run ARA-C block should not be interrupted. However, should it be postponed or interrupted, then 6-thioguanine (TG) also must be withheld for the same period of time. The omitted TG doses should be subsequently delivered to make up the planned cumulative total dose of 840 mg/m^2 (14 x 60 mg/m^2).

Treatment schedule

CPM **Cyclophosphamide** 1,000 mg/m^2/d, PI, over 1 h, on day 36.

- Hyperhydration 3,000 ml/m^2 over 24 hours: G5% + NaCl 0.45% + 90 mEq/m^2 KCl
- Give Mesna (Uromitexan®) 400 mg/m^2/dose, IV, x3, before and at 4 and 8 hours from the start of the CPM infusion.
- Furosemide 0.5-1 mg/kg i.v. if input > output + 400 ml/m^2/12h.

TG **6-Thioguanine** 60 mg/m^2/day, PO, day: 36 – 49 (= 14 days), to be taken in the evening on a fasting stomach without milk.

ARA-C **Cytarabine** 75 mg/m^2/dose, IV, in 2 blocks, over 4 days each, days 38-41 and 45-48.

IT MTX **Intrathecal methotrexate** at age-adjusted dosage on the same day as the first dose of ARA-C in block 1 (day 38) and block 2 (day 45).

Age (yr)	≥ 1 < 2	≥ 2 < 3	≥ 3
Dosage (mg)	8	10	12

Tilt head-down position for at least 2 h after IT MTX.

6.3 HR Therapy Branch

6.3.1 Preface

Patients assigned to the HR group are managed with chemotherapy while transplant represents the consolidation of choice in a minor proportion of patients.

Induction therapy consists of Protocol I (IA+IB). After a rest period of 2 weeks, consolidation therapy follows, with 3 highly intensive short blocks (HR-1', HR-2', HR-3'), always with a recovery period of about 2 weeks following the 6th day of each block. Thereafter, patients are addressed to continue treatment with chemotherapy or to transplant. Patients who do not present criteria for transplant proceed to the reinduction phase, consisting in two courses of Protocol II and a 4-week interim maintenance phase in between, followed by maintenance therapy (up to 104 weeks of treatment).

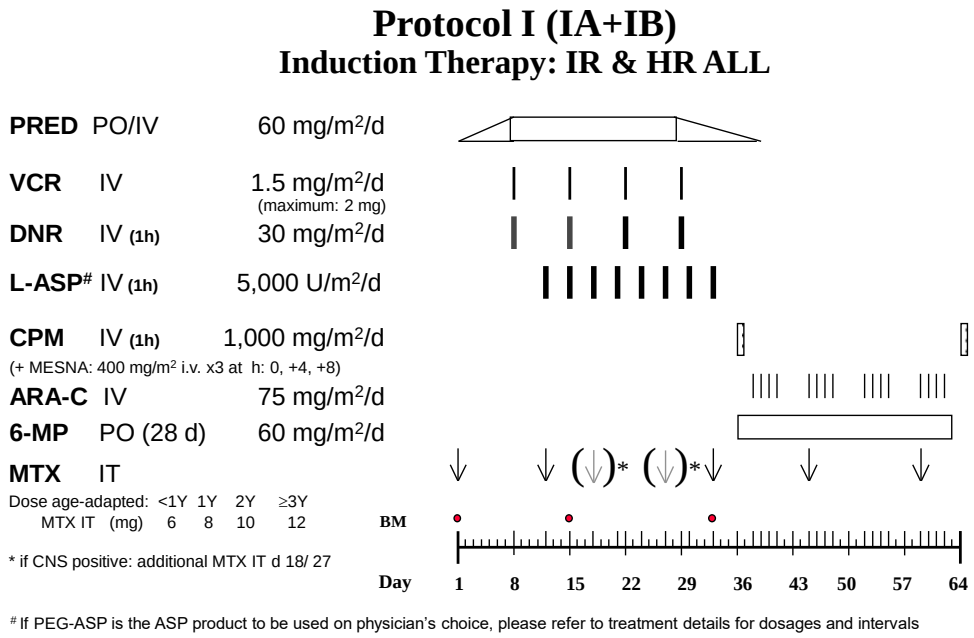
On the other hand, a selected subset of the HR patients will be managed with allogeneic HSCT, see section 6.6.1.

6.3.2 Induction Therapy (HR ALL)

6.3.2.1 Protocol I

Patients with HR features receive both parts of Protocol I (IA and IB) as induction and consolidation therapy. Treatment is discussed in detail in section 6.2.2.1 of this chapter.

Fig. 9: Protocol I induction and Consolidation Therapy in HR patients



If PEG-ASP is the ASP product to be used on physician's choice, please refer to treatment details for dosages and intervals

Treatment schedule

For treatment details see chapter 6.2.2.1

6.3.3 Re-consolidation Therapy (HR ALL)

6.3.3.1 Outline of Re-Consolidation Therapy

Re-Consolidation therapy begins 2 weeks following the conclusion of induction and consolidation therapy (Protocol IA+B), provided that they fulfill the entry criteria outlined below. It consists in 3 highly intensive, multi-agent chemotherapy elements condensed in brief blocks (HR-1', HR-2', HR-3'), delivered approximately 2 weeks apart, which is the interval from the 6th day of the outgoing block to the 1st day of the ingoing one. From a prognostic point of view, therapy should be realized as quickly as possible. The BSA must be updated in order to actualize dosage at the start of every HR block.

6.3.3.2 Regulation of Therapy

General Guidelines

- Treatment should be as quick as possible, because dose intensity will have hopefully a favorable impact on prognosis. The interval between 2 blocks (from the 6th day of the previous block through the 1st day of the next one) is about 2 weeks.
- Whenever possible, once begun, a block should not be interrupted.
- As far as possible, the prescribed intervals between the individual drugs within each block should be maintained, e.g. 7 h between MTX and CPM or IFO.
- Dosage is a function of BSA determined always at the start of each HR block.

Requirements for entry of a HR block

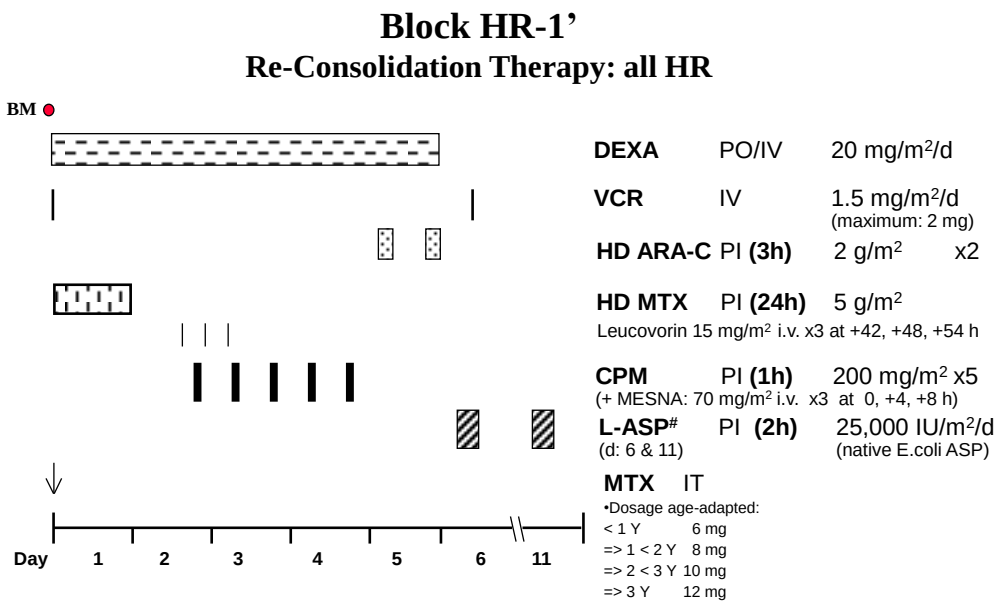
- Adequate clinical condition and no serious infection

- No urinary obstruction
- Intact mucous membranes
- Recovering (increasing) blood counts
  - Granulocytes ≥ 500/µl
  - Platelets ≥ 50 000/µl
- No essential organ dysfunction:
  - Normal renal function. Dose adjustments of HD-Methotrexate and High-dose Cytarabine are recommended in the case of reduced creatinine clearance. Dose adjustment of Cyclophosphamide is recommended in the case of severe renal dysfunction (creatinine clearance < 25 ml/min/1.73 m²).
  - GOT/GPT <10 x upper normal limit
  - Bilirubin <3 x upper normal limit with normal direct bilirubin

6.3.3.3 Block HR-1'

The HR-1' block is given to all patients of the HR group. It starts 2 weeks after the end of Protocol IB.

Fig. 10: Block HR-1' for Re-Consolidation Therapy in HR patients



If PEG-ASP is the ASP product to be used on physician's choice, please refer to treatment details for dosages and intervals

Treatment schedule

DEXA **Dexamethasone** 20 mg/m²/day, PO/IV, divided into 3 doses, days 1 to 5 (5 days).

VCR **Vincristine** 1.5 mg/m²/dose, IV, (maximal single dose 2 mg), on days 1 and 6.

- The first dose of VCR (on day 1) should be given 1 h before starting HD MTX. This sequence of delivery should avoid an accidental intrathecal administration of VCR as well as drug interactions that would dampen the efficacy of MTX.
- The second dose of VCR (on day 6) should be delivered 12 h prior to the hepatotoxic ASP in order to reduce the risk of VCR-induced neurotoxicity by washing out VCR.

HD MTX **High-dose methotrexate** 5,000 mg/m²/d, PI, over 24 h, on day 1.

1/10 of the total MTX dose (= 500 mg/m²) should be infused over 30 minutes as a loading dose. 9/10 of the total MTX dose (= 4,500 mg/m²) is to be infused over 23.5 h.

- Alkalinization aiming at and maintaining a urine pH ≥ 7 from –4 h through +72 h as of the start of the MTX infusion, checking every urine portion by dipstick.
- Intense IV hydration with crystalloids (5% G/0.45% NaCl/7.45% KCl) from –4 h through +72 h as of the start of the MTX infusion, checking fluid balance q 12 h, and administering furosemide 0.5 mg/kg (maximum: 20 mg) IV if input > output by > 400 ml/m2 /12h.
- Leucovorin rescue: 15 mg/m^2 i.v. of the racemic product or 7.5 mg/m^2 of the levo product are given at 42, 48 and 54 hrs after the start of the Methotrexate infusion. The leucovorin dose depends on the Methotrexate plasma level. For details of Methotrexate level monitoring and regulation of leucovorin rescue see Appendix II.
- For detailed guidelines of the management of impaired Methotrexate excretion see Appendix II.

CPM **Cyclophosphamide** 200 mg/m^2/dose, PI, over 1 h, every 12 hours on days 2 to 4 (5 doses), beginning 7 h after the end of HD MTX.

- Hyperhydration 3,000 ml/m^2 over 24 hours: G5% + NaCl 0.45% + 90 mEq/m^2 KCl
- Give Mesna (Uromitexan®) 400 mg/m^2/dose, IV, x3, before and at 4 and 8 hours from the start of the CPM infusion.
- Furosemide 0.5-1 mg/kg i.v. if input > output + 400 ml/m^2/12h.

HD ARA-C **Cytarabine** 2,000 mg/m^2/dose, PI, over 3 h, every 12 hours on day 5 (2 doses).

- For supportive care and monitoring during HD ARA-C therapy see protocol appendix II.

ASP **E. coli L-asparaginase** 25,000 U/m^2/dose, PI, over 2 h, on days 6 and 11.

Alternatively, PEG-L-Asparaginase should be used at 2,500 U/m^2, PI, over 1 h, on day 6 (1 dose) (maximal single dose 3,750 IU).

- If the patient has not yet displayed an overt hypersensitivity reaction to the drug, it is preferable to administer the same formulation as in Protocol I'/I (native E. coli L-asparaginase or PEG-L-asparaginase).

In case of a HSR to the native E. coli ASP formulation, which leads to discontinuation of treatment, two options are offered:

- A PEG-L-asparaginase, to be used at 2,500 U/m^2, PI, over 1 h, on day 6;
- Erwinia chrysanthemi ASP should be given at a dosage of 20,000 U/m^2/dose PI (1 h) or IM every second day for 2 weeks (6 doses).

IT MTX **Intrathecal methotrexate** at age-adjusted dosage, administer 1 h after starting HD-MTX infusion on day 1.

Age (yr)	≥ 1 < 2	≥ 2 < 3	≥ 3
Dosage (mg)	8	10	12

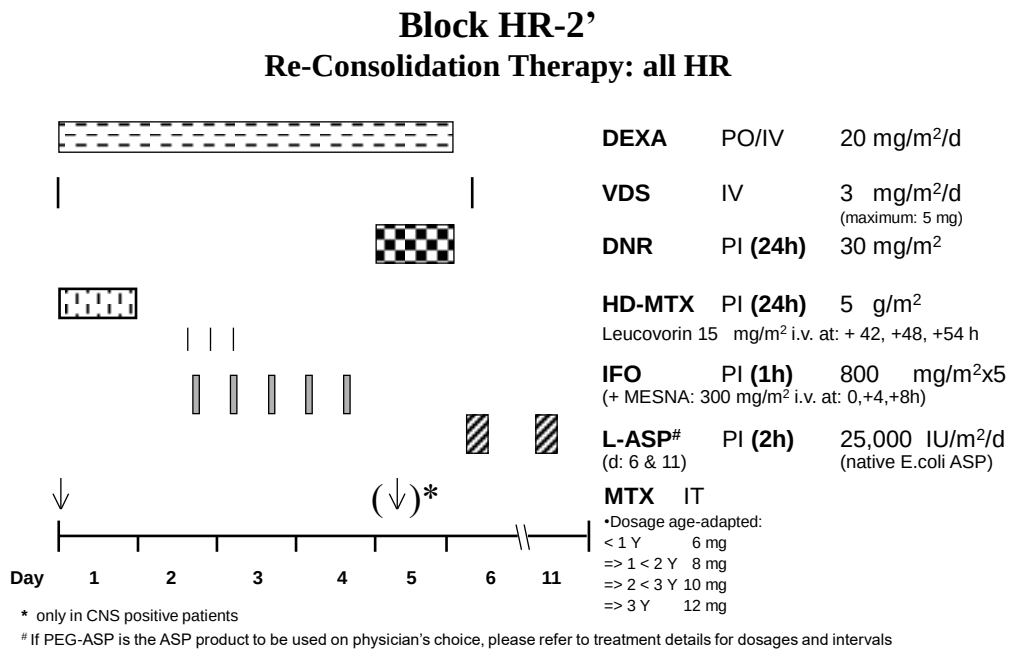
Tilt head-down position for at least 2 h after IT MTX.

G-CSF **Granulocyte colony stimulating factor** 5 µg/kg/dose SC or PI once a day from day 11 until the neutrophil count exceed 5,000/µl.

6.3.3.4 Block HR-2'

Block HR-2' usually begins 2 weeks after the 6th day of block HR-1', provided the fulfillment of entry criteria.

Fig. 11: Block HR-2' for Re-Consolidation Therapy in HR patients



Treatment schedule

DEXA **Dexamethasone** 20 mg/m²/day, PO/IV, divided into 3 doses, days 1 to 5 (5 days).

VDS **Vindesine** 3 mg/m²/dose (maximal single dose 5 mg), slowly IV, on days 1 and 6 (2 doses). Since ASP retards the clearance of VDS leading to prolonged exposure and hence enhanced risk of VDS-induced neurotoxicity, it is prudent to administer ASP 12 h after VDS (on day 6).

HD MTX **High-dose methotrexate** 5,000 mg/m²/d, PI, over 24 h, on day 1.

1/10 of the total MTX dose (= 500 mg/m²) should be infused over 30 minutes as a loading dose. 9/10 of the total MTX dose (= 4,500 mg/m²) is to be infused over 23.5 h.

- Alkalinization aiming at and maintaining a urine pH ≥ 7 from –4 h through +72 h as of the start of the MTX infusion, checking every urine portion by dipstick.
- Intense IV hydration with crystalloids (5% G/0.45% NaCl/7.45% KCl) from –4 h through +72 h as of the start of the MTX infusion, checking fluid balance q 12 h, and administering furosemide 0.5 mg/kg (maximum: 20 mg) IV if input > output by > 400 ml/m2 /12h.
- Leucovorin rescue: 15 mg/m² i.v. of the racemic product or 7.5 mg/m² of the levo product are given at 42, 48 and 54 hrs after the start of the Methotrexate infusion. The leucovorin dose depends on the Methotrexate plasma level. For details of Methotrexate level monitoring and regulation of leucovorin rescue see Appendix II.
- For detailed guidelines of the management of impaired Methotrexate excretion see Appendix II.

IFO **Ifosfamide** 800 mg/m²/dose, PI, over 1 h, every 12 hours on days 2 to 4 (5 doses).

- Give Mesna (Uromitexan®) 400 mg/m^2/dose, IV, x3, before and at 4 and 8 hours from the start of the CPM infusion.
- For hydration and cystitis prophylaxis see protocol Appendix II.

DNR **Daunorubicin** 30 mg/m^2/dose, PI, over 24 h, on day 5 (1 dose).

ASP **E. coli L-asparaginase** 25,000 U/m^2/dose, PI, over 2 h, on days 6 and 11.

Alternatively, PEG-L-Asparaginase should be used at 2,500 U/m^2, PI, over 1 h, on day 6 (1 dose) (maximal single dose 3,750 IU).

- If the patient has not yet displayed an overt hypersensitivity reaction to the drug, it is preferable to administer the same formulation as in Protocol I'/I (native E. coli L-asparaginase or PEG-L-asparaginase).

In case of a hypersensitivity reaction to the native E. coli ASP formulation, which leads to discontinuation of treatment, two options are offered:

- A PEG-L-asparaginase, to be used at 2,500 U/m^2, PI, over 1 h, on day 6;
- Erwinia chrysanthemi ASP should be given at a dosage of 20,000 U/m^2/dose PI (1 h) or IM every second day for 2 weeks (6 doses).

IT MTX **Intrathecal methotrexate** at age-adjusted dosage, administer 1 h after starting HD-MTX infusion on day 1. Only in case of initial CNS involvement another dose is administered on day 5.

Age (yr)	≥ 1 < 2	≥ 2 < 3	≥ 3
Dosage (mg)	8	10	12

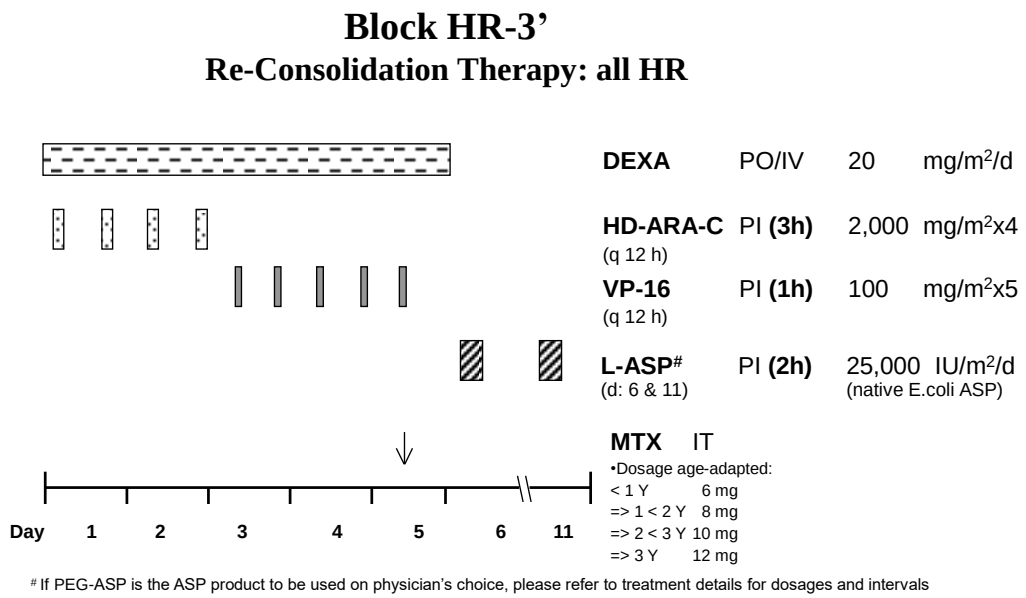
Tilt head-down position for at least 2 h after IT MTX.

G-CSF **Granulocyte colony stimulating factor** 5 µg/kg/dose SC or PI once a day from day 11 until the neutrophil count exceed 5,000/µl.

6.3.3.5 Block HR-3'

Block HR-3' starts 2 weeks following the 6th day of the preceding block HR-2', once criteria are met.

Fig. 12: Block HR-3' for Re-Consolidation Therapy in HR patients



If PEG-ASP is the ASP product to be used on physician's choice, please refer to treatment details for dosages and intervals

Treatment schedule

DEXA **Dexamethasone** 20 mg/m²/day, PO/IV, divided into 3 doses, days 1 to 5 (5 days).

HD ARA-C **Cytarabine** 2,000 mg/m²/dose, PI, over 3 h, every 12 hours on day 1 to 2 (4 doses).

- For supportive care and monitoring during HD ARA-C therapy see protocol appendix II.

VP-16 **Etoposide** 100 mg/m²/dose, PI, over 1 h, every 12 hours on day 3 to 5 (5 doses).

- Etoposide is preferably administered as Etoposide phosphate (Etopophos®), if available, due to the lower infusion-related toxicity compared to its original ancestor Etoposide (Vepesid®); 100 mg Etoposide correspond to 113.6 mg etoposide phosphate.
- For monitoring during Etoposide therapy see protocol appendix II.

ASP **E. coli L-asparaginase** 25,000 U/m²/dose, PI, over 2 h, on days 6 and 11.

Alternatively, PEG-L-Asparaginase should be used at 2,500 U/m², PI, over 1 h, on day 6 (1 dose) (maximal single dose 3,750 IU).

- If the patient has not yet displayed an overt hypersensitivity reaction to the drug, it is preferable to administer the same formulation as in Protocol I'/I (native E. coli L-asparaginase or PEG-L-asparaginase).

In case of a hypersensitivity reaction to the native E. coli ASP formulation, which leads to discontinuation of treatment, two options are offered:

- A PEG-L-asparaginase, to be used at 2,500 U/m^2, PI, over 1 h, on day 6;
- Erwinia chrysanthemi ASP should be given at a dosage of 20,000 U/m^2/dose PI (1 h) or IM every second day for 2 weeks (6 doses).

IT MTX

Intrathecal methotrexate at age-adjusted dosage, administer 1 h after starting HD-MTX infusion on day 1.

Age (yr)	≥ 1 < 2	≥ 2 < 3	≥ 3
Dosage (mg)	8	10	12

Tilt head-down position for at least 2 h after IT MTX.

G-CSF

Granulocyte colony stimulating factor 5 µg/kg/dose SC or PI once a day from day 11 until the neutrophil count exceed 5,000/µl.

6.3.4 Reinduction Therapy (HR ALL)

6.3.4.1 Introduction

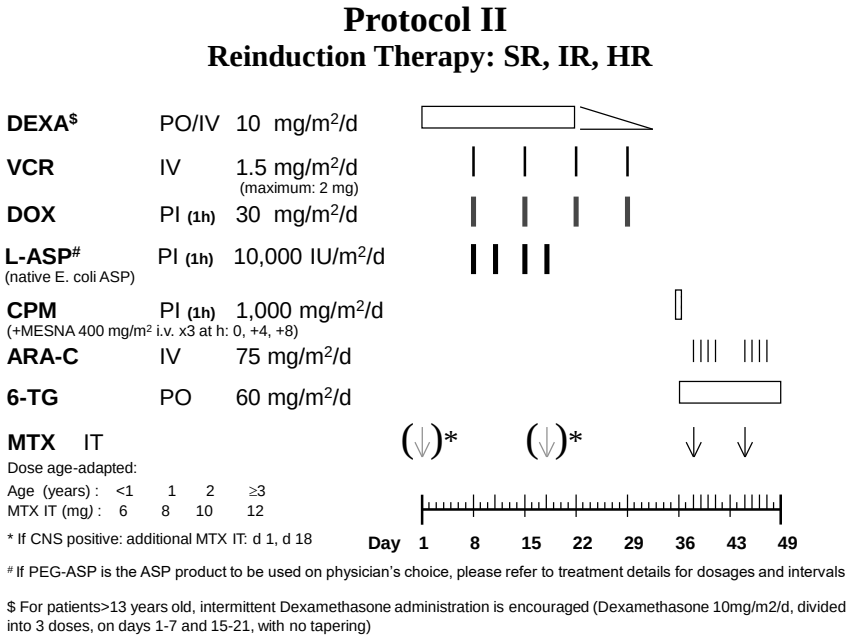
Reinduction therapy is administered to all HR patients who do not undergo to alloHSCT present indication for transplant. It begins 2 weeks after the 6th day of the first HR-3' block provided that the entry requirements are fulfilled.

Reinduction phase consists in the administration of two cycles of Protocol II, over 7 week each, that are harnessed by a 4-week interim maintenance phase of oral MP and weekly MTX. A rest period of 1 week on either side is interposed between the individual elements.

6.3.4.2 Protocol II

Protocol II is the first phase administered in the reinduction phase of HR patients. It begins 2 weeks after the 6th day of the first HR-3' block provided that the entry requirements are fulfilled.

Fig. 13: Protocol II for Reinduction Therapy in SR, IR and HR patients



If PEG-ASP is the ASP product to be used on physician's choice, please refer to treatment details for dosages and intervals

$ For patients>13 years old, intermittent Dexamethasone administration is encouraged (Dexamethasone 10mg/m2/d, divided into 3 doses, on days 1-7 and 15-21, with no tapering)

Requirements for beginning Protocol II

- Adequate clinical condition and no serious infection
- Recovering (increasing) blood counts
  - Granulocytes ≥ 500/µl
  - Platelets ≥ 50 000/µl

Protocol II is administered to HR patients similarly to SR and IR patients. See chapter 6.2.4.1 for drugs and dosage specifications.

6.3.4.3 Interim Maintenance Therapy

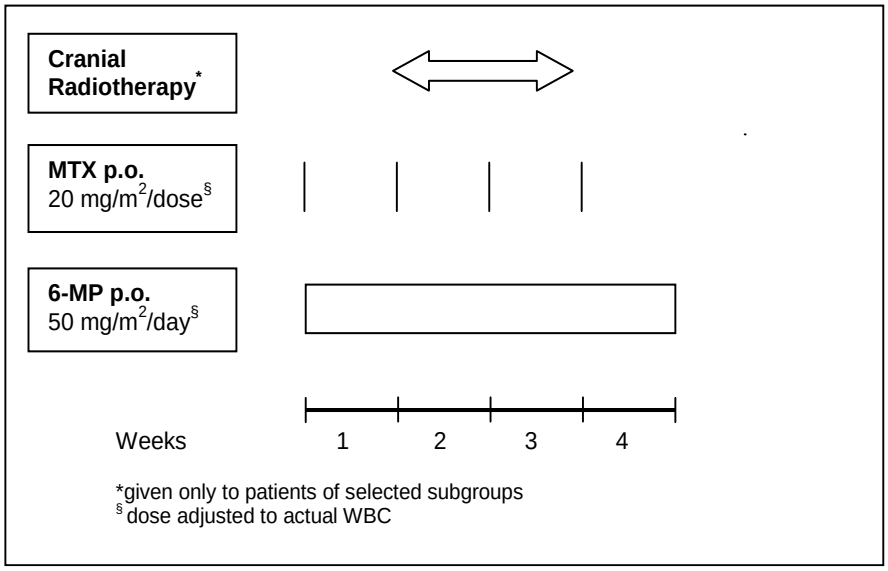
After one week from the conclusion of the first Protocol II, HR patients begin the 4-week phase of Interim Maintenance Therapy (IMT), with oral daily 6-MP and weekly MTX interspacing the

intensive elements. Drug dosing should be adapted to the BSA as updated at the beginning of each phase.

If indicated, pCRT/tCRT should be delivered during the first 1.5/2.5 weeks of the interim maintenance phase.

As late reinduction therapy is overall intensive, and the patient has already become markedly immunocompromised, prophylaxis of Pneumocystis carinii pneumonia (PCP) is mandatory.

Fig. 14: Interim Maintenance Therapy in HR patients



Requirements for the start of the Interim Maintenance

- Adequate clinical condition and no serious infection
- Recovering (increasing) blood counts
  - Granulocytes ≥ 500/µl
  - Platelets ≥ 50 000/µl
- No essential organ dysfunction:
  - GOT/GPT <10 x upper normal limit
  - Bilirubin <3 x upper normal limit with normal direct bilirubin

Therapy modulation during Interim Maintenance

Dosage of 6-MP & MTX should be adapted to the WBC count & differential as shown in **Tab. 11**.

A CBC should be performed 1x weekly, preferably on the same day as the MTX dose. Routine measurement of liver parameters during Interim Maintenance is not necessary in patients without symptoms. In case of symptoms, dose reductions should be based on a rise in direct bilirubin to more than three times the upper normal limit or aminotransferase levels more than 10 times the upper normal limit and rising. Stop Interim Maintenance 4 weeks after start of the phase regardless of the total cumulative doses of drugs given.

Table 11: Dosage of MP & MTX by WBC & Differential during IMT

WBC/µL	<1,000	1,000-2,000	>2,000-3,000	>3,000	
Lymphocytes/µL					<300
% MP/MTX dose	0	50	100	up to 150	50

Interruption of Interim Maintenance

- WBC < 1,000/µL
- Infection
- Grade ≥ 3 liver toxicity:

  (virologic studies mandatory)
  - GOT/GPT > x10 ULN for age
  - Bilirubin > x3 ULN for age
- Long-standing diarrhea
- Lung changes on CXR

Treatment schedule

MP **6-Mercaptopurine** 50 mg/m^2/d, PO, daily in the evening on an empty stomach without milk.

Adjust dose using tablets at 50 mg or at 10 mg and/or different doses on alternating days in order to attain the daily target dose on average, or use 6-MP as suspension. The drug should be taken in one single dose in the afternoon on an empty stomach (at least 30 min before the evening meal) without milk.

MTX **Methotrexate** 20 mg/m^2, PO, x1 weekly (always on the same day of every week), to be taken also in the evening on an empty stomach without milk.

CRT **Cranial Radiotherapy** during the 1st Interim Maintenance only for selected subgroups of patients. See section 6.5.1.1 for dosage and administration.

6.4 Maintenance Therapy (MT)

6.4.1 Maintenance Therapy General Outline

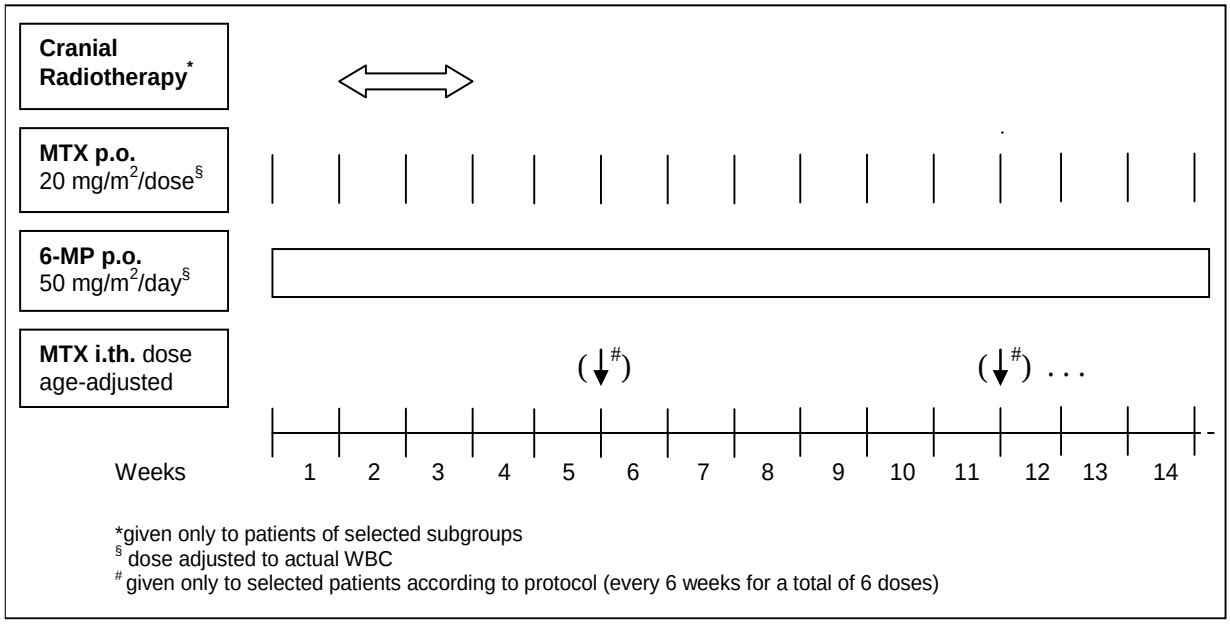
Two weeks after the conclusion of the last intensive therapy element, meeting the entry criteria outlined below, all patients with ALL (except those HR patients who have undergone allogeneic SCT) are put on oral Maintenance Therapy (MT) with daily 6-MP and weekly MTX. The overall duration of treatment from the start of induction through the end of MT is uniformly 104 weeks (24 months). Approximately, SR and IR patients receive 30 weeks of intensive therapy and 74 weeks of maintenance, whereas HR patients undergo to 42 weeks of intensive chemotherapy and 62 of maintenance therapy.

Locoregional therapy with IT MTX is indicated to selected patients (see below 6.4.2).

6.4.2 Realization of Therapy

- MT begins upon recovery of hemopoiesis and with the patient being in a good clinical standing, which is usually the case 2 weeks after the end of the last intensive therapy element.
- Pneumocystis carinii prophylaxis (PCP) is mandatory in all patients.
- The BSA should be updated, and the dosage of MP/MTX adjusted accordingly x1/month.
- A CBC and differential must be performed once monthly, preferably on the same day as the weekly MTX, in order to adapt the dosage of MP & MTX.
- GOT, GPT, AST, LDH, ALP, bilirubin, albumin, creatinine and urinalysis must be checked regularly q 8 – 12 weeks.
- Additional investigations may be necessary at the discretion of the physician in charge.
- Long treatment breaks should be avoided if possible. A dose reduction or quick resumption of the chemotherapy at reduced dose is generally to be favored over longer intervals without therapy.

Fig. 14: Maintenance Therapy in SR, IR and HR patients



Requirements for the start of Maintenance

- Adequate clinical condition and no serious infection
- Recovering (increasing) blood counts:
  - Granulocytes ≥ 200/µL
  - Platelets ≥ 50,000/µL

- No essential organ dysfunction:
  - GOT/GPT <10 x upper normal limit
  - Bilirubin <3 x upper normal limit with normal direct bilirubin

Regulation of MT by WBC count

The dosage of 6-MP and MTX should be modified according to the WBC count and differential, which must be checked regularly q 4 weeks, preferably on the same day as the weekly MTX, and as required- see **Tab. 12** below.

Table 12: Dosage of MP & MTX by WBC & Differential during MT

WBC/µL	<1,000	1,000-2,000	>2,000-3,000	>3,000	
Lymphocytes/µL					<300
% MP/MTX dose	0	50	100	up to 150	50

Interruption of therapy by

- WBC < 1,000/µL
- Infections
- Grade ≥ 3 liver toxicity
  - GOT/GPT > 10 x ULN for age
  - Bilirubin > 3 x ULN for age
- Long-standing diarrhea
- Lung changes on CXR (? MTX pneumonitis)

Treatment schedule

All patients receive uniform oral antimetabolite therapy with daily 6-MP and weekly MTX. It is desirable to schedule the blood test onto the same day as the regular MTX dose.

MP **6-Mercaptopurine** 50 mg/m^2/d, PO, once daily in the evening on a fasting stomach without milk.

Adjust dose using tablets at 50 mg or at 10 mg and/or different doses on alternating days in order to attain the daily target dose on average, or use 6-MP as suspension. The drug should be taken in one single dose in the afternoon on an empty stomach (at least 30 min before the evening meal) without milk.

MTX **Methotrexate** 20 mg/m^2, PO, once weekly (always on the same day of every week), to be taken also in the evening on a fasting stomach without milk.

IT MTX **Methotrexate intrathecally**, starting at week 6 every 6 weeks up to a total of 6 doses is given to:

- Patients without initial CNS disease (i.e. CNS status CNS 1 or CNS 2) and with:
  - T-ALL (HR or non-HR) and age < 4 years at start of CRT,
  - T-ALL, non-HR and initial WBC < 100,000/µl and age ≥ 4 years at start of (interim) maintenance, or
  - pB-ALL (or unknown immunophenotype) and risk group HR
- Patients with initial CNS disease (i.e. CNS status CNS 3) and < 4 years of age at start of Maintenance/Interim Maintenance

Age (yr)	≥ 1 < 2	≥ 2 < 3	≥ 3
Dosage (mg)	8	10	12

6.5 CNS directed treatment

6.5.1 Intrathecal therapy

All patients receive intrathecal therapy at specific time points across the treatment. Patients with initial CNS disease (CNS3) receive additional injections during Protocol I'A/IA (2 doses), as well as in Protocol IIA (2 doses) or during the HR2' block (1 dose), if the patient is treated in the HR group.
Injections of intrathecal MTX are administered during Protocol I/I', Protocol mM/M, Protocol II and during the three HR blocks.
During maintenance, the following patient groups receive 6 additional intrathecal MTX injections with a 6-week intervals and starting week 6 from start of maintenance:

- Patients without initial CNS disease (i.e. CNS status CNS 1 or CNS 2) and with
  - T-ALL (HR or non-HR) and age < 4 years at start of CRT,
  - T-ALL, non-HR and initial WBC < 100,000/µl and age ≥ 4 years at start of (interim) maintenance, or
  - pB-ALL (or unknown immunophenotype) and HR

- Patients with initial CNS disease (i.e. CNS status CNS 3) and < 4 years of age at start of Maintenance/first Interim Maintenance.

	Intrathecal injections for all patients	**Additional injection in subgroups**	
		Selected CNS-negative (CNS1 or CNS2) subgroups*	**CNS3**
Prot. I'A/IA	days 1, 12, 33		days 18, 27
Prot. IB	days 45, 59		
Prot. mM/M	days, 8, 22, 36, 50		
Prot. II	days 38, 45		days 1, 18
HR-1'	day 1		
HR-2'	day 1		day 5
HR-3'	day 5		
Maintenance		6 additional doses in 6-week interval	Patients < 4 years of age: 6 additional doses in 6-week interval

*as described above

6.5.2 Cranial Irradiation

6.5.2.1 Indication for cranial radiotherapy (CRT)

Due to the high rate of potential irradiation associated late effects in young children, patients younger than 4 years of age at the start of irradiation do no longer receive CRT.
CRT will be used at the dose of 12 Gy when given for therapeutic or preventive purposes and will be limited to patients ≥ 4 years old with:

- CNS involvement at diagnosis (CNS 3, therapeutic CRT, tCRT)
- CNS-negative (CNS 1 or CNS 2) patients with T-ALL and initial WBC of 100,000/µl (prophylactic CRT, pCRT)

Patients with T-ALL and HR patients who are not irradiated will receive intensified intrathecal therapy during maintenance.

Table 13: Indication for cranial radiotherapy (not applicable to patients undergoing alloHSCT)

Age	Risk group	CNS neg (CNS 1 or CNS 2)			CNS pos (CNS 3)
		pB-ALL	T-ALL		
			init. WBC <100 000/µl	init. WBC ≥100 000/µ	
< 4 years	**non-HR**	0 Gy	0 Gy + i.th. MTX in MT*	0 Gy + i.th. MTX in MT*	0 Gy + i.th. MTX in MT*
	HR	0 Gy + i.th. MTX in MT*	0 Gy + i.th. MTX in MT*	0 Gy + i.th. MTX in MT*	0 Gy + i.th. MTX in MT*
≥ 4 years	**non-HR**	0 Gy	0 Gy + i.th. MTX in MT*	12 Gy	12 Gy
	HR	0 Gy + i.th. MTX in MT*	0 Gy + i.th. MTX in MT*	12 Gy	12 Gy

6.5.2.2 Timing of CRT

CRT is given from day 8 to day 22 of interim maintenance or maintenance phase in parallel with chemotherapy, about 2 weeks after conclusion of the preceding chemotherapy element (upon conclusion of the first or the single therapeutic element of reinduction therapy).

Requirements for start of CRT:

- Good general condition
- No serious infection
- Recovering (increasing) blood counts o WBC ≥ 1000/µl
- Granulocytes ≥ 200/µl
- Platelets ≥ 50 000/µl
- No signs of cerebral disorder

6.5.2.3 Technique of CRT

Cranial irradiation is performed with a high-voltage Telecobalt-60 apparatus or linear accelerator. The exact reproducibility of the daily setting, e.g. with a mask-technique, must be possible. CNS irradiation must include the complete neurocranium, including the first two cervical vertebrae (C1 and C2), the retro bulbar space, and the complete skull base, including the middle cranial fossa. This implies the use of individual screens and the performance of a field-verification shooting. The dose-distribution during radiation therapy should be homogeneous. All fields must be irradiated in each sitting. The daily single dose is 1.5 Gy. This is given in 5 sittings a week until the total dose has been applied.

6.5.3 Other indications for RTX

- Testicular involvement:

  In case of biopsy-proven unilateral/bilateral testicular leukemia persisting beyond consolidation chemotherapy, fractionated local RTX to a total dose of 18 Gy is necessary.
- Rarely, additional specific situations may be encountered that could be considered for palliative or adjuvant radiotherapy. This should be done on a strictly individual basis after discussion with and approval by the national study coordinator.

6.5.4 RTX Side Effects

- Radiation-induced headache is the most common side effect occurring already during CRT. Short-term management with dexamethasone 15 mg/m^2/d is often helpful in relieving symptoms.

- Apathy/somnolence syndrome may be encountered 4 – 6 weeks post CRT. Patients with the syndrome may need a long time of convalescence to recover, and should not be exposed to physical overstrain or psychic overload. Adequate sleep, recreation and protection from direct sunlight are helpful in facilitating recovery.
- Specific, non-verbal intellectual deficits have been also described after CRT. These cases need to be approached on an individual basis with special emphasis on tactful psychologic/pedagogic care.
- Individuals with pre-existent cerebral damage are particularly at augmented risk of developing second brain tumors following CRT. They need careful consideration of the risk to benefit ratio of CRT against an extended course of IT MTX as an alternative option. Long-term follow-up is a prerequisite.

6.6 Stem Cell Transplantation

Conduction of allogeneic hematopoietic stem cell transplantation (alloHSCT) including diagnostics, donor selection, conditioning regimen, immunosuppression, and supportive management is not part of this protocol.

6.6.1 Indications for alloHSCT

In table 15, indications for performing alloHSCT are reported.

Table 15: Indications for alloHSCT

	PCR-MRD				
	TP1 neg	TP1 pos and TP2 <5x10-4	MRD-HR		No MRD
			TP1 pos and TP2 ≥5x10^{-4} <5x10^{-3}	TP1 pos and TP2 ≥5x10^{-3}	
No CR d33	No	MD	MMD	MMD	MMD
Hypodiploidy < 44 chr or DNA index < 0.8	No	MD	MD	MMD	MD
***KMT2A/AFF1* t(4;11)**	No	MD	MD	MMD	MD
TCF3-HLF	MMD	MMD	MMD	MMD	MMD
***IKZF1plus* MRD≥10% on day 15**	No	MD	MD	MMD	MD
***IKZF1plus* MRD<10% on day 15**	No	No	MD	MMD	MD
T-ALL + PPR	No	No	MD	MMD	MD
T-ALL + FCM-MRD d15 ≥10%	No	No	MD	MMD	MD
None of the above	No	No	MD	MMD	No

No allo HSCT not indicated
MD permitted donor: HLA-matched sibling or non-sibling donor
MDD permitted donor: HLA-matched or HLA-mismatched donor

6.6.2 Timing of alloHSCT

If a suitable donor were available and so far as the logistics allow, SCT is to be performed after the third HR block.

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NCT00764907: https://clinicaltrials.gov/ct2/show/NCT00764907

NCT03643276: https://clinicaltrials.gov/ct2/show/NCT03643276

CHAPTER 2

Philadelphia-chromosome Positive (Ph+) Acute Lymphoblastic Leukemia (ALL)

1. BACKGROUND AND RATIONALE

1.1 Background

Philadelphia chromosome positive (Ph+) Acute Lymphoblastic Leukemia (ALL) is characterized by the presence of t(9;22)(q34;q11.2) and accounts for approximately 3-5% of children and adolescents with ALL. Historically, Ph+ ALL has been associated with poor prognosis despite intensive chemotherapy approach and the use of hematopoietic stem cell transplantations, with rate of event-free survival of 25-30% (Arico 2000, Arico 2010). Subsequently, collaborative and international studies have demonstrated the benefit of the association of tyrosine kinase inhibitors, i.e. imatinib and dasatinib, with conventional chemotherapy backbones (Schultz 2009, Slayton 2018, Biondi 2012, Biondi 2018, Biondi 2019).
Recently, the randomized European intergroup Phase III study of post-induction treatment of Philadelphia/chromosome positive ALL (EsPhALL2010) has showed a clear advantage from early, continuous and protracted exposure to TKI combined with EsPhALL chemotherapy backbone (Biondi 2018), challenging the indications for allogeneic hematopoietic stem cell transplantation according to end of induction minimal residual disease (MRD). The results of the study demonstrated the safety and efficacy of the continuous administration of imatinib, with an 5-year event-free survival of 57·0% (95% CI 48.5–64.6) and a 5-year overall survival of 71·8% (63.5–78.5), similar to the previous EsPhALL2004 study but with a reduced number of patients undergoing transplant in first remission. Moreover, MRD assessment at post-induction timepoint has been demonstrated to improve patient risk group stratification and reduce the number of patients receiving transplant without affecting the survival (Cazzaniga 2018).
Given the published results and the well-established expertise of the European countries of treating pediatric Ph+ ALL with the EsPhALL backbone approach, these guidelines are based on the most recent indications and achievements of the EsPhALL study group in order to provide the best standard of care for these patients, reduce the toxicities, and offer therapeutic options for relapse.

2. PATIENT GROUP

After the Induction IB phase, patients are stratified in two risk groups based on minimal residual disease (MRD) levels at specific timepoints, assessed by PCR of immunoglobulin/ T cell receptor (IgH-TCR) rearrangements. Patients who are classified as Standard Risk (SR) continue on chemotherapeutic treatment while High Risk (HR) patients should be addressed to transplant after the third consolidation block (HR-3).

The following criteria will be used for the stratification in the two risk groups:

1) Standard risk (SR): patients with MRD < $5x10^{-4}$ at end of induction IB performed at count recovery;
2) High-risk (HR): patients with MRD ≥ $5x10^{-4}$ at end of induction IB performed at count recovery.

Table 1. Criteria used for risk group assignment.

Standard risk (SR)	High risk (HR)
MRD < $5x10^{-4}$ or negative at end of induction IB	MRD ≥ $5x10^{-4}$ at end of induction IB

3. ELIGIBILITY AND DIAGNOSTIC CRITERIA

The target population includes patients aged more than 365 days and less than 21 years with documented newly diagnosed Ph+ ALL (BCP-ALL or T-ALL).
Evidence of t(9;22)(q34;q11) determined by institutional cytogenetics or FISH and/or the presence of BCR-ABL fusion transcript identified by RT-PCR or FISH is mandatory.
The basic diagnostic program regarding diagnosis, biological characterization and main definitions are described in the ESCP part dedicated to Philadelphia-chromosome negative ALL.

4. TREATMENT

4.1 Treatment overview

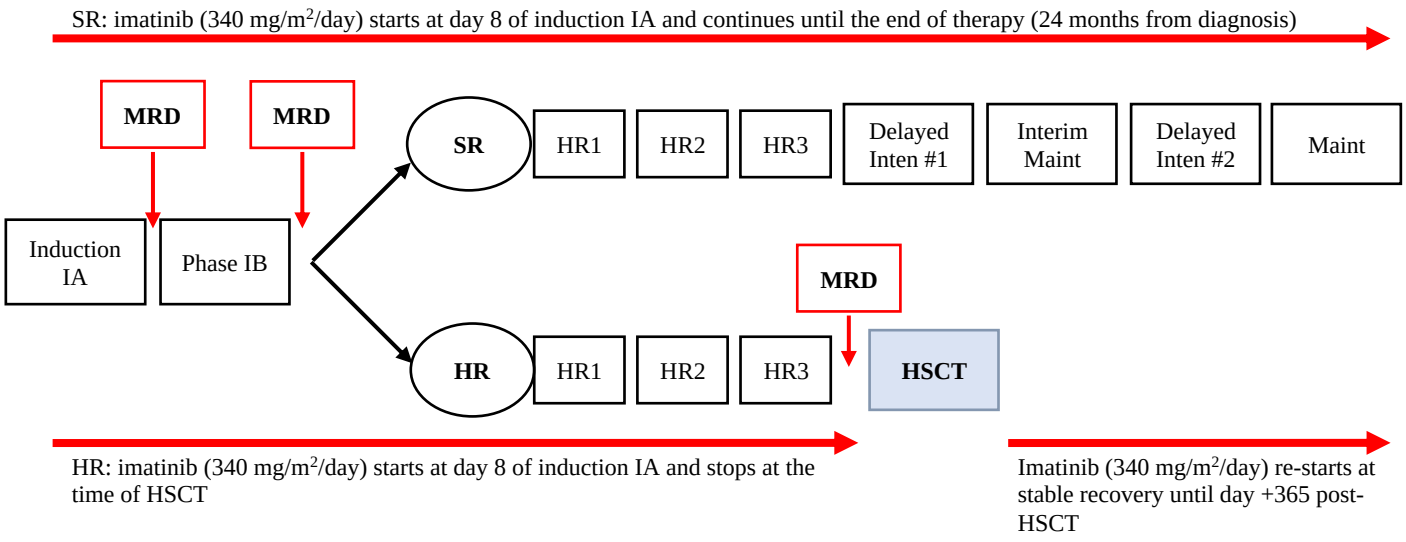
Treatment for Ph+ ALL consists in the administration of the standard EsPhALL chemotherapeutic backbone in association with the early introduction and continuous administration of imatinib.

Patients will receive the following phases of treatment:

- Induction phase IA: Section 4.2
- Phase IB: Section 4.3
- Three high-risk (HR) blocks: Section 4.4, 4.5, 4.6
- Delayed Intensification #1: Section 4.7
- Interim Maintenance phase: Section 4.8
- Delayed Intensification #2: Section 4.9
- Maintenance phase: Section 4.10

Total duration of therapy will be 24 months from the start of Induction IA. SR and HR patients will receive the same chemotherapeutic backbone. Consolidative HSCT is recommended for HR patients and should be performed as soon as possible after the third HR block.

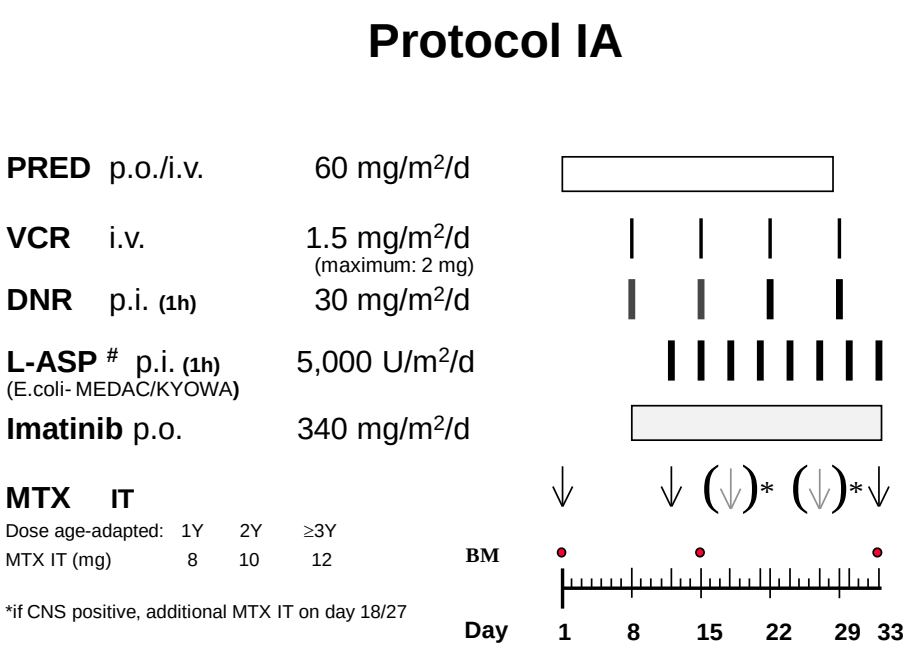
Fig. 1 Treatment plan according to ESCP Ph+ ALL guidelines



4.1.1 Frontline Induction (Induction IA)

All patients will receive the first induction (IA) described in this ESCP ALL in the chapter n1, dedicated to the Ph neg ALL (section 6.1). Imatinib 340 mg/m^2 daily (maximum daily dose 800 mg/m^2) will be introduced early on day +8 in all patients on top of the ongoing chemotherapy till day + 33.

Figure 2. Induction phase (IA) in Ph+ ALL



If PEG-ASP is the ASP product to be used based on physician's choice, please refer to induction treatment details for dosages and intervals

Treatment schedule

PRED **Prednisone/Prednisolone** 60 mg/m^2/d, PO/IV, in 3 single doses per day.

IMATINIB **Imatinib** 340 mg/m^2/day orally from day 8, given daily without interruption.

VCR **Vincristine** 1.5 mg/m^2/d, IV (maximal single dose 2 mg), on day: 8, 15, 22, 29 (4 doses).

DNR **Daunorubicin** 30 mg/m^2/d, PI, over 1 h on day: 8, 15, 22, 29.

ASP **E. coli L-asparaginase** at 5,000 I.U./m^2/d, PI, over 1 h, on day: 12, 15, 18, 21, 24, 27, 30, 33 (8 doses).

Alternatively, PEG-L-Asparaginase could be used at 2,500 I.U./m^2, PI, over 1 h, on day 12 and 26 (2 doses) (maximal single dose 3,750 I.U.).

In case of a hypersensitivity reaction to the native E. coli ASP formulation, which leads to discontinuation of treatment, two options are offered:

- Peg Asparaginase to be used at 2,500 I.U./m^2, PI, over 1 h. One dose of PEG-ASP 2,500 U/m^2 substitutes 4 doses of the native E. coli ASP.
- Erwinia Asparaginase could be given at a dosage of 20,000 I.U./m^2/dose p.i. (1 h) or i.m. every other day (6 doses)

IT MTX **Intrathecal methotrexate** at age-adjusted dosage on day 1, 12 and 33.

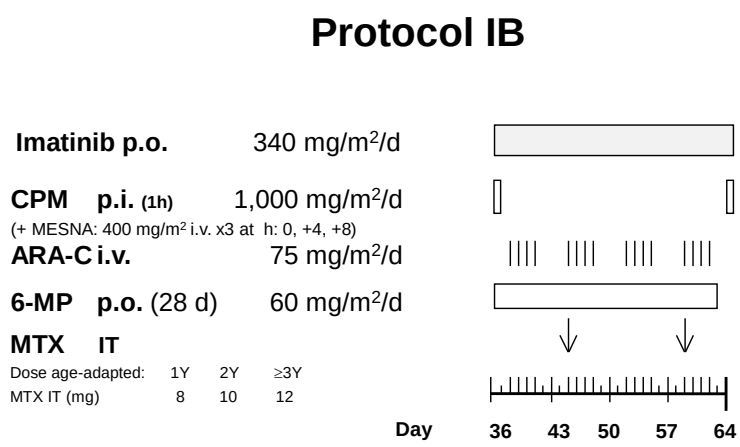
- In case of initial CNS involvement (CNS 3), see chapter 3.3.1.1, additional MTX IT is administered on days 18 and 27.
- LP is urgently necessary for the assessment of initial CNS status. Therefore, the first LP may be postponed only in exceptional situations.

Age (yr)	≥ 1 < 2	≥ 2 < 3	≥ 3
Dosage (mg)	8	10	12

Tilt head-down position for at least 2 h after LP.

4.1.2 Phase IB

Figure 3. Phase IB in Ph+ ALL



Starting time: After completion of Induction IA

Starting criteria:

Requirements for beginning of phase IB:

- Good general condition without serious infections;
- Creatinine level within normal limits according to age;
- WBC> 2000/µl, ANC >500/µl; platelets >50,000/µl.

Dosages, schedules and routes:

IMATINIB **Imatinib** 340 mg/m^2/day orally from day 36 to 64 (total 28 days).

Note: all patients will receive IMATINIB. To allow close patient monitoring, patient will attend outpatient clinics at least weekly because of ARA-C injections.

CPM **Cyclophosphamide** 1000 mg/m^2/dose i.v. (1 hour) on days 36 and 64**.**
Associate:
- Hyperhydration 3.000 ml/m^2 over 24 hours: G 5%+ NaCl 0.45%+ 90 mEq/m^{2}KCl
- MESNA (1/3 of CPM dose, hours 0, 4, 8 from CPM start).
- Furosemide 0.5-1 mg/kg i.v. if input > output +400 ml/m^2/12h.

Please consider adequate anti-emetic supportive therapy.

6-MP **6-Mercaptopurine** 60 mg/m^2/day p.o. from days 36-64 (total 28 days)
Note: to be taken in the evening on an empty stomach (1 hour before or after dinner), not together with milk.

ARA-C **Cytosine arabinoside** 75 mg/m^2/day s.c. o i.v. in one daily dose on days 38-41, 45-48, 52-55, 59-62
Note: each 4-day cycle should be started when WBC >500µl and platelets >30.000/µl; each cycle when started should not be stopped unless for acute infection (please consider that fever might be also induced by the drug).

IT MTX **Intrathecal methotrexate** on days 38, 52 (together with ARA-C cycles 1 and 3), dose by age, as table below

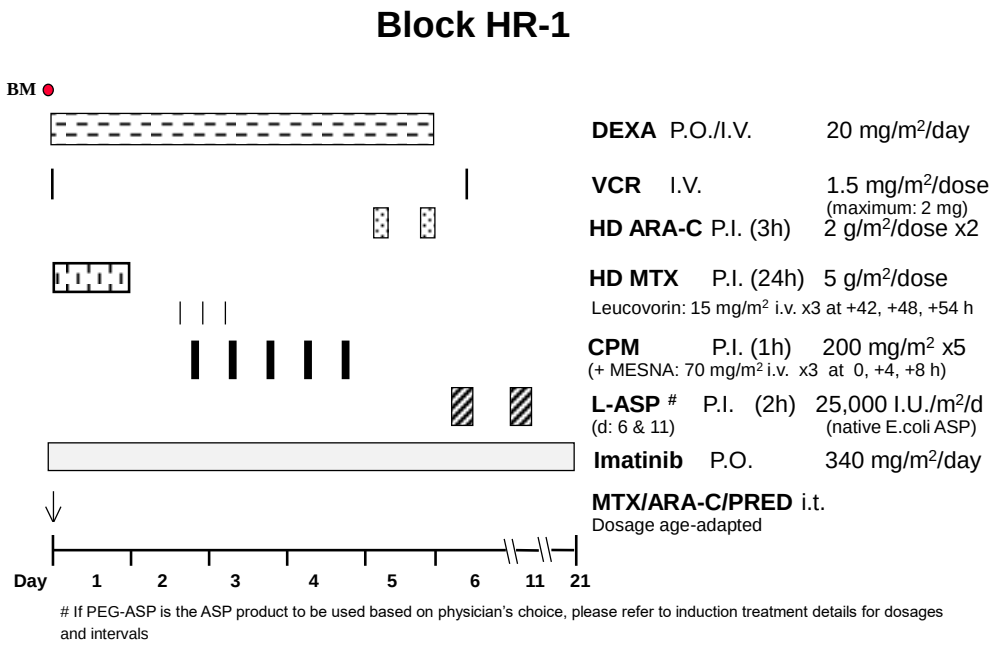
Age (yr)	≥ 1 < 2	≥ 2 < 3	≥ 3
Dosage (mg)	8	10	12

Tilt head-down position for at least 2 h after IT MTX.

4.1.3 Consolidation Block 1 (HR-1)

Imatinib administration will not be interrupted between the end of phase IB and the beginning of the first HR block. **The interval between each block element should be 21 days (counting from day 1 of HR-1 to day 1 of HR-2).**

Figure 4. HR-1 block in Ph+ ALL



If PEG-ASP is the ASP product to be used based on physician's choice, please refer to induction treatment details for dosages and intervals

Starting time: Start on day 78 of therapy

Starting criteria:

Requirements for beginning of HR-1 block:

- Good general condition
- Creatinine level within normal limits according to age
- Documented adequate ANC (≥ 500/µl) and platelet counts (≥ 50.000/µl) (rising counts)

Dosages, schedules and routes:

DXM **Dexamethasone** 20 mg/m^2/day p.o. or i.v. in 3 doses on days 1-5 (no tapering).

VCR **Vincristine** 1.5 mg/m^2/day i.v. (max dose: 2 mg) on day 1 and 6.

HD-MTX **High-dose methotrexate** 5 g/m^2/dose i.v. over 24 hours (1/10 in 30 minutes, the remaining 9/10 in 23.5 hour-infusion) on day 1.

Associate:

- Hyperhydration: 3.000 ml/m^2 over 24 hours: Gluc. 5%+ NaCl 0.45%+ 90 mEq/m^2/KCl+ NaHCO3 90 mEq/m^2.
- Urine pH>7.0 over the time of infusion.

Serum levels of MTX must be determined at hours 24, 42, 48 from start of MTX infusion. For monitoring of MTX serum levels and intensification of LCV rescue, see Appendix II.

CF **Citrovorum factor (Folinic acid)** 7.5 mg/m^2 i.v (Levo form) or 15 mg/m^2 i.v (Racemic form), at hours 42, 48, 54 from start of HD-MTX infusion
Note: For monitoring of MTX serum levels and intensification of LCV rescue, see Appendix II. Starting from hour 60, Citrovorum Factor is needed only if serum levels at hour 48 exceed 0.5 µmol/l. In this case, see nomogram for therapeutic adjustments.

HD-ARA-C **High-dose cytarabine** 2 gr/m^2/iv in 3-hour infusion, repeated after 12 hours on day 5
Note: The use of prednisolone eye drops is suggested.

HD-L-ASP **High-dose L-asparaginase (**E. coli - medac or kidrolase) 25,000 I.U./m^2/dose, over 2 h i.v., 3 hours after completion of the infusion of the second dose of HD-ARA-C.

Note: If PEG-ASP is the ASP product to be used based on physician's choice or in case of an allergic reaction, PEG-ASP may be used in a single dose of 2,500 I.U./m^2 over 1 h i.v. instead of the whole ASP schedule planned.

In case of a HSR to the native E. coli ASP formulation, which leads to discontinuation of treatment, two options are offered:

- A PEG-L-asparaginase, to be used at 2,500 I.U./m^2, PI, over 1 h, on day 6;
- Erwinia chrysanthemi ASP should be given at a dosage of 20,000 I.U./m^2/dose PI (1 h) or IM every second day for 2 weeks (6 doses).

CPM **Cyclophosphamide** 200 mg/m^2 i.v. in 1 hour q 12 hours, 5 doses. Start immediately after the completion of HD-MTX infusion on days 2-4.
Note: Associate MESNA 70 mg/m^2 hours 0, 4 and 8 from start of CPM.

IT ARA-C/PDN/MTX Intratechal therapy at age-adjusted dosage on day 1, 2 hours after start of HD-MTX

Age	1-1.99yrs	2-2.99yrs	≥3yrs
Methotrexate	8 mg	10 mg	12 mg
Ara-C	20 mg	26 mg	30 mg
Prednisone	6 mg	8 mg	10 mg

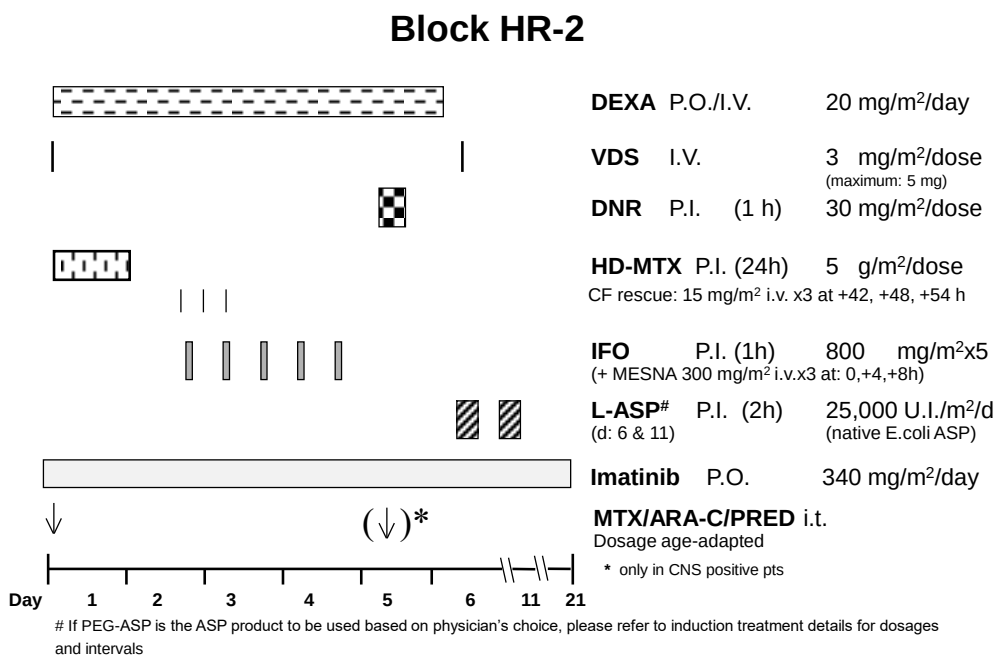
Tilt head-down position for at least 2 h after IT MTX.

G-CSF **Granulocyte-colony stimulating factor** 5 µg/kg/day s.c. from the 5th day after completion of the block.
Note: continue until the WBC count is >20.000/µl

IMATINIB **Imatinib** at 340 mg/m^2/day orally from days 1 to 21 (total 21 days).
Note: IMATINIB should not be given longer than 21 days if next chemotherapy block has to be postponed.

4.1.4 Consolidation Block 2 (HR-2)

Figure 5. HR-2 block in Ph+ALL



Starting time: after the end of HR-1 block

Starting criteria:

Requirements for beginning of HR-2 block:

- Good general condition
- Documented adequate ANC (≥ 500/µl) and platelet counts (≥ 50.000/µl) (rising counts)

Dosages, schedules and routes:

DXM **Dexamethasone** at 20 mg/m^2/day p.o. or i.v. in 3 doses on days 1-5 (no tapering).

VDS **Vindesine** at 3 mg/m^2/day i.v. (max 5 mg) on days 1 and 6.

HD-MTX **High-dose methotrexate** 5 g/m^2/dose i.v. over 24 hours (1/10 in 30 minutes, the remaining 9/10 in 23.5 hour-infusion) on day 1.
Associate:
- Hyperhydration: 3.000 ml/m^2 over 24 hours: Gluc. 5%+ NaCl 0.45%+ 90 mEq/m^2 KCl+ NaHCO3 90 mEq/m^2.
- Urine pH>7.0 over the time of infusion.

Serum levels of MTX must be determined at hours 24, 42, 48 from start of MTX infusion. For monitoring of MTX serum levels and intensification of CF rescue, see Appendix II.

CF **Citrovorum factor (Folinic acid)** 7.5 mg/m^2 i.v (Levo form) or 15 mg/m^2 i.v (Racemic form) at hours 42, 48, 54 from start of HD-MTX infusion
Note: For monitoring of MTX serum levels and intensification of CF rescue, see Appendix II.

Starting from hour 60, Citrovorum Factor is needed only if serum levels at hour 48 exceed 0.5 µmol/l. In this case, see nomogram for therapeutic adjustments.

IFO **Ifosphamide** at 800 mg/m^2 i.v. over 1-hour infusion, q 12 hours, 5 doses, on days 2-4, start immediately after completion of HD-MTX infusion.
Note: Associate MESNA 300 mg/m^2 i.v hour 0, 4 and 8 from start of infusion.

HD-L-ASP **High-dose L-asparaginase** (E. coli - medac or kidrolase) at 25,000 I.U./m^2/dose, over 2 h i.v. on day 5.

Note: If PEG-ASP is the ASP product to be used based on physician's choice or in case of an allergic reaction, PEG-ASP may be used in a single dose of 2,500 I.U./m^2 over 1 h i.v. instead of the whole ASP schedule planned.

In case of a HSR to the native E. coli ASP formulation, which leads to discontinuation of treatment, two options are offered:

- A PEG-L-asparaginase, to be used at 2,500 I.U./m^2, PI, over 1 h, on day 6;
- Erwinia chrysanthemi ASP should be given at a dosage of 20,000 I.U./m^2/dose PI (1 h) or IM every second day for 2 weeks (6 doses).

DNR **Daunorubicin** 30 mg/m^{2}m over 1-hour infusion on day 5.

IT ARA-C/PDN/MTX **Intratechal therapy** at age-adjusted dosage on day 1, 2 hours after start of HD-MTX. Patients with CNS3 at diagnosis receive another dose on day 5.

Age	1-1.99yrs	2-2.99yrs	≥3yrs
Methotrexate	8 mg	10 mg	12 mg
Ara-C	20 mg	26 mg	30 mg
Prednisone	6 mg	8 mg	10 mg

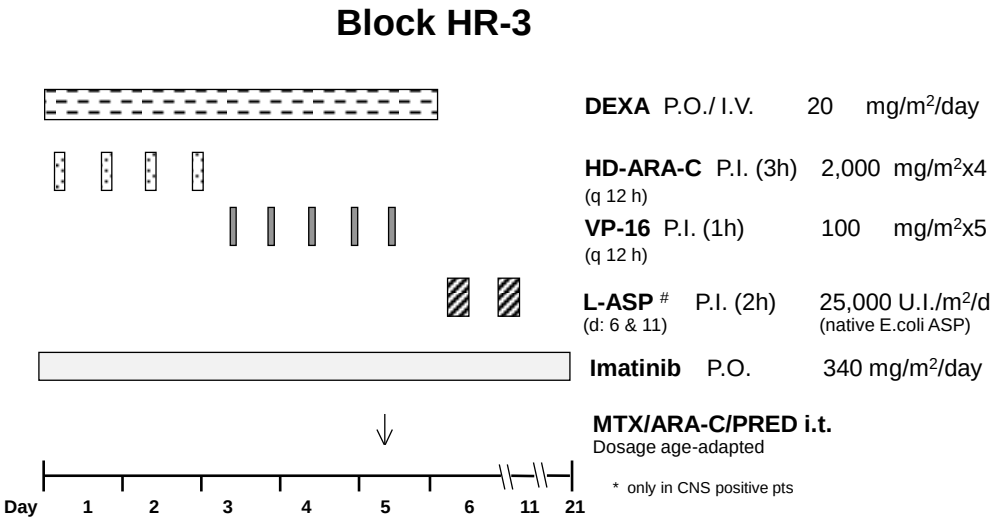
Tilt head-down position for at least 2 h after IT MTX.

G-CSF **Granulocyte-colony stimulating factor** 5 µg/kg/day s.c. from the 5th day after completion of the block.
Note: continue until the WBC count is >20.000/µl

IMATINIB **Imatinib** at 340 mg/m^2/day orally from days 1 to 21 (total 21 days).
Note: IMATINIB should not be given longer than 21 days if next chemotherapy block has to be postponed.

4.1.5 Consolidation Block 3 (HR-3)

Figure 6. HR-3 block in Ph+ ALL



If PEG-ASP is the ASP product to be used based on physician's choice, please refer to induction treatment details for dosages and intervals

Starting time: after the end of HR-2 block

Starting criteria:

Requirements for beginning of HR-3 block:

- Good general condition
- Documented adequate ANC (≥ 500/µl) and platelet counts (≥ 50.000/µl) (rising counts)

Dosages, schedules and routes:

DXM **Dexamthasone** at 20 mg/m^2/day p.o. or i.v. in 3 doses on days 1-5 (no tapering).

HD-ARA-C **High-dose cytarabine** 2 gr/m^2/iv in 3-hour infusion, q 12 hours, 4 total doses on days 1-2. **Note:** The use of prednisolone eye drops is suggested.

VP-16 **Etoposide** 100 mg/m^{2}m i.v. in 1 hour , q 12 hours, 5 total doses, days 3-5.

HD-L-ASP **High-dose L-asparaginase** (E. coli - medac or kidrolase): 25,000 I.U./m^2/dose, over 2 h i.v., day 5.
Note: If PEG-ASP is the ASP product to be used based on physician's choice or in case of an allergic reaction, PEG-ASP may be used in a single dose of 2,500 I.U./m^2 over 1 h i.v. instead of the whole ASP schedule planned.

In case of a HSR to the native E. coli ASP formulation, which leads to discontinuation of treatment, two options are offered:

- A PEG-L-asparaginase, to be used at 2,500 I.U./m^2, PI, over 1 h, on day 6;
- Erwinia chrysanthemi ASP should be given at a dosage of 20,000 I.U./m^2/dose PI (1 h) or IM every second day for 2 weeks (6 doses).

IT ARA-C/PDN/MTX **Intratechal therapy** at age-adjusted dosage on day 5, 2 hours after start of HD-MTX.

Age	1-1.99yrs	2-2.99yrs	≥3yrs
Methotrexate	8 mg	10 mg	12 mg
Ara-C	20 mg	26 mg	30 mg
Prednisone	6 mg	8 mg	10 mg

Tilt head-down position for at least 2 h after IT MTX.

G-CSF **Granulocyte-colony stimulating factor** 5 µg/kg/day s.c. from the 5th day after completion of the block.
Note: continue until the WBC count is >20.000/µl

IMATINIB **Imatinib** at 340 mg/m^2/day orally from days 1 to 20 (total 20 days).
Note: IMATINIB should not be given longer than 2 days if next chemotherapy block has to be postponed.

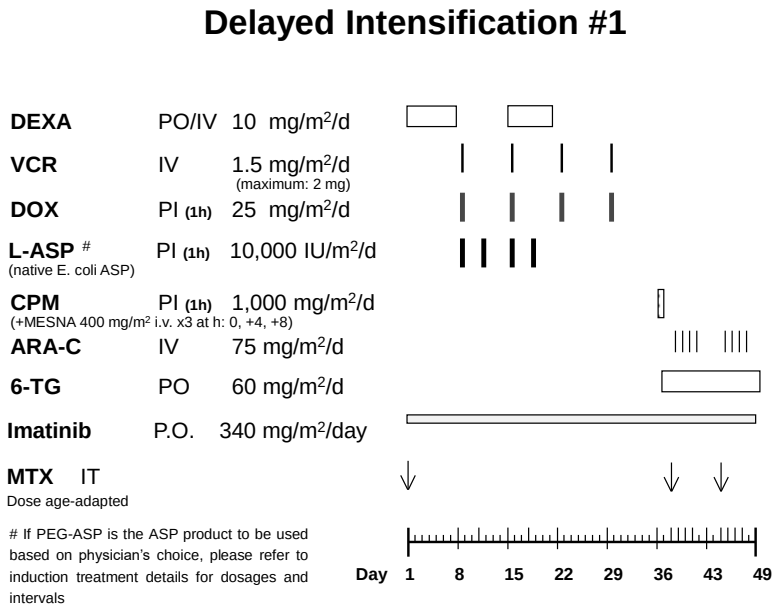
4.1.6 Delayed Intensification #1 (First Protocol II)

Reinduction with delayed intensification starts 14 days after completion of consolidation block-therapy (intended as day 5 of HR block 3). Patient good general condition and adequate ANC (≥ 500/µl) and platelet counts (≥ 50.000/µl) are required.

Protocol II comprises two phases:

- Phase IIa: days 1-35
- Phase IIb: days 36-64

Figure 7. First Delayed Intensification in Ph+ ALL



4.1.6.1 Delayed Intensification #1, part 1

Starting time: 14 days after completion of consolidation block-therapy (intended as day 5 of HR-3 block)

Starting criteria:

Requirements for beginning:

- Good general condition
- Documented adequate ANC (≥ 500/µl) and platelet counts (≥ 50.000/µl)

Dosages, schedules and routes:

DXM **Dexamethasone** 10 mg/m^2/day p.o. in 2 doses from day 1 to 7 and from day 15 to 21 (no tapering).

VCR **Vincristine** 1.5 mg/m^2/dose i.v. (maximum dose 2 mg/dose), days 8, 15, 22, 29.

DOX **Doxorubicin** 25 mg/m^2/dose i.v. to be infused over 1h on days 8, 15, 22, 29.

L-ASP **L-asparaginase** (E. coli - medac or kidrolase) 10,000 I.U./m^2/dose, over 1 h i.v**,** days 8, 11, 15 and 18.

Note: If PEG-ASP is the ASP product to be used based on physician's choice or in case of an allergic reaction, PEG-ASP may be used in a single dose of 2,500 I.U./m^2 over 1 h i.v. instead of the whole ASP schedule planned.

In case of a HSR to the native E. coli ASP formulation, which leads to discontinuation of treatment, two options are offered:

- A PEG-L-asparaginase, to be used at 2,500 I.U./m^2, PI, over 1 h, on day 6;
- Erwinia chrysanthemi ASP should be given at a dosage of 20,000 I.U./m^2/dose PI (1 h) or IM every second day for 2 weeks (6 doses).

IT MTX **Intrathecal methotrexate** at age-adjusted dosage, administer on day 1.

Age (yr)	≥ 1 < 2	≥ 2 < 3	≥ 3
Dosage (mg)	8	10	12

Tilt head-down position for at least 2 h after IT MTX.

IMATINIB **Imatinib** 340 mg/m^2/day orally, days 1-35 (total 35 days).

4.1.6.2 Delayed Intensification #1, part 2

Starting time: day 36

Starting criteria:

Requirements for beginning:

- Good general condition
- Documented adequate ANC (≥ 500/µl) and platelet counts (≥ 50.000/µl)

Dosages, schedules and routes:

CPM **Cyclophosphamide** 1000 mg/m^2/dose i.v. (1 hour), day 36.
Associate:
- Hyperhydration 3.000 ml/m^2 over 24 hours: G 5%+ NaCl 0.45%+ 90 mEq/m^2/KCl;
- MESNA (1/3 of CPM dose, hours 0, 4, 8 from CPM start);
- FUROSEMIDE 0.5-1 mg/kg i.v. if input > output +400ml/ m^2/12h.

Please consider adequate anti-emetic supportive therapy.

TG **Thioguanine** 60 mg/m^2/day p.o., days 36-49 (total 14 days). It should be taken in the evening on the empty stomach without milk (1 hour before or after dinner)

ARA-C **Cytosine arabinoside** 75 mg/m^2/day s.c. o i.v. in one daily dose, days 38-41 and 45-48.
Note: Each 4-day cycle should be started when ANC >200/µl and platelets >50.000/µl; each cycle when started should not be stopped unless for acute infection (please consider that fever might be also induced by the drug).

IT MTX **Intrathecal methotrexate** at age-adjusted dosage, administer on days 38 and 45 (together with ARA-C cycles).

Age (yr)	≥ 1 < 2	≥ 2 < 3	≥ 3
Dosage (mg)	8	10	12

Tilt head-down position for at least 2 h after IT MTX.

IMATINIB: **Imatinib** 340 mg/m^2/day orally, days 36-63 (total 28 days).

4.1.7 Interim Maintenance

This short phase is aimed to allow administration of cranial irradiation to patients with CNS disease at diagnosis during antimetabolite-based non-intensive chemotherapy. It will start on day 64 of Delayed Intensification #1 or when the starting criteria are met. Interim Maintenance will last 4 weeks i.e. the time comprised between the first and the second Delayed Intensification. Imatinib administration will not be interrupted between the end of Delayed Intensification and the beginning of interim maintenance.

Starting time: on day 64 of Delayed Intesification #1

Starting criteria:

Requirements for beginning of interim maintenance:
- Good general condition
- Documented adequate ANC (≥ 500/µl) and platelet counts (≥ 50.000/µl) (rising counts)

Dosages, schedules and routes:

6-MP **6-Mercaptopurine** 50 mg/m^2/day p.o., days 1-28. It should be taken in the evening on the empty stomach without milk (1 hour before or after dinner).

MTX **Methotrexate** 20 mg/m^2/dose p.o**.** once a week days 8, 15, 22, 29.

IMATINIB **Imatinib** 340 mg/m^2/day orally, days 1-28 (total 28 days).

In selected cases it could be necessary to start the two drugs with increasing doses according to the patient's compliance and/or haematological reconstitution.

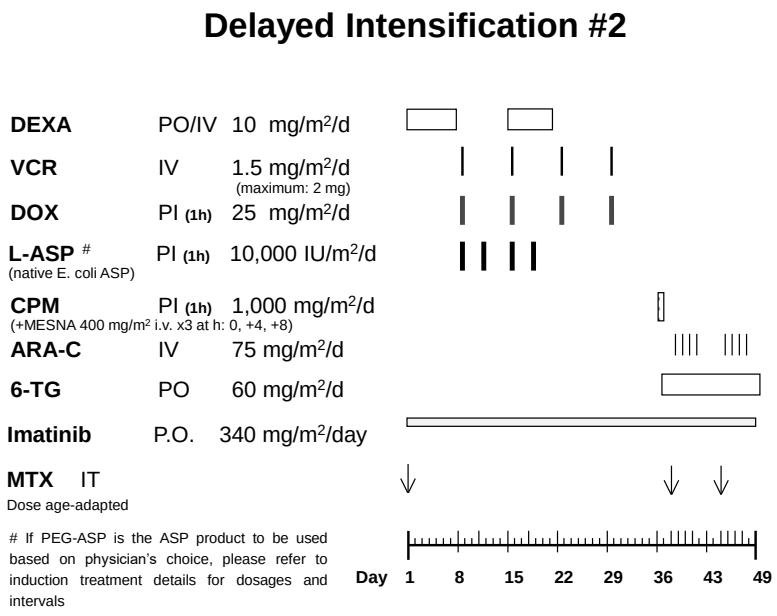
4.1.7.1 Cranial irradiation

Cranial irradiation will be administered to patients with CNS3 disease at diagnosis.
Days of administration: during the first week of interim Maintenance therapy. It should be completed within 14 days of starting.
Dose: dose of 18 Gy (single dose: 1.4-1.7 Gy) in 10 fractions, no dose modification depending on age.

4.1.8 Delayed Intensification #2

It will start immediately after the interim maintenance provided the patient is in good general condition and adequate ANC (≥ 500/µl) and platelet counts (≥ 50.000/µl) are documented.

Figure 8. Second Delayed Intensification in Ph+ ALL



4.1.8.1 Delayed Intensification #2, part 1

Starting time: begin on day 29 of Interim Maintenance or when criteria are met

Starting criteria:

Requirements for beginning:

- Good general condition
- Documented adequate ANC (≥ 500/µl) and platelet counts (≥ 50.000/µl) (rising counts)

Dosages, schedules and routes:

DXM **Dexamethasone** 10 mg/m^2/day p.o. in 2 doses from day 1 to 7 and from day 15 to 21 (no tapering).

VCR **Vincristine** 1.5 mg/m^2/dose i.v. (maximum dose 2.0 mg/dose), days 8, 15, 22, 29.

DOX/ADR **Doxorubicin/Adriamycin** 25 mg/m^2/dose i.v. to be infused over 1h, days 8, 15, 22, 29.

L-ASP **L-asparaginase** (E. coli - medac or kidrolase) 10,000 I.U./m^2/dose, over 1 h i.v., days 8, 11, 15 and 18.

Note: If PEG-ASP is the ASP product to be used based on physician's choice or in case of an allergic reaction, PEG-ASP may be used in a single dose of 2,500 I.U./m^2 over 1 h i.v. instead of the whole ASP schedule planned.

In case of a HSR to the native E. coli ASP formulation, which leads to discontinuation of treatment, two options are offered:

- A PEG-L-asparaginase, to be used at 2,500 I.U./m^2, PI, over 1 h, on day 6;
- Erwinia chrysanthemi ASP should be given at a dosage of 20,000 I.U./m^2/dose PI (1 h) or IM every second day for 2 weeks (6 doses).

IMATINIB **Imatinib** 340 mg/m^2/day orally, days 1-35 (total 35 days).
Note: IMATINIB should not be given longer than 20 days if next chemotherapy block has to be postponed.

4.1.8.2 Delayed Intensification #2, part 2

Starting time: day 36

Starting criteria:

Requirements for beginning:

- Good general condition
- Documented adequate ANC (≥ 500/µl) and platelet counts (≥ 50.000/µl)

Dosages, schedules and routes:

CPM **Cyclophosphamide** 1000 mg/m^2/dose i.v. (1 hour), day 36.
Associate:
- Hyperhydration 3.000 ml/m^2 over 24 hours: G 5%+ NaCl 0.45%+ 90 mEq/m^{2}KCl
- MESNA (1/3 of CPM dose, hours 0, 4, 8 from CPM start).
- FUROSEMIDE 0.5-1 mg/kg i.v. if input > output +400ml/ m^2/12h.
Please consider adequate anti-emetic supportive therapy.

TG **6-Thioguanine** 60 mg/m^2/day p.o., days 36-49 (total 14 days). It should be taken in the evening on the empty stomach without milk (1 hour before or after dinner).

ARA-C **Cytosine arabinoside** 75 mg/m^2/day s.c. o i.v. in one daily dose, days 38-41 and 45-48.
Note: Each 4-day cycle should be started when ANC >200/µl and platelets >50.000/µl; each cycle when started should not be stopped unless for acute infection (please consider that fever might be also induced by the drug).

IT MTX **Intrathecal methotrexate** at age-adjusted dosage, administer on days 38 and 45 (together with ARA-C cycles).

Age (yr)	≥ 1 < 2	≥ 2 < 3	≥ 3
Dosage (mg)	8	10	12

Tilt head-down position for at least 2 h after IT MTX.

IMATINIB **Imatinib** 340 mg/m^2/day orally, days 36-63 (total 28 days)

4.1.9 Maintenance

Maintenance therapy will start 2 weeks after completion of delayed intensification and is based on antimetabolite drugs. It consist in multiple 12-week cycles (84 days) of oral chemotherapy for a total duration of therapy of 2 years (104 weeks) from the start of Induction IA therapy.

Starting time: begin Maintenance therapy 2 weeks after completion of DI#2 or when criteria to start are met. Each cycle should start on day 85 of the previous Maintenance cycle.

Starting criteria:

Requirements for beginning of Maintenance cycle:

- Good general condition
- Documented adequate ANC (≥ 500/µl) and platelet counts (≥ 50.000/µl)

Dosages, schedules and routes:

MP **6-Mercaptopurine** 50 mg/m^2/day p.o., days 1-84 (total 84 days). It should be taken in the evening on the empty stomach without milk (1 hour before or after dinner).

MTX **Methotrexate** 20 mg/m^2/weekly p.o. on day 1, 8, 15, 22, 29, 36, 43, 50, 57, 64, 71, and 78 (once weekly)

IT MTX **Intrathecal methotrexate** at age-adjusted dosage, administer on days 1 and 43 of the first 3 cycles.

Age (yr)	≥ 1 < 2	≥ 2 < 3	≥ 3
Dosage (mg)	8	10	12

Note: intrathecal MTX should be administered only during the first 3 cycles of Maintenance. **Do not administer to CNS-3 patients who received cranial irradiation.**

Tilt head-down position for at least 2 h after IT MTX.

IMATINIB **Imatinib** 340 mg/m^2/day orally, days 1-84 (total 84 days).
Note: do not interrupt between cycles

Dose modifications

Chemotherapy will be modulated to keep WBC count between 1,000 and 3,000/µl. The dosage of the two drugs will be adjusted accordingly:

Leukocyte count /µl	Dose of 6-MP/MTX
<1.000	0%
1.000-2.000	50%
2.000-3.000	100%

>3.000	consider to increase the dosage to achieve a WBC count between 2000 and 3000/µl
ANC < 500/µl	0%
Lymphocytes < 300/µl	50%

4.1.10 Completion of treatment

Total duration of chemotherapy will not exceed 24 months from the start of Induction IA therapy. At the time of treatment completion confirm CR by bone marrow aspirate and CSF examination.

4.2 Imatinib

Source and pharmacology

Imatinib is a selective and potent tyrosine kinase inhibitor of the *BCR-ABL1* fusion gene of the Philadelphia chromosome. Imatinib has also shown activity in blocking other tyrosine kinases as c-kit and platelet-derived growth factor receptor (*PDGFR*). The activity of imatinib is related to its occupancy of the kinase pocket of the protein, which blocks access to ATP and prevents substrate phosphorylation inhibiting Bcr- Abl dependent cellular proliferation.
Imatinib is well-absorbed after oral administration; maximum plasma concentrations are achieved 2 to 4 hours after administration. The elimination half-lives of imatinib and its major active metabolite, the N-desmethyl derivative, are approximately 18 and 40 hours, respectively. The elimination half-life of the parent drug in children is approximately 15 hours. Imatinib is approximately 95% protein-bound, mostly to albumin and alpha-1- acid glycoprotein. CYP3A4 is the major enzyme responsible for metabolism. CYP1A2, CYP2D6, CYP2C9, and CYP2C19 play minor roles in metabolism. Drugs metabolized by these same enzymes should be avoided or used with caution to avoid unwanted drug interactions. Severe hepatic impairment (bilirubin >3-10 times ULN) increases AUC by 45% to 55% for imatinib and its active metabolite, respectively. Elimination is predominately in the feces, mostly as metabolites.

Administration guidelines

Imatinib is the recommended tyrosine kinase inhibitor for the treatment of Ph+ ALL. Imatinib should be administered each morning together with breakfast. It is a local irritant and must be taken in a sitting position with a large glass of water (250 ml/8oz; at least 100 ml/4 oz for children < 3 years of age).
If the child/patient can not swallow the capsules the drug should be administered according to the following guidelines: pour the contents of a capsule by small portions into 20 ml of water, milk or apple juice. Stir with a spoon and administer the suspension immediately afterwards. Do not use any other beverage like Coca-Cola or orange juice. Note: the excipients used in the capsule will not dissolve. However, they are white whilst the active substance is yellow. Thus, if a white solid residue remains in the glass, it does not matter as long as the capsule has been slowly added and well dispersed to allow the active substance to dissolve during stirring. If a yellow residue is observed, it means that the active substance was not completely dissolved and only a fraction of the dose has been swallowed. Medications, which interfere with P-450 cytochrom metabolism (see Appendix II), should be avoided and recommended doses of acetaminophen, should not be exceeded. The following medications and foods can interfere with P-450 metabolism: grapefruit juice, erythromycin, azithromycin, clarithromycin, rifampin and its analogs, fluconazole, ketoconazole, itraconazole, cimetidine, cannabinoids (marijuana or dronabinol) and the leukotriene inhibitors used in asthma such as zafirleukast and zileuton. In addition, drug interactions in patients receiving prochlorperazine (Compazine) and coumadin are possible. Patients who require prochlorperazine during therapy should be monitored for extrapyramidal symptons and those on coumadin should have weekly prothrombin times while on therapy. These medications should not be used during Imatinib administration unless there is

unavoidable medical need and no other appropriate alternative agents are available. Also, acetaminophen should not be taken in greater than the recommended dose.
For dose modifications, see Appendix II.

Toxicities
The use of Imatinib is associated with the following toxicities:

- Common (>20% patients): nausea, vomiting, diarrhea, abdominal pain, fluid retention/edema, fatigue/weakness, rash, myalgia, arthralgia, muscle cramps, musculoskeletal or joint pain, headache, infection
- Occasional (4-20% of patients): fever, rigors/chills, flu-like symptoms, hemorrhage, anemia, neutropenia/leukopenia, thrombocytopenia, dyspepsia/heartburn, flatulence, dizziness, insomnia, constipation, night sweats, weight gain, dysgeusia, anorexia, dysphagia/odynophagia, mucositis/stomatitis, esophagitis, cough, epistaxis, pruritis, ascites, paresthesias, pigmentation changes (vitiligo), alopecia, hypokalemia, hypoalbuminemia, hypophosphatemia, hypeglycemia, lymphopenia, alanine aminotransferase increased, aspartate aminotransferase increased, alkaline phosphatase increased, bilirubin increased, creatinine increased
- Rare (<3% of patients): angioedema, increased intracranial pressure/ cerebral edema, dehydration, hepatotoxicity, pleural effusion, pulmonary edema, pneumonitis, dyspnea, pericardial edema, exfoliative dermatitis, steven johnson syndrome, erythema multiforme, dress syndrome (fever, severe skin eruption, lymphadenopathy, hematologic abnormalities (eosinophilia or atypical lymphocytes), and internal organ involvement), conjunctivitis, blurred vision, dry eye, thrombosis/thromboembolism, left ventricular systolic dysfunction, tumor lysis syndrome, growth retardation in children

Effective contraception and the discontinuation of breast feeding is recommended.

4.3 Cranial irradiation

4.3.1 SR patients

Cranial irradiation will be administered to SR patients with CNS3 disease at diagnosis. The total dose of 18 Gy will be divided in 10 fractions during the first two weeks of the Interim Maintenance phase and completed by Day 14.

4.3.2 HR patients

HR patients with CNS3 leukemia will not receive cranial irradiation during chemotherapy phases administered prior to preparative regimen for HSCT in CR1. It is recommended that HR patients going to HSCT who were CNS-3 at diagnosis receive a cranial boost prior to total body irradiation (TBI). If the TBI dose is 14 Gy TBI, a 4 Gy boost is recommended within two weeks of the preparative regimen. If TBI dose is 12 Gy, a higher boost (6 Gy) is recommended. If the myeloablative regimen does not include TBI, cranial irradiation (18 Gy) is recommended prior to the preparative regimen.

HR patients who for some reasons do not proceed to HSCT in CR1 and who were CNS-3 at diagnosis should receive cranial radiation (18 Gy) during the first two weeks of Interim Maintenance phase. All other HR patients who do not proceed to HSCT will be treated WITHOUT cranial radiation and will receive intrathecal methotrexate during the first 3 cycles of Maintenance.

4.4 Hematopoietic Stem Cell Transplantation (HSCT)

4.4.1 Transplant indications

At diagnosis, every patient should be screened for matched familiar donor (MFD) or matched unrelated donor (MUD).
HSCT is indicated in HR patients defined by MRD assessments at specific timepoints. HSCT should be performed after the Consolidation block HR3. If a patient is not able to receive transplant after HR3 block, he should continue chemotherapy and imatinib until he is ready to undergo transplant. HR patients who will not receive transplant will continue on standard chemotherapy in combination with imatinib until the completion of 24 months of therapy.
Transplant is not recommended in SR patients.

4.4.2 Donor and conditioning regimen

Allogeneic transplant from MFD or MUD is recommended. The choice of an alternative donor should be based on centre expertise.

Myelo-ablative conditioning should be used (preferentially TBI-based), depending on patient's performance status and comorbidities.

4.4.3 Use of imatinib after transplant

There are still limited data on the use of TKIs after HSCT in Ph+ ALL in childhood. The toxicity of post-HSCT TKI and its impact on GVHD and relapse risk has not been defined in the pediatric Ph+ ALL population. However it is suggested thethe use of TKIs post HSCT from day +56 provided a satisfactory PTL and WBC count with stable neutrophils engraftment (PTL > 50 x 109/L; WBC > 1.5 x 109/L; neutrophils > 0.5 x 109/L for at least 15 days)
till day +365 from HSCT.
It is recommended that a bone marrow aspirate with morphologic evaluation and PCR detection of BCR/ABL is done before the beginning of post-transplant IMATINIB and every three months for the first year post-transplantation. During IMATINIB post-transplant administration weekly assessment of hepatic function and complete blood count is required.

4.4.4 Disease monitoring after transplant

BM with morphologic evaluation and PCR detection of BCR/ABL is recommended before the beginning of post-transplant Imatinib and regularly for the first year post-transplantation.

5. ASSESSMENTS

5.1 Definition

See the ESCP part dedicated to the Philadelphia negative ALL.

5.2 MRD assessment at diagnosis and during treatment

The use and the implementation of MRD techniques are strongly suggested to ensure the best standard of care, to limit toxicities related to over-treatment and to offer an affordable option for second-line treatment. Therefore, the MRD evaluation should be performed in each patient, as:

1) MRD should be preferentially assessed with the PCR for Ig-TCR rearrangements.
2) If Ig-TCR PCR is not available, MRD should be evaluated using validated flow cytometry. The routinely use of RQ-PCR for BCR-ABL is discouraged, and limited to the rare cases where both Ig-TCR PCR and flow cytometry are not available or failed.
3) MRD assessment should be performed at mandatory timepoint after count recovery to determine patient risk group, specifically:
    a. End of induction IA: even if this timepoint is not included in the risk group classification, MRD level at end of induction is highly recommended because it is one of the strongest prognostic factor in BFM-based protocols;
    b. End of Phase IB/Start HR1 block: all patients are required to have a MRD assessment at this timepoint;
    c. End of HR3 block: it is required for those patients with an intermediate MRD (MRD ≥ $5x10^{-5}$ to < $5x10^{-4}$) at end IB/start of block HR1.
    d. Post-HSCT: discussed in Section 3.12.3.
4) The MRD assessment at other timepoint, as end of HR1 and HR2 block or after delayed intensification, is not mandatory.

Table 2. Proposed MRD assessment timeline

	End IA	End IB	End HR1	End HR2	End HR3
Mandatory					
BM	X	X			X (those not in CR after IB; HR patients as pre-HSCT BM);
MRD	X	X			X (those not in CR after IB; HR patients as pre-HSCT BM);
Not Mandatory					
BM			X (those not in CR after IB)	X (othose not in CR after IB)	X (SR patients)
MRD			X (those not in CR after IB)	X (those not in CR after IB)	X (SR patients)

6. DOSE MODIFICATION AND DELAYS

6.1 Therapy modifications for IMATINIB toxicity

Non-Hematologic Toxicity

Grade 3/4:

If a patient experiences unexpected non hematological Grade 3-4 toxicity, IMATINIB must be withheld until the toxicity has resolved to < Grade 2 or lower. An assessment of possible drug interactions should be performed, and any drug that is suspected as contributing to the toxicity should be reevaluated regarding its dosage and/or necessity of administration. When toxicity is grade 2 or lower, IMATINIB should be reintroduced at 20% less dose.

Hepatic Toxicity:

Patients with a total serum bilirubin < 1.5 x ULN at baseline who experience Grade 3-4 elevations should be managed using the criteria detailed above for non-hematological toxicity

Hematologic Toxicity

Patients developing anemia are transfused at the discretion of the treating centerr. No dose reductions are foreseen for any grade of anemia, except for Grade 3 and 4 anemia resulting from an acute cause considered to be related to administration of IMATINIB (e.g. severe gastrointestinal hemorrhage).

In the event of Grade 3 thrombocytopenia (platelets 10.000<50.000/µl) or neutropenia (500<1000 /µl) not accompanied by clinical manifestations, i.e. significant bleeding or neutropenic fever, potential causes of the cytopenias should be considered (e.g, leukemic progression, infection); if the thrombocytopenia or neutropenia are attributed to IMATINIB, the dose will be reduced at 20% less dose.

With the occurrence of Grade 4 thrombocytopenia (platelets <10.000/µl), not accompanied by clinical manifestations, platelets support and continuation of the drug are suggested.

G-CSF is permitted in the case of neutropenic fever or suspected drug induced neutropenia. If Grade 3 or 4 thrombocytopenia or neutropenia are accompanied by clinically significant bleeding or evidence of an infection, IMATINIB will be interrupted until toxicity has resolved to grade 2 or lower and the clinical situation has stabilized; IMATINIB will then be restarted at 240 mg/m^2 daily.

6.2 Supportive Treatment

For supportive treatment during treatment, refer to Appendix III.

7. REFERENCE LIST

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CHAPTER 3

KMT2A-rearranged Infant Acute Lymphoblastic Leukemia (ALL)

1. BACKGROUND AND RATIONALE

1.1 Background

Infant acute lymphoblastic leukemia (ALL) is a rare disease and comprises about 4% of childhood ALL. Whereas the outcome of older children with ALL has improved to >85% event-free survival (EFS) infants with ALL have a poor prognosis. The Interfant study group was formed in 1999 and is a collaborative group that consists of all major European study groups and several large pediatric study groups outside Europe. The 4-year EFS of the first Interfant trial, called Interfant-99 was 47% (Pieters 2007) while previous studies reported long-term EFS rates of 28-45%. Relapses occur early, 90% of all events will occur in the first 2 years after diagnosis in infant ALL, and survival after relapse is only 20%. (Driessen 2015)

Infant ALL is characterized by a high frequency (~75%) of abnormalities in the chromosome 11q23 that affect the histone lysine methyltransferase 2A (*KMT2A*) gene, formerly known as mixed lineage leukemia (*MLL*) gene. *KMT2A* rearrangements occur in only 2% of older children with ALL. Infant ALL has a very immature CD19-positive B-cell phenotype (pro-B ALL) without CD10 expression, and a high tumor load at diagnosis.

Infant ALL cells are more resistant to chemotherapy than ALL cells of older children, but are sensitive in vitro to cytarabine. Therefore the **Interfant** treatment protocols contain cytarabine on top of an ALL regimen.

The first Interfant protocol, Interfant-99, was satisfying compared to the previous protocols (4 yr EFS 47 %, and OS 54%). In **Interfant-99**, patients were randomized to receive or not an intensification with VIMARAM before maintenance. This study closed before the target number was reached because no difference between the 2 arms was found. Thus this intensification block with high-dose cytarabine and high-dose methotrexate at a later stage did not improve the outcome. Another important aim of the Interfant-99 protocol was to determine which factors have independent prognostic value. Analysis showed that infants with four risk factors (*KMT2A* rearrangement, age <6 months and WBC ≥ 300 at diagnosis and/or poor prednisone response) had a 4-year EFS of only 20%. These patients were stratified as high risk in the subsequent Interfant-06 protocol and included about 25% of all patients. The remaining *KMT2A* rearranged patients had a 4-yr EFS of 45% and were stratified as medium risk in the interfant-06 protocol and included about 50% of all patients. Low risk patients were patients with *KMT2A* germline ALL and had a 4-yr EFS of 75%.

The **Interfant-06** tested whether “myeloid”-style consolidation chemotherapy is superior to “lymphoid”-style consolidation. But early intensification with post-induction myeloid-type chemotherapy courses did not significantly improve outcome for infant ALL compared to the lymphoid type course protocol IB. Outcome for infant ALL on Interfant-06 did not improve compared to that on Interfant-99 (Pieters 2019).

The role of SCT for infant *KMT2A*-rearranged ALL is limited. However high risk patients seem to benefit from HSCT. (Kosaka 2004, Dreyer 2011). In Interfant-99, medium risk patients with molecular disease after MARMA, all experienced relapse and died (Mann 2010, vdVelden 2009). Therefore in Interfant-06 these patients, in addition to high risk patients, were eligible for HSCT.

Since infants with *KMT2A* rearranged ALL seem to mainly benefit from a hybrid protocol; the ESCP decided to develop a separate protocol, based on Interfant-06, for infants with *KMT2A* rearranged ALL.

2. PATIENT GROUP

The current ESCP recommendations apply to:

- ✓ Patients with newly diagnosed B-precursor acute lymphoblastic leukemia (ALL) or mixed-phenotype acute leukemia according to WHO 2016 criteria.
- ✓ < 365 days of age at time of diagnosis of ALL
- ✓ 11q23/ *KMT2A* rearrangement
- ✓ Admission, diagnosis and therapy performed by experienced centers

3. DEFINITIONS

Please refer to the ESCP section dedicated to the Philadelphia Chromosome negative ALL.

4. DIAGNOSTICS

See ESCP Ph- ALL protocol.

For this protocol on *KMT2A* rearranged infant ALL, diagnostics of *KMT2A* (formerly known as *MLL*) rearrangement is solely important.

5. PROGNOSTIC RISK STRATIFICATION

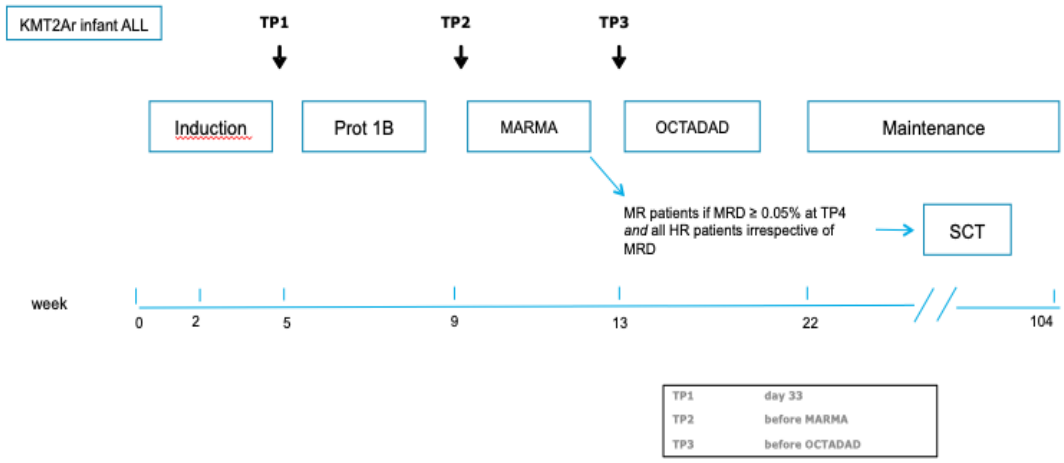
Risk Stratification

High risk (HR)	*KMT2A* rearranged AND
	• age at diagnosis < 6 months AND • WBC≥300 x 10^9/L and/or prednisone poor response
Medium risk (MR)	All other cases so including those with:
	• *KMT2A* rearranged AND age ≥ 6 months OR • *KMT2A* rearranged AND age < 6 months AND WBC< 300 x 10^9/L AND prednisone good response

6. TREATMENT

6.1 Chemotherapy schedule – treatment overview

Treatment schedule



General treatment modifications

Calculate the body surface area (BSA) at the start of each treatment block and then adjust:

Age	Dose modification
<6 months	2/3 of the calculated dose based on BSA
6 through 12 months	3/4 of the calculated dose based on BSA
>12 months	full dose

Dose reductions are for all drugs including glucocorticoids, but **not for intrathecal drugs, glucocorticosteroids, PEGasparaginase.**

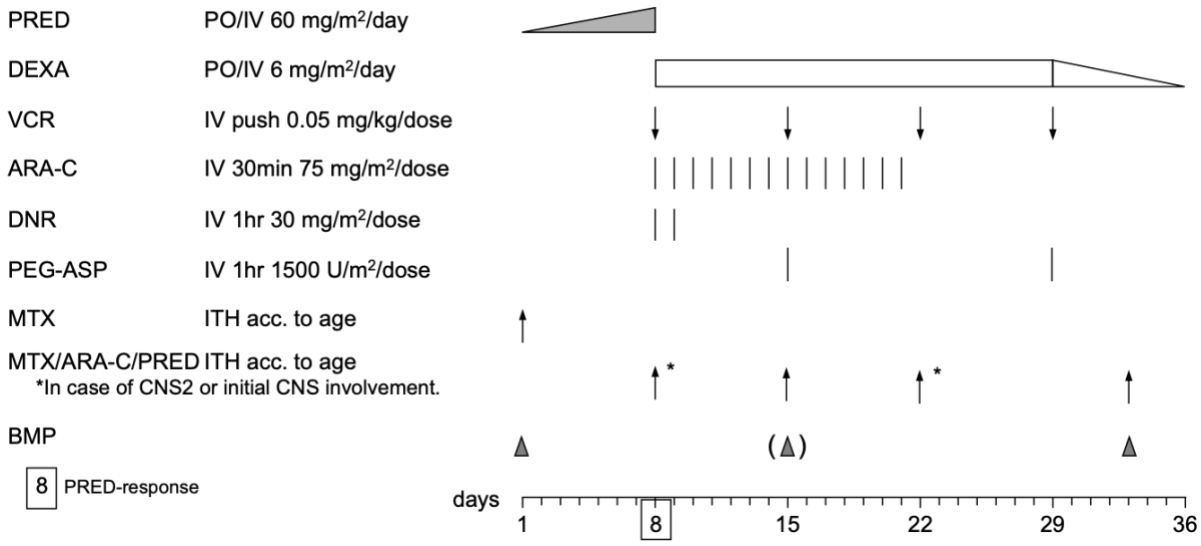
Intrathecal doses are according to age as indicated in the schemes and do not depend on surface area.

Intrathecal therapy

	<1 year	≥ 1 year	≥ 2 year
MTX	6 mg	8 mg	10 mg
Cytarabine	15 mg	20 mg	25 mg
Prednisone*	3 mg	4 mg	5 mg

*If prednisone is not available for intrathecal use, this can be replaced by 12 mg, 16 mg or 20 mg hydrocortisone, respectively.

6.2 Induction



6.2.1 Prednisone Phase (day 1-7)

PRED **Prednisone** 60 mg/m^2 daily divided into 3 doses orally or iv on 7 consecutive days, i.e day 1-7. In case of a high risk of tumor lysis it is advisable to start at a lower dose which is increased each day as quickly as possible:

Initial WBC:	***Advised starting dose of prednisone at day 1***
50-100x 10^9/L	30 mg/m^2 daily
>100x10^9/L	15 mg/m^2 daily

The total dose of prednisone in 7 days should be at least 200 mg/m^2 (optimal 420 mg/m^2). Previous experience with the BFM regimens shows that sometimes a rise in WBC can be seen during the first 2 days of treatment with prednisone, followed by a decrease thereafter. If the patient remains in good condition there is no need to introduce other drugs. If the patient's condition deteriorates or if the leucocyte count continues to rise after three days then the other induction drugs should be started

IT therapy **Methotrexate** is given on day 1. For dose see table, page 88

6.2.2 Rest of Induction (day 8-33)

DEXA **Dexamethasone** 6 mg/m^2 daily divided into 3 doses iv or orally on 21 consecutive days, i.e. day 8-28, followed by one week in which the drug is reduced stepwise to zero.

VCR **Vincristine** 0.05 mg/kg daily iv push on 4 days at day 8, 15, 22, 29. In case age ≥ 12 months or weight ≥ 10 kg 1.5 mg/m^2 per iv.

ARA-C **Cytarabine** 75 mg/m^2 daily iv in 30 min on 14 consecutive days from days 8-21.

DNR **Daunorubicin** 30 mg/m^2 daily iv in 60 min on 2 consecutive days at day 8 and 9. If local protocols advise other infusion times of daunorubicin, it is acceptable to give it iv over a minimum of 30 min up to a maximum of 6 hrs.

PEG **PEG-asparaginase** 1,500 IU/m2 iv in 60 min at day 15 and 29. In case of clinical allergy to PEG-asparaginase 6 doses of Erwinia asparaginase (20,000 IU/m2 in 60 min iv or im) can be give every other day.

*If centers use native **E-Coli asparaginase** instead of PEG-asparginase then following dose of L-Asparaginase is recommended: 10.000 U/m^2 daily iv in 1 hr or im on day 15, 18, 22, 25, 29, 33.. If only Erwinase is available, the dose should be adjusted to 20.000 U/m^2, 3 times a week so a total of 9 doses.*

*The recommended dose of **PEG-asparaginase** is 1500 U/m2. It is allowed to use a dose of 1000-2500 U/m2 iv or im according to local/national guidelines*

IT MTX/PRED/ARA-C **Intrathecal methotrexate cytarabine and prednisone** at day 15 and at day 29. In case of CNS2 or CNS involvement at initial diagnosis extra intrathecal doses should be given at day 8 and 22. If CNS leukemia is still present at day 29 then weekly intrathecal MTX until the CNS is free of leukemia. For doses see table, page 88

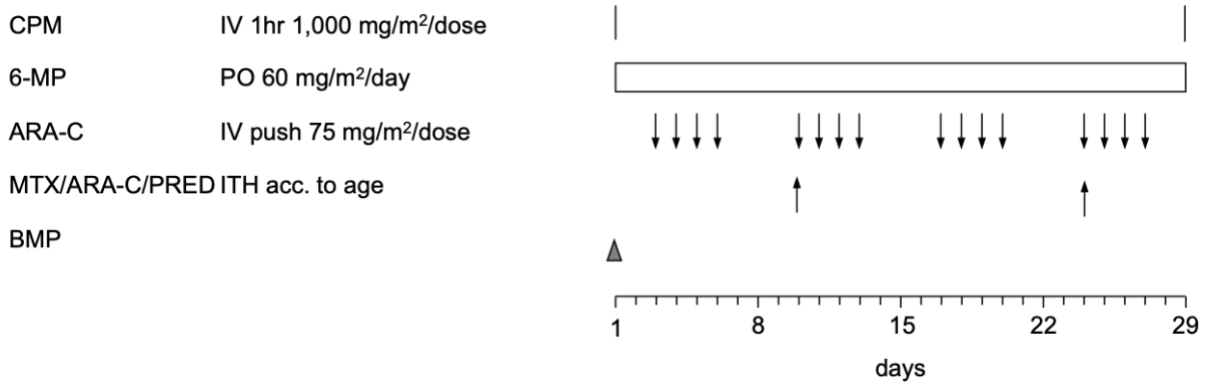
6.2.3 Treatment of CNS involvement

For central nervous system (CNS) disease at diagnosis (CNS+) weekly intrathecal doses in induction are scheduled, at least two but more if needed to clear the spinal fluid from blasts. Patients with CNS2 or TLP+ are not defined as CNS+ but should be treated as CNS+ so these patients also get at least two extra intrathecal doses of therapy.

6.2.4 Treatment of testicular involvement

Unilateral or bilateral testis enlargement at diagnosis constitutes tentative testicular involvement of ALL. Testicular infiltration should be confirmed by ultrasound (for subsequent follow-up), but a biopsy should not be performed at diagnosis. An ultrasound should also be performed after protocol IB. A testicular biopsy is indicated if the patient still has testiculomegaly or suspicion of leukemic infiltration by bilateral ultrasound after protocol IB. Biopsy-proven leukemic infiltration at that time-point will need to also consider orchidectomy and irradiation of the contralateral testis.

6.3 Consolidation – Protocol IB



Requirements for the start of protocol IB

- Complete remission after blinatumomab
- Good clinical condition without serious infections
- Creatinine within normal limits according to age
- WBC > 2 x 10^9/L, AND Neutrophil count > 0.4 x 10^9/l
- Platelets > 50 x 10^9/L

Requirements starting each block of cytosine arabinoside (ARA-C)

- WBC > 0.5 x 10^9/L
- Platelets > 30 x 10^9/L

Requirements for the second cyclophosphamide dose at day 64

- WBC > 1 x 10^9/L
- Neutrophil count > 0.3 x 10^9/L
- Platelets > 50 x 10^9/L

If possible, the ARA-C blocks should not be interrupted. If nevertheless an ARA-C block has to be postponed or interrupted because of clinical problems, the 6-mercaptopurine should also be interrupted. Omitted 6-mercaptopurine doses should be made up until the planned cumulative total dose of 1680 mg/m^2 (28 x 60 mg/m^2) is reached.

CPM **Cyclophosphamide** 1,000 mg/m^2/dose, i.v. over 1 hour, day 1 and 29.

Requirements during administration:

- Hydration and cystitis prophylaxis: 3,000 ml/m^2 fluid/24 hr for a minimum of 6 hours;
- Mesna (Uromitexan®): 400 mg/m^2/dose i.v. before and 3 and 6 hours after the start of the CPM-infusion;
- In case of (microscopic) hematuria: increase i.v. fluid and Mesna;
- Furosemide 0.5 mg/kg i.v., 6 hours and 12 hours after CPM only if required for diuresis.

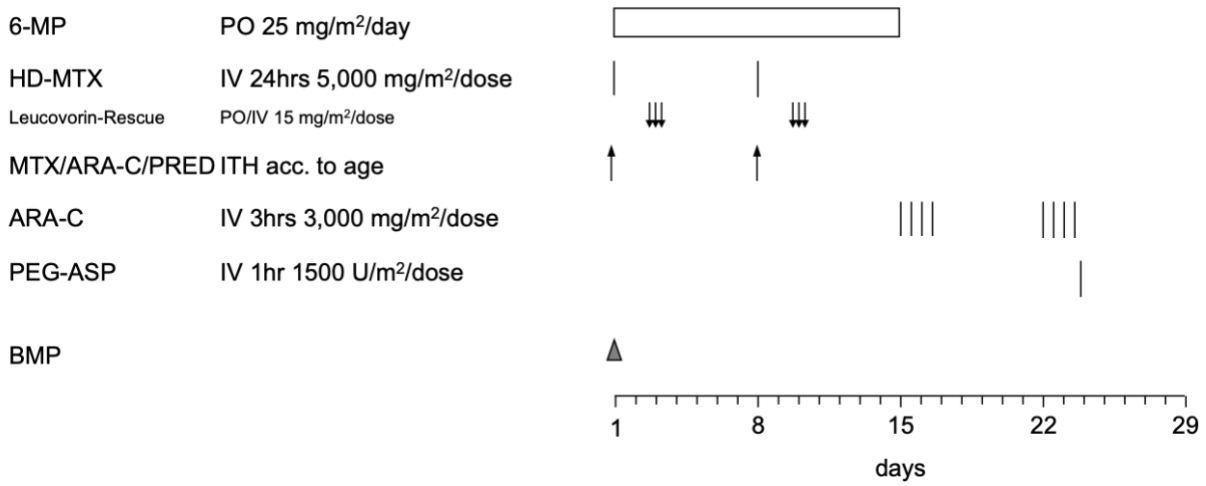
6MP **6 Mercaptopurine** 60 mg/m^2/day p.o., days 1-28 (28 days in total).

- Administration: with empty stomach, in the evening, not with milk.
- Omitted 6 MP-doses should be made up until the planned cumulative total dose of 1680 mg/m^2 (28 x 60 mg/m$^{2)}$ is reached.

ARA-C **Cytosine Arabinoside** 75 mg/m^2/dose i.v. push in four blocks, of 4 days each: days 3-6, 10-13, 17-20, 24-27.

IT MTX/PRED/ARA-C **Intrathecal methotrexate cytarabine and prednisone** at day 10 and at day 24. For doses see table, page 88.

6.4 MARMA



Requirements for the start of MARMA:

Neutrophil count > 0.5 x 10^9/l and platelets > 50 x 10^9/l and rising, and should not start earlier than two weeks after cyclophophamide day 64.

MP **6-Mercaptopurine** 25 mg/m^2 daily in 1 dose orally on 14 consecutive days, i.e. day 1-14. If day 8 MTX is delayed due to toxicity then stop 6-MP and recommence with second dose to complete 14 days.

HD MTX **Methotrexate** 5000 mg/m^2 iv as 24 hour infusion on day 1 and 8; 10% (500 mg/m^2) of the dose in 30 minutes iv followed by 90% (4500 mg/m^2) of the dose in 23.5 hrs. Cotrimoxazole should be stopped from 48 hours prior to methotrexate and until methotrexate plasma level is <0.4 uM. The second dose of HD-MTX may be given regardless of the blood count but not regardless of the condition of the patient, e.g. mucositis.

LCV **Leucovorin rescue** 15 mg/m^2 (racemic form, 7,5 mg/mq/dose if levo-form) orally/ iv at 42, 48 and 54 hrs after the start of the MTX infusion. Plasma levels of MTX should be determined 24 hrs and 48 hrs after the start of the MTX infusion. If the plasma MTX level is > 0.4 uM at 48 hrs after the start of MTX infusion then continue the leucovorin doses every 6 hours until MTX plasma level is <0.25 uM.

IT MTX/PRED/ARA-C **Intrathecal methotrexate cytarabine and prednisone** at day 1 and 8. For doses see table, page 88.

The second phase of MARMA consisting of high dose cytarabine and asparaginase may start only when there is no mucositis and when the neutrophil count > 0.5 x 10^9/l and platelets > 100 x 10^9/l. *The high dose cytarabine at day 22 can be started irrespective of the blood counts*.

ARA-C **Cytarabine** 3000 mg/m^2 iv in 3 hrs infusion twice daily with 12 hrs interval on 4 days, i.e. day 15, 16, 22, 23.

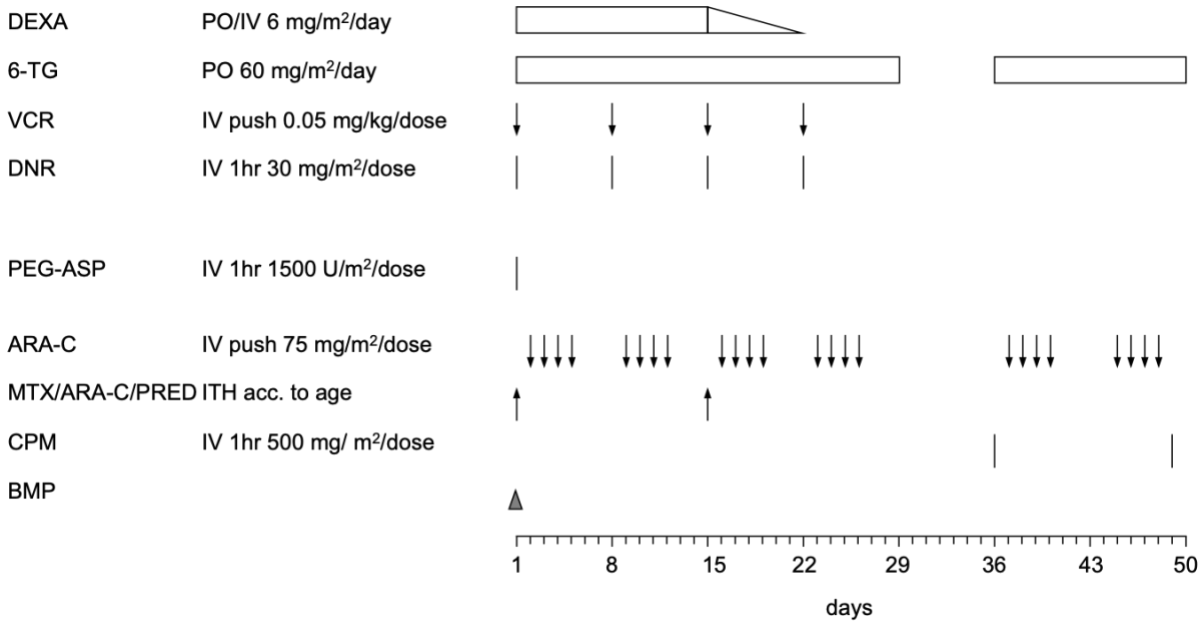
PEG-ASP **PEG-asparaginase** 1500 U/m^2 on day 23 IV in 1 hr or IM. The asparaginase is given 3 hrs after completion of the last araC infusion on day 23 because of its supposed synergistic effects. The asparaginase should not be given before or during araC infusion because of supposed antagonistic effects in that case.

In case of clinical allergy to PEG-asparaginase 6 doses of Erwinia asparaginase (20,000 IU/m2 in 60 min iv or im) can be given every other day.

*If centers use native **E-Coli asparaginase** instead of PEG-asparginase then following dose of L-Asparaginase is recommended: 10.000 U/m^2 daily iv in 1 hr or im on day 15, 18, 22, 25, 29, 33.. If only Erwinase is available, the dose should be adjusted to 20.000 U/m^2, 3 times a week so a total of 9 doses.*

*The recommended dose of **PEG-asparaginase** is 1500 U/m2. It is allowed to use a dose of 1000-2500 U/m2 iv or im according to local/national guidelines.*

6.5 OCTADAD



Requirements for the start of OCTADAD

OCTADAD starts when neutrophil count > 0.5 x 10^9/l and platelets > 50 x 10^9/l and both are rising but should not start earlier than 2 weeks after the end of MARMA. *The first part of this course takes 4 weeks after which there is one week without chemotherapy.* Neutrophils and platelets should be measured at the start of each week. Application of VCR, DNR and the start of an ARA-C bloc should be delayed and 6TG interrupted when neutrophils drops < 0.3x10^9/L and/or platelets < 50x10^9/L but if a 4-day course of cytarabine has started, then this should not be interrupted.

First part

DEXA **Dexamethasone** 6 mg/m^2 daily divided into 3 doses orally on 14 consecutive days, i.e. day 1-14, followed by one week in which the drug is reduced stepwise to zero at day 21.

TG **6-Thioguanine** 60 mg/m^2 daily in 1 dose orally on 28 consecutive days, day 1-28.

VCR **Vincristine** 0.05 mg/kg iv push on 4 days at day 1, 8, 15, 22. In case age ≥ 12 months or weight ≥ 10 kg 1.5 mg/m^2 per iv.

DNR **Daunorubicin** 30 mg/m^2 iv over 60 min on 4 days at day 1, 8, 15, 22. If local protocols advise other infusion times of daunorubicin, it is acceptable to give it iv over a minimum of 30 min up to a maximum of 6 hrs. Only for patients treated with Protocol IB.

PEG **PEG-asparaginase** 1,500 IU/m2 IV in 1hr or IM at day 1.
In case of clinical allergy to PEG-asparaginase 6 doses of Erwinia asparaginase (20,000 IU/m2 in 60 min iv or im) can be given every other day.
*If centers use native **E-Coli asparaginase** instead of PEG-asparginase then following dose of L-Asparaginase is recommended: 10.000 U/m^2 daily iv in 1 hr or im on day 15, 18, 22, 25, 29, 33.. If only Erwinase is available, the dose should be adjusted to 20.000 U/m^2, 3 times a week so a total of 9 doses.*

*The recommended dose of **PEG-asparaginase** is 1500 U/m2. It is allowed to use a dose of 1000-2500 U/m2 iv or im according to local/national guidelines.*

IT MTX/PRED/ARA-C **Intrathecal methotrexate cytarabine and prednisone** at day 1 and 15. For doses see table page 88.

Second part

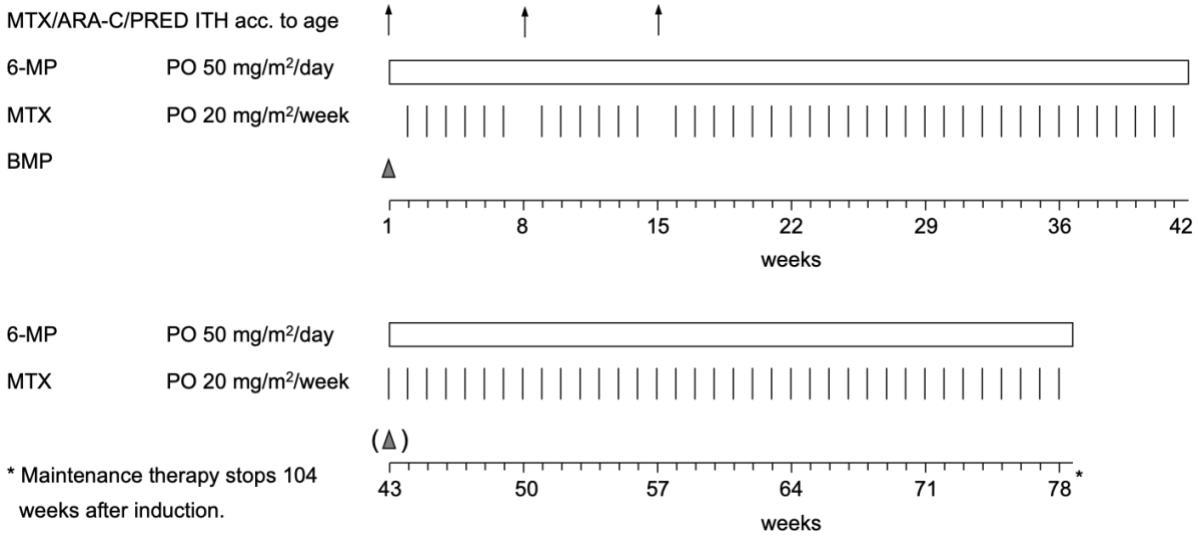
The second part of this course should only start when neutrophils > 0.5 x 10^9/l and platelets > 50 x 10^9/l.

TG **6-Thioguanine** 60 mg/m^2 daily in 1 dose orally on 14 consecutive days, day 36-49.

ARA-C **Cytarabine** 75 mg/m^2 daily given iv push on day 37-40 and day 45-48.

CPM **Cyclophosphamide** 500 mg/m^2 in 1 hr iv on day 36 and 49.

6.6 Maintenance



Requirements for the start of maintenance

This phase starts when the neutrophil count > 0.5 x 10^9/l and platelets > 50 x 10^9/l and rising but not earlier than 2 weeks after the end of the previous course of chemotherapy.

This part of the maintenance consists of daily 6-MP plus weekly MTX and 3 administrations of intrathecal medication (week 1, 8, 15).

IT MTX/PRED/ARA-C **Intrathecal methotrexate cytarabine and prednisone** in week 1, 8 and 15. For doses see table page 88.

6-MP **6-Mercaptopurine** 50 mg/m^2 daily in 1 dose orally in the evening, on an empty stomach avoiding milk products.

MTX **Methotrexate** 20 mg/m^2 once a week orally on the same day of each week.

Maintenance therapy stops 104 weeks after initial diagnosis. The duration of this phase varies according to the length of previous consolidation.

Dose adjustments during maintenance

During maintenance the doses of 6-MP and MTX should be adjusted upward (with no upper dose limit) to obtain a total white blood cell count below 3.0 x 10^9/L. The drugs should be reduced in dosage or withdrawn if the white blood cell count falls below 1.5 x 10^9/L, the absolute neutrophil count below 0.3 to 0.5 x 10^9/L, or the platelet count below 50 x 10^9/L.

Routine measurements of liver function are not necessary in patients without symptoms. In case of symptoms, dose reductions should be based on a rise in bilirubin to more than three times the upper normal limit or aminotransferase levels more than 10 times the upper normal limit and rising. In such cases, other causes such as viral hepatitis or Gilbert syndrome should be considered.

6.7 Stem cell transplantation

6.7.1 Indication

In view of the uncertainties about the efficacy and risks of SCT only MR patients with MRD positive BM after MARMA and all HR patients will be eligible for SCT and only if the donor criteria are met.

The primary eligibility criteria for SCT are:

- HR patients AND
- First complete remission

OR

- MR patients with positive MRD levels (≥ 0.05%) after MARMA AND
- First complete remission

6.7.2 Conditioning therapy

Before start of conditioning the remission status must be documented by a bone- marrow and lumbar puncture, which should not be older than 14 days. The conditioning regimen consists of intravenous Treosulfan, Fludarabine and Thiotepa.

For more detailed information, see ESCP HSCT protocol

7. MODIFICATIONS FOR TOXICITY

Asparaginase

Asparaginase should be discontinued in the presence of clinically evident pancreatitis. In most of the cases, hyperglycaemia in induction will be due to steroids rather than asparaginase.
In case of allergic reactions each PEG-asparaginase product given at 1.500 U/m2 once every 2 weeks could be replaced by Erwinase 20.000 U/m2 3 times per week per 2 weeks.
In case of clinically significant hemorrhagic or thrombotic complications, withhold asparaginase and restart asparaginase under anticoagulation (LMWH) therapy once symptoms resolve.

Cyclophosphamide

It is unlikely that hematuria will occur at the dose of 500 mg/m^2. If it does occur, hyperhydration and Mesna 500 mg/m^2 continuous infusion for 24 hrs after a loading dose of 150 mg/m^2 is advisable.

High Dose Cytarabine (AraC, Cytosar)

If during cytarabine nystagmus occurs as an isolated event, stop araC for 24 hours. If nystagmus and other cerebellar signs occur, stop cytarabine and do not proceed with this course. Conjunctivitis should be treated or prevented with prednisone eyedrops (e.g. 0.5% frequently).

Daunorubicin

If cardiac function is low (left ventricular shortening fraction (LVSF) (repeatedly) lower than 27%) daunorubicin needs to be delayed to OCTADAD. When LVSF is 27%-30% or higher, the normal dose of daunorubicin can be given.

Dexamethasone and Prednisone

When clinical overt diabetes mellitus develops after introduction of steroids, use insulin. In case of hypertension, first use antihypertensive drugs and sodium restriction. If further treatment of hypertension is absolutely necessary, reduction of the dose with 30%-50% of the glucocorticoids may be indicated.

High Dose -Methotrexate (5000 mg/m^2)

1. Stop cotrimoxazole 48 hours before HD-MTX until 24 hours after the plasma MTX level < 0.4 uM.

2. If creatinine is above the upper normal limit for age or increased >30% from baseline value, it is advised to measure the glomerular filtration rate (GFR) or creatinine clearance before giving MTX. If the GFR is below the upper normal limit for age, consider 50% dose reduction for MTX. If GFR < 30 ml/min/1.73m^2 omit MTX. Subsequent renal function can be measured with plasma creatinine and correlating these with the creatinine, obtained at the first GFR measurement.

3. MTX levels: these will be determined at the end of the MTX infusion, i.e. 24hrs after the start of the MTX infusion (T24), and 48 hrs after the start of the MTX infusion (T48). If the MTX level is > 0.4 uM at T48, repeat MTX level determinations every 6 to 24 hrs until the level is < 0.25 uM.

4. Hydration and alkalinization: Pre-hydration with e.g. glucose 5%/NaCl 0.45% (+ 50 mmol Sodium Bicarbonate/L) at the rate of 125 ml/m2/hour during 6 hours. Urine pH needs to be > 7.0. If urine pH < 7.0, increase Sodium Bicarbonate to 75 mmol/L infusion fluid. Hyperhydration and alkalinization should be continued during MTX infusion and after infusion until MTX plasma level is < 0.4 uM.

5. Leucovorin rescue: 15 mg/m^2 (racemic form, 7,5 mg/mq/dose if levo-form) orally or iv at 42 (T42), 48 (T48) and 54 (T54) hrs after the start of the MTX infusion. If the plasma MTX level is > 0.4 uM at T48, then continue these doses every 6 hours until MTX plasma level is <0.25 uM.

Vincristine

The dose of vincristine may be reduced to 2/3 of the recommended dose, when severe paresis or constipation develop. If the symptoms disappear, re-introduce vincristine at a full dosage. In case of a dropping foot or when an ileus is present, vincristine is withheld until the clinical signs are completely resolved. Re-introduce vincristine at 2/3 of the recommended dose. Do not modify the dose for jaw pain but use analgesics.

8. SUPPORTIVE CARE

For supportive treatment during treatment, See Appendix III. Since this protocol is very intensive guidelines for prevention and treatment of infections in infants is given.

Preventive measures

It is strongly recommended to give antibiotic and antifungal prophylaxis during and after the intensive chemotherapy courses Induction, protocol IB, MARMA and OCTADAD until recovery of neutrophils. Advise is to use ciprofloxacin and itraconazol but alternative prophylaxis can be used according to national or institutional guidelines. Some clinicians may regard this as conflicting with the SPC stating that there is an interaction between ciprofloxacin and MTX. This is based upon case reports that report higher MTX levels resulting in toxicities. The “expert center for pharmacists” indicates that there is no relevant interaction but the SPC for ciprofloxacin advises not to use ciprofloxacin and MTX in combination. Please note also that the HD-AraC part and not the HD-MTX part of the MARMA course puts patients at a high risk of infections. So we leave the choice to administer ciprofloxacin during MTX courses up to the treating physician and advise antibiotic prophylaxis during MARMA according to the national or institutional guidelines taking into account the possible interaction between MTX and ciproxin. (guidelines Aug 2013)

Itraconazol should *not* be given in combination with weekly vincristine (Induction, first weeks of OCTADAD) because the interaction may lead to increased neurotoxicity.

- Mouth and skin care should be provided, especially in the diaper region.
- Because of the high risk of Pneumocystis Carinii Pneumonitis (PCP), it is strongly recommended to start PCP prophylaxis not later than at day 28 of the induction therapy. The prophylaxis should be interrupted in the HD-MTX courses as indicated in section 4.2 and 4.4.

- Preventive measures against bacterial infections should be taken according to the local policy of each centre.
- During neutropenia after HD-ARA-C, infections with particularly the gram positive Streptococcus viridans can occur, complicated by ARDS. (Centres may wish to consider prophylaxis during the neutropenic phase after HD-AraC with oral penicillin).
- Because of their young age and the intensive chemotherapy regimen, most of the children develop severe hypogammaglobulinemia that often lasts until the end of maintenance. IVIG may be administered at the investigator's discretion.
- A potential large number of infants will not have been infected previously with the Varicella-Zoster Virus (Herpesvirus Varicellae). If there has been an exposure of the patient with an individual with varicella, Varicella Zoster Immunoglobulin (VZIG) needs to be administered within 72 hours of exposure. The administration of VZIG to a patient extends the incubation period to 18-21 days. Nevertheless specific VZIG is not available in many countries. In that case, chemoprophylaxis with acyclovir 40-80 mg/kg/day PO in 4 divided doses starting 7-9 days after exposure (second viremic phase) is recommended. In case of manifest varicella infection, complications with pneumonia or encephalitis can be avoided by prompt treatment with intravenous acyclovir (500 mg/m2 q 8 hr IV). In case of active disease the chemotherapy should be stopped, till all lesions have dried. Vaccination of household members against varicella is recommended if they have not developed previous natural infection.
- Measles is potentially the most serious infection during treatment as measles is not treatable and leads to progressive interstitial pneumonia and death. The high level of immunisation in the population has rendered this problem less common. The non-immunised child should avoid contact at all cost. In case of contact, hyperimmune immunoglobulin, if available, or standard Ig preparation is strongly recommended. In case of measles, IV Ribavarin is recommended.

Patients should be kept in the hospital after intensive chemotherapy courses or they should be checked at the outpatient clinic at least twice a week. Patients can only be discharged or treated in the outpatient setting if they are in clinically perfect condition and no signs of upcoming infection are present.

9. REFERENCES

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APPENDIX I

AIEOP-BFM CONSENSUS GUIDELINES 2016 FOR FLOW CYTOMETRIC IMMUNOPHENOTYPING OF PEDIATRIC ACUTE LYMPHOBLASTIC LEUKEMIA

Table 1 (Appendix I):The AIEOP-BFM consensus antibody panel for pediatric ALL

[Dworzak M, et al. Cytometry Part B (Clinical Cytometry) 94B:82–93 (2018)]

Mandatory and optional markers (each combined with CD45)	
Intracellular[a,b]	iCD3, iCD22, iCD79a, iIgM (l-chain), iLysozyme, iMPO
Surface[a]	CD2[c], CD3, CD5, CD7; CD10, CD19, CD20; CD11c, CD11b, CD13, CD14, CD15, CD33, CD64, CD65[d], CD117; CD34, (CD45), CD56, HLA-DR if T-ALL: CD1a, CD4, CD8, TCRab, TCRgd if B-IV suspected: j-chain, k-chain (surface staining after pre-washing or intracellular)
Optional / Recommended	all cases: NG2[e], CD371[c,f] if BCP-ALL: CD11a[c], CD22, CD24, CD38, CD44, CD58, CD66c, CD123[c], CRLF2[c,g] if T-ALL: CD99, iTdT if BAL according to general panel: CD24, iTdT
[a]Mandatory markers for WHO, EGIL, ETP classifications. [b]Prefix "i" stands for intracellular staining. [c]Phycoerythrin-conjugate (PE) recommended. [d]Available only labelled with fluorescein isothiocyanate (FITC). [e]Clone 7.1. [f]Clone 50C1. [g]Clone 1D3.	

Table 2 (Appendix I): The AIEOP-BFM dominant lineage assignment[a]

[Dworzak M, et al. Cytometry Part B (Clinical Cytometry) 94B:82–93 (2018)]

Lineage	Criteria	Antigens
BCP-ALL	≥2 positive of:	CD19[b]; CD10, (i)CD22, iCD79a
T-ALL	all 3 of:	(i)CD3[pos,c] CD7[pos]; iMPO negative or weak
AML	≥2 positive of: and	iMPO, CD13, CD33, CD64, CD65, CD117 BCP-/T-ALL criteria not met

[a]These markers are relevant for dominant lineage assignment, but are insufficient for a thorough description of leukemic immunophenotypes.
[b]BCP-ALL needs strong positivity in ≥2 of the four antigens– in the rare case of CD19-negativity, specifically CD10 must be strong positive. Mind that rare cases of MLL-rearranged BCP-ALL may drop out of this scheme due to biology inherent lack of CD10, as well as weak (i)CD22 and iCD79a expression (CD19 is then usually strong positive).
[c]For T-ALL, iCD3 positivity must be either strong, or if rated weak, CD2 and/or CD5 should be any positive in addition. Surface CD3 expression needs to be tested in addition.

Table 3 (Appendix I):The AIEOP-BFM subclassification of ALL

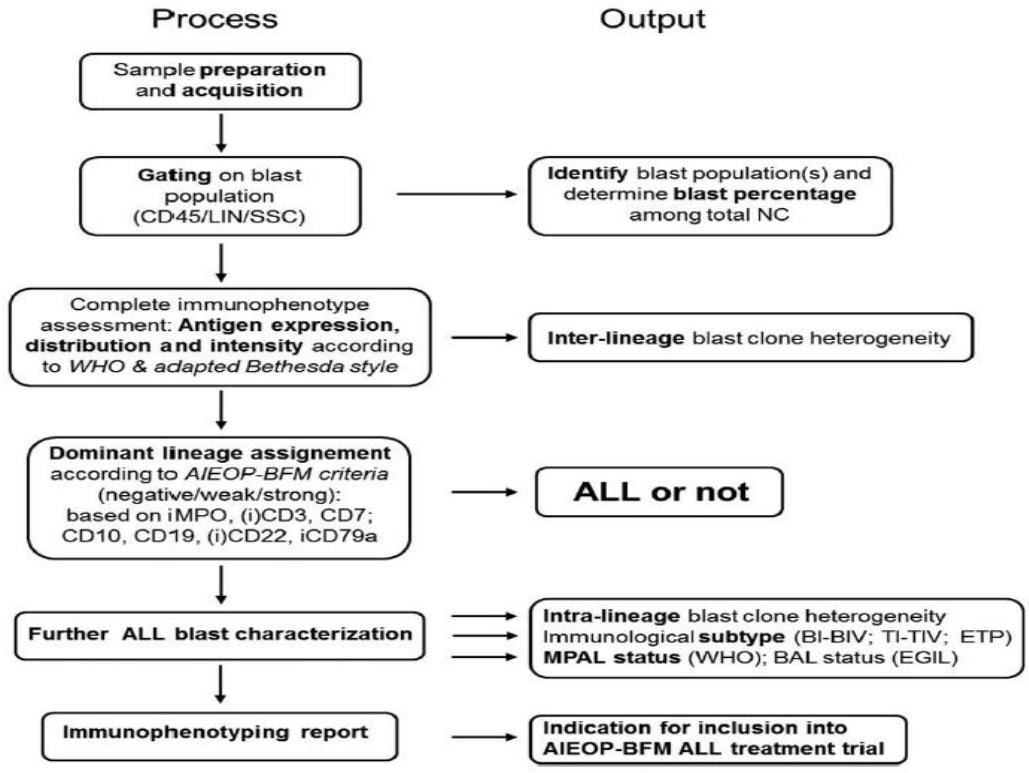
[Dworzak M, et al. Cytometry Part B (Clinical Cytometry) 94B:82–93 (2018)]

Subtype	Discriminators	Remarks
B-I *(pro-B)*	*$CD10_{neg}$*	*BCP-ALL lineage criteria fulfilled*
B-II *(common)*	*$CD10_{pos}$*	
B-III *(pre-B)*	*$iIgM_{pos}$*	*$CD10_{neg\ or\ weak\ pos}$ may occur[a]*
B-IV *(mature B)*	*κ- or λ-$chain_{pos}$*	*may occur with FAB L1/L2 morphology[b]*
T-I *(pro-T)[c]*	*only $iCD3_{pos}$ and $CD7_{pos}$*	*T-ALL lineage criteria fulfilled*
T-II *(pre-T)*	*≥1 of $CD2_{pos}$, $CD5_{pos}$, $CD8_{pos}$*	*surface (s) $CD3_{weak\ pos}$ allowed[d]*
T-III *(cortical T)*	*$CD1a_{pos}$*	*$sCD3_{weak}$ may occur[e]*
T-IV *(mature T)*	*$CD1a_{neg}$ and $sCD3_{pos}$*	*$sCD3_{strong}$, or $sCD3_{weak\ pos}$ with TCR_{pos}*
ETP (only additive to *T-I or T-II)*	$CD1a_{neg}$, $CD8_{neg}$ usually $CD5_{neg\ or\ weak\ pos}$ and ≥1_{pos} of *HLADR, CD11b,13,33,34,65,117*	if $CD5_{strong\ pos}$: _2_{pos} of HLADR, CD11b,13,33,34,65,117; *$sCD3_{weak\ pos}$ may occur[e]*

[a]$CD10_{neg/weak}$ B-III is frequently associated with MLL-rearrangements.
[b]Light-$chain_{pos}$ cases without FAB L3-morphology and without *MYC*-translocation are eligible for conventional ALL treatment, and thus must be separated from Burkitt-type mature B-ALL .
[c]T-I is very rare and can be reported together with T-II (as T-I/II).
[d]Dim or even more frequently partial surface positivity with CD3 (e.g., in a minor blast subpopulation) occurs when sensitive methodology is used and should not mislead to diagnose mature T-ALL in the absence of TCR expression.

Figure 1 (Appendix I): Diagnostic algorithm based on the AIEOP-BFM consensus guidelines. This algorithm is used to define the leukemic immunophenotype as prerequisite for treatment initiation. Note that dominant lineage assignment can lead to a diagnosis of ALL according to the AIEOP-BFM consensus despite fulfilled MPAL criteria according to WHO 2008/2016. LIN refers to usage of a lineage marker for blast cell gating as appropriate. A prefix in parenthesis indicates that the respective marker needs to be tested both on surface as well as intracellular.

[Dworzak M, et al. Cytometry Part B (Clinical Cytometry) 94B:82–93 (2018)]



APPENDIX II

MANAGEMENT OF TOXICITY AND MODIFICATIONS FOR TOXICITY

Management of toxicity and modifications for toxicity

1.1 Cyclophosphamide, Ifosfamide

1.1.1 Hematuria

The following measures are recommended in the event of microhematuria, macrohematuria, dysuria or stranguria with or after administration of Cyclophosphamide:

- Increased fluid intake of 4 500 ml/m^2/24 h
- Regular balancing (every 4-6 hours), furosemide administration if necessary
- Pain therapy as required
- Dose intensification and therapy prolongation with Mesna may be considered. The objective and success of this measure depend on the elimination half-life of cyclophosphamide and its metabolites, which can vary widely, and on the degree of pre- existing urothelial damage.

With past hematuria, strict hydration should be conducted prior to introducing Cyclophosphamide

1.1.2 Neurotoxicity/ encephalopathy (Ifosfamide)

CNS symptoms (encephalopathy with confusion, somnolence, rarely with seizures and coma) may develop within several hours to days after initiation of Ifosfamide treatment and generally resolve spontaneously several days after the drug has been discontinued. If such events occur, Ifosfamide treatment should be discontinued and replaced by cyclophosphamide (at a dose of 25% of ifosfamide). Methylene blue may contribute to a better resolution of clinical symptoms and should be considered for all patients with Grade 2 neurotoxicity and is indicated for patients with Grade 3 and 4 toxicity. It is administered at a dose of 1-2 mg/kg every 4 to 8 hours, i.v. slowly over several minutes until the patient no longer experiences symptoms (Ajithkumar*, et al* 2007). Methylene blue is contraindicated in patients with Glucose-6-phosphate dehydrogenase deficiency, known sensitivity to the drug and severe renal impairment

1.2 Cytarabine

1.2.1 Low-dose Cytarabine blocks

After a cytarabine block has been started, it should not be interrupted due to uncomplicated myelosuppression. Fever following Cytarabine injections is frequently due to the drug itself and therefore no reason to interrupt treatment, as long as infections can be excluded (blood cultures, infection parameters). In case of suspected or confirmed infection, Cytarabine therapy as well as 6-Mercaptopurine (or Thioguanine) should be interrupted.

1.2.2 High-dose Cytarabine

To prevent toxic keratoconjunctivitis, the administration of artificial tears is recommended every 6 hours until 48 hours after the last Cytarabine dose. During HD-ARA-C infusion, patients should be monitored closely for any signs of neurotoxicity. The infusion should be stopped immediately if there are any signs of nystagmus and/or ataxia. If neurological symptoms persist or recur with repeat administration of HD-ARA-C, HD-ARA-C may no longer be given.

1.3 Daunorubicin, Doxorubicin

Echocardiograms are recommended prior to therapy introduction and after each cumulative anthracycline dose of 120 mg/m^2. In case of clinical signs of heart failure and/or significant decrease in left ventricular function (left ventricular shortening fraction (LV-SF) below 30% or the ejection fraction below 50%), the reduction or discontinuation of anthracycline should be considered.

1.4 Etoposide

Adverse events as hypotension or allergic reaction are mainly due to etoposide additives (Vepesid®) and can largely be avoided by administering etoposide phosphate (Etopophos®).

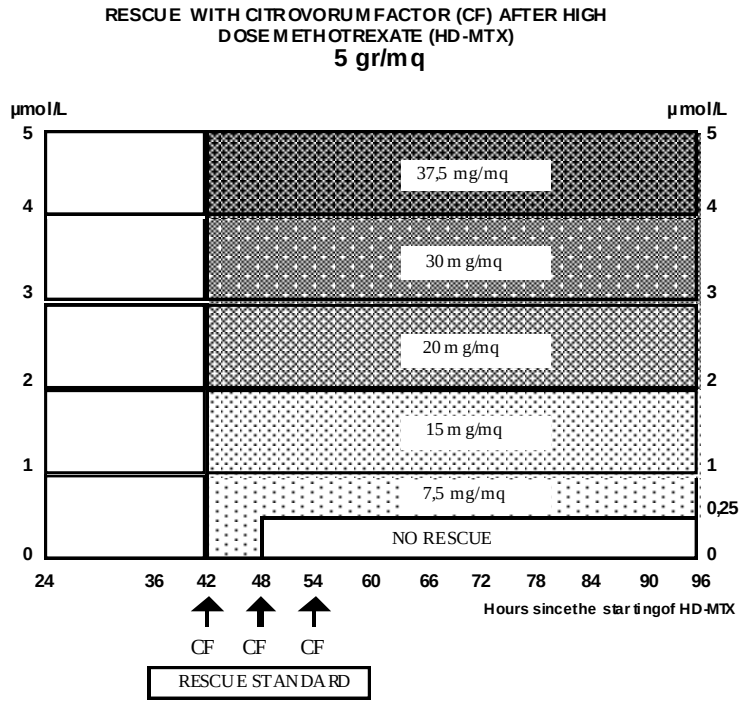
1.5 Methotrexate

During the administration of HD-MTX, renal function (serum creatinine) and MTX level should be monitored at 24, 42 and 48 hours from the start of the infusion. Delayed MTX excretion should be suspected in case of onset of diarrhea or heavy vomiting during the MTX infusion, significant increase in serum creatinine over the baseline value 24 hours after the start of the MTX infusion and/or MTX serum concentration >150 µmol/l at the end of the MTX infusion (24 hours after the start of the infusion).
In order to minimize the risk of delayed excretion, the following precautions should taken:

- Stop co-trimoxazole 48 hours before HD-MTX until 24 hours after the plasma MTX level < 0.5 uM.
- If creatinine is above the upper normal limit for age or increased >30% from baseline value, it is advised to measure the glomerular filtration rate (GFR) or creatinine clearance before giving MTX. If the GFR is below the upper normal limit for age, consider 50% dose reduction for MTX. If GFR < 30 ml/min/1.73m^2 omit MTX.
- MTX levels: these will be determined at the end of the MTX infusion, i.e. 24hrs after the start of the MTX infusion (T24), 42 hrs (T42) and 48 hrs after the start of the MTX infusion (T48). If the MTX level is > 0.5 uM at T48, repeat MTX level determinations every 6 to 24 hrs until the level is < 0.25 uM.
- Adequate hydration and alkalinisation
- Leucovorin rescue: 15 mg/m2 orally or iv at 42 (T42), 48 (T48) and 54 (T54) hrs after the start of the MTX infusion. If the plasma MTX level is > 0.5 uM at T48, then continue these doses every 6 hours until MTX plasma level is <0.25 uM.

If the MTX level at the end of the MTX infusion (at 24 h) exceeds 150 µmol/l and/or there is a significant increase in serum creatinine over the baseline value, fluid intake is to be increased to 4500 ml/m^2/24 h. It is also recommended to determine the MTX level at 36 h. If the MTX level at 36h exceeds 3 µmol/l, the patient should be given 30 mg/m2 i.v. Leucovorin immediately upon receipt of the findings.

Table 16. Recommended Leucovorin doses for elevated MTX levels, applicable to levels from 42 hours onwards.



In case of delayed MTX excretion, i.e. if the MTX levels indicated in Table 16 are at all exceeded, the following procedure applies.

- Increased fluid intake of 4500 ml/m²/24 h.
- Stabilisation of urine pH at >7.0.
- Careful fluid balancing, frequent electrolyte tests.
- Administration of Leucovorin every 6 hours, adjustment of Leucovorin dose to the MTX level determined 6 hours prior, as specified in Table 16.
- If the MTX serum level at 54 hours is still ≥ 0.25 µmol/l, continue hydration and 6-hourly Leucovorin rescue and conduct repeat measurements at 6 - 12h intervals until the MTX serum level has dropped below 0.25 µmol/l.

Down Syndrome patients
Although Down Syndrome patients are known to suffer from a higher toxicity related to treatment, a general dose reduction is not advisable for the risk of recurrence. However, these patients are particularly susceptible to methotrexate induced toxicity and therefore, an *a priori* dose reduction of methotrexate is recommended in Protocol M. The first HD-MTX (Protocol M or HR-1') should be administered at a dose of 500 mg/m^2/24 h in all patients. If it has been tolerated without significant toxicity, dose increase to 2000 mg/m^2/24 h and 5000 mg/m^2/24 h should be considered for the following courses. In the case of relevant systemic toxicity (myelosuppression or mucositis) due to the intrathecal methotrexate, intrathecal dose should not be reduced.

1.6 6-Mercaptopurine and Thioguanine (intensive therapy phase)

Thiopurine analogues are used in Protocol I and II as well as in Protocol M (at a lower dose). If chemotherapy needs to be interrupted during these therapy components, for instance due to myelosuppression or infection, treatment with 6-Mercaptopurine/Thioguanine should also be interrupted. It should be ensured that missed doses are subsequently given so that the scheduled cumulative dose is reached. In Protocol M, 6-Mercaptopurine therapy should be administered until day 56 (i.e. one week after the fourth HD-MTX block), even if this means that the scheduled cumulative dose for this component is exceeded.

In case of TPMT deficiency reduce dose to 10% of the standard dose.

1.7 Vincristine/ Vindestine

The dose of vincristine may be reduced to 2/3 of the recommended dose, when severe paresis or constipation develop. If the symptoms disappear, re-introduce vincristine at a full dosage. In case of a dropping foot or when an ileus is present, vincristine is withheld until the clinical signs are completely resolved. Re-introduce vincristine at 2/3 of the recommended dose. Do not modify the dose for jaw pain but use analgesics
The level of bilirubin should be carefully monitored before vincristine administration.

1.8 Asparaginase (PEG-Asparaginase)

1.8.1 Anaphylaxis, allergic reaction, silent inactivation

If patients develop allergic reactions (urticaria, bronchial obstruction, laryngospasm, hypotension gastrointestinal symptoms etc.) during or after PEG-L-asparaginase administration, use of the same asparaginase preparation should be discontinued. It generally makes no sense to continue administering the preparation with the "protection" of antihistamines or glucocorticoids. Even if the drug is subsequently well tolerated, it must be generally assumed that it has been immunologically inactivated and that asparaginase has therefore ceased to be effective.

In the event of an allergic reaction to PEG-L-ASP or if there is clinical suspicion of an allergy, it is recommended that asparaginase activity is measured. In order to do this, a blood sample (serum) should be taken from the patient immediately after the event (approx. 1 hour after the end of the asparaginase infusion) as well as 7 days after, provided the patient has not been given another asparaginase preparation in the meantime. Some patients develop abdominal pain with asparaginase infusion, which is probably an equivalent to an allergic reaction. In these cases asparaginase activity should also be measured and the preparation be changed in case of insufficient activity. The latter

also applies to unconfirmed suspicion of allergic reaction

Administration of Erwinia asparaginase as replacement preparation in case of allergic reaction to PEG-L-ASP:

If patients develop an allergy to PEG-L-ASP, they are given Erwinia asparaginase as replacement preparation in a dose of 20.000 IU/m^2/single dose p.i. (1h) or i.m. every other day for the remaining duration of scheduled asparaginase therapy in the current and subsequent therapy components. The assumed length of effect of PEG-L-ASP is 2 weeks, so that 7 doses of Erwinia asparaginase replace one dose of PEG-L-ASP.

Administration of Erwinia asparaginase as replacement preparation in case of silent inactivation of PEG-L-ASP:

To detect silent inactivation measure serum asparaginase activity at 7 days after asparaginase administration following every re-introduction after a gap in asparaginase (Protocol II/III, HR blocks). Following evidence of insufficient asparaginase levels on day 7, indicating silent PEG-L-ASP inactivation, (level on day 7 after dose <100 IU/l) the preparation is changed to Erwinia asparaginase (procedure as per allergic reaction).

Allergic reactions to asparaginase are to be reported as SAEs or medically relevant AEs, respectively. Besides clinical signs of the allergic reaction, documentation should include the times of start and stop of asparaginase infusion, infused amount of asparaginase and time of first symptoms of allergic reaction.

The administration of native E.coli-L-ASP after reactions to PEG-L-ASP is discouraged due to the high risk of cross-reaction.

Please refer also to (van der Sluis*, et al* 2016).

1.8.2 Pancreatitis

The Asparaginase dose should not be modified in case of asymptomatically elevated amylase and/or lipase levels. If there is confirmed pancreatitis (see section “Safety” in the protocol), Asparaginase therapy has to be discontinued. As a matter of principle it is not recommended to continue Asparaginase therapy (including with other preparations, e.g. Erwinia Asparaginase) after past pancreatitis. In case of very mild forms of pancreatitis (no correlation in image morphology, improvement after only a few days with conservative treatment), reinstatement of Asparaginase may be considered following consultation with the study coordination center.

Please refer also to (Raja*, et al* 2014, Samarasinghe*, et al* 2013, Wolthers*, et al* 2017).

1.8.3 Coagulation disorders

When taking blood via the central catheter for coagulation parameter analysis, the possibility of artificial activation of coagulation and contamination by heparin should be taken into account, especially in case of remarkable or implausible findings. In doubtful cases another blood sample must be taken from a peripheral vein through venipuncture.

Remarkable coagulation values without clinical symptoms are *by themselves* no indication for postponing or modifying asparaginase doses. To date there is no evidence of the usefulness of substituting coagulation factors or fresh plasma in clinically unremarkable patients and therefore no relevant, clear recommendations can be given in this regard.

1.8.4 Thrombosis

Non-cerebral deep vein thrombosis: Thrombosis should be treated according to applicable clinical standards. If they are accompanied by significant clinical symptoms, asparaginase therapy should be interrupted until there is improvement; antithrombotic therapy/secondary prophylaxis should be continued for the remainder of intensive therapy or at least as long as the patient has a central vein catheter. Asparaginase therapy should not be interrupted, and asparaginase doses should not be modified due to pathological coagulation parameters without clinical event. Following thrombotic

complications asparaginase therapy should only be discontinued after consultation with the study coordination center. If relevant thromboses occur that are not associated with the central catheter, diagnostics for hereditary thrombophilia should be considered.

CNS thrombosis/sinus vein thrombosis: For diagnostics and therapy of cerebral thrombosis, please refer to the current recommendations (Appendix II). Asparaginase therapy should be suspended until clinical symptoms have improved and should be resumed at the full dose. Any missed doses should be administered, if possible. Antithrombotic therapy/secondary prophylaxis should be continued for the remainder of intensive therapy or at least as long as the patient has a central vein catheter. Diagnostics for hereditary thrombophilia should be considered

Please refer also to (Grace*, et al* 2011)**.**

1.8.5 Hyperglycemia

No dose modification of Asparaginase (or glucocorticoids). Insulin treatment should be administered, if indicated.

1.8.6 Hyperlipidemia

Transient hypertriglyceridemia can be adequately managed with dietary modifications and close monitoring without altering chemotherapy. In case of highly elevated levels (e.g. triglycerides >2000 mg/dl *or >20 UNL*), closely scheduled pancreas enzyme controls are recommended due to the increased risk of pancreatitis. Patients with hypertriglyceridemia have a possible increased risk of thrombosis. Pseudohyponatriemia is commonly observed.

1.9 Therapy modifications for Imatinib toxicity

1.9.1 Non-Hematologic Toxicity

Grade 3/4:
If a patient experiences unexpected non hematological Grade 3-4 toxicity, IMATINIB must be withheld until the toxicity has resolved to < Grade 2 or lower. An assessment of possible drug interactions should be performed, and any drug that is suspected as contributing to the toxicity should be reevaluated regarding its dosage and/or necessity of administration. When toxicity is grade 2 or lower, IMATINIB should be reintroduced at 20% less dose.

Hepatic Toxicity:
Patients with a total serum bilirubin < 1.5 x ULN at baseline who experience Grade 3-4 elevations should be managed using the criteria detailed above for non-hematological toxicity

1.9.2 Hematological Toxicity

Patients developing anemia are transfused at the discretion of the treating centerr. No dose reductions are foreseen for any grade of anemia, except for Grade 3 and 4 anemia resulting from an acute cause considered to be related to administration of IMATINIB (e.g. severe gastrointestinal hemorrhage).
In the event of Grade 3 thrombocytopenia (platelets 10.000<50.000/µl) or neutropenia (500<1000 /µl) not accompanied by clinical manifestations, i.e. significant bleeding or neutropenic fever, potential causes of the cytopenias should be considered (e.g, leukemic progression, infection); if the thrombocytopenia or neutropenia are attributed to IMATINIB, the dose will be reduced at 20% less dose.
With the occurrence of Grade 4 thrombocytopenia (platelets <10.000/µl), not accompanied by clinical manifestations, platelets support and continuation of the drug are suggested.
G-CSF is permitted in the case of neutropenic fever or suspected drug induced neutropenia. If Grade 3 or 4 thrombocytopenia or neutropenia are accompanied by clinically significant bleeding or evidence of an infection, IMATINIB will be interrupted until toxicity has resolved to grade 2 or lower and the clinical situation has stabilized; IMATINIB will then be restarted at 240 mg/m^2 daily.

APPENDIX III
SUPPORTIVE CARE

1 Toxicity with therapy, general recommendations

Supportive care is needed because of the likelihood of infections, metabolic disturbances and organ damage especially in the time period between diagnosis and start of maintenance therapy. Most centres will have their own supportive care protocols, which should be followed.

Some relevant guidelines are set out in the protocol; however, these recommendations do not claim to be complete. Additionally these recommendations always need to be interpreted in light of the individual patient's specific situation and to be modified or extended, if necessary.

It is essential to monitor carefully not only specific side effects of therapy, but also patients' clinical conditions. Such monitoring should always be supervised by an experienced pediatric oncologist. Patients' parents need to be given detailed instructions and information about the importance of certain precautions and measures. They should be informed in great detail about critical clinical signs requiring immediate presentation at a pediatric oncology clinic.

2 Acute tumor lysis syndrome

2.1 Pathophysiology

Tumor lysis syndrome (TLS) is the consequence of rapid cytolysis, resulting in the release of various cell components. It may occur spontaneously without treatment or within a few days up to a week after initiation of therapy. In this process, the purine metabolites hypoxanthine, xanthine and uric acid are released in particular, as well as phosphate and potassium, which are then excreted via the kidneys.

Metabolic disorders typical of TLS are **hyperuricemia**, **hyperpotassemia** and **hyperphosphatemia** as well as, consequently, **hypocalcemia**.

Hyperuricemia: The effect of xanthine oxidase helps converting purines released during cytolysis into xanthine and uric acid via hypoxanthine. Uric acid does not dissolve readily, especially in acidic environments, and may crystallize in the distal renal tubuli, the collecting ducts and the renal parenchyma. Hypoxanthine and xanthine generally dissolve much more readily than uric acid and can be eliminated more easily via the kidneys. However, the solubility of hypoxanthine and xanthine decreases significantly with pH values > 7.5, so that renal precipitation in an alkaline environment can result in xanthine/hypoxanthine nephropathy.

Hyperpotassemia: Hyperpotassemia may develop early on during TLS and can rapidly progress to life-threatening complications. Renal insufficiency may intensify an existing hyperpotassemia, and an on-setting acidosis additionally causes extracellular potassium levels to rise. Clinical symptoms of hyperpotassemia are muscle weakness, lethargy, ECG changes and eventually cardiac irregularities in the form of arrhythmia and sudden cardiac death.

Hyperphosphatemia and **hypocalcemia:** Phosphate serum levels usually only increase several hours after hyperpotassemia develops. High phosphate levels cause the solubility product of calcium and phosphate to be exceeded, particularly with alkaline pH levels, and consequently calcium phosphate to precipitate in soft tissues and organs, especially the renal parenchyma. This in turn causes acute nephrocalcinosis with renal failure and hypocalcemia with clinical symptoms such as muscle spasms, tetany, seizures and cardiac irregularities.

The resulting functional kidney and heart disorders cause acidosis, which in turn intensifies the metabolic disorders and electrolyte imbalances through a vicious circle.

Definitions of laboratory and clinical TLS (Cairo and Bishop 2004):

Laboratory TLS:

2 or more of the following factors within 3 days before or 7 days after initiation of therapy:

- Uric acid: ≥ 480 µmol/l (8 mg/dl) or 25% increase from base lin
- Potassium: ≥ 6 mmol/l or 25% increase from base line
- Phosphate: ≥ 2.1 mmol/l (6.5 mg/dl) or 25% increase from base line
- Calcium: ≤ 1.75 mmol/l (7.0 mg/dl) or 25% decrease from base line

Clinical TLS:

Laboratory TLS and any of the following factors without any other obvious explanation:
- Creatinine: ≥ 1.5 x upper limit of normal
- Cardiac arrhythmia
- Seizures

2.2 TLS prevention

The risk of developing TLS depends on the leukemia cell mass and the apoptosis rate of leukemia cells, both spontaneously before and after initiation of therapy. It can be estimated on the basis of the following factors:

- WBC (white blood cell count):
  - high risk: ≥ 100.000/µl and LDH ≥ 2x ULN (upper limit of normal) < 100.000/µl and LDH ≥ 2x ULN
  - intermediate risk: < 100.000/µl and LDH < 2x ULN

- Organomegaly: size of liver and spleen as well as any mediastinal or other tumor
- Initial LDH and LDH after start of therapy elevated
- Initial uric acid and uric acid after start of therapy elevated

Patient-related factors associated with the development of TLS:

- Renal function disorder, pre-existing or due to leukemic infiltration of the kidney
- Obstruction of efferent urinary tracts, e.g. due to lymphomas
- Pre-existing constitutional hyperuricemia
- Exsiccosis or insufficient hydration
- Excessively low urine pH (risk of urate nephropathy)
- Excessively high urine pH (risk of nephrocalcinosis and xanthine/hypoxanthine nephropathy due to calcium phosphate and xanthine/hypoxanthine precipitation\
- Simultaneous administration of potentially nephrotoxic substances.

The above suggests the following preventive and therapeutic measures:

Patient monitoring:

- Monitoring of fluid intake and output with balancing every (3-)6-12hours
- Once to twice daily weight checks
- Vital parameter checks at least every 6 hours
- Closely scheduled laboratory tests, particularly of uric acid, potassium, phosphate, calcium, sodium, chloride, creatinine, blood glucose and LDH, coagulation parameters incl. D-Dimers, blood counts (every 8-12-24h, in critical cases and initially also more frequently).
- In case of clinical TLS manifestation intensive care unit monitoring is recommended.

Preservation and improvement of renal function:

- Sufficient fluid intake: 3000-5000ml/m^2/day (5%glucose in half-isotonic NaCl solution), target for urine output 100 - 250 ml/m^2/h.
- Slight alkalinisation of urine (Bicarbonate: $NaHCO_3$ 120-200mmol/m2/day, ideally as a parallel infusion)
  - Control of sodium bicarbonate intake depending on urine pH
  - Target urine pH of 7.0
  - No alkalinisation with administration of Rasburicase (Fasturtec®).

- In case of insufficient output: Furosemide: up to 10 mg/kg/day in several individual single i.v. doses or as a continuous infusion.

• Avoidance of potentially nephrotoxic substances (aminoglycosides, X-ray contrast medium, non-steroidal antirheumatics)

Correction of electrolyte imbalances:

- Initially no addition of potassium to the infusion solution
- For the treatment of electrolyte imbalances see below

Therapy medication

- Allopurinol 10 mg/kg/day or 300 mg/m^2/day p.o. in (maximum absolute dose 400 mg/day) 2 - 3 single doses
  - Allopurinol and its metabolite oxipurinol, which is formed by xanthine oxidase, inhibit xanthine oxidase and thus conversion of xanthine to hypoxanthine and eventually to uric acid. Hypoxanthine and xanthine dissolve much more readily than uric acid and can be eliminated more easily via the kidneys.
  - In case of renal insufficiency the allopurinol dose must be adjusted, although with
- For special indications (see below): Rasburicase (Fasturtec®) 0.2 mg/kg/day in 50 ml NaCl 0.9% as single dose over 30 min i.v.
  - Rasburicase (recombinant uratoxidase) metabolizes already formed uric acid to form allantoin, which dissolves approximately 10x more readily.
  - No urine alkalinisation which may increase the risk for xanthine/hypoxanthine and calcium/phosphate nephropathy and no additional allopurinol which may withdraw the substrate (uric acid) of Rasburicase during Rasburicase therapy.
  - Serum to be stored and transported on ice until uric acid analysis!
  - Acute and delayed allergic reactions are possible. Keep emergency medication available at the bedside!
  - Rasburicase is contraindicated in patients with G6PD deficiency as it may cause hemolytic anemia and methemoglobinemia!
  - **Rasburicase therapy is to be considered in case of**
    - Clinical TLS (as defined above!)
    - high risk for TLS (Cairo and Bishop 2004): peripheral leukocytes ≥ 100.000/µl and LDH ≥ 2x ULN peripheral leukocytes < 100.000/µl and LDH ≥ 2x ULN
    - intermediate risk for TLS (Cairo and Bishop 2004):
      - peripheral leukocytes < 100.000/µl and LDH < 2x ULN **and**
      - large mediastinal tumor or other massively enlarged organs **or**
      - elevated or rising uric acid levels despite hydration

2.3 Therapy of electrolyte imbalances

2.3.1 Hyperpotassemia

- Potassium ≥ 6 mmol/l: Consider/prepare for hemodialysis (or transfer patient to an intensive care unit)

- Potassium ≥ 7 mmol/l: Hemodialysis

Exclude pseudo-hyperpotassemia due to hemolysis during or after sampling.

Acute measures:

1. Increase of urine production through hydration (only with sufficient renal function):
   - NaCl 0.9%: 10 - 20 ml/kg as short infusion over 1h, followed by Furosemide: 1 - 10 mg/kg/day i.v.
   -
2. Potassium shift into intracellular spaces

- 1g glucose/kg/h + 1IU normal insulin/4gglucose **or** 100 ml glucose 10% + 2-3 IU normal insulin, of which 2 ml/kg are given as a bolus and then 10 ml/kg/h as a continuous infusion
- In case of metabolic acidosis:1-2 ml/kg $NaHCO_3$ 8.4% over 20 min i.v
- Salbutamol
    - Inhalation: 0.1 mg/kg (1 drop/year of age), repeat if necessary (max. 10 drops/single dose)
    - Intravenous: 1 - 2 µg/kg slowly i.v., continuous infusion: 0.1 µg/kg/min if necessary

Effect within15-30min., length of effect approximately 2-4 hours, since potassium then returns to extracellular spaces.

3. Potassium elimination via cation exchanger resins:
    - ResoniumA®:0.5-1g/kg/day in several single doses rectal/p.o.(no rectal enema in patients with neutropenia!).

4. Functional antagonisation of potassium effect: Calcium is the fastest way for reversing cardiac effects of hyperpotassemia! The onset of action is within minutes, but the duration of working is only about 30 minutes.
    - Calcium gluconate10%: 0.5-1ml/kg slowly i.v.
        - In case of symptomatic hyperpotassemia with ECG changes
        - Injection of calcium gluconate only with ECG monitoring. Warning: bradycardia!
        - In case of persisting changes possibly repeat administration after 5 - 10 min.
        - Calcium gluconate does not decrease serum potassium levels!

2.3.2 Hyperphosphatemia

- Increased fluid intake (up to 5000 ml/m2/24h).
- Urine pH should not increase to > 7.0 which may increase the risk for calcium/phosphateprecipitation in soft tissues and organs.
- Stop enteral food, replace by phosphate-free parenteral feeding in order to reduce exogenous phosphate intake, alternatively aluminium hydroxide p.o. 0.1 g/kg to bind phosphate from food.

2.3.3 Hypocalcemia

- Correction by intravenous calcium doses only in case of symptomatic hypocalcemia and/or normal serum phosphate levels.
- Administer calcium gluconate 10% 0.5 - 1 ml/kg slowly i.v. only with ECG monitoring. Warning: Bradycardia.
- Calcium levels cannot be normalized if magnesium levels are too low. Therefore magnesium levels also need to be determined and corrected.

2.4 Renal failure, hemodialysis

Definition of oliguria/anuria:

Urine output < 50 ml/m^2/h despite Furosemide therapy 10 mg/kg/day i.v. and fluid supply of 130 - 200 ml/m^2/h.

The “standard” definition of anuria (urine output < 5ml/m^2/h) is not useful in this situation, as output needs to be assessed in relation to fluid intake, and potassium release can be so intense that life-threatening serum potassium levels may be reached before urine output is reduced this far.

Diagnostics with decreasing urine output:

- Sonography: urinary tract obstruction (due to lymphoma), renal infiltration
- Serum: levels of calcium, phosphate, potassium, sodium, creatinine, urea (BUN) and uric acid.
- Urine: levels of calcium, phosphate, potassium, sodium and creatinine, uric acid crystals, calcium phosphate crystals

Indications for hemodialysis:

- Potassium: > 7 mmol/l, or > 6 mmol/l and increasing despite fluid therapy and diuretics
- Phosphate: > 7 mmol/l, or > 6 mmol/l and increasing despite fluid therapy and diuretic
- Creatinine: > 10 x normal range for age.
- Urine output: < 50 ml/m^2/h despite Furosemide therapy 10 mg/kg/d i.v. and fluid supply of 130- 200 ml/m^2/h.
- High-grade or full urinary tract obstruction on both sides

Excessively elevated uric acid levels (> 600 µmol/l or 10 mg/dl) do not inevitably require dialysis as long as there still is sufficient kidney function. It should be attempted first to reduce uric acid concentrations with Rasburicase under intense monitoring.

3 Infections

(Simon, et al 2016) (Lehrnbecher, et al 2017) (Lehrnbecher, et al 2018)

The treating physician is responsible for infection prophylaxis and therapy. The following information should therefore be seen as guidance rather than as binding guidelines. As already emphasized in section 1.1 of this appendix, careful patient monitoring, closely scheduled examinations by experienced pediatric oncologists and thorough instruction of parents are essential. Patients should be kept in the hospital especially during the neutropenic episodes of induction therapy (Protocol I) or they should be checked at the outpatient clinics every or at least every 2nd day. During induction therapy (Protocol IA), patients can only be discharged or treated in an outpatient setting if they are in a clinically perfect condition and no signs of upcoming infection are present. Similar precautions should be taken during the neutropenic episodes of re-induction chemotherapy elements.

3.1 General preventive measures

Consequent hand hygiene is one of the most relevant measures preventing communicable infection. All persons must wash and, in particular, disinfect their hands thoroughly before patient contact. Patients need to wash hands regularly throughout the day, especially before meals and after attending bathroom. During intensive therapy, crowds (i.e. public transport, super markets), attendance at pre-school or school and contact with persons not immunized against common childhood diseases (varicella zoster, measles, mumps, rubella) should be avoided. Contact with non-infected and fully immunized friends, peers and siblings should be supported.

The greatest infectious risk for patients stems from their endogenous colonization with potential pathogens. The microbial spectrum of life-threatening infections suggests that intestinal microorganisms are an important potential source of infection. Constipation and (sub)ileal conditions promote the growth of bacteria and fungi in the intestinal lumen and the invasion of mucous membranes, particularly where these are already affected by cytostatic therapy and/or infiltration. It is therefore important to ensure regular bowel movements, i.e., by administering oral Lactulose or Polyethylenglycol (Osmotica). Hydragoga such as Natrium-picosulfate or Bisacodyl may also be used. If these measures remain unsuccessful, enemas need to be given; however, in neutropenic patients these are to be avoided.

Due to potential colonization with pathogens (fungi, bacteria), certain foods are to be avoided during intensive therapy phases, i.e., fresh nuts, unpasteurized milk, blue vein cheese, raw milk cheese as well as raw meat and fish. In general food should be fresh and preparation should avoid contamination with environmental pathogens (i.e. soil).

3.2 Medication for infection prophylaxis

3.2.1 Prophylaxis against bacterial infection

There is no evidence that the administration of non-absorbable antibiotics (e.g. colistin, gentamycine, polymyxin, paromomycine) for total or selective intestinal decontamination reduces incidence and severity of infectious complications in adult or pediatric patients (review (Alexander*, et al* 2012, Simon*, et al* 2001)).

Various meta-analyses of cotrimoxazole (trimethoprim/sulfamethoxazole) and fluoroquinolone (i.e.,

ciprofloxacin) have confirmed the effectiveness of systemic, oral antibiotic prophylaxis in adult oncology patients with neutropenia (Gafter-Gvili, *et al* 2012) (van de Wetering, *et al* 2005), with fluoroquinolones having been the preferred drugs since the 1990s due to their better side effect profile, although a decrease of mortality has not been observed (Bucaneve, *et al* 2005). As these drugs have been associated with cartilage damage in growing puppies and because of the associated high risk of bacterial resistance development (Bucaneve, *et al* 2005, Castagnola, *et al* 2005), fluoroquinolones are not generally recommended for children and adolescents. At this stage the usefulness of cotrimoxazole prophylaxis (except for pneumocystis jirovecii prophylaxis) cannot be confirmed for pediatric oncology patients, and for this reason no recommendation is given for antibacterial prophylaxis in pediatric oncology on the basis of currently available studies (Alexander, *et al* 2012).

3.2.2 Prophylaxis against fungal infection

Although oral prophylaxis with Amphotericin B suspension or tablets reduces colonization with most Candida species, its effectiveness in preventing invasive fungal infections, particularly infections with Aspergillus and mucormycetes, has not yet been confirmed (review in (Groll, *et al* 2001) and (Cornely, *et al* 2003)). A meta-analysis failed to confirm the effectiveness of oral Nystatin (Gotzsche and Johansen 2002). According to published studies, fluconazole appears to be effective in the prophylaxis against invasive Candida albicans infections (Groll, *et al* 2001).

However, Fluconazole is not effective in the prophylaxis against or the therapy of non- albicans Candida infections (i.e., Candida krusei, Candida glabrata) and of mold infections (i.e., Aspergillus and mucormycetes species), cause the majority of invasive fungal infections (Hale, *et al* 2010). The selection of primarily fluconazole-resistant Candida species as well as the development of secondary resistances are additional potential problems of fluconazole prophylaxis.

According to the European Conference on Infections in Leukaemia (ECIL) (Groll, *et al* 2014) antifungal prophylaxis is indicated for patients with high risk (>10 %) for invasive fungal diseases (IFDs).

Although there is a lack of well-performed randomized studies in children and adolescents with ALL showing in which treatment phase which type of drug in which dosage is effective in anti-fungal prophylaxis, an increased risk of IFD is observed for older children and adolescents in therapy phases with glucocorticoids (mainly with Dexamethasone) in particular in association with prolonged neutropenia. An antifungal prophylaxis might therefore be indicated for selected patients in the treatment phases with glucocorticoids (Protocol IA, IIA, IIIA and after the HR-1', HR-2' and HR-3' blocks). In these therapy phases liposomal Amphotericin B, Voriconazole, Itraconazole, Posaconazole or Micafungin may be used at the discretion of the treating centers. For Voriconazol, Itraconazol and Posaconazol suspension, therapeutic drug monitoring is recommended. Azoles should not be used with vinca alkaloids or Bortezomib due to the risk of increased neurotoxicity.

The following drugs and schedules are recommended for antifungal prophylaxis according to the ECIL-4 guidelines (Groll, *et al* 2014). Treating centers should implement the prophylaxis having regard to the chemotherapy phase and their local policy:

- Liposomal Amphotericin B: 1 mg/kg i.v. every other day or 2.5 mg/kg, twice weekly
- Micafungin : 1 mg/kg/day (max. 50 mg) i.v. in 1 single dose or 4 mg/kg every 3 - 4 day
- Posaconazole: ≥13years and adults:2x300mgonday1,followedby1x 300 mg p.o. (TDM for suspension recommended)
- Voriconazole:

  ≥ 2 - 14 years: 18 mg/kg/day i.v. in 2 single doses on day 1, followed by 16 mg/kg/day i.v. in 2 single doses or 18 mg/kg/day p.o. in 2 single doses (max. 350 mg/d);

  ≥ 15 years: (or > 50 kg age ≥ 2 years) 12 mg/kg/d i.v. in 2 single doses on day 1, followed by 8 mg/kg/d i.v. in 2 single doses i.v. or 800 mg/d p.o. on day 1, followed by 400 mg/d in 2 single doses. (TDM is recommended, target trough level > 1 and < 5,5 mg
- Itraconazole: ≥ 6 months: 5 mg/kg/d p.o. in 2 single doses. (TDM is recommended, target trough level > 0,5 mg/l)
- Fluconazol:

1 month - 12 years: 8-12 mg/kg/d p.o. or i.v. in 1 single dose (max 400 mg/d);

≥ 13 years and adults: 400 mg/d p.o. or i.v. in 1 single dose.

3.2.3 Pneumocystis jirovecii prophylaxis

For prevention of Pneumocystis jirovecii pneumonia (PCP), all patients should receive stringent prophylactic treatment with trimethoprim/sulfamethoxazole (TMP/SMZ, cotrimoxazole) for the entire duration of therapy until about three months after maintenance therapy.

Cotrimoxazole p.o.: 5 mg trimethoprim portion/kg/day p.o. in 2 single doses on two or three subsequent days of the week.

Alternatively in case of cotrimoxazole intolerance:

Pentamidine aerosol: < 4 years: 150 mg/month in 5 ml aqua dest. over 20 - 30 min ≥4 years: 300mg/month in 5ml aqua dest. over 20-30 min every four weeks

(If necessary, use of a ß2_sympathomimetic before and after inhalation in order to prevent broncho-obstructive reactions).

Dapsone (children over the age of 1 month): 2 mg/kg (max. 100 mg)/day p.o.

Atovaquone for patients with G6PD deficiency (only off-label use in children): age < 3 months and ≥ 24 months: 30 mg/kg/d; age: ≥3 - <23 months 45 mg/kg; age ≥ 13 years and adults 30 mg/kg (max. 1500 mg) daily in a single dose p.o.

3.2.4 Varicella zoster virus (VZV) prophylaxis

Patients and parents must be informed that contact with persons with florid Varicella or Herpes zoster is to be strictly avoided throughout the entire therapy (including maintenance), as any Varicella infection during therapy, with its associated immune compromisation, continues to be a potentially life-threatening disease despite available antiviral treatment. Patients and parents must be informed that treating physicians are to be immediately notified of suspected contact with Varicella so that they can introduce appropriate post-exposure prophylaxis. If there has been contact, there is a risk of disease for at least 28 days, regardless of the serological status. This risk is, however, considerably lower for sero- positive patients (Gershon and Steinberg 1989). There have been individual reports on patients who developed Varicella again during immunosuppressive therapy, despite a history of this condition (Feldman and Lott 1987). So far no controlled study has been conducted on the best post-exposure procedure. The standard response continues to involve passive immunoprophylaxis with VZV immunoglobulin or eventually polyvalent immunoglobulins. There are, however, references in literature to acute forms of the disease developing within 72 hours of exposure, even where prophylaxis was administered in time, and in some of these cases the disease took a severe course (Rössig*, et al* 1997). Post-exposure prophylaxis in the form of preventive acyclovir therapy has been investigated in sero- negative children (Asano*, et al* 1993) (Suga*, et al* 1993), but not in patients undergoing chemotherapy.

The following recommendations are made in consideration of available data:

All patients who have been exposed to Varicella should be isolated from the 8th until 28th day from exposure if there is any inherent or other reason for being in the hospital in order to protect other hematologic-oncologic patients.

VZV IgG negative:

Varicella zoster immunoglobulins [0.2 - 0.5 ml/kg i.m. (max. 5 ml) or 1 ml/kg i.v.: 1 ml/min], administration within 24 h - 96 h from exposure. Chemoprophylaxis is recommended in addition to VZV immunoglobulins:

Acyclovir 60 - 80 mg/kg/d p.o. in 3 - 4 single doses over at least 14 days from day 7 from exposure, if necessary introduction of i.v. therapy over 2 - 3 days: 30 - 45 mg/kg/day in 3 single doses.

alternatively: For age ≥ 12 years valgacyclovir 2 x 1000 mg/d p.o.

alternatively: Brivudine 2 mg/kg/day p.o. in 1 single dose (max. 1 x 125 mg) over at least 14 days from day 7 from exposure (off-label use in children, warning: interaction with 5-FU and probably also 5-Flucytosine).

VZV IgG positive

No clear prophylaxis recommendations can be given. Since it is uncertain whether there is full protection against re-infection with chemotherapy, prophylaxis as above with Acyclovir is recommended

With chemotherapy (including maintenance), VZV immunization is not recommended due to the risk of infection with the vaccine virus (Schrauder*, et al* 2007)

3.2.5 G-CSF

Therapy with recombinant human granulocyte growth factor (rh-G-CSF) reduces the extent and duration of neutropenia with chemotherapy as well as the incidence of febrile phases during this period (Welte*, et al* 1996), but not the risk fatal infectious complications.

All HR patients receive G-CSF from the 11th day of initiation of each therapy block. G-CSF treatment should be given until neutrophil granulocytes exceed 3.000-5.000/µl in peripheral blood, since levels frequently drop sharply once G-CSF is discontinued. In case of infection, G-CSF treatment should be continued, depending on severity. G-CSF should be discontinued by two days before the start of the next chemotherapy block.

Dose: G-CSF 5 µg/kg/day s.c. in 1 single dose or, in exceptional cases, G-CSF 5 µg/kg/day p.i. (1-4 h).

During protracted neutropenia with severe, life-threatening septicemia and/or systemic mycosis, G-CSF can be used generously in all patients, regardless of their risk group.

3.3 Fever in neutropenia without a focus (febrile neutropenia)

With granulocyte levels <500/µl and temperatures (taken orally, through the ears or by forehead) ≥38.5°C or repeated measurements ≥38.0°C, relevant diagnostics as well as

systemic antibiotic therapy must be introduced immediately. In patients with high therapy risk (induction therapy, other therapy phases involving glucocorticoids [re-induction, HR blocks]), broad-spectrum antibiotic therapy and, if necessary, rapid escalation are required in order to prevent severe courses.

3.3.1 Diagnostics

- Anamnesis current medical history
- Full clinical examination of the patient
- Cultures: blood from all catheter tubes, stool (incl. clostridium difficile), respiratory exsudates, wounds, drains, possibly urine.
- Swabs taken from throat, skin and mucous membrane lesions and anus
- If clinically indicated lumbar puncture to assess cerebrospinal fluid
- Depending on diagnostic options of the treating centers: possibly pan-fungal and pan-bacterial PCRs, Candida Ag, Aspergillus Ag (Galactomannan), ß-D-Glucan
- If clinically indicated, PCRs from blood for HSV, VZV, CMV, Adenovirus and EBV and possibly. respiratory and gastro-intestinal viruses
- Virus isolations from mucosal and skin lesions, urine, stool and cerebrospinal fluid. Chest X-ray; if clinically indicated, chest computer tomography (CT)
- Abdominal sonography; if clinically indicated, abdominal magnetic resonance imaging (MRI)
- If clinically indicated, MRI of the neurocranium (possibly of the whole neuroaxis)
- Laboratory diagnostics (blood counts and chemistry) as performed at the treating centers

3.3.2 Therapy

Antibiotic therapy, which initially will usually be empirical, should be modulated according to the patient's individual situation (symptoms, prior infections, treatment phase) and the hospital's specific pathogen and resistance spectrum. As a matter of principle, initial antibiotic therapy should be broad and cover pathogens commonly found in oncology patients. Analyses of serious infections during the ALL-BFM 2000 study revealed that approx. three quarters of all life-threatening and fatal bacterial infections were caused by gram-negative rods, predominantly by E. coli and Pseudomonas aeruginosa, followed by gram-positive cocci (Staphylococcus aureus, Enterococcus and Viridans Streptococci). Gram-positive rods (Bacillus spp.) were considerably less common, but caused infections with particularly fulminant clinical courses. Any initial, empirically selected treatment should definitely cover these pathogens, taking into account the local resistance spectrum.

If there is no clinical improvement, persistent fever and signs of inflammation with the initially chosen therapy, treatment should be escalated or changed at the latest after 48-72 hours, depending on the patient's clinical situation. Fungal infections should be considered during therapy phases involving glucocorticoids in particular, after prolonged neutropenia or with pulmonary or central nervous symptoms. The infectious high-risk phases of neutropenia are in Protocol I-A, II-A, and after the HR1', HR2' and HR3' blocks). Relevant diagnostics should be introduced early and anti-infectious therapy adjusted accordingly, if necessary.

The following is a recommended initial treatment plan with subsequent escalation; an initial combination therapy is to be preferred:

Broad spectrum beta-lactam antibiotics:

- Ceftazidim: 100 - 150 mg/kg/day (max. 6 g/d) in 3 single doses ***OR***
- Cefepime: 100 - 150 mg/kg/day (max. 6 g/d) in 3 single doses ***OR***
- Meropenem: 60 mg/kg/day (max. 4 g/d) in 3 single doses ***OR***
- Imipenem-Cilastatin: 60 mg/kg/day (max. 4 g/d) in 3 single doses ***OR***
- Piperacillin/Tazobactam: 240-300 mg/kg/day (max. 4 x 4 g/d) in 3-4 single doses

***PLUS* an aminoglycoside:**

- Amikacin: 15 - 20 mg/kg/day (max. 1,5 g/d) in 1 single dose ***OR***
- Tobramycin: 7 - (10) mg/kg/day (max. 400 mg/d) in 1 single dose ***OR***
- Gentamicin: 5 - 10 mg/kg/day (max. 400 mg/d) in 1 single dose

If there is no improvement within 48 – 72 h, glycopeptide treatment should be considered:

- Vancomycin: 40 - 60 mg/kg/day (max. 2 g/d) in 3 single doses ***OR***
- Teicoplanin: initially 3 x 10 mg/kg every 12 hours, then 10 mg/kg/day in 1 single dose (max. 400 mg/dose)

If there is no improvement within another 48h, an antifungal drug covering Aspergillus species should be added (Fluconazole is not effective against Aspergillus!)**.**

For recommendations of empirical antifungal therapy according to ECIL-4 guidelines see section **3.5.2** of this appendix (Groll*, et al* 2014):

This plan is only an example and cannot replace any national or center-specific guidelines for the treatment of fever in neutropenia without a focus. It should be adjusted to the relevant local pathogen and resistance spectrum in consultation with local microbiologists and needs to be extended or modified in keeping with individual requirements, clinical evidence and microbiological findings. If there is suspicion or evidence of an atypical pneumonia, antibiotic therapy is to be supplemented by a macrolide or tetracycline antibiotic. Moreover, due to the lack of efficacy against Pseudomonas

aeruginosa, the 3rd generation cephalosporine ceftriaxone should not be used as for initial monotherapy of febrile neutropenia. Fluoroquinolones such as Ciprofloxacin or Levofloxacin are also not to be used as for initial antibiotic therapy due to the selection of especially multi-resistant gram-negative bacte

3.4 Pneumocystis jirovecii pneumonia

Despite cotrimoxazole prophylaxis, some, even though only few, infections with Pneumocystis jirovecii were reported during the ALL-BFM 2000 study. It could not be determined in all cases whether there had been unreliable compliance with prophylaxis. Pneumocystis jirovecii usually manifests in the lungs; extra-pulmonary manifestations are extremely rare. The disease is characterized by fever and a restrictive respiratory disorder with progressing hypoxia. Auscultation is often unremarkable. Chest X-rays show striated and nodular interstitial changes beginning in the center, which are later overshadowed by alveolar changes; in CT ground glass opacity due to inflammatory infiltration of the alveoli, linear and reticular opacities (interlobular septal lines) as well as patchy opacities are seen.

3.5 Invasive/systemic fungal infections

Extensive diagnostics should be initiated if there is clinical suspicion such as no reduction of fever with antibiotic therapy, pulmonary or cerebral clinical symptoms.

3.5.1 Diagnostics

- Cultures: blood from all catheter tubes, stool, respiratory exsudates, wounds, drains
- Swabs taken from nose, throat, skin and mucous membrane lesions and anus If clinically indicated, lumbar puncture to assess cerebrospinal fluid
- Depending on diagnostic options of the treating centers: pan-fungal PCRs
- Aspergillus Ag (Galactomannan), ß-D-glucan, Candida Ag,
- Imaging diagnostics (depending on individual risk and clinical symptoms: o ChestCT o AbdominalsonographyandabdominalMRI o XCT of nasal sinuses o MRI of the neuroranium (possibly of the whole neuroaxis)
- Biopsy or resection of the lesions for cytomorphological analysis, histopathological diagnosis, cultures and PCRs
- Laboratory diagnostics (blood counts and chemistry) as per performed at the treating centers

3.5.2 Therapy

Empirical therapy (according to the policy of the treating center):**Recommended antifungal therapy according to ECIL-4 guidelines** (Groll*, et al* 2014)**:**

- Invasive aspergillosis (first line):
- Invasive candidosis:

Voriconazole, liposomal Amphotericin B, Amphotericin B lipid complex or antifungal combination therapy with Echinocandin plus polyene or triazole.

Caspofungin, Fluconazole, liposomal Amphotericin B, Micafungin, Voriconazole or Amphotericin B lipid complex.

3.6 Varicella and herpes zoster

As a rule, new infections with VZV take a very severe course during immunosuppression and represent a very dangerous condition. The presentation is frequently atypical; patients often develop signs of hepatitis through to acute liver failure, pneumonia and/or encephalitis even during the initial viremic stage. Rash, if present at all, often occurs later in the course of the disease and is frequently atypical. This also applies if the disease occurs during maintenance, where efflorescences initially often have an atypical appearance.

If there is suspected Varicella infection, antiviral treatment should be initiated immediately. Since virus serology is often not useful with chemotherapy, the pathogen can frequently only be identified by repeated PCR analyses of blood and, where applicable, vesicle content. Past chickenpox infection or a positive VZV IgG status do not exclude re-infection during chemotherapy.

Therapy of manifest disease:

• Acyclovir i.v. 30 - 45 mg/kg/day in 3 (-4) single doses p.i. (1 h)

The duration of therapy depends on the severity of infection; 5 days (uncomplicated Herpes zoster) to at least 21 days (systemic VZV infection).

• Varicella zoster immunoglobulin or polyvalent immunoglobulins In case of suspected acyclovir resistance: Foscarnet

4 Sinus vein thrombosis; Recommendations for diagnostics and therapy symptoms

The clinical signs of cerebral SVT are often subtle, uncharacteristic and diffuse and may develop over several hours or days.

Each unclear neurological change during induction therapy, no matter how subtle, must always be viewed from the aspect of potential SVT and therefore requires **immediate** imaging diagnostics and introduction of appropriate anti-thrombotic therapy.

The most common symptoms in children aged above 1 year are:

- Headaches
- Seizure-like phenomena
- Vomiting
- Alterations of consciousness
- Focal neurological signs, incl. hemiparesis and cranial nerve paresis
- Diffuse neurological signs
- Possibly fever

4.1 Radiological diagnostics

Objective: Detection of thrombus/thrombi and/or reduced or absent flow in the cerebral venous system.
Immediate cerebral computer tomography (CCT) => exclusion of bleeding
Then as quickly as possible, but no later than within 24 hours Magnetic resonance tomography including venogram.

Important:
The **problem of potential SVT** must be noted on the CT/MR request.
A normal CT **does NOT exclude** SVT!

4.2 Laboratory

Only **platelets**, **AT III**, possibly **protein C** and current **PTT**, if unfractioned heparin is used, are of therapeutic relevance (see E. Therapy). D-dimer levels are only suitable as individual course parameters in order to document the efficiency of the antithrombotic therapy administered more extensively. **Neither do normal D-dimer levels exclude venous thrombosis, nor do elevated levels confirm it!**

4.3 Anticoagulation, general comments

Unless there is extensive, severe bleeding (see E), anticoagulation therapy is indicated. The neurological outcome in particular is poorer for children receiving purely supportive treatment. For this reason every SVT should be treated with appropriate antithrombotic therapy.

Unfractioned heparin (UFH) and **low-molecular weight heparin (LMWH)** are available for this purpose; in individual cases also possibly antithrombin or protein C* (see E: Severe post- thrombotic bleeding).

Thrombolysis is not indicated during induction therapy.

According to currently available evidence, the two forms of heparin are to be treated as equivalent in treating SVT, but they differ in their practical application and side effect profile. The decision whether to use UFH or LMWH is to be taken at the discretion of the treating hospital or primary physician respectively, taking into account the patient's individual clinical situation as well as the following criteria:

4.4 Practical therapeutic procedure

4.4.1 Time of therapy start

Immediately after CCT, if primary cerebral bleeding and severe post-thrombotic bleeding have been excluded

4.4.2 Heparinisation

In case of SVT during induction regardless of any hereditary thrombophilia

4.4.2.1 Low-molecular weight heparin

Initial dose (therapeutic, for children > 1 year)
Enoxaparin (Clexane®): 100 - 120 IU/kg, 2x daily s.c
1.0 - 1.2 mg/kg, 2x daily s.c
(1 mg Enoxaparin = 110 anti-Xa units)
alternatively:
Enoxaparin (Clexane®) 1.5 mg/kg, 1x daily s.c.
Dalteparin (Fragmin®) 120-150IU/kg,1x daily s.c

Monitoring:
- anti-Xa level 4 hours after dose
- Target range 0.4 - 0.6 IU/ml

Duration of therapy
The decision on how long antithrombotic therapy should be continued must be taken on an individual basis. The following aspects need to be taken into account:

- Extent of thrombosis:
- Extent of recanalisation or course documented by imaging procedures
- Current treatment phase of lymphoma/leukemia therapy with associated changes in blood count and coagulation to be expected

General recommendation:
6 weeks, if full recanalisation within this period 3 months, if only partial recanalisation after 6 weeks.

4.4.2.2 Unfractionated heparin

Initial dose: 10 IU/kg/h

Monitoring: PTT target range 1.5x upper normal range of age

Duration of therapy: max 6 days of UFH, then transfer to low-molecular heparin

4.4.2.3 Antithrombin/platelets/Protein

AT – activiteit: 80 - 100%

AT- dosis : $(AT_{target} - AT_{actual}) \times k$

Platelets: > 40G/l

Protein C target activity : 80 - 100%

Protein C – Dosis: $(PC_{target} - PC_{actual}) \times k$

4.4.3 Heparin neutralisation

Only to be conducted by an experienced haemostasis specialist or intensive care physician!)

4.4.3.1 Low-molecular weight heparin (LMWH)

It is generally sufficient to leave out injections in order to stop anticoagulation. Protamine hydrochloride (Protamine® ICN) does not result in full heparin neutralisation, but achieves partial neutralisation. Yet the administration of protamine hydrochloride is indicated in cases of very high LMWH doses and severe bleeding that cannot be controlled otherwise.

1 mg protamine hydrochloride slowly i.v. (hypotension!) per 1 mg or 100 IU administered LMWH within 3 - 4 hours after LMWH administration.

Warning: Excessive protamine hydrochloride itself acts as an anticoagulant!

4.4.3.2 Unfractioned heparin

It is generally sufficient to stop the infusion in order to stop anticoagulation (short half-life of UFH). Protamine hydrochloride (Protamine^{R} ICN) results in practically full heparin neutralisation within only a few minutes.

Since excess protamine hydrochloride itself acts as an anticoagulant, **only neutralise the quantity of UFH administered during the past 2 hours:**

0.5 mg protamine hydrochloride slowly i.v. (hypotension!) per 100 IU UFH given over the past 2 hours.

4.5 Severe post-thrombotic bleeding

Minor **localised bleeding** around the area of venous infarction is not to be considered severe bleeding and is therefore **no contraindication for anticoagulation.** In view of the better side effect profile and particularly low tendency to cause bleeding, **low molecular weight heparins are preferable** to unfractioned heparin in this situation.

In case of major postthrombotic bleeding, **AT III** may initially be substituted **up to an activity of 100%** and anticoagulation initiated once the clinical situation has stabilised.

In individual cases protein C* may also be substituted up to an activity of 100% and anticoagulation initiated once the clinical situation has stabilised.

Major bleeding demands an individual, interdisciplinary treatment decision in all cases!

5 Blood product substitution

All blood products given should be leukocyte depleted and irradiated at 30 Gy for GvHD prophylaxis. The use of CMV-negative blood products only appears to be useful with replacement transfusions as long as leukocyte depletion is adequate (Bowden*, et al* 1995, Hillyer*, et al* 1994, Saarinen*, et al* 1993, van Prooijen*, et al* 1994).

Erythrocyte transfusions:

Erythrocyte concentrates are the standard product for erythrocyte transfusions. No generally applicable recommendation can be given for an absolute Hb threshold below which a transfusion is indicated. An Hb level of <8g/dl can be used as a guideline. However, a relevant decision should take into account the patient's age and clinical condition, the dynamics of Hb decline and the timing within the therapy schedule as far as any hematological regeneration can be expected. The quantity to be transfused is 10 - 15 ml/kg given over 3 - 4 hours.

In patients with extreme hyperleukocytosis at the time of diagnosis, erythrocyte transfusions may only be given in case of life-threatening anemia due to the risk of leukostasis. Hemoglobin levels should remain below 8 g/dl

Platelet transfusions:

Platelet concentrates are available as pooled concentrates or single-donor concentrates. Pathogen-inactivated plasma-depleted platelet concentrates not irradiated may be used at discretion of the treating centers. Single-donor concentrates should preferably be used, particularly if platelet levels increase insufficiently after transfusion, if there is intolerance or if there is a primary SCT indication. No absolute threshold can be given for transfusions. Platelet levels of 10000 to 20000/µl can be used as a guideline. Clinical signs of bleeding (petechiae, epistaxis, mucous membrane bleeding) as well as any existing infection should be taken into account in making this decision.

In case of hyperleukocytosis > 100 000/µl the platelet level should be > 50 000/µl for the initial lumbar puncture at diagnosis, For lumbar punctures during the further course platelet counts should be at least 30000/µl. If platelet counts fall below 30000-50000/µl any heparin prophylaxis should be stopped or a platelet transfusion initiated. For surgical interventions (i.e., central catheter placement, lung or brain operations due to invasive mycosis) a minimum thrombocyte count of 50 000 - 80 000 /µl is required.

In patients with extreme hyperleukocytosis at the time of diagnosis, platelet transfusions may only be given in case of life-threatening thrombocytopenia or bleeding due to the risk of leukostasis.

6 Gastritis

In case of gastritis or gastritic complaints (due to glucocorticoid therapy, stress, non-steroidal antirheumatics, etc.), the generous use of histamine 2 antagonists or protone pump inhibitors is recommended.

7 Mucositis

Oral and dental care:
The peridontium should regularly be cleaned without causing lesions. For this purpose an extra-soft tooth brush or a dental water jet are recommended. In case of thrombocytopenia or extremely vulnerable mucous membranes it is recommended not to use a tooth brush but rather to rinse the mouth with Chlorhexamed® (Chlorhexidine), which, in contrast to pure hexidine, dissolves and prevents plaques.

Diagnostics:
In case of severe mucositis, it is recommended to investigate a culture for fungi and bacteria, including an antibiogram, and HSV-PCR, so that therapy can be better targeted, if necessary.
The mucous membranes underneath the tongue are usually representative of the condition of the entire gastro-intestinal tract. Even with considerable swelling and pain this area usually remains accessible for inspection and assessment.

Therapy:

- At least 4 x daily application of mouth wash using chamomile solution or Lidocaine viscous 2 % /

Dexpanthenol solution. 5 % 1 : 1 are indicated.

- Local treatment of open sores with astringents (i.e., aqueous solution of methylene blue). If there are open sores, hexidine is not recommended since it inhibits fibroblast growth. Leucovorin (=tetrahydrofolate) rinses are also not recommended.
- In case of extensive thrush resistant to intensive topical treatment, systemic anti-Candida albicans therapy is recommended, e.g. fluconazole 6 mg/kg/day in one single dose or liposomal Amphotericin B 3 mg/kg/day p.i. (1 h) over 5 - 7 days. Do not apply fluconazole or any other azole derivates in therapy phases with Vinca alkaloids due to the risk of increased neurotoxicity.
- In case of confirmed Herpes simplex virus infection: Aciclovir 30 (-45mg/kg/d) in 3 single doses .i.v. (1 h) or 80 mg/kg/d in 3 - 4 single doses p.o.
- In case of extensive inflammation/necrosis of the periapical gingiva, systemic therapy with anti-anaerobic antibiotics, e.g. metronidazole/clindamycin should be considered.
- In case of large, severe ulcerations of the oral mucous membranes it should be kept in mind that these are usually not limited to the mouth. Such situations also require close monitoring as well as consistent, early balancing of protein and electrolyte losses. Provisions must be made for sufficient pain therapy, if necessary also with opioids.

8 Antiemetic treatment

With strongly emetogenic therapy components such as high-dose ARA-C, alkylating agents (Ifosphamide, Cyclophosphamide) or anthracyclines, prophylactic administration of a serotonin receptor (5-HT) antagonist is envisaged:

- Ondansetron: 0.15 mg/kg/dose or 5 mg/m^2/dose (max. 8 mg) i.v. or p.o. every 12 hours
- Tropisetron: 0.2 mg/kg/dose (max. 5 mg) i.v. or p.o. once per day
- Granisetron:10 - 40 µg/kg/dose (max. 3 mg) i.v. once or every 12 hours

Dexamethasone may also be given in order to optimize the effect if it is not already administered together with the chemotherapy elements. If the effect remains insufficient, especially with adolescents, additional treatment with Dimenhydrinate (1.25 mg/kg i.v. every 8-24 hours) may be useful. Antiemetic treatment is not always necessary during the low- dose ARA-C cycles in Protocols I, and II. In case of poor tolerance, Dimenhydrinate, if necessary also Ondansetron/Tropisetron/Granisetron may be given once p.o. one hour before the ARA-C injection.

Metoclopramide and Aprepitant [neurokinin 1 (NK-1) receptor antagonist] may also be used in preventing chemotherapy-induced nausea and vomiting.

9 Hyponatremia and SIADH

Hyponatremia (serum sodium < 130 mmol/l) is common with chemotherapy due to a negative sodium balance (loss > intake), fluid retention or a combination of both. One of the causes of hyponatremia may be the Syndrome of an Inappropriate ADH Secretion (SIADH), which results from excessive ADH (anti-diuretic hormone, vasopressin) release. Some drugs are known to be associated with SIADH, particularly vinca alkaloids (Vincristine, Vindesine), but also Morphine, Carbamazepine and alkylating agents (Cyclophosphamide, Ifosphamide).

SIADH diagnosis rests on the following findings:

- Hyponatremia (serum sodium < 130 mmol/l) and hypochloremia
- Reduced serum osmolarity (< 270 mOsm/l)
- Elevated urine osmolarity (> 300 mOsm/l)
- Elevated sodium elimination in urine (> 20 mmol/l)
- Clinical euvolemia, generally no edemas or ascites
- Normal renal function

In most cases chemotherapy-related SIADH is probably not associated with clinical symptoms and not

diagnosed. However, clinical symptoms such as nausea, vomiting, reduced urine output, sometimes irritability and personality changes may also develop. With very low serum sodium levels there is the risk of neurological symptoms, alterations of consciousness and seizures. Laboratory diagnostics: Serum: levels of sodium, chloride, phosphate, calcium, magnesium, albumin, urea Urine: pH level, specific gravity/osmolarity, sodium, potassium, chloride Early therapeutic intervention should aim at maintaining serum sodium levels at > 130 mmol/l.

10 Adrenal insufficiency

Secondary adrenal cortex insufficiency, which may cause clinical problems particularly during stress situations, must be expected during the phase of glucocorticoid reduction in IA, IIA and IIIA as well as for an indeterminate period after completion of tapering of the glucocorticoids. For this reason a glucocorticoid stress dose is recommended for patients in these therapy phases (Protocol IA, IIA or IIIA from glucocorticoid reduction until approx. 4 weeks after), if they have fever >38.5°C, other clinical symptoms of hypocortisolism or are exposed to other stress situations. For intensive-care patients the administration of an initial hydrocortisone bolus is recommended (age < 6 months 25 mg, 6 months to 6 years 50 mg, > 6 years 100 mg) followed by a continuous hydrocortisone infusion of 150 mg/m^2/day. For non-intensive care patients substitution by 30-50mg/m^2/day hydrocortisone p.o. or i.v., distributed over 4 even single doses, is recommended.