

ALL IC-BFM 2009

A Randomized Trial of the I-BFM-SG for the Management of Childhood non-B Acute Lymphoblastic Leukemia

Final Version of Therapy Protocol from August-14-2009

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FOREWORD

In 2002, when the first intercontinental randomized clinical trial of the International BFM Study Group, ALL-IC BFM 2002, had been launched, many observers and some of us were quite sceptic if this new and large endeavour would succeed. Nearly 8 years later and with more than 4000 patients treated on that protocol, one can only congratulate the group to their large success. Diagnostics were more successful than anticipated, all randomizations reached their target numbers, toxicities remained in the expected range, and relapse rates were rather low. It is also certainly fair to say that the implementation of such a demanding protocol together with the regular and intensive exchange of experiences and results was of major benefit to many groups comprised in trial ALL-IC BFM 2002.

The new study protocol ALL-IC BFM 2009 is the final result of very comprehensive data analyses and discussions over the last few years. Clinical research performed within the ALL-IC BFM group but also in parallel in the context of trial AIEOP-BFM ALL 2000 produced important evidence for the new stratification. The systematic evaluation of minimal residual disease (MRD) provided the basis for a fine-tuning of the previous stratification system which combined both initial clinical and biological factors with dynamic parameters derived from the response evaluation. The ALL-IC group has agreed to utilize only flow-cytometry (FCM) for MRD analysis for this trial as it appeared to difficult in most participating countries to establish PCR-based methods. Nevertheless, quality standards and reproducibility of this new approach will be important hallmarks for this study.

ALL-IC BFM 2009 will define a small low-risk group in which clearly less than 10%, maybe even less than 5% of the patients will have to expect a relapse. These patients will be spared of any treatment intensification, and will not be randomized. In the large intermediate risk (IR) and in the high risk (HR) group, however, there will be randomized questions which address the direct comparison of the BFM-type induction consolidation (Protocol IB) with the COG-derived augmented BFM-consolidation. In addition, pcB-ALL patients of the IR group will be randomized to determine the optimal dose of MTX in Protocol M.

Results in all three risk groups will be difficult to be compared to results of trial ALL-IC-BFM 2002 as MRD will change the risk group assignment quite significantly. This is an unavoidable side effect of any modified stratification. Some comparisons to trial AIEOP-BFM ALL 2009 will, however, be possible, e.g. in the question which induction consolidation is more efficacious in HR patients. That trial will utilize both FCM- and PCR-based MRD analysis for risk stratification. Thus, I expect again a very fruitful exchange of experiences between these two large trials in childhood ALL under the umbrella of the I-BFM-SG.

Last but not least I want to thank Jan Starý, all trial steering committee members, and the individual group chairs for their major achievements in ALL-IC BFM 2002. Myriam Campbell, Luis Castillo, Gabor Kovacs, Jerzy Kowalczyk, Martin Zimmermann, Janos Kappelmayer, Michael Dworzak and many others have to be applauded for their large efforts to get the new protocol finalized.

Kiel, February 2010

Martin Schrappe
Chairman, I-BFM-SG

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1 Chapter GENERAL PART

1.1 Introduction

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 20 years, which were derived from the original "BFM backbone". 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 25 years ago was the recognition that early treatment response, in particular the response to the prednisone pre-phase (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). The results of early response assessment are presented in this protocol proposal. It appears that the BM d15 response can add some 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.

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.

As it was expected it will take some years to have the answers, other I-BFM-SG members developed a trial, ALL-IC- BFM 2002, 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 might provide the tool to adjust treatment intensity accordingly. Such an approach would also allow the combination of forces to put some common randomized questions together with the AIEOP/BFM 2000 trial. In addition, prospective studies on MRD by using various techniques, mainly FCM, were developed.

1.2 DATA BASIS FOR THE STRATIFICATION SYSTEM

1.2.1 As used in ALLIC 2002, without MRD analysis

The possible stratification criteria for a new international trial without the use of the PCR-based minimal residual disease (MRD) detection were evaluated. For the purpose of generating a new stratification system data from two major groups (AIEOP & BFM) were analyzed by the number of blasts on PB smears on day 8 ($ABC/\mu L = \% \text{ blasts} \times WBC/\mu L$) and the morphologic results of BM smears (% blasts) on day 15 of induction.

1.2.1.1 AIEOP

For 90% of the children day 15 bone marrow data were available. All patients with t(4;11) and t(9;22) were excluded. Only 20% of the children had a central review of the bone marrow, while the other were evaluated locally. Distribution of the morphologic data was: M1 60%, M2 26%, M3 14%, equally for the local and central assessment. Some slides were locally evaluated as M1 and centrally as M3 and vice versa (concordance 79.5%). Day 8 PB smears were locally evaluated, but in case of doubt slides were sent for central review. The data were reproducible and rather consistent over the different trials.

1.2.1.2 BFM

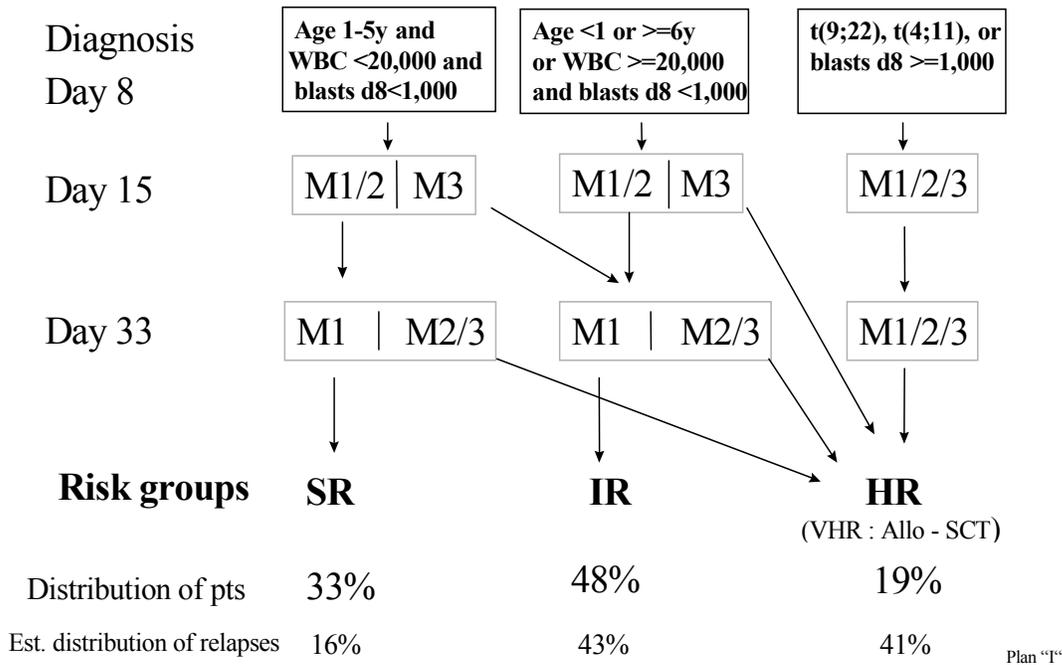
The distribution of morphology on day 15 was similar to the Italian group, but the evaluation was entirely made in the reference center: M1 61%, M2 28%, M3 11%-excluding NR d33, t(9;22) & t(4;11) from this calculation. In the subset of 0-100 blasts/ μL in PB d8 there were 10% more children in the BFM group than in the AIEOP group. Data were reproducible over the different trials, too.

Based on these data the stratification proposal was defined including translocations t(9;22) and t(4;11) and/or detection of the corresponding fusion genes (BCR/ABL and MLL/AF4) and the BM response at time of remission control (day 33). This classification is schematically summarized in **Fig. 1**. The aim of this stratification was to generate a standard-risk group with 30%, an intermediate-risk group with about 50% and a high-risk group with 20% of the patients. Up to 50% of the relapses should be included in the high-risk group to provide sufficient specificity as to justify major treatment modifications and/or intensifications. An aplastic d15 marrow might prohibit a correct response assessment. Therefore, these patients should be stratified according to their remaining criteria.

Fig. 1: Risk Group Definition Based on Age, WBC, Response on d8 (PB) & d15/d33 (BM).
 Figures at the Bottom Give the Estimated Distribution of the Patients & Relapses

Febr. 23, 2002

ALL IC-BFM 2002 : CLASSIFICATION

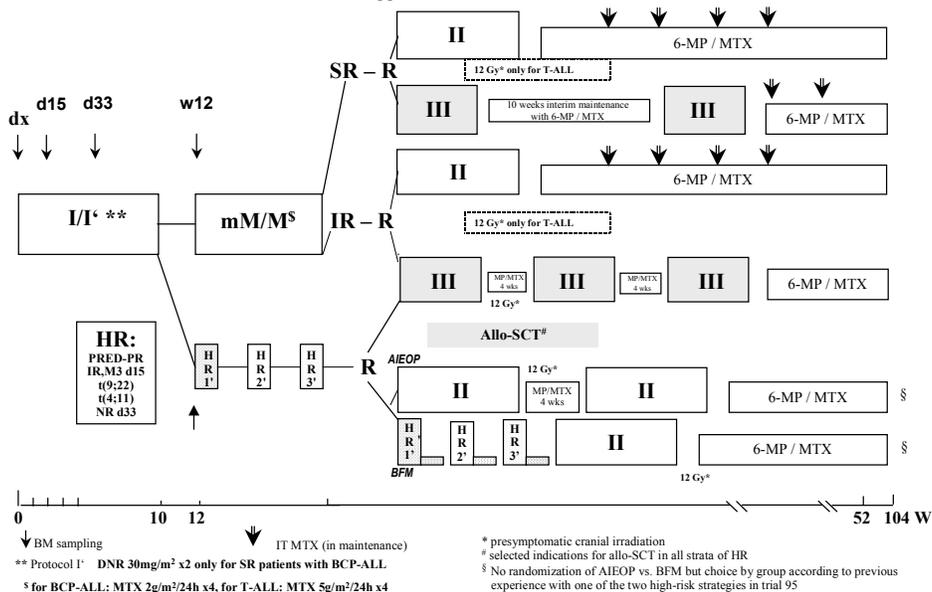


The intercontinental, randomised multicenter trial, ALL-IC- BFM 2002 (Fig. 2), run from November 2002 - 2007 in 13 cooperative or national groups, 3 of them as non regular members.

Fig 2

ALL IC-BFM 2002 : TREATMENT

Version approved in Hannover on 23.02.2002



Most groups enrolled patients < 1 year of age in the INTERFANT 99 or INTERFANT06 trial. T immunophenotype was not a stratification criterion in this study. However, patients with SR/IR T-ALL received Daunorubicin x4 q 30 mg/m² in induction (Protocol I), HD MTX x4 q 5 g/m² in consolidation (Protocol M), and finally prophylactic/therapeutic cranial radiotherapy (pCRT/tCRT by CNS status). HR T-ALL was managed within the HR strategy.

Fig 3.

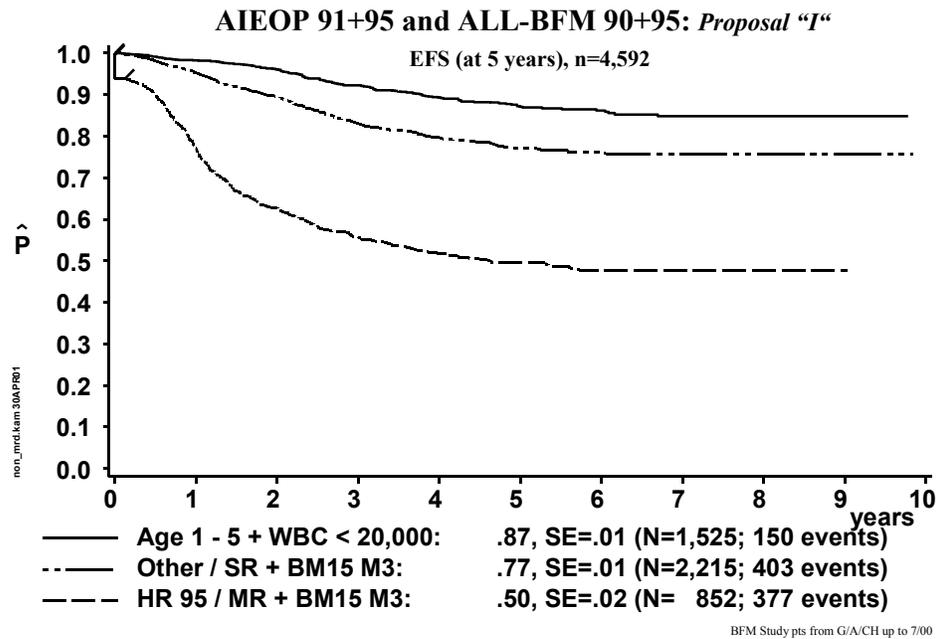
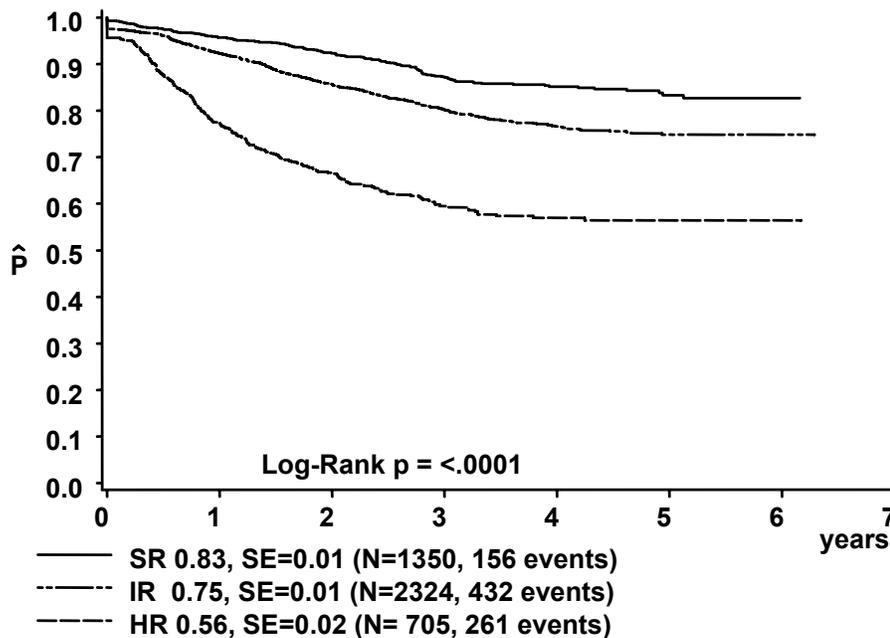


Fig 4. ALLIC 2002. Risk groups 5 years EFS (regular members)



As can be seen in Figure 3 and 4, distribution of the risk groups and the results were similar in study ALLIC 2002 as compared to those from the combined studies AIEOP LLA 90 and 95 and BFM 90 and 95 (patients with data on BM day 15 only).

1.2.2 Data Basis for the Stratification with FC-MRD in ALLIC 2009

As a research project in study AIEOP-BFM 2000 MRD-detection by Flow Cytometry (FC) was analysed. Also a collateral research, "mini-mini" project developed within ALLIC 2002, assessed MRD by FC in BM at day 15 and 33. It could be shown that this method is useful to define risk groups but a high level of logistics and laboratory skill is needed. As ALLIC 2002 turned out to be a successful project it was decided to develop a second trial, on the basis of the same stratification as in ALL IC 2002 but using in addition MRD analysis by FC, as this could be available and also it could be afforded by a majority of the participant groups. Based on data of FC- MRD in BM day15 from the mini-mini project and the AEIOP-BFM-ALL 2000 trial (Dworzak et al 2002, Dworzak et al 2008, Basso et al 2009, Ratei et al 2009, Fronkova et al 2008) ^(1,2,3,4,5) the distribution of the risk groups by FC in ALLIC 2009 was estimated.

Table 1. Risk groups distribution by FC-MRD within risk groups according to ALLIC 2002

| | <0,1 % | Other levels | ≥ 10% |
|----|--------|--------------|-------|
| | % | % | % |
| SR | 45 | 53 | 3 |
| MR | 37 | 57 | 6 |
| HR | 9 | 41 | 50 |

In order to be on the safe side for the patients and to allow for the participation of study groups who could not introduce FC-MRD for the majority of patients it was decided to use FC-MRD only on top of the risk criteria of ALLIC 2002.

- Standard Risk patients should have less than <0,1% to remain in this group
- Patients of other risk groups with MRD<0.1% stay in their risk group
- All patients with >10% will be included in HR
- Patients with genetic abnormalities as hipodiploidy (≤ 45 Cr), translocations t(9;22) and t(4;11) and/or detection of the corresponding fusion genes (BCR/ABL and MLL/AF4) are classified as HR irrespective of age, WBC and response

***NOTE:**

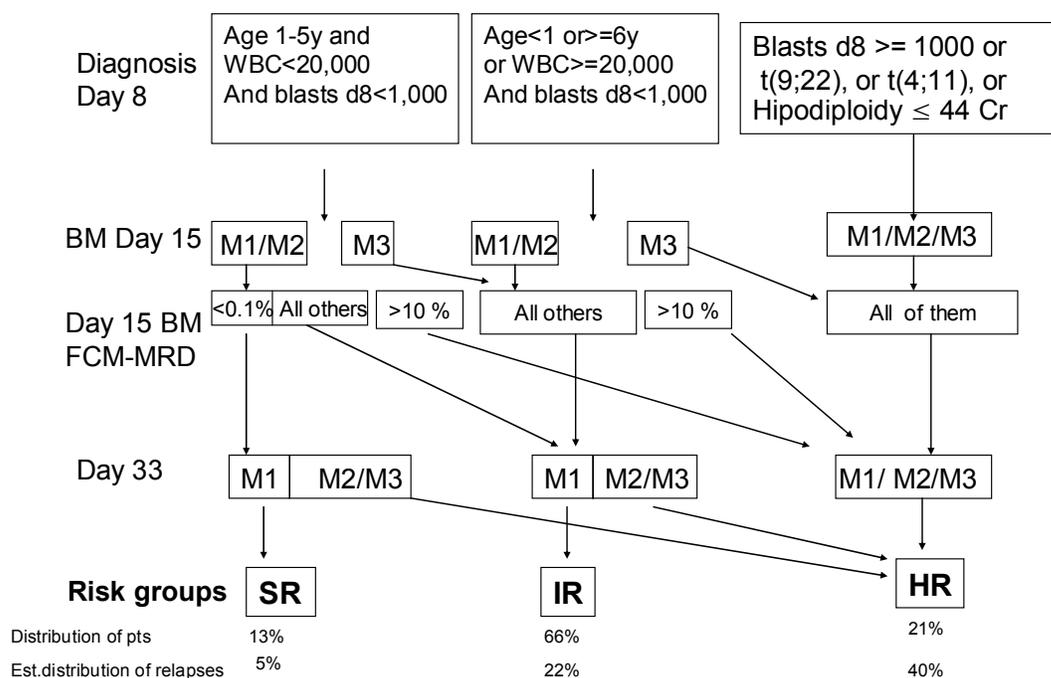
- Until FC- MRD analysis are standardized and fully incorporated in the study, only the BM day 15 morphology will be used to stratify, as in ALLIC 2002.
- After FC-MRD is incorporated, it will prevail over morphology for stratification.
- An aplastic BM d15 might prohibit a correct assessment of FC-MRD and morphology. Therefore, these patients should be stratified according to their remaining criteria, but this patient cannot be included in SR if it is the case

This classification is schematically summarized in **Fig. 5**.

Fig. 5: Risk Group Definition Based on Age, WBC, cytogenetics, Response on day 8 (PB), day15/d33 (BM) and FC-MRD.

Figures at the Bottom Give the Estimated Distribution of the Patients & Relapses

ALL IC-BFM 2009 CLASSIFICATION



Based on this classification considering one thousand new patients / year and a study duration of 5 years the distribution by risk groups and the expected EFS is described in **Table 2**.

Table 2. Expected distribution by risk groups and pEFS in ALLIC 2009

| | N° patients | % patients | pEFS |
|----|-------------|------------|------|
| SR | 672 | 13 | 95 |
| MR | 3277 | 66 | 78 |
| HR | 1051 | 21 | 55 |

1.3 Study Questions

Based on the stratification shown in **Fig. 5** (pag 15), three distinct risk groups are derived. In the total group the main study question focuses on the the separation of a standard risk group without change of treatment and the improvement of outcome in the intermediate and the high risk group by randomized intensifications of therapy. Until FC-MRD is available and standardized, the stratification will be based only on the criteria of ALL-IC 2002 .When

finally FC could be introduced it will prevail over morphology on day 15 and it will be used for the stratification. At the end in each group we will have patients with and without MRD. As the final group SR-MRD will be small, no study question will be asked except the confirmation of the expected high pEFS. In IR and HR the type and intensity of early intensification, phase 2 of protocol I (protocol IB), will be evaluated by a randomized question. In IR-B Cell Progenitor- ALL (BCP-ALL) the optimal dose of MTX will be randomly evaluated in consolidation: The study questions are:

- **SR:** Will the outcome of SR patients, defined by SR according to ALLIC2002 criteria and a FC-MRD load <0.1% on day 15, be better than the result which can be expected by stratification with the criteria of ALL-IC 2002?
- **IR:** and **HR:** Can the addition of an early intensification ,protocol IB (Augmented BFM), improve the outcome?
- **IR-BCP ALL:** Are 5 gr/m² of MTX more efficient than 2 gr/m² ?

1.3.1 RESEARCH PROJECTS. See Appendix 5

For interested centers/national groups participating in this study two research projects are planned. It is intended solely for research purposes, and should not serve as a basis for making clinical decisions whatsoever.

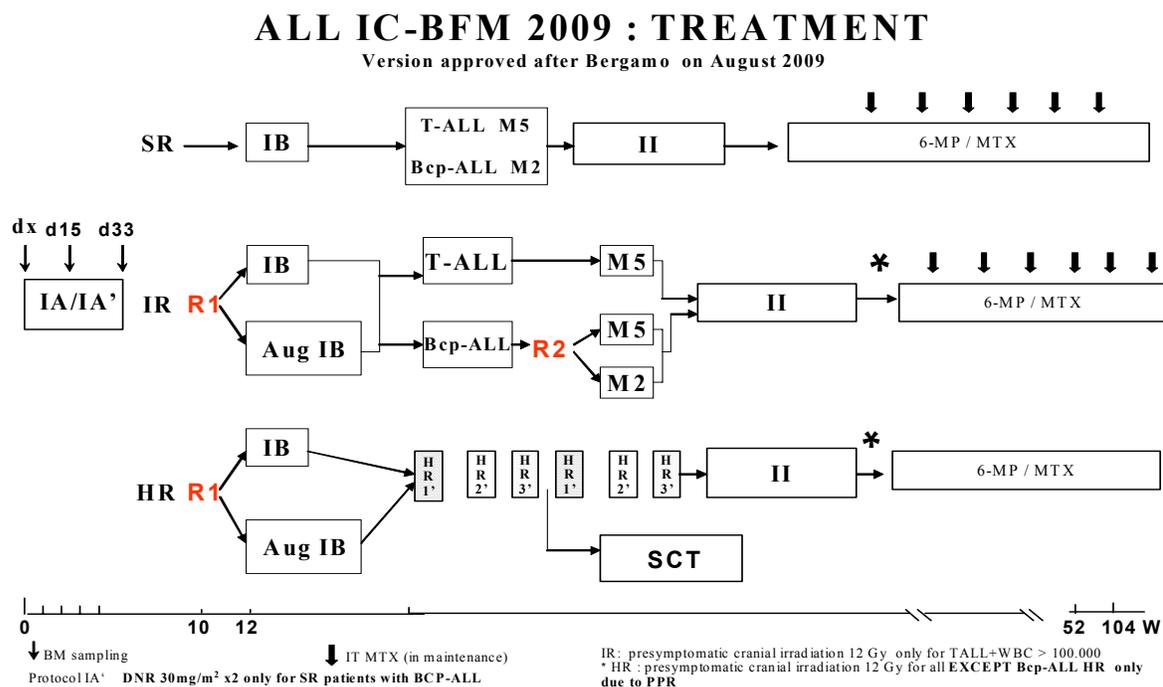
A.- Role of gene polymorphisms in the acute side effects of all therapy

B.- Facultative MTX-pharmacokinetic study

1.4 Treatment Principles (See Fig. 6 below).

1. SR patients will not be randomized. All patients in IR and HR will participate in randomized evaluations of early intensification therapy, which are considered to be the key for further improvement.
2. For early intensification therapy the following randomizations are scheduled:
 - **IR** Protocol IB Augmented (**IR-2**) vs. Protocol IB (**IR-1**)
 - **HR** Protocol IB Augmented (**HR-2**) vs. Protocol IB (**HR-1**)
3. In consolidation of IR BCP-ALL patients, MTX 2 g/m² (x 4) will be randomly evaluated with MTX 5 g/m² (x 4). More IT MTX in early maintenance therapy will compensate for the lower dosage of IV MTX. However, MTX at 5 g/m² x 4 will be used in T-ALL (in SR and IR), as this is the group where pharmacodynamic and clinical data have demonstrated the largest benefit from HD MTX (Barredo JC et al. 1994, Belkov VM et al. 1999, Reiter A et al. 1994, Rots MG et al. 1999, Rots MG et al. 1999, Synold TW et al. 1994)⁽⁶⁻¹¹⁾. This MTX dose has been introduced also by SJCRH and COG for their T-ALL treatment protocols. Preventive radiotherapy with 12 Gy will be maintained for T-ALL + WBC > 100.000.
 - **IR BCP-ALL** Protocol M5 (**IR-4**) vs. Protocol mM (**IR-3**)

Fig.6:



1.5 Treatment Strategy in SR, IR and HR Groups

1.5.1 STRUCTURE OF THERAPY ELEMENTS

All treatment elements used for early intensification are based on therapy elements that have been used previously. Phase IB Augmented has been used by COG (CCG 1961), being effective in patients with slow response, even it was evaluated in a different type of stratification it is expected that survival can also be improved in this trial. The composition of the various treatment arms is also found in the new AIEOP/BFM 2009 trial, however, for differently defined risk groups. The structure of the study questions will nevertheless allow the combined analysis of patient subsets from both trials. See Fig. 7 below for outline of trial AIEOP/BFM 2009.

1.5.2 ADDRESSING THE CNS

As in ALLI-IC 2002 preventive cranial radiotherapy (pCRT) was omitted for BCP-ALL patients in the standard- and intermediate-risk groups. In ALLIC 2009 the difference is that it will be omitted for BCP-ALL with HR due only to PPR. It also will be omitted for T-ALL with WBC < 100.000. A dose of 12 Gy will be applied to all other high-risk patients and to T-ALL ≥ 100.000. Therapeutic irradiation of the neurocranium will be given to all CNS-positive patients at age-adjusted dosage of 12/18 Gy. Consequently, in SR and IR patients with BCP-ALL the number of intrathecal injections will be equalized [17 (+ 4 if CNS-positive)] to minimize any risk

of CNS relapse that might be associated with omitting pCRT and with the relatively lower dose level of systemic MTX in Protocol mM. Post consolidation, *BCP-ALL patients with a CNS status 1/2* will receive 4 IT shots of MTX, as in ALL-IC 2002, T ALL with WBC < 100.000 will receive 6 IT shots of MTX

1.5.3 HR GROUP

The high-risk group will be larger than in the previous ALLIC 2002 trial. As the MRD at day 15 is incorporated in the risk assignment process those patients with >10% in SR and MR will be upgraded to HR. Hypodiploidy ≤ 44 Cr has been associated with poor outcome (Nachman et al 2007)⁽¹²⁾ and it is evaluated as HR feature in AEIOP/BFM 2009 and other trials, and it will also be in ALLIC2009

1.5.4 STEM-CELL TRANSPLANTATION SCT

For SCT it was decided to recommend matched-family-donor (MFD) transplantation in selected subgroups of HR patients on the basis of prognostically unfavorable constellations of disease biology and response quality, basically similar to the previous recommendations of the BFM & AIEOP trials. For matched-unrelated-donor (MUD) transplantation a high level of experience has to be first gained with the procedure, aiming at a transplantation-related morbidity/mortality (TRM/M) comparable to that of MFD transplantation, so their inclusion will be a decision of each country. In any case the decision to treat a patient with SCT and treatment with SCT itself is not part of the protocol but is based on the individual decision of the responsible physician.

1.6 Items Common to ALL IC-BFM 2009 & AIEOP/BFM ALL 2009

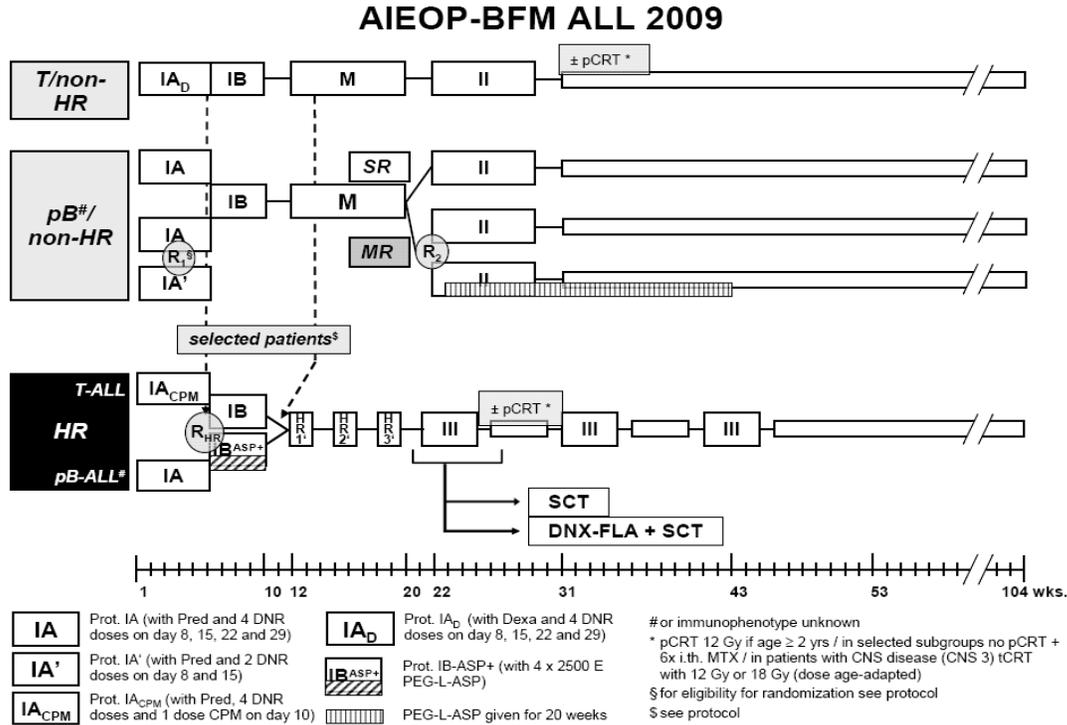
In ALLIC 2009 it was decided to include MRD in stratification as in AIEOP/BFM, although with a different technique and different time points.

In AEIOP/BFM 2009, T-ALL non HR patients will receive the same treatment but Dexamethasone instead of Prednisone.

In both trials a randomized question in HR group includes an early intensification in phase IB even though with a different schema.

Other subgroups will also be comparable. For comparison see **Fig. 6 & Fig. 7**.

Fig. 7: AEIOP/BFM 2009 treatment



1.7 MINIMAL RESIDUAL DISEASE by Flow Cytometry

As it has been defined MRD detection by FC will be incorporated as stratification criteria only after it is standardized and evaluated within most of the participant laboratories.

1.7.1. MONITORING AND ADJUSTMENT OF SETTINGS ON THE FLOW CYTOMETER

1.7.1.1 MONITORING

- **Aim:**

Performing MRD on a flow cytometer affords that multiple samples of an individual patient taken at several occasions in the follow-up period of weeks or months are to be studied. Due to treatment, expression of antigens may differ by biological causes between time-points and cell numbers in samples may change. To assure stability of results at least with respect to the methodological approach, monitoring of technical performance and device set up is of utmost importance. The following guidelines are intended to secure this issue and to make comparisons of data possible.

- **Intralaboratory monitoring:**

This is a sequence of quality controls that should be performed on flow cytometers used for MRD assessment in each institution. Besides yearly check up through an accredited company, controls must be carried out every two weeks. In the following a proposal containing BD

applications will be made, but this may be performed with corresponding tools on other flow cytometers than FACSCalibur.

The automated FACSComp program has to be run every two weeks. For running FACSComp, Calibrite 3 beads (BD#340486) extended with APC beads (BD#340487) are necessary. FACSComp controls device set up including time delay (for technical support and interpretation of data ask local BD support).

- **Interlaboratory monitoring:**

MRD determination by flow leads to results which are examinable and must meet certain criteria. To achieve this aim, appropriate teaching and longitudinal quality control of involved staff must be assured. Standards concerning sample preparation and performance must be adhered to. For quality control, discussion of problematic cases as well as teaching a common platform is highly recommendable to warrant a rapid LMD file exchange. Special attention has to be put to new staff.

In order to expect uniform data analysis it is required that ALL IC 2009 participants successfully complete 2 sendarounds of LMD file analysis.

Ring test trials based on exchange of non-selected (spotted by time points) LMD files, or of patient samples and spiked specimens (mixture of leukemic cells from normal samples at diagnosis with normal PB or BM are of great value to determine the quality of performance when multiple laboratories co-operate. LMD file exchange is particularly useful to access the ability of staff in terms of post-acquisition skills, which is most crucial in MRD assessment because depending on the human factor in dot plot interpretation. Technically, evaluation of exchanged LMD files can be hampered by interlaboratory differences in the technical devices used for acquisition which may also disturb appropriate import of files into cross-company software for analysis.

Recently, cell stabilizing reagents (TransFix from Cytomark, distributed by Invitrogen) became available which makes sample exchange feasible even under consideration of several days of time lapse between sampling and analysis due to transport over borders. About the use of TransFix further investigations have to be performed.

The most promising alternative for external quality control monitoring will be the participation in the UK-NEQAS MRD analysis sendarounds.

1.7.1.2 ADJUSTMENT OF SETTING THE FLOW CYTOMETER (E.G. FACSCALIBUR)

- **Aim:**

Standardized flow cytometer setup secures comparable results in participating institutions. Nevertheless, settings can not be transferred from one cytometer to the other, because each device has its own peculiarities. Certainly each institution has its own approach, but adherence to a backbone of common guidelines is recommended.

- **Guidelines:**

General: Stain PB cells with mAbs in FITC, PE, PerCP, APC, and SYTO16 (per tube only one mAb) plus extra tube without staining. Make sure to have enough cells and choose mAbs giving bright signals (3rd log); prefer antigens positive on lymphocytes (e.g. CD3+). Importantly, the preparation of the samples must be used for MRD monitoring.

Setting:

1. put all compensation values to zero
2. show histograms for FL1 to FL4
3. show dot plots according to compensation possibilities
4. acquire unstained tube
5. adjust FSC and SSC (P1 and P2) linear values to optimize gates
6. gate on lymphocytes, exclude debris
7. adjust PMT (P3 to P7 (APC)) in log values to put auto-fluorescence of unstained cells into first log.
8. acquire FITC tube and adjust FL2-FL1 (second compensation)
9. acquire PE tube and adjust FL1-FL2 (first comp) and FL3-FL2 (fourth comp)
10. acquire PerCP tube and adjust FL4-FL3 (sixth compensation)
11. acquire APC tube and adjust FL3-FL4 (fifth compensation)
12. acquire SYTO16 tube (show all cells) and optimize FL2-FL1 (second compensation)
13. values for SYTO16 must be noted and used according to mAb composition in tubes

1.7.1.3 DETERMINATION OF MABS USED IN MRD MONITORING

- **Aim: All participants should use same combinations of essential mAbs but may use different dyes.**

The combination of mAbs per tube is based on a backbone of two mAbs: in BCP-ALL CD19 (as primary gate), CD10 (as immature/blast cell marker); and in T-ALL CD7 (as primary gate, positive in T and NK cells), CD3 (mature T-cells). Backbone staining warrants that in each tube out of the series of three tubes the relevant cells can be addressed by similar phenotypic appearance, so that the additional markers can be evaluated as if all markers were combined in one tube. Notably, the expression of antigens frequently changes under treatment. Phenotypic patterns during follow up should therefore not simply be considered similar as at diagnosis. Instead, it is recommended to compare phenotypes rather with the immediately preceding time point. In analyzing the data the primary gate should be set in CD19 versus SSC correlations in BCP-ALL samples, and in CD7 versus SSC in T-ALLs, hence, it is recommended to use similar mAb conjugates for these two antigens throughout all tubes.

Note: It is necessary to use the same clone for CD3 cytoplasmatic and surface staining in order to prevent re-staining of surface epitopes during cytoplasmic staining (after prior surface staining) because of differences in epitopes. It is NOT necessary to engage negative control antibodies because PB and BM are sufficiently heterogeneous cell compartments, allowing for in-sample control by normal cell types which are negative for the antigens under investigation on MRD cells.

ALL IC 2009 SUGGESTED PANELS:

Tab 3. Suggested panels of MABS for BCP-ALL MRD

| | | | | |
|---------------|-----------------------|----------------|-------------------------|-----------------|
| Tube 1 | SYTO 16 | CD10-PE | CD45-PerCP | CD19-APC |
| Tube 2 | CD20-FITC | CD10-PE | CD34-PerCP-Cy5.5 | CD19-APC |
| Tube 3 | CD58-FITC | CD10-PE | CD45-PerCP | CD19-APC |
| Tube 4 | CD10/CD20-FITC | CD38-PE | CD45-PerCP | CD19-APC |

Tab 4. Suggested panels of MABS for T-ALL MRD

| | | | | |
|---------------|------------------|---------------|------------------------|------------------|
| Tube 1 | SYTO 16 | CD7-PE | CD45-PerCP | CD3-APC |
| Tube 2 | CD99-FITC | CD7-PE | CD5-PerCP-Cy5.5 | CD3-APC |
| Tube 3 | TdT-FITC | CD7-PE | CD3-PerCP | cyCD3-APC |
| Tube 4 | CD4-FITC | CD8-PE | CD45-PerCP | CD3-APC |

ALL IC 2009 will use the following time points for MRD analysis:

1. **de novo sample**
2. **day 15 sample**

Optionally: day33 and week12 (before MTX) are recommended for research purposes.

1.7.1.4 SYTO

- **Aim:**

SYTO16 is DNA>RNA dye which readily stains nucleated cells without a need for permeabilization. This dye is used to determine all nucleated cells in samples in order to exclude non-nucleated events like erythrocytes, platelets and debris. Weakly it also stains reticulocytes (hence exclude these by gating on SYTO16 clearly positive events). SYTO 16 may be used like a mAb (but not added to cocktails) or added to already lysed material.

SYTO16 is measured in FL1-channel although is not FITC, therefore it needs a bit different strategies in compensation (see chapter adjustment). Ideally SYTO positive cells should lie in the 3rd log (not higher), to prevent overspill into other channels and to clearly separate positive from negative events.

- **Procedure:**

SYTO 16 is delivered frozen (Invitrogen, molecular probes #S7578). The thawed stock solution can be aliquoted and refrozen, e.g. at -20°C. Working solutions of SYTO 16 should be prepared as 1:1000 dilution preferably with PBS/BSA2%/0.1%NaN3 and can be stored at 4°C in dark vials. This dilution is stable for months. If SYTO 16 working solution is prepared using isotonic saline, it is recommended to prepare the solution fresh every week. Add 2 µl of the 1:1000 working solution to 200 µl of sample volume, e.g. immediately before acquisition. If necessary, increase the input by 0,5 µl steps to find the optimum. Respective optimum amount per 700 000 cells can be added to individual MRD tubes either along with mAbs (in this case double the amount) or after the final wash immediately prior to acquisition (1x amount). If location is too low more SYTO16 can be added without the need for extra incubation time. Notably, intensity might decrease with storage of the dilution over weeks, discard dilution when values arrive in the 2nd log.

1.7.1.5 Usage of mAbs – amounts for staining

- **Aim:**

Warrant saturating staining for best resolution of blast versus normal cells.

- **General procedure:**

The amount of mAb as indicated by manufacturer

Note: mAb amounts as recommended on data sheets from companies are adapted for staining specimens with homogeneous cell populations at saturating conditions. In case, mAb amounts can be optimized by titration. If mAb amounts for usage are determined by titration, documentation of experimental evidence supporting the use of mAb at the relevant dilution must be available in the center. This documentation should include the type of antibody, the amount determined for usage per MRD tube, the amount recommended by the manufacturer, and a summary of the titration experiment (date, cell type, and results). If titration is used to determine the amount of mAb insert instead of the manufacturer recommendation, mind that titration is needed with each new lot of mAb – compare to previous lot.

- **Titration procedure:**

For titration of ALL-associated mAbs like TdT, CD5, CD7, CD10, CD19, CD34 BM or PB derived from de novo leukemia samples with respective antigen positivity should be used.

Make sure that the tested mAb are positive and the sample contains over 90% blasts. Frozen samples (in liquid nitrogen) can also be used but lower fluorescence may occur. The viability should be $\geq 90\%$. In case a cell line is used mind the usually higher autofluorescence.

For titration of mAbs as CD3, CD20, CD45, CD58 and CD99 PB of a healthy donor can also be used, cytoplasmatic CD3 staining must be determined separately.

Note: It is necessary to use the same clone for CD3 cytoplasmatic and surface staining in order to prevent re-staining of surface epitopes during cytoplasmic staining (after prior surface staining) because of differences in epitopes.

- **Detailed description:**

1. Stain approximately 1 million cells per test in a maximum of 100 μL volume.
2. Preferably use step-wise longitudinal dilution technique for staining.
3. Use a negative control (this could be cells without mAb or with matched irrelevant mouse mAbs conjugated with respective fluorochromes).
4. Use a positive control (stain with the amount of mAb indicated on bottle or data sheet.)
5. Dilute mAb with PBS or just use lower input. The lowest step should be at least the tenth part of the positive control (example: positive control 20 μL ; lowest titration step 2 μL).
6. Incubate, lyse, measure according to the protocol.
7. Analyze results in a histogram and compare Geomean MFIs. Choose the optimal concentration of mAb by recording the lowest Ab concentration that still saturates binding. In case of variable positivity in non-homogeneous samples (relevant for broadly expressed antigens: CD11a, CD38, CD45, CD58, CD99), gate on distinct subpopulation with highest expression.
8. Use then the double amount of the first saturating concentration of mAb!
9. Test lot-to-lot changes using the optimal amount of mAb of the old lot as positive control, and compare the result with those from the new lot: 1x, 2x, 0.5x the amount of the old lot (example: optimum is 2 μL of old lot; check 2 μL , 4 μL and 1 μL of the new reagent); compare MFI results, make changes if necessary.
10. Save files or print outs of titrations and record expiry dates and lot numbers of mAbs.

1.7.1.6 Work with mAb cocktails

- **Aim:**

Working with mAb cocktails has several advantages towards economization of work and avoidance of errors: pipetting steps can be reduced and expedited, and mAb input is assured

and standardized. On the other hand, since tandem fluorochrome conjugates as PE-CY7 tend to instability their inclusion into cocktails is NOT recommended. In addition, manufacturers do not evaluate performance of their mAbs sold and stored as single reagents for performance when stored in cocktails. Therefore, accreditation of these reagents for in-vitro-diagnostics is limited to storage as recommended by the vendor. Usage of cocktails depends upon individual decisions and are recommended as long as the cocktails are rapidly used and prepared fresh again weekly or provided that stability is monitored.

Tandem conjugates and SYTO dyes must NOT be included in cocktails.

- **Procedure:**

10 μ L of cocktail must contain all mAb in the optimal concentration plus PBS/BSA/NaN₃ (Example: 2 μ L FITC+ 2 μ L PE+ 1 μ L PerCP+ 1 μ L APC+ 4 μ L PBS/BSA/NaN₃).

Note:

Use cocktail according to recommended input of each single antibody (as indicated by manufacturer). In case mAb amounts for staining are determined by titration, use respective volumes: e.g. 10 μ L of cocktail should contain mAbs for up to 1-2 million cells to be stained in a final volume of 50-100 μ L. For volumes bigger than 100 μ L (in low cellularity material) input of cocktail must be adjusted. This is 20 μ L of cocktail for up to 200 μ L end volume, and 30 μ L for up to 300 μ L or more.

Check stability of cocktails (preferably at bi-weekly intervals) using BD CompBeads: for details of procedure see below (chapter on Settings and Compensation).

Save files or print outs of stability assessments and record dates of evaluation together with records of the date of cocktail preparation.

1.7.1.7 Calculation of utilized volumes (cell input)

- **Aim:**

For MRD detection down to the 0.01% level (with a required resolution of 30 MRD events minimum to refer a sample as positive), an optimum of 300 000 nucleated cells need to be acquired. Since preparation leads to cell loss it is recommended to stain at least 700 000 cells at all time points except at diagnosis. At diagnosis, stain 100 000 and acquire 30 000 cells. Cell loss depends on storage and age of materials and on cell content.

- **Procedure:**

1. Determine nucleated cell (NC) count. This can be performed using an automated hemocounter (note that this value includes also normoblasts!)
2. Calculate necessary volume by formula (see also example below):
Input in μ L = intended number of input cells/counter cell count (cells per μ L)
3. Round up as necessary
4. Record NC count

- **Example:**

If cell count in the sample is 2100 / μ L (= 2.1 G/L) of PB/BM; therefore input per staining tube is 333 μ L of original sample. (Formula: 700 000:2100=333)

1.7.1.8 Staining and lysing of MRD samples

- **Aim:**

Staining for MRD assessment should be done with a stain-lyse (=red cell lysis) approach.

- **Procedure surface staining:**

1. Add mAbs into tube (e.g. falcon BD# 352052)
2. Add SYTO16 in appropriate tube

3. Add BM (volume according to calculation; minimum is 50 μ L- in case of very high cellularity dilute BM with PBS up to 50 μ L volume)
4. Vortex, incubate at RT, in dark for 15 min.
5. Add 2 ml of BD lysing solution (BD# 349202 predilution 1:10 with demin. water) Vortex, leave for 10 to 15 min at RT in the dark
6. Centrifuge, discard supernatant
7. Top up with washing solution, e.g. 4ml cold PBS
8. Centrifuge at 400g for 10 minutes
9. Tip off/aspirate supernatant
10. Resuspend in 200 μ L PBS
11. Alternatively SYTO staining can be carried out at this poin (see chapter SYTO)
12. Proceed to Flow cytometer

Remarks:

To steps 1 and 11:

SYTO16 can also be added early together with mAbs (step 1), so that step 12 can be cancelled.

To step 4:

Add 1 ml lysis buffer, vortex, add second ml of buffer, doing so will prevent overspill of fluid.

2 ml of lysing buffer are sufficient for lysing up to 400 μ L of BM in cases of low cellularity with $>>$ 400 μ L of input, the whole volume should be split into several tubes (for example 3 tubes with 400 μ L input each); add lysing solution per tube as indicated above, and merge tubes before measurement.

If after 10 minutes of incubation with lysing buffer a sample does not appear clear extend incubation time up to 15 minutes; in very reluctant cases (lots of red cells after step 6) one may continue at step 7 (instead of 1x PBS) with hypotonic lysis (add 1 ml of deionized water for 30 sec and then add 1 ml of double concentrated PBS).

We have no experience with NH_4Cl but according to literature data are available showing that NH_4Cl -lysis renders comparable results to BD lysis solution. Usage of pharmacy-grade NH_4Cl is therefore an acceptable option.

Intracellular staining:

1. Execute point 1 to 4 from surface staining protocol
2. Add reagent A from IntraStain (Dako), increase reagent A volume proportionally to sample volume (per 50-100 μ L BM staining volume add 100 μ L of reagent A; increase in increments of 100 μ L as necessary up to a maximum of 300 μ L of reagent A)
3. Incubate for 15 min at RT in the dark
4. Wash once in 4 mL cold PBS
5. Centrifuge (400g 10 minutes), discard supernatant, vortex to fully resuspend the cell pellet
6. Add reagent B (similar volumes as in point 2 according to volume of resuspended pellet) and add mAbs for intracellular staining (i.e. TdT, cy-CD3)
7. Vortex and incubate 20 minutes at RT in the dark
8. Wash with 4 mL cold PBS
9. Adjust volume to 200 μ L
10. Add SYTO (see chapter SYTO)
11. Proceed to acquisition on flow cytometer.

Remarks:

See tips for surface staining, like splitting into more tubes. Hypotonic lysis may be also applied as step 8

1.7.1.9 Acquisition of cells at diagnosis and in follow up for MRD

- **Aim:**

Adequate numbers of acquired events are a prerequisite for sufficient test sensitivity.

- **Guidelines:**

A number of 30 cellular events (dots) of similar overall features is the minimum amount to judge a sample MRD-positive. Hence, to warrant that a sample can reliably be judged at the threshold of 0.01% it is necessary that acquired events contain as much as possible relevant events. The proportion of relevant cells among all events is assessed by the SYTO16 stain (also needed of the calculation of MRD% among NC=SYTO16+). Hence, to keep the number of non-relevant events low a certain standard in sample preparation is necessary (see before for optimized lysis procedure). At time points in follow up with a low cellularity of supplied samples the number of tube to be investigated should be kept as low as necessary in order to warrant enough sample per relevant tube. Notably, aplastic samples sometimes also tend to poor lysis efficacy, which should be concentrated by increasing the cell input into the most relevant tubes, or by acquiring a higher than standard number of events. Gated acquisition of CD19+ or CD7 + events, respectively is a further alternative to be done in the very rare problematic cases.

At diagnosis acquire 30 000 events only

Acquisition in BCP- ALL follow up:

First tube: SYTO16/CD10/CD45/CD19

Acquire 10 000 events (SYTO-count among all events)

Define gate on SYTO+ cells

Acquire 300 000 events in SYTO gate

Record the number of total acquired events in the first tube

Second tube: CD20/CD10/CD34/CD19

Acquire the number of events recorded by tube 1

Third tube: CD58/CD10/CD45/CD19

Acquire the number of events recorded by tube 1

Fourth tube: CD10-CD20/CD38/CD45/CD19

Acquisition in T- ALL follow up:

Follow the above listed steps adjusted to T-ALL panel.

Control:

Evaluate percentage of SYTO16+ cells in tube 1. Collect SYTO%/all events data in a spread sheet to monitor lysis performance over time. The percentage of SYTO+ cells must be higher than 50%. Record simultaneously how often it is possible to acquire 300 000 events in the SYTO gate of tube 1. If this is a frequent event cell input must be increased.

MRD report form is found in appendix 4.1

1.8 STUDY DESIGN & ORGANIZATION

1.8.1 STUDY DESIGN

ALL IC-BFM 2009 is a prospective randomized trial of the I-BFM-SG (International BFM Study Group) for the management of children and adolescents (up to 18 years of age) with *de novo* non-B acute lymphoblastic leukemia. Part of the patients will be randomized to receive therapy of varying intensity. It is strongly encouraged to enroll infants < 1 year of age into the INTERFANT 06 trial.

1.8.2 TRIAL PARTICIPANTS

At the BFM meetings held in Brugge 2007, Glasgow 2008 and Bergamo 2009 cooperative or national groups from 13 countries have expressed interest to take part in ALL IC-BFM 2009 trial. These are: Argentina, Chile, Croatia, Cuba, Hungary, Serbia, Slovakia, Slovenia, Poland, Moscow, Turkey, Ukraine (Kiev) and Uruguay. Additional candidate groups interested to participate can join the trial in the future. It has been agreed that these groups should first submit to the Trial Statistics Center complete data on all their patients accrued during the first year of the trial. Separate analysis of their data will be done, and the Trial Steering Committee will then decide on their participation as regular members of the trial. A list of the participating groups, national study coordinators & vice-coordinators, data managers & reference labs for immunophenotyping is found in Appendix 5.0. It has been estimated that approximately 1,000 patients with a newly diagnosed ALL will be accrued on the trial annually. Taking into account the intercontinental dimensions, heterogeneous traditions, cultures and legislations, different socioeconomic backgrounds, GNPs and political conditions as well as the different experience of the individual participating groups, again this trial will be a very large and demanding one, with demands on communication, organization, data collection, management and statistics. With the experience obtained in ALLIC-2002, the group has decided to continue with this second intercontinental trial intended to improve on the results of each participating group in managing the most prevalent childhood cancer.

1.8.3 DIAGNOSIS & RISK STRATIFICATION

The diagnosis should stand firm with the aid of conventional diagnostic tools along with basic flow cytometry and cytogenetics/molecular biology. The evolved classification system is based on age at diagnosis, initial WBC count, early treatment response and some genetic markers: t(9;22) and t(4;11) and/or the molecular equivalents (BCR/ABL and MLL/AF4) just as in ALLIC 2002 including as **new criteria**: hipodiploidy ≤ 44 and FC MRD on day 15 of the therapy. Three risk groups (SR, IR & HR) should be readily discriminated on the basis of these criteria. It is essential that the cytomorphology, immunophenotyping, MRD and genetics be performed at experienced laboratories accredited and approved by the individual national groups for these purposes. MRD will be evaluated in a pilot phase for at least one year before the final decision to use it for stratification in each participating group.

1.8.4 TREATMENT STRATEGY

With regard to treatment, effort has been exercised to get as close as possible to the AIEOP/BFM 2009 trial, which is based on MRD criteria, but it should be borne in mind however that they are not identical, with different MRD techniques used for stratification at different time points. The philosophy of the trial lies in the evaluation

of a new classification including FC MRD and the use of early intensification being those the principal questions; also the dose of MTX will be evaluated.

1.8.5 CONDITIONS & PREREQUISITES FOR PARTICIPATION

To be accepted as a regular member of ALL IC-BFM 2009 the following conditions & prerequisites must be satisfied:

- Adequate experience with BFM-type therapy.
- Adequate supportive care infrastructure and experience.
- Adherence to the prescribed diagnostic requirements all through the protocol.
- Fidelity to the scheduled therapy and therapy regulation/modification guidelines.
- Preferably, only well organized cooperative or national study groups could participate. A minimum of 30 patients with newly diagnosed ALL per year is required for a single center to be accepted as a participating member in this trial.
- All eligible patients should be enrolled in the trial. Patient selection of any type is not acceptable. Registration of the patients at the national data management office must be done once the diagnosis of ALL has been made (within 24 – 72 h). The registration form and the informed consent form must be sent to the national data manager at diagnosis.
- However, to be included in the trial, every patient and/or his/her parent(s)/guardian(s) must be properly acknowledged with the trial concept and should provide an informed consent, either written or verbal (in keeping with the Helsinki Declaration as well as the culture and laws of each country taking part in the trial and of the patient's homeland) with the diagnostics and therapy prescribed in the protocol, with randomization as well as with the anonymous and confidential collection, management, storage, communication and processing of data, aiming finally at publication of the results of the individual participating groups and of the global patient population. The same rules apply for the research projects, too. However these are optional, and refusal to participate in them does not preclude entry of an otherwise eligible patient into the trial. Information for the Patient/Parents/Guardians and Informed Consent Forms are found in Appendices 2.0 – 2.5.
- It should be clear to all participants and patients/parents/guardians that ALL IC-BFM 2009 is a prospective randomized trial of early intensification therapy for the treatment of children with non-B ALL aiming at improving the outcome of these patients, with pEFS being the primary end point. In addition, the protocol might be associated with acute toxicities of unpredictable outcome and with potential late effects of varying severity. An interim analysis will be performed in 2011 and another in 2013. Should these demonstrate excessive toxicity and/or significantly inferior outcome for those patients assigned to the more intensive arms compared to the controls, the modification of the therapy and eventually closure of the respective arm will be considered.
- However, it should be realized that the I-BFM-SG as a whole as well as its individual bodies and committees (separately or combined) carry no liability neither for therapy-related damage/sequels whatsoever or fatality, nor for a deterioration of the ultimate outcome on final analysis or in the long-term.
- It is the responsibility of each participating group to obtain approval for the trial from the local competent authorities, e.g. the ministry of health and/or institutional ethics committees (review boards), and to ensure the trial *pro re nata*, depending on the legislation of the country in question.

- Each group has to have its own national study coordinator, and preferably at least one vice-coordinator, with great clinical experience in the management of ALL, who must be entitled the power and responsibilities to discuss problematic issues and to make difficult decisions in particularly critical, precarious or dubious situations.
- Randomization should be done at a national level by a uniform method (automatic permuted blocks). Provided that informed consent has been obtained, all patients who are eligible for randomization should be randomized. The time point for randomization is the end of induction (day 33) for the first randomization in MR and HR and for the second randomization in MR.
- Full documentation of the patient's status at every visit, completion of the therapy flow sheets and toxicity forms are required for the success of the trial. Within 2 weeks after the end of each therapy element a completed copy of the corresponding flow sheet and toxicity form should be sent to the national data manager. He/she should be notified of whether consent with randomization has been obtained. Should the culture and laws of the country allow for verbal consent only, this must be clearly stated in the consent form by the physician in charge and 2 witnesses. In case of severe toxicity/toxic death the SAE form must be sent to the national study coordinator within 24-72 h.
- Adequate experience with data collection and management as well as with data exchange is essential. Each participating group should collect and manage its own data, entering them into a national database and exporting them to the central database common to the entire trial. Exchange of the basic clinical, outcome and toxicity data between the individual national databases and the central database should be done on an annual basis, i.e. in mid January each year beginning by 2011.

1.8.6 DATA MANAGEMENT

- Each group should have its own national data manager who will be responsible for ensuring complete reports from the individual centers of the group.
- The national study coordinator will review the data for completeness and correctness before sending them to the Trial Statistics Center.
- The national data manager should send to the Trial Statistics Center the data from his/her group on a computerized file in the format described in the protocol once annually. The data must be always updated as of December 31 (beginning by 2010) and pooled in mid January (beginning by 2011) of each calendar year.

1.8.7 TRIAL STEERING COMMITTEE'S (TSC) RESPONSIBILITIES

- To supervise the entry of patient data in the common database;
- To initiate and facilitate basic-research studies;
- To maintain regular contact with the individual centers participating in the trial;
- To decide on admission of new participants to the trial in close liaison with the Trial Management Committee (TMC);
- To evaluate together with the TMC the results of the interim analyses and decide on necessary changes of the protocol in case of excessive toxicity and/or significantly inferior outcome for those patients assigned to the more intensive arms compared to the controls;
- To approve presentations and publications of the results of the trial as well as of the research projects by members of ALL IC-BFM 2009, who should be authorized to do so by the TSC;
- To nominate a writing committee for the purpose of publication of the results of the trial;

- To approve the use of data from the central database by national groups/centers participating in the trial for the purpose of presenting their own results.

1.8.8 TRIAL MANAGEMENT COMMITTEE'S (TMC) RESPONSIBILITIES

- To supervise the progress of the trial;
- To organize and conduct annual meetings of the TSC;
- To propose admission of new participants to the trial;
- To evaluate the results of the interim analyses and propose necessary changes of the protocol in case of excessive toxicity and/or significantly inferior outcome for those patients assigned to the more intensive arms compared to the controls.

1.8.9 TRIAL SCIENTIFIC COMMITTEE'S RESPONSIBILITIES

- To initiate and facilitate research among members of the trial;
- To work out common protocol(s) and firm rules for handling patient material, which will be binding on all members of the trial;
- To collaborate with members of the TSC in the design of clinical trials.

1.8.10 PUBLICATION RULES

- Final results of the study will be published irrespective of whether the aims of the study have been reached or not. Publication will follow the CONSORT Statement and include a thorough safety analysis. Data relating to the study must not be reported or published without prior consultation with and approval by the Trial Steering Committee.
- All participating groups contributing to ALL IC-BFM 2009 will be entitled to claim authorship of publications emanating from this trial.
- Publications by participating groups are not allowed before the overall study results have been published..
- As the modalities of publication may have to differ slightly according to the aim of the study, the effort, contribution or interest of the investigators, the mode of authorship will be chosen from two versions as spelled out below:
 - 1 Investigators having performed active research are listed as authors. All other participants are listed alphabetically on the first page of the publication in a footnote of the citation: "and members of the ALL IC-BFM 2009 trial: ..."
 - 2 A writing committee is nominated by the TSC. The members of the writing committee are the authors of the publication. All other participants are listed alphabetically on the first page of the publication in a footnote of the citation: "and members of the ALL IC-BFM 2009 trial: ..."

1.9 Statistics

1.9.1 Study questions;

- **SR**: Will the outcome of SR patients, defined by SR according to ALLIC2002 criteria and a FC-MRD load <0.1% on day 15, be better than the result which can be expected by stratification with the criteria of ALL-IC 2002?
- **IR**:and **HR**: Can the addition of an early intensification ,protocol IB (Augmented BFM), improve the outcome?
- **IR-BCP ALL** : Are 5 gr/m² of MTX more efficient than 2 gr/m² ?

1.9.2 END POINTS

The evaluation of outcome will be performed in terms of event-free survival (EFS) from diagnosis, in which case death in induction, resistance, death in CR, relapse, and second malignancy will be counted as events, with resistance being an event as of the date of diagnosis. Also the overall survival (OS), time from diagnosis to death from any cause or last follow-up will be evaluated.

The main end point for the randomized questions will be the disease-free survival (DFS) defined as time from the randomization to first event (relapse, death or second malignancy) or last follow up. DFS, EFS and OS will be estimated using the Kaplan-Meier method.

1.9.3 METHODS OF ANALYSIS

For the three study questions an intent-to-treat analysis as the main principle applied on all randomized patients and a per-protocol analysis as a secondary analysis will be performed.

The null-hypothesis test (no difference) for all randomizations will be carried out by comparing the DFS in the two treatment arms with the two-sided log-rank test, stratified by participating group. Combined estimates of treatment effect and the confidence intervals will be given, adjusting by participating group, if no significant heterogeneity of the effects will be detected. The α is 5% for all comparisons.

1.9.4 RANDOMIZATION

Each child who enters the protocol (all inclusion criteria fulfilled, no exclusion criterion given) is eligible for randomization. Patients who are otherwise eligible for allogeneic SCT must be also randomized (for the case of not performing the transplant). Randomization will be performed by the data management office of each national group, so that the treatment arms will be balanced at each risk level. At the time of randomization (i.e. by the end of induction phase), at least the registration and diagnostic data as well as note of appropriately expressed consent should be available at the data management office in order to verify eligibility. The random assignment will be produced by an automatic procedure based on random permuted blocks.

If the patient/parents or guardians refuse randomization, they or the treating physician can choose the treatment arm, fully respecting the Declaration of Helsinki.

1.9.5 SAMPLE SIZE

Tab. 5 gives the estimated number of patients per year in the individual national groups/centers that may participate in ALL IC-BFM 2009. Under the assumption that about 10% of the patients will not be randomized because of very early events or refusal, approximately 5,000 patients will be randomized within the study period of 5 years. Of these about 13 % (N = 672) are assumed to be SR, 66% (N = 3,277) IR and 21% (N = 1051) HR. An analysis of the data from trial ALLIC 2002 had estimated the probability of 5-year pEFS at 95% for SR, 78% for MR and 55% for HR (**Tab 2**, pag 15). With a first type error $\alpha = 0.05$, there will be a power of about 80% to detect a difference of 5% in SR & IR, and 10% in HR. (Freedman LS 1982)⁽¹³⁾.

Table 5: Estimated Annual Number of Patients

| Country | Patients/year |
|-----------|---------------|
| Argentina | 210 |
| Chile | 100 |
| Croatia | 30 |

| | |
|----------------|--------------|
| Cuba | 30 |
| Hungary | 60 |
| Moscow | 30 |
| Poland | 220 |
| Serbia | 60 |
| Slovakia | 30 |
| Slovenia | 30 |
| Turkey | 200 |
| Ukraine (Kiev) | 70 |
| Uruguay | 30 |
| Σ | 1,100 |

1.9.6 INTERIM & FINAL ANALYSES

Three interim analyses will be performed 2 and 3 years after the start of the trial and at the end of the accrual period. A final analysis will be carried out 3 years after stopping accrual. The significance levels will be adjusted according to O'Brien and Fleming (O'Brien PC et al. 1979)⁽¹⁴⁾.

Toxicity will be monitored for each treatment branch (SR, IR, HR) with a Wald sequential plan, whereby $H_0: q \leq p_0$ and $H_1: q \geq p_1$ (q : probability of therapy-related death). The probabilities for p_0 (lower limit for "acceptable" probability of therapy-related death) and p_1 (upper limit thereof) are: SR: $p_0 = 0.02$, $p_1 = 0.03$; IR: $p_0 = 0.03$, $p_1 = 0.05$; HR: $p_0 = 0.08$, $p_1 = 0.12$. All cases of death suspected to be therapy-related within the time from start of treatment to therapy duration of 40 weeks are included in the analysis.

If one of the interim analyses shows a significant result, or if limits of the sequential plans are reached, the DSMC will propose, and the TSC together with the TMC will decide on whether the trial as a whole or its part(s) could be continued or should be stopped or modified.

1.9.7 DATA COLLECTION

The set of data to be collected and pooled by the participants is listed in Appendix 1.3 (Variables in ALL IC-BFM 2009 Database). Each group will centralize the forms for input and quality checks in its own data office according to the approach routinely used by that group.

Every national data management office will have to ask the clinical center(s):

- To report immediately events (relapse, death, SMN).
- To send on a regular basis the forms on diagnosis, response, randomization, treatment, and toxicity as well as notification of consent, as soon as they can be completed. It should be made clear to the clinical centers that randomization can be obtained from their national data management office only if at least registration, diagnosis, response to prednisone, induction and induction-response data as well as note of consent have been previously sent.
- To update follow-up at the end of each calendar year.

1.9.8 DATA POOLING

A common coding system and format is specified in Appendix 1.3 for use by the data managers and statisticians in order to minimize mistakes in data exchange. This coding system is defined for the purpose of data pooling only: it is required that a data set including every registered patient is prepared in this format by the data managers and statisticians of each group. The data have to be periodically (by mid January of

every year from 2011 through 2016) sent for pooling to the Trial Statistics Center. This in collaboration with the contact person and the statistician of each group pools the data and circulates a report on them.

The data managers and statisticians of the participating groups will define a set of data-checking procedures, which have to be performed before sending the data to the Trial Statistics Center. The national study coordinator/vice-coordinator should supervise the quality-control process of data collection within the group so as to ensure correct and complete data be then submitted in the appropriate format to the trial central database.

The data pooling will be done in the following steps:

- Each group will send (preferably by E-mail) to the Trial Statistics Center the data file by mid January of each calendar year (beginning by 2011), with data frozen and follow-up updated as of December 31, the previous year;
- The data files will be pooled in a common database, to be used for the trial aims only;
- A report on the trial progress will be produced annually (recruitment, toxicity, and so on), and the interim analysis performed when planned;
- Reports will be circulated by the Trial Statistics Center to the contact persons of the groups and to the Data & Safety Monitoring Committee (DSMC);
- Interim analyses will be submitted (blinded) to the contact persons of the groups, the TMC, and (unblinded) to the DSMC.

Contact persons are the national data managers/statisticians.

Members of the DSMC are experienced researchers (two clinicians and one statistician) not involved in the trial, who will be responsible for providing the investigators with guidance on the conduct of the trial, and, in case of problems, on whether the trial should be stopped, modified or continued.

1.10 Patient Enrollment & Eligibility

1.10.1 STUDY PATIENTS

All patients with ALL diagnosed in one of the centers/hospitals participating in ALL IC-BFM 2009 trial of the I-BFM-SG will be enrolled as study patients, if the following criteria are met:

1. Age at diagnosis \leq 18 years.
2. Start of induction therapy within the enrollment period of the trial: from April 1, 2010 through March 31, 2015.
3. Diagnosis of ALL ensured by all the diagnostic criteria defined in the protocol.
4. Informed consent to participate in ALL IC-BFM 2009 trial available.
5. Admission, diagnosis and therapy performed by a center participating in the study.

Infants < 1 year of age should be treated according to INTERFANT 06 trial in the majority of the participating countries

Patients that are enrolled according to these criteria must not be excluded except for proven misdiagnosis of ALL. All study patients are separated into observed patients and protocol patients.

Observed patients are study patients who meet at least one of the following criteria:

1. The disease is a second malignancy.
2. The disease is a relapse of a previously unrecognized ALL, which was treated inadequately.

3. The patient suffers from a major other disease that prohibits treatment according to the protocol (e.g. severe congenital heart disease).
4. The patient was treated with steroids or cytostatic drugs within the four weeks preceding the start of protocol therapy.
5. Essential data are missing that are needed for the differential diagnosis of ALL vs. AML, or for selection of the proper therapy arm.
6. Death prior to start of diagnostic & therapeutic procedures according to the protocol, e.g. due to intervention or complications (at a department not taking part in the trial), for which the patient is referred to a participating center and expires upon admission.

All other patients are protocol patients who are included in each study report and the final analysis.

1.11 Basis for Stratification

1.11.1 EARLY TREATMENT RESPONSE

The early response to therapy is the most important stratification principle in ALL IC-BFM 2009 trial of the I-BFM-SG. The cut-off limits for the definition of bone marrow (BM) status are shown in **Tab. 6** below.

Table 6: Definition of BM Status

| BM Status | M1 | M2 | M3 |
|----------------|-----|------------|------|
| % Blasts in BM | < 5 | ≥ 5 – < 25 | ≥ 25 |

- **Prednisone response**

This is assessed by the absolute blast count (ABC) in the peripheral blood (PB) on day 8 after 7 days of prednisone pre-phase and one dose of IT MTX on day 1 with or without allopurinol. The day of the first dose of prednisone is day 1.

$$\boxed{ABC/\mu\text{L PB} = \text{Blasts (\%)} \times \text{WBC}/\mu\text{L PB}}$$

Patients with an ABC on day 8 of < 1,000/μL PB are *prednisone-good responders* (PGR). Depending on the concrete constellation of prognostic factors, they may fall into the SR, IR or HR group- see **Fig. 5** (p 15) and further discussion in this section as well as section 1.12 .

By contrast, patients with ≥1,000 blasts/μL PB are *prednisone-poor responders* (PPR), and should be treated according to the HR arm, no matter what profile they may otherwise exhibit.

- **M3 bone marrow with ≥ 25% blasts on day 15**

The bone marrow status should be assessed on day 15 after 2 weeks of prednisone, one dose each of vincristine, daunorubicin and asparaginase and two doses of IT MTX. Patients that would have been classified into either the SR or IR arm according to the initial WBC count, age, and prednisone response day 8, will be for an M3 day 15 "upstaged" and treated within the IR (originally SR) or HR (originally IR) arm. Patients with non-assessable BM day 15, due to severe aplasia, will be stratified according to their remaining criteria.

- **FC MRD on BM day 15.**

Even though FC MRD should be determined since the beginning of the trial it will be incorporated as a stratification criteria only after the Trial Management Committee decides it for each study group separately

Patients classified as SR should have < 0,1% to remain as SR. Those with FC MRD >0,1 and < 10% will be upgraded to IR; those SR with >10% will be included in HR

Patients classified as IR with FC MRD >10% will be included in HR, all others will remain as IR. No patient will be “downgraded”.

Patients classified as HR will remain in this group no matter the level of MRD. They will not be “downgraded”

- **Bone marrow status after 33 days of induction therapy**

If the morphologic criteria for M1 bone marrow (M1: < 5% blasts) and features of a regenerating hematopoiesis are not fulfilled on day 33, the patient will be classified into the HR arm.

1.11.2 EXCEPTIONS

- Patients with Ph₁ + (BCR/ABL +) ALL, t(4;11) (MLL/AF4 +) ALL or hipodiploidy ≤ 44 are classified into the HR arm irrespective of treatment response.

1.11.3 SPECIAL CONSIDERATIONS

- An anterior mediastinal tumor or enlarged thymus with or without pleural effusion at diagnosis is not *per se* decisive for initial stratification. Indeed, 50 – 60% of patients with T-ALL, often adolescents, present with such findings. The maximal width of the mediastinum at the level of the body of Th 5 on a PA CXR should be measured at baseline and on day 33. If the tumor has disappeared altogether by day 33, the patient will continue on with the assigned therapy. However, if it has not receded completely by that time, the patient should be rigorously reevaluated by MRI/CT one week after completing the induction therapy. A suspected residual structure post induction should be resected and carefully examined by histology as well as with the aid of molecular biology techniques. If the excised tissue contains viable blasts, the patient must be further handled within the HR group. In addition, local radiotherapy to the mediastinum may be also considered. If no blasts could be demonstrated as well as in case of normal imaging findings upon induction, the patient is to be managed along the already begun treatment strategy.

- A similar approach applies for overt testicular leukemia at diagnosis. Up to 25% of males with ALL have infraclinical infiltration of their gonads with leukemic blasts. However, only 2% present with overt disease, the majority being infants or adolescents with T-ALL, high WBC count, and usually a mediastinal mass. With contemporary therapies, the disease is rare at relapse, too (historically it accounted for 10% of all relapses). First of all, an inflammatory, vascular or other origin of the testicular swelling must be ruled out. In addition to history, physical examination, by a urologist if need be, is adequate, and US quite informative. Primary orchiectomy is by no means an option, and biopsy is rather the exception.

A testicular tumor at diagnosis has no input to the initial risk classification. Nonetheless, if the size of the testicle(s) had not quite normalized by day 33 induction, the local status should be thoroughly reevaluated after completing phase I/2 (I/2). In case of persistent space-occupying lesion/infiltration, biopsy becomes a necessity. Should leukemic blasts be shown in the biopsy specimen, the patient must be further managed within the HR strategy. A persistent testicular leukemia may ultimately need local radiotherapy (RTX) at 18 Gy to eradicate- see section 1.16.1.5 (p 39).

- Skeletal involvement is not a stratifying criterion either since it does not affect the prognosis adversely. However, florid leukemic skeletal lesions as suggested by MRI, and with blasts proven in the curettage biopsy, which persist post induction therapy, should be considered for further management approach- see section 1.16.1.4 (p 38).
- CNS status does not by itself allocate a patient to this or the other risk level. Nonetheless, tailored to age, management (prophylaxis/therapy) of covert/overt disease within the CNS sanctuaries is governed by that status, the risk group (HR vs. non-HR) as well as by the immunophenotype (T-ALL vs. BCP-ALL). This is discussed later in this chapter- see section 1.14 (p 33) and permeates chapter 2.
- Co-expression of My markers is not a stratification criterion on its own. Provided that they display otherwise comparable features and managed properly, patients with My (+) ALL fair the same as those with My (-) ALL, as shown by a recent analysis of the BFM data (Schrappe M et al. 2000)⁽¹⁵⁾ and supported by other investigators (Pui CH et al. 1998)⁽¹⁶⁾.
- T immunophenotype *per se* is not a stratification criterion. Nevertheless, patients with T-ALL deserve within the framework of SR/IR more intensive treatment than those with BCP-ALL, as discussed elsewhere in this chapter as well as in chapter 2. HR T-ALL is managed as such, i.e. within the HR strategy.

1.12 Risk Group Assignment

1.12.1 STANDARD-RISK GROUP (SR)

- PB day 8: < 1,000 blasts/ μ L
and Age \geq 1 yr – < 6 yr
and Initial WBC < 20,000/ μ L
and if available FC MRD < 0,1% **or** M1/ M2 marrow on day 15
and no M 2/3 marrow on day 33

All criteria must be fulfilled.

1.12.2 HIGH-RISK GROUP (HR)

1. IR and, if available FC MRD >10% **or** M3 marrow on day 15
2. SR if available FC MRD >10%
3. PB on day 8: \geq 1,000 blasts/ μ L
4. M2 or M3 marrow on day 33
5. Translocation t(9;22) [BCR/ABL] or t(4;11) [MLL/AF4]
6. Hipodiploidy \leq 44

At least one criterion must be fulfilled.

1.12.3 INTERMEDIATE-RISK GROUP (IR)

All patients who are not stratified to SR or HR are intermediate risk patients.

1.13 Indications for Allogeneic SCT in first Complete Remission

1.13.1 DEFINITION

In ALL IC-BFM 2009 trial of the I-BFM-SG, the general term stem-cell transplantation (SCT) will be used for bone-marrow transplantation.

1.13.2 INDICATIONS

Allogeneic SCT from an HLA-identical family donor (MFD = matched family donor), if available, is a therapeutic option only in a subgroup of HR patients. In general, SCT can be considered in patients with a prognosis < 50% pEFS (Balduzzi et al 2005, Schrauder et al 2006).^(17,18)

Only in exceptional cases may family members with a one-antigen difference be used as donors. SCT from a matched unrelated donor (MUD) need to be discussed and approved by the national study coordinator on a strictly individual basis. The indication for allogeneic SCT in these cases is the responsibility of every national group. One has to consider his/her own experience/expertise with the procedure, the local infrastructure, resources, and the availability of comprehensive supportive care as well as transplant-related morbidity/mortality (TRM/M).

The procedure of alloSCT is not part of the ALL IC BFM 2009 study.

The indications for allogeneic SCT in HR ALL are the same as in ALLIC 2002 (Tab. 7).

Table 7: Indications for Allogeneic SCT in ALL IC-BFM 2009

| INDICATION | | MFD [†] SCT |
|-------------------------------|--------------------------|----------------------|
| NR d33 | | + |
| Hypodiploidy < 44 chromosomes | | + |
| PPR | + T-ALL or + pro B-ALL | + |
| | + WBC > 100,000/ μ L | + |
| | + t(9;22) or BCR/ABL | + |
| | + t(4;11) or MLL/AF4* | + |
| PGR | + t(9;22) or BCR/ABL | + |
| HR | + M3 d15 | + |

[†] MFD matched family donor

* Infants < 1 yr only

1.14 CNS Therapy

1.14.1 RATIONALE & PRINCIPLES

Since its introduction in the mid 60's and early 70's, CNS-targeted therapy addressing occult or manifest disease within the CNS, being a "natural reservation" favored by the malignant lymphoblast, has become commonplace in all protocols designed for the management of ALL. This strategy has led to a dramatic descent in the rate of CNS relapses from about 70% to less than 5%, thus improving the overall prognosis of children with this disease. On the other hand, the acute and late, often debilitating, side effects of that therapy on the growing and developing child have since been driving the pediatric hematologists/oncologists towards critical revision, sophistication and refinement of their approaches to CNS leukemia. For more details refer to section 2.1.2, section 2.3.3.6 on locoregional drug therapy and section 2.5 on radiotherapy. The generally accepted principles of CNS-targeted therapy in pediatric ALL can be formulated as follows:

1. Improved definition of CNS leukemia.
2. Therapy adapted to CNS status as defined at diagnosis (or relapse).
3. Therapy tailored to age attained by the time of its delivery.
4. Therapy adjusted to level of risk:
 - HR vs. non-HR.
 - T-ALL vs. BCP-ALL.

5. Choice of the safest formulations of effective drugs for locoregional therapy.
6. Avoidance of potential incompatibility between drugs used for locoregional therapy.
7. Use of certain antineoplastic drugs at high dosage for systemic therapy to overcome the BBB (HD MTX, HD ARA-C).
8. Omission or dose reduction of cranial radiotherapy (CRT).
9. Improved technique/quality control of CRT.
10. Careful observation of the patient on therapy (acute toxicity) and LTFU off therapy (subacute & delayed side effects, CNS relapse, SMN).

1.14.2 DEFINITION OF CNS STATUS

Refer to section 1.16.1.3 for definition of initial CNS status.

1.14.3 CHANGES vs. ALLIC 2002

With minor modifications, the recommendations of ALLIC 2002 are in principle applicable to the current trial as well. As in that trial infants < 1 year of age receive neither prophylactic nor therapeutic cranial radiotherapy (pCRT/tCRT), nor those HR patients younger than 2 years undergoing allogeneic SCT receive any form of RTX. On the other hand, like in ALL-BFM 95, those HR patients ≥ 2 years undergoing allogeneic SCT do receive TBI, and, if indicated, local RTX as well. However, contrary to that trial, no prophylactic boost will be given within the framework of conditioning. While in ALLIC 2002 patients with T-ALL assigned to SR & IR with CNS status 1/2, received pCRT, in ALLIC 2009 only T-ALL+ WBC > 100000 will receive it. A second change in pCRT is in HR as BCP-ALL only due to PPR will not receive pCRT (they will receive 6 doses of IT MTX in maintenance). The dosage of pCRT as well as tCRT is uniform across the given indications all through the trial. With regard to CNS-targeted chemotherapy, HD MTX (5 g/m²/24h) is reserved for SR/IR T-ALL and HR patients and it will be randomly evaluated in IR BCP-ALL. However, to be on the safe side, SR/IR patients with BCP-ALL will receive extra IT MTX during maintenance, a strategy that should counteract the lower dose level of systemic MTX (2 g/m²/24h) employed for the consolidation of those patients.

1.14.4 INDICATIONS & MODALITIES FOR CNS THERAPY

CNS therapy is briefly outlined in sections 1.14.4.1 through 1.14.5. For more details refer to section 2.5 on radiotherapy

1.14.4.1 PROPHYLACTIC CNS THERAPY (CNS STATUS 1) - No tCRT

- SR and IR patients
- ❖ BCP-ALL: MD MTX; no CRT
- ❖ T-ALL: HD MTX; 12 Gy only for those with WBC > 100.000 (pts aged ≥ 1 year)
- HR patients :HD MTX + HD ARA-C; 12 Gy (pts aged ≥ 1 year) **except those BCP ALL with a HR due only to PPR, they will not receive pCRT**
- All patients: prophylactic shots of single/triple IT therapy (by risk group & arm)

1.14.4.2 PROPHYLACTIC CNS THERAPY (CNS STATUS 2) - No tCRT

- The same as for CNS status 1 above +
- 2 additional IT MTX doses on days 18 & 27 of Protocol I/I'

1.14.4.3 CNS THERAPY IN CNS STATUS 3 - all patients undergo tCRT

- Patients aged $\geq 1 < 2$ years: 12 Gy

- Patients aged ≥ 2 years: 18 Gy
- Locoregional therapy:
 - Prophylactic shots of single/triple IT therapy (by risk group & arm) +
 - Additional doses of IT MTX in Protocol I/I'/II
 - Additional TIT in block HR-2'
- Systemic chemotherapy:
 - SR/IR BCP-ALL: MD MTX
 - SR/IR T-ALL: HD MTX
 - HR: HD MTX + HD ARA-C

1.14.5 TIMING OF CRT

- Upon the conclusion of the reinduction therapy (protocol II) in all but:
- Prior to allogeneic SCT:
 - TBI within the conditioning regimen, if indicated
 - Local RTX pre-conditioning, if indicated

1.15 Duration of Therapy & Maintenance Therapy

The duration of overall therapy in all patients is 104 weeks (24 months). All patients receive uniform oral maintenance therapy composed of 6-mercaptopurine (50 mg/m²/d in a SD on a fasting stomach without milk in the evening), and MTX (20 mg/m², x 1 weekly, also in a SD on a fasting stomach without milk in the evening). Patients with BCP-ALL allocated either to arm SR or arm IR receive 4 doses of IT MTX. This supplemental locoregional therapy is intended to compensate for the lower dose level of systemic MTX in Protocol mM in this trial, compared to Protocol AEIOP/BFM 2009. Two other groups, HR due only to PPR and T-ALL+ < 100.000 WBC, will receive 6 doses of IT MTX in maintenance, as they will not receive RTX in this trial.

1.16 DIAGNOSTICS

1.16.1 INITIAL DIAGNOSTICS

1.16.1.1 DIAGNOSIS & BIOLOGIC CHARACTERIZATION OF ALL

The diagnosis of ALL is made based primarily on the cytologic examination of the bone marrow (BM), peripheral blood (PB) and cerebrospinal fluid (CSF). Well spread BM smears should be stained with Romanowsky dyes, preferably May-Grünwald-Giemsa (MGG). The myelogram and FAB^(19,20) 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 \pm NaF) is not routinely required, but may be helpful in otherwise uncertain cases. Usually, panoptic staining, again preferably with MGG, is the all needed for the cytologic evaluation of PB films and CSF cytopsin preparations. For this purpose, it is preferable to prepare native PB smears and CSF cytopsin, i.e. without EDTA, heparin, albumin, etc so that the cytologic detail could be better appreciated. A diagnosis of ALL can be made if $\geq 25\%$ of the nucleated cells in the BM are lymphoblasts. In every case, the diagnosis is to be confirmed by the reference national lab accredited or approved for this purpose by the participating groups in the individual countries.

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 RT-PCR) to investigate prognostically important fusion genes (BCR/ABL, MLL/AF4 and TEL/AML1) are indispensable. If available, techniques of molecular cytogenetics (CGH, FISH) are encouraged, as they may reveal changes that would otherwise escape detection (Jarosova M et al. 2000)⁽²¹⁾.

Immunophenotyping by FC, for its direct therapeutic and other implications, is an integral part of the initial work-up of every patient with ALL.

The basic diagnostic program aimed at making the diagnosis of ALL and exploring its biologic profile, which is essential for the choice of proper therapy that will in turn affect the ultimate prognosis, is outlined in **Tab. 8**, and shown in Appendix 1.0, where addresses of the reference labs must be completed in the void box by each participating group. Preparation & shipping of samples should meet routine standards to ensure safety and maximum efficiency. The immunologic classification of ALL⁽²²⁾, and criteria for its separation from AML & B-ALL/NHL are given in Appendix 4.0.

Table 8: Obligatory Diagnostics for the Biologic Characterization of ALL

| | |
|-----------------------|--|
| 1. Cytomorphology | <ul style="list-style-type: none"> • BM: MGG • PB: CBC & Differential (MGG) • CSF: Cell count (chamber) & Cytology (MGG-stained cytopsin preparation) |
| 2. Flow Cytometry | <ul style="list-style-type: none"> • BM/PB: Immunophenotype & DNA index* MRD |
| 3. Cytogenetics | <ul style="list-style-type: none"> • BM/PB: High-resolution G-banding (Numerical & structural aberrations) |
| 4. Molecular Genetics | <ul style="list-style-type: none"> • BM/PB: RT-PCR for selected fusion genes (BCR/ABL, MLL/AF4, TEL/MLL1) |

* DNA Index is optional in this trial. If no conventional cytogenetics is available and the DNA Index is lower than 0.8 it can be accepted as equivalent of < 45 Cr.

1.16.1.2 GENERAL DIAGNOSTICS OF EXTRAMEDULLARY DISEASE

For the documentation of the patient's baseline status as well as of any extramedullary manifestations of the disease, the following diagnostic work-up is indispensable and obligatory, i.e. should be performed on every study patient:

1. Careful history.
2. Physical examination including sites of involved or suspected lymph nodes, size of liver, spleen, and testes.
3. Lumbar puncture (LP) before beginning the cytoreductive pre-phase of therapy.
4. Chest X-ray (CXR) in two views (PA & lateral). The maximal diameter of the mediastinum at the level of the body of the 5th thoracic vertebra is to be measured.
5. In case of an uncertain finding, then thorax MRI or CT is necessary.
6. X-ray of the left hand in dorsovolar projection.
7. X-ray of the lumbar vertebral column in lateral view.
8. Ultrasonography (US) of the neck, mediastinum, abdomen (& testicles, if need be).
9. In case of proven & suspected CNS disease, or if blasts in CSF: cranial MRI or CT.

10. ECG & Echocardiography (Echo-CG).

11. Neurologic examination & EEG.

12. Fundoscopy.

Additional investigations may be indicated in specific situations that should be determined by the treating center.

1.16.1.3 DIAGNOSIS OF CNS INVOLVEMENT & DEFINITION OF CNS STATUS

LP at diagnosis is an essential part of the initial evaluation of patients with ALL. It should be carried out prior to starting the cytoreductive prednisone pre-phase. Even hyperleukocytosis $> 100,000/\mu\text{L}$, under conditions of a still effective hemostasis, good general standing and absence of a severe infection etc, is not a contraindication for LP, so far as MTX is administered intrathecally at the same time. Needless to say however, that the diagnosis of ALL should already stand for MTX to be administered. LP is urgently necessary for the assessment of initial CNS status. Therefore, the first LP may be postponed only in exceptional situations. In addition to chemistry (total protein, glucose) and cell count in Fuchs-Rosenthal' or Nageotte's chamber, cell morphology and differential must be assessed on a high-quality cytopspin preparation made by a standard technique. Cytopspin preparations should be always worked out, regardless of the CSF cell count, and at least one slide (with data on CSF cell count & CBC) be sent to the reference laboratory for expert reading and assent.

CNS status can be defined on clinical/imaging grounds and/or on the basis of the cell count and cytomorphology of the CSF. The following definitions will be used in this study:

1. CNS status 1, i.e. negative:
 - No clinical evidence of a CNS disease, including cranial nerve palsy that would be unequivocally attributable to leukemia.
 - No imaging (CT/MRI) evidence of a CNS abnormality that would be unequivocally attributable to leukemia.
 - Normal fundoscopic finding.
 - Blast-free CSF along with absence of any other evidence of CNS leukemia.

2. CNS status 2, i.e. negative:
 - Blasts unambiguously identified and RBC : WBC $\leq 100:1$ on cytopspin preparation of CSF with a cell count of $\leq 5/\mu\text{L}$. With this RBC : WBC ratio, the LP is considered to be non-traumatic, and the CSF uncontaminated with blood (Lauer SJ et al. 1989, Mahmoud HH et al. 1993, McIntosh S et al. 1986)⁽²³⁻²⁵⁾.
 - Lymphoblasts identified and RBC : WBC $> 100:1$ on cytopspin preparation of CSF. With this RBC : WBC ratio, the LP is considered to be traumatic, and the CSF contaminated with blood ⁽²³⁻²⁵⁾.
 - A traumatic LP (blood-contaminated CSF) is combined with an initial WBC of $> 50,000/\mu\text{L}$.

3. CNS status 3, i.e. positive:
 - A mass lesion in the brain and/or meninges on CT/MRI.
 - Cranial nerve palsy unrelated to other origin, even if the CSF is blast-free, or no circumscribed space-occupying lesion could be demonstrated within the neurocranium on MRI/CT scan (Ingram LC et al. 1991, Smith M et al. 1996)⁽²⁶⁻²⁷⁾.

- Pure retinal involvement, i.e. with a blast-free CSF, and no mass on MRI/CT scan ⁽²⁷⁾.
- A non-traumatic LP yielding a CSF with a cell count of $> 5/\mu\text{L}$ and a majority of blasts on the cytospin slide (Gilchrist GS et al. 1994, Tubergen DG et al. 1994)⁽²⁸⁻²⁹⁾.
- If contamination with blood is doubtful, the diagnosis of CNS involvement can be still made on the basis of either of the following 2 constellations of findings:
 - Cell count $> 5/\mu\text{L}$ (chamber)
+ majority of blasts (cytospin)
+ RBC : WBC $\leq 100:1$ (cytospin)
 - Cell count $> 5/\mu\text{L}$ (chamber)
+ higher % of blasts in CSF than PB

In case of a CNS status 2/3, the CSF should be controlled carefully at subsequent therapeutic LPs until it is definitively blast-free. In case of a questionable initial or follow-up finding, it is recommended to repeat the LP two weeks later (Odom LF et al. 1990)⁽³⁰⁾. The national study coordinator is always available for discussing any dubious cases.

The diagnostic criteria of a **CNS relapse** are generally those of the initial diagnosis.

Not qualifying for initial CNS involvement, patients with a CNS status 1 receive limited locoregional therapy and NO therapeutic cranial radiotherapy (tCRT).

Cases defined as CNS status 2 do not meet the qualification for initial CNS involvement either. They will receive additional IT MTX only, on day **18** and **27** of Protocol **I/I'**, but should not undergo tCRT.

However, those HR patients not undergoing SCT (except BCP+ PPR) as well as patients with T-ALL + WBC > 100.000 , who qualify for a CNS status 1 or 2 should receive prophylactic cranial radiotherapy (pCRT) at age-adjusted dosage- see section 2.5.6.1 & **Tab. 13** (p 49).

All cases with initial CNS involvement as defined on the basis of assessment of the CSF and/or on clinical/imaging grounds will be for the purpose of this trial and for the sake of simplicity generally labeled as CNS status 3. In an effort to sterilize the CNS, these patients should receive extra doses of IT MTX in Protocol I/I/II & extra TIT in block HR-2' as well as tCRT at age-adjusted dosage- refer to section 2.5 (p 101) on radiotherapy, also HR-CNS 3 patients with SCT receive it the week before TBI.

It should be borne in mind, that the CNS status is not otherwise a stratifying criterion in this trial. Nor does it *per se* envisage any modifications in the dose level of systemic chemotherapy.

1.16.1.4 DIAGNOSIS OF SKELETAL STATUS & INVOLVEMENT

A skiagram of the chest (PA & lateral view), the left wrist (dorsovolar projection) as well as the lumbar vertebral spine (lateral view) must be performed on all patients. These baseline investigations are rather required for the sake of prospective evaluation of potential adverse effects of therapy, particularly steroids and MTX, on the skeleton, although they may reveal leukemia-related changes and manifestations, such as a mediastinal mass, pleural or pericardial effusions, and a variety of skeletal abnormalities. Clinical indications for further X-rays are, e.g. complaints of pain or signs of skeletal instability. If the results are doubtful, MRI of the site in question is

often helpful. It should be borne in mind that it will usually take several months for the radiographic changes of the skeleton to normalize.

Initial skeletal involvement has no impact on prognosis, and is not incorporated within risk stratification. However, a skeletal affection persisting beyond induction, e.g. an expanding osteolytic lesion with an MRI signal compatible with neoplastic infiltration should be curettaged, stuffing the defect with spongiosa. If histology/cytology of the curettage specimen shows viable blasts, and the lesion does not heal properly on continued antileukemic therapy, one is facing a refractory disease that needs individual consideration and discussion with the national study coordinator as to further management.

1.16.1.5 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 also section 1.11.3 .

1.16.1.6 BASELINE DIAGNOSTICS OF HEMOSTASIS

The risk for thrombosis or bleeding is increased mainly in phase 1 of Protocol I/I', and to a lesser extent in phase 1 of Protocol II (Sutor AH et al. 1999)⁽³¹⁾. The risk factors are concomitant corticosteroid and asparaginase therapy, especially in patients with central venous catheters (CVC). Particularly patients with inherited prothrombotic defects are at high risk for these complications (Sutor AH et al. 1999, Nowak-Göttl U et al. 1999, Wermes C et al. 1999)⁽³¹⁻³³⁾. Global coagulation tests (Quick & aPTT) as well as assessment of fibrinogen, D dimers, protein C, protein S, antithrombin III, and APCR are obligatory, whereby blood should be sampled already prior to hydration/alkalinization and any specific antileukemic therapy. It is strongly recommended to explore by techniques of molecular biology the presence of the C677T methylenetetrahydrofolate reductase (MTHFR), prothrombin G20210A and F. V Leiden (G1691A) mutations. In case of a family history positive for or suggestive of a hemorrhagic or thrombophilic diathesis, additional tests may be also considered, e.g. vWF, lipoprotein (a), and homocysteine.

1.16.2 DIAGNOSTICS DURING THE COURSE OF THERAPY

In the course of therapy, evaluation of the initial therapy response, MRD and remission is in the center of interest. Further indications for additional investigations depend on the initial results (e.g. extramedullary manifestations, previous illnesses, HR group) or on the participation in parallel research studies .

1.16.2.1 PREDNISONE RESPONSE

The leukemic cell count per μL PB is determined on protocol day 8 after 7 days of exposure to prednisone (plus MTX IT on day 1) with or without allopurinol, day 1 being the first day of prednisone therapy. Being a supreme prognostic factor with

direct implications with regard to risk stratification and treatment, great attention should be paid to this parameter of early blast clearance.

- Native PB smears, i.e. without EDTA, must be prepared for this purpose.
- It is useful to compare the cytologic close-up of PB₈ with that of PB₁ in order to appreciate the blast features more precisely in the case under study.
- 4 – 6 unstained PB smears along with providing data on the total WBC count on day 8 should be submitted promptly to the national reference laboratory.

1.16.2.2 BM MORPHOLOGY EVALUATION AT DAY 15

The initial blast reduction in the BM is assessed on day 15 of the protocol. The national reference laboratory requires 4 unstained BM smears be sent without delay.

1.16.2.3 MRD by FC in BM AT DAY 15

A BM sample – should be at least one syringe of 3-4 mL stabilized with Heparin or EDTA should be sent as soon as possible to the lab. The first sample taken, if there are others studies running, should be for MRD analysis

FLOW-day 15 material has first priority both in transportation (time from hospital to lab) and in amount of material delivered

1.16.2.4 REMISSION STATUS ON PROTOCOL I DAY 33

A CBC, BMP, LP, clinical and imaging examinations of localized infiltrates (if initially present, e.g. a thymus tumor) are necessary for assessment of the remission status on day 33. For confirmation the national reference laboratory should receive 4 unstained BM smears, and, if initially CNS +, additionally 2 unstained & native CSF cytospin preparations.

Complete remission (CR) is defined as:

1. M1 marrow: < 5% blasts with normal or only slightly decreased cellularity, i.e. with signs of recovering hemopoiesis.
2. No localized leukemic infiltrates/masses on clinical examination and/or by imaging studies.
3. No leukemic cells in the CSF obtained by the therapeutic LP on day 33.

If CR is not achieved by protocol day 33, then the remission status should be reassessed on day 52 of Protocol I, and, if need be, before 1. HR-1' block, and *pro re nata* before 1. HR-2' block. The results obtained at the latter time point serve to separate late responders (LR) who have achieved remission by that time and who can continue on conventional HR therapy or may be preferably scheduled to allogeneic SCT, if a suitable donor were available, from non-responders (NR), for whom the national study coordinator should decide on the most appropriate therapeutic option available or otherwise.

The assessment of remission can be difficult in the following situations:

1. The BM contains between 5 and 25% suspected cells:
The question with regard to continuation of therapy is whether these are leukemic blasts or an extremely immature stage of regenerating hematopoietic cells (hematogonia), which is of supreme value for risk differentiation, and hence for allocation to further post-induction therapy. In this case, BMP should be repeated after 1 week without intervening therapy, and the BM smears submitted to the reference laboratory for assessment. *Flow cytometry and molecular biology (clone-*

specific probes for IRG) may be ultimately helpful in differentiating a malignant clone from a benign cell population.

2. The BM contains no blasts due to significantly decreased cellularity:
If the blood count shows thrombocytes $\geq 50,000/\mu\text{L}$ and leukocytes $\geq 2,000/\mu\text{L}$, BM dilution with PB is probable. BM must be repeated.
3. The BM contains no blasts due to aplasia:
True BM aplasia should be reflected by profound thrombocytopenia, leukopenia and reticulocytopenia in the blood. In this case, therapy must be withheld for one week, and the BM thereupon repeated. Recovering hemopoiesis is one of the defining features of CR.
4. A mediastinal tumor has not completely receded or, in case of initial testicular involvement, the size of the testicle(s) has not quite normalized by day 33:
These patients should be rigorously reevaluated by imaging techniques (US/MRI/CT) after completing phase I'/B (I/B). If these studies demonstrate by that time point a significant finding of a persistent space-occupying lesion/infiltration, biopsy (excisional: mediastinum; wedge: testis) is then necessary. Should leukemic blasts be shown in the biopsy specimen, these patients must be further handled within the HR strategy. Persistent testicular leukemia may ultimately need local radiotherapy (RTX) at 18 Gy. For more details refer to section 1.11.3 & section 1.16.1.5 .
5. The CSF contains ambiguous cells:
Not infrequently it is difficult to discriminate by routine morphology between malignant lymphoblasts and benign activated lymphocytes on a cytospin slide of the CSF. Provided that the CSF is not contaminated with blood, immunophenotyping should be helpful in this regard (Bradstock KF et al. 1980 & 1981, Hooijkaas H et al. 1989, Kranz BR et al. 1989, Peiper S et al. 1980)⁽³⁴⁻³⁸⁾.
 - Monoparametric (TdT) is sufficient in the overwhelming majority of cases.
 - Multiparametric on poly-L-lysine-coated slides for the minority of TdT-negative cases.

1.16.2.5 SUMMARY OF KEY BMPs / LPs

An overview of the timetable of BMPs/LPs for all as well as for specific patient populations at key time points (at diagnosis, on-therapy and off-therapy) is shown in **Tab. 9**. For more details refer to the individual therapy elements in chapter 2.

The timetable within research projects is discussed in Appendix 5

Table 9: Baseline, Check-point & Follow-up BMPs / LPs

| Pt Population | BMP | LP |
|------------------------------|--|---|
| All pts: SR, IR, HR | At Dx, , 15, 33, Start of Consolidation (mM/M, 1. HR-1'), End of Maintenance (wk: 104) | Every Therapeutic LP: Cell Count & |
| HR: NR 33 | Day 52 protocol I | |
| HR: NR 52 | Start of 1. HR-1' Block | |
| HR: NR 1. HR-1' | Start of 1. HR-2' Block | |
| All pts: MRD by FC | At Dx, , 15 | |

1.16.2.6 EXAMINATION OF HEMOSTASIS ON THERAPY

During phase 1 of Protocol I/I/II parameters of hemostasis including PT, aPTT, AT III, fibrinogen and D dimers should be assayed *pro re nata*, i.e. as deemed necessary on clinical grounds. This may help guide the choice of proper therapeutic interventions. Refer to discussion sub section 3.13 on disorders of hemostasis.

1.16.3 DIAGNOSIS OF RELAPSE

The diagnostics for relapse usually follows that of the primary diagnosis. If a relapse is diagnosed, the patient should be promptly enrolled on the relapse trial with which the participating center has the best experience. **Tab. 10** summarizes the definitions for various kinds of relapse seen in ALL.

Table 10: Definition of Relapse by Site

| Type of Relapse | Defining Criteria/Prerequisites |
|-------------------------------------|---|
| Isolated BM | 1. Lymphoblasts $\geq 25\%$ of nucleated BM cells |
| Isolated CNS | 2. Cells $> 5/\mu\text{L}$ CSF & unambiguous lymphoblasts identified on cytopspin preparation 3. Intracerebral mass on CT/MRI w/o lymphoblasts in CSF, PB or BM: Biopsy is necessary to establish the Dx |
| Isolated testicular | 4. Uni/bilateral painless hard swelling of testicle(s) (Testicle volume > 2 deviation scores by Prader orchidometer): The Dx should be confirmed by biopsy |
| Isolated infiltrates at other sites | 5. Biopsy is necessary to make the Dx |
| Combined | 6. Simultaneous involvement of ≥ 2 compartments or localizations 7. BM considered involved if it contains $> 5\%$ lymphoblasts |

Identification of blast cells by flow cytometry but morphological remission is Not sufficient for diagnosis of relapse.

2 Chapter TREATMENT STRATEGY

2.1 Introduction & General Remarks

2.1.1 MEDICAL NOMENCLATURE

The nomenclature and abbreviations used in ALL IC-BFM 2009 trial are widely coined in the English-language medical literature. In addition, efforts have been made to harmonize as far as possible the terminology with that of the ALL-BFM 2009 and with the culture of the maternal BFM family in order to facilitate communication and to address more easily the questions common to both studies. It should be borne in mind however that, though too similar, these studies are not identical.

2.1.2 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. For SCT in this trial, body weight is used directly as the basis for dosage with the exception of MTX for GVHD prophylaxis in mismatched-family-donor SCT. For **obese patients** with an actual body weight of > 2 SD or > 125% their ideal body weight (IBW), the adjusted IBW must be taken as the basis for dosage in the setting of allogeneic SCT- see later. The dosage of drugs in many other conditioning regimens however is also based on the BSA. The BSA should be calculated always at the start of each phase of treatment (I/A; I/A; I/B; II/1; II/2; Protocol mM & Protocol M; every HR block: HR-1', HR-2', HR-3'; maintenance therapy). In addition, the BSA must be calculated before each MTX infusion in Protocol mM/M (x4), q 4 weeks during the maintenance therapy. Dosage should be updated at each of these time points.

The overwhelming majority of infants will be probably managed within the Interfant 06 study. However, if a participating center decides to enroll infants on ALL IC-BFM 2009 trial, dosage should be adjusted to age as follows (**Tab. 11**):

Table 11: Reduced Cytostatic Dosage for Infants – by Age

| Age | Dosage according to BSA | |
|---------------|-------------------------|------------------------|
| ≤ 6 months | 2 / 3 | of the calculated dose |
| 7 - 12 months | 3 / 4 | |
| ≥ 1 year | 1 / 1 | |

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

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

- **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:

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

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 (Bleyer W 1977, Bleyer W et al. 1983, Zimm S et al. 1984, Bekassy AN 1990)⁽³⁹⁻⁴²⁾. The age-adjusted dosage of the drugs used IT in this trial is shown in **Tab. 12**.

Table 12: Dosage of IT Medications by Age Attained at Time of Therapy

| Age (yr) | MTX (mg) | ARA-C (mg) | Prednisone (mg) | 0.9% NaCl (ml) |
|----------|----------|------------|-----------------|----------------|
| < 1 | 6 | 16 | 4 | 1.5 |
| ≥ 1 < 2 | 8 | 20 | 6 | 2.0 |
| ≥ 2 < 3 | 10 | 26 | 8 | 2.5 |
| ≥ 3 | 12 | 30 | 10 | 3.0 |

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

- In contrast to the AML protocol, hyperleukocytosis > 100,000/ μ L, under conditions of adequate hemostasis, good general status, and absence of a severe infection etc, is not a contraindication for LP, so far as MTX is administered intrathecally at the same time. LP is urgently necessary for the evaluation of initial CNS status- an article of direct therapeutic implications-
- Therefore, the first LP may be postponed only in exceptional situations.
- 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 cytopspin preparation made by a standard technique
- The findings should be always carefully documented and sent along with 2 unstained cytopspin 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
- A water-soluble formulation of a prednisone derivative (methyl/prednisolone, e.g. Urbason soluble or Solu-Decortin H) are preferable in order to minimize the risk of adverse events
- As MTX and ARA-C are physically and chemically incompatible with each other, they need not get together in the same syringe *. On the other hand, ARA-C and prednisone are mutually compatible; hence they can be mixed in a single syringe. For triple intrathecal medication, the following guidelines are recommended:
 - In addition to an LP needle, tube(s), a plastic tubing, a clamp and three 5-ml syringes (one each for: MTX, ARA-C/Pred mixture, saline) are needed
 - A sample of CSF for diagnostic purposes is to be obtained first. This should be done always (also if a single drug will be administered)

- MTX can be administered first, then flushed with sterile, isotonic saline at the age-tailored volume
- Next, the ARA-C/Pred mixture should be instilled IT, again flushing it with sterile isotonic saline at the age-tailored volume
- Care should be taken so that the total volume delivered IT (MTX + ARA-C/Pred + saline) be almost the same as that of the CSF removed
- Tilt head-down position for at least 2 h after LP

In addition to avoiding the potential incompatibility, these measures aim at ensuring a better spread of the drugs and their more even disposition within the liquor space, along with improved access to the upper parts of the CNS.

* MTX and ARA-C can get together in the same syringe only if it is prepared in situ and it is administered immediately.

Besides the specific indication or the strategic subgroup, age by the time of radiotherapy is also a decisive factor with regard to dosage. A more detailed account is given elsewhere in this chapter- see section 2.5 on radiotherapy **Tab. 13** feature the RTX dosage by age in the setting of conventional therapy.

Table 13: Dosage of pCRT & tCRT by Age at Time of CRT

| Age by time of RTX (yr): RG | pCRT (Gy) CNS Status 1/2 | tCRT (Gy) CNS Status 3 |
|-----------------------------|-----------------------------|---------------------------|
| < 1 : all risk groups | 0 | 0 |
| ≥ 1 < 2 : SR, IR | 0 | 12 |
| ≥ 2 : SR, IR | 0 | 18 |
| ≥ 1 :T+WBC>100000 | 12 | 12 |
| ≥ 2 :T+WBC>100000 | 12 | 18 |
| ≥ 1 HR with a exception* | 12* | 18 |
| ≥ 2 HR with a exception* | 12* | 18 |

* BCP ALL with HR due only to PPR do not receive pCRT

N.B. CNS status 2 patients receive extra IT MTX on day 18 & 27 of Protocol I/I', while those with CNS status 3 receive (by risk group/arm) extra IT MTX in Protocol I/I'/II/ & TIT in block HR-2'.

2.1.3 TIMETABLE

Each patient should be registered at the national data management office once the diagnosis of ALL has been made, i.e. within 24 – 72 h. **Randomization takes place by the end of induction.** Those HR patients who are eligible for allogeneic SCT should be also randomized (for the case of not performing the transplant) at the proper time.

The first complete blood count and differential available upon admission prior to hydration, transfusion, and antineoplastic treatment including prednisone will 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, so as to avoid any confusion or misinterpretation.

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. 6** (pag 17) and Appendix

3.0.a. **After Induction (day 33) patients in IR & HR groups are randomized in 2 arms of early intensification therapy. IR-BCP ALL are also randomized for two different doses of MTX in consolidation.** Radiotherapy is contemplated in certain subgroups of patients after the reinduction phase of treatment (protocol II), or as a part of the conditioning regimen before allogeneic SCT (TBI + local RTX a week earlier in case of testicular and/or CNS involvement) in selected HR patients who are at least 2 years old. 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.

2.1.4 DOCUMENTATION OF THERAPY & Follow Up DATA

Documentation of remission, realization of treatment, and of severe therapy-associated side effects is mandatory. For better data recording and quantification this should be done only according to the therapy plan forms and a simplified toxicity report form (based on the NCI Common Toxicity Criteria as modified by SIOP and corresponding to the recommendations of the German Society of Pediatric Oncology and Hematology- see Appendix 3.2.a & Appendix 3.2.b).

The individual therapy elements have overviews (sub Appendix 3.0). On these overviews, the doses given, dates of administration, delays, modifications or omissions, and data on remission status should be recorded. One toxicity report per each phase of every therapy element should be filled out within approximately two weeks after its completion (phase A/B/Aug B of Protocol I' / I / II, Protocol mM/M x4, HR-1'/ HR-2'/ HR-3'/ x2, maintenance). This should ensure the most important data regarding the course of therapy be always available to the treating center in a compact and succinct form. The maintenance therapy will be documented on a uniform sheet for all risk groups. A properly completed copy of all these documents and reports should be sent on a regular basis to the national data manager, who should be also notified of whether consent has been obtained or not.

The obligation with respect to the national data manager is solely that a copy of the updated and correctly completed Variables in ALL IC-BFM 2009 Database & SAE Form (both peer-reviewed by the national study coordinator) be sent to the Trial Statistics Center in mid January every year beginning by 2010 in a computerized file and in the format described in the protocol. Also, the follow-up data must be entered into the national database, and imported annually to the Trial Statistics Center as well. In addition, therapy-related death or life-threatening adverse events, overdose and accidents should be notified without delay (within 24 – 72 h) to the national study coordinator.

2.2 SR, IR & HR Therapy Branch

2.2.1 PREAMBLE

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. All the other, i.e. SR non-BCP, IR and HR patients should receive Protocol I with 4 doses of the drug. Patients in IR and HR will be randomized to receive standard phase IB (arms IR-1,HR-1) or phase IB Augmented (arms IR -2, HR-2). Two weeks after protocol I, the consolidation phase of therapy is diversified by immunophenotype. Patients with T-ALL of either risk group (SR & IR) should receive Protocol M, i.e. HD MTX (5g/m²/24h x 4 q 2 weeks), as this is the population for which pharmacodynamic and

clinical data have demonstrated the largest benefit from this dose level (Barredo JC et al. 1994, Belkov VM et al. 1999, Reiter A et al. 1994, Rots MG et al. 1999, Rots MG et al. 1999, Synold TW et al. 1994)⁽⁶⁻¹¹⁾. IR patients with BCP-ALL will be randomized to receive the standard Protocol mM (arm IR-3) i.e. MD MTX (2g/m²/24h x4 q 2 weeks) or Protocol M (arm IR-4), i.e. HD MTX (5g/m²/24h x 4 q 2 weeks). On the other hand SR BCP-ALL will receive the standard Protocol mM, i.e. MD MTX (2g/m²/24h x4 q 2 weeks). Two weeks following consolidation, all patients of each risk group will receive the standard reinduction (protocol II). Finally, 2 weeks after the reinduction, all patients, except those with SCT, are put on oral maintenance therapy with daily 6-MP and weekly MTX for the remainder months until 24 months (104 weeks) of overall treatment.

To compensate for the lower dose level of systemic MTX in Protocol mM, patients with BCP-ALL will receive IT MTX x 4 during maintenance therapy, as it was in ALLIC 2002. T-ALL patients with WBC < 100000, of either risk group (SR & IR), consolidated with HD MTX (Protocol M), but as they will not receive pCRT they will receive IT MTX x 6 during maintenance therapy. However, all patients with initial CNS involvement, who are at least 1 year old, do receive therapeutic cranial irradiation (tCRT).

An overview of the treatment plan is illustrated in **Fig. 6** (pag 17). The individual elements of therapy are discussed in the following sections and are also featured in Appendix 3.0.

2.2.2 INDUCTION THERAPY SR / IR / HR- ALL

2.2.2.1 **PROTOCOL I' A SR - BCP ALL** **PROTOCOL I A SR – T / IR / HR- ALL**

Protocol I' A is designed for the induction therapy of SR patients with BCP-ALL only, whereas Protocol I A should be used for induction in all the others. The former prescribes only 2 doses, and the latter 4 doses of DNR q 30 mg/m². The early intensification for SR patients is phase IB. For IR and HR patients phase IB is randomized with IB Augmented

Dosage should be accommodated to the BSA at the beginning of each phase, i.e. on day 1 and 36. Protocol I' A & Protocol IA are illustrated in **Fig. 8 & Fig. 9- vide infra**, and in Appendix 3.0.b 2 & Appendix 3.0.b 1, respectively. See also the corresponding infusion plans.

Protocol I'/A

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- see chapter 3 on supportive therapy. Severe anemia with Hb below 80 g/L, and severe thrombocytopenia (platelets < 30,000/ μ L) with or without bleeding should be corrected to a safe limit, particularly in case of infection and prior to LP. On the other hand, however, 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 exceptional conditions. The absolute granulocyte count should not fall below (100)- 200/ μ L during the initial phase, if

possible. Severe granulocytopenia is not *per se* in the absence of infection a contraindication for intensive induction chemotherapy in ALL.

Protocol I' A
Induction Therapy: SR - Bcp ALL

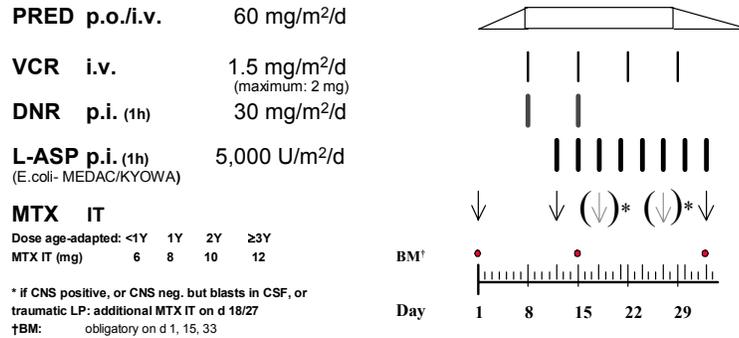


Fig. 8: Protocol I' A for Induction Therapy in SR – BCP ALL

Protocol I A
Induction Therapy: SR –T ALL, IR, HR

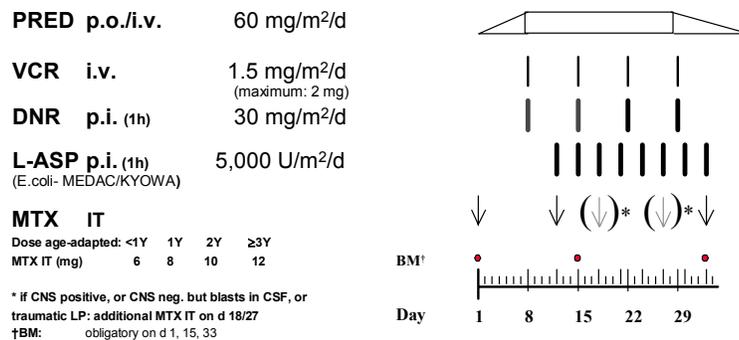


Fig. 9: Protocol I A for Induction Therapy in SR – T, IR and HR

MTX IT: Intrathecal methotrexate on day: 1, 12, 33.

- In case of initial CNS involvement, or if there is uncertainty in this regard as well as if blasts are found on a cytospin preparation of the first CSF which is not contaminated with blood but with a cell count $\leq 5/\mu\text{L}$, and in case of a traumatic LP with blasts on the cytospin preparation, and finally, when the initial LP is traumatic in a patient with hyperleukocytosis $> 50,000/\mu\text{L}$, additional MTX IT is administered on days 18 and 27. See section 1.16.1.3 on diagnosis of CNS involvement & definition of CNS status; section 1.14 on CNS therapy.
- In contrast to the AML protocol, hyperleukocytosis $> 100,000/\mu\text{L}$, under conditions of normal hemostasis, good general status and absence of a severe infection etc, is not a contraindication for LP, as far as MTX is given intrathecally at the same time. LP is urgently necessary for the assessment of initial CNS status. Therefore, the first LP may be postponed only in exceptional situations.
- Tilt head-down position for at least 2 h after LP.
- The dosage of IT MTX is adjusted to age (**Tab. 14**):

Table 14: Dosage of IT MTX by Age at Time of LP

| Age (yr) | < 1 | $\geq 1 < 2$ | $\geq 2 < 3$ | ≥ 3 |
|-------------|-----|--------------|--------------|----------|
| Dosage (mg) | 6 | 8 | 10 | 12 |

- PRED:** **Prednisone/Prednisolone** 60 mg/m²/d, PO/IV, in 3 single doses per day.
- Day 1 – 7: Depending upon clinical condition, begin with total or 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/m²/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/m².
- For patients with a great tumor burden** (high leukocytosis, significant organomegaly) a lower starting dosage (0.1– 0.2 – 0.5 mg/kg/d) must be chosen in order to forestall acute tumor lysis syndrome.
- Patients with increasing WBC count while on prednisone, being prednisone-poor responders, are by definition HR patients, and should be switched over to the HR arm. Progressive/refractory disease must be upon discussing the issue with the national study coordinator handled differently, e.g. with cyclophosphamide and the earlier initiation of HR-oriented therapy.
- Day 8 – 28: Prednisone 60 mg/m²/d, in 3 single doses PO.
- Because of the risk of developing ulcer disease under prednisone therapy, prophylaxis with an H2 blocker is indicated. In case of persistent abdominal pain, a proton pump inhibitor must be considered- see section 3.14.2.3 (p 156). If severe, life-threatening complications occur, the national study coordinator must be informed within 24 h.
- From day 29: Tapering to withdrawal of prednisone over 9 days by halving the dosage q 3 days x3, with the highest dose given in the morning.
- VCR:** **Vincristine** 1.5 mg/m²/d, IV (dsm: 2 mg), on day: 8, 15, 22, 29.
- Mild to moderate VCR-induced neuropathy should not lead to withdrawing the drug in this phase. Individual cases of severe neuropathy can be discussed with the national study coordinator. VCR may also not infrequently cause a syndrome of inappropriate antidiuretic-hormone secretion (SIADH), which is

usually accompanied by neurotoxic manifestations, suggesting action on the hypothalamic supraoptic nuclei. The following guidelines may be helpful in managing this adverse event:

- Since the differential diagnosis of SIADH is quite broad, it is necessary to rule out any other cause of the disorder. Drug-induced SIADH is a diagnosis made *per exclusionem*. This is particularly important to know, if one has to decide on further therapy with the drug in question.
- Monitoring of vital functions (continuous), body weight (x2/24h), fluid balance (on 1 h basis), S/U electrolytes (Na, K) and S/U osmolality (q 6-8 h).
- Fluid restriction as far as possible.
- Cautious correction of hyponatremia at a rate of 2 mmol/L/h with 1.5 – 3% saline given at 50% the urine output during the preceding hour. This approach creates a desirable negative water balance and gradually replaces the sodium loss. (Too rapid and aggressive correction of hyponatremia is dangerous, as it leads to life-threatening brain damage such as pontine myelinolysis).
- Replacement of the lost potassium.
- Furosemide 1 mg/kg, IV, to be repeated as required.
- Deoxycorticosterone acetate (DOCA) 4 mg/m²/d (twice the regular therapeutic dose)- may be repeated if needed (Weizman Z et al. 1982)⁽⁴³⁾.
- Rarely, other measures may be required, e.g. lithium carbonate 15 – 60 mg/kg/d, PO, in divided doses q 6 – 8 h aiming at a target plasma level of 0.6 – 1.5 mmol/L, or demeclocycline 7 – 13 mg/kg/d, PO, in divided doses q 6 – 12 h.
- Osmotic diuresis with urea or mannitol cannot be generally recommended, as the too dynamic shifts of water and electrolytes may be devastating to the brain (risk of pontine myelinolysis), and may precipitate cardiac failure with pulmonary edema.
- Cases of severe VCR-induced neurotoxicity must be discussed with the national study coordinator to decide on further therapy with the drug.

DNR: Daunorubicin 30 mg/m²/d, PI, over 1 h

- x2 on day: 8, 15 (Protocol IA')- in SR BCP-ALL only.
- x4 on day: 8, 15, 22, 29 (Protocol IA)- in SR T, IR & HR ALL.

An ECG and Echo-CG should be performed at baseline. If the shortening fraction (SF) < 30% or if signs of cardiac insufficiency are present, e.g. an ejection fraction (EF) < 35%, DNR may be delivered only after consulting the national study coordinator.

ASP: E. coli L-asparaginase

A native formulation of the drug, preferably with a known pharmacokinetics and pharmacodynamics such as that of Medac/Kyowa Hakko or Elspar of Merck, Sharp & Dohme, should be used first.

This must be given at 5,000 U/m²/d, PI, over 1 h, on day: 12, 15, 18, 21, 24, 27, 30, 33 (8 doses).

In case of a hypersensitivity reaction to the native E. coli ASP formulation, which leads to discontinuation of treatment, 2 options are offered:

- A pegylated E. coli asparaginase- pegaspargase (Oncaspar[®] Medac/Enzon), to be used at 2,500 U/m² (max 3,750 U), PI, over 1 h, x1 or x2 (2 weeks apart)

depending on the time point of this adverse event. One dose of PEG-ASP substitutes 4 doses of the unmodified E. coli ASP.

- Erwinia chrysanthemi ASP (Erwinase[®] Speywood) if it is available can be used, however at 10,000 U/m²/d every other day **IM** on day 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, i.e. each 6 doses of Erwinase[®] substitute 4 doses of native E. coli ASP. Unless the manufacturer of the drug settles down the issue of safety definitively, the IV route should be avoided.

A test dose of 10-50 U or 0.2 U/kg body weight, to be administered PI over 15 minutes at the beginning of the first dose of any ASP preparation (so far as it is approved for IV use) may be considered. If no reaction occurs during the test drip and within 30 minutes thereafter, the rest of the prescribed dose may be delivered. However, while a positive test dose is predictive, an uneventful one does not exclude the subsequent development of an allergic reaction. Therefore, the patient must be observed closely during each ASP infusion.

In case of allergy, it is unacceptable to continue with the same ASP preparation under coverage of antihistaminics and/or steroids, since this will only lead to loss of its efficacy through inactivation by the formed antibodies.

ASP is implicated in both thrombotic and bleeding complications. In particular, the combination of ASP and steroids may be associated with an increased risk of thrombosis. Permanent central venous catheters in general are another risk factor for thrombotic events, although the thrombogenic potential may vary with different materials.

Careful history regarding a thrombophilic as well as hemorrhagic diathesis should be taken prior to therapy with ASP. At baseline, i.e. already before hydration and prednisone, global coagulation tests (PT, aPTT), and measurement of fibrinogen, D dimers, AT III, protein C, protein S as well as APCR should be performed on all patients. If needed, F.V mutation (Leiden: G1691A) & F.II mutation (G20210A) should be investigated. It may be warranted to assess also the level of vWF, its functional activity & multimeric composition. In selected cases (a family history positive for or suggestive of thrombophilia) assessment of Lp (a) and homocysteine is indicated as well. During therapy with ASP and prednisone, hemostasis (PT, aPTT, fibrinogen, AT III and D dimers) should be checked as clinically indicated. As far as possible, e.g. in older children, it is advisable to defer the insertion of a central line until phase 2 of protocol I/I in order to avoid therapy-associated thromboembolic complications. Until the results of randomized trials will be available, thrombophilic patients should not have central lines and may need other measures as well. For more details see discussion on disorders of hemostasis sub section 3.13.

ASP is hepatotoxic. On the other hand, VCR is metabolized in the liver. Hence, it is prudent to administer ASP at least 12 h after VCR. This sequence allows sufficient time for VCR to be cleared out, making severe VCR-associated neuropathy unlikely. In Protocol I/I, this may be the case on day 15 (if E. coli ASP is used) or day 22 (if Erwinase[®] is used).

Hyperglycemia may be encountered during therapy with L-asparaginase, and blood/urine sugar should be therefore monitored also. However, this must be

approached with caution, since the hyperglycemia may be due to concomitant therapy with prednisone. Anyhow, this complication is manageable with insulin.

In case of unequivocal pancreatitis (based on clinical findings, lipase/amylase elevation and US/CT imaging), L-asparaginase should be omitted altogether and never resumed.

2.2.2.2 EARLY INTENSIFICATION

PROTOCOL I B SR / IR -1/ HR-1
PROTOCOL I B Augmented IR-2 / HR-2

All SR patients will have it as early intensification.

It will be randomly assigned as early intensification : IR (arm IR-1)
HR (arm HR-1)

Protocol I B
Early intensification: SR , IR (arm-1), HR (arm-1)

CPM p.i. (1h) 1,000 mg/m²/d
 (+ MESNA: 400 mg/m² i.v. x3 at h: 0, +4, +8)
ARA-C i.v. 75 mg/m²/d
6-MP p.o. (28 d) 60 mg/m²/d
MTX IT
 Dose age-adapted: <1Y 1Y 2Y ≥3Y
 MTX IT (mg) 6 8 10 12
 †BM: obligatory on d 52 (only HR if NR d 33)

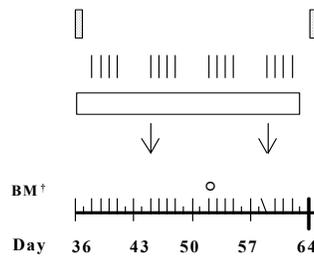


Fig. 10 : Early intensification Therapy in SR , IR -1 and HR -1

It starts at day 36 of protocol I'A or IA.

Requirements for beginning protocol IB

- Good general status
- No severe infection
- Creatinine level within normal range for age
- Recovering blood counts, at least:
 - WBC ≥ 2,000/μL
 - Granulocytes ≥ 500/μL
 - Platelets ≥ 50,000/μL

Therapy regulation in protocol IB

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

- WBC $\geq 500/\mu\text{L}$
- Platelets $\geq 30,000/\mu\text{L}$

As far as possible, a run 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 $1,680 \text{ mg/m}^2$ ($28 \times 60 \text{ mg/m}^2$).

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

- WBC $\geq 1,000/\mu\text{L}$
- Granulocytes $\geq 300/\mu\text{L}$
- Platelets $\geq 50,000/\mu\text{L}$
- Creatinine level within normal range for age

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

- Diuresis and cystitis prophylaxis: IV hydration $3,000 \text{ ml/m}^2/24\text{h}$ (5% G/0.45% NaCl aa + $90 \text{ mmol/m}^2/24\text{h}$ 7.45% KCl).
- Check overall fluid balance q 12 h! If fluid intake exceeds output by more than $400 \text{ ml/m}^2/12\text{h}$, then give furosemide 0.5 mg/kg body weight (maximum: 20 mg) IV.
- Check every urine portion by dipstick for 24 h.
- Uromitexan[®] 400 mg/m^2 , IV, x3 at: 0, +4 and +8 h from the start of the CPM infusion.
- Hematuria (red urine, dipstick positive for RBCs) or painful micturition indicate first of all hemorrhagic cystitis. In these cases, intensify diuresis (IV fluids up to $4,500 - 5,000 \text{ ml/m}^2/24\text{h}$, furosemide $0.5 - 1 \text{ mg/kg}$, IV, q 4 – 6 h), relieve pain by analgesics, and, if need be, give additional doses of Uromitexan[®]. Irrigation of the urinary bladder or derivation of the urine is rarely needed.

N.B. It should be borne in mind however that the implementation and outcome of supplemental MESNA will depend on the elimination $t_{1/2}$ of CPM/metabolites, which is in turn governed by many factors, as well as on the nature of the damage the urothelium has already sustained.

The terminal elimination $t_{1/2}$ of CPM is variable, ranging from 108 to 960 minutes (mean: 7 h in adults, shorter in children). Additional risk factors including drug interactions or obesity must be taken into account, too (The Chemotherapy Source Book, 3rd edition, Perry MC ed., Williams & Wilkins 2001).

CPM may rarely cause a syndrome of inappropriate antidiuretic-hormone secretion (SIADH) by reducing free-water clearance. Considering the need for aggressive hydration to prevent hemorrhagic cystitis, this is a particularly problematic issue to manage. If severe, with CNS symptoms/signs, the following guidelines may be helpful:

- Considering the quite broad spectrum of differential diagnosis of SIADH, every effort should be made to rule out other causes of the disorder. The diagnosis of drug-induced SIADH is one of exclusion.

This is particularly important for future decision-making as to further therapy with the offending drug.

- Careful monitoring of vital functions (continuous), fluid balance (on 1 h basis), body weight (x2/24h), S/U electrolytes (Na, K) and S/U osmolality (q 6-8 h) is mandatory.
- Thoughtful fluid restriction, if any- this should be done on an individual basis.
- Although osmotic diuresis with urea in 5% G or with mannitol (both at: 0.5 – 1 – 1.5 – 2 g/kg, given PI over 30 – 60 min, q 6 – 12 h, if needed) may obviate the need for fluid restriction, which would be beneficial in CPM-induced SIADH, this cannot be generally recommended. Osmotic diuresis leads to too rapid and profound shifts of water and electrolytes, which may prove disastrous to the brain (pontine myelinolysis) and hazardous to the heart (cardiac failure with associated pulmonary edema). A 10% mannitol solution, for example, has an osmolality of 549 mmol/kg. Moreover, both drugs cause large losses of sodium (and potassium), which would further aggravate the pre-existent hyponatremia of SIADH.
- A loop diuretic, e.g. furosemide 1 mg/kg, IV, to be repeated as required (q 4 – 6 – 8 – 12 – 24 h) may be used instead.
- Guarded correction of hyponatremia at a rate of 2 mmol/L/h with 1.5 – 3% saline given at 50% the urinary output during the previous hour is a reasonable measure which will create a desired negative fluid balance and cautiously replace the lost sodium.
- The expanded plasma volume in SIADH is inherently associated with hypoaldosteronism leading to suppressed reabsorption of sodium in the distal tubule of the kidney. Therefore, the mineralocorticoid deoxycorticosterone acetate (DOCA) given at twice the regular therapeutic dose, i.e. 4 mg/m²/d, to be repeated if need be, will improve sodium reabsorption in the distal tubule. DOCA will thus decrease the urinary losses of sodium and contribute to elevation of the plasma sodium level (Weizman Z et al. 1982)⁽⁴³⁾.
- An intensified MESNA protocol with Uromitexan[®] given at a higher dosage for a sufficiently long period of time to more efficiently prevent hemorrhagic cystitis may be considered (Haas A et al. 1986)⁽⁴⁴⁾.
- Additional measures, e.g. lithium carbonate 15 – 60 mg/kg/d, PO, in divided doses q 6 – 8 h aiming at a target plasma level of 0.6 – 1.5 mmol/L, or demeclocycline 7 – 13 mg/kg/d, PO, in divided doses q 6 – 12 h are rarely needed.
- Further therapy with CPM, if any, should be consulted with the national study coordinator. It is usually safe with the infusion of isotonic or slightly hypertonic crystalloids (0.9% NaCl/5% G), regular furosemide (0.5 – 1 mg/kg, maximum: 20 mg, IV, at +6 h & +12 h from the start of CPM infusion) along with careful monitoring.

MP: **6-Mercaptopurine** 60 mg/m²/d, PO, day: 36 – 63 (=28 days), to be taken in the evening on a fasting stomach without milk.

ARA-C: **Cytarabine** 75 mg/m²/d, IV, in 4 blocks, over 4 days each, on day:
38 – 41; 45 – 48; 52 – 55; 59 – 62.

MTX IT: **Intrathecal methotrexate** at age-adjusted dosage. See **Tab. 14** (pag 53)

- On the same day as the first dose of ARA-C in:

- block 2 (day 45) & block 4 (day 59).
- Tilt head-down position for at least 2 h after IT MTX.

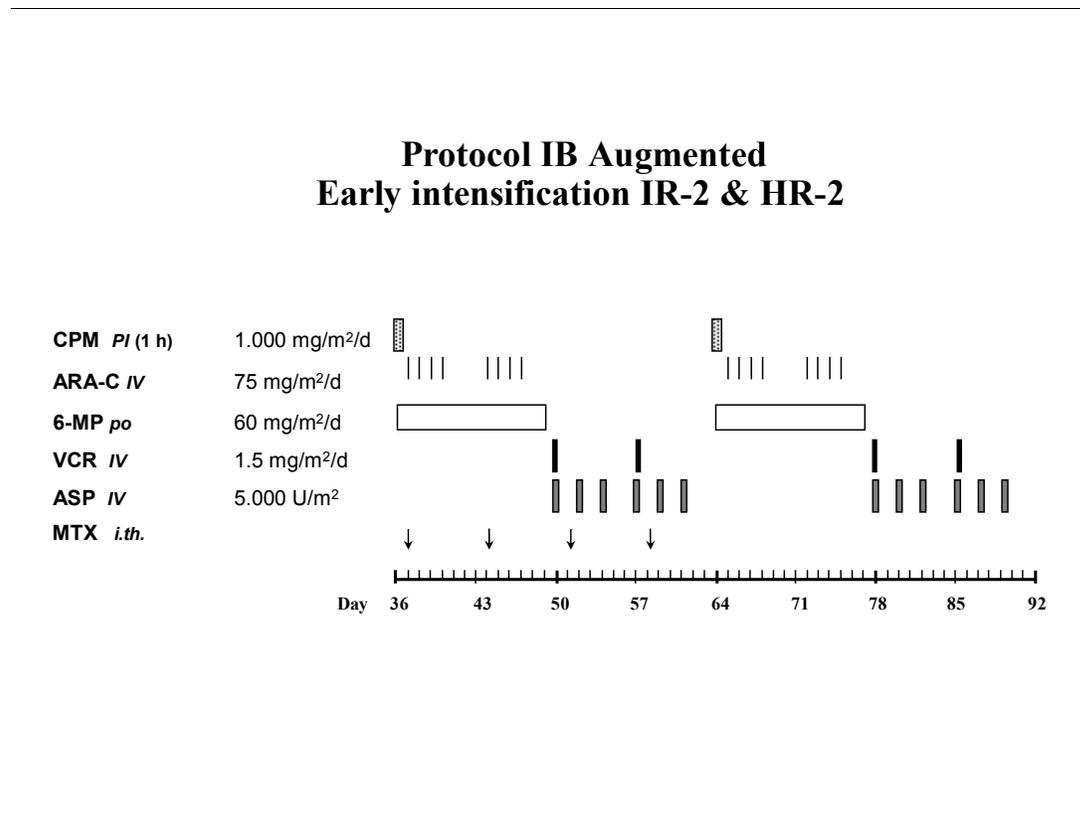


Fig. 11: Early intensification Therapy in IR-2 and HR -2

Protocol IB Augmented

As early intensification will be randomly assigned : IR (arm IR-2) and HR (arm HR-2)

It starts on day 36 of protocol IA

Requirements for beginning protocol IB Augmented

- Good general status
- No severe infection
- Creatinine level within normal range for age
- Recovering blood counts, at least:
 - WBC $\geq 2,000/\mu\text{L}$
 - Granulocytes $\geq 500/\mu\text{L}$
 - Platelets $\geq 50,000/\mu\text{L}$

Therapy regulation in protocol I/B Augmented

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

- WBC $\geq 500/\mu\text{L}$
- Platelets $\geq 30,000/\mu\text{L}$

As far as possible 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/m² (28 x 60 mg/m²).

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

- WBC $\geq 1,000/\mu\text{L}$
- Granulocytes $\geq 300/\mu\text{L}$
- Platelets $\geq 50,000/\mu\text{L}$
- Creatinine level within normal range for age

CPM: **Cyclophosphamide** 1,000 mg/m²/d, PI, over 1 h, on day: 36, 64.

- Diuresis and cystitis prophylaxis: IV hydration 3,000 ml/m²/24h (5% G/0.45% NaCl aa + 90 mmol/m²/24h 7.45% KCl).
- Check overall fluid balance q 12 h! If fluid intake exceeds output by more than 400 ml/m²/12h, then give furosemide 0.5 mg/kg body weight (maximum: 20 mg) IV.
- Check every urine portion by dipstick for 24 h.
- Uromitexan[®] 400 mg/m², IV, x3 at: 0, +4 and +8 h from the start of the CPM infusion.
- Hematuria (red urine, dipstick positive for RBCs) or painful micturition indicate first of all hemorrhagic cystitis. In these cases, intensify diuresis (IV fluids up to 4,500 – 5,000 ml/m²/24h, furosemide 0.5 – 1 mg/kg, IV, q 4 – 6 h), relieve pain by analgesics, and, if need be, give additional doses of Uromitexan[®]. Irrigation of the urinary bladder or derivation of the urine is rarely needed.

N.B. It should be borne in mind however that the implementation and outcome of supplemental MESNA will depend on the elimination $t_{1/2}$ of CPM/metabolites, which is in turn governed by many factors, as well as on the nature of the damage the urothelium has already sustained.

The terminal elimination $t_{1/2}$ of CPM is variable, ranging from 108 to 960 minutes (mean: 7 h in adults, shorter in children). Additional risk factors including drug interactions or obesity must be taken into account, too (The Chemotherapy Source Book, 3rd edition, Perry MC ed., Williams & Wilkins 2001).

CPM may rarely cause a syndrome of inappropriate antidiuretic-hormone secretion (SIADH) by reducing free-water clearance.

Considering the need for aggressive hydration to prevent hemorrhagic cystitis, this is a particularly problematic issue to manage. If severe, with CNS symptoms/signs, the following guidelines may be helpful:

- Considering the quite broad spectrum of differential diagnosis of SIADH, every effort should be made to rule out other causes of the disorder. The diagnosis of drug-induced SIADH is one of exclusion. This is particularly important for future decision-making as to further therapy with the offending drug.
- Careful monitoring of vital functions (continuous), fluid balance (on 1 h basis), body weight (x2/24h), S/U electrolytes (Na, K) and S/U osmolality (q 6-8 h) is mandatory.

- Thoughtful fluid restriction, if any- this should be done on an individual basis.
- Although osmotic diuresis with urea in 5% G or with mannitol (both at: 0.5 – 1 – 1.5 – 2 g/kg, given PI over 30 – 60 min, q 6 – 12 h, if needed) may obviate the need for fluid restriction, which would be beneficial in CPM-induced SIADH, this cannot be generally recommended. Osmotic diuresis leads to too rapid and profound shifts of water and electrolytes, which may prove disastrous to the brain (pontine myelinolysis) and hazardous to the heart (cardiac failure with associated pulmonary edema). A 10% mannitol solution, for example, has an osmolality of 549 mmol/kg. Moreover, both drugs cause large losses of sodium (and potassium), which would further aggravate the pre-existent hyponatremia of SIADH.
- A loop diuretic, e.g. furosemide 1 mg/kg, IV, to be repeated as required (q 4 – 6 – 8 – 12 – 24 h) may be used instead.
- Guarded correction of hyponatremia at a rate of 2 mmol/L/h with 1.5 – 3% saline given at 50% the urinary output during the previous hour is a reasonable measure which will create a desired negative fluid balance and cautiously replace the lost sodium.
- The expanded plasma volume in SIADH is inherently associated with hypoaldosteronism leading to suppressed reabsorption of sodium in the distal tubule of the kidney. Therefore, the mineralocorticoid deoxycorticosterone acetate (DOCA) given at twice the regular therapeutic dose, i.e. 4 mg/m²/d, to be repeated if need be, will improve sodium reabsorption in the distal tubule. DOCA will thus decrease the urinary losses of sodium and contribute to elevation of the plasma sodium level (Weizman Z et al. 1982)⁽⁴³⁾.
- An intensified MESNA protocol with Uromitexan[®] given at a higher dosage for a sufficiently long period of time to more efficiently prevent hemorrhagic cystitis may be considered (Haas A et al. 1986)⁽⁴⁴⁾.
- Additional measures, e.g. lithium carbonate 15 – 60 mg/kg/d, PO, in divided doses q 6 – 8 h aiming at a target plasma level of 0.6 – 1.5 mmol/L, or demeclocycline 7 – 13 mg/kg/d, PO, in divided doses q 6 – 12 h are rarely needed.
- Further therapy with CPM, if any, should be consulted with the national study coordinator. It is usually safe with the infusion of isotonic or slightly hypertonic crystalloids (0.9% NaCl/5% G), regular furosemide (0.5 – 1 mg/kg, maximum: 20 mg, IV, at +6 h & +12 h from the start of CPM infusion) along with careful monitoring.

MP: **6-Mercaptopurine** 60 mg/m²/d, PO, day 36 – 49 and 64 -77 (=28 days), to be taken in the evening on a fasting stomach without milk.

ARA-C: **Cytarabine** 75 mg/m²/d, IV, in 4 blocks, over 4 days each, on day: 37– 40; 43 – 46; 65 – 68; 72 – 75.

MTX IT: **Intrathecal methotrexate** at age-adjusted dosage. See **Tab. 14** (pag 53)

- 4 doses in days 37,44,51,58
- Tilt head-down position for at least 2 h after IT MTX.

VCR: **Vincristine** 1.5 mg/m²/d, IV (dsm: 2 mg), on day: 50, 57, 78, 85.

ASP: **E. coli L-asparaginase** 5,000 U/m²/d, PI, over 1 h, on days 50, 52, 54, 57, 59, 61,78,80,82,85,87,89 (12 doses).

In case of a hypersensitivity reaction to the native E. coli ASP formulation, which leads to discontinuation of treatment, 2 options are offered:

- A pegylated E. coli asparaginase- pegaspargase (Oncaspar[®] Medac/Enzon), to be used at 2,500 U/m² (max 3,750 U), PI, over 1 h, x1 or x2 (2 weeks apart) depending on the time point of this adverse event. One dose of PEG-ASP 2,500 U/m² substitutes 4 doses of the unmodified E. coli ASP
- Erwinia chrysanthemi ASP (Erwinase[®] Speywood) if it is available .

**2.2.3 CONSOLIDATION THERAPY
SR / IR ALL**

2.2.3.1 PROTOCOL mM SR / IR BCP- ALL (arm IR-3)

As it was used in ALLIC 2002 this treatment element, with a period of 56 days, is designed for consolidation therapy of both SR and IR patients with BCP-ALL. The fundamental difference versus ALL-IC 2002 consists in MTX dose for **IR BCP-ALL will be randomized in two branches: arm IR-3 with MTX 2 g/m²/24h x4 q 14 days; and arm IR-4 with MTX 5 g/m²/24h x4 q 14 days.** On the other hand, the potential risk of inferior efficacy in addressing disease within the CNS sanctuaries will be compensated for by additional doses of IT MTX during the subsequent intensification therapy. Otherwise, the schedule including leucovorin rescue is identical in both studies.

Protocol mM begins 2 weeks following the end of Protocol I'B/IB. 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. 12** below, and detailed in Appendices 3.0.c 1 & 3.0.c 1.1.

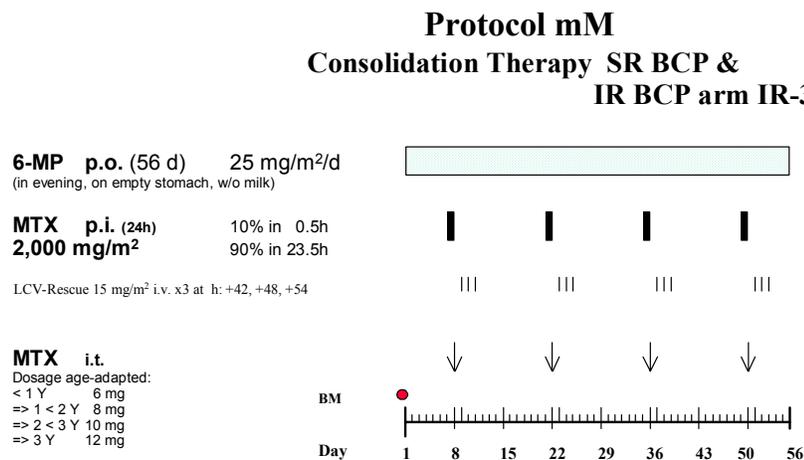


Fig. 12: Protocol mM for Consolidation Therapy in SR/IR BCP-ALL arm IR-3

Requirements for beginning Protocol mM

- Complete cytomorphicologic remission (BMP mandatory)
- Satisfactory general condition

- No severe infection
- Creatinine/ CL_{cr} within normal range for age
- No urinary obstruction
- Acceptable LFTs:
 - ALT/AST $\leq 5 \times N$ for age
 - Bilirubin $\leq 3 \times N$ for age
- Blood count showing an ascendant trend, at least:
 - WBC $\geq 1,500/\mu\text{L}$
 - Granulocytes $\geq 500/\mu\text{L}$
 - Platelets $\geq 50,000/\mu\text{L}$

MP: **6-Mercaptopurine** 25 mg/m²/d, PO, day: 1 – 56, in the evening on a fasting stomach without milk.

MD MTX: **Medium-dose methotrexate** 2,000 mg/m²/d, PI, over 24 h, q 14 days (x4) on day: 8, 22, 36, 50. Refer to flow sheet in Appendix 3.0.c 1.1.

- 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.
- 1/10 of the total dose (200 mg/m²) should be administered PI over 30 minutes as a loading dose.
- 9/10 of the total dose (1,800 mg/m²) is to be given PI over 23.5 h.
- 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).
- An acute neurotoxicity syndrome may be encountered within 24 h of systemic MTX exposure whatever the dose and route with or without LP (IT MTX). A brief account of acute MTX-induced neurotoxicity is given in the section on Protocol M- *vide infra*. See also chapter 4 on chemotherapy side effects.

MTX IT: **Intrathecal methotrexate** 1 h after the start of MTX infusion:

- Dosage adjusted to age. See **Tab. 14** (pag 53).
- Tilt head-down position for at least 2 h after IT MTX.

LCV: **Leucovorin Ca** 15 mg/m², IV, x3 at: 42 h, 48 h, 54 h after the start of MTX infusion.

2.2.3.2 PROTOCOL M SR & IR T-ALL / IR BCP-ALL (arm IR-4)

Patients with either SR or IR T-ALL should be consolidated with HD MTX, i.e. 5 g/m²/24h x4 q 14 days, as they are most likely to benefit from this dose level of the

drug (Barredo JC et al. 1994, Belkov VM et al. 1999, Reiter A et al. 1994, Rots MG et al. 1999, Rots MG et al. 1999, Synold TW et al. 1994)⁽⁶⁻¹¹⁾. *HD MTX (q 5 g/m²/24h x1)* is also an item of therapy in the HR group (block HR-1' & block HR-2'). In this trial IR Bcp ALL will randomly receive it (arm IR 4). Therapy is detailed in a separate flow sheet- see Appendix 3.0.c 2.1. In addition, a diagram depicting LCV rescue according to the plasma level of MTX within specified time zones is also provided-see Appendix 3.1. Finally, guidelines for managing the catastrophic scenario of poor MTX elimination are given as well- see Appendix 3.1.

This section is intended to summarize and emphasize a few common, generally applicable points, clarify LCV rescue so as to avoid misinterpretation, and finally to draw attention to the issue of acute MTX-induced neurotoxicity, which is possibly underestimated.

- MTX is notorious for its too common drug interactions. These are of varying mechanisms, and their clinical relevance in terms of impact on the reciprocal efficacy and toxicity of the drugs in question also varies greatly. In order to avoid any potential confounding problem or complication, it is strongly recommended to withdraw/withhold any dispensable medications from -24 h to +72 h as of the MTX infusion. If need be, the choice of drug(s) must be minimized and thoughtfully considered, bearing in mind the risk to benefit ratio of the available options and selecting as far as possible the most active and safest drug(s).
- Monitoring of the plasma concentration of MTX is *conditio sine qua non* for HD MTX therapy. It is desirable, but not obligatory, to monitor also the most important metabolite of the drug, i.e. 7-OH MTX. Monitoring is essential for the diagnosis and management of life-threatening and potentially lethal MTX-induced toxicity. It is recommended to start the MTX infusion at such a time (e.g. at 12⁰⁰ h or 14⁰⁰ h), which would ensure the optimal set-up of the timetable for intrathecal drug administration, measurement of plasma MTX concentrations as well as for LCV rescue. This will depend on the local conditions of the participating center; however, the prescribed intervals between infusions/injections, e.g. 7 h between MTX and CPM, must be kept. **Tab. 15** below shows the cut-off values for plasma MTX level at key time points, along with the standard LCV rescue protocol for the HD MTX (5 g/m²/24h) prescribed in Protocol M as well as in blocks HR-1' & HR-2'.

Table 15: Regular Plasma MTX Concentrations & Corresponding LCV Rescue

| Time as of MTX infusion (+ h) | Cut-off MTX level (µmol/L) | LCV Dosage: IV (mg/m ²) |
|-------------------------------|----------------------------|-------------------------------------|
| 24 | ≤ 150.0 | |
| 36 | ≤ 3.0 | |
| 42 | ≤ 1.0 | 15 |
| 48 | ≤ 0.4 | 15 |
| 54 | ≤ 0.4 | 15 |

Plasma MTX concentration at +24 h, +42 h and +48 h after starting the MTX infusion should be always measured in a *statim* regime. As to +36 h, blood must be sampled and properly stored for *ex post* assessment together with that from +42 h. However, if the plasma MTX₂₄ exceeds 150 µmol/L and/or if poor MTX elimination is suspected on clinical grounds (significant increase of creatinine, out-of-proportion reduction in diuresis, edema, hypertension, etc), forced diuresis, alkalization, close monitoring of vital functions and more stringent fluid balance control should be initiated without delay. In this case, MTX₃₆ must be also assessed *statim*. Should MTX₃₆ exceed 3

$\mu\text{mol/L}$, it is imperative to start LCV rescue immediately (**according to the diagram in Appendix 3.1**), while continuing with the measures mentioned above. Since MTX_{42} will be still pending at that time, the dose of LCV_{42} should be initially based on the already known MTX_{36} . When MTX_{42} becomes available, the LCV dose can be made up *ex post*, if need be. These measures should be continued until the plasma MTX concentration falls to at least $0.25 \mu\text{mol/L}$. The same strategy applies for "abnormal" MTX_{42} , 48, 54. If MTX_{42} or later $> 5 \mu\text{mol/L}$, the dose of LCV must be calculated according to the formula:

| |
|--|
| $\text{Leucovorin [mg]} = \text{plasma MTX concentration } [\mu\text{mol/L}] \times \text{Body Weight [kg]}$ |
|--|

If the dose of LCV $> 20 \text{ mg/kg}$, it should be administered by IV infusion over 1 h to avoid Ca-induced bradycardia/cardiac arrest, because folinic acid is formulated as calcium folinate. For more details refer to the guidelines and diagram of LCV rescue-Appendix 3.1.

Although the available data are still too limited to allow for definitive conclusions to be made, the bacterial enzyme carboxypeptidase G2 (CPD G2), which hydrolyzes MTX to its inactive metabolite 2,4-diamino- N_{10} -methylpteroic acid (DAMPA), should be strongly considered in case of too high and symptomatic plasma MTX level. Very poor MTX elimination manifests itself by acute hyperemesis within 24 – 48 h of exposure, passing yellow loose stools and neurologic symptoms including confusion, visual disturbances and seizures. CPD G2 may be helpful as a potent pharmacokinetic rescue agent, providing an alternative route for the elimination of MTX. It is capable of lowering the plasma MTX concentration by 2 logs within minutes (Adamson PC et al. 1992, Widemann BC et al. 1995, DeAngelis LM et al. 1996)⁽⁴⁵⁻⁴⁷⁾. However, the molecule is too large to cross the blood-brain barrier, which limits its utility in the systemic rescue from HD MTX-induced CNS toxicity. *On the other hand, it has been experimentally shown that CPD G2 is also active within the CNS upon intrathecal administration. This suggests the drug might prove useful in case of IT MTX overdose (Adamson PC et al. 1991)⁽⁴⁸⁾.*

N.B. CPD G2 and a protocol for its use are available at a few centers within the BFM Study Group. It is recommended to keep the drug readily available in a central store at a national or regional level.

- An acute neurotoxicity syndrome with stroke-like features including headache, anorexia, nausea, emesis, arterial hypertension, confusion, dizziness, blurred vision, aphasia, agitation, lethargy, convulsions, obtunded sensorium to coma and hemiparesis has been described during MTX therapy (parenteral as well as oral, and at variable dose levels). It is usually associated with MTX-induced nephrotoxicity or otherwise impaired renal function leading to poor MTX elimination. It has been shown that therapy with aminophylline is effective and safe in managing this adverse event, with recovery being prompt and complete or almost complete in the overwhelming majority of the patients. It has been used at 2.5 mg/kg PI over 45 – 60 minutes, or 0.5 mg/kg/h by continuous infusion for 12 h, or in the form of a rapid-release oral theophylline preparation to yield a plasma concentration of $10 - 30 \mu\text{mol/L}$. Since this event is reversible with aminophylline treatment, further therapy with MTX is usually feasible and uneventful (Bernini JC et al. 1995, Peyriere H et al. 2001)⁽⁴⁹⁻⁵⁰⁾.

Protocol M begins also 2 weeks following the end of Protocol I B. 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. 13** & in Appendices 3.0.c 2 & 3.0.c 2.1.

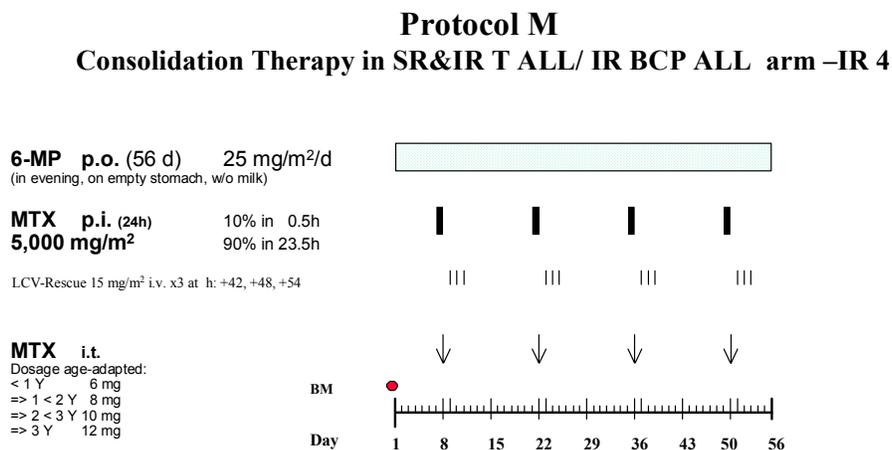


Fig. 13: Protocol M for Consolidation in T-ALL SR/IR /IR BCP-ALL (arm IR-4)

Requirements for beginning Protocol M

- Complete cytomorphicologic remission (BMP mandatory)
- Satisfactory general condition
- No severe infection
- Creatinine/CL_{cr} within normal range for age
- No urinary obstruction
- Acceptable LFTs:
 - ALT/AST ≤ 5 x N for age
 - Bilirubin ≤ 3 x N for age
- Blood count showing an ascendant trend, at least:
 - WBC ≥ 1,500/μL
 - Granulocytes ≥ 500/μL
 - Platelets ≥ 50,000/μL

MP: **6-Mercaptopurine** 25 mg/m²/d, PO, day: 1 – 56, in the evening on a fasting stomach without milk.

HD MTX: **High-dose methotrexate** 5,000 mg/m²/d, PI, over 24 h, q 14 days (x4) on day: 8, 22, 36, 50. Refer to flow sheet in Appendix 3.0.c 2.1.

- 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 alkalization.
- 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.

- 1/10 of the total dose (500 mg/m²) should be administered PI over 30 minutes as a loading dose.
 - 9/10 of the total dose (4,500 mg/m²) is to be given PI over 23.5 h.
- MTX IT:** **Intrathecal methotrexate** 1 h after the start of MTX infusion:
- Dosage adjusted to age. See **Tab. 14** (pag 53)
 - Tilt head-down position for at least 2 h after IT MTX.
- LCV:** **Leucovorin Ca** 15 mg/m², IV, x3 at: 42 h, 48 h, 54 h after the start of MTX infusion.

2.2.4 REINDUCTION THERAPY

SR / IR / HR

2.2.4.1 PROTOCOL II

As far as dosage, composition and schedule are concerned, this therapy element is identical to Protocol II of ALL-IC-2002 /AEIOP-BFM 2009, and consists of 2 phases, beginning 2 weeks after Protocol mM/M.

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.

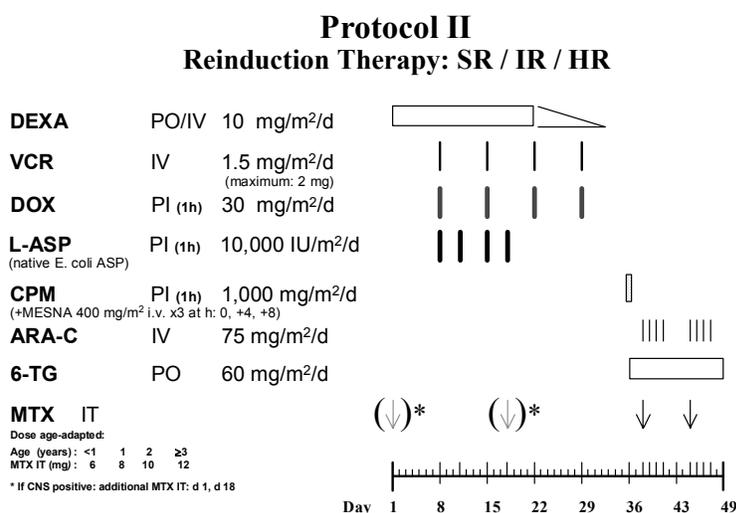


Fig. 14 : Protocol II for Late Reinduction Therapy in SR /IR /HR

Requirements for beginning Protocol II

- Continuous complete remission
- Satisfactory general status
- No severe infection
- Increasing trend in blood count, at least:
 - WBC $\geq 2,500/\mu\text{L}$
 - Granulocytes $\geq 1,000/\mu\text{L}$
 - Platelets $\geq 100,000/\mu\text{L}$

Phase II/1

Regulation of therapy in Phase II/1

- In case of severe neuropathy, VCR may be deleted.

- By insufficient granulopoiesis (WBC < 500/ μ L or granulocytes < 200/ μ L), doxorubicin/vincristine doses can be postponed.

- DEXA:** **Dexamethasone** 10 mg/m²/d, PO/IV, in 3 single doses, on day: 1 – 21.
By day 22 taper down stepwise to withdrawal over 9 days by halving the dose q 3 days x3, giving the highest dose in the morning.
While dexamethasone is almost void of mineralocorticoid effects (by virtue of α methylation at C 16), it exhibits at equipotent doses 5- to 6-fold higher glucocorticoid activity than 6 α -methylprednisolone and prednisone/prednisolone, respectively. The latter 3 compounds demonstrate extensive, dose-dependent protein binding, mainly to transcortin (> 90 – 95%) and to some extent to albumin. By contrast, protein binding of dexamethasone is minimal and dose-independent. Furthermore, substitution by fluorine at C 9 in dexamethasone renders its metabolic degradation less efficient, and hence its t_{1/2} substantially longer.
Hyperglycemia is not uncommon, in particular with concomitant ASP therapy. Glycemia/glycosuria should be monitored during phase II/1. Steroid diabetes mellitus is manageable with insulin, and is not by no means *per se* a contraindication for treatment with dexamethasone. However, both dexamethasone and L-asparaginase have been implicated in pancreatitis, which prohibits further therapy with the offending drug.
Because of the risk of developing ulcer disease under dexamethasone therapy, prophylaxis with an H2 blocker is justified. In case of persistent abdominal pain, a proton pump inhibitor must be considered- see section 3.14.2.3. If severe, life-threatening complications occur, the national study coordinator must be notified within 24 h.
As with prednisone, weight gain/fluid retention with or without hypertension is the rule during dexamethasone therapy, although the mineralocorticoid effects of the latter are relatively attenuated. Occasionally, antihypertensive medication may be required.
Changes in mood to include euphoria, dysphoria and depression are also frequent. However, severe agitation or even (paranoid) psychotic reactions and depression with/without suicidal intents are occasionally encountered, particularly in patients with premorbid history (predisposition), in which case consulting a psychiatrist is necessary.
If the side effects are severe enough as to cause withdrawal of dexamethasone, the national study coordinator should be notified without delay.
- VCR:** **Vincristine** 1.5 mg/m²/d, IV, (dsm: 2 mg), on day: 8, 15, 22, 29.
VCR may occasionally cause severe neuropathy or a syndrome of inappropriate antidiuretic-hormone secretion (SIADH). In case of severe VCR-induced neurotoxicity, the drug should be suspended and measures undertaken to manage it- see section 3.5 .
- DOX:** **Doxorubicin** 30 mg/m²/d, PI, over 1 h, on day: 8, 15, 22, 29.
- Before the first and third dose, ECG and Echo-CG should be performed.
 - If there are signs of cardiac insufficiency, EF < 35% or its deterioration by more than 10% against baseline value as well as in case of SF < 30% or its remarkable decline versus baseline value, doxorubicin may be administered only after discussing the issue with and approval by the national study coordinator.
- ASP:** **E. coli L-asparaginase** 10,000 U/m²/d, PI, over 1 h, on day: 8, 11, 15, 18.

- If the patient has not yet displayed an overt hypersensitivity reaction to the drug, a native formulation of E. coli ASP is to be used first, preferably the same preparation as in Protocol I/I, however with a 2-fold dosage given biweekly in the hope to overwhelm a possible covert or silent hypersensitivity. On the other hand, accumulating evidence has shown that the risk of allergic reactions is particularly high at this stage of therapy (ca 30%), as the patient might have been already primed during antecedent exposure.
- In Protocol II an allergic reaction mostly occurs by the first dose of ASP due to previous exposure. This argues for performing a hypersensitivity test already at the beginning of ASP therapy. A test dose of 10-50 U or 0.2 U/kg body weight is administered PI over 15 minutes. The patient should be observed closely during and over 30 minutes following the test dose. If no reaction occurs, the prescribed dose may be then continued. However, an uneventful test dose does not exclude the subsequent emergence of an allergic reaction, and therefore the personnel must be alert and prepared to this possibility.
- In case of a severe hypersensitivity reaction to the native E. coli ASP formulation, a pegylated E. coli asparaginase- pegaspargase (Oncaspar[®] Medac/Enzon) may be used at 2,500 U/m² PI(max 3,750 U) over 1h x1. Hence, a dose of Oncaspar[®] substitutes 4 doses of the unmodified E. coli ASP.
- If available, Erwinia chrysanthemi ASP (Erwinase[®] Speywood) can be used, however at 10,000 U/m²/d alternate day **IM** x7 on day: 8, 10, 12, 14, 16, 18, 20, i.e. 7 doses of Erwinase[®] substitute 4 doses of native E. coli ASP.

MTX IT: **Intrathecal methotrexate** at age-adjusted dosage. See **Tab. 14** (pag 53).

- In case of **initial CNS** involvement on day **1** and **18** of Protocol II.
- Tilt head-down position for at least 2 h after IT MTX.

Phase II/2

Requirements for starting phase II/2

- Satisfactory general status
- No severe infection
- Creatinine within normal limits for age
- Increasing trend in blood counts with:
 - WBC $\geq 2,000/\mu\text{L}$
 - Granulocytes $\geq 500/\mu\text{L}$
 - Platelets $\geq 50,000/\mu\text{L}$

Therapy regulation in phase II/2

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

- WBC $\geq 500/\mu\text{L}$
- Platelets $\geq 30,000/\mu\text{L}$

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/m² (14 x 60 mg/m²).

- CPM:** **Cyclophosphamide** 1,000 mg/m²/d, PI, over 1 h, on day: 36.
- Diuresis and cystitis prophylaxis: IV hydration 3,000 ml/m²/24h (5% G/0.45% NaCl aa + 90 mmol/m²/24h 7.45% KCl).
 - Check overall fluid balance q 12 h. If fluid intake exceeds output by more than 400 ml/m²/12h, then give furosemide 0.5 mg/kg body weight (maximum: 20 mg) IV.
 - Check every urine portion by dipstick for 24 h.
 - Uromitexan[®] 400 mg/m², IV, x3 at: 0, +4 and +8 h from the start of the CPM infusion.
 - Hematuria (red urine, dipstick positive for RBCs) or painful micturition indicate first of all hemorrhagic cystitis. In these cases, intensify diuresis (IV fluids up to 4,500 – 5,000 ml/m²/24h, furosemide 0.5 – 1 mg/kg IV q 4 – 6 h), relieve pain by analgesics, and, if need be, give additional doses of Uromitexan[®]. Irrigation of the urinary bladder or diversion of the urine is rarely needed.

N.B. It should be borne in mind however that the implementation and outcome of supplemental MESNA will depend on the elimination t_{1/2} of CPM/metabolites, which is in turn governed by many factors, as well as on the nature of the damage the urothelium has already sustained.

The terminal elimination t_{1/2} of CPM is variable, ranging from 108 to 960 minutes (mean: 7 h in adults, shorter in children). Additional risk factors including drug interactions or obesity must be taken into account, too (The Chemotherapy Source Book, 3rd edition, Perry MC ed., Williams & Wilkins 2001).

CPM may rarely cause a syndrome of inappropriate antidiuretic-hormone secretion (SIADH) by reducing free-water clearance. Considering the need for aggressive hydration to prevent hemorrhagic cystitis, this is a particularly problematic issue to manage. If severe, with CNS symptoms/signs, the following guidelines may be helpful:

- Considering the quite broad spectrum of differential diagnosis of SIADH, every effort should be made to rule out other causes of the disorder. The diagnosis of drug-induced SIADH is one of exclusion. This is particularly important for future decision-making as to further therapy with the offending drug.
- Careful monitoring of vital functions (continuous), fluid balance (on 1 h basis), body weight (x2/24h), S/U electrolytes (Na, K) and S/U osmolality (q 6-8 h) is mandatory.
- Thoughtful fluid restriction, if any. This should be done on an individual basis.
- Although osmotic diuresis with urea in 5%G or with mannite (both at: 0.5 – 1 – 1.5 – 2 g/kg, given PI over 30 – 60 min, q 6 – 12 h, if needed) may obviate the need for fluid restriction, which would be beneficial in CPM-induced SIADH, this cannot be generally recommended. Osmotic diuresis leads to too rapid and profound shifts of water and electrolytes, which may prove disastrous to the brain (pontine myelinolysis) and hazardous to

the heart (cardiac failure with associated pulmonary edema). A 10% mannite solution, for example, has an osmolality of 549 mmol/kg. Moreover, both drugs cause large losses of sodium (and potassium), which would further aggravate the pre-existent hyponatremia of SIADH.

- A loop diuretic, e.g. furosemide 1 mg/kg, IV, to be repeated as required (q 4 – 6 – 8 – 12 – 24 h) can be used instead.
- Guarded correction of hyponatremia at a rate of 2 mmol/L/h with 1.5 – 3% saline given at 50% the urinary output during the previous hour is a reasonable measure which will create a desired negative fluid balance and cautiously replenish the lost sodium.
- The expanded plasma volume in SIADH is inherently associated with hypoaldosteronism leading to suppressed reabsorption of sodium in the distal tubule of the kidney. Therefore, the mineralocorticoid deoxycorticosterone acetate (DOCA) given at twice the regular therapeutic dose, i.e. 4 mg/m²/d, to be repeated if need be, will improve sodium reabsorption in the distal tubule. DOCA will thus decrease the urinary losses of sodium and contribute to elevation of the plasma sodium level (Weizman Z et al. 1982)⁽⁴³⁾.
- An intensified MESNA protocol with Uromitexan[®] given at a higher dosage for a sufficiently long period of time to more efficiently prevent hemorrhagic cystitis may be considered (Haas A et al. 1986)⁽⁴⁴⁾.
- Additional measures, e.g. lithium carbonate (15 – 60 mg/kg/d, PO, in divided doses q 6 – 8 h, aiming at a target plasma level of 0.6 – 1.5 mmol/L), or demeclocycline (7 – 13 mg/kg/d, PO, in divided doses q 6 – 12 h) are rarely needed.
- Further therapy with CPM, if any, should be consulted with the national study coordinator. It is usually safe with the infusion of isotonic or slightly hypertonic crystalloids (0.9% NaCl/5% G), regular furosemide (0.5 – 1 mg/kg, maximum: 20 mg, IV, at +6 h & +12 h from the start of CPM infusion) along with careful monitoring.

TG: **6-Thioguanine** 60 mg/m²/d, PO, day: 36 – 49 (= 14 days), to be taken in the evening on a fasting stomach without milk.

ARA-C: **Cytarabine** 75 mg/m²/d, IV, in 2 blocks, over 4 days each, on day:
38 – 41 & 45 – 48.

MTX IT: **Intrathecal methotrexate** at age-adjusted dosage. See **Tab. 14** (pag 53).

- Prophylaxis in **all** patients on the same day as the first dose of ARA-C in:
block 1 (day 38) & block 2 (day 45)
- Tilt head-down position for at least 2 h after IT MTX.

2.3 HR Therapy Branch

2.3.1 PREFACE

Patients assigned to the HR group are managed with chemotherapy as well as radiotherapy. Induction therapy consists of Protocol IA. As a difference with ALLIC 2002 the early intensification will be randomized between standard phase IB (arm HR-1) and phase IB Augmented (arm HR-2). After a rest period of 2 weeks, consolidation therapy follows, as a difference with ALLIC 2002 instead of 3, with 6 highly intensive short blocks (HR-1', HR-2', HR-3') x 2, always with a recovery period of about 2 weeks following the 6th day of each block. After 2 weeks of the last block

intensification will be with standard Protocol II. Finally, two weeks after reinduction, all patients are put on maintenance therapy, provided they are in a satisfactory general condition, infection-free and demonstrating findings of recovering hemopoiesis.

A global overview of therapy is outlined in **Fig. 6** (pag 17) & Appendix 3.0.a. The specific changes introduced in this trial against ALL-IC-2002 are dealt with *ad hoc* in the corresponding sections on the individual stages of treatment.

The overall duration of treatment from the start of induction through the end of maintenance therapy is 24 months (104 weeks).

A selected subset of the HR patients will be managed with allogeneic SCT- see section 1.5.4, section 1.13.2 and section 2.6 on stem-cell transplantation. As these patients will undergo a myeloablative pretransplant conditioning, they should receive reduced conventional intensive chemotherapy. In addition, the strategy of radiotherapy, if any, for these patients is also different, and conceivably they will receive no maintenance therapy.

In addition to chemotherapy, the majority of the HR patients (except HR BCP-ALL due only to PPR) receive also radiotherapy (RTX) at age-adjusted dosage, whereby age attained as of the start of RTX or conditioning is the arbiter:

- Prophylactic cranial radiotherapy (pCRT): CNS-negative patients ≥ 1 year of age.
- Therapeutic cranial radiotherapy (tCRT): CNS-positive patients ≥ 1 year of age.

2.3.2 INDUCTION THERAPY

HR ALL

2.3.2.1 PROTOCOL I

As in ALL IC-BFM 2002 protocol IA is to be used for induction therapy of the HR patients. The main difference is that phase IB (arm HR-1) will be randomized with phase IB Augmented (arm HR-2). In case of excess or persistence of blasts, the patient should receive phase IB or IB Aug with an evaluation of remission status at day 52, or later in HR-1' or HR-2'. If the remission is not obtained at the later point, the matter should be discussed with the national study coordinator to make another decision on an individual basis. Therapy is discussed in detail in section 2.2.2.1. Protocol I is composed of 2 phases (I/A & I/B) **Fig. 9 & 10 & 11** (pag 52,56,59 repeated below) & Appendix 3.0.b 1.

Protocol I A Induction Therapy: SR –T ALL, IR, HR

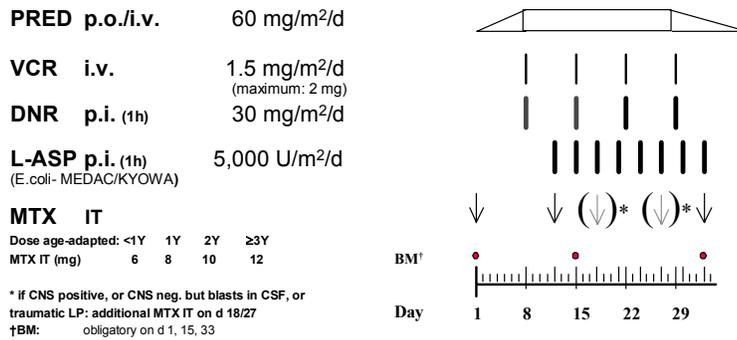


Fig. 9: Protocol I A for Induction Therapy in SR – T, IR, HR- ALL

Protocol I B Early intensification: SR , IR-arm 1, HR-arm 1

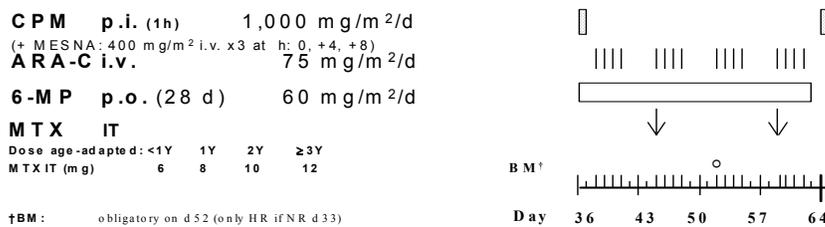


Fig. 10: Early intensification Protocol IB for SR, IR-arm1, HR-arm1

Protocol IB Augmented Early intensification IR-arm2, HR-arm2

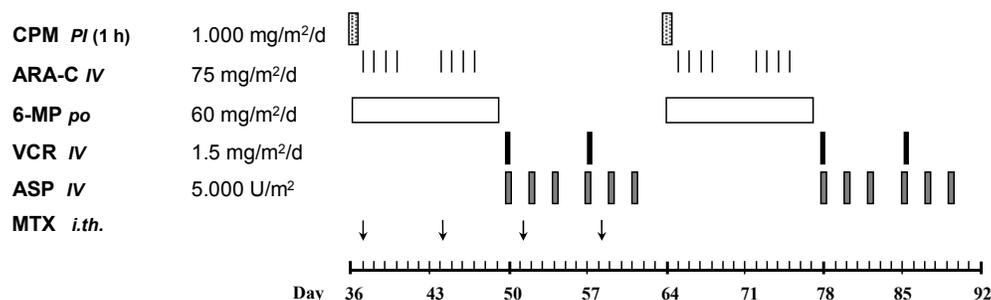


Fig. 11: Early intensification Protocol IB Augmented for IR -arm2 and HR-arm2

2.3.3 CONSOLIDATION THERAPY HR ALL

2.3.3.1 OUTLINE OF CONSOLIDATION THERAPY

All HR patients receive identical consolidation treatment, which begins 2 weeks following induction therapy (Protocol I), provided that they fulfill the entry criteria outlined below. As in ALLIC 2002 it contains 3 different highly intensive, multi-agent chemotherapy elements condensed in brief blocks (HR-1', HR-2', HR-3'), but in this trial they are **given twice**, with a total of 6 blocks delivered approximately 2 weeks apart, which is the interval from the 6th day of the outgoing block to the 1st day of the ongoing 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.

2.3.3.2 POST-INDUCTION REMISSION STATUS

In this trial also, the concept of remission continues to be defined on the basis of the generally accepted conventional cytomorphic criteria- see section 1.16.2.3 on definition of remission. If CR has not been achieved by day 33 of the induction therapy, BM puncture must be performed before starting the 3rd ARA-C block of phase I/B, i.e. on day 52. If still not in CR, it should be repeated on the first day of the first, and if need be, of the second HR block. Patients not yet in CR as of day 1 of the second HR block (1. HR-2') are classified as non-responders, while those who have achieved CR not until after the first HR block (1. HR-1') are ranked as late responders.

The further management of these late responders and non-responders is up to the participating center. They are otherwise eligible for allogeneic SCT, and this option should be considered.

Similarly, patients with a mediastinal tumor that had not receded completely, or in case of an initial testicular involvement, the size of the testicle(s) had not quite normalized by day 33 induction, should be rigorously reevaluated by imaging techniques (US/MRI/CT) after completing phase I/B. If these studies demonstrate a significant finding of a persistent space-occupying lesion/infiltration, biopsy is then necessary. Should leukemic blasts be shown in the biopsy specimen, these patients must be further handled within the HR strategy.

2.3.3.3 REGULATION OF THERAPY

General Guidelines

- The realization of treatment should be as quick as possible, because dose intensity will have hopefully a favorable impact on prognosis. This is particularly relevant to the first 3 HR blocks. 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.
- No reductions in dosage are allowed without approval by the national study coordinator. If needed be, a drug can be rather pushed farther or omitted, i.e. either everything or nothing.
- 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

- Satisfactory general status
- No severe infection
- Intact mucous membranes
- Free urinary tract
- No essential organ dysfunction:
 - Creatinine/CL_{cr} within normal limits for age
 - ALT/AST ≤ x5 ULN for age
 - Bilirubin ≤ x3 ULN for age
- Acceptable blood coagulation parameters:
 - aPTT ≤ x1.6 ULN for age
 - Fibrinogen ≥ x0.75 LLN for age
 - AT III ≥ x0.75 LLN for age
- Findings of recovering hemopoiesis:
 - Rising trend in granulocyte & platelet counts
 - Granulocytes ≥ 200/μL
 - Platelets ≥ 50,000/μL
- TC O₂ saturation by pulse oxymetry ≥ 94%

2.3.3.4 G-CSF POST HR BLOCKS

It is obligatory that all HR patients receive supportive therapy with G-CSF upon the conclusion of every HR block, however not after Protocol II. G-CSF has to be given at 5 μg/kg/d, SC, or exceptionally PI over 4 h, once daily, from day 7 of a HR block and continued until a neutrophil count exceeding 5,000/μL is achieved. This high cut-off

level is necessary, as the neutrophil count often drops markedly upon withdrawing the drug. In case of a severe infection, intervention therapy with G-CSF should be continued even above that limit.

2.3.3.5 LOCOREGIONAL DRUG THERAPY

An integral component of every HR block is triple intrathecal therapy (TIT) with methotrexate, cytosine arabinoside and prednisone. This part of therapy and route of delivery are all too important as to deserve special attention. The information and guidelines most relevant to this form of treatment are therefore summarized in one section, to be referred to whenever need be.

- Soft, short-acting analgesia/sedation may be considered, namely in infants/toddlers and poorly cooperating children, taking into account all possible drug interactions, first of all with MTX.
- The dosage of intrathecal medications (MTX, ARA-C, prednisone, 0.9% NaCl) is according to age at the time of treatment delivery (Bleyer W 1977, Bleyer W et al. 1983, Zimm S et al. 1984, Bekassy AN et al. 1990)⁽³⁹⁻⁴²⁾ see **Tab. 16** below.

Table 16: Dosage of IT Medications by Age Attained at Time of Therapy

| Age (yr) | MTX (mg) | ARA-C (mg) | Prednisone * (mg) | 0.9% NaCl (ml) |
|----------|----------|------------|-------------------|----------------|
| < 1 | 6 | 16 | 4 | 1.5 |
| ≥ 1 < 2 | 8 | 20 | 6 | 2.0 |
| ≥ 2 < 3 | 10 | 26 | 8 | 2.5 |
| ≥ 3 | 12 | 30 | 10 | 3.0 |

* If prednisone is not available for IT therapy it can be replaced by dexamethasone in dose according to age: <1y= 1 mg; ≥ 1 < 2y = 2mg; ≥ 2 < 3y =3 mg; ≥ 3y =4 mg.

- The 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 cytopspin preparation made by a standard technique.
- The findings should be always carefully documented, and sent along with 2 unstained cytopspin 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.
- A water-soluble formulation of a prednisone derivative (methyl/prednisolone, e.g. Urbason soluble or Solu-Decortin H) is preferable in order to minimize the risk of adverse events.
- As MTX and ARA-C are physically and chemically incompatible with each other, they need not get together in the same syringe*. On the other hand, ARA-C and prednisone are mutually compatible; hence they can be mixed in a single syringe. For triple intrathecal medication, the following guidelines are recommended:
 - In addition to an LP needle, tube(s), a plastic tubing, a clamp and three 5-ml syringes (one each for: MTX, ARA-C/Pred mixture, saline) are needed.
 - A sample of CSF for diagnostic purposes is to be obtained first. This should be done always (also if a single drug will be administered).
 - MTX can be administered first, then flushed with sterile, isotonic saline at the age-tailored volume

- Next, the ARA-C/Pred mixture should be instilled IT, again flushing it with sterile, isotonic saline at the age-tailored volume
- Care should be taken so as the total volume delivered IT (MTX + ARA-C/Pred + saline) be almost the same as that of the CSF removed
- Tilt head-down position for at least 2 h after LP.

In addition to avoiding the potential incompatibility, these measures aim at ensuring a better spread of the drugs and their more even disposition within the liquor space, along with improved access to the upper parts of the CNS.

* MTX and ARA-C can get together in the same syringe if the preparation is made in situ and it is administered immediately

2.3.3.6 SUPPORTIVE CARE DURING HR BLOCKS

- Hydration with IV crystalloids at least for the first 6 days of each HR block is necessary. This is accomplished by 3,000 ml/m²/24h 5% G/0.45% NaCl aa + 90 mmol/m²/24h 7.45% KCl. In case of HD MTX (HR-1' & HR-2'), alkalization of the urine is also needed to prevent MTX from crystallizing within the renal tubules and to facilitate its elimination. This is done by adding sodium bicarbonate (NaHCO₃) *quantum satis ad U-pH > 7* to the hydration infusion or along with it from -4 h to +72 h as of the start of HD MTX. For details refer to the corresponding flow sheet in Appendices 3.0.f 1, 3.0.g 1 & 3.0.h 1.
- Control of fluid balance q 12 h while on hydration/alkalinization.
- In case of a positive fluid balance by > 400 ml/m²/12h, furosemide must be given at 0.5 mg/kg body weight (maximum: 20 mg) IV to forestall fluid overload.
- Leucovorin rescue from HD MTX (HR-1' & HR-2'). Refer to Protocol M, where HD MTX/LCV rescue is discussed- section 2.2.3.2 & **Tab. 15** (pag 64) .
- Uroprotection by MESNA IV (Uromitexan[®]) to prevent hemorrhagic cystitis, which could be induced by the oxazaphosphorine metabolites, namely 4-OH CPM, 4-OH IFO & acrolein (HR-1' & HR-2'). The MESNA to CPM/IFO ratio is usually 1 – 1.2:1. The drug is given in 3 equal doses at 0, +4, +8 h as of the start of CPM/IFO.
- Prevention of toxic keratoconjunctivitis (HD ARA-C in HR-1' & HR-3') by an eye ointment/drops containing dexamethasone along with eye hygiene for 3 days beginning by the first dose of HD ARA-C.
- Reducing the risk of neurotoxicity (HD ARA-C in HR-1' & HR-3') by HD vitamin B₆ at 150 mg/m², IV/PO, q 12 h for 3 days beginning by the first dose of HD ARA-C.
- Effective antiemetic therapy with an HT₃ blocker is usually necessary.
- Monitoring of vital functions, paying attention to specific symptoms/signs as described in the individual blocks- *vide infra*.

2.3.3.7 HIGH-DOSE METHOTREXATE HD MTX

HD MTX (5 g/m²/24h) is an item of 2 blocks: HR-1' & HR-2'. A brief account of HD MTX is given in either block. Therapy is detailed in separate flow sheets (Appendices 3.0.f 1 & 3.0.g 1). In addition, a diagram depicting LCV rescue according to the plasma level of MTX within specified time zones is also provided (Appendix 3.1). Finally, guidelines for managing the catastrophic scenario of poor MTX elimination are given as well. Further information on HD MTX/LCV rescue is discussed under Protocol M in section 2.2.3.2 & **Tab. 15** (pag 64).

2.3.3.8 BLOCK HR-1'

Block HR-1' begins 2 weeks upon completion of phase I/2, provided that the patient is in a good clinical standing, free from severe infection, with recovering hemopoiesis and adequate hemostasis, and without essential major-organ toxicity or urinary tract obstruction- see section 2.3.3.3 above.

Block HR-1' is shown in **Fig. 15** below, and detailed in Appendices 3.0.f & 3.0.f 1.

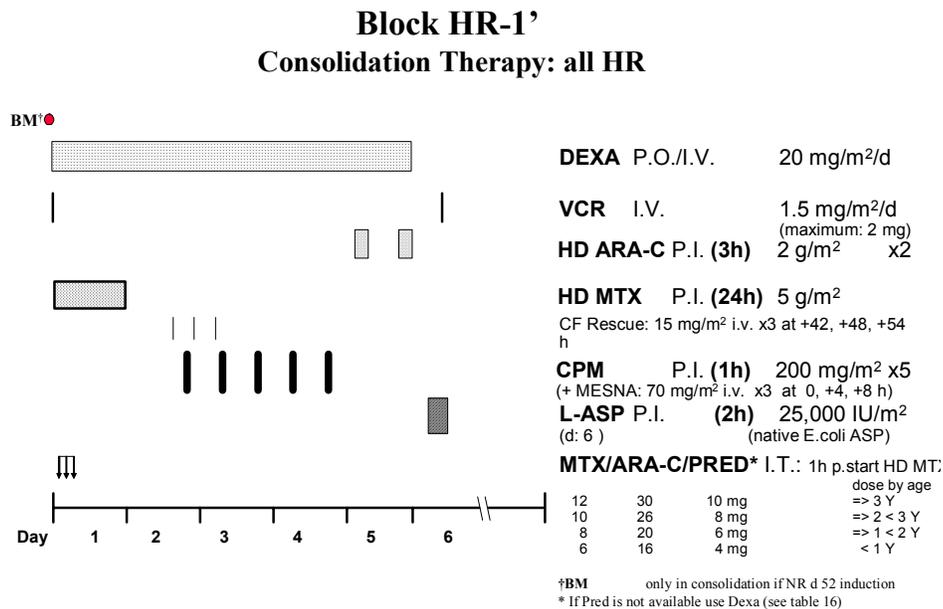


Fig. 15: Block HR-1' for Consolidation Therapy in all HR

DEXA: **Dexamethasone** 20 mg/m²/d, PO/IV, in 3 divided doses, on day: 1 – 5.

VCR: **Vincristine** 1.5 mg/m² *pro dosi*, IV, (dsm: 2 mg), on day: 1, 6.

- Compared to ALL-BFM 95, both phases of Protocol I are used for induction therapy of HR patients. Hence, the interval between block HR-1' and phase I/1 is long enough to minimize the risk of VCR-induced neuropathy. Therefore, VCR can be safely administered on day 1 of the first HR-1' block, too.
- 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.

See flow sheet- Appendix 3.0.f 1.

- 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.

MTX is compatible with G, NaCl, KCl & NaHCO₃, so that they can be mixed together or flow in parallel.

- 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.
- LCV:** **Leucovorin Ca** 15 mg/m² *pro dosi*, IV, x3 at: +42 h, +48 h+54 h after the start of HD MTX.
- See the general guidelines on HD MTX/LCV rescue under the heading of Protocol M in section 2.2.3.2 & **Tab. 15** (pag 64), the diagram for LCV rescue- Appendix 3.1, and the guidelines on the management of poor MTX elimination- Appendix 3.1.
- CPM:** **Cyclophosphamide** 200 mg/m²/d, PI, over 1 h, on day: 2 – 4.
- See flow sheet- Appendix 3.0.f 1.
- 5 doses q 12 h apart, beginning 7 h after the end of HD MTX.
 - Adequate diuresis/hemorrhagic cystitis prophylaxis: IV fluids 3,000 ml/m²/24h, checking fluid balance q 12 h, administering furosemide 0.5 mg/kg (maximum: 20 mg) IV, should input exceed output by > 400 ml/m²/12h, and checking every urine portion by dipstick during the hospital stay- see flow sheet in Appendix 3.0.f 1.
- The hydration/alkalinization protocol used with HD MTX provides fluid coverage for CPM for 41 h as well, whereupon another infusion (without NaHCO₃) may be given instead. The latter infusion should be continued until day 6 in order to cover also HD ARA-C.
- MESNA (Uromitexan[®]) 70 mg/m² *pro dosi*, IV, x3 at: 0 h, +4 h+8 h from the start of the CPM infusion.
 - At the dose level and schedule prescribed in this block it is unlikely to encounter CPM-induced SIADH.
 - If symptoms/signs of hemorrhagic cystitis occur (macroscopic or microscopic hematuria, dysuria/stranguria):
 - Forced diuresis with up to 4,500 – 5,000 ml/m²/24h 5% G/0.45% NaCl aa/± 90 mmol/m²/24h 7.45% KCl. **CAVE: fluid overload.**
 - More rigorous fluid balance control, i.e. q 4 – 6 h.
 - Furosemide 0.5 – 1 mg/kg (maximum: 20 mg), to be given IV as needed, i.e. if input > output by > 150 – 200 ml/m²/4 – 6h.
 - An intensified MESNA protocol should be considered, e.g. Uromitexan[®] 150 – 200 mg/m² *pro dosi*, IV, q 4 – 6 h until reliable control of this complication. However, the implementation and outcome of this measure will depend on the elimination t_{1/2} of CPM/metabolites, which is in turn governed by many factors, as well as on the nature of the damage the urothelium has already sustained.
- The terminal elimination t_{1/2} of CPM is variable, ranging from 108 to 960 minutes (mean: 7 h in adults, shorter in children). Additional risk factors including drug interactions or obesity must be taken into account, too (The Chemotherapy Source Book, 3rd edition, Perry MC ed., Williams & Wilkins 2001).*
- Pain relief with analgesics.
 - Irrigation of the urinary bladder or derivation of the urine is rarely needed.
- HD ARA-C:** **Cytarabine** 2,000 mg/m² *pro dosi*, PI, over 3 h, on day: 5.
- See flow sheet- Appendix 3.0.f 1.
- 2 doses 12 h apart.

- Prevention of toxic keratoconjunctivitis by eye hygiene along with eye ointment/drops containing dexamethasone to be introduced into both conjunctival sacs x3 daily for at least 2 days beginning by day 5.
- Prevention of neurotoxicity by high-dose vitamin B₆: 150 mg/m², IV/PO, q 12 h for at least 2 days beginning by day 5.
- Careful observation of the patient for signs of neurotoxicity during the ARA-C infusion is obligatory. If nystagmus and/or ataxia are encountered, the infusion should be withheld. If these signs do not disappear, or if they reappear again upon resuming the ARA-C infusion, the drug must be withdrawn immediately and forever. Otherwise, an irreversible Purkinje cell damage would ensue.

ASP: E. coli L-asparaginase 25,000 U/m², PI, over 2 h, on day 6

- The drug of first choice should be a native formulation of E. coli L-asparaginase, whenever possible the same preparation as in Protocol I/1, and preferably of well established pharmacokinetics/pharmacodynamics, e.g. that of Medac/Kyowa Hakko, or Elspar of MSD. The unmodified formulation of E. coli ASP, even of as yet not well characterized pharmacologic profile, is to be given at 25,000 U/m² by 2-h infusion, x 1: on day 6
- A test dose of ASP at 10 – 50 U or 0.2 U/kg body weight, to be infused over 15 minutes before therapy with ASP, is recommended. The patient should be closely observed during the test infusion and for additional 30 minutes thereafter. If no reaction occurs, the prescribed therapeutic dose can be continued. However, although a positive test is predictive, a negative one does not guarantee a smooth course of subsequent ASP therapy.
- In case of a severe allergic reaction to the native E. coli ASP preparation, it is recommended to use the pegylated E. coli ASP formulation- pegaspargase (Oncaspar[®], Medac/Enzon). This is given at 2,500 U/m² (max 3,750 U), PI, over 1 h, x1 on day 6 only.
It is expected that this dose should ensure ≥ 100 U/L enzyme activity in the plasma over the inter-block period in ca 66 – 75% of the patients, effecting complete or almost complete asparagine depletion.
- If available, Erwinase[®], Speywood can be used, however at 10,000 U/m²/d, **IM**, on alternate day: 6, 8, 10.
- Should a severe hypersensitivity reaction be encountered again, then switching over to the available, so far unused ASP preparation is necessary, i.e. Erwinase[®] post Oncaspar[®] or *vice versa*.

Additional information on L-asparaginase is given in Protocol I, section 2.2.2.1 and chapter 4 on chemotherapy side effects.

MTX/ARA-C/Pred* IT: Intrathecal methotrexate/cytarabine/prednisone

See section 2.3.3.6 & **Tab. 16** (pag 76) of this chapter on locoregional therapy.

- Age-adjusted dosage.
- Administration 1 h after starting HD MTX.
- Choice of the safest preparations/formulations.
- CAVE: incompatibility between MTX & ARA-C.
- Tilt head-down position for at least 2 h after IT therapy.
- * If Pred is not available for IT, use Dexamethasone, see **Tab 16**.

2.3.3.9 BLOCK HR-2'

This element is a component of the consolidation treatment of all HR patients. Block HR-2' usually begins 2 weeks after the 6th day of block HR-1', provided that the entry

criteria outlined in section 2.3.3.3 of this chapter are met- *vide supra*. Additionally, specific demands on the side of cardiac function from the viewpoint of potential DNR-induced cardiotoxicity are required.

Echocardiographic entry criteria

- SF within normal limits for age & without remarkable descent vs. baseline value.
- EF > 35%. A decrease of the EF by < 10% vs. baseline value is still acceptable.

Block HR-2' is shown in **Fig. 16** below, and detailed in Appendices 3.0.g & 3.0.g 1.

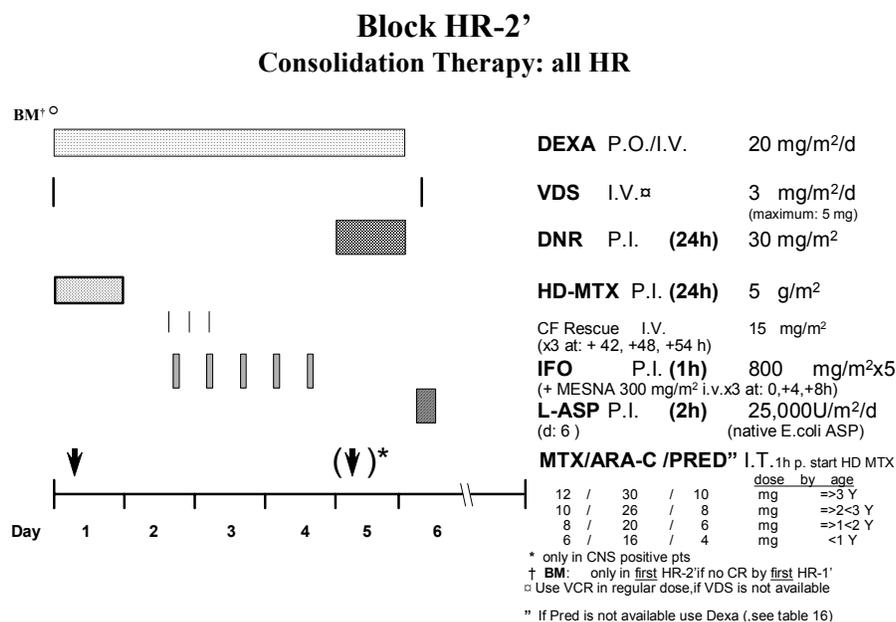


Fig. 16: Block HR-2' for Consolidation Therapy in all HR

DEXA: Dexamethasone- the same as in block HR-1'.

HD MTX: High-dose methotrexate- the same as in block HR-1'.

LCV: Leucovorin Ca- the same as in block HR-1'.

MTX/ARA-C/Pred* IT: Intrathecal methotrexate/cytarabine/prednisone

See section 2.3.3.6 & **Tab. 16** (pag 76) of this chapter on locoregional therapy.

- Age-adjusted dosage.
- CNS status-differentiated intensity:
 - CNS-negative patients: x1, on day 1 only (1 h after starting HD MTX).
 - CNS-positive patients: x2, on day 1 (1 h after starting HD MTX), and 5.
- Choice of the safest preparations/formulations.
- CAVE: incompatibility between MTX & ARA-C.
- Tilt head-down position for at least 2 h after IT therapy.
- * If Pred is not available for IT, use Dexa ,see **Tab 16**.

ASP: L-asparaginase- the same as in block HR-1'.

VDS: Vindesine 3 mg/m²/d (dsm: 5 mg), slowly IV, x2, on day: 1, 6.

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).

If not available, replace with **Vincristine** as in HR-1': 1,5 mg/m²/d (max 2 mg), slowly IV, x 2 days, on day: 1, 6.

IFO: **Ifosfamide** 800 mg/m² *pro dosi*, PI, over 1 h, on day: 2 – 4.

See flow sheet- Appendix 3.0.g 1.

- 5 doses, q 12 h, beginning 7 h after the end of HD MTX.
- Both oxazaphosphorines (CPM & IFO) used in this trial share many features in common. However, at equipotent doses IFO is potentially neurotoxic and more urotoxic than CPM. The intermediate metabolite chloroacetaldehyde is held responsible for the IFO-associated encephalopathy. In addition, IFO may cause damage to the renal proximal tubules manifesting itself as a Fanconi-like wasting syndrome not manageable with MESNA. Mostly, encephalopathy and Fanconi syndrome have been associated with HD IFO/MESNA usually in the context of multi-agent chemotherapy regimens.

For more details refer to: A Clinician's Guide to Chemotherapy Pharmacokinetics and Pharmacodynamics, 1st edition, Grochow LB & Ames MM ed., Williams & Wilkins 1998.

- Adequate diuresis and hemorrhagic cystitis prophylaxis:
 - Hydration with IV crystalloids. Initially, this is covered by the hydration/alkalinization protocol prescribed for HD MTX. After +72 h as of the start of HD MTX, NaHCO₃ is usually no longer required, so that an infusion of 3,000 ml/m²/24h 5% G/0.45% NaCl aa + 90 mmol/m²/24h 7.45% KCl can be used instead. The latter infusion should be continued until day 6.
 - MESNA (Uromitexan[®]) 300 mg/m² *pro dosi*, to be given IV, x3, q 4 h at: 0 h, +4 h, +8 h as of the start of the IFO infusion.
 - Control of fluid balance q 12 h. If positive by > 400 ml/m²/12h, furosemide 0.5 mg/kg body weight (maximum: 20 mg) must be given IV.
 - Monitoring of symptoms/signs of hemorrhagic cystitis is mandatory, i.e. painful micturition, red urine or dipstick positive for blood.
- If hemorrhagic cystitis is proven or merely suggested, it should be managed promptly and intensively:
 - Careful monitoring of vital functions (**Cave:** fluid overload).
 - More vigorous hydration with up to 4,500 – 5,000 ml/m²/24h of 5% G/0.45% NaCl aa ± 90 mmol/m²/24h of 7.45% KCl.
 - More rigorous control of fluid balance, i.e. q 4 – 6 h, administering furosemide 0.5 mg/kg body weight (maximum: 20 mg) IV should input exceed output by > 150 – 200 ml/m²/4 – 6 h.
 - Intensified/prolonged MESNA protocol, e.g. Uromitexan[®] 500 – 600 mg/m², IV, q 4 h until reliable control of this serious side effect. However, as with CPM, the implementation and outcome of this measure will depend on the elimination *t*_{1/2} of IFO/metabolites, which is in turn governed by many variables, as well as on the nature of the damage the urothelium has already sustained.

*The mean elimination *t*_{1/2} of IFO fluctuates in the range of 5.5 to 7.7 h (The Chemotherapy Source Book, 3rd edition, Perry MC ed., Williams & Wilkins 2001).*

- Pain relief with analgesics is necessary.
- Urinary bladder irrigation or urine diversion is rarely needed.

DNR: **Daunorubicin** 30 mg/m², PI, over 24 h, on day: 5.

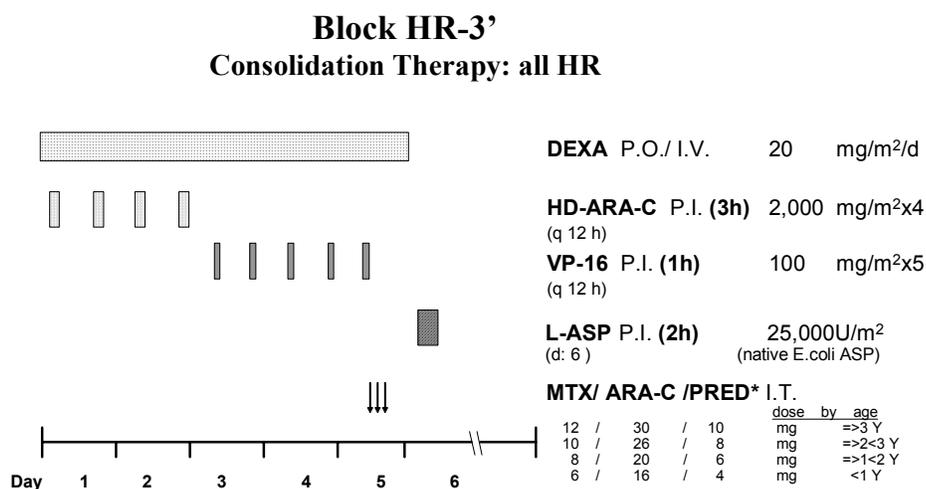
- ECG and Echo-CG are mandatory prior to DNR.
- If symptoms/signs of cardiac insufficiency are present, or if the SF falls below 30% and/or markedly declines against baseline level as well as in case of EF under 35% or should it decrease by > 10% in comparison with baseline value, DNR can be delivered only after discussion with and approval by the national study coordinator.

2.3.3.10 BLOCK HR-3'

Block HR-3' is a part of the consolidation therapy of all HR patients. It is usually launched 2 weeks following the 6th day of block HR-2', so far as the patient qualifies for the entry criteria outlined in section 2.3.3.4 of this chapter- *vide supra*. In addition to the changes in L-asparaginase therapy discussed in general in section 2.3.3.3 and in depth in section 2.3.3.9 on block HR-1', VP-16 is infused over 1 h. Moreover, besides the parent drug, a novel, water-soluble derivative of VP-16 (etoposide phosphate = Etopophos[®], Bristol-Myers Squibb) is currently available on the market in many countries. This formulation is *de facto* void of the notorious infusion-related toxicity of its original ancestor that should be dissolved in organic vehicula and additives (Tween 80, PEG, ethanol, etc) to improve solubility. It is just these vehicula/additives that are rather held responsible for the infusion-associated toxicity observed with first-generation etoposide preparations.

One vial of Etopophos[®] contains 113.6 mg etoposide phosphate, corresponding to 100 mg etoposide. Etopophos[®] contains no benzyl alcohol or other additives, needs a substantially smaller volume of 0.9% NaCl or 5% G to dissolve (11.36 mg Etopophos/ml) and is more stable compared to the classic compound. Furthermore, the risk of phlebitis or local irritation on extravasation is negligible. Also allergic reactions occur less frequently. Nonetheless, to avoid hypotension and anaphylaxis as well as phlebitis it is recommended to infuse the drug over 1 h, too.

Block HR-3' is depicted in **Fig. 17** and detailed in Appendices 3.0.h & 3.0.h 1.



*If Pred is not available use Dexa (see table 16)

Fig. 17: Block HR-3' for Consolidation Therapy in all HR

DEXA: Dexamethasone- the same as in block HR-1'.

ASP: **L-asparaginase**- the same as in block HR-1'.

MTX/ARA-C/Pred IT: Intrathecal methotrexate/cytarabine/prednisone

See section 2.3.3.6 (p 80) & **Tab 16** (pag 76) on locoregional therapy.

- Age-adjusted dosage.
- Choice of the safest preparations/formulations.
- CAVE: incompatibility between MTX & ARA-C.
- Reduction of the risk of neurotoxicity via scheduling IT therapy apart from HD ARA-C, i.e. on day 5.
- Tilt head-down position for at least 2 h after IT therapy.
- * If Pred is not available for IT, use Dexa, see **Tab 16**.

HD ARA-C: High-dose cytarabine 2,000 mg/m² *pro dosi*, PI, over 3 h, on day: 1, 2.

See flow sheet- Appendix 3.0.h 1.

- 4 doses 12 h apart.
- Prevention of toxic keratoconjunctivitis by eye hygiene along with eye ointment/drops containing dexamethasone to be introduced into both conjunctival sacs x3 daily for 3 days beginning by day 1.
- Prevention of neurotoxicity by high-dose vitamin B₆: 150 mg/m², IV/PO, q 12 h for 3 days beginning by day 1.
- Careful observation of the patient for signs of neurotoxicity during the ARA-C infusion is obligatory. If nystagmus and/or ataxia are encountered, the infusion should be withheld. If these signs do not disappear, or if they reappear again upon resuming the ARA-C infusion, the drug must be withdrawn immediately and forever. Otherwise, an irreversible Purkinje cell damage would ensue.
- Hydration with IV crystalloids for at least 6 days beginning by day 1, i.e. 3,000 ml/m²/24h of 5% G/0.45% NaCl aa + 90 mmol/m²/24h of 7.45% KCl, checking fluid balance q 12 h, and administering furosemide 0.5 mg/kg body weight (maximum: 20 mg) IV *pro re nata*, i.e. if input > output by > 400 ml/m²/12h.

VP-16: Etoposide 100 mg/m² *pro dosi*, PI, over 1 h, on day: 3 – 5.

- 5 doses q 12 h.
- Dilution with 0.9% NaCl at 1:50 (v/v).
- CAVE: fall in BP, arrhythmias, allergic reactions/anaphylaxis, bronchospasm, phlebitis/local irritation.
- Monitoring of the patient during and for 5 h after the infusion.
- **N.B.** Etopophos[®] at 113.6 mg/m² *pro dosi* may be used in lieu of Vepesid[®]. In this case, follow the manufacturer's instructions.

2.3.4 REINDUCTION THERAPY HR ALL

2.3.4.1 PROLOGUE

By the end of consolidation, patients with HR ALL, including those who are otherwise eligible for allogeneic SCT (for the case of not allografting them) receives a reinduction phase with protocol II. Reinduction therapy usually begins ca 2 weeks after the 6th day of the second HR-3' block provided that the entry requirements set forth are satisfied. Each therapy element and phase of treatment has a specified set of entry criteria that should be fulfilled. Occasionally, specific prerequisites must be first met for a dose of a drug to be delivered or a component of therapy to be launched, e.g.

good cardiac performance in case of adriamycin and daunorubicin, a blood count acceptable enough to initiate an ARA-C block, etc.

It should be borne in mind, that the reinduction therapy prescribed for patients with HR ALL in this trial is too intense and comes up at a stage, when the hemopoietic reserve is limited and the immune status already severely compromised. These patients are particularly vulnerable, and should be carefully monitored for toxicity. Consequently, they need comprehensive supportive care. Pneumocystis carinii pneumonia (PCP) prophylaxis is also indispensable. In addition, it is expected that overt or covert hypersensitivity to L-asparaginase might become more frequent and more urgent as therapy progresses.

Drug dosing is to be tailored to the BSA updated at specified key time points along the flow of treatment, namely at the start of every therapy element and phase thereof.

In addition to chemotherapy, the overwhelming majority of HR patients are managed with radiotherapy (RTX), delivered after the reinduction therapy, at dosage adjusted to their age at the time of treatment delivery. With regard to cranial radiotherapy (CRT), it is indicated either prophylactically (pCRT at 12 Gy) in CNS-negative (CNS status 1/2) or therapeutically (tCRT at 12 Gy or 18 Gy- by age) in CNS-positive (CNS status 3) patients. **Patients with HR BCP-ALL due only to PPR will not receive pCRT**, instead they will have 6 IT MTX in maintenance (every 4 weeks). Infants less than 1 year of age receive neither form of CRT. Those HR patients programmed to allogeneic SCT, who receive TBI as part of the preparative regimen with/without (by status) local RTX to the testicle(s) and/or CNS. If indicated, local RTX is given during the week preceding conditioning. Refer to section 1.14 on CNS therapy; section 1.16.1.3 on the diagnosis of CNS involvement & definition of CNS status; section 1.16.1.5 on the diagnosis of initial testicular involvement; section 2.5 on RTX. A global overview of the therapy plan is given in Fig. 6 (pag 17) & Appendix 3.0.a.

2.3.4.2 REINDUCTION THERAPY

Three weeks after the 6th HR block all patients receive one Protocol II over 7 weeks

Protocol II is shown in Fig. 14 (pag 67) & below, and dealt with in depth elsewhere in this chapter- refer to section 2.2.4.1 & section 2.2.6 above.

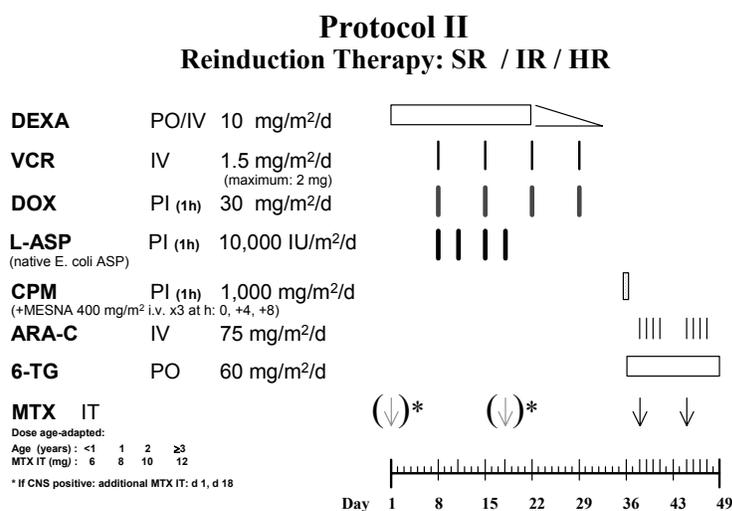


Fig. 14: Protocol II for Late Reinduction Therapy in Arms SR, IR, HR

2.4 Maintenance Therapy. MT SR / IR / HR

2.4.1 MT GENERAL OUTLINE

Two weeks after the conclusion of protocol II, 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) for all patients enrolled on this trial. Since the intensive therapy, including the recovery pauses spans a somewhat different period of time in the individual arms and options, the duration of MT will also vary.

Locoregional therapy with IT MTX during the maintenance phase of treatment is indicated for those patients with BCP-ALL, who are assigned to : SR or IR-3, (4 doses)

T-ALL with WBC < 100.000 & HR only for PPR ,6 doses, as they will not receive pCRT.

2.4.2 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.
- Therapy is to be documented on the corresponding sheet for MT- see Appendix 3.0.i.
- Pneumocystis carinii prophylaxis (PCP) is mandatory in all patients:
 - Co-trimoxazol (SMZ/TMP) 25/5 mg/kg/d, PO, in 2 divided doses q 12 h on 3 consecutive days per week, apart from MTX as possible, e.g. if MTX is given on Tuesday, then SMZ/TMP must be taken on Friday- Saturday- Sunday.
 - In case of hypersensitivity to SMZ/TMP, an attempt at desensitization according to the protocol detailed in section 3.14.1.2 (p 151) may be made. Moreover, a number of alternative effective approaches are available for PCP prophylaxis or treatment, and can be used should intolerance or allergy to SMZ/TMP emerge, e.g. pentamidine aerosol to be inhaled over 20 – 30 minutes x1 monthly:
 - Patients < 4 years of age: 150 mg/5 ml distilled water.
 - Patients > 4 years of age: 300 mg/5 ml distilled water.

In case of bronchospasm, 1 – 2 puffs of a selective β_2 -sympatomimeticum applied before and after pentamidine inhalation will be needed.
- 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.
- If, on the basis of the blood count, dosage has been modified in either direction, then the patient should be seen, and the blood count checked 2 weeks later in order to avoid inappropriate dosage for undue period of time.
- ALT, 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.

Requirements for starting MT

- Satisfactory general status
- No severe infection
- Recovering hemopoiesis, at least:
 - WBC $\geq 1,000/\mu\text{L}$
 - Granulocytes $\geq 200/\mu\text{L}$
 - Platelets $\geq 50,000/\mu\text{L}$

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. 17** & **Fig. 18** below.

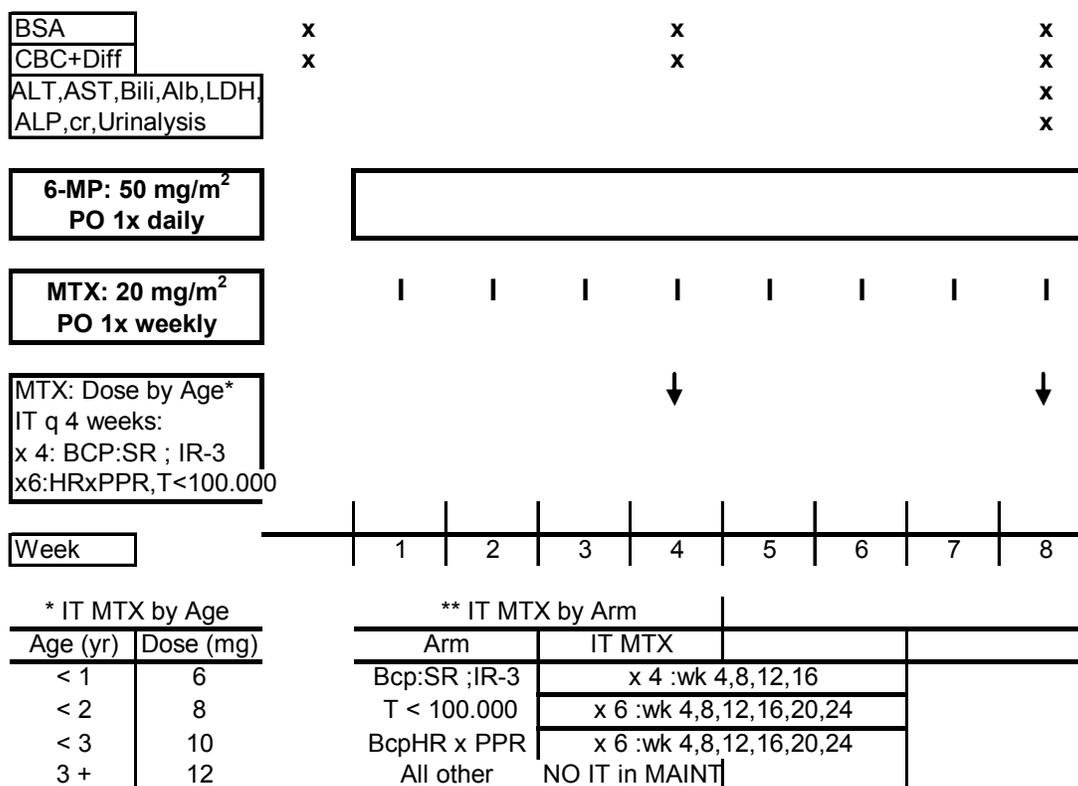
Table 17: Dosage of MP & MTX by WBC & Differential during MT

| 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
(virologic studies mandatory):
 - ALT/AST > 5 x ULN for age
 - Bilirubin > 3 x ULN for age
- Long-standing diarrhea
- Lung changes on CXR (? MTX pneumonitis)

The maintenance therapy is illustrated in an abbreviated form in **Fig. 18** below.



2.4.3.1 MT COMMON TO ALL

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/m²/d, PO, x1 daily in the evening on a fasting stomach without milk.

MTX: **Methotrexate** 20 mg/m², PO, x1 weekly (always on the same day of every week), to be taken also in the evening on a fasting stomach without milk.

2.4.3.2 Intrathecal MTX in MT in SR & IR-arm 3

Patients with BCP-ALL assigned to either arm SR or arm IR receive **4 age-adjusted doses of IT MTX** q 4 weeks beginning by week 4 of the maintenance therapy to counterbalance the lower dose level of systemic MTX in consolidation. For these patients this approach should ensure the risk of CNS relapse be not increased in this trial against ALL-BFM 95.

MTX IT: **Intrathecal methotrexate**

- At age-adjusted dosage q 4 weeks into the maintenance phase, beginning by week 4 (week: 4, 8, 12, 16), **4 doses** overall- see **Tab. 14** (pag 53)& below.
- Tilt head-down position for at least 2 h after IT MTX.

Table 14: Dosage of IT MTX by Age at Time of LP

| Age (yr) | < 1 | ≥ 1 < 2 | ≥ 2 < 3 | ≥ 3 |
|-------------|-----|---------|---------|-----|
| Dosage (mg) | 6 | 8 | 10 | 12 |

2.4.3.3 Intrathecal MTX in MT in T-ALL + WBC < 100.000 & HR due to PPR

Patients with T ALL + WBC < 100.000 and BCP-HR due ONLY to PPR will receive **6 age-adjusted doses of IT MTX** q 4 weeks beginning by week 4 of the maintenance therapy to counterbalance the elimination of pCRT.

MTX IT: Intrathecal methotrexate

- At age-adjusted dosage q 4 weeks into the maintenance phase, beginning by week 4 (week: 4, 8, 12, 16, 20, 24), **6 doses** overall- see **Tab. 14** (pag 53) & below.
- Tilt head-down position for at least 2 h after IT MTX.

Table 14: Dosage of IT MTX by Age at Time of LP

| Age (yr) | < 1 | ≥ 1 < 2 | ≥ 2 < 3 | ≥ 3 |
|-------------|-----|---------|---------|-----|
| Dosage (mg) | 6 | 8 | 10 | 12 |

2.4.3.4 T - ALL & OTHER ARMS / OPTIONS

Since covert as well as overt leukemia within the CNS sanctuaries has been already addressed adequately via intensive therapy (± CRT by indication), patients managed along IR-4, HR and T- ALL +WBC > 100.000 should not receive IT MTX during maintenance therapy.

2.5 Radiotherapy RTX

2.5.1 CHANGES vs. ALL-IC 2002

- 1 To compensate for the lower dose level of MTX (2 g/m²/24h versus 5 g/m²/24h), additional IT MTX is to be given during maintenance therapy. Those will be 4 doses IT MTX ,as in ALLIC 2002, for patients with BCP-ALL in SR, BCP-ALL IR-3.
- 2 **Patients with T ALL + WBC > 100.000 and BCP HR due to PPR do not receive pCRT**, instead they will have 6 IT MTX in maintenance
- 3 IR patients with T-ALL+ WBC > 100000 and a CNS status 1/2 ,should receive pCRT following the reinduction therapy.

For the sake of overview, a global comparison of CNS-targeted chemotherapy between ALL-BFM 95 and ALL IC-BFM 2002 is summarized in **Tab.18, Tab 19 & Tab. 20**.

Table 18: CNS-targeted Chemotherapy in ALL-BFM 95

| RG | Systemic Chemotherapy | | Locoregional Chemotherapy | | |
|----|----------------------------|----------------------------|---------------------------|--------------------|----------|
| | HD MTX | HD ARA-C | # IT MTX [†] | # TIT [†] | ∑ # |
| SR | 5 g/m ² /24h x4 | | 11 + (4) | | 11 + (4) |
| MR | 5 g/m ² /24h x4 | | 11 + (4) | | 11 + (4) |
| HR | 5 g/m ² /24h x4 | 2 g/m ² /3h x12 | 5 + (3) | 6 + (2) | 11 + (5) |

Table 19: CNS-targeted Chemotherapy in ALL IC-BFM 2002

| RG | Systemic Chemotherapy | | Locoregional Chemotherapy | | |
|-------------------------------------|----------------------------|----------------------------|---------------------------|-----------------------|----------|
| | MD/HD MTX | HD ARA-C | IT MTX [†] # | TIT [†] # | ∑ # |
| BCP: SR-1 BCP: SR-2 BCP: IR-1 | 2 g/m ² /24h x4 | | 15 + (4) | | 15 + (4) |
| T: SR-1 T: IR-1 | 5 g/m ² /24h x4 | | 11 + (4) | | 11 + (4) |
| T: SR-2 | 5 g/m ² /24h x4 | | 13 + (4) | | 13 + (4) |
| BCP: IR-2 | 2 g/m ² /24h x4 | | 15 + (5) | | 15 + (5) |
| T: IR-2 | 5 g/m ² /24h x4 | | 15 + (5) | | 15 + (5) |
| BCP/T: HR-1 | 5 g/m ² /24h x2 | 2 g/m ² /3h x6 | 11 + (5) | 3 + (1) | 14 + (6) |
| BCP/T: HR-2A | 5 g/m ² /24h x2 | 2 g/m ² /3h x6 | 9 + (6) | 3 + (1) | 12 + (7) |
| BCP/T: HR-2B | 5 g/m ² /24h x4 | 2 g/m ² /3h x12 | 7 + (4) | 6 + (2) | 13 + (6) |

Legend to Tab. 18 & Tab. 19

† Intrathecal methotrexate and triple intrathecal therapy (MTX/ARA-C/Pred) at age-adjusted dosage.

+ (#) Number of additional doses in initially CNS-positive patients (CNS status 3). These patients received/will receive tCRT at age-adjusted dosage.

N.B. ALL-BFM 95: Cases defined as CNS status 2 received extra IT MTX only: on day 18 & 27 of Protocol I' (SR) & I (MR) or day 18 of Protocol IA (HR). However, these cases received no tCRT.

ALL IC-BFM 2002: Cases defined as CNS status 2 will receive extra IT MTX on day 18 & 27 of Protocol I' (BCP SR) & Protocol I (all other), but no tCRT. However, SR/IR T-ALL & all non-transplant HR patients with a CNS status 1/2 should receive pCRT.

Table 20: CNS-targeted Chemotherapy in ALL IC-BFM 2009

| RG | Systemic Chemotherapy | | Locoregional Chemotherapy | | |
|----------------------|----------------------------|-------------------------|---------------------------|-----------------------|----------|
| | MD/HD MTX | HD ARA-C | IT MTX [†] # | TIT [†] # | ∑ # |
| BCP: SR BCP: IR-3 | 2 g/m ² /24h x4 | | 15 + (5) | | 15 + (5) |
| BCP: IR- 4 | 5 g/m ² /24h x4 | | 11 + (5) | | 11 + (5) |
| T<100000: IR | 5 g/m ² /24h x4 | | 17 + (5) | | 17 + (5) |
| T>100000: IR | 5 g/m ² /24h x4 | | 11 + (5) | | 11 + (5) |
| BCP/T HR | 5 g/m ² /24h x4 | 2 g/m ² x 12 | 7 + (5) | 6 + (6) | 13 +(6) |

N.B Patients assigned to arm IR-2 and HR-2 receive 2 extra doses of IT MTX

2.5.2 CONTRAINDICATIONS & PRECAUTIONS

- 1 Infants under 1 year of age do not receive any form of CRT.
- 2 Cases defined as CNS status 2 receive additional IT MTX on day 18 & 27 of Protocol I' (BCP SR) & Protocol I (all other), but no therapeutic cranial radiotherapy (tCRT). However, in case of CNS status 1/2 in patients with SR/IR T-ALL +WBC > 100.000 as well as in those HR patients not delegated to allogeneic SCT, prophylactic cranial radiotherapy (pCRT) should be given- *vide infra*.
- 3 Children less than 2 years of age undergoing allogeneic SCT receive neither TBI nor local radiotherapy to involved site(s).
- 4 Serious, non-leukemic CNS (psycho/neurologic) disorders including chronic leukoencephalopathy post MTX, a history of intracranial thrombosis or hemorrhage, etc contraindicate CRT.
- 5 Patients with a chromosomal instability/fragility syndrome and/or a defective DNA repair machinery, e.g. ataxia-telangiectasia or Nijmegen breakage syndrome should not receive any form of RTX. Being primarily immunodeficient and in the face of severe orointestinal mucositis with potentially lethal infections, these patients may receive MTX at 1 g/m² in lieu of 5 g/m². In addition, because of their clastogenic effects, alkylating agents (CPM, IFO) and topoisomerase II inhibitors (VP-16) ought to be either omitted altogether or reduced to 20 – 80% of the prescribed dosage. The decision should be made on an individual basis, i.e. according to tolerance and complications. Careful surveillance, comprehensive and meticulous supportive care as well as life-long follow-up including genetic monitoring are essential (Irsfeld H et al. 2000, Seidemann K et al. 1999 & 2000)⁽⁵¹⁻⁵³⁾.
- 6 RTX is also contraindicated in the nevoid basal cell carcinoma (Gorlin) syndrome.
- 7 The patient should be free of symptoms/signs of a CNS disorder or a severe infection before initiating CRT.

2.5.3 DOSAGE BY AGE

The dosage is adjusted to age attained at:

- Start of irradiation in non-transplant patients.

2.5.4 TIMING OF RTX

- Upon conclusion of reinduction therapy in all but:
- Prior to allogeneic SCT

2.5.5 TECHNIQUE OF RTX

- CRT

CRT should be carried out under high-energy conditions (6 MV) with a linear accelerator or a telecobalt-60 device. The reproducibility of the daily set-ups (settings) must be ensured, usually through a mask technique. The target volume should encompass the entire neurocranium to include all the intracranial structures, both retrobulbar spaces and the entire base of the skull with its deep-seated middle groove, as well as the upper 2 segments of the cervical spine (C1 & C2). This assumes the use of individual shielding tools (custom-made blocks/filters) and control through verification films. Great attention should be paid to the homogeneity of dose distribution with both contralateral fields being irradiated always at the same session. The daily dose per fraction is 1.5 Gy, which is delivered 5 sessions per week (on weekdays) until the total target dose is reached. In 1- to 2-year-old children hyperfractionated irradiation (x2 q 0.8 Gy or x2 q 1.0 Gy at least 6 h apart) may be considered.

2.5.6 INDICATIONS FOR RTX (tab 13,pag 49)

In addition to chemotherapy, certain patient with ALL need radiation therapy to prevent or treat their disease. The specific indications for this therapeutic modality in pediatric non-B ALL within this trial will be discussed in the following 4 sections.

2.5.6.1 PROPHYLACTIC CRANIAL RADIOTHERAPY pCRT

pCRT will be given to patients with a CNS status 1/2 and:

- T-ALL+ WBC > 100.000
- HR non transplanted (except Bcp HR only x PPR)

- At age-adjusted dosage, with age attained at the start of irradiation being determinative: Age $\geq 1 = 12$ Gy
- Timing: Upon completion of Protocol II , first 1.5 wks post II

2.5.6.2 THERAPEUTIC CRANIAL RADIOTHERAPY tCRT

All CNS positive patients (CNS status 3) receive tCRT:

- At age-adjusted dosage, with age attained at the start of irradiation being determinative: Age $\geq 1 < 2$ y = 12 Gy
 $\geq 2 = 18$ Gy
- Timing: Upon completion of Protocol II , first 2.5 wks post II

Table 13: Dosage of pCRT & tCRT by Age at Time of CRT

| Age by time of RTX (yr): RG | pCRT (Gy) CNS Status 1/2 | tCRT (Gy) CNS Status 3 |
|-------------------------------|-----------------------------|---------------------------|
| < 1 : all risk groups | 0 | 0 |
| $\geq 1 < 2$: SR, IR | 0 | 12 |
| ≥ 2 : SR, IR | 0 | 18 |
| ≥ 1 :T+WBC>100000 | 12 | 12 |
| ≥ 2 :T+WBC>100000 | 12 | 18 |
| ≥ 1 HR with a exception* | 12* | 18 |
| ≥ 2 HR with a exception* | 12* | 18 |

2.5.6.3 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.

2.5.6.4 RTX SIDE EFFECTS

- CRT:
 - Radiation-induced headache is the most common side effect occurring already during CRT. Short-term management with dexamethasone 15 mg/m²/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.
-

2.6 Stem - Cell Transplantation SCT

2.6.1 INDICATIONS for allogeneic stem cell transplantation (alloSCT) as specified in section 1.13 and **Tab 7** (pag 37) & below:

Table 7: Indications for Allogeneic SCT in ALL IC-BFM 2009

| INDICATION | | MFD [†] SCT |
|------------------------------|--------------------------|----------------------|
| NR d33 | | + |
| HYPODIPOIDY < 44 CHROMOSOMES | | + |
| PPR | + T-ALL | + |
| | + pro B-ALL | + |
| | + WBC > 100,000/ μ L | + |
| | + t(9;22) or BCR/ABL | + |
| | + t(4;11) or MLL/AF4* | + |
| PGR | + t(9;22) or BCR/ABL | + |
| HR | + M3 d15 | + |

[†] MFD **matched family donor**

* Infants < 1 yr only

HR patients due only to M3 at day 15 are not eligible for SCT

2.6.2 THE PROCEDURE of alloSCT is not part of the AIEOP-BFM ALL 2009 study.

Conducting of alloSCT including diagnostics, donor selection, conditioning regimen, immunosuppression, and supportive management is not part of this protocol.

Treatment details are specified in the protocols of current SCT trials (e.g. ALL-SZT BFM 2003, ALL-SZ BFM International).

2.6.3 SCHEDULING A SCT

If a suitable donor were available and so far as the logistics allow, SCT is to be performed after the third HR block. The latest deadline for performing the procedure is after the sixth HR block.

3 Chapter SUPPORTIVE THERAPY

3.1 Foreword

Some medical and pediatric hematologists/oncologists may not fully agree with many details of the supportive therapy measures described in this chapter, as the clinical experience and the level of evidence will vary. Indeed, not infrequently, the validity and utility of many approaches are fraught with a variable degree of controversy. On the other hand, everybody recognizes that current antineoplastic therapy and adequate supportive care should go hand in hand in order to ensure a smooth and timely handling of the underlying malignancy as far as possible and to improve the immediate as well as the ultimate outcome of anticancer treatment. This is particularly relevant to pediatrics by virtue of the high cure rates of the majority of childhood cancers with the therapies available nowadays and owing to the long life expectancy of the survivors.

The recommendations described in this chapter should be merely conceived as general guidelines intended to help clinicians forestall and manage a number of specific emergency situations and complications/consequences associated with the underlying disease or its basic treatment. They can be neither exhaustive nor dogmatic. It is the responsibility of the physician caring for the child with cancer to undertake and implement the proper measures deemed by him/her necessary and effective to prevent or treat any disease as well as therapy-related problems.

3.2 Acute Tumor Lysis Syndrome ATLS

3.2.1 BASIC ASPECTS

- 1 The intense lysis of blasts leads to a massive release into the systemic circulation of intracellular constituents, of which the purine metabolites- hypoxanthine (**HX**), xanthine (**X**) & uric acid (**UA**) as well as potassium (**K**) & phosphate (**P**) are most relevant to the pathophysiology and clinical features of ATLS. The kidneys play a major role in eliminating these substances.
- 2 The lab/ancillary findings and clinical symptoms/signs of ATLS are variable, depending on the rate of cell breakdown, the amount of substances poured out, the status of organ function, particularly of the kidneys and heart, co-morbid conditions, and complications of the underlying disease as well as of ATLS itself.
- 3 Although well recognized, the true incidence of ATLS is not known. However, a number of factors will put the patient at enhanced risk for developing the syndrome:
 - o Type of malignancy: acute & chronic leukemias in general, malignant LPDs (first of all high-grade NHL including Burkitt's, B-ALL, T-ALL).
 - o Exquisite responsiveness to antineoplastic therapy.
 - o Tumor burden/extent of disease: hyperleukocytosis (WBC > 100,000/ μ L), lymphoma-leukemia syndrome, huge organomegaly, bulky mediastinal, abdominal or retroperitoneal masses.
 - o Proliferative rate: great growth fraction or high rate of cell turnover.
 - o LDH > 1,500 U/L (> 25 μ cat/L).
 - o Volume contraction/low urine output.
 - o Acidic/concentrated urine.
 - o Pre-existing renal dysfunction/obstructive uropathy.
 - o Infiltration of the kidneys by tumor.
 - o Post-treatment ARF.

- o Other factors may precipitate or aggravate ATLS: sepsis/DIC, exogenous excess K, P & purines, nephrotoxic/uricosuric drugs, agents with a hyperkalemic effect.
- 4 The solubility of the individual end products of purine catabolism depends on pH as shown in **Tab. 21**. Overall, **X** is the least soluble purine metabolite, while **UA** is best soluble at alkaline pH. Although the solubility of **HX** & **UA** are fairly comparable at pH 5 – 7, **HX** is poorly soluble at pH > 7.5.

Table 21: Solubility Limits of Purine Metabolites by pH

| pH | Uric Acid (mg/L) | Xanthine (mg/L) | Hypoxanthine (mg/L) |
|-----|------------------|-----------------|---------------------|
| 5.0 | 150 | 50 | 115 |
| 7.0 | 2,000 | 130 | 1,500 |

- 5 At higher concentrations, the purine metabolites crystallize out within the renal distal tubules, collecting ducts and renal parenchyma. This causes a decrease in the GFR and can lead to full-blown ARF.
- 6 Similarly, **P** binds to **Ca**, and may in the form of Ca phosphate also precipitate in the renal tubules as well as in soft tissues (renal & extrarenal), resulting in nephrocalcinosis, renal dysfunction/ARF, hypocalcemia, and tissue damage with associated organ dysfunction. On the other hand, in highly proliferating tumors the pre-treatment level of serum **P** might be normal or even low due to its massive uptake by the newly formed neoplastic cells.
- 7 However, contrast to **X** & **UA**, the solubility of Ca phosphate is poor in alkaline milieu. Therefore, alkalinization of the urine can also favor the precipitation of cell lysis products (**P** & **HX**).
- 8 The differential solubility of the individual purine metabolites and Ca phosphate at different pH advocates for targeting a "compromise" urine pH of 7 – 7.5 for the period at highest risk of ATLS (24 – 48 h, no longer than 72 – 96 h). Other measures should prove efficacious in the meantime. As far as possible, chemotherapy is to be started with the patient off alkalinization. However, it should not be unduly postponed.
- 9 Hyperkalemia is the most frequent, immediately life-threatening complication of ATLS that can evolve within 6 – 72 h. Close surveillance and prompt action are *conditio sine qua non* to forestall a disaster.
- 10 Increased protein catabolism is often present at diagnosis, which leads to elevated levels of blood urea. A compromised renal function will only further aggravate the azotemia with its attendant symptoms/signs. Mostly, azotemia goes hand in hand with hyperphosphatemia.
- 11 The patient should be first stabilized prior to instituting antileukemic treatment. If the UA, K, P and/or creatinine levels are already increased before the start of cytoreductive therapy, measures to put metabolic derangements under control should be undertaken first. This goal can be accomplished usually within 24 h, so that the cytoreductive pre-phase can be then safely launched.
- 12 **The most important measure is the initiation and maintenance of a high urine output** (100 - 250 ml/m²/h), keeping the urine specific gravity ≤ 1,010. If this is working well, then metabolic imbalances, which would require intervention, are infrequent.
- 13 If, in spite of sufficient liquids and diuretics, it is not possible to induce and maintain a satisfactory urine output, then timely preparation for hemodialysis must be started. This situation is probably due to extensive infiltration of the kidneys by neoplastic cells, obstruction of the urinary tract by tumor, advanced urate or Ca phosphate

nephropathy, or a combination thereof. The diagnosis can be easily made by imaging studies (**Cave: IV contrast media are contraindicated**).

3.2.2 DRUG MANAGEMENT OF HYPERURICEMIA

Hyperuricemia is defined as a plasma UA level of ≥ 7 mg% (≥ 420 $\mu\text{mol/L}$). The drugs that have been used over the last 2 – 3 decades to control hyperuricemia are allopurinol and the natural urate oxidase (Uricozyme[®], Sanofi-Synthelabo). More recently, a recombinant formulation of the latter- rasburicase (Fasturtec[™], Elitek[™], Sanofi-Synthelabo) has been introduced.

3.2.2.1 ALLOPURINOL

- 1 Allopurinol is oxidized by the liver xanthine oxidase to oxypurinol, which binds tightly to the enzyme inhibiting its activity more potently than the parent compound. This leads to accumulation in the plasma and massive excretion in the urine of HX & X. Higher levels of UA precursors will further inhibit purine biosynthesis via a negative feedback loop, thereby possibly exerting an antileukemic effect (Lascari AD 1991, Nelson SC et al. 1993, Maheboob Basade et al. 1995)⁽⁵⁴⁻⁵⁶⁾. The $t_{1/2}$ of allopurinol at 300 mg is ca 1.3 h (PO) to 1.2 h (IV), whereas that of oxypurinol is about 24 h independent of dose (100/300 mg) & route of administration (PO/IV).
- 2 The recommended dosage of allopurinol (PO/IV) is 200 – 400 mg/m²/d (300 mg/m²/d = 10 mg/kg/d on average) to be given in 3 divided doses, usually for 3 – 8 days. There is flattening of the dose-response curve beyond 600 mg/d. Dosing should be adjusted to the level of uricemia as well as to renal function- see **Tab. 22**

Table 22: Dosage of Allopurinol by Renal Function

| GFR = CL _{cr} (ml/min/1.73 m ²) | Dosage (% dosis pro die) |
|---|-----------------------------|
| > 20 | 100% pro die |
| ≤ 20 | 50 – 66% pro die |
| ≤ 10 | 10 – 25 – 33% pro die |
| < 3 | 10 – 25% q 36 – 48 h |

- 3 The IV route (Apurin[®], Aloprim[®]: 30 ml vial = 500 mg allopurinol equivalent; lyophilized, sterile, preservative-free) is indicated whenever the patient is unable to take oral medications. The IV formulation may be reconstituted with 5% G or 0.9% NaCl up to 6 mg/ml. The reconstituted solution is stable for 10 h, and should not be refrigerated. The drug is incompatible with NaHCO₃.
- 4 Allopurinol is generally well tolerated, the most common adverse event (1%) being a mild to moderate skin rash or urticaria (Feusner J et al. 2001)⁽⁵⁷⁾. Occasionally, headache, mild GI side effects (emesis, diarrhea) or reversible hepatotoxicity may be encountered. More severe hypersensitivity reactions are rather the exception. A rare, sometimes fatal, syndrome (fever, hepatitis, skin rash, eosinophilia) has been reported. Alopecia is scarce also, being an article of long-term use of the drug. A number of drug interactions are known (reviewed by Feusner J et al. 2001)⁽⁵⁷⁾.
- 5 The pattern of ARF associated with ATLS has changed with the advent of allopurinol. The prevalence of urate-nephropathy-induced ARF, occasionally seen prior to therapy, has decreased with the drug given before cytoreductive chemotherapy or radiation. On the other hand, hyperphosphatemia, uncommon pre-treatment, has become the usual cause of ARF following chemotherapy (Flombaum CD 2000, Stapleton FB 1988)⁽⁵⁸⁻⁵⁹⁾. In addition, the role of xanthine in inducing nephropathy may become more prominent, although the pathogenesis of renal damage/dysfunction associated with the

purine metabolites probably extends beyond their physicochemical properties (Andreoli SP et al. 1986)⁽⁶⁰⁾.

- 6 Allopurinol does not act on pre-existing UA, and it usually takes 3 – 4 days for the elevated UA levels to normalize, which is critical at this stage of the illness and its therapy. Treatment delays could be associated with greater morbidity including ARF requiring dialysis and might prove even fatal. In addition, there are patients who are at extraordinarily high risk for ATLS. With classic measures, as many as 20 – 25% of these patients would need some form of hemodialysis that, not infrequently, should be repeated q 12 – 24 h in order to control the life-threatening metabolic derangements and ARF consequent on ATLS. Additionally, these patients are more likely to die during or shortly after the first course of chemotherapy (Seidemann K et al. 1998)⁽⁶¹⁾. For this patient population with or at highest risk for foudroyant and potentially lethal ATLS more efficient and rapidly acting strategy must be urgently considered.

3.2.2.2 URATE OXIDASE

- 1 Urate oxidase is a natural uricolytic enzyme that catalyzes the oxidation of UA to 5-hydroxyisourate and finally to allantoin, which is 5 – 10-fold more soluble than UA, rendering it readily excretable by the kidneys. Urate oxidase is one of the public enzymes occurring in many species including most mammals. However, by virtue of a nonsense mutation in the encoding gene, the enzyme was deleted from the repertoire of higher primates and humans (Yeldandi AV et al. 1991)⁽⁶²⁾.
- 2 Originally, the tetrameric enzyme containing 4 catalytic units q 34 kDa each was purified from industrial cultures of *Aspergillus flavus*, and has been available for clinical use under the name *Uricozyme*[®] (Sanofi-Synthelabo) in France and Italy since 1975 and 1984, respectively. The drug proved effective at doses of 50 – 100 U/kg/d IV/IM (Masera G et al. 1982, Jankovic M et al. 1985, Pui C-H et al. 1997)⁽⁶³⁻⁶⁵⁾. However, the yield was limited, and possibly because of impurities allergic reactions, sometimes severe (bronchospasm, anaphylaxis, hypoxemia), occurred in 4 – 5% of the cases.
- 3 Recently, the enzyme was produced by recombinant technology via expressing the urate oxidase cDNA from *Aspergillus flavus* in the yeast *Saccharomyces cerevisiae*, yielding large amounts of a highly pure and precisely quantifiable product- rasburicase (Fasturtec[™], Elitek[™], Sanofi-Synthelabo). 100 U/kg *Uricozyme*[®] is equivalent to 0.15 mg/kg of the recombinant form. The drug is effective and safe in the prophylaxis and therapy of ATLS both in children as well as adults. Given at 0.15 or 0.20 mg/kg in 50 ml normal saline PI over 30 min q 24 h (exceptionally q 12 h during the first 24 – 72 h), almost all patients (99%) responded by about 88% reduction in UA levels as early as 4 h after the first dose, with sustained response of normal or even undetectable levels in virtually all patients over 5 – 7 days of treatment (Goldman SC et al. 2001, Pui C-H et al. 2001 & 2001)⁽⁶⁶⁻⁶⁸⁾. By contrast, 5 – 12% of the patients could not achieve a decline in their uricemia by $\geq 60 \mu\text{mol/L}$ following IV allopurinol (Smalley RV et al. 2000)⁽⁶⁹⁾. Moreover, it has been shown in a small comparative trial that exposure to UA as expressed by AUC_{0-96} was ca 2.6-fold higher with oral allopurinol 10 mg/kg/d TID vs. rasburicase 0.20 mg/kg infused over 30 min q 24 h, and that the reduction in uricemia 4 h after the first dose was only 12% in the former vs. 86% in the latter arm (Goldman SC et al. 2001)⁽⁶⁶⁾.
- 4 The mean plasma $t_{1/2}$ of rasburicase at the steady state is dose-dependent, ranging between 16 h for 0.15 mg/kg and 21.1 h for 0.20 mg/kg. The drug does not accumulate in the body, and once-daily dosing is adequate. In some but not all studies, antibodies to the foreign protein were found in up to 7 – 14% of the patients (Lascombes F et al.

- 1998)⁽⁷⁰⁾ & (Pui C-H et al. 2001)⁽⁶⁷⁾. The significance of these antibodies in terms of impact on efficacy or hypersensitivity is not clear so far. However, of 15 subsequent courses given to 12 patients with antibodies all but one were effective and two were associated with a possible hypersensitivity reaction (Pui C-H et al. 2001 & 2001)⁽⁶⁷⁻⁶⁸⁾.
- 5 Advantages of rasburicase:
 - o As rasburicase is a potent and fast-acting uricolytic agent, antineoplastic therapy can be safely instituted within 4 – 24 h in the overwhelming majority of cases.
 - o HX & X will not accumulate, eliminating the risk of nephropathy due to these purine metabolites. Indeed, renal function improved in those patients receiving the drug (Goldman SC et al. 2001, Pui C-H et al. 2001)⁽⁶⁶⁻⁶⁸⁾.
 - o No alkalinization is needed, which in addition to avoiding the potential problems inherent in artificial metabolic alkalosis would facilitate the solubility of Ca phosphate and its excretion by the kidneys, thus minimizing the risk of nephropathy or metastatic calcifications. However, 10/245 highly selected patients (5 children or adolescents, 5 adults), 8 of whom presented with renal insufficiency, required dialysis after the start of rasburicase because of hyperphosphatemia and/or azotemia. All of them had very low UA levels after rasburicase as well as at the time of hemodialysis, and all recovered (Pui C-H et al. 2001)⁽⁶⁸⁾.
 - o Although the experience is still limited, rasburicase has been associated with a low incidence of allergic reactions (1.5 – 2%) that are mostly mild to moderate and manageable with epinephrine, diphenhydramine or promethazine (bolus corticoids should be avoided as far as possible, as they may precipitate ATLS).
 - o No drug-drug interactions including those mediated via cytochrome P450 are anticipated within the dosage range recommended (Sanofi-Synthelabo: data on file).
 - 6 Rasburicase should be considered preventatively in patients at particularly high risk of developing ATLS and/or with pre-/co-existent renal dysfunction- see section 3.2.1 (p 113). In already established ATLS with UA level ≥ 7 mg% (≥ 420 $\mu\text{mol/L}$) or with decreased renal function, the drug is indicated therapeutically.
 - 7 FasturtecTM/ElitekTM is supplied in vials containing 1.5 mg lyophilized rasburicase (with excipients) and ampoules with 1 ml solvent, both preservative-free, to be stored at +2° to +8° C. Following gentle reconstitution, the drug should be further diluted with 50 ml saline to be given immediately at 0.15 or 0.20 mg/kg by infusion over 30 min q 24 h for up to 5 – 7 days. A separate line without filter should be used or, if this were not available, the line must be flushed out with 15 ml 0.9% NaCl before and after delivering rasburicase. The drug should not be admixed with dextrose. If need be, the reconstituted or diluted solution can be stored for up to 24 h at +2° to +8° C (don't freeze).
 - 8 Adverse events observed in association with rasburicase therapy in highly selected patients include fever, nausea, emesis, diarrhea, headache, skin rash of variable extent, itching, hives, facial or lip swelling, edema, fever, myalgia, and wheezing or respiratory discomfort/distress. Adverse events of allergic nature were reported in 1.5 – 2% of cases, occurring within the first minutes of the first dose or during the first day of therapy. However, only 2/347 patients discontinued treatment due to a systemic reaction (Sanofi-Synthelabo: data on file). Other side effects likely to be related to rasburicase occurred in < 10% of patients. A by-product of the conversion of UA into allantoin by urate oxidase is H₂O₂, which is scavenged by antioxidants. However, in G-6-PD-deficient patients H₂O₂ can precipitate a hemolytic crisis or methemoglobinemia. Rasburicase is therefore contraindicated in G-6-PD deficiency.

Safety in other inherited anemias as well as in patients with a history of allergy remains to be established.

3.2.3 PREVENTION OF ATLS

- 1 Eliminate/minimize all factors that might contribute to ATLS or worsen renal function - see section 3.2.1.
- 2 Allopurinol: 10 mg/kg/d (300 ± 100 mg/m²/d) PO/IV in 2 - 3 SD for 3 - 8 days
- 3 IV hyperhydration: 3,000 – 5,000 ml/m²/d (5% G + 0.45% NaCl aa)
- 4 Initially, no extra KCl may be added. Slight hypokalemia does not matter
- 5 IV alkalization of urine for 24 – 48 h (exceptionally longer):
NaHCO₃ 40 – 80 mmol/L (100 – 200 mmol/m²/d)
- 6 Monitoring & regulation of therapy:
 - o Abdominal US/CT/MRI (w/o IV contrast media):
 - ? Infiltration of kidneys by neoplastic cells
 - ? Urinary tract obstruction by tumor
 - ? Changes suggestive for urate or Ca phosphate nephropathy
 - ? Uropoietic anomalies
 - o Balance: output = input - perspiration
 - o Check body weight twice daily
 - o Keep urine output at 100 – 250 ml/m²/h
 - o For inadequate output: furosemide IV 1 – 10 mg/kg/d (**Cave:** fluid overload)
 - o Control of NaHCO₃ supply: urine pH 7.0 – 7.5 is optimal
 - o Maintain urine specific gravity $\leq 1,010$
 - o Lab tests: CBC, mineralogram, Ca, P, UA & creatinine q 12 - 24 h, in critical cases- initially more often
- 7 If risk of ATLS is enhanced: Fasturtec™/Elitek™ (in lieu of allopurinol & w/o alkalization) 0.15 – 0.20 mg/kg q 24 h PI over 30 min for 5 – 7 days

3.2.4 THERAPY OF ATLS

The principles of prevention of ATLS discussed in section 3.2.3 form the basis for the treatment of already established ATLS. However, therapy should be intensified and/or extended in the face of the often critical to life-threatening manifestations and complications of the syndrome.

3.2.4.1 HYPERURICEMIA

- General measures (Tab. 23)

Table 23: General Measures for Management of Hyperuricemia

| | |
|----------------------|---|
| 1. Normal excretion | • Hyperhydration up to 5,000 ml/m ² /24h |
| 2. Euphosphatemia | • Urine pH 7.0 – 7.5 |
| 3. Hyperphosphatemia | • Urine pH 7.0 |

- Specific therapy with rasburicase

Recombinant urate oxidase is indicated in:

- Hyperuricemia ≥ 7 mg% (≥ 420 μ mol/L)
- Initially decreased renal function
- Large tumor burden/high proliferative rate or WBC $\geq 100,000/\mu$ L

Fasturtec™/Elitek™ (in lieu of allopurinol & w/o alkalization) 0.15 – 0.20 mg/kg q 24 h PI over 30 min for 5 – 7 days

3.2.4.2 HYPERKALEMIA

- This is an immediately life-threatening condition that should prompt action without delay. However, it is important to distinguish *statim* between false and true hyperkalemia, as the decision to be made is serious- see **Tab. 24**. Pseudohyperkalemia results from hemolysis during or after blood sampling, particularly in patients with elevated WBC and platelet counts.

Table 24: Spurious Hyperkalemia vs. Authentic Hyperkalemia

| Hyperkalemia | Serum K | Plasma K | ECG Changes |
|--------------|-----------|-----------|------------------|
| • False ↑ K | increased | normal | none |
| • True ↑ K | increased | increased | wide QRS, high T |

- Exclude exogenous K from enteral & parenteral nutrition.
- $K \geq 6$ mmol/L: prepare for dialysis/transfer patient to dialysis ward.
- $K \geq 7$ mmol/L: immediate hemodialysis.
- Urgent measures to be carried out instantly (solo or combined):
 - Provided that renal function is adequate, forced diuresis:
 - 0.9% NaCl: 10 – 20 ml/kg by IV infusion over 1 h
 - + Furosemide: 1 – 3 mg/kg IV push or preferably PI over 15 – 30 min.
 - Salbutamol (2 options are available):
 - Solution for inhalation: 0.1 mg/kg (q 4 – 6 h, if needed)
 - IV formulation: 1 – 2 µg/kg slowly IV or 0.1 µg/kg/min by IV drip
 - Hypertonic G (20 – 50%):
 - 1 g/kg G + 0.3 U/kg regular insulin to be infused over 0.5 h.

Measures sub 2 & 3 lead to redistribution of K from extra- to intracellular compartment. However, K will efflux back within 2 – 4 h. These are merely temporary measures useful to bridge the gap until dialysis is started.

- Catex (Calcium Resonium[®], Resonium A[®], Kayexalate[®]):
 - 0.5 – 1 – (2) g/kg/d PO thoroughly dissolved in water (to avoid bowel obstruction). Fruit juices or potassium-containing beverages must not be used, as they would decrease the catex exchanging efficiency.
 - The same dose may be used PR also, however the drug should be dissolved in methylcellulose or 20% sorbitol to minimize the risk of rectal ulceration.
 - Rectal enema is contraindicated in neutropenic patients, as it would set the stage for cellulitis/septicemia (Gram-negative or mixed flora).

Additionally:

In case of ECG changes (wide QRS, high T):

- Ca gluconate 10% slowly (over 5 – 10 min) IV: 0.5 – 1 – (2) ml/kg.
Cave: bradycardia! (ECG monitoring is necessary).

In case of metabolic acidosis only:

- NaHCO₃: 1 – 2 mmol/kg IV push.

3.2.4.3 HYPERPHOSPHATEMIA

- Exclude exogenous P from the diet. Aluminum hydroxide (or Ca carbonate, if hypocalcemia is also present) $\times 4$ q 0.5 – 0.15 g/kg PO can be added to bind out alimentary P. Parenteral nutrition may be given, if need be.
- Top hyperhydration (5,000 ml/m²/24h), provided that excretion is normal.
- Urine pH should not exceed 7.
- Hypertonic G (20 – 50%):
1 g/kg G + 0.3 U/kg regular insulin to be infused over 0.5 h.
- Hemodialysis: if $P > 5$ mmol/L $\pm P \times Ca > 6.4$.
- Avoid Ca as far as possible to forestall metastatic calcifications.
- However, if hypocalcemia is symptomatic \pm changes on ECG:
10% Ca gluconate: 0.5 – 1 – (2) ml/kg slowly (over 5 – 10 min) IV.
Cave: bradycardia! (ECG monitoring is necessary).

3.2.4.4 HYPOCALCEMIA & HYPOMAGNESEMIA

- Only symptomatic hypocalcemia \pm changes on ECG may be corrected, as in the face of hyperphosphatemia ($\pm P \times Ca > 6.4$) Ca phosphate would precipitate in tissues. Hypocalcemia should resolve with control of hyperphosphatemia, which is the cornerstone of management.
- However, if need be:
10% Ca gluconate: 0.5 – 1 – (2) ml/kg over 5 – 10 min IV.
Cave: bradycardia! (ECG monitoring is necessary).
- Check serum Mg also.
- If hypomagnesemia is present, then MgSO₄: 0.2 – 0.8 mmol/kg slowly IV.
This may alleviate neuromuscular symptoms/signs.

3.2.4.5 OLIGURIA / ANURIA

- Definition
From a therapeutic/prognostic point of view the usual limit of urinary output of < 5 ml/m²/h is not useful for the definition of oliguria/anuria in cancer patients at presentation and during the first 7 – 10 days of antineoplastic treatment, particularly if ATLS is already established or anticipated. Adhering to this definition could put the patient at the just edge of disaster and might even lead to death first of all due to hyperkalemia.
In this context oliguria should be defined as:
Urine output of < 50 ml/m²/h in spite of fluid input of 130 – 200 ml/m²/h & IV furosemide as high as 10 mg/kg/d. Excretion must be assessed in relation to intake while ruling out urinary retention.
- Differential diagnosis & management guidelines
The preventative and therapeutic measures to be undertaken should be based on the cause and mechanism underlying the oliguria/anuria. This is important in order to avoid harm to the patient and to improve the outcome of these measures. Three major levels of origin must be considered, although it is recognized that they are not mutually exclusive.

1 Prerenal:

Low urinary outflow is secondary to either true volume constriction associated with extrarenal salt losses or edematous states accompanied by an increase in total body water and salt content. In both situations $U\text{-Na} < 10$ mmol/L.

Management should be tailored to the patient's individual needs following comprehensive evaluation of the condition that led to prerenal oliguria/anuria. In the

former scenario, volume repletion with saline is necessary to suppress non-osmotic ADH release, thus increasing free-water excretion by the kidneys and correcting the hyponatremia. By contrast, in the latter scenario, both water and salt must be restricted, as hypotonic fluids will aggravate the hyponatremia, while saline will worsen the edema/ascites. Refer also to section 3.5 .

2 Renal:

ARF may be caused by extensive involvement of the kidneys by tumor and/or by renal damage secondary to the metabolic derangements of ATLS ± nephrotoxic drugs.

Thoughtful treatment of the underlying malignancy with a judicious cytoreductive pre-phase should be an integral part of the overall management of ARF due to extensive infiltration of the kidneys by neoplastic cells. In addition to reduced dosage the extent of protein binding of the cytotoxic agents employed must be taken into account when scheduling their delivery in relation to hemodialysis, as dialyzability is inversely proportional to the bound fraction.

By contrast, if oliguria/anuria is caused solely by urate or Ca phosphate nephropathy, measures should be undertaken to stabilize the patient first, aiming at rectifying the metabolic derangements and improving renal function. Usually, this can be achieved within 24 h. Proceeding with great caution, the cytoreductive pre-phase can be then commenced. However, it should not be delayed unduly.

Regardless of the cause of ARF, the urgent measures discussed within the guidelines for treatment of ATLS in sections 3.2.4.2 through 3.2.4.4 should be undertaken *pro re nata* while preparing for hemodialysis. In ARF rasburicase is indicated, while hyperhydration/alkalinization are contraindicated. Dialysis is discussed in section 3.2.4.6 .

3 Postrenal:

Bulky abdominal/retroperitoneal tumors may cause obstructive uropathy that, if complete or high-grade, can lead to renal failure, too.

In this case also, deliberate cytoreductive therapy is the most reliable measure for relieving the obstruction. Radiotherapy at 4 – 8 Gy is effective as well, but this is rather an exceptional option, although not infrequently used in adults.

The principles of dosage reduction and timing of drug delivery in relation to dialysis, the ladder of priorities, the urgent measures, indications and contraindications discussed at the "renal level" and in sections 3.2.4.2 through 3.2.4.4 are applicable at the "postrenal level" of ARF, too. Moreover, insertion of a ureteral double pig-tail catheter for a few days should be considered. Dialysis is discussed in section 3.2.4.6 .

3.2.4.6 INDICATIONS FOR DIALYSIS

The specific dialysis protocol is up to the nephrologist, and will depend on the size of the patient, the facilities for and experience in the management of pediatric patients as well as on the phosphate load that reflects the tumor burden and proliferative rate. Peritoneal dialysis is substantially less efficient than hemodialysis. In addition, it is often technically difficult to perform, especially in the case of huge hepatosplenomegaly or bulky tumor masses within the abdomen. Conventional hemodialysis is the most often employed procedure that can be repeated q 12 – 24 h, if need be, e.g. in case of hyperphosphatemia or rebounding hyperkalemia. Continuous, high-dialysate-flow-rate A – V hemodialysis (Pichette V et al. 1994)⁽⁷¹⁾ and continuous V – V hemofiltration (Sakarcan A et al. 1994)⁽⁷²⁾ are particularly efficacious in eliminating large quantities of phosphate.

Hemodialysis is indicated in the following situations:

- Potassium > 7 mmol/L or:
> 6 mmol/L and rising in spite of:
hyperhydration & forced diuresis
- Phosphate > 5 mmol/L or: Ca x P > 6.4
- Creatinine > 10-fold the ULN for age
- Uric acid > 600 μ mol/L
- Urine output < 50 ml/m²/h in spite of:
fluid input 130 – 200 ml/m²/h & furosemide 10 mg/kg/d IV
- High-grade or complete urinary tract obstruction on both sides

3.2.4.7 ATLS-RELATED DIC

The management of disseminated intravascular coagulation associated with ATLS is essentially the same as in other situations- see sub section 3.13 (p 143) on disorders of hemostasis. However, the setting of ATLS, particularly in case of concomitant ARF, is much more dramatic and demanding. The patient will be best managed at PICU with the joined endeavor of members of a dedicated team (pediatric hematologist/oncologist, intensivist, nephrologist).

3.3 Complications of Mediastinal Tumor

Overall, mediastinal tumor and central intrathoracic lymphadenopathy, often with a pleural and/or pericardial effusion, are relatively frequent in pediatric pathology, and may be caused by a variety of benign as well as malignant disorders. The superior vena cava (SVC) and the major central airways are quite readily compressible in children, particularly by anterior/superior mediastinal masses. Although the clinical relevance will vary, the degree and dynamics of occlusion of these structures determine its symptomatology, which can be exacerbated or augmented by supine and flexed position (as for LP), physical exertion, emotional stress, conscious sedation, induction to anesthesia, and general anesthesia. Problems may arise not only at intubation but also during extubation.

So, in addition to considering the differential diagnosis, the patient should be evaluated for anesthetic risk. This is done by careful history, thorough physical examination, CBC + Diff., global coagulation tests, routine blood chemistry & gases, specific ancillary tests as deemed necessary, CXR, CT/MRI, ECG & Echo-CG. Spirometry may add useful information, however it is not always feasible or available. Should the anesthetic hazard be considered high, the diagnosis must be made by the least invasive techniques under local anesthesia in semi-Fowler's position, whenever possible from extramediastinal site(s). However, if general anesthesia is inevitable, then mask narcosis with support of spontaneous ventilation performed also semi-Fowler is the method of choice. Endotracheal anesthesia must be rather the exception. LP must be performed without or with local anesthesia (EMLA), the patient lying on his/her side as least flexed as possible in the trunk and lower extremities while inhaling O₂. In severe, life-threatening situations, the diagnostic procedures should be minimized in order to be completed after the patient had been stabilized.

Four complications secondary to mediastinal tumor or central intrathoracic lymphadenopathy are known, although it is recognized that they may be due to a range of other pathologic conditions. These are: the superior-vena-cava syndrome (SVCS), tracheo/bronchial compression (TBC), the superior mediastinal syndrome (SMS), which is a constellation of SVCS & TBC, and cardiac tamponade. As the majority of pediatric patients with SVCS have also central airway compression, exhibiting both

upper and lower respiratory symptoms/signs, the terms SVCS and SMS have become *de facto* synonymous in pediatrics (Rheingold SR et al. 2002)⁽⁷³⁾.

3.3.1 SUPERIOR-VENA-CAVA SYNDROME SVCS

Although overall rare, the true incidence of SVCS is not known, and will vary with the underlying malignancy as well as the stage of disease. In a retrospective analysis of 3661 pediatric patients admitted for malignancy at SJCRH over a 16-year period (1973 – 1988), a mediastinal mass was found in 576 patients (15.7%), of whom SVCS developed in merely 24 (4.2%). This was the case at initial presentation in 16 patients (all with a mediastinal mass), and occurred as a late complication in 8 (5 with a demonstrable mediastinal mass) (Ingram L et al. 1990)⁽⁷⁴⁾.

The syndrome *per se* is not an emergency unless there be a concomitant TBC. Although blood return is embarrassed and will be more so upon analgesedation-induced peripheral vasodilatation, the cardiac output will be still satisfactory enough to allow for a patient with SVCS alone and with a Hb > 100 g/L to undergo procedures under general anesthesia, particularly if he/she had already developed collateral veins. Management of SVCS is that of the underlying disorder or disease- see **Tab. 25**

Table 25: Management of SVCS

| Indication | Therapeutic Measure(s) |
|---|---|
| <ul style="list-style-type: none"> • Malignancy | <ul style="list-style-type: none"> • Antineoplastic therapy[†] |
| <ul style="list-style-type: none"> • Benign tumor | <ul style="list-style-type: none"> • (Sub)total resection |
| <ul style="list-style-type: none"> • Infection | <ul style="list-style-type: none"> • Antimicrobial therapy |
| <ul style="list-style-type: none"> • SVC thrombosis ± CVC | <ul style="list-style-type: none"> • Thrombolytic/antithrombotic therapy[‡] • ? Removal of CVC • ? Oral anticoagulant therapy |
| <ul style="list-style-type: none"> • VA shunt ± SVC thrombosis | <ul style="list-style-type: none"> • Removal of VA shunt • Alternative shunting for hydrocephalus (VP) • ? Thrombolytic/antithrombotic therapy[‡] |
| <ul style="list-style-type: none"> • Miscellaneous <ul style="list-style-type: none"> • LCH • Sarcoidosis • Castleman disease • Rosai-Dorfman disease • Inflammatory pseudotumor | <ul style="list-style-type: none"> • Prednisone 40 –60 mg/m²/d tid • ? Other agents |

Legend

- SVC Superior vena cava
 SVCS Superior-vena-cava syndrome
 CVC Central venous catheter
 VA Ventriculo-atrial
 VP Ventriculo-peritoneal
 LCH Langerhans'-cell histiocytosis
 † Potential risk for developing ATLS
 See sections 3.2.3 & 3.2.4 (p 118)
 ‡ See sub section 3.13.3 (p 146)

3.3.2 TRACHEOBRONCHIAL COMPRESSION TBC

Tracheo/bronchial compression of variable degree occurs in about half of the cases with a mediastinal tumor, being severe in ca 10%. It is just this compression (with or w/o SVCS) that should be handled as a true medical emergency (Ribeiro RC et al. 1999)⁽⁷⁵⁾.

Again, the differential diagnosis should be soundly considered, and the anesthetic risk assessed- see section 3.3. However, general anesthesia is almost always contraindicated, and the diagnosis should be made by the least invasive procedures, e.g. CBC + Diff., BMP with adequate local anesthesia while the patient is inhaling O₂ in semi-Fowler's position. The diagnostics can be completed only after the patient has been stabilized, which is usually the case within 24 – 48 h. Guidelines for the management of TBC/SMS are summarized in **Tab. 26**

Table 26: Management of TBC & SMS

| | |
|---|---|
| 1. Start cytoreductive therapy immediately | <ul style="list-style-type: none"> • Prednisone 0.5 mg/kg/d TID |
| 2. Unsatisfactory improvement of respiratory distress after ca 48 h | <ul style="list-style-type: none"> • + CPM 100 mg/m²/d |
| 3. Prevention/therapy of ATLS | <ul style="list-style-type: none"> • See sections 3.2.3 & 3.2.4 (p 118) |
| 4. Massive pleural effusion | <ul style="list-style-type: none"> • Drainage with 16 G Teflon needle; no continuous drainage |
| 5. Critical pericardial effusion | <ul style="list-style-type: none"> • Drainage under ECG/Echo-CG guidance & fluoroscopic control • Drainage fluid for diagnostics • FFP 20 – 30 ml/kg |
| 6. SVCS | <ul style="list-style-type: none"> • See section 3.3.1 (p 123) |

Comments

- 1 Manage at PICU.
- 2 Avoid IV bolus corticosteroids (risk of life-threatening to lethal ATLS).
- 3 CPM will confound the initial evaluation of the prednisone pre-phase:
 - Discuss immediately with the national study coordinator.
 - Decide always in the patient's best interest.
- 4 If need be, give blood products prior to drainage to ensure:
 - aPTT ≤ x1.60 ULN for age
 - PT ≥ 75%
 - Fbg ≥ x0.75 LLN for age
 - AT III ≥ x0.75 LLN for age
 - Platelets > 50,000/μL
 - Hb > 100 g/L if WBC ≤ 100,000/μL
 - Hb > 80 g/L if WBC > 100,000/μL
- 5 Assess the effusion fluid for:
 - Protein content & specific gravity
 - Triglycerides
 - LDH
 - Cell count & cytology

- Immunophenotype & biologic markers
 - Gram stain & cultures
- 6 Should ATLS be already established and/or if renal failure is impending as well as in case of pre- or coexistent renal dysfunction:
- Treat ATLS & renal failure- refer to sections 3.2.4.1 through 3.2.4.7 (pp 118 – 122). However, fluid overload should be avoided. Therefore, the timely initiation of hemodialysis becomes an urgent task.
 - As the lymphoblasts are exquisitely radiosensitive, emergency low-dose mediastinal irradiation can be considered in lieu of CPM in the face of full-blown ATLS & ARF or in patients at particularly high risk for those complications- see section 3.2.1 (p 113). Consult the national study coordinator immediately.

There is appealing experience with this modality applied empirically pre-biopsy in cases of solid tumors involving solely the mediastinum that were associated with TBC/SMS (Loeffler JS et al. 1986)⁽⁷⁶⁾. Although RTX rendered histology *ex post* uninterpretable, it would have not changed the subsequent management for the presumed diagnosis.

Two field arrangements have been employed (Rheingold SR et al 2002)⁽⁷³⁾:

- 1 One small field centered on the trachea excluding the lateral edges of the tumor.
- 2 Two contralateral small fields with a target volume including the trachea, SVC and proximal right auricle.

Two doses of 1 + 1 or 1 + 2 Gy delivered 10 – 12 h apart is the all needed to kill the blasts that merely cell ghosts and debris could be recognized. Many patients will improve within 12 h after a single dose.

Exclusion of the tumor margins from the radiation field could preserve some viable tissue to make the histologic diagnosis *ex post* in those solid tumors confined completely to the mediastinum, which is not relevant to ALL, where the diagnosis is made from the PB & BM. The major rationale is however to minimize the potential risk of post-radiation respiratory deterioration due to swelling and obstruction of the small-caliber bronchial/bronchiolar airways that cannot accommodate enough edema, a problem almost exclusively limited to children and adolescents. This is usually not a concern in ALL either, as the post-radiation edema will be efficiently eliminated by prednisone. Yet another advantage of local RTX to the mediastinum vs. CPM is that it will not compound evaluation of the prednisone response (ABC on d 8).

3.3.3 CARDIAC TAMPONADE

Although cardiac infiltration or hemorrhage was found at autopsy in as many as 69% of 420 cases of acute leukemia, only 4% had exhibited cardiac symptoms *ante mortem* (Roberts WC et al. 1968)⁽⁷⁷⁾. Whether this is indeed the case, or whether these symptoms are rather commonly overlooked or simply misinterpreted and attributed to the "general status" and complicating conditions of the often critically sick cancer patient is not clear. Similarly, cardiac tamponade as defined by failure, owing to extrinsic or intrinsic factors, of the LV to expand adequately during diastole, leading to its impaired diastolic filling and inability to maintain cardiac output, is too rare compared to pericarditis associated with mediastinal tumors. So far, about 30 pediatric cases of cardiac tamponade occurring at presentation of acute leukemia have been

reported in the English-language medical literature, these being most commonly T-ALL, less frequently M4/M5-AML (reviewed by da Costa CML et al. 1999 & Arya LS et al. 2002)⁽⁷⁸⁻⁷⁹⁾.

The etiology and pathophysiology of pericardial and pleural effusions are diverse. The subjective symptoms, objective signs, imaging findings, ECG & Echo-CG changes are also multiform and protean, evolving over time. Hence, the differential diagnosis should be considered, and close monitoring and serial evaluation of the patient including his/her cardiopulmonary & hemodynamic status are necessary (Keefe DL 2000)⁽⁸⁰⁾. However, catheterization of the right heart, although useful for the accurate assessment of the degree of hemodynamic compromise, is too invasive and hazardous. It is therefore CONTRAINDICATED in the setting under study.

The management guidelines to be followed in this trial are:

- 1 Management at PICU.
- 2 Prompt cytoreductive therapy with prednisone 0.5 mg/kg/d tid.
- 3 Supportive care including hydration, O₂, positioning, digoxin, and analgesics. Fluid overload must be however avoided.
- 4 As they may worsen venous return, diuretics are usually contraindicated unless there be fluid overload or renal insufficiency despite fluid repletion.
- 5 Only critical pericardial effusion, where cardiac tamponade is deemed imminent or already established, may be managed by drainage, as the procedure is not void of hazard.
- 6 Adequate hemostasis as outlined in section 3.3.2 should be ensured prior and during drainage.
- 7 Drainage can be performed under local anesthesia (1 – 2% lidocaine) with the patient in the astronaut position (at 45° - 60° backward tilt) through a subxiphoid approach. Usually, an 18 G, thin-walled, conductive, short-beveled (30°), long (up to 20 cm) needle is used. It is inserted through a small incision in the angle between processus xiphoideus and the left rib margin (0.5 cm below and left to xiphoid process), advanced posteriorly until its tip is just behind the bony rib cage. Then, with the hub flattened toward the abdomen, the needle is advanced cephalad at approximately 15°, targeting the patient's head or either shoulder. However, the needle may need be redirected, e.g. in case of injury current. Once the needle's tip is properly positioned in the pericardial cavity, a floppy-tip guide wire is passed through the needle and wrapped around the heart. The needle is then removed, and a 6 F or 7 F soft, tapered, standard or pig-tail catheter with central and side holes is advanced over the wire. Finally, the wire is removed.
- 8 In general, 2 – 3 days of closed-system drainage will be needed.
- 9 The effusion fluid should be assessed for:
 - Protein content & specific gravity
 - Triglycerides
 - LDH
 - Cell count & cytology
 - Immunophenotype & biologic markers
 - Gram stain & cultures

3.4 Hyperleukocytosis

Leukocytosis is a well-recognized unfavorable prognostic factor in pediatric as well as adult ALL, contributing to a worse outcome (inferior pEFS, higher relapse rate). With varying cut-off values (mostly 20,000/ μ L, 25,000/ μ L or 50,000/ μ L), it is therefore incorporated within the risk stratification systems of all clinical trials on ALL. Overall,

the incidence of hyperleukocytosis, arbitrarily defined as a total WBC or ABC of $\geq 100,000/\mu\text{L}$, ranges between 6% and 18% of the cases, with 2 age peaks in pediatric patients being strikingly prevalent, i.e. infants < 1 year of age and teenagers. Similarly, it is more frequent in young adults with the disease than in the elderly.

Usually, ATLS, metabolic derangements, and ARF are the most common problems at presentation and/or upon commencing cytoreductive therapy in ALL with hyperleukocytosis. Bacterial or fungal sepsis is not uncommon during the induction therapy of these patients. Although coagulopathy may be encountered at presentation, first of all in T-ALL, it is in general mild to moderate and resolves rapidly with chemotherapy. On the other hand, compared to AML the leukostasis syndrome, particularly as far as the lungs are concerned, is too rare in ALL with hyperleukocytosis (Porcu P et al. 2000, Eguiguren JM et al. 1992, Maurer HS et al. 1988, Bunin NJ et al. 1985, Wald BR et al. 1982)⁽⁸¹⁻⁸⁵⁾.

Guidelines for the management of hyperleukocytosis in ALL are presented in **Tab. 27**

Table 27: Management of Hyperleukocytosis in ALL

| Problem | Measures |
|---|--|
| ❖ ATLS | <ul style="list-style-type: none"> • Deliberate cytoreduction: Prednisone (0.1) – 0.2 – (0.5) mg/kg/d • Prevention & therapy of ATLS: See sections 3.2.3 & 3.2.4 (p 118) • Steering of therapy according to: <ul style="list-style-type: none"> • Therapeutic response • Parameters of ATLS • Parameters of renal function |
| ❖ Leukostasis | <ul style="list-style-type: none"> • IV hydration: 5% G/0.45% NaCl aa 3,000 – 5,000 ml/m²/d • IV alkalization: NaHCO₃ 100 – 200 mmol/m²/d • Allopurinol: 300 ± 100 mg/m²/d PO/IV bid or tid • Exact fluid balancing • U-pH = 7 – 7.5; U-specific gravity $\leq 1,010$ • No transfusion of EC unless there be severe anemia (Hb < 80 g/L) • Exchange transfusion or leukapheresis: See sections 3.4.1 – 3.4.3 (pp 127 – 128) |
| ❖ Hemorrhage <ul style="list-style-type: none"> • Platelets < 20,000/μL • Coagulopathy | <ul style="list-style-type: none"> • Transfusion of PC • Transfusion of FFP |

3.4.1 INDICATIONS FOR EXCHANGE TRANSFUSION (ET) & LEUKAPHERESIS

The indication for exchange transfusion or leukapheresis is not and should not be rigid or binding. The decision should be rather made on an individual basis, whereby the WBC count is by far a less important criterion than the clinical situation. Namely, by pulmonary involvement (hypoxia, respiratory distress, lung infiltrates, etc) and/or CNS symptoms and signs suggestive of cerebral leukostasis, the indication is liberal. In those cases, the procedure should be performed as early as possible, as the morbidity and mortality is high. By contrast, asymptomatic patients and those responding quickly to prednisone can be successfully managed conservatively even in case of too high

WBC counts. As a rule, controlled cytoreduction with prednisone is adequate for a WBC count $< 500,000/\mu\text{L}$. If indicated, exchange transfusion is to be preferred, particularly in small children (< 12 to 15 kg body weight).

3.4.2 TECHNIQUE OF ET

Total exchange volume: ca $100 - 150$ ml/kg body weight.

Exchange blood: fresh, blood group matched erythrocyte concentrate (EC < 5 days old, leukocyte-depleted, CMV negative, irradiated with 30 Gy) + isoagglutinin-free FFP blood group AB. The EC should be diluted with the FFP via a 3-way spigot in a ratio of ca $1:3$ so that the resultant hematocrit is lower than the patient's, and carefully brought up to body temperature before administration. Alternatively, the procedure may be accomplished by the alternate exchange of 1 portion EC and ca 3 portions FFP. During and after the exchange the patient's hematocrit should not rise above the baseline value. Should the platelet count fall below $60,000/\mu\text{L}$, transfusion of platelet concentrates will be necessary.

Exchange portion: $10 - 50$ ml aliquots according to patient's size, to be exchanged by slow aspiration and injection, withdrawing first to a safe volume deficit. As far as possible, 2 large-caliber venous lines should be employed.

3.4.3 CONTRAINDICATIONS OF ET & PRECAUTIONS

Exchange transfusion is generally contraindicated in shock, oliguric/anuric renal failure, and severe liver disease. Extreme caution must be exercised in case of cardio/pulmonary compromise.

Only fresh, leukocyte-depleted, irradiated and whenever possible CMV-negative blood products should be used. Leukocyte depletion is indispensable, particularly if the patient's CMV status is not known or when CMV-negative blood components are not available. In these cases, hyperimmune CMV globulin (Cytotect[®], Megalotect[®], Cytogam[®]) should be given post exchange (50 U/kg q $2 - 3$ weeks, up to $\times 6$).

The procedure is to be carried out very slowly, usually over $12 - 24$ h. In addition to the slow exchange rate, $0.5 - 1 - (2)$ ml 10% Ca gluconate should be given slowly IV for each 100 ml of exchanged volume to forestall citrate intoxication.

If transfusion-related acute lung injury (TRALI) is suspected, the exchange should be abandoned immediately. This is a rare, yet life-threatening complication that must be managed promptly by rapidly acting antihistaminics (e.g. Benadryl N or Phenergan), O_2 , and sometimes by mechanical ventilation. Bolus corticosteroids are contraindicated in the context of the underlying hyperleukocytosis, as this will precipitate or worsen ATLS.

The patient should be closely monitored during and after the procedure (ECG monitor, BP, body temperature & urine output). CBC, Hct & Hb, global coagulation tests, electrolytes, Ca & P, creatinine, BUN, UA, blood sugar & urinalysis must be checked frequently. Blood gases are not reliable, as TC oxymetry may be normal, while arterial pO_2 low on Astrup, even if the sample had been placed on ice immediately.

3.5 HYPONATREMIA & SIADH

Defined as $\text{S-Na} < 130$ mmol/L, hyponatremia is the most common metabolic disorder in cancer patients. The degree of the deficit and mainly the rate at which it evolves are the pacemakers of its clinical relevance in terms of morbidity and mortality as well as of therapeutic intervention. Hyponatremia may be due to a negative sodium balance (losses $>$ intake), water retention, or the combination of both. However, hyponatremia can also result from excessive losses of Na relative to those of water. Yet more

intriguing is the hyponatremia (\pm hypokalemia) associated with a number of edematous conditions (cardiac failure, obstruction of IVC by tumor, nephrotic syndrome, acute/chronic renal failure, severe liver diseases, e.g. cirrhosis, VOD) in the face of increased total body water and sodium content. Finally, hyponatremia is often encountered in severe illnesses and in the terminally ill patient, probably due to redistribution of total body sodium. True hyponatremia should be distinguished from the pseudohyponatremia of hyperlipidemia and paraproteinemia as well as from so-called water-shift hyponatremia of diabetes mellitus.

One of the causes of hyponatremia is the syndrome of inappropriate antidiuretic-hormone secretion (SIADH = Schwartz-Bartter syndrome), which is the result of increased ADH (vasopressin) release, either from ectopic sites (ADH-producing tumors), or from the hypothalamus-neurohypophysis in response to "non-physiologic", i.e. non-osmotic stimuli. In the latter situation, baroreceptors located in the systemic circulation sense changes (in this case decrease) in the effective intravascular volume to mediate ADH release. In some cases, the syndrome is possibly caused by impaired free-water handling at the level of the renal tubule/collecting duct (reduced free-water clearance, enhanced ADH effect). Among other causes, SIADH may be also induced by a number of drugs, e.g. morphine, carbamazepine, VCR, VBL, melphalan, CDDP, IFO, CPM. CPM-induced SIADH has been described most commonly at high dosage (30 – 50 mg/kg or 6 g/m²), less frequently after lower doses (10 – 15 mg/kg) of the drug. The diagnosis of SIADH is made on the basis of the following constellation of findings:

- S-Na < 130 mmol/L (either asymptomatic or symptomatic).
- S-osmolality < 280 mmol/kg.
- U-osmolality > S-osmolality (usually \geq 500 mmol/kg).
- U-Na > 20 mmol/L (w/o diuretics).
- Clinical euvolemia (BP & HR within normal limits for age).
- Normal renal function (by age-adjusted creatinine & height-adjusted GFR estimate) (a la Schwartz et al. 1976)⁽⁸⁶⁾.
- No evidence of adrenal dysfunction.
- No evidence of thyroid dysfunction.

The differential diagnosis of hyponatremia and SIADH is too broad that only general guidelines for investigation can be provided:

- Careful history: underlying disease (stage, extent, treatment), co-morbid conditions, diet, PN, drugs, head and chest trauma/surgery, complaints & duration of symptoms, etc. Is the patient on mechanical ventilation?
- Thorough physical examination: BW, HR, RR, BP, temperature, general & nutritional status, signs related to a CNS, cardiac, broncho/pulmonary, GIT, liver, renal/uropoietic tract, or endocrine (thyroid, adrenal) disease.
- Imaging studies as deemed necessary on the basis of history & physical examination: ECG, EEG, CT/MRI, US, CXR, etc.
- Lab tests as would be indicated by history & physical examination, at least: CBC + Diff; serum- electrolytes, Ca, P, albumin, G, urea, creatinine, UA & osmolality; urine- pH, specific gravity/osmolality, Na (< 10 mmol/L vs. > 20 mmol/L), K, Cl, albumin & casts.
- Strict record on: oral intake, parenteral fluids & output from all sources.

The management of SIADH complicating VCR & CPM has been discussed within Protocol I/I/II- refer to section 2.2.2.1 & section 2.2.4.1 . Freely adapted from (van Oosterom AT et al. 1995)⁽⁸⁷⁾, (Adelman RD et al. 1996)⁽⁸⁸⁾, (Jakob A et al. 1999)⁽⁸⁹⁾ & (Flombaum CD 2000)⁽⁵⁸⁾, the following general recommendations can be made to meet the needs of pediatric patients (See also **Tab. 28**):

- 1 Treatment of the underlying disease, if available, as well as management of the primary disorder upsetting water and electrolyte homeostasis should be given top priority.
- 2 Asymptomatic patients with S-Na > 125 mmol/L of recent onset can be initially observed closely. They may recover spontaneously. However, as soon as they would go downhill, appropriate therapy must be started without delay.
- 3 Patients with severe (S-Na ≤ 125 mmol/L) and/or symptomatic hyponatremia should be managed immediately.
- 4 Symptomatic hyponatremia of acute onset (24 – 48 h) should be managed promptly and decisively in order to prevent irreversible damage to the brain and to restore normal neurologic function.
- 5 Symptomatic hyponatremia of duration longer than 48 – 72 h is to be approached cautiously with less vigorous treatment, for a too rapid correction of the deficit would inevitably lead to chronic demyelination syndrome (pontine myelinolysis) with permanent brain damage, and may even prove fatal.
- 6 Patients with long-standing hyponatremia that has evolved slowly are usually asymptomatic or have but little symptoms, tolerating S-Na levels as low as 105 – 115 mmol/L by virtue of adaptive mechanisms of the brain neurons against swelling. They can be managed on outpatient basis with oral salt tablets, furosemide and fluid restriction. Aggressive therapy would be life threatening.
- 7 Severe and/or symptomatic cases refractory to standard strategy should be managed with ADH antagonists to induce nephrogenic diabetes insipidus. Yet another candidate for treatment with these alternative agents is SIADH complicating oxazaphosphorine chemotherapy (CPM, IFO) in the face of the hyperhydration necessary to prevent hemorrhagic cystitis, as fluid restriction can be safely obviated.
Either lithium carbonate 15 – 60 mg/kg/d PO in divided doses q 6 – 8 h aiming at a target plasma level of 0.6 – 1.5 mmol/L, or demeclocycline 7 – 13 mg/kg/d PO in divided doses q 6 – 12 h can be used. The latter is more effective, yet more toxic to the kidneys, and azotemia may be a concern, particularly when hepatopathy is also present.
- 8 As S-Na concentration is regulated rather indirectly, i.e. via water content, hyponatremia must be always judged in the context of the hydration status. Hyponatremia may coexist with hypovolemia, euvoemia as well as hypervolemia. Special attention should be paid to the edematous states that may be associated with oliguria, where hyponatremia (± hypokalemia) is a typical feature despite increased total body water and salt content. In these conditions, both water and NaCl are contraindicated.
- 9 A concurrent hypokalemia must be also rectified, as this will help correct the hyponatremia. **Cave:** Not applicable in ATLS!
- 10 The amount of Na required can be calculated according to the following formula:

$$\boxed{\text{Na [mmol]} = C \times \text{BW [kg]} \times \Delta \text{Na [mmol/L]}}$$

where: C Constant = 0.80 to 0.75 for infants 0 – 6 months of age

= 0.75 to 0.70 for infants 6 – 12 months of age
 = 0.70 to 0.65 for children 1 – 12 yr old
 = 0.65 to 0.60 for children > 12 yr old
 = 0.60 for adult men
 = 0.55 for adult women

BW Body weight Use ideal BW in obese children
 Δ Na Difference in S-Na concentration Na (desired) – Na (actual)

Table 28: Management of Hyponatremia

| Volume Status | Hyponatremia | | |
|---|---|---|--|
| | Asymptomatic | Symptomatic 24 – 48 h | Symptomatic > 48 (72) h |
| Hypovolemia | NaCl PO / 0.9% NaCl PI | 3% NaCl PI | 3% NaCl PI |
| Euvolemia (SIADH) | Fluid restriction | Fluid restriction 3% NaCl PI ± Furosemide | Fluid restriction 3% NaCl PI ± Furosemide |
| Hypervolemia <i>See text</i> | Fluid restriction Furosemide q 4 – 24h 0.5 – 1 mg/kg IV | Fluid restriction 3% NaCl PI Furosemide q 4 – 12h 1 mg/kg IV | Fluid restriction 3% NaCl PI Furosemide q 4 – 12h 1 mg/kg IV |
| maximal rate of serum Na correction | 0.5 mmol/L/h, 10 – 12 mmol/L/24h | 1.5 – 2 mmol/L/h over first few hours, 12 – 15 mmol/L/24h | 0.5 – 1 mmol/L/h over first few hours, 10 mmol/L/24h, 18 mmol/L/48h |
| other measures | | ? Dialysis | ? Dialysis |

3.6. LACTIC ACIDOSIS LA

LA is a quite common life-threatening form of metabolic acidosis in pediatric cancer patients in view of the wide array of clinical presentations as well as complications of the underlying disease and its toxic therapy. Most of the time, it is associated with tissue hypoxia, albeit this is not always the case as in extensive infiltration of the liver by tumor cells. Sepsis may or may not lead to poor tissue perfusion and hypoxia, yet LA can develop in both situations, although more likely in septic shock (as in shock of any cause). Occasionally, LA w/o hypoxia has been observed as a manifestation of the malignancy itself, this being most commonly ALL, AML, high-grade NHL or HD.

The diagnosis of LA should be made promptly:

- ❖ Clinical signs suggestive of LA: malaise, anorexia, emesis, confusion, disorientation, hyperventilation, cardiac instability
- ❖ Think of LA in any case of metabolic acidosis
- ❖ Anion gap \geq 18 mmol/L (normal range: 12 ± 4)
- ❖ Decreasing S-bicarbonate + widening anion gap (incipient LA)
- ❖ S-lactate \geq 5 mmol/L (definitive diagnosis)
Normal range: arterial 0.5 – 1.0; venous 0.6 – 1.2

Management of LA:

- 1 Immediate treatment of the underlying disease or disorder that has led to LA.
- 2 Elimination of potential factors that could contribute to metabolic acidosis, e.g. hyperalimentation, drugs, etc.

- 3 The use of bicarbonate is controversial and rather contraindicated with the exception of cardiac arrest. In "paraneoplastic" LA, bicarbonate may even lead to an increase in S-lactate level.
- 4 If the patient is not exhausted and not yet overbreathing to the level of Kussmaul, aminophylline to support hyperventilation along with humidified O₂ given by mask may be of benefit.
- 5 Similarly, pentoxyphylline may improve tissue perfusion.
- 6 Na dichloroacetate (DCA), an investigational compound, is capable of ameliorating LA via inhibiting the production of lactate as well as by increasing its uptake in peripheral tissues. Furthermore, it exhibits a positive inotropic effect. However, in a controlled clinical trial, the drug failed to alter survival or hemodynamics in critically ill adults (Stacpoole PW et al. 1992)⁽⁹⁰⁾.
- 7 If feasible, attempt at dialysis is justified in severe, life-threatening cases, while managing the underlying disease/disorder properly.

3.7 HYPERCALCEMIA OF MALIGNANCY HM

Compared to adults, HM is rare in children, with an incidence ranging between 0.4 – 0.7% (McKay C et al. 1993 & Leblanc A et al. 1984)⁽⁹¹⁻⁹²⁾. Occurring at different stages of the disease, it is usually associated with significant morbidity and may be severe enough to threaten life. Most commonly, it has been related to tumor-derived PTHrP (the humoral hypercalcemia of malignancy = HHM). Conversion of 25-OH-vitamin D₃ into 1,25-(OH)₂-vitamin D₃ (calcitriol) by tumor cells is held responsible for the hypercalcemia sometimes encountered in HD and NHL (Seymour JF et al. 1993)⁽⁹³⁾. As in adults, it is likely that locally acting mediators may cause some cases of HM and osteolysis in children also.

The diagnosis of HM is usually straightforward based on the clinical picture (although non-specific) and the demonstration of hypercalcemia in the context of malignoma. PTH should be low. Specific assays such as PTHrP, U-cAMP, etc are not necessary most of the time. However, a complete mineralogram (Na, K, Cl, Ca, Mg, P), albumin, creatinine, urea, UA, S/U-osmolality, urinalysis and abdominal US should be obtained at baseline, and repeated until safety post treatment. In addition, fluid balance, BW, BP, HR & ECG should be monitored.

Management guidelines: These are governed by the severity and symptomatology of the hypercalcemia as well as by cardiac & renal function (**Tab. 29** & text- *vide infra*).

Table 29: Management of HM (*See also text below*)

| Therapeutic Measure | Hypercalcemia | | |
|---------------------|--|--|---|
| | Mild/Asymptomatic S-Ca = 2.75 – 3.0 mmol/L | Moderate S-Ca = 3.0 – 3.5 mmol/L | Severe/Symptomatic S-Ca > 3.5 mmol/L |
| Hydration | PO/IV: x1.5 – x2 maintenance needs | PI: 2.5 – 3 L/m ² /24h 0.9% NaCl | PI: 4 – 5 L/m ² /24h 0.9% NaCl |
| Fluid balancing | q 12 h | q 12 h | q 6 h |
| Furosemide | 0.5 – 1 mg/kg PO/IV q 12 – 24 h | 1 mg/kg IV if input > output by + 400 ml/m ² /12h | 1 mg/kg IV if input > output by + 200 ml/m ² /6h |
| KCl | PO/IV quantum satis | PI: 90 – 120 mmol/m ² /24h | PI: 150 – 200 mmol/m ² /24h |

| | | | |
|----------------|--|---|---|
| Pamidronate | | 1 mg/kg PI 2 – 4 h | 1.5 mg/kg PI 2 – 4 h |
| + Calcitonin | | ? 4 – 8 U/kg SC/IM q 6 – 12 h for 2 – 3 d | 4 – 8 U/kg SC/IM q 6 – 12 h for 2 – 3 d |
| Other measures | <ul style="list-style-type: none"> • Ca-poor diet • ? Mg PO/IV | <ul style="list-style-type: none"> • Ca-poor diet • ? Mg IV • ? Dialysis | <ul style="list-style-type: none"> • Ca-poor diet • ? Mg IV • ? Dialysis • ? Other agents |

- 1 Effective antineoplastic therapy is the mainstay of management to guarantee long-term control of HM. However, it is often necessary to stabilize the patient first by managing the HM itself, which has to be tailored to the severity and spectrum of metabolic disturbances, taking also into account co-morbid conditions.
- 2 Diuretics are of limited value in HM. They are rather intended to prevent fluid overload. Only loop diuretics may be used, and only after volume has been already replenished.
- 3 In CHF & in renal failure, controlled hydration with hypotonic crystalloids instead of NS is necessary to avoid pulmonary edema and hypertension.
- 4 50% of patients already present with hypokalemia. Furosemide causes additional K be lost in the urine, which should be supplemented *quantum satis* to maintain S-K between 3.5 – 4.5 mmol/L.
- 5 In case of hypomagnesemia, being present *a priori* or evolving post treatment, Mg must be supplemented (0.2 – 0.8 mmol/kg/d) to maintain S-Mg at 0.7 – 1.0 mmol/L.
- 6 Phosphate is contraindicated in the management of HM, as it may entail ARF & Ca phosphate precipitation/metastatic calcifications.
- 7 Salmon calcitonin is a safe, fast acting (within 2 – 4 h), but weak anticalcemic agent with a dual mode of action, promoting intense calciuria on the one hand, and inhibiting osteoclasts on the other. Furthermore, it displays a potent analgesic effect. However, tachyphylaxis developing quickly is the rule. Therefore, the drug is suitable for either urgently acute or intermittent management.
- 8 The geminal bisphosphonates (gBP) have become the gold standard in the drug management of HM in adults. A dozen of them have been evaluated in clinical trials for efficacy and safety. However, the experience in pediatrics is too limited so far, with pamidronate (aminohydroxypropylidene bisphosphonate = AHPPrBP) being the most commonly tried member of this family. At a dosage ranging between 0.66 – 2 mg/kg (mostly 1 – 1.5 mg/kg) given by IV infusion, the drug proved consistently efficacious and safe in the treatment of HM in pediatric patients, too (Jabali Y et al. 2000)⁽⁹⁴⁾.
- 9 Particularly severe cases refractory to other modalities may be considered for alternative agents such as plicamycin (mithramycin M) or Ga nitrate. Although effective, these agents are associated with significant toxicities (Bilezikian JP 1992)⁽⁹⁵⁾ & (Flombaum CD 2000)⁽⁵⁸⁾. Therefore, they can be used only as ultimate refuge.
- 10 Mechanical ventilation to protect the airways is indicated in patients with significantly altered sensorium.
- 11 Indications for dialysis in HM:
 - Severe hypercalcemia refractory to conservative therapy.
 - Severe hypercalcemia associated with profound mental changes.
 - CHF (risk of pulmonary edema).
 - Renal failure.

3.8 IDIOPATHIC HYPERAMMONEMIA IHA

Since mild cases could well escape attention, while other fatal ones might have been not recognized as such or merely not reported on, the true incidence of IHA in cancer patients is not known. In one retrospective study, the incidence was 2.4% in leukemia and 1.2% post SCT (Mitchell RB et al. 1988)⁽⁹⁶⁾, while it was 0.5% in the latter population in another study (Davies SM et al.)⁽⁹⁷⁾. The overwhelming majority of reported cases are hematologic malignancies managed with intensive chemotherapy or SCT. The mortality rate has been high, with 75% of the patients dying of hyperammonemia. Aside from L-asparaginase therapy, where hyperammonemia is an inherent feature of the drug's effect, the mechanism underlying IHA remains enigmatic. However, the disorder developed abruptly a median of 3.5 weeks post treatment, with the patient being severely neutropenic (WBC < 500 / μ L), in a presumed catabolic state, mostly receiving TPN, and often with GI hemorrhage.

The diagnosis should be made urgently:

- Dramatic progressive encephalopathy and tachypnea developing suddenly in a patient severely neutropenic consequent on intensive chemotherapy or SCT.
- ? Potential risk factors: catabolic state, TPN, GIT hemorrhage, infection.
- Family & personal history negative/inconclusive for an inborn error of metabolism that may be associated with hyperammonemia. If the patient recovers, however, this should be investigated *ex post*.
- Other cause of encephalopathy excluded (Reye's syndrome, portosystemic shunt, fulminant hepatitis, chronic active hepatitis, cirrhosis, VOD, hypoglycemia, etc).
- Drug-induced hyperammonemia ruled out (valproate, L-asparaginase).
- LFTs within normal limits or only modestly abnormal.
- Hyperventilation/respiratory alkalosis.
- S-NH₃ > 2-fold ULN & rising.

Therapy of IHA should be started immediately; otherwise irreversible brain damage will develop to inexorably kill the patient. The optimal treatment of this disorder is yet to be defined. However, deriving from the experience in managing a number of urea-cycle disorders and hepatic encephalopathy (Batshaw ML 1984, Breningstall GN 1986)⁽⁹⁸⁻⁹⁹⁾, the following guidelines may be recommended:

- 1 Stop proteins altogether; later on (upon regression of neurologic symptoms/signs and control of S-NH₃ to a safe limit) 2% solution of essential AA including valine, leucine & isoleucine can be provided. Synthetic, N-free analogues of AA, i.e. ketoacids may be used.
- 2 Supply calories (100 – 120 kcal/kg/24h) with 10 – 20% G & regular insulin at 1 U per 3 – 4 g G by IV infusion.
- 3 Alternative pathways for waste nitrogen (N) excretion:
 - 10% arginine HCl: 0.2 g/kg/24h by IV infusion.
 - Na benzoate: 0.25 g/kg/1.h + 0.25 – 0.5 g/kg/24h by IV infusion. The drug binds glycine to form hippuric acid excretable by the kidney at x4 – x5 the GFR, removing one nitrogen.
 - Na phenylacetate or its prodrug phenylbutyrate: 0.25 g/kg/1.h + 0.25 – 0.5 g/kg/24h by IV infusion. The drug binds glutamine to form phenylacetylglutamine, which is also cleared rapidly by the kidney. Two nitrogens will be removed by this way.
- 4 Identify and treat bleeding from the GIT.

- 5 Decontaminate the gut & promote its motility with lactulose 10 – 20 ml q 6 h via NGT or retention enema adjusting dosage to yield 2 – 3 soft stools/d. Alternatively, gut decontamination may be accomplished with neomycin (50 – 100 mg/kg/d) and/or colistin (50,000 – 100,000 U/kg/d) given via NGT tid or qid.
- 6 If N-trapping therapy is contraindicated (e.g. in hypernatremia), or is not available, then dialysis should be employed to eliminate NH₃. However, hemodialysis is 15-fold less efficient than the former in this regard. On the other hand, both techniques can be combined.
- 7 Institute mechanical ventilation/hyperventilation in case of altered sensorium/intracranial hypertension or brain edema. Urgently consider additional antiedematous measures, e.g. controlled mannitol therapy, along with an optimal relaxation protocol, e.g. pancuronium bromide.
- 8 Rectify additional electrolyte imbalances.
- 9 Provide antiinfective prophylaxis/therapy.

3.9 MTX – INDUCED ACUTE ENCEPHALOPATHY

MTX exhibits a wide range of acute, subacute & chronic toxicities. A stroke-like acute neurotoxicity syndrome has been described to occur at different dosages and with different routes of administration- discussed in section 2.2.3.2. It is usually associated with MTX-induced nephrotoxicity or otherwise impaired renal function leading to poor MTX elimination.

It has been also shown that therapy with aminophylline is effective and safe in managing this adverse event, with recovery being prompt and complete or almost so most of the time. The conventional formulation of the drug has been used either at 2.5 mg/kg by IV infusion over 45 – 60 min, or 0.5 mg/kg/h as continuous infusion for 12 h. Equally effective is the rapid-release oral formulation of theophylline. The aim should be a plasma concentration of 10 – 30 µmol/L, which is safe in asthma patients. Since this event is reversible with aminophylline treatment, further therapy with MTX is usually feasible and uneventful (Bernini JC et al. 1995, Peyriere H et al. 2001)⁽⁴⁹⁻⁵⁰⁾.

3.10 POOR ELIMINATION OF MTX

A definite minority of patients will turn out to be intrinsically poor eliminators of MTX. Unfortunately, the only test available to reliably define this population is the just exposure to the first high dose of the drug. The personnel should be extremely vigilant, and close clinical and lab monitoring of the patient on MD/HD MTX is essential. However, all patients- not merely the intrinsically poor eliminators- should be considered at risk for delayed MTX elimination. Similarly, any high dose of the drug- not only the first one- could be poorly eliminated. The great inter- and intra-individual variability, the complex pharmacokinetics of MTX as well as its many drug interactions preclude predicting a firm pharmacokinetic profile for a given patient. Therefore, monitoring of plasma MTX concentrations is *conditio sine qua non* whenever the drug is given at high dosage.

The major route of MTX elimination is the kidney, which would also be the main victim of MD/HD drug toxicity unless efficient prophylactic measures (hyperhydration/alkalinization) are undertaken. Hence, renal function should be also monitored on a regular basis before and during MD/HD MTX therapy.

A minor fraction of MTX is cleared through the bile. Although less efficient compared to the renal route, biliary excretion of the drug becomes more important as renal function deteriorates. The origin notwithstanding, renal insufficiency or failure will

lead to increased systemic exposure to MTX and its metabolites, thereby enhancing marrow, GI & CNS toxicity.

This topic has been discussed in part in section 2.2.3.2 . Rescue guidelines along with a diagram are also found in Appendix 3.1. In addition, the following discussion is particularly useful in the case of MTX-induced nephrotoxicity and impaired elimination of the drug.

- 1 Adjust MTX dosage to GFR as assessed either by the classic technique of CL_{cr} or ^{51}Cr -EDTA/ ^{99m}Tc -DTPA scintigraphy, or as estimated a la Schwartz et al. for children > 6 months of age (Schwartz GJ et al. 1976)⁽⁸⁶⁾. See **Tab. 30** below (Powis G 1982)⁽¹⁰⁰⁾. See Appendix 3.2.b for age-dependent changes in CL_{cr} (GFR).

$$GFR \left[ml / min / 1.73 m^2 \right] = \frac{0.55 \times Length [cm]}{Creatinine [mg\%]} = \frac{48.5 \times Length [cm]}{Creatinine [\mu mol/L]}$$

Table 30: Dose Adjustment of MD/HD MTX & MD/HD CPM by GFR

| Drug | GFR [ml/min/1.73m ²] | | | |
|------|----------------------------------|---------|---------|------|
| | > 60 | 30 – 60 | 10 – 30 | < 10 |
| MTX | 100% | 50% | 0 | 0 |
| CPM | 100% | 100% | 100% | 50% |

- 2 MTX-induced renal failure should be managed conservatively, as it usually resolves within 2 – 3 weeks after withdrawing the drug.
- 3 However, in case of uremia or electrolyte abnormalities, dialysis is indicated, although MTX itself is not readily dialyzable.
- 4 Alternative pathways of MTX elimination or detoxification should be considered:
 - Cholestyramine (Cuemid, Questran) is a hydrophilic resin, acting as an anion exchange resin. It is capable of binding bile acids with the admixed MTX in the gut via exchange for chloride anions, thus interrupting the enterohepatic cycle so that MTX will be excreted in the feces (Shinozaki T et al. 2000)⁽¹⁰¹⁾. The drug is given at 2 g q 3 – 6 h PO (preferably via NGT). Depending on dose, it may cause hyperchloremic acidosis and constipation- both manageable. Active charcoal is possibly also effective via "passive" adsorption of MTX.
 - Carboxypeptidase G₂ (CPD G₂) is another effective option. It is indicated in 2 closely related settings:
 - i. High plasma MTX level defined as follows:
 1. MTX₃₆ > 10 μmol/L
 2. MTX₄₂ > 5 μmol/L
 3. MTX₄₈ > 3 μmol/L
 - ii. Rise of S-creatinine by > 50% vs. baseline

Therapy with CPD G₂ should be commenced within 5 days from the start of MTX.

Dosage: 50 U/kg slowly IV (5 min). Occasionally, 2 doses are needed.

LCV should be withheld from 4 h before until 1 h after CPD G₂ injection.

Efficacy: Compared to baseline, plasma MTX concentration falls to < 5% by HPLC or to 20 – 50% by immunoassay. Measurement with HPLC is necessary.

Safety: The drug is well tolerated.

Availability: It is advised the drug be deposited in a central store at a national or regional level.

3.11 DRUG EXTRAVASATION

Compared to the general population, the cancer patients are for many reasons more prone to a variety of local drug side effects. Of these, extravasation is the most stressful and most serious from the viewpoint of both the patient and caregiver, for the harm it could entail may have large and far-reaching consequences (anatomic, functional, cosmetic, socio-psychologic & economic). The overall incidence of extravasation accidents is 0.1 – 6%. Although any drug may hurt the vessel wall, cross it or leak out to permeate the surrounding tissues, mechanical injury sustained via a penetrating venepuncture is certainly more important, and with vesicant or otherwise aggressive agents being particularly dangerous. In this regard, attention should be paid to daunorubicin, doxorubicin, vincristine, vindesine & etoposide.

3.11.1 PREVENTION OF EXTRAVASATION

- Continue in learning & training.
- Choose an easily visualized vein of adequate caliber, optimally in the forearm.
- Place a CVC if peripheral venous access is problematic.
- Don't administer cytostatics distal to a site of recent venepuncture.
- Allow ample time for a recently accessed vein to recover.
- Use flexible cannulas rather than rigid needles.
- Inject agents adequately diluted or infuse via a collateral to a well-running drip.
- Watch the patient carefully for any symptoms and signs of a local reaction during administration.
- Stop infusing the drug immediately upon extravasation and if this were merely suggested or suspected. However, leave the cannula *in situ* and manage immediately- *vide infra*.
- Document every local adverse event thoroughly and follow up the patient long enough.

3.11.2 MANAGEMENT OF EXTRAVASATION

General measures:

- The best management is prevention- see section 3.11.1.
- Aspirate 3 – 5 ml of blood via the original cannula, discarding the content.
- Try to aspirate and discard the content of blisters with the aid of a thin needle.
- Then remove the original cannula.
- In case of vinca, infiltrate the lesion with hyaluronidase (Hylase[®], Wyeth), in part through the original cannula before removing it, in part via additional thin needle(s). You will need 1 – 6 ampoules of the drug (150 – 900 U).
- Elevate the involved arm in a sling until swelling resolves.
- Immediately undertake the specific measures as described in **Tab. 31** below.
- Cover the lesion with a sterile dry dressing.
- Check locoregional vital parameters frequently and on a regular basis.

- If in spite of local measures gangrene develops, with absent erythema being often an early sign thereof, the plastic surgeon should be consulted. Early revision of the necrotic lesion and surrounding inflamed-looking tissues, with wide excision, plastic covering and delayed grafting should be considered, particularly in case of anthracycline extravasation. The extent of the latter is best delineated by exposure of the lesion to UV light.
- Follow up the patient for months, as necrosis may not develop until several weeks.
- Document the evolution of this adverse event and the outcome of the undertaken measures thoroughly.

Specific measures:

A number of specific measures and actions have been shown to be useful in the management of extravasation of vesicant or aggressive cytotoxic agents. Those relevant to this trial are summarized in **Tab. 31** below.

Table 31: Specific Measures for Management of Extravasation

| Drugs | Measures | Comments |
|-----------|--|--|
| DNR, DOX | <ul style="list-style-type: none"> • Cooling • DMSO (Dimethylsulfoxide) | <ul style="list-style-type: none"> • Ice-packs for 15 min q 4 – 6 h over several days (Cave: frostbite, chilblain) • 99% solution: 4 gtts/10 cm² involved skin area to air dry freely, w/o bandage • Alternatively: DMSO ointment (Dolobene[®]) in thin layer onto lesion |
| VCR, VDS | <ul style="list-style-type: none"> • No cooling • Hyaluronidase | <ul style="list-style-type: none"> • Rather warm mildly for 1 – 2 h (dry heat) • Hylase[®]: 150 – 900 U diluted in NS to be injected SC/ID into lesion |
| Etoposide | <ul style="list-style-type: none"> • No cooling • DMSO (Dimethylsulfoxide) • HC | <ul style="list-style-type: none"> • Rather warm mildly for 1 – 2 h (dry heat) • 99% solution: 4 gtts/10 cm² involved skin area to air dry freely, w/o bandage • Alternatively: DMSO ointment (Dolobene[®]) in thin layer onto lesion • Hydrocortisone cream: thin film over involved skin area x2/day |

3.12 HEMOTHERAPY

This is an indispensable part of supportive therapy that is fraught with overall infrequent yet important hazards (blood-borne infections, a variety of transfusion reactions, TRALI, GVHD, allosensitization, etc). The cons & pros should be therefore carefully considered from case to case when deciding on this type of therapy.

Blood products:

- Standard, properly stored units of erythrocyte concentrates (EC) should be used for substitution of erythrocytes. One transfusion unit (TU) with CPDA-1, ACD or additive solution has a volume of 250 – 350 ml, a hematocrit in the range of 0.50 – 0.75, and contains < 1 x 10⁹ platelets, up 10¹⁰ WBC (< 5 x 10⁶ to 1 x 10⁷ WBC,

if leukodepleted), and < 15% plasma. The storage shelf life at 1° – 6° C is 35 – 42 days, depending on the stabilizer used and licensing conditions.

Dosage: Usually, transfusion of 1 ml/kg EC should increase the Hct by 1%.

Children ≤ 20 kg 10 – 15 ml/kg

Children > 20 kg up to 300 ml

- In case of repeated febrile and/or allergic reactions in spite of using leukocyte-poor (w/o buffy coat) or leukodepleted (filtered) EC as well as for the prevention of severe allergic reactions/anaphylaxis in IgA-deficient patients mounting anti-IgA antibodies, washed EC resuspended in NS should be used. This process will remove the plasma including IgA & C, and will reduce the WBC content to 10^8 – 10^9 /1 TU. The volume and hematocrit will also vary according to the amount of added NS. Stored at 1° – 6° C the shelf life is 24 h.
- Standard, properly stored random-donor platelet concentrates (RD-PC) are usually used to substitute thrombocytes. The platelets are retrieved from 1 whole-blood donation and suspended in 50 ml plasma. Usually, 4 – 8 RD-PCs are pooled together in 200 – 400 ml plasma. One pooled RD-PC contains 2×10^{11} – 4×10^{11} platelets, a maximum of 2 – 4 ml erythrocytes, and up to 4×10^8 – 8×10^8 WBC (usually < 1×10^7 WBC, if leukodepleted).
- Standard, properly stored apheresis single-donor platelet concentrates (SD-PC) should be used in patients refractory to RD-PC and in primary candidates for SCT. One SD-PC is roughly equivalent to 6 RD-PCs, and contains 2×10^{11} – 4×10^{11} platelets, trace to 5 ml erythrocytes, and depending on the apheresis technique/separator from 10^6 to 10^9 WBC (mostly < 1×10^7 WBC upon leukodepletion) in 250 – 300 ml plasma.
- SD-PC from ABO-identical & Rh (D)-compatible donors should be ordered first. If these are not available, units with plasma compatible with the recipient's erythrocytes are to be chosen. Should the latter be not available either, then units with the lowest anti-A/anti-B isoagglutinin titer can be used, or alternatively volume reduction of the PC may be considered. Sometimes, HLA class I-matched (the best match available) and/or platelet-cross-match compatible units may be needed to ensure satisfactory post-transfusion platelet recovery. The rare patient who has been alloimmunized at platelet-specific antigens will need platelets lacking the corresponding antigen. Persistent refractoriness to platelet transfusions is a problematic albeit not inevitably desperate challenge, and should be discussed with the national study coordinator.
- As platelets are too vulnerable, both RD-PC & SD-PC should be delivered as soon as they have been dispatched from the blood bank, and as rapidly as clinically tolerated (within 4 h at the latest).
- A starting dose of 1 RD-PC/10 kg BW or 5 ml SD-PC/kg BW is expected to raise the platelet count by about 50,000/ μ L. However, in splenomegaly and conditions associated with increased platelet consumption, e.g. sepsis or DIC, larger doses are usually required.
- The standard product for plasma substitution, if any, is fresh-frozen plasma (FFP). The volume of 1 TU of FFP obtained from a whole-blood donation is 160 – 250 ml, whereas 400 – 600 ml can be harvested via plasmapheresis. It is also encouraged to employ in-line pre-storage leukodepletion, as it has been shown that immunocompetent leukocytes are always admixed in this preparation, too. Dosage will depend on the underlying indication, the size of the patient and other considerations. Aside from exchange/massive transfusion, FFP is usually given at (10) – 20 – (30) ml/kg.

- To prevent transfusion-related GVHD in the severely immunocompromised host, all blood products should be irradiated with a minimum of 30 Gy.
- To prevent allosensitization and to minimize the risk of infection, mainly CMV, 3rd generation bedside leukocyte filters should be used, or, preferably, highly efficient leukodepletion is to be integrated within the manufacturing process at the blood bank. This step might also reduce the incidence of TRALI.
- If 3rd generation leukocyte filters are used, then CMV seronegative blood products seem useful only in patients primarily indicated for allogeneic SCT or in case of massive/exchange transfusion (Saarinen UM et al. 1993, Prooijen HC van et al. 1994, Hillyer CD et al. 1994, Bowden RA et al. 1995)⁽¹⁰²⁻¹⁰⁵⁾.

Management considerations:

1 Erythrocyte transfusion: The child with ALL will most often exhibit a subacute to chronic anemia consequent on infiltration of the BM with blasts and failure of normal hemopoiesis, myelosuppressive therapy or GVHD. Acute *de novo* anemia is encountered rather rarely, e.g. due to massive bleeding or infection, notably HPV B19 and overwhelming sepsis. More frequently, "episodes" of worsening are superimposed on a background of chronic anemia, e.g. secondary to repeated blood draws in a small kid. One has to consider a number of factors to facilitate a rational decision to be made:

- The degree of anemia as judged by the Hb level: There is no doubt that severe anemia (Hb < 70 – 80 g/L) deserves substitution therapy, although the goal needn't be correction to normal. One may decide to substitute a moderate degree of anemia (Hb = 80 to 100 g/L), particularly if it is likely to become soon more severe. The majority of pediatric hematologists/oncologists will not probably give treatment in case of asymptomatic mild anemia.
- The rate at which the anemia has developed: An acute blood loss as low as 10 – 15% in a patient receiving chemotherapy for malignancy should be substituted, while another with a long-standing iron-deficiency anemia will tolerate a Hb level as low as 40 – 50 g/L to be safely and effectively managed with medicinal iron.
- Co-morbid conditions: Particularly the patient with a heart disease poorly tolerates anemia because of compromised cardiovascular compensation capability. On the other hand, to forestall cardiac failure in this case, transfusion should be given at a low dose, slow rate and, if need be, fractionated, i.e. 3 – 5 ml/kg over 3 – 4 h, while closely monitoring vital functions.

Complications of the underlying disease and of its treatment, e.g. severe infection or major hemorrhage must be also taken into account, as the anemia may have different dimensions in this context.

- Alternative therapy: It is unlikely that medicinal Fe could keep pace with the chronic blood losses; and the role of rhEPO has not been clearly defined in pediatric ALL.
- Exchange transfusion is indicated in the leukostasis syndrome- see section 3.4.1 (p 127), whereas transfusion of EC is contraindicated in hyperleukocytosis, unless the Hb < 80 g/L- see section 3.4 .

2 Plasma transfusion:

- Exchange transfusion in the leukostasis syndrome- see section 3.4.1 .
- Massive hemorrhage: 20 – 30 ml/kg or 1 TU FFP per 4 TU EC.

- DIC/sepsis (+ bleeding & pathologic global coagulation tests): 20 – 30 ml/kg.
 - Drainage of critical pericardial effusion: 20 – 30 ml/kg.
- 3 **Platelet transfusion:** BM puncture/biopsy can be safely performed at any platelet count with local measures only. A framework for considering platelet transfusion is proposed in **Tab. 32**. It should not be however interpreted dogmatically. Although some questions have been addressed in randomized clinical trials, overall the available information does not allow for providing a simple and clear-cut solution for every situation that may be encountered. Often the evidence has been merely an article of experience or expert opinion, and the physician in charge has therefore to rely on clinical judgment in many cases.

Table 32: Recommended Indications for Platelet Transfusion

| Platelet Count [x 10 ³ /μL] | + Situation(s) to be Considered |
|--|--|
| < 10 | <ul style="list-style-type: none"> • Almost always indicated |
| < 20 | <ul style="list-style-type: none"> • Infection, T > 38.5°C • Sepsis • DIC • Significant hemorrhage: <ul style="list-style-type: none"> ❖ Gastrointestinal ❖ Urogenital ❖ Mucosal ❖ Retinal • Severe mucositis • Mechanical ventilation • Fibroscopy • Fbg < 1 g/L • Thrombolytic/anticoagulant therapy • WBC > 100,000/μL + hemorrhage • IV amphotericin B • Platelets expected to fall further prior to forthcoming visit |
| < 30 – 40 | <ul style="list-style-type: none"> • DIC, particularly during induction in APL • LP • Placement of CVC • Stem-cell harvest • Minor surgery/biopsy |
| < 50 – 60 | <ul style="list-style-type: none"> • Exchange/massive transfusion • CNS hemorrhage • Drainage of pericardial effusion • Major surgery |
| < 100 | <ul style="list-style-type: none"> • Neurosurgery • Ophthalmic surgery |

- Corrected platelet count increment (CCI):
This is used to assess the number of platelets recovered in vivo in relation to the patient's BSA [m²] following infusing a known amount of platelets [N-

fold of 10^{11}]. In addition to the baseline platelet count [C_B], this is performed usually 10 – 60 min and 18 – 24 h post transfusion [C_A].

$$CCI = \frac{\{C_A [n/\mu L] - C_B [n/\mu L]\} \times BSA [m^2]}{N [\times 10^{11}]}$$

CCI provides valuable information with regard to:

- i. Efficacy of platelet transfusion, whereby response is defined as:
 - Adequate = $CCI_{1h} > 7,500 - 10,000/\mu L$ & $CCI_{24h} > 4,500 - 7,500/\mu L$.
 - Inadequate = $CCI < 5,000/\mu L$ following at least 2 separate transfusions of an adequate number of platelets.
 - ii. Likely mechanism of inadequate response (refractoriness):
 - ▶ If $CCI_{1h} < 7,000/\mu L$, the likely mechanism is immune, i.e. the patient is alloimmunized to HLA antigens, platelet-specific antigens or has an autoantibody to platelets.
 - ▶ If $CCI_{1h} > 10,000/\mu L$, but $CCI_{24h} < 7,000/\mu L$, then the likely mechanism is non-immune, e.g. splenomegaly, fever, sepsis/DIC or drugs (amphotericin B, vancomycin, ciprofloxacin, etc).
- 4 Post-transplant transfusion strategy: This is a complex issue involving a number of specific immunohematologic profiles of the recipient and a range of constellations of recipient-donor relations including, among others, the potential evolution of chimerism. The transplantation center in cooperation with the blood bank should adopt the optimal transfusion policy on a strictly individual basis. When the patient is discharged, he/she should be equipped with a report, which will also include guidelines as to his/her specific transfusion requirements.

3.13 DISORDERS OF HEMOSTASIS

Data from the Canadian registry of venous thromboembolism (VTE) in children had shown that the incidence of deep-vein thrombosis (DVT) & pulmonary embolism (PE) was 5.3/10,000 hospital admissions or 0.07/10,000 Canadian children aged 1 month to 18 years. There was a bimodal distribution of thrombotic events, with infants < 1 year of age and teenagers being most frequently affected, and significant prevalence of upper-venous-system involvement (50/137) compared to adults, which could be attributed to the more common use of that system for placement of central venous lines (CVLs). The overwhelming majority of children had associated condition(s) that might have contributed to thrombotic risk, with 33% having a CVL accounting for 45 events. The complications were thrombotic, not hemorrhagic, and the mortality rate was 2.2% (Andrew M et al. 1994)⁽¹⁰⁶⁾.

The incidence of thromboembolism (TE) in children with ALL reported in 15 studies oscillates, for several reasons, over a wide range from less than 1% to 15%. In 2 prospective cohort studies, the incidence was reported to be 11.5% and 13% (Mitchell L et al 1994 & 1994)⁽¹⁰⁷⁻¹⁰⁸⁾, whereas that of hemorrhage and thrombosis was merely 2.8% in ALL-BFM 1990 (Sutor AH et al. 1999)⁽³¹⁾.

Of the 150 thrombotic events reported in the literature as of 1994, the location could be determined in 135 (90%), with approximately 2/3 affecting the CNS (Andrew ML et al. 1994)⁽¹⁰⁹⁾. By contrast, the incidence of CVL-associated DVT has been reported to be about 4% only. While the former are probably upbiased by virtue of their seriousness, the latter could be well downbiased due to the use of less sensitive

diagnostic tools, thus underscoring the need for prospective cohort studies to better define TE events and their distribution in pediatric ALL (Mitchell LG et al. 1995)⁽¹¹⁰⁾.

A number of inherited defects have been linked to enhanced risk of TE disease in the general pediatric population (Andrew M et al. 1994)⁽¹⁰⁹⁾ as well as in patients with ALL (Nowak-Göttl U et al. 1999 & Wermes C et al 1999)⁽³²⁻³³⁾. These include F.V Leiden (G1691A mutation), mutation of F.II gene (G20210A), mutation of the gene for methylenetetrahydrofolate reductase (MTHFR), congenital deficiency of AT III, protein C, protein S, and vWF-cleaving protease. Increased plasma levels of Lp (a) and homocysteine as well as obesity have been also described as potential risk factors. In a study of 301 patients enrolled on ALL-BFM 90/95 trials, 55 (+18%) were shown to have at least one of those inherited defects. Almost one half of these 55 developed a TE complication ($p < 0.0001$). However, the proportion of patients with CVC was not reported in that paper (Nowak-Göttl U et al. 1999)⁽³²⁾.

Bleeding is a common phenomenon at presentation, i.e. prior to antileukemic therapy. Most often it is due to thrombocytopenia, but rarely to coagulopathy. Indeed, the plasma concentrations of most coagulation proteins are within the normal range for children, although they may be increased (vWF, F.VIII, F.IX, Fbg, α_2 -macroglobulin, protein S) or decreased (protein C, prekallikrein, F.XIIIa, F.XIIIb). On the other hand, the true incidence of pre-treatment thrombosis in pediatric ALL, a quite common harbinger of covert or overt cancer in adults, is not known. However, both hemorrhage and thrombosis are apparently more prevalent in hyperleukocytosis, particularly in the case of leukostasis, as they are notorious in APL.

Many studies have evaluated the hemostatic system in ALL on-therapy. The best studies to build on come from DFCI (Mitchell L et al. 1994)⁽¹⁰⁶⁾ and from BFM SG (Sutor AH et al. 1992)⁽¹¹¹⁾. Single-agent chemotherapy with E. coli asparaginase elicits a significant decrease in the plasma concentrations of 11 coagulation proteins, notably AT III. Despite some variability across studies, the most consistent effect of combination chemotherapy including L-asparaginase (w/o prednisone) is a significant decrease in plasma levels of AT III, Fbg & Plg, but marked changes may be seen in 7 coagulation factors. Combination chemotherapy including no L-asparaginase but usually prednisone leads to significant changes in the plasma concentrations of 13 coagulation proteins (increase in 10; decrease in 3), occurring evidently in temporal relation to prednisone. In regimens combining both agents, prednisone mostly offsets the hemostatic effects of L-asparaginase in this regard, although the net action is agonistic for a number of coagulation proteins such as Fbg.

3.13.1 COAGULATION DIAGNOSTICS

This is discussed in sections 1.16.1.6, section 1.16.2.5, section 2.2.2.1, section 2.2.4.1 & 2.2.5.1. Three points should be made in this regard:

- When sampling from a CVC, particularly in case of pathologic or questionable results, a possible artificial activation of coagulation or contamination with heparin should be kept in mind. If in doubt, the blood sample must be drawn gently from a peripheral vein. The sample for coagulation studies should rank first in a series of blood tests.
- Even if every thing has been done *lege artis*, the findings should be interpreted within the context of the underlying disease, associated conditions, treatment, and complications.
- Interpretation of the results should be made using pediatric reference values (Hathaway W et al. 1991, Andrew M et al. 1988, 1987, 1990 & 1992)⁽¹¹²⁻¹¹⁶⁾.

3.13.2 THERAPEUTIC CONSIDERATIONS

- As bleeding complications under L-asparaginase/steroids in the absence of additional risk factors are rather the exception across ALL-BFM studies, the prophylactic substitution of coagulation factors is not routinely recommended. Nor the prophylactic substitution of individual coagulation components, e.g. AT III, or heparinization without evidence of activation of the coagulation cascade can be recommended as a standard approach (Sutor AH et al. 1992 & 1999)^(111, 31). In fact, there is no evidence that children are at greater risk for hemorrhagic complications than adults for any given insult, and the risk for thrombotic complications is considerably less than for adults (Andrew M et al. 1992)⁽¹¹⁶⁾. However, whenever possible, e.g. in older children, implantation of a CVC should be deferred until phase 2 of protocol I/I'.
- In case of documented activation of coagulation, the following approach is recommended (**Tab. 33**). Management of the triggering cause(s), e.g. infection is a MUST.

Table 33: Intervention in Selected Disorders of Hemostasis

| Pathologic Condition | Management |
|---|---|
| Marked asynchronous ↓ of: <ul style="list-style-type: none"> • Fbg • AT III Parallel ↑ of: <ul style="list-style-type: none"> • D dimers | <ul style="list-style-type: none"> • AT III by continuous or short infusion (1h) <p style="text-align: center;">Dosage</p> $\text{AT III [U]} = [\text{AT III}_{80-100\%} - \text{AT III}_{\text{actual}}] \times \text{BW [kg]}$ |
| <ul style="list-style-type: none"> • Overt bleeding | <ul style="list-style-type: none"> • Targeted substitution of deficient factor(s) according to results of blood coagulation analysis • Concentrates or recombinant formulations of coagulation factors should be used whenever available • For Fbg < 1 g/L: Fbg concentrate 100 mg/kg BW or cryoprecipitate 1 bag/5 – 10 kg BW • FFP is indicated only if thermolabile factors (F.V or F.XI) are deficient or when concentrates and recombinant preparations of coagulation proteins are not available • Platelet transfusion, if need be: See section 3.12 (p 139) • Erythrocyte transfusion, if deemed necessary: See section 3.12 (p 139) • Dosage adequate to achieve hemostatically effective levels and control of bleeding |
| <ul style="list-style-type: none"> • Massive hemorrhage / DIC / sepsis | <ul style="list-style-type: none"> • FFP 20 – 30 ml/kg BW • AT III by continuous infusion Dosage: <i>vide supra</i> • For Fbg < 1 g/L: Fbg concentrate 100 mg/kg BW or cryoprecipitate 1 bag/5 – 10 kg BW • Vitamin K 2.5 – 5 – 10 – (20) mg/d IM or slowly IV, may be repeated, if need be Cave: G-6-PD deficiency |

| | |
|--|---|
| | <ul style="list-style-type: none"> • Platelet transfusion: See section 3.12 (p 139) • Erythrocyte transfusion, if deemed necessary: See section 3.12 (p 139) • ? LMWH 100 – 150 U/kg BW q 24 h SC/CI |
|--|---|

- 3 Patients at enhanced risk for TE or hemorrhagic complications, e.g. those with inborn errors of the coagulation system, metabolic and other disorders predisposing to thrombophilic or hemorrhagic diathesis as well as patients with congenital heart disease, liver failure, etc require an individualized approach. Should any problems arise, the national study coordinator is to be contacted for discussion and advice.

3.13.3 THROMBOLYTIC (TL) & ANTITHROMBOTIC (AT) THERAPY

3.13.3.1 GENERAL REMARKS ON TL / AT THERAPY

- 1 The immediate efficacy and the long-term outcome of thrombolytic and antithrombotic (anticoagulant) therapy most likely depend on the early recognition of the thrombotic event, identification of thrombogenic risk factors, prompt initiation and adequate duration of proper treatment and prophylaxis.
- 2 The experience with this type of therapy in pediatrics is still limited due to the overall rarity of TE events in children as compared with adults. However, they are common enough as to entail dilemmas in decision making with regard to therapeutic intervention. Recommendations based on the available level of evidence have been developed at the 4th, 5th & 6th ACCP Consensus Conference on Antithrombotic Therapy (Michelson AD et al. 1995, 1998, Monagle P et al. 2001)⁽¹¹⁷⁻¹¹⁹⁾. Although a number of randomized clinical trials addressing specific questions of this field in pediatric patients have been or are being conducted in the meantime, those recommendations have been primarily derived and mostly extrapolated from the experience in adults. However, it is likely that the optimal strategy for children, particularly infants, with TE events will diverge from that for adults, primarily because of different ontogenetic features of hemostasis that could affect the pathophysiology of thrombosis as well as the response to therapy.
- 3 There is no therapeutic range for systemic thrombolytic therapy. The correlation between hemostatic parameters and efficacy/safety of thrombolytic agents is too weak to have useful predictive value (Bovill E et al. 1992)⁽¹²⁰⁾. Although it is often recommended to monitor hemostasis by global coagulation tests, Fbg & FDPs/DD, only Fbg may help guide the need for replacement hemotherapy with FFP, cryoprecipitate or Fbg concentrate, the generally accepted threshold for this purpose being a level < 1 g/L. FDPs/DD will merely indicate whether a fibrinolytic effect is present.

3.13.3.2 SELECTED TL / AT DRUGS

- 1 Although effective, streptokinase (SK), a bacterial protein, is associated with severe allergic reactions/anaphylaxis in children, particularly on repeated use. This is due to sensitization by streptococcal infections that are prevalent at young age. Therefore, the drug should be avoided as far as possible, even at low dosage, in the pediatric population.
- 2 Urokinase (UK), a plasminogen activator derived from cultures of human fetal renal cells harvested post mortem, is also effective. However, although no

transmissible infections that would be related to the product (Abbokinase, Abbott Laboratories, Abbott Park, IL) have been ever reported, its production has been suspended by the U.S. FDA quality control regulations on January 25, 1999. The drug (Ukidan, Serono, Italy/Switzerland) may be still available or become so in the future on the pharmacy formulary in some countries. Like SK, UK acts not only *in situ*, but also systemically. On the other hand, it is essentially non-allergenic, and can be used repeatedly.

- 3 Alteplase is a recombinant tissue plasminogen activator (rtPA) that exerts its action directly on the thrombus, as it converts plasminogen to plasmin only upon specific binding to fibrin. So, the systemic effects of the drug (Actilyse, Activase) are negligible. In addition, hypersensitivity adverse events are too rare. Although the biologic $t_{1/2}$ is only 5 minutes, its action *in situ*, i.e. at the thrombus lasts for several hours. Compared to SK, rtPA performs substantially more rapidly and more efficiently. The drug is expensive, and experience in pediatrics is just emerging (Johnson TR et al. 2000)⁽¹²¹⁾.
- 4 Unfractionated heparin (UFH) is a cheap antithrombotic/anticoagulant agent with proven efficacy and well known, rare but important side effects. It is still legitimate to use, if indicated judiciously. However, it is not suitable for long-term therapy, i.e. for at least 3 months.
- 5 Low-molecular-weight heparins (LMWHs) are an attractive alternative both to UFH and oral anticoagulants (OAs). Their advantages include: SC route of administration & negligible protein binding (exquisite bioavailability at low dosage, predictable dose-response relation); lack of binding to endothelial cells and macrophages (2 to 4-fold longer plasma $t_{1/2}$ compared to UFH, more comfortable dosing schedule); predictable pharmacokinetics on fixed dosing (minimal need for monitoring activity to optimize treatment); reduced interaction with platelets (? lower incidence of HIT/HITT than with UFH); low affinity to vWF (only weak inhibition of vWF-dependent platelet activation with apparently attenuated hemorrhagic potential compared to equipotent doses of the parent compound); and lack of interference (compared to warfarin) with other drugs or diet. It is also possible that the risk of osteoporosis following long-term use of LMWH could be lesser than with standard heparin or OAs.

These advantages render the LMWHs particularly suitable for use in pediatrics so that they might supplant conventional heparin and OAs for short-term and long-term antithrombotic/anticoagulant therapy, respectively. The bulk of evidence from clinical trials in adults and the available data from the few trials so far accomplished in children (Massicotte MP et. al. 1996 & 1997, Punzalan RC et al. 2000, Dix D et. 2000)⁽¹²²⁻¹²⁵⁾ have shown that the LMWHs are at least as effective as, yet safer than their classic ancestor (UFH).

Like UFH, ca 1/3 of the molecules of LMWH is active. However, contrast to UFH, only 25 – 50% of those molecules exhibit full activity, i.e. against both F.IIa & F.Xa. Hence, while the ratio of anti-F.IIa:anti-F.Xa is 1:1 for UFH, it is only 1:2 to 1:4 for LMWH. For both, 1 mg is approximately equivalent to 110 U.

- 6 Of the several oral anticoagulants (OAs) available, **warfarin** (coumadin, 4-OH-coumarin) is the most widely used one.

ALL *per se*, the antineoplastic therapy, complications of the underlying disease and its treatment, the multiple medications used, changes in oral intake and diet, TPN, age-related differences of the hemostatic system, and problematic monitoring issues render OAs unsafe and unreliable in children. Although protocols and nomograms for OA therapy in pediatrics have been proposed at the

last 3 ACCP Consensus Conferences (Michelson AD et al. 1995, 1998, Monagle P et al. 2001)⁽¹¹⁷⁻¹¹⁹⁾, the overall experience in pediatric cancer is hitherto too limited as to be recommended for use on a routine basis in this setting.

3.13.3.3 OCCLUSION OF CVLs

If the line is occluded, an attempt at restoring its patency should be first made:

- Prime each lumen with 1.5 – 3 ml UK diluted to 5,000 IU/ml; keep *in situ* for 2 – 4 h. Then try to aspirate. If the line has recanalized, which is usually the case, flush it out with 10 – 20 ml NS, and lock with additional dose of UK without further aspiration.
- Alternatively, UK at 150 IU/kg/h per lumen may be infused over 12 – 48 h. This approach is also successful most of the time.
- Yet another recently recommended option is rtPA. Guidelines for its local use are given in **Tab. 34** (Monagle P et al. 2001)⁽¹¹⁹⁾. This modality has been validated for efficacy and safety at MSKCC. To make it cost effective, the content of the original vial was divided into aliquots q 2.5 ml with 1 mg/ml of the drug, which were frozen at -20 °C for up to 30 days and thawed *ad hoc*. The technique proved effective in clearing occluded central venous access devices in 136/168 (81%) of the attempts in 121 patients without loss of activity of rtPA or adverse events, becoming a standard practice at MSKCC (Timoney JP et al. 2002)⁽¹²⁶⁾.

Table 34: Guidelines for Local Use of rtPA to Unblock CVLs

| BW (kg) | Treatment with rtPA by Type of CVL | | |
|---------|--|--|---------------------------|
| | 1-lumen CVL | 2-lumen CVL | SC Port |
| ≤ 10 | 0.5 mg diluted in NS qs to fill line | 0.5 mg per lumen diluted in NS qs to fill line. Treat 1 lumen at a time | 0.5 mg diluted in 3 ml NS |
| > 10 | 1 mg in 1 ml NS, qs to fill line, max: 2 mg/2 ml | 1 mg in 1 ml NS, qs to fill line, max: 2 mg/2 ml. Treat 1 lumen at a time | 1 mg diluted in 3 ml NS |

- Should the block persist, the CVL must be removed.

3.13.3.4 SYSTEMIC AT / TL THERAPY FOR DVT / PE

1 Antithrombotic therapy:

The overall duration of systemic antithrombotic (anticoagulant) therapy for VTE ± PE ranges from 3 months to indefinite, depending on associated risk factors, and whether the case is that of a single isolated episode or a recurrent event.

Heparin is the most commonly used anticoagulant in children.

i. UFH

- Loading dose: 75 U/kg IV over 10 min.
- Initial maintenance dose (infants < 1 yr of age): 28 U/kg/h CI.
- Initial maintenance dose (children > 1 yr of age): 20 U/kg/h CI.
- Dosage adjustment to maintain aPTT within 60 – 85 s (0.30 – 0.70 U/ml anti-F.Xa level)- refer to the nomogram proposed in the original article (Michelson AD et al. 1995)⁽¹¹⁷⁾.
- aPTT monitoring: 4 h post loading dose & any change in infusion rate.
- When aPTT is therapeutic, CBC & aPTT should be checked daily.

- The minimum duration of heparin therapy is 5 days in case of DVT, and 7 – 10 days or longer for extensive DVT/PE.
- ii. LMWH
- Extrapolated from adults, the therapeutic range for LMWH is an anti-F.Xa level of 0.5 – 1.0 U/ml 4 – 6 h after a SC injection.
 - Validated nomograms for monitoring enoxaparin & reviparin in pediatric patients to adjust therapeutic dosing by anti-F.Xa level have been also developed (Massicotte MP et. al. 1996 & 1997)⁽¹²²⁻¹²³⁾. However, this is usually necessary in the first few days of therapy. Once the targeted therapeutic range has been achieved, further monitoring should be rather guided by the clinical situation (extension of a thrombus, embolism on therapy, significant hemorrhage), otherwise 1 week after starting treatment and monthly thereafter.
 - Infants < 2 months of age or < 5 kg in weight have relatively increased requirements for LMWH than those > 2 months old or > 5 kg, likely reflecting altered heparin pharmacokinetics (larger V_d) and/or lower AT III levels during the first 2 months of life (0.50 U/ml at birth, reaching adult values by 3 months of age). **Tab. 35** shows the starting therapeutic and prophylactic dosing of enoxaparin and reviparin.
 - The duration of therapy with LMWH is at least 3 months.

Table 35: Starting Dosage of Enoxaparin / Reviparin by Age / Weight

| Starting Dosage | Enoxaparin | | Reviparin | |
|-----------------|-----------------|-----|----------------|-----|
| | mg/kg q 12 h SC | | U/kg q 12 h SC | |
| | Age (months) | | Weight (kg) | |
| | < 2 | > 2 | < 5 | > 5 |
| Therapeutic | 1.5 | 1.0 | 150 | 100 |
| Prophylactic | 0.75 | 0.5 | 50 | 30 |

- 2 OAT: The experience is lacking to make any recommendations in pediatric ALL.
- 3 It is expected that the conservative approach described could be effective. Vascular surgery (thrombectomy) should be rather the exception.
- 4 Systemic thrombolytic therapy (Michelson AD et al. 1995, 1998, Monagle P et al. 2001)⁽¹¹⁷⁻¹¹⁹⁾:
 - UK 4,400 IU/kg slowly IV as a loading dose followed by 4,400 IU/kg/h PI for 6 – 12 h.
 - Alternatively, rtPA can be given without loading dose at 0.1 – 0.6 mg/kg/h PI over 6 h.
 - Heparin may be started w/o a loading dose during or immediately upon completion of thrombolytic therapy- *vide supra*.

3.13.3.5 SINOVENOUS THROMBOSIS SVT

All children with or without prothrombotic risk factors who will be managed according to ALL IC-BFM 2002 are expected to be at increased risk for developing SVT. In one study, this has been reported to be 20% for children with and 2.2% for those without a known prothrombotic risk factor (Wermes C et al. 1999)⁽³³⁾. Specifically, this complication occurred more frequently under induction than reinduction therapy (14/17 vs. 3/17). In that study, the impact of CVC, otherwise a known prothrombotic risk factor, was negligible. The majority of leukemic patients

with cerebral venous sinus thrombosis initially presented with acute clinical symptoms such as headache, vomiting, hemiparesis and seizures, stupor or coma. Most commonly, the superior sagittal sinus was affected (Wermes C et al. 1999)⁽³³⁾.

The diagnosis of SVT can be made by MRI or CT using a contrast medium (Einhäupl KM et al. 1996)⁽¹²⁷⁾. Undoubtedly, the interventionalist will require a complete angiogram be performed.

Different therapeutic options are available. However, there are no randomized trials addressing the management of this event in children as to allow for providing firm recommendations with regard to the optimal strategy. One study randomizing heparin vs. placebo in 20 adult patients demonstrated a significant advantage for the heparin group in terms of survival and complete recovery (Einhäupl KM et al. 1991)⁽¹²⁸⁾. From a Canadian pilot study involving 30 children, on whom UFH was tested against LMWH, it follows that both drugs can be used to treat SVT. The study called for starting a randomized multi-center trial to assess the need for and feasibility of heparin therapy in the management of SVT (de Veber G et al. 1998)⁽¹²⁹⁾.

Yet another option is systemic thrombolytic therapy similar to that employed in DVT/PE- see section 3.13.3.4 .

Finally, many interventionalists would rather advocate for aggressive management with thrombolytic agents infused *in situ* via selective catheterization of the involved sinus or deep-seated vein. As of 1995, individual case reports and small series on 26 patients, mostly adults, have been published in the English-language literature. Among these, there were many young adults and very few children, the youngest being a 6-week-old infant. Most importantly, the technique proved to be highly effective and safe in the overwhelming majority of the patients so treated, even in those with pre-treatment, usually hemorrhagic infarction (Horowitz M et al. 1995)⁽¹³⁰⁾. This modality was also successful in a 4-yr-old boy with ALL and extensive DVT in the upper trunk that evolved into SVCS despite systemic thrombolytic therapy (Savage ShA et al. 2000)⁽¹³¹⁾.

Taken together, under these conditions and with the available knowledge, no standard approach can be recommended for use in pediatric patients with SVT. However, at least the data from studies on adults suggest that anticoagulation even in SVT associated with bleeding is warranted (Einhäupl KM et al. 1991)⁽¹²⁸⁾.

3.14 INFECTION PROPHYLAXIS & THERAPY

Children treated for leukemia are rendered significantly immunocompromised through chemotherapy. Infection is still a major cause of death in leukemia patients. Infection prophylaxis and therapy is the responsibility of the physician in charge. The discussion in this part is not intended to take over that responsibility, nor it should be interpreted as firm rules or binding guidelines. Many of the discussed issues may be also relevant to the SCT setting. However, these issues are the daily bread of the transplantation team, and are outlined in section 2.6.6.5 . Therefore, the discussion will be limited to infection prophylaxis & therapy in patients on conventional chemotherapy.

In addition to ANC & CRP it is useful to obtain pre-treatment a microbiologic survey including:

- Oral/mesopharynx & rectal swabs
- Swabs from mucocutaneous lesions
- Blood, urine & effusion cultures
- Ab profile: VZV, HSV₁, CMV, EBV, measles, HAV, HBV, HCV, HIV₁ & 2 (consent is required in some countries), Candida/Aspergillus Ab titer (unreliable)

- Candida Ag & galactomannan test (*Aspergillus* spp.)

On-therapy monitoring, particularly in HR patients, should include:

- ANC & CRP
- Oral/mesopharynx swabs
- Swabs from mucocutaneous lesions
- Blood cultures from every lumen of CVC
- If available: panfungal/specific fungal PCR, Candida Ag, galactomannan test (*Aspergillus* spp.)
- Candida/*Aspergillus* Ab titer (unreliable)

3.14.1 INFECTION PROPHYLAXIS

3.14.1.1 INTRODUCTORY REMARKS

The most effective yet least expensive infection prophylaxis measure lies in everybody meticulously washes hands before and following any contact with the patient. Detailed information of the patient/parents about neutropenia and about the risk of infection is also essential. Inverse isolation (single ward or cubicle, gown, gloves, mouth mask) does not reduce patient colonization with new agents significantly (Nauseef WM et al. 1981)⁽¹³²⁾. The major threat of infection for the patient comes from his/her own endogenous, potentially pathogenic flora. Constipation and (sub)ileus favor the growth of bacteria and fungi within the gut lumen and their invasion of the mucosa, especially if it is damaged by cytostatics or tumor infiltration. It is therefore important to promote bowel motion to ensure the daily passage of stool, e.g. with the aid of oral lactulose. In case of failure of softeners and prokinetic agents, then enemas must be early considered. However, care should be taken in the severely neutropenic patient to avoid injury of the anorectal mucosa, which could lead to cellulitis.

Let alone non-compliance, the use of oral non-absorbable antibiotics (colistin, polymyxin, gentamicin, neomycin, etc) for total or selective decontamination of the digestive tract can promote the emergence of resistant strains, and its efficacy has not been proven (Pizzo P 1993, Feusner JH et al. 1995, Graubner UB et al. 1999)⁽¹³³⁻¹³⁵⁾. Although oral antimycotic chemoprophylaxis with nystatin, amphotericin B or fluconazole can decrease colonization by most *Candida* spp., it does not reduce the incidence of systemic *Candida* and *Aspergillus* infections (Winston DJ et al. 1993)⁽¹³⁶⁾. Only systemic administration of low-dose AmB, e.g. 0.3 mg/kg/d or 1 – 1.5 mg/kg alternate day seems, at least in studies performed on adults, to reduce the incidence of systemic mycoses (Graubner UB et al. 1999)⁽¹³⁵⁾. The azoles can significantly enhance VCR-induced peripheral/autonomic neuropathy including constipation. Hence, neither selective digestive tract decontamination nor antifungals can be generally recommended as a standard for infection prophylaxis. Furthermore, the recommended use of SMZ/TMP for PCP prophylaxis offers possibly an equally good alternative for gut decontamination (Hughes WT et al. 1987, Feusner JH et al. 1995)^(137, 134).

3.14.1.2 PNEUMOCYSTIS CARINII PNEUMONIA (PCP) PROPHYLAXIS

All patients should receive prophylaxis against *Pneumocystis carinii* pneumonia with SMZ/TMP all through the intensive phase of chemotherapy (Hughes WT et al. 1987)⁽¹³⁷⁾. PCP prophylaxis should be provided also during the maintenance phase of treatment and continued for 3 – 6 months off therapy. In case of intolerance or

hypersensitivity to SMZ/TMP, pentamidine isethionate can be used via inhalation (Weinthal J et al. 1994)⁽¹³⁸⁾- see **Tab. 36**.

Table 36: Selected Options for PCP Prophylaxis

| Medication: Route | Dosage & Schedule |
|---|--|
| 1 SMZ/TMP: PO | <ul style="list-style-type: none"> • 25/5 mg/kg/d bid on 3 consecutive days/week • Apart from MTX as possible |
| 2 Pentamidine isethionate aerosol: 20 – 30 min inhal. | <ul style="list-style-type: none"> • < 4 yr: 150 mg/5 ml distilled water x1 monthly • > 4 yr: 300 mg/5 ml distilled water x1 monthly • In case of bronchospasm, 1 – 2 puffs of a selective β_2-sympatomimeticum applied before and after pentamidine inhalation are needed |

However, a number of other options of varying efficacy, side effects and cost are available for *Pneumocystis carinii* pneumonitis prophylaxis and treatment (Masur H 1992 & Groll AH et al. 2001)⁽¹³⁹⁻¹⁴⁰⁾. Alternatively, in case of allergy to SMZ/TMP, an attempt at desensitization may be made. Originally, a desensitization protocol was proposed and validated for sulfasalazine, i.e. salazosulfapyridine (Holdsworth CD 1981 & Purdy BH et al. 1984)⁽¹⁴¹⁻¹⁴²⁾, and later developed *per analogiam* for SMZ/TMP (Smith RM et al. 1987)⁽¹⁴³⁾, and successfully applied in a number of HIV-infected TMP/SMZ-intolerant patients (Gluckstein D et al. 1995)⁽¹⁴⁴⁾. The following approach may be recommended:

- ❖ If no skin rash emerges during desensitization, then the protocol detailed in **Tab. 37** should be completed.

Table 37: Standard Sulfa Desensitization Protocol for Uneventful Course

| Age [yr] | Day | SMZ Dose [ml] | SMZ Dose [mg] | Doses per Day | Stock Strength |
|----------|---------|---------------|---------------|---------------|--|
| | 1 | 0.25 | 1 | x1 | Pediatric suspension 5 ml = 200 mg SMZ diluted 1:9 in syrup: 1 ml = 4 mg SMZ |
| | 2 | 0.50 | 2 | x1 | |
| | 3 | 1 | 4 | x1 | |
| | 4 | 2 | 8 | x1 | |
| | 5 | 4 | 16 | x1 | |
| | 6 | 0.50 | 20 | x1 | Pediatric suspension 5 ml = 200 mg SMZ undiluted: 1 ml = 40 mg SMZ Use adult formulation 400 mg SMZ in 1 pill where appropriate |
| | 7 | 1 | 40 | x1 | |
| | 8 | 2 | 80 | x1 | |
| | 9 | 4 | 160 | x1 | |
| ≤ 12 | 10 | 5 | 200 | x1 | |
| > 12 | 10 | 10 | 400 | x1 | |
| ≤ 12 | 11 – 30 | 5 | 200 | x2 | |
| > 12 | 11 – 30 | 10 | 400 | x2 | |
| ≤ 12 | > 30 | full | full | x2 | |
| > 12 | > 30 | full | full | x2 | |

- ❖ If the desensitization process is interrupted for reasons not likely due to sulfa allergy, then a test dose of 100 mg SMZ for children ≤ 12 yr of age and 200 mg SMZ for those > 12 yr old may be given. If this dose is well tolerated, then dosing is restarted at 200 mg SMZ bid for the former and 400 mg SMZ bid for

the latter group and continued for 30 days. If uneventful, full dosage is to be given thereupon.

- ❖ If a skin rash appears during desensitization that is most likely due to sulfa, the protocol should be stopped. An alternative PCP program must be pursued.
- ❖ Transplant patients with sulfa allergy should undergo desensitization as early as possible. If this could not be performed before transplantation, then the desensitization procedure must be started post transplant when ANC > 500/ μ L has been sustained for 72 h or by day + 30 at the latest

3.14.1.3 VZV PROPHYLAXIS

Contact of ALL patients during chemotherapy with individuals sustaining varicella or zoster infection must be avoided (patient/parent instruction). However, if exposure does occur, there is a risk to contract the infection for 28 days, regardless of the serologic status, although seropositive patients do have a markedly decreased risk (Gershon A et al. 1989)⁽¹⁴⁵⁾. With regard to the post-exposure approach, there are no controlled trials so far that the prophylactic administration of specific immune globulin (VZIG) would provide any advantage compared to aciclovir or brivudin alone. In the individual case, the degree of immunosuppression at the time of exposure determines the therapeutic measures to be undertaken- see **Tab. 38** (Feldman S et al. 1987, Nyerges G et al. 1988, Asano Y et al. 1993, Wagstaff AJ et al. 1994, Rössig C et al. 1998)⁽¹⁴⁶⁻¹⁵⁰⁾. Children under intensive ALL-BFM chemotherapy should be considered at least intermediately immunosuppressed. During the incubation period, chemotherapy and steroids should not be interrupted. However, the patient who had come into contact with VZV infection must be separated from other immunocompromised hosts, while undertaking the measures outlined in **Tab. 38** and keeping the BC within the protocol guidelines.

Table 38: VZV Infection Prophylaxis

| Patient Status | Measures |
|---|---|
| <ul style="list-style-type: none"> • Patient had gone through varicella: history, scars, IgG (IgM) Ab titer • \pm Moderate-to-severe immunosuppression | <ul style="list-style-type: none"> • Close observation |
| <ul style="list-style-type: none"> • Patient had not gone through varicella • \pm Moderate-to-severe immunosuppression | <ul style="list-style-type: none"> • Quarantine for the period at risk of transmitting infection • 4 alternative options: <ol style="list-style-type: none"> 1) Aciclovir 80 mg/kg/d qid PO for at least 7 d, beginning by d 7 post exposure 2) Start with IV aciclovir for 2 – 3 d at 30 – 45 mg/kg/d tid, then continue PO as sub 1) 3) Brivudin 15 mg/kg/d tid PO for at least 7 d, beginning by d 7 post exposure 4) VZIG (12.5) – 25 – (50) U/kg IM/IV within 24 – 96 h post exposure; may be repeated after 5 d; close observation & quarantine for 28 d |

Although some have advocated active immunization for seronegative children with leukemia, the prerequisites for that (remission > 12 months; WBC > 1,200/ μ L; withholding chemotherapy for at least 2 weeks) make these recommendations impracticable. On the other hand, it is strongly recommended that all contact persons, first of all parents and siblings, who have not yet gone through the infection receive varicella vaccine (Schmitt HJ et al. 2000)⁽¹⁵¹⁾.

3.14.1.4 INFECTION PROPHYLAXIS IN HR PATIENTS

HR therapy entails considerable immunosuppression and mucosal damage that may in individual patients cause notable delays in treatment delivery. Realization of therapy as scheduled in the protocol could have a favorable impact on the overall prognosis of patients suffering an ALL relapse (Hartmann R et al. 1992)⁽¹⁵²⁾. Although the advantage of infection prophylaxis has not been proven with certainty, it is recommended that HR patients receive more intensive prophylaxis than those from the SR or IR group.

❖ G-CSF (Filgrastim[®])

Therapy with the recombinant human granulocyte growth factor (rh-G-CSF) reduces both the degree and duration of neutropenia following chemotherapy as well as the incidence of fever episodes during that period (Welte K et al. 1996)⁽¹⁵³⁾.

All HR patients receive G-CSF from the 7th day of each HR block. Therapy with G-CSF should be continued until the granulocyte count in PB exceeds 5,000/ μ L, for so high values will often sharply sink down as G-CSF is withdrawn. In case of a severe infection, G-CSF should be given for a longer period. By the start of the subsequent HR block, G-CSF must be withdrawn. G-CSF will not however be used in HR patients during or following Protocol I/II.

G-CSF will be given at a dose of 5 μ g/kg/d SC or exceptionally PI over 4 h.

In the context of protracted neutropenia with severe, life-threatening septicemias and/or systemic fungal infections, G-CSF must be generously used irrespective of the risk-group stratification or stage of treatment. In this case also, it should be given at 5 μ g/kg/d SC or exceptionally PI over 4 h.

❖ INFECTION PROPHYLAXIS – HR

Infection prophylaxis in HR patients should be implemented from the outset of through ca 4 weeks after the end of intensive therapy. PCP prophylaxis must however span the maintenance therapy as well. The prophylactic elements most relevant to the HR group are found in **Tab. 39**.

Table 39: Infection Prophylaxis in HR Patients

| Medication: Route | Dosage & Schedule |
|--|---|
| 1 SMZ/TMP: PO PCP | <ul style="list-style-type: none"> • 25/5 mg/kg/d bid on 3 consecutive days/week • Apart from MTX as possible |
| 2 Pentamidine aerosol: Inhalation over 20 – 30 min PCP, if hypersensitivity or intolerance to SMZ/TMP | <ul style="list-style-type: none"> • < 4 yr: 150 mg/5 ml distilled water x1/month • > 4 yr: 300 mg/5 ml distilled water x1/month • In case of bronchospasm: 1 – 2 puffs of a selective β_2-sympatomimeticum applied before and after pentamidine inhalation |
| 3 Colimycine: PO or Paromomycine: PO | <ul style="list-style-type: none"> • 100,000 U/kg qid • 25 – 50 – 75 mg/kg tid – qid |

| | |
|--|--|
| SDD, if hypersensitivity or intolerance to SMZ/TMP | |
| 4 | Amphotericin B: PO [†] or Natamycine (Pimafucin): PO [†] |
| 5 | Oral hygiene |
| 6 | Dental hygiene |
| 7 | Fare |

- < 3 yr: 100 mg (1 ml) q 6 h
- > 3 yr: 200 mg (2 ml) q 6 h
- 12.5 – 25 mg q 4 – 6 h

- Wash oral cavity with disinfectant & alkaline solutions
- Cover lesions with astringents
- Careful & regular cleaning of periodontium & teeth w/o causing injury, using a soft toothbrush or a water pick
- In severe thrombocytopenia or vulnerable gingiva:
Don't use toothbrush
Rinse with chlorhexamed to dissolve plaques
Apply astringents
- During HR block & subsequent neutropenia:
Only germ-poor, well cooked food
No fresh fruit, salads or vegetables

† To be washed in mouth & swallowed

3.14.2 SELECTED COMPLICATIONS OF THERAPY

Chemotherapy is associated with many adverse effects & complications of varying severity & clinical relevance. It is the responsibility of the physician in charge & nursing staff to anticipate, early recognize, correctly assess, and properly manage these events, and closely cooperate with other experts, if need be. The national study coordinator should stand ready to help in particularly precarious situations.

3.14.2.1 UDT / URT MUCOSITIS & INFECTION

Although the symptoms and signs as well as the clinical context usually pinpoint exactly the diagnosis, swabs from oropharyngeal lesions for bacterial and fungal cultures and for antibiogram should be always performed. HSV must be also investigated through PCR & culture (lesion swabs & pharyngeal washings). Hoarse voice will often suggest laryngeal moniliasis, while dysphagia and retrosternal pain indicate either mucositis (esophagitis) or infection involving the esophagus (Candida spp., HSV, less frequently CMV). Esophagoscopy is helpful in at least suggesting the diagnosis via a close-up view of the lesions, and in obtaining material for cultures.

Management guidelines

1. Don't use hexetidine on open lesions, as it inhibits fibroblast sprouting.
2. Don't rinse the mouth with leucovorin, as it could be resorbed locally & via GIT.
3. Wash the mouth e.g. with a mixture of Maalox suspension : 2% viscous lidocaine : 5% panthenol solution (1 : 1 : 1).
4. Cover open lesions with astringents.
5. Extensive thrush lesions refractory to intensive topical therapy including AmB solution x6 daily are indicated for low-dose systemic AmB, i.e. 0.1 – 0.5 mg/kg/d PI over 4 h for 5 – 7 days (Büchner T et al.)⁽¹⁵⁴⁾.
6. For definitively proven HSV: aciclovir 30 – 45 mg/kg/d tid PI over 1 h for 5 days.
7. In case of extensive inflammation or necrosis of the periapical gingiva, an antibiotic active against anaerobes (metronidazole, clindamycin) must be given.
8. If Candida spp. laryngitis is suspected, consider either fluconazole 10 – 12 mg/kg qd PO, or low-dose AmB as sub 5.
9. Adequate analgesia to relieve pain.

3.14.2.2 ABDOMINAL PAIN

The differential diagnosis of abdominal pain is too broad already in the general pediatric population and is still broader in children with malignant diseases undergoing complex and toxic treatments. Many medical and surgical situations may arise that will range from trivial and benign to debilitating, life-threatening or even fatal events. Great attention should be paid to abdominal complaints and signs, prompting performing diagnostic tests and procedures quickly in an effort to establish the diagnosis as accurately as possible. Appropriate therapy must be provided without undue delays, not infrequently side by side with the ongoing diagnostic work-up. This is necessary to avoid a catastrophe and to improve the outcome.

Every effort should be made to prevent weight loss of > 10% against baseline. In general, enteral nutrition is preferred. However, virtually all GI and medical or surgical abdominal problems such as nausea, vomiting, diarrhea, subileus/ileus, pancreatitis, surgical interventions within the abdominal cavity or on the gut, etc require some degree of oral-nutrition restriction, diet, or, not infrequently, absolute bowel rest for a variable period of time, in which case either partial or total parenteral nutrition (PPN/TPN) should be instituted without delay. This, in addition to realimentation, is in the realm of the dietician and pharmacist.

The following 4 sections (3.14.2.3 – 3.14.2.6) will focus only on few abdominal therapy-related complications. The reader is encouraged to refer to many excellent widely available textbooks, monographs and review articles.

3.14.2.3 GASTRITIS & GASTRODUODENAL ULCER

1. In general, medications used to reduce HCl secretion in the stomach as well as mucoprotective agents and prokinetic drugs should be used 0.5 – 1 h before meals and at bedtime. By contrast, it is preferable to administer analgesics and antipyretics postprandially.
2. The dosage & schedule of the individual drugs should be adjusted to the patient's individual needs, however capped to *dosis singula maxima et dosis maxima pro die*. Follow the manufacturer's instructions in this regard.
3. Ranitidine is an H₂-receptor antagonist, generally well tolerated with minimal side effects and limited drug interactions at the level of cytochrome P450. Dosage: 1 mg/kg q 12 h IM, or preferably slowly IV/IV infusion over 30 min (risk of bradycardia & I° AV block), or 2 mg/kg q 12 h PO. By GFR < 50 ml/min, give at 50% dosing, e.g. qd.
4. Alternatively, in children > 6 yr of age, famotidine 0.5 mg/kg (15 mg/m²) q 12 – 24 h PO/IV (slowly or preferably by IV infusion over 30 min) can be used. Famotidine has a longer t_{1/2} compared to ranitidine, and its dosage should be halved by GFR < 30 ml/min.
5. Proton-pump inhibitors (omeprazol, esomeprazol, pantoprazol, lansoprazol) via binding to the sulfhydryl groups block irreversibly H⁺/K⁺ ATPase in gastric parietal cells. Interference at P450 is overall limited (relatively most marked for omeprazol). Omeprazol is given at 0.25 mg/kg/d qd by brief IV infusion, or 0.5 – 1 mg/kg/d bid PO. Pantoprazol may be given at 0.25 – 0.5 mg/kg (max 40 mg) PO before breakfast.
6. Sucralfate (saccharose aluminum octasulfate) is a mucoprotective agent of complex mode of action. It is most effective when given on empty acidic stomach, so that it makes no sense to use the drug together with antacids, H₂-receptor blockers or proton-pump inhibitors. In addition, sucralfate can "buffer" many drugs, thereby reducing their bioavailability. Hence, it may be only used 2 – 3 h

apart from other oral medications. Side effects are rare (2%). Long-term use is contraindicated in end-stage renal failure because of the risk of aluminum nephropathy. Dosage: 0.25 – 2 g PO bid – qid. The suspension and granules should be given priority before pills.

3.14.2.4 ACUTE PANCREATITIS AP

In pediatric ALL, acute pancreatitis will be most commonly caused by drugs (L-ASP, steroids, 6-MP, H₂-receptor blockers, furosemide, sulfonamides, metronidazole, erythromycin, tetracycline, pentamidine, paracetamol, salicylates, etc), or infection (mumps, measles, AV, ECHO-V, EBV, CMV, HAV, HBV, HCV, Mycoplasma, bacteria, fungi or parasites). It may be also encountered within the multi-organ failure syndrome (MOFS), which can in turn evolve from AP. Hypercalcemia of malignancy (HM) may be complicated by pancreatitis, too. Finally, ALL does not spare any organ, so that the pancreas may be infiltrated by blasts either at presentation, relapse or *ante finem*.

The disease may vary in severity from mild and transient, which is the case in 80 – 90% of L-ASP-induced AP, to necrotizing and hemorrhagic in a minority of the patients (Garrington T et al. 1998)⁽¹⁵⁵⁾. Particularly the latter form is notorious in its dramatic clinical picture and catastrophic course with shock, MOFS and death. Kept in mind, the diagnosis is easy to make in time.

Management

Based on several randomized trials in adults, almost all elements of therapy of AP are at best controversial or even of no proven benefit (Steinberg W et al. 1994)⁽¹⁵⁶⁾. Nevertheless, the current "good clinical practice" includes:

1. Withdrawal of the offending drug.
2. Management tailored to severity & complications.
3. Management at PICU (severe cases).
4. Bowel rest & gastric drainage via NGT.
5. TPN with sufficient calories & regular insulin.
6. Broad-spectrum antibiotics & antifungals.
7. H₂ blocker.
8. Aprotinin: 200,000 – 500,000 IU by short IV infusion as loading dose, followed by 100,000 IU infused over 3 – 5 h, then 100,000 IU qd.
9. Somatostatin-14 or preferably its superactive analogue octreotide: The optimal dosage for children with acute pancreatitis remains to be defined or possibly individualized. It will probably be 125 – 250 µg slowly IV as loading dose, followed by continuous IV infusion at 4 µg/kg/h up to 250 µg/h for the former, and ca 5 – 10 µg/kg/d bid or tid SC for the latter. Therapy may be needed for 3 – 5 – 7 days.
10. In case of hypocalcemia: 10% Ca gluconate slowly IV.
11. Peritoneal lavage ± intraperitoneal aprotinin.
12. Organ-system targeted therapy as needed, e.g. mechanical ventilation in case of lung injury & respiratory failure, drainage of pleural effusion, management of shock, DIC, etc.
13. Surgical débridement of the infected or all necrotic tissue; sump drainage; marsupialization of pancreatic pseudocyst.

3.14.2.5 TYPHLITIS

Known also as neutropenic enterocolitis, necrotizing enterocolitis (enteropathy), or ileocecal syndrome, typhlitis is a relatively rare, but well-recognized clinico-pathologic entity most commonly encountered during periods of protracted and profound neutropenia in leukemic patients, who within the antecedent 4 weeks have received or are just undergoing aggressive chemotherapy. Typhlitis has been also described a median of 2 weeks following allogeneic or, less frequently, autologous SCT, again mostly performed for hematologic malignancies. However, a minority of cases occurred under similar conditions in aplastic anemia, Letterer-Siwe disease, solid tumors, & HIV infection. In addition to individual case reports, small series and retrospective reviews have been published, and the literature reviewed (Wagner ML et al. 1970, Shamberger RC et al. 1986, Katz JA et al. 1990, Sloas MM et al. 1993, Urbach DR et al. 1999, Otaibi A et al. 2002)⁽¹⁵⁷⁻¹⁶²⁾.

The pattern of incidence has changed over the last 4 decades. Historically, the diagnosis of typhlitis was made more often *ante finem* and/or *post mortem*. Although this still occurs, the predominant scenario has become intensive-chemotherapy-induced BM aplasia/severe neutropenia. Only rarely the disease has been reported to occur at presentation of acute leukemia prior to cytoreductive therapy or during maintenance chemotherapy. The common denominator for all patient subgroups is a status of immune compromise and neutropenia. It seems that ARA-C, VP-16, vinorelbine, taxol & docetaxel are particularly enterotoxic.

The cecum is always, although not necessarily exclusively, involved; hence the name typhlitis. However, the terminal ileum and/or the ascending colon may be also affected. Sometimes, the appendix is involved as well. Elsewhere, discrete or confluent ulcers may be scattered throughout the intestine.

Typhlitis should be always considered within the differential diagnosis in the neutropenic, immunocompromised patient presenting with right-lower-quadrant or diffuse abdominal pain, tenderness and distension, along with fever, often watery or bloody diarrhea, and, not infrequently, with nausea or vomiting. Imaging studies (CT, US, plain radiography) are helpful in 50 – 85% of the time. Blood & stool cultures, CBC, CRP, S-electrolytes, urea, creatinine, albumin, glucose, S/U-osmolality & urinalysis are mandatory. The patient should be closely monitored for vital functions and complications (sepsis, septic shock, DIC, appendicitis, peritonitis, bowel perforation, persistent enterorrhage, etc).

Management

1. The mainstay of management in the overwhelming majority of cases is conservative treatment for the typhlitis itself and some of its medical complications. In one study (Shamberger RC et al. 1986)⁽¹⁵⁸⁾, typhlitis was identified in 25/77 children with AML. Of the 5 patients who underwent surgery, 1 died on the 14th postoperative day due to miliary TB. Of the 20 patients managed medically, 2 died: one, for whom surgery was deferred because of refractory underlying disease, died of typhlitis with necrosis extending from the cecum to the sigmoid; the other, whose disease was also unresponsive, died of fungal pneumonia. In another study from SJCRH (Sloas MM et al. 1993)⁽¹⁶⁰⁾, 24 children treated for cancer (mostly acute leukemia) were diagnosed to have typhlitis. Of these, only 1/21 managed medically vs. 1/3 taken to surgery expired.

- Bowel rest
- TPN

- Broad-spectrum antibiotics to cover first of all Gram-negative intestinal flora including Pseudomonas, Klebsiella, coliform bacilli, Enterococci, etc
 - Amphotericin B (Fungi are found substantially more frequently at autopsy than *in vivo*, i.e. in blood cultures)
 - Therapy of shock
 - Therapy of DIC
 - Other: analgesics, antipyretics, ice bags
2. Surgical exploration should be reserved for some complications, e.g. appendicitis, peritonitis, bowel perforation, liver abscess, adhesions, intractable enterorrhage or hematochezia persisting beyond resolution of neutropenia and thrombocytopenia and in spite of adequate hemostasis, etc. The type and extent of surgery will be dictated by the concrete complication and findings, and may include appendectomy, bowel resection, peritoneal lavage with instillation of antibiotics, drainage, etc.

3.14.2.6 HEPATOSPLENIC CANDIDIASIS HSC

A retrospective analysis involving 562 adults with acute leukemia revealed 38 (6.8%) cases of hepatosplenic candidiasis (chronic systemic candidiasis). The incidence was more than 2-fold higher in ALL (11.3%) than AML (5.1%), and increased 5-fold during the 14-yr study period (1980 – 1993). A total of 11 patients died within 3 months after the diagnosis of HSC, with the infection being confirmed in all at autopsy: 7/16 with newly diagnosed leukemia, 4/10 with refractory or relapsed leukemia, but 0/12 if the disease was in remission prior to infection, thus underscoring the impact of the disease status on outcome (Anttila VJ et al. 1997)⁽¹⁶³⁾. The true incidence of HSC in children is not known. However, all investigators agree that it has been growing over the last 20 years in both adults and children (Sobel JD et al. 1990, Sallah S 1999)⁽¹⁶⁴⁻¹⁶⁵⁾. On the one hand, the increased astuteness and experience of clinicians along with the availability of more sophisticated diagnostic techniques as well as the better expertise in imaging & pathology have resulted in more patients being diagnosed with HSC. On the other hand, the wider use of aggressive chemotherapy regimens and SCT to treat hematologic malignancies leads to profound immunosuppression, prolonged periods of severe neutropenia and more frequently causes serious damage to epithelial cells. Under these conditions, *Candida* spp. more efficiently colonize the GI mucosa and more easily seed the portal vein in order to settle in the liver, spleen, kidneys and other organs.

By pooling the most relevant data from the literature (von Eiff M et al. 1990, Semelka RC et al. 1992, Anttila VJ et al. 1994, Gorg C et al. 1994, Anttila VJ et al. 1997, Sallah S et al. 1998, Colovic M et al. 1999, Hebart H et al. 2000)⁽¹⁶⁶⁻¹⁷³⁾, a number of conclusions and recommendations can be drawn:

The criteria for definitive diagnosis of HSC are:

1. Blood culture positive for *Candida* spp.
2. and/or liver biopsy positive for *Candida* spp. or pseudohyphae.
(Laparoscopy-guided biopsy is substantially more rewarding than random liver biopsy).
3. Imaging studies showing focal lesions in the liver and/or spleen that are consistent with HSC.
(Order of sensitivity & efficiency: MRI > CT > US).

However, from a practical point of view, a presumptive diagnosis of HSC can be made, based on compatible evidence from imaging studies within the appropriate clinical context, which justifies the initiation of pre-emptive antifungal therapy. Clinical features and lab tests to be taken into account include:

- Fever unresponsive to appropriate broad-spectrum antibiotic therapy in an immunocompromised and neutropenic patient consequent on intensive chemotherapy.
- Fever emerging or recurring about 2 weeks post chemotherapy, when the patient is just recovering from neutropenia.
- Abdominal symptoms and signs that may include pain, discomfort, dyspepsia, nausea, anorexia, occasionally vomiting, hepatomegaly, splenomegaly.
- Focal lesions in the liver, spleen, and sometimes in the kidneys, best appreciated approximately 2 weeks after chemotherapy, coinciding with onset of regeneration of granulopoiesis. The sensitivity & efficiency of imaging studies depend on the timing of the study as well as on the technique and protocol applied, ranging from > 90% for Gd-FLASH MRI to < 50% for US.
- Positive LFTs: Particularly valuable is elevation of ALP & GGT, which occurs early in Candida septicemia, often already during aplasia, being typically out of proportion to elevation of ALT, AST or bilirubin.
- Although non-specific, a CRP > 50 mg/L may be also an early indicator of HSC.
- Panfungal PCR-based assay is also highly sensitive and predictive of systemic fungal infection including deep-seated candidiasis such as HSC. In one prospective study, the assay was positive in > 1/3 of the neutropenic episodes at risk for invasive fungal infection, with all patients subsequently developing a proven infection found to be positive. In addition, PCR positivity was the earliest indicator of invasive fungal infection, preceding its clinical manifestation by a mean of 5.75 days (range: 0 – 14).

Principles of management

1. The optimum duration of treatment has not been, and possibly cannot be determined fix for all patients. However, long-term therapy for many weeks to months is usually needed. An occasional patient may require > 12 months of treatment. Therapy should be continued until all lesions reliably have cleared up or healed over- *vide infra* (sub 6).
2. Antifungal therapy should be individualized as to choice of drugs, too.
3. Since it is clear that the infection could not be eradicated within a couple of weeks, the conventional (desoxycholate) formulation of AmB is probably not optimal by virtue of its prohibitive nephrotoxicity. Therefore, ABCD (Amphocil[®]), ABLC (Abelcet[®]) or liposomal AmB (Ambisome[®]) should be considered at 2.5 – 5 mg/kg/d by IV infusion for at least the first 6 – 8 weeks of treatment.
4. Alternatively, fluconazole may be given at 8 – 12 mg/kg (max 16 mg/kg) PO (or initially by IV infusion over 0.5 – 1 h) qd for children up to 12 yr old and 400 – 800 mg/d for children > 12 yr of age.
5. Only in case of proven fluconazole-resistant *Candida* spp., along with deterioration of the patient's status that is clearly related to progression of HSC, itraconazole cyclodextrin suspension at 2.5 mg/kg bid PO (for children > 6 months of age) may be tried. Voriconazole and other novel antifungals are now available in some countries. The experience in pediatrics is however too limited as to allow for any recommendations to be made.

6. Therapy should be monitored at 2-week intervals initially (for the first 6 – 8 weeks) and at 4 – 6-week intervals thereafter with the aid of high-resolution imaging techniques, first of all specific MRI protocols or contrast-enhanced CT (Semelka RC et al. 1992)⁽¹⁶⁵⁾ & (Sallah S et al. 1998)⁽¹⁶⁹⁾.
7. If after the initial 2 weeks of therapy the patient is in a satisfactory condition, tolerating the treatment well, and the lesions are at least stable, the same therapy can be continued.
8. Should there be, at any time, evidence of disease progression and/or serious therapy-related toxicity, the patient must be switched over from AmB to fluconazole, or *vice versa* (sub 3 & sub 4).
9. If the patient has been improving and the lesions receding steadily over the first 6 – 8 weeks, the initial therapy may be continued. However, the majority will rather continue with fluconazole as sub 4.
10. Exceptionally, drainage of liver abscess(es) or splenectomy may be ultimately needed.
11. Provided the patient is stable and HSC is being adequately managed or well controlled, he/she can safely continue with intensive chemotherapy and even proceed to SCT (Katayama K et al. 1994, Bjerke JW et al. 1994, Groll AH et al. 2001)⁽¹⁷⁴⁻¹⁷⁶⁾.

3.14.2.7 FEVER WITH NEUTROPENIA

Definition

- A single oral temperature $\geq 38.5^{\circ}\text{C}$, or $> 38^{\circ}\text{C}$ lasting for at least 1 h, or 3 spikes of $> 38^{\circ}\text{C}$ at least 4 h apart during a 24-h period. Axillary temperature is a median of 0.5°C lower, whereas the rectal is a median of 0.6°C higher than oral temperature. Core body temperature is close to the rectal. Rectal temperature measurement is discouraged in the neutropenic patient because of the potential risk of perirectal cellulitis (Hughes WT et al. 1997)⁽¹⁷⁷⁾.
- ANC $< 500/\mu\text{L}$ in PB.

Diagnostics

The extent of diagnostic tests is dictated by the clinical situation, and may include:

- Cultures: blood (PB & from every CVC lumen), stool (including *Clostridium difficile* toxin), urine.
- Swabs: throat, anus, skin & mucous membrane lesions.
- By availability: fungal PCR, *Candida* spp. Ab/Ag, *Aspergillus* spp. Ab/Ag, direct identification of fungi (Ab titer assessment cannot be relied on).
- Ab titer: HSV, CMV, EBV, etc (whenever possible: IgA, **IgM**, IgG).
- Virus isolation: mucocutaneous lesions, urine, feces.
- Imaging studies: CXR/CT for pulmonary symptoms/signs, US in case of abdominal problems.
- In case of pulmonary symptomatology, a pathologic finding on CXR/CT and fever unresponsive to appropriate antibiotic therapy, then a diagnostic bronchoalveolar lavage is recommended.

Therapy: broad-spectrum antibiotics

Antibiotic therapy must be tuned to the individual patient's unique situation and to the specific antibiotic-resistance pattern of the given institution, department and hematology/oncology unit or division. The following are only examples of possible options:

- Consider one of the following alternatives to start with:

- Aminoglycoside + 3rd generation cephalosporin, e.g. cefotaxime, ceftazidime, ceftriaxone, ceftizoxime (? cefoperazone, cefsulodine).
- Monotherapy with 3rd or 4th generation cephalosporin, e.g. cefotaxime, ceftazidime, ceftizoxime, ceftriaxone, cefepime, ceftiprome, provided that ESBL strains are not of concern.
- Aminoglycoside + broad-spectrum/extended-spectrum penicillin, i.e. either a carboxypenicillin ± β -lactam inhibitor or an ureidopenicillin ± β -lactam inhibitor, e.g. ticarcillin/co-ticarcillin (ticarcillin : clavulanic acid = 15:1) or piperacillin/co-piperacillin (piperacillin : sulbactam = 8:1), mezlocillin, azlocillin.
- In case of known or suspected β -lactam-resistant Staph. aureus / Staph. mitis strains or other virulent Gram-positive bacterial species/strains (mucositis, CVC, abdominal symptoms), then:
 - Add a glycopeptide antibiotic to the initial empiric antibiotic regime: either vancomycin 40 mg/kg/d qid by IV infusion over 30 – 60 min, or teicoplanin, which is less toxic and pharmacokinetically more favorable than vancomycin. Teicoplanin is given at 10 mg/kg (IM/IV/brief IV infusion) q 12 h x3 as a loading dose, and then continued at 6 – 10 mg/kg qd. The dosage of both glycopeptides should be tailored to renal function (follow manufacturer's instructions).
 - Consider removal of a colonized CVC.
- If after 48 h the patient has not defervesced, or the parameters of inflammation and/or clinical symptoms have not receded, antibiotic therapy should be extended to include e.g. imipenem/meropenem + vancomycin/teicoplanin.
- If in spite of (3) – 5 – 7 days of appropriate antibiotic therapy the fever persists or reappears, then IV AmB must be added.
- Should anaerobic infection be suspected: + clindamycin or metronidazole.
- In case of a Gram-negative infection, the monobactam antibiotic aztreonam (100 – 150 mg/kg/d q 6 or 8 h IM/IV/brief IV infusion) can provide a less toxic alternative to aminoglycosides, particularly if nephrotoxicity and/or ototoxicity are of concern. Since aztreonam is not cross-reactive with other β -lactams, it may be used also in penicillin- or cephalosporin-allergic patients.
- The duration of antibiotic therapy is guided by the clinical status. Usually, 7 – 14 days are needed. However, this will vary considerably from one case to another. Factors to be considered when deciding on whether to discontinue antibiotics are:
 - The patient afebrile for > 24 h.
 - Good general condition.
 - Inflammatory markers significantly receding or inverting to negative.
 - ANC > 500/ μ L in PB and showing a rising trend even though the site of infection has not been ascertained.
- In recent years IV or even oral antibiotics are being explored in the outpatient setting in low-risk patients who are only mildly-to-moderately immunocompromised and without serious co-morbid conditions, and who are clinically stable and expected to recover their neutrophil count within 5 – 7 days. However, this approach cannot be recommended for use on a routine basis, and should be rather the exception.

3.14.2.8 SYSTEMIC (INVASIVE) FUNGAL INFECTION

If a systemic mycosis is merely suspected or proven as well as in case of fever unresponsive to appropriate antibiotic therapy given for (3) – 5 – 7 days, then AmB

should be initiated without delay (**Tab. 40**) while utilizing every available means to identify the yeast and document the site(s) of infection. Other assays are also useful, particularly *Candida* spp. Ag, *Aspergillus* spp. Ag and fungal PCR. On the other hand, antifungal Ab titers are of limited value.

The mainstay of management of invasive mold infection is AmB. If renal function is intact, the conventional (desoxycholate) formulation of the drug can be safely used. By contrast, in case of pre-existent renal dysfunction, or if the patient is being on therapy with additional nephrotoxic medications as well as if nephrotoxicity develops during therapy with standard AmB, then a lipid formulation of the drug should be used. These include ABCD (Amphocil[®]), ABLC (Abelcet[®]) and liposomal AmB (Ambisome[®]). The optimal dosage for these formulations in different types and locations of systemic fungal infection has not been defined with certainty. However, most commonly they are applied at 2.5 – 5 mg/kg/d qd by IV infusion.

Therapy with AmB should be continued until resolution of all pathologic findings. In addition, many patients will need some form of surgical treatment to eradicate the infection, e.g. débridement or resection of residual lesions.

Provided that the infection is curtailed, with evidence of at least partial response to appropriate antifungal therapy, systemic mycosis does not present an absolute contraindication for further chemotherapy. Under these conditions, some patients could proceed to SCT. However, the decision should be made on an individual basis (Groll AH et al. 2001)⁽¹⁷⁶⁾.

Table 40: Amphotericin B (AmB) for Systemic Mold Infection

| | |
|--|--|
| <ul style="list-style-type: none"> • AmB desoxycholate | <ul style="list-style-type: none"> • IV infusion (4 h) • Starting dose: 0.1 – 0.5 mg/kg/d • Stepwise increments: 0.1 – 0.5 mg/kg/d • Target dose: 0.5 – 1 – 1.5 mg/kg/d • Severe infection: increase directly from starting dose (0.1 – 0.5 mg/kg/d) to target dose (0.5 – 1 – 1.5 mg/kg/d) • AmB is incompatible with NaCl • AmB should be given at least 8 – 10 h apart from granulocyte transfusion to avoid risk of pulmonary leukostasis & associated ALI/ARDS • Substitute KCl & NaCl losses as needed • Adequate hydration to prevent nephrotoxicity • Some authors advocate prophylactic measures to reduce risk of nephrotoxicity: <ol style="list-style-type: none"> 1. Paracetamol 10 mg/kg PO 0.5 h prior to AmB 2. ± Hydrocortisone 25 mg IV 0.5 h prior to AmB 3. Pentoxifylline 5 – 7 mg/kg/d by CI |
| <ul style="list-style-type: none"> • Liposomal AmB (Ambisome[®]) | <ul style="list-style-type: none"> • (0.5) – (1.5) – 4 – (6) mg/kg/d qd IV infusion (4 h) |

3.14.2.9 THERAPY OF VZV & HSV INFECTION

Profound defects in cell-mediated immunity consequent on chemotherapy or SCT put patients at high risk for developing primoinfections and reactivation of hitherto latent viral infections that may cause serious morbidity, e.g. pneumonitis, hepatitis,

encephalitis, etc, and can even kill the patient. This is particularly true of, but not limited to herpesviridae.

The diagnosis of VZV & HSV infection is based on the clinical picture (\pm imaging studies), Ab titers, PCR assay & cultures.

Duration of treatment of an active infection (primary & secondary as well) is variable, and will range from (5) – 7 – 10 days for a simple disease, e.g. shingles to 14 – 21 days for a complicated one, such as encephalitis. Therapy of active VZV or HSV infection is outlined in **Tab. 41** below.

Table 41: Therapy of VZV & HSV Infection

| | |
|--|--|
| <ul style="list-style-type: none"> • Manifest/breakthrough infection: <ul style="list-style-type: none"> ❖ Varicella ❖ Herpes zoster ❖ Herpes simplex | <ul style="list-style-type: none"> • Strict isolation until all blisters have dried up • The drug of first choice is aciclovir: 30 – 45 mg/kg/d tid by IV infusion (1 h) or 20 mg/kg (max 800 mg) q 6 h PO for a minimum of 5 d (until all blisters crust dry) • Alternative virostatic agents include: <ol style="list-style-type: none"> 1. Valaciclovir = L-valin ester of aciclovir: 250 – 500 – 1000 mg q 8 h PO (by weight) 2. Brivudin 5 mg/kg q 8 h PO 3. Famciclovir: 125 – 250 – 500 mg q 8 h PO (by weight) • Aciclovir-resistant infection: <ul style="list-style-type: none"> Foscarnet: 40 mg/kg q 8 h by IV infusion over 2 h Cave: nephrotoxic; adjust dosage to CL_{cr}. Cave: risk of hypocalcemia; drug interaction with pentamidine (severe hypocalcemia) |
|--|--|

3.14.2.10 THERAPY OF SEVERE CMV INFECTION / CMV PNEUMONITIS

CMV may cause a number of distinct diseases in the immunocompromised host. These include the infectious mononucleosis syndrome, esophagitis, colitis, hepatitis, pneumonitis, retinitis & encephalitis.

Depending on the site of infection, the diagnosis is made on the basis of a constellation of clinical features, results of imaging, ophthalmoscopic or fibroscopic examination, lab findings, CMV Ab titers (**IgM** & IgG by ELISA), CMV Ag, particularly the matrix phosphoprotein pp65 with Clonab CMV, cytopathic effect in cell culture, histology, demonstration of viral DNA by in situ hybridization or genomic PCR technique. Particularly for patients undergoing intensive chemotherapy or SCT, the most useful assays to assist in decision-making with regard to institution of antiviral therapy and for monitoring its efficacy are those that are highly sensitive (to detect active infection early and predict the potential development of disease), quick enough (within few hours to one day) as to aid in the timely initiation of treatment prior to evolution of the secondary, often devastating immune response cascade, as is the case in CMV pneumonitis, efficient and (semi)quantitative as to allow for monitoring and optimizing therapy. The prognostic value of the viral load has been firmly established. These criteria are best met by RQ-PCR-based techniques, and assays that detect specific viral proteins, such as pp65, by monoclonal antibodies. The intelligent interpretation of the results is also important in discriminating between primary and secondary infection (reinfection or reactivation), which is especially pertinent for serologic tests (de Jong MD et al. 1998)⁽¹⁷⁸⁾.

Management of severe CMV infection is summarized in **Tab. 42** below.

Table 42: Management of Severe CMV Infection

| Medication | Dosage & Schedule |
|--|---|
| <ul style="list-style-type: none"> Ganciclovir | <ul style="list-style-type: none"> 5 mg/kg q 12 h by IV infusion over 1 h Adjust dosage to CL_{cr} Follow manufacturer's instructions For $ANC < 1,000/\mu L$, 3 options available: <ol style="list-style-type: none"> Withhold ganciclovir until recovery (usually impractical) Give G-CSF or GM-CSF & continue ganciclovir Switch over to foscarnet |
| <ul style="list-style-type: none"> Foscarnet | <ul style="list-style-type: none"> Indicated in case of intolerance, allergy or resistance to ganciclovir as well as if $ANC < 1,000/\mu L$ Start at 20 mg/kg by IV drip over 30 min as loading dose Continue at 60 mg/kg q 8 h or 90 mg/kg q 12 h over 2 h or 200 mg/kg/d by continuous IV infusion Cave: nephrotoxic; adjust dosage to CL_{cr} Follow manufacturer's instructions Cave: risk of hypocalcemia & drug interaction with pentamidine (severe hypocalcemia) |
| <ul style="list-style-type: none"> 7S hyperimmune Ig = 25 – 50 PEI/ml[†] | <ul style="list-style-type: none"> 100 U/kg/d = 200 mg/kg/d for several days Slow IV infusion (max at 60 ml/h) Follow manufacturer's instructions |

[†] Unit of the Paul-Ehrlich-Institute (PEI) reference preparation

1 ml = 25 U = 50 mg (e.g. Cytogam[®])

1 ml = 50 U = 100 mg (e.g. Cytotect[®] / Megalotect[®])

3.14.2.11 THERAPY OF PNEUMOCYSTIS CARINII PNEUMONIA PCP

Reclassified among molds on the basis of molecular genetic and phylogenetic studies, *Pneumocystis carinii* is the most important causative agent of opportunistic infection in the immunocompromised host with severe quantitative or qualitative defects in cell-mediated immunity, particularly those involving T-cell number and/or function. The historical experience at SJCRH from the pre-chemoprophylaxis era had shown morbidity rates as high as 22 – 43% across the individual subgroups of patients with primary ALL (Hughes WT et al. 1975)⁽¹⁷⁹⁾, and 25% in STS patients receiving CPM, DOX, AMD & VCR (Hughes WT et al. 1978)⁽¹⁸⁰⁾. Although less well surveyed, the infection has been described in adult or pediatric patients who had received dose-intensive chemotherapy, pharmacologic doses of steroids ± radiation therapy for lymphoma or brain tumors as well as following treatment with FDA, DCF or CDA. The disease has been also reported after allogeneic and, less frequently, autologous SCT as well as in ALL during maintenance therapy. The majority of patients have been taking pharmacologic doses of corticosteroids prior to the development of infection (Sepkowitz KA 1993)⁽¹⁸¹⁾. Like the situation in AIDS patients, the incidence of opportunistic infections including CMV has been shown to correlate with the degree and duration of T-cell depletion (Mackall CL et al. 1994 & 2000)⁽¹⁸²⁻¹⁸³⁾. As the disease is inevitably lethal without therapy, effective strategies for PCP prophylaxis and treatment have been developed, rendering the infection almost a historical one despite the increasing use of aggressive immunosuppressive regimens and SCT for

malignant and non-malignant diseases (Hughes WT et al. 1978)⁽¹⁸⁰⁾ & (Hughes WT et al. 1987, Wienthal J et al. 1994, Masur H 1992, Groll AH et al. 2001)⁽¹³⁷⁻¹⁴⁰⁾.

The diagnosis of PCP is made on the basis of the following criteria:

- Peracute onset of respiratory symptoms with tachypnea and fever
- Progressive respiratory distress with nasal flaring and intercostal retractions that evolves into respiratory failure with hypoxemia & hypocapnia within a few days
- *Contrast to patients severely immunocompromised upon chemotherapy or SCT, the clinical course of PCP in newborns and small infants with severe inborn cellular immunodeficiency as well as in patients with AIDS is rather subacute*
- Absence of rales on auscultation
- Bilateral infiltrates on CXR
- Demonstration of the organism in sputum, BAL fluid, via percutaneous needle biopsy or open lung biopsy. The organism is stainable with Gomori methenamine silver. *Contrast to the murine *Pneumocystis carinii*, the human strain does not grow on culture media, and hence sensitivity testing is not possible*

Management of PCP

A number of drugs and drug combinations with multifarious mechanisms of action, activity, pharmacokinetic profiles, tolerability and toxicities are now available for the prophylaxis and treatment of PCP. The bulk of experience has been gained during the AIDS epidemic in the 80's, and often extrapolated from patients with AIDS, mostly adults, to other populations at risk for or with PCP. Potential targets for drug therapy are DHFR (TMP, PMA, trimetrexate, piritrexim); DHPS (SMZ, sulfadiazine, sulfadoxine, diaminodiphenylsulfone = dapsone); cytochrome b complex (atovaquone); ornithine decarboxylase (eflornithine); thymidylate synthase; topoisomerase; and β -1, 3-glucan synthetase. For some agents (primaquine, clindamycin, pentamidine), the mechanism of action has not been defined with certainty (Masur H 1992, Groll AH 2001)⁽¹³⁹⁻¹⁴⁰⁾. Selected chemotherapeutic regimes of proven efficacy in treating PCP are listed in order of choice in **Tab. 43** below.

- ❖ The drug combination of first choice should be TMP/SMZ. In case of intolerance/allergy to, or serious toxicity/failure of TMP/SMZ, then pentamidine is to be given. If the latter drug fails or is associated with severe adverse effects, the combination of TMP/dapsone offers an alternative option.
- ❖ Severe forms of the disease should be managed at the PICU. Mild-to-moderate forms can be managed on an outpatient basis. However, as the disease often runs a dramatic course, it would be prudent to begin therapy in the hospital, for at least 3 – 4 days, and then decide according to initial response.
- ❖ The patient should be observed for any adverse events. In general, mild-to-moderate drug reactions, e.g. maculopapulous skin rash, mild-to-moderate GI side effects or elevation of liver transaminases, hypoglycemia or hyperglycemia, etc are not necessarily clinically relevant and are usually manageable so that therapy may be continued. However, Lyell-Stevens-Johnson syndrome, anaphylaxis, hypotension (systolic BP < 100 torr), cardiac dysrhythmia, acute pancreatitis, nephritis, hemolysis or methemoglobinemia (? **G-6-PD deficiency**), etc are contraindications for further therapy with the offending drug(s).
- ❖ Attention should be paid to possible drug interactions. To be on the safe side, only indispensable medications may be given while following the manufacturer's instructions.
- ❖ Dosage should be adjusted to CL_{cr}. Refer to the manufacturer's instructions.

- ❖ The duration of treatment is usually 21 days.

Table 43: Treatment of PCP

| Drug / Drug Combination | Dosage & Schedule | Duration of Therapy [days] |
|----------------------------|--|----------------------------|
| 1. TMP/SMZ | 20/100 mg/kg/d qid by IV infusion over 1 h or PO | 21 |
| 2. Pentamidine isethionate | 4 mg/kg/d qd by IV infusion over 3 h | 21 |
| 3. TMP + Dapsone | 20 mg/kg/d qid PO + 100 mg/d qd PO | 21 |

3.15 OTHER SERIOUS COMPLICATIONS OF THERAPY

Aggressive chemotherapy, conditioning regimens and SCT, particularly allogeneic, as well as RTX are often associated with many immediate, early, delayed and late side effects, complications or sequels. The most important adverse events with the necessary prophylactic and therapeutic supportive measures have been discussed within the therapy elements themselves, in the previous sections of this chapter, in section 2.5.6.5, and are outlined in sections 2.6.6.3 through 2.6.6.6. Furthermore, the majority of drug untoward effects and relevant drug interactions are listed in chapter 4. Many of these issues would deserve discussion, but the scope and extent are prohibitive for them to be dealt with in depth in a cookbook for daily use. Nevertheless, at least two of these issues, namely ALI/ARDS & ABN, are quite problematic, and will be discussed in the following two sections.

3.15.1 ALI / ARDS

Often lethal, acute lung injury (ALI) and acute respiratory distress syndrome (ARDS) may be occasionally encountered in patients with ALL undergoing intensive chemotherapy or SCT. The true incidence of ALI/ARDS is however unknown, neither in the general population (adults & children), nor in pediatric ALL. This is because of, among others, the lack of uniform definition criteria for these conditions. Since ALI/ARDS are associated with serious morbidity & too high mortality rate, the best management is prevention, insofar as this is possible.

1. Definitions

It is recommended to accept the definitions for ALI/ARDS as proposed by the American-European Consensus Conference on ARDS in 1992 (Bernard GR 1994)⁽¹⁸⁴⁾ - see **Tab. 44** & Comments below.

Table 44: Recommended Criteria for ALI & ARDS

| | Timing | Oxygenation PaO ₂ : FiO ₂ [torr] (regardless of PEEP) | CXR | PAWP [torr] / Clinical LA Hypertension |
|------|-------------|---|-------------------------------|--|
| ALI | Acute onset | ≤ 300 | Diffuse bilateral infiltrates | ≤ 18 / absent |
| ARDS | Acute onset | ≤ 200 | Diffuse bilateral infiltrates | ≤ 18 / absent |

Comments

- ❖ ALI & ARDS are the extreme ends of a continuum that can be defined as a syndrome of inflammation and increased permeability of the alveolo-capillary membrane, which is associated with a constellation of clinical, radiologic and physiologic abnormalities that cannot be explained by, but may coexist with, left atrial or pulmonary capillary hypertension. The syndrome is acute in onset and persistent (lasting for days to weeks), and is associated with a number of risk factors. Pulmonary edema consequent on CHF alone is not, by definition, neither ALI nor ARDS.
- ❖ Substitute 0.21 for FiO_2 (room air).
- ❖ PEEP = 0, i.e. not applicable prior to mechanical ventilation.
- ❖ Pulmonary infiltrates may be initially mild.
- ❖ Pulmonary artery wedge pressure (PAWP) is but exceptionally measured. Instead, left atrial (LA) hypertension must be ruled out clinically (auscultation, CXR, Echo-CG). However, if present, LA hypertension could not *per se* explain the condition.

2. Risk factors

These may be direct or indirect. The most common risk factors for ALI/ARDS in pediatric ALL are:

- Sepsis syndrome with or without clinically significant hypotension, with or without evidence of infection outside the lung.
- Diffuse pneumonia (bacterial, viral, fungal including PCP).
- Multiple-organ-failure syndrome (MOFS), with severe infection being the most common cause.
- Acute (hemorrhagic) pancreatitis.
- Aspiration.
- Pulmonary leukostasis syndrome. This syndrome is however more common in AML & CML either prior to or early upon initiation of chemotherapy (Tryka AF et al. 1982, Würthner JU et al. 1999)⁽¹⁸⁵⁻¹⁸⁶⁾.
- Transfusion of non-leukodepleted blood, massive transfusion, namely exchange transfusion (TRALI).
- Transfusion of granulocyte concentrates, particularly if given with or too close to AmB.
- MTX-induced ARDS: MTX may cause pneumonitis, bronchiolitis, interstitial lung fibrosis, lung nodules, pleurisy (\pm pleural effusion) and non-cardiogenic pulmonary edema (ARDS).
- "Drug-induced" ARDS apparently related to MD (1 g/m^2) and HD (3 g/m^2) ARA-C, with *Streptococcus viridans* possibly playing a pathogenetic role. The Dutch experience is quite compelling in this regard (Peters WG et al. 1987, Tjon A Tham RTO et al. 1987)⁽¹⁸⁷⁻¹⁸⁸⁾: Ten courses were complicated by interstitial lung disease caused by HSV (2), AV (1), disseminated candidiasis (2), diffuse pulmonary hemorrhage (1), and ARDS during a Gram-negative septicemia (4). Additional 12 courses were complicated by interstitial pneumonitis of unknown origin, developing a median of 16 days (range: 8 – 20) after the start of ARA-C therapy. In 8/12 a *Strept. viridans* septicemic episode of brief duration (1 – 3 days) preceded full-blown lung changes. No relation with the treatment schedule ($1 \text{ g/m}^2/2\text{h} \times 12$ vs. $3 \text{ g/m}^2/2\text{h} \times 8$), previous chemotherapy or radiotherapy was found. The course was fatal in 2/12 cases, while 10/12 patients completely recovered after a median of 7 days (range: 4 – 11), with clinical recovery coinciding with hemopoietic

regeneration in 8/10 patients. Of note, 2/12 patients received HD methylprednisolone (10 mg/kg IV) immediately upon the onset of pneumonitis and partially improved within 1 day.

3. Prevention

- Identify the patients at risk for developing ALI/ARDS.
- Eliminate or at least curtail potential risk factors by appropriate preventative measures as deemed necessary, e.g. effective antiemetic and prokinetic therapy; energetic antimicrobial treatment; prophylactic oral penicillin post MD/HD ARA-C; judicious transfusion policy; use of leukodepleted and irradiated blood products; blood exchange with extreme caution and *lege artis*; avoidance of granulocyte transfusions as far as possible, delivering them otherwise *lege artis* and at least 8 – 10 h apart from AmB; complex and intensive management of acute hemorrhagic (necrotizing) pancreatitis and MOFS; etc.

4. Management

- ? Methylprednisolone 10 mg/kg x 1 by brief IV infusion over 0.5 – 1 h for "drug-induced" ALI/ARDS.
- Withdraw the offending drug (e.g. MTX) without delay.
- Transfer the patient to the PICU immediately.

3.15.2 OSTEOPENIA / OSTEOPOROSIS & OSTEONECROSIS

Many skeletal abnormalities, notoriously known to every pediatric hematologist/oncologist, may occur already at presentation of ALL. Although very important, they will not be dealt with in this section. Rather, skeletal changes that may be encountered during and after treatment will be discussed in some detail, as these can cause long-term serious and even disabling morbidity with negative impact on the quality of life and social integration of survivors of pediatric ALL.

There is no doubt that particularly corticosteroids induce profound alterations in bone metabolism and structure. The role of MTX in skeletal pathology is increasingly appreciated, giving birth to so-called MTX osteopathy. MTX inhibits osteoblasts, stimulates osteoclast recruitment and hampers healing. Importantly, the drug preferentially affects cortical bones and relatively spares trabecular ones. Therefore, when assessing bone mineral density (BMD) by dual-energy X-ray absorptiometry (DXA), it is necessary to sample also on the distal radius (cortical bone), not merely on the lumbar spine (LS) or hip (trabecular bones). However, it is debatable whether MTX can cause ABN. In one randomized study in children with ALL (Kardos G et al. 1995)⁽¹⁸⁹⁾, it was shown that patients receiving 5 g/m² MTX were at higher risk for developing ABN compared to those who received 2 g/m² MTX. On the other hand, it is due to note that over the long history of the osteosarcoma studies, in which HD MTX has been employed, no increase in the incidence of ABN has been observed.

In addition to prolonged hospitalization and restricted physical activity with their well-known negative effects on bone metabolism, it is possible that additional cytotoxic agents and other medications might also adversely affect the skeleton. Heparin-induced osteoporosis, for example, is now a well-recognized, clinically relevant complication of long-term therapy with the drug. Heparin binds firmly to bone matrix proteins, where it is detained for a long time. Histomorphometric, metabolic & radionuclide studies have demonstrated in animal models a significant reduction in the number of osteoblasts and in the amount of osteoid (for both UFH & LMWH) along with a remarkable increase in the surface of osteoclasts (for UFH only) in cancellous

bone at drug concentrations commonly achievable with the prophylactic and therapeutic dosages used in humans. In parallel, the level of S-ALP decreased, and continued to decline after cessation of heparin, while there was a transient increase in urinary type 1 collagen cross-linked pyridinoline. The results of these experiments are consistent with inhibition of bone formation by both UFH & LMWH as well as stimulation of bone resorption by UFH only. They are also compatible with the clinical experience of ca 30% incidence of BMD reduction and 2 – 3% rate of symptomatic vertebral fractures in patients receiving heparin for at least 1 month. LMWHs may carry a lower risk of osteoporosis than UFH, as shown in one study randomizing patients with DVT to dalteparin (5,000 U) or UFH (10,000 U) SC bid for 3 – 6 months. The spinal fracture rate was 1/40 in the former vs. 6/40 in the latter group (reviewed by Hirsh J et al. 2001)⁽¹⁹⁰⁾.

One of the problems of long-term survivors following therapy for childhood ALL is reduced bone density and possibly osteoporosis, compared to healthy controls (Arikoksi P et al. 1998)⁽¹⁹¹⁾. Osteonecrosis (aseptic bone necrosis = ABN) is clearly more frequently observed in children on antileukemic therapy than in their healthy peers. A recent work from the CCG (Mattano LA et al. 2000)⁽¹⁹²⁾ has described an incidence of $14.2 \pm 1.3\%$ in 10 – 20-yr-old ALL patients enrolled on CCG-1882 Study. In that study, patients < 10 yr of age at the time of diagnosis demonstrated a significantly lower incidence of only $0.9 \pm 0.4\%$. Osteonecrosis was encountered most commonly during the first 3 years after diagnosis. In addition, contrast to the general pediatric population, where M. Perthes is 3-fold more frequent in boys than in girls, males are affected less often than females in ALL ($11.7 \pm 1.6\%$ vs. $17.4 \pm 2.1\%$). The study provided evidence for an increased incidence among those children who had received double reinduction with Protocol II ($23.2 \pm 4.8\%$ vs. $16.4 \pm 4.3\%$). The increased incidence of osteonecrosis on ALL therapy could be in part explained by the high dexamethasone dose, but at the same time, it remains unclear why the incidence had increased markedly in comparison with the 80's.

In a Finnish study (Ojala AE et al. 1999)⁽¹⁹³⁾, osteonecrosis mostly affecting the lower extremities was shown on MRI in 9 (6 M, 3 F) out of the 24 children examined (38%), of whom however 6 were asymptomatic. Hence, the incidence of occult osteonecrosis should rank substantially higher. In that study, the incidence was most frequent immediately following reinduction until several months thereafter- a median of 12 months (range: 8 – 25) from ALL diagnosis. None of the patients received aggressive treatment for ABN, which markedly improved in 6 and completely resolved in 3 of them.

A retrospective analysis of the Czecho-Slovak experience (Plevova P et al. 1999)⁽¹⁹⁴⁾ identified 9/665 (1.4%) cases of osteonecrosis among pediatric ALL patients, but none among 76 AML patients, over a 10-yr period spanning 1987 – 1996. ABN occurred a median of 13 months (range: 5 – 25) from the diagnosis of ALL, i.e. mostly during maintenance therapy. ABN was significantly more frequent in children ≥ 10 yr old ($8/205 = 3.5\%$) than in those < 10 yr of age ($1/536 = 0.2\%$) at the time of leukemia diagnosis ($p = 0.0002$). As in the Finnish study, although only 2 boys vs. 7 girls were affected, the difference was not statistically significant ($p = 0.087$).

A recent Dutch study (van den Heuvel-Fibrink MM et al. 2002)⁽¹⁹⁵⁾ involving 61 children with ALL documented reduced bone-formation markers and significantly decreased BMD_{LS} already at initial presentation, increased bone-turnover markers (both formation & resorption) and still decreased BMD_{LS} during intensive chemotherapy, and a 6-fold higher fracture rate against healthy controls during and shortly after treatment, whereby not just the BMD_{LS} Z score itself, but rather a

decrease in BMD_{LS} during the first 6 months of intensive chemotherapy that was associated with a higher fracture risk. Total-body bone mineral density (BMD_{TB}) was normal at presentation, in order to show a fast descent mainly over the first 8 months of treatment. Furthermore, the study demonstrated a decreased lean body mass at baseline, and a significant increase in % body fat during therapy. Following therapy, BMD and body composition tended to improve slightly.

Tab. 45 below provides an overview of the total dose of steroids by arm in ALL IC-BFM 2009. As the randomized elements do not include steroids there is a dose homogeneity in SR and IR. The total steroid dose in HR decreased is 100 mg/m^2 lower than the one used in ALLIC 2002.

Table 45: Cumulative Steroid Dose by Arm in ALL IC-BFM 2009

| RG – Arm | Σ Prednisone mg/m^2 | Σ Dexamethasone mg/m^2 |
|----------|---------------------------------|------------------------------------|
| SR | 1,727.5 – 1,937.5 | 236.25 |
| IR – 1 | | 236.25 |
| IR – 2 | | 236.25 |
| HR – 1 | | 600.25 |
| HR – 2 | | 600.25 |

Unfortunately, the available literature offers no preventative measures for adolescents who are at highest risk for developing these complications. The use of the geminal bisphosphonates (gBP) is still controversial in this regard. In general pediatrics, these drugs may be considered in certain indications such as hypercalcemia, osteogenesis imperfecta or idiopathic osteoporosis (Shoemaker LR 1999, Srivastava T et al. 1999, Rauch F et al. 2000)⁽¹⁹⁶⁻¹⁹⁸⁾. Only single case reports on the long-term effects of this therapy are so far available. Likewise, no controlled studies on upfront bisphosphonate treatment have been ever published (van Persijn van Meerten EL et al. 1992, Brumsen C et al. 1997)⁽¹⁹⁹⁻²⁰⁰⁾. A small pilot study involving 10 osteopenic children who were receiving maintenance therapy for ALL according to the DFCI protocol 95-01 was presented recently (Wiernikowski J et al. 2000)⁽²⁰¹⁾. According to that study, almost 80% of the children on DFCI protocols are osteopenic by the end of treatment, and 2/3 have reduced BMC, which predicts for fractures that are sustained by 40% of all children on therapy. The 10 patients received 3 doses of IV pamidronate delivered on 3 consecutive days. A gain in BMC was shown in all of them 3 months later. The side effects of bisphosphonates, often administered by the IV route, such as flu-like syndrome, hypocalcemia, and, in single cases, acute renal failure support the so far held view that these drugs are not generally indicated for the management of post-ALL-therapy skeletal changes, whatsoever, in children and adolescents.

An early diagnosis and therapy (immobilization, rest, surgical measures) can reduce the extent of destruction of the affected bone and hasten healing as is the case in ABN of other origin, e.g. M. Perthes.

Diagnosis of ABN

For this reason, in case of pain, limp on walking or running, or restricted joint mobility, a skiagram, CT scan or preferably MRI, which is the gold standard, should be performed to confirm or refute ABN. ^{99m}Tc skeletal scan & US are additional useful techniques.

Management of ABN

Treatment should be determined by the orthopedist. Only conservative measures (rest, immobilization, corset) are often the all needed, as spontaneous improvement or even complete resolution of the lesions are features of the natural history of ABN (Ojala AE et al. 1999, Plevova P et al. 1999)⁽¹⁹³⁻¹⁹⁴⁾. Surgical intervention (forage, relieving osteotomy, auto- /alloplasty, total endoprosthesis, etc) may be indicated for but the most severe cases, first of all those involving the weight-bearing low extremities, and should be decided on individually. In severe cases, reduction or omitting MTX must be consulted with and approved by the national study coordinator.

Data Collection & Analysis of ABN

Each documented osteonecrosis, whether symptomatic or not, should be reported to the national data manager on the SAE Form & Acute-Toxicity Form (Appendix 1.4, Appendix 3.2a). Ultimately, it must be recorded in the FU Form (Appendix 1.2). The national data manager will in turn send a specific questionnaire to the treating center in order to obtain and retrieve adequate information on the course and management of every individual case of ABN. The data will be regularly pooled in the central database at the Trial Statistics Center. The ultimate goal will be to perform central analysis of the global data for the entire population as well as within the individual arms, aiming at comparing the incidence of this complication as a function of the different late-reintensification therapeutic strategies. Should evidence for excessive toxicity emerge on interim analysis, then the TMC/TSC will decide on the fate of the trial as a whole or its individual arms, and notify all the participating centers in due time in this regard.

4 Chapter

CHEMOTHERAPY SIDE EFFECTS

Recommendations on supportive measures for the management and prevention of specific side effects of the individual cytostatic drugs are given in the body of the therapy elements themselves, and in part in chapter 3 on supportive therapy. In this section, the most important side effects of those drugs are reviewed, as far as they can be expected in the light of current knowledge for the dosages specified in this trial. One should be aware of the many interactions between cytotoxic drugs and other medications that may enhance or attenuate the actions of the drug(s) in question. Some of these interactions are useful through enhancing efficacy, while others may be noxious via interfering with efficacy or augmenting toxicity. On the other hand, the drugs may behave mutually indifferently, or they may exhibit interactions that are not clinically relevant. The concepts of (sub)additive, synergic, interfering and antagonistic effects of antineoplastic drug combinations should be also well known to every hematologist/oncologist. It is the responsibility of the physician caring for the child with malignancy to enrich and update his/her know-how through systematic study of the medical and pediatric literature, particularly in the scope of hematology/oncology. In addition to continuous medical education (CME), close contact with people from the pharmaceutical industry and the intelligent use of information technology are very important. A number of excellent monographs are now available in the bookstores, e.g.:

- The Chemotherapy Source Book, 3rd edition, Perry MC ed., Williams & Wilkins 2001
- A Clinician's Guide to Chemotherapy Pharmacokinetics and Pharmacodynamics, 1st edition, Grochow LB & Ames MM ed., Williams & Wilkins 1998
- Prevention and Management of Anticancer Drug Toxicity by Lipp H-P, published by Universitätsverlag, Jena, Germany 1995

In addition, one may resort to Drug Interactions in the Therapy of Malignant Diseases by Bornmann L, Herdrich K & Illiger HJ, edited under the auspices of Asta Medica in the form of a booklet as well as on a CD ROM, or can browse through the homepage of a renowned (inter)national society/group of pediatric hematology/oncology, e.g. www.gpoh.de.

L-ASPARAGINASE (ASP)

By splitting the amino acid asparagine to aspartic acid and ammonia, the pool of asparagine, which is essential for blasts, is depleted. The drug is available as E. coli asparaginase (CO-ASP[®] Medac/Kyowa Hakko), Erwinia asparaginase (Erwinase[®] Speywood), which will be probably approved for use via the IM route only, and PEG-asparaginase (Oncaspar[®] Medac/Kyowa Hakko/Enzon). The preparation of first choice in this trial should be a native formulation of E. coli asparaginase of known pharmacokinetics such as that of Medac/Kyowa Hakko. In case of an allergic reaction that would interfere with further therapy, it is then necessary to switch over to an alternative product, either a pegylated E. coli asparaginase (Oncaspar[®]) or a native formulation from Erwinia chrysanthemi (Erwinase[®]).

In recent years, Erwinase[®] has been approved for **IM** use only. The TMC undertakes to acknowledge all centers taking part in this trial of any approved change in the route of administration.

Most important side effects

Allergic reaction: 5-35% (serious in up to 10%)- slight erythema, urticaria, serious bronchospasm, anaphylactic shock. Upset of the hemostatic system with bleeding and thrombotic complications, mostly intracranial. Hepatotoxicity- hyperbilirubinemia, depressed protein synthesis with significantly decreased levels of 11 anti- & pro- coagulants. Pancreatic dysfunction- endocrine (hyperglycemia, ketoacidosis), exocrine (diarrhea), (hemorrhagic)

pancreatitis. Encephalopathy (somnolence, seizures, coma), cerebral dysfunction with diffuse slowing on EEG, disturbed attention. Hyperventilation, alkalosis, feeling hot. Kidney damage. Transient secondary hypothyroidism, hypoparathyroidism.

Contraindication: acute or chronic pancreatitis.

Test dose: A test dose of 10-50 IU or 0.2 IU/kg body weight PI over 15 min is recommended before ASP infusion. The patient should be closely observed during and for 30 min after the test dose. However, an uneventful test dose does not preclude a subsequent allergic reaction.

Interactions: ASP enhances the effect of vincristine, vindesine & etoposide.

CYCLOPHOSPHAMIDE (CPM)

Alkylating agent, oxazaphosphorine; cytotoxic effect in S phase of the cell cycle, metabolized in the liver to phosphoramidate mustard and acrolein (urotoxic), the metabolites can bind covalently to DNA or proteins.

Cave: Dose reduction in case of decreased CL_{cr} - see **Tab. 30** (p 118).

Most important side effects: myelosuppression, hemorrhagic cystitis (prophylactic administration of MESNA and hyperhydration/forced diuresis are necessary), impaired free-water clearance (SIADH), renal tubular (Fanconi syndrome) and glomerular damage, nausea, vomiting, stomatitis, carcinogenic potential, disturbance of spermatogenesis, ovarian dysfunction, alveolitis, cardiotoxicity, taste alterations, anaphylaxis, bronchospasm, Stevens-Johnson syndrome, dermatitis, hair loss, neurotoxicity, liver damage.

Interactions: CPM effect increased with allopurinol, cimetidine, paracetamol, barbiturates.

Special interactions (in part individual cases described):

| | |
|--------------------|----------------------------------|
| CPM + AmB: | hypotension, bronchospasm. |
| CPM + insulin: | increased effect of insulin. |
| CPM + anesthetics: | increased effect of anesthetics. |

CYTARABINE (ARA-C)

Antimetabolite, pyrimidine antagonist; cytotoxic effect in G1 phase of the cell cycle, inhibition of pyrimidine synthesis and induction of strand breakage through insertion of a "false" base.

Cave: lethal overdose IT: 1,000 mg vial (intervention possibilities at: www.gpoh.de, recommendations for daily work in pediatric oncology).

Cave: Strictly controlled use in case of liver malfunction.

Most important side effects: myelosuppression, orointestinal mucous membrane toxicity, enteritis, intestinal wall necrosis, erythema, fever, myalgia, bone and joint pain, facial „flush“, liver malfunction, hair loss, CNS disturbances, leukoencephalitis (rare), paraplegia (rare), disorder of spermatogenesis and ovarian dysfunction, heart rhythm abnormalities, pulmonary edema, kidney malfunction, allergic reaction.

HD ARA-C: keratoconjunctivitis (prevention with dexamethasone eye drops), tachyarrhythmia, somnolence, cerebellar ataxia, aphasia, nystagmus. When ataxia and/or nystagmus appear immediately stop the HD ARA-C infusion/no further HD ARA-C infusion (Purkinje cell degeneration). Pneumonitis, veno-occlusive disease (VOD, rarely), fatal peripheral neuropathy (axonal degeneration).

Synergism: with 6-mercaptopurine.

DAUNORUBICIN (DNR)

Anthracycline, cytotoxic antibiotic; works by means of intercalation, biotransformation to active metabolites in the liver (semichinon radicals), formation of superoxides, hydrogen peroxide and hydroxyl radicals, inhibition of topoisomerase II and tyrosine kinase.

Cave: incompatible with solutions of pH > 8.0; light protection necessary, particularly when infused over a longer period.

Cave: delayed breakdown and increased toxicity in case of significantly reduced liver function.

Most important side effects: acute and chronic cardiotoxicity with cardiomyopathy (regular monitoring of cardiac function is necessary, when marked reduction in comparison with initial finding, e.g. a decrease in EF by > 10% vs. baseline/antecedent value or EF < 35%, daunorubicin should be withheld, dysrhythmias, myelosuppression, tissue necrosis after paravasation- see sections 3.11 – 3.11.2 , phlebitis, orointestinal mucous membrane toxicity, hair loss, dermatitis, nephrotoxicity (tubular damage), carcinogenic potential, gastrointestinal disorders (nausea, vomiting, diarrhea), liver damage, spermatogenesis and ovulation disturbances.

Note: red discoloration of urine, possible pigmentation disturbances on sun exposure (avoid sun exposure).

Interaction: + antibiotics (amikacin, gentamicin, clindamycin, vancomycin): loss of antibiotic efficacy through reduced intracellular uptake.

Contraindications: NYHA IV, myocarditis, pericarditis, in NYHA III use only under close cardiologic control.

DOXORUBICIN/ADRIAMYCIN (DOX)

Anthracycline, cytotoxic antibiotic; mechanism of action- see DNR.

Most important side effects: see DNR, pneumonitis.

Threshold dose of DOX for cardiomyopathy: **400 mg/m²**.

Interactions: + AmB: increased effect of DOX (cellular uptake probably increases).

Special drug interactions (in part only single case reports):

DOX + barbiturates: reduced effect of DOX (increased degradation).

DOX + anticonvulsants: decreased efficacy of anticonvulsants.

DOX + foscarnet/ganciclovir: additive hematologic toxicity.

ETOPOSIDE (VP-16) & ETOPOSIDE PHOSPHATE

Podophyllotoxin; blockage of mitosis, stabilization of topoisomerase II-DNA complex, DNA strand breakage, phase-specific action in late S and early G2 phase, inhibition of protein synthesis.

Cave: dosage of etoposide phosphate is 1.136-fold that of conventional etoposide; discuss with local pharmacy.

Cave: should not be diluted with glucose or buffered solutions with pH > 8 (precipitation); if flocculation (precipitation, cloudiness) occurs, stop infusion immediately & prepare a new one.

Most important side effects: hypotension on fast infusion, allergic reaction, anaphylactic shock, bronchospasm, myelosuppression, mucositis, enterotoxicity, peripheral neuropathy, arrhythmia, cholestasis, defluvium/alopecia, local tissue and vein toxicity, carcinogenic (particularly secondary AML), hepatotoxicity.

Synergism: with cytarabine.

Interactions: VP 16 + phenytoin: reduced effect of etoposide.
 Etoposin + calcitonin: decreased transformation into etoposide.

IFOSFAMIDE (IFO)

Alkylating agent; cytotoxic effect in G2 phase of the cell cycle, the active 4-OH-ifosfamide produced by activation in the liver is split into ifosfamide mustard and acrolein (urotoxic), additionally chloroacetaldehyde is produced (probably neurotoxic).

Most important side effects: see CPM, but more nephrotoxic (Fanconi syndrome) and more urotoxic (hemorrhagic cystitis) than CPM, potential neurotoxicity (reversible encephalopathy, manageable with methylene blue), psychotic states, visual disturbances.

Interactions: (in part, description of individual cases): see CPM.

IFO + oral anticoagulants: increased effect of anticoagulants.

IFO + dexamethasone: increased effect of dexamethasone.

IFO + N-acetylcysteine: loss of efficacy of IFO (ACC is taken up by cells, where it can interact with active IFO metabolites).

METHOTREXATE (MTX)

Antimetabolite (folic acid antagonist); cytotoxic effect in S phase of the cell cycle, active uptake by cells, intracellular accumulation of polyglutamates, specific reversible inhibition of dihydrofolate reductase (DHFR).

Cave: lethal overdose with IT administration due to dilution errors or use of false vial.

Cave: pooling and/or delayed MTX excretion in case of preexisting body-cavity effusions, ileus or reduced kidney function.

Most important side effects: orointestinal mucositis, dermatitis (erythema, desquamation), nephrotoxicity (renal tubular necrosis, augmented by crystallization in the kidney when urine pH < 7 and urine flow is low during 24-h infusion), acute, subacute and chronic encephalopathy, cerebral atrophy, visual disturbances, liver toxicity (elevation of transaminases, icterus), liver fibrosis on low-dose long-term therapy; pneumonitis, pulmonary nodules, diffuse interstitial lung fibrosis, non-cardiogenic pulmonary edema/ARDS, pleurisy ± pleural effusion, malabsorption, myelosuppression, hair loss, osteoporosis, spermatogenesis and ovulation disturbances, vasculitis, fever, metabolic disorders.

Note on IT administration: feeling cool and pain irradiating downward may occur (in radicular pain - repositioning of the needle), on headache - no air injection, a dose of 0,9% NaCl may be considered.

Interactions:

Increased MTX effect: non-steroid antiphlogistics (reduced renal clearance and reduced metabolic degradation in the liver), cephalosporins, aminoglycosides (increased nephrotoxicity), sulfonamides, probenecid (reduced elimination), phenytoin (release of protein-bound MTX metabolites), barbiturates (relevant only under special clinical conditions), tetracycline, sulfonamides, trimethoprim-sulfamethoxazole (restricted renal elimination), metamizole, piperacillin, vitamin A, vitamin C, theophylline (however, aminophylline and a theophylline derivative were successfully used to manage acute MTX-induced neurotoxicity: see section 3.9, L-asparaginase (questionable, the interaction possibly depends on the sequence of delivery), salicylates (restricted elimination and competition for protein binding), cyclosporine A.

Decreased MTX effect: folic acid, L-asparaginase (questionable, the interaction possibly depends on the sequence of delivery), allopurinol, glucocorticoids (diminished uptake by

cells), penicillin G (restricted intracellular uptake), Vinca alkaloids (therefore in HR-1' block administer VCR at least 1 h before MTX), thymidylate infusion.

Special interactions (in part casuistic):

MTX + AmB: increased MTX effect (increased intracellular MTX uptake).
 MTX + oral anticoagulants: increased anticoagulant effect.
 MTX + amoxicillin/mezlocillin: reduced MTX renal clearance via competitive tub. secretion.
 MTX + omeprazol: reduced plasma MTX clearance.
 MTX + pristinamycin, possibly also erythromycin: prolongation of terminal $t_{1/2}$ to > 200 h.
 MTX + valproate: decreased valproate level.
 MTX + theophylline: decelerated breakdown & reduced clearance of theophylline.
 MTX + 6-MP: synergism (enhanced effect of 6-MP, inhibition of xanthine oxidase, accumulation of phosphoribosylpyrophosphate).

Increased sensitivity to MTX in trisomy 21 and disorders of homocysteine metabolism.

6-MERCAPTOPURINE (MP)

Antimetabolite (purine antagonist); cytotoxic effect in G1 & S phase of the cell cycle, transformation by hypoxanthine phosphoribosyltransferase into triphosphate nucleotides. These are incorporated as blocks into DNA, leading to strand breakage and disruption of DNA neosynthesis.

Cave: bioavailability improved when taken in the evening on empty stomach (without milk).

Most important side effects: myelosuppression (dose-limiting, cave: particularly endangered are homozygote thiopurine methyltransferase –TPMT- carriers), nausea, vomiting, stomatitis, diarrhea, liver damage, hyperuricemia with nephropathy, drug-induced fever, skin rash, pancreatitis.

Drug interactions:

Increased MP effect: allopurinol - increased bioavailability because of inhibited degradation.
 anticoagulants - reduced anticoagulant effect.

Decreased MP effect: Co-trimoxazole (limited absorption and cytotoxicity).

PREDNISONE & DEXAMETHASONE (PRED / DEXA)

Glucocorticoids, induction of apoptosis through glucocorticoid receptor (exact mechanism unclear).

Most important side effects: Cushing syndrome, diabetes mellitus, sodium retention or hyponatremia, potassium loss, hypertension, gastrointestinal ulcers and bleeding, myopathy, osteoporosis, aseptic bone necrosis, psychic alterations (euphoria or depressive states, particularly in the dose-tapering phase and with DEXA use), rise in Hb, erythrocytes, neutrophils and thrombocytes, decline in lymphocytes, growth delay.

Interactions (in part only single cases reported):

PRED + oral anticoagulants: decreased effect of oral anticoagulants.
 PRED + NSAIDs: increased incidence of gastrointestinal ulcerations (enhanced inhibition of prostaglandin synthesis).
 PRED + barbiturates: decreased effect of glucocorticoid (enzyme induction).
 PRED + cyclosporine: increased toxicity of glucocorticoid.
 PRED + erythromycin: enhanced methylprednisolone effect and toxicity.
 PRED + salicylates: reduced effect of salicylates.

6-THIOGUANINE (TG)

Antimetabolite (purine antagonist); mechanism of action similar to that of 6-MP.

Most important side effects: see 6-MP, VOD.

No relevant interaction with allopurinol.

VINCRISTINE (VCR)

Historically so-called Vinca alkaloid (Vinca rosea) is in fact a Catharanthus alkaloid (extracted from leaves of Catharanthus roseus of the Apocyanaceae family); cytotoxic effect in M phase of the cell cycle, blocks mitosis via inhibition of intracellular tubulin synthesis. Administration strictly intravenously, max SD 2 mg, necrosis on paravasation, **accidental IT administration is lethal** (also in ALL-BFM 95 study 1 death, in the Netherlands study group 3 deaths).

Most important side effects: peripheral neuropathy (weakening of peripheral reflexes), paresis, myopathy, fever, neuralgiform pain, constipation, paralytic ileus (maintain regular stool), SIADH, seizures, myelosuppression, hair loss, cardiovascular disturbances, photosensitivity, headache, dysphagia, polyuria, dysuria, dysfunction of cranial nerves including palsy, rarely optic nerve atrophy with blindness and temporary cortical blindness.

Interactions (in part individual cases described):

| | |
|-----------------------------------|---|
| VCR + phenytoin: | decreased phenytoin level. |
| VCR + cyclosporine A: | increased neurotoxicity. |
| VCR + barbiturates: | faster VCR clearance. |
| VCR + H ₂ antagonists: | slower VCR elimination. |
| VCR + itraconazole: | enhanced polyneuropathy. |
| VCR + etoposide: | synergic effect and increased neurotoxicity (presumed). |
| VCR + acetyldigoxin: | reduced digoxin effect. |
| VCR + INH (isoniazid): | increased neurotoxicity (individual cases). |
| VCR + metronidazole: | increased neurotoxicity (individual cases). |
| <u>Contraindication:</u> | Charcot-Marie-Tooth syndrome. |

VINDESINE (VDS)

"Vinca alkaloid", mitosis blocker; cytotoxic effect in M phase of the cell cycle.

Administration strictly intravenously, max SD 5 mg.

Cave: incompatible with solutions of pH > 6.0.

Most important side effects: see VCR.

Interactions:

VDS + MTX: loss of MTX efficacy (infuse 1 h prior to MTX in HR-2' block).

VDS + foscarnet/ganciclovir: additive hematologic toxicity.

GRANULOCYTE-COLONY STIMULATING FACTOR (G-CSF)

Cytokine; SC administration, exceptionally by IV infusion over 4 h.

Most important side effects: bone & joint pain, occasionally hypersensitivity reactions, modest hypotension, dizziness, paresthesias, slight fever, cellular infiltrates at injection site, allergic reactions.

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6 Appendices

APPENDIX 1

LOGISTICS & KEY DATA ON PATIENT & DIAGNOSIS

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- 1.1 Baseline-Data & Initial-Response Form
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APPENDIX 2

PATIENT / PARENT INFORMATION & CONSENT

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APPENDIX 3

THERAPY , TOXICITY & OUTCOME

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APPENDIX 6

- 6.0 Participating Cooperative / National Groups

7 Abbreviations

| | | | |
|---------------------|---|-------------------|---|
| A | | APRO | Association of Pediatric Radiation Oncology |
| aa | ana partes aequales | aPTT | activated partial thromboplastin time |
| AA | amino acid | ARA-C | cytosine arabinoside (cytarabine) |
| Ab | antibody | ARDS | acute respiratory distress syndrome |
| ABC | absolute blast count | ARF | acute renal failure |
| ABN | aseptic bone necrosis | ASP (L-ASP) | L-asparaginase |
| ABCD | amphotericin B colloid dispersion | AST (GOT) | aspartate transaminase |
| ABL | amphotericin B lipid complex | AT | antithrombotic |
| ACC | N-acetylcysteine | AT III | antithrombin III |
| ACD | a blood-stabilizing additive mixture | ATB | antibiotic(s) |
| AcP | acid phosphatase | ATG | antithymocyte globulin |
| ADH | antidiuretic hormone | ATLS | acute tumor lysis syndrome |
| Ag | antigen | ATPase | adenosine triphosphatase |
| AHL | acute hybrid (biphenotypic) leukemia | AUL | acute undifferentiated leukemia |
| AHP _r BP | aminohydroxypropylidene bisphosphonate | AV | adenovirus; atrioventricular block |
| AIDS | acquired immunodeficiency syndrome | A – V | arterio-venous |
| AIEOP | Associazione Italiana Ematologia Oncologia Pediatrica | B | |
| Alb (alb) | albumin | BAL | bronchoalveolar lavage |
| ALI | acute lung injury | BBB | blood-brain barrier |
| ALL | acute lymphoblastic leukemia | BC | blood count /chemistry |
| Allo-SCT | allogeneic stem-cell transplantation | BCP | B-cell precursor |
| ALP | alkaline phosphatase | BFM | Berlin-Frankfurt-Münster Study Group |
| ALT (GPT) | alanine transaminase | bid | twice daily (usually q 12 h) |
| AmB | amphotericin B | Bili | bilirubin |
| AMD | actinomycin D (dactinomycin) | BM | bone marrow |
| AML | acute myeloid leukemia | BMC | bone mineral content |
| ANAE | α -naphthol acetate esterase | BMD | bone mineral density |
| ANBE | α -naphthol butyrate esterase | BMD _{LS} | lumbar-spine BMD |
| ANC | absolute neutrophil count | BMD _{TB} | total-body BMD |
| a./o. | and/or | BMP | bone marrow puncture |
| AP | acute pancreatitis | BMT | bone marrow transplantation |
| APCR | activated protein C resistance | BP | blood pressure |
| APL | acute promyelocytic leukemia | BSA | body surface area |
| | | BU-CY | busulfan-cyclophosphamide |
| | | BUN | blood urea nitrogen |
| | | BW | body weight |

| C | | D | |
|------------------|--|------------------|--|
| C | constant in a formula; complement (protein) | d | day |
| °C | degree centigrade (Celsius) | DAH | diffuse alveolar hemorrhage |
| ca | circa | DAMPA | 2,4-diamino-N ₁₀ - methylpterotic acid |
| cAMP | cyclic adenosine – 3',5' – monophosphate | DCA | Na dichloroacetate |
| CBC | complete blood count | DCF | 2'-deoxycoformycin |
| CCI | corrected platelet count increment | DCLSG | Dutch Childhood Leukemia Study Group |
| CD | cluster of differentiation | DD | D dimers |
| CDA | 2-chlorodeoxyadenosine (cladribine) | dd/mm/yyyy | day/month/year |
| CDDP | cis-dichlorodiamine platinum II (cisplatin) | DEXA | dexamethasone |
| cDNA | complementary deoxyribonucleic acid | DFCI | Dana-Farber Cancer Institute |
| CF | citrovorum factor | DFS | disease-free survival |
| CGH | comparative genomic hybridization | DHFR | dihydrofolate reductase |
| CHF | congestive heart failure | DHPS | dihydropteroate synthetase |
| CI | continuous infusion | DIC | disseminated intravascular coagulation |
| CL _{cr} | creatinine clearance | Diff. | differential blood count |
| cm | centimeter | ditto | the same as (idem) |
| CME | continuous medical education | dL | deciliter |
| CML | chronic myeloid leukemia | DL _{CO} | lung diffusion capacity |
| CMV | cytomegalovirus (cytomegaly virus) | DMSO | dimethylsulfoxide |
| CNS | central nervous system | d/m/y | day/month/year |
| COG | Children's Oncology Group | DNA | deoxyribonucleic acid |
| CPDA-1 | blood-stabilizing additive mixture | DNR | daunorubicin |
| CPD G2 | carboxypeptidase G2 | DOB | date of birth |
| CPM | cyclophosphamide | DOCA | deoxycorticosterone acetate |
| CR | complete remission | DOX | doxorubicin (adriamycin) |
| cr (crea) | creatinine | dsm | dosis singula maxima |
| CRP | C-reactive protein | DSMC | Data & Safety Monitoring Committee |
| CRT | cranial radiotherapy | DTPA | diethylenetriamine pentaacetic acid |
| CSA | cyclosporine A | DVT | deep-vein thrombosis |
| CSF | colony stimulating factor | Dx | diagnosis |
| CT | computerized tomography | DXA | dual-energy-X-ray absorptiometry |
| CTC | common toxicity criteria | E | |
| CVC | central venous catheter | EBMT | European Group for Blood & Bone-Marrow Transplantation |
| CVL | central venous line | EBV | Epstein-Barr virus |
| CXR | chest skiagram | EC | erythrocyte concentrate |
| | | ECG | electrocardiogram |
| | | Echo-CG | echocardiography |

| | | | |
|------------------|--|---------------------|--|
| ECHO-V | enterocytopathogenic human orphan virus | ft3 | triiodothyronine free fraction |
| EDTA | ethylenediamine tetraacetic acid | ft4 | tetraiodothyronine (thyroxine) free fraction |
| EEG | electroencephalogram | FU | follow-up |
| EF | ejection fraction | | |
| EFS | event-free survival | G | |
| e.g. | exempli gratia | | |
| EGIL | European Group for Immunological Characterization of Leukemias | | |
| ELISA | enzyme-linked immunosorbent assay | g | gram |
| ELTEC | Early-&-Late-Toxicity Education Committee | G | glucose (dextrose); gauge (standard measure, esp. of width or thickness, e.g. for needles, cannulae) |
| EORTC | European Organization on Research & Treatment of Cancer | GA | general assembly |
| ESBL | extended-spectrum β -lactamase | gBP | geminal bisphosphonate |
| ESR | erythrocyte sedimentation rate | G-CSF | granulocyte-colony stimulating factor |
| ET | exchange transfusion | Gd-FLASH | gadolinium-enhanced fast low-angle short (an MRI protocol) |
| et al. | et alii / alia | GFR | glomerular filtration rate |
| etc | et cetera | GGT (γ -GT) | γ -glutamyl transferase |
| F | | GI | gastrointestinal |
| F | female; French (standard measure for catheters) | GIT | gastrointestinal tract |
| F. | coagulation factor | GM-CSF | granulocyte-macrophage-colony stimulating factor |
| Fa. | firm (company) | GNP | gross national product |
| FAB | French-American-British | GOT (AST) | glutamate-oxalacetate transaminase |
| Fbg | fibrinogen | G-6-PD | glucoso-6-phosphate dehydrogenase |
| FC | flow cytometry | GPOH | Gesellschaft für pädiatrische Onkologie / Hämatologie |
| FDA | fludarabine; USA Food & Drug Administration | GPT (ALT) | glutamate-pyruvate transaminase |
| FDPs | fibrin/fibrinogen degradation products | gtts | guttae |
| FFP | fresh-frozen plasma | GVHD | graft-versus-host disease |
| Fig. | figure | Gy | Gray |
| FiO ₂ | fraction of inspired oxygen | H | |
| FISH | fluorescence in situ hybridization | h | hour |
| FSc | forward scatter | H ₂ | histamine receptor 2 |
| FSH | follicles stimulating hormone | HAV | hepatitis A virus |
| | | Hb | hemoglobin |
| | | HBV | hepatitis B virus |
| | | HC | hydrocortisone |
| | | Hct | hematocrit |
| | | HCV | hepatitis C virus |

| | | | |
|--------------------------------------|---|----------------|--|
| HD | high-dose; Hodgkin's disease | inhal. | (via) inhalation |
| HEPA | high-efficiency particulate air | IR | intermediate-risk |
| HHM | humoral hypercalcemia of malignancy | IRG | immunoreceptor gene |
| HIT | heparin-induced thrombocytopenia | IT (i.t.) | intrathecal |
| HITT | heparin-induced thrombocytopenia with thrombosis | IU | international unit |
| HIV | human immunodeficiency virus | IV (iv.) | intravenous |
| HLA | human leukocyte antigen | IVC | inferior vena cava |
| HM | hypercalcemia of malignancy | K | |
| HPLC | high-performance liquid chromatography | <hr/> | <hr/> |
| HPV B19 | human parvovirus B19 | κ | kappa light chain of Ig |
| HR | high-risk | L | |
| HSC | hepatosplenic candidiasis | <hr/> | <hr/> |
| HSV | herpes simplex virus | L | liter |
| HT ₃ (5-HT ₃) | 5-hydroxytryptamine (serotonine) receptor 3 | λ | lambda light chain of Ig |
| HUS | hemolytic uremic syndrome | LA | lactic acidosis; left-atrial, left atrium |
| H-VOD | hepatic veno-occlusive disease | LAF | laminar airflow |
| HX | hypoxanthine | L-ASP (ASP) | L-asparaginase |
| I | | LCH | Langerhans'-cell histiocytosis |
| <hr/> | | LCV | leucovorin |
| I | input (enteral + parenteral intake) | LDH | lactate dehydrogenase |
| I°. | first-degree | LESS | Late-Effects-Surveillance Study |
| I-BFM-SG | International Berlin- Frankfurt-Münster Study Group | LFTs | liver function tests |
| IBW | ideal body weight | LH | luteinizing hormone |
| ID | intradermal | LLN | lower limit of normal |
| i.e. | id est | LMWH | low-molecular-weight heparin |
| IFO | ifosfamide | LP | lumbar puncture |
| IgA | immunoglobulin A | Lp (a) | lipoprotein (a) |
| IgG | immunoglobulin G | LPD | lymphoproliferative disease / disorder |
| IgH | immunoglobulin heavy chain | LR | late responder |
| IgM | immunoglobulin M | LS | lumbar spine |
| IHA | idiopathic hyperammonemia | LTFU | long-term follow-up |
| IM (i.m.) | intramuscular | LVEF | left-ventricle ejection fraction |
| IMT | interim maintenance therapy | LVSF | left-ventricle shortening fraction |
| INH | isoniazid | Ly | lymphoid marker |
| | | M | |
| | | <hr/> | <hr/> |
| | | M | male |
| | | M. | morbus |
| | | m ² | square meter |

| | | | |
|-----------|--|------------------|--|
| M1 | BM with < 5% blasts | neg. | negative |
| M2 | BM with \geq 5% & < 25% blasts | NGT | nasogastric tube |
| M3 | BM with \geq 25% blasts | NHL | non-Hodgkin lymphoma |
| mAbs | monoclonal antibodies | NR | non-responder/non-response |
| max | maximum, maximal | NS | normal saline (0.9% NaCl) |
| MD | medium-dose | NSAIDs | non-steroid antiinflammatory drugs |
| MDS | myelodysplastic syndrome | NYHA | New York Hear Association |
| MESNA | 2-mercaptoethane sulfonate sodium | O | |
| MFD | matched family donor | | |
| mg | milligram | O | output |
| μ g | microgram | OA(s) | oral anticoagulant(s) |
| MGG | May-Grünwald-Giemsa | OAT | oral anticoagulant therapy |
| min | minute | | |
| ml | milliliter | P | |
| μ L | microliter | | |
| MLC | mixed lymphocyte culture | | |
| μ M | micromole per liter | p | page |
| MMFD | mismatched family donor | P450 | cytochrome enzyme superfamily catalyzing oxidation, dehalogenation & S-, O-, N- dealkylation |
| mmol | millimole | PA | postero-anterior |
| μ mol | micromole | PAS | periodic acid-Schiff |
| MMP | mini-mini project | PaO ₂ | partial pressure (tension) of oxygen in arterial blood |
| MOF | multiple-organ failure | PAWP | pulmonary-artery wedge (occlusion) pressure |
| MOFS | multiple-organ failure syndrome | PB | peripheral blood |
| MP (6-MP) | 6-mercaptopurine | PBSC | peripheral-blood stem cell |
| MPO | myeloperoxidase | PC | platelet concentrate |
| MR | medium-risk (ALL-BFM 95) | PCP | Pneumocystis carinii pneumonia |
| MRD | minimal residual disease | PCR | polymerase chain reaction |
| MRI | magnetic resonance imaging | pCRT | prophylactic cranial radiotherapy |
| MRP | mini-risk project | PE | pulmonary embolism |
| MSKCC | Memorial Sloan-Kettering Cancer Center | PEEP | positive end-expiratory pressure |
| MT | maintenance therapy | PEG | polyethyleneglycol |
| MTHFR | methylenetetrahydrofolate reductase | PEG-ASP | pegylated L-asparaginase (pegaspargase) |
| MTX | methotrexate | pEFS | estimated probability of event-free survival |
| MUD | matched unrelated donor | PEI | Paul-Ehrlich Institute |
| My | myeloid marker | PGR | prednisone-good response/responder |
| N | | Ph ₁ | Philadelphia chromosome |
| N | normal; nitrogen | PI (p.i.) | per infusionem |
| n (N) | number | PICU | pediatric-intensive-care unit |
| NACE | naphthol AS-D chloracetate esterase | | |
| N.B. | nota bene | | |
| NCI | National Cancer Institute | | |

| | | | |
|-------------|---|-----------|--|
| PINDA | National Chilean Pediatric Oncology Group | RT-PCR | reverse-transcription polymerase chain reaction |
| Plg | plasminogen | RTX | radiotherapy |
| PLT | platelet | S | |
| PMA | pyrimethamine | | |
| PN | parenteral nutrition | | |
| PO (p.o.) | per os | | |
| pos. | positive | s | second (time unit) |
| pp | pages | S | serum |
| pp65 | CMV matrix phosphoprotein | Σ | total number, sum |
| PPN | partial parenteral nutrition | SAA | severe aplastic anemia |
| PPR | prednisone-poor response/responder | SAE | severe adverse event |
| PR | partial remission; per rectum | SBB | Sudan Black B |
| PRED (Pred) | prednisone | SC (s.c.) | subcutaneous |
| Prot. | Protocol | SCT | stem-cell transplantation |
| Pt | patient | SD | single dose; standard deviation |
| PT | prothrombin time (Quick) | SDD | selective digestive-tract decontamination |
| PTHrP | parathormone-related peptide | SD-PC | single-donor platelet concentrate |
| P-VOD | pulmonary veno-occlusive disease | SE | standard error |
| | | SF | shortening fraction |
| | | SIADH | syndrome of inappropriate antidiuretic-hormone secretion |
| Q | | SIOP | Société Internationale D'Oncologie Pédiatrique |
| q | quite (every) | SK | streptokinase |
| qd | once daily | SMN | second malignant neoplasm |
| qid | x4 per day (usually q 6 h) | SMS | superior mediastinal syndrome |
| qs | quantum satis | SMZ/TMP | sulfamethoxazole / trimethoprim |
| R | | Spp. | species |
| RBC | red blood cell | SR | standard-risk |
| RD-PC | random-donor platelet concentrate | SSc | side scatter |
| RF | BFM risk factor | STS | soft-tissue sarcoma |
| RG | risk group | sulfa | sulfonamide |
| rhEPO | recombinant human erythropoietin | SVC | superior vena cava |
| rh-G-CSF | recombinant human granulocyte-colony stimulating factor | SVCS | superior-vena-cava syndrome |
| RQ-PCR | real-time polymerase chain reaction | SVT | sinovenous thrombosis |
| RR | respiratory rate | T | |
| rtPA | recombinant tissue plasminogen activator | $t_{1/2}$ | half-life |
| | | Tab. | Table |
| | | TB | tuberculosis |
| | | TBC | tracheobronchial compression |

| | | | |
|-------------------|--|----------|-------------------------------------|
| TBI | total-body irradiation | VHR | very-high-risk |
| TC | transcutaneous | VOD | veno-occlusive disease |
| TC O ₂ | transcutaneous O ₂ saturation | VP | ventriculo-peritoneal |
| TCR | T-cell receptor | VP-16 | etoposide |
| tCRT | therapeutic cranial radiotherapy | vs. | versus |
| TdT | terminal deoxynucleotidyl transferase | VTE | venous thromboembolism |
| TE | thromboembolism | V – V | veno-venous |
| TG (6-TG) | 6-thioguanine | vWF | von Willebrand's factor |
| TID (tid) | thrice daily (usually q 8 h) | VZIG | varicella-zoster immune globulin |
| TIT | triple intrathecal therapy | VZV | varicella-zoster virus |
| TL | thrombolytic | | |
| TLS | tumor lysis syndrome | W | |
| TMC | Trial Management Committee | WBC | white blood cell / count |
| TMP/SMZ | trimethoprim / sulfamethoxazole | wk(s) | week(s) |
| torr | mm Hg | w/o | without |
| TPMT | thiopurine methyltransferase | WMA | World Medical Association |
| TPN | total parenteral nutrition | X | |
| TRALI | transfusion-related acute lung injury | X | xanthine |
| TRM/M | transplantation-related morbidity/mortality | Y | |
| TSC | Trial Steering Committee | | |
| TSH | thyroid stimulating hormone | yr | year(s) |
| TU | transfusion unit | | |
| Tx | treatment | | |
| U | | | |
| U | unit; urine | | |
| UA | uric acid | | |
| UDT | upper digestive tract | | |
| UFH | unfractionated heparin | | |
| UK | urokinase | | |
| ULN | upper limit of normal | | |
| UPN | unique patient number | | |
| URT | upper respiratory tract | | |
| US | ultrasonography | | |
| UV | ultraviolet | | |
| V | | | |
| VA | ventriculo-atrial | | |
| VBL | vinblastine | | |
| VCR | vincristine | | |
| V _d | volume of distribution | | |
| VDS | vindesine | | |