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AIEOP-BFM ALL 2017

International collaborative treatment protocol for children and adolescents with acute lymphoblastic leukemia

A randomized phase III study conducted by the AIEOP-BFM study group

Protocol version 1.2

Date: 25.08.2017

EudraCT Number AIEOP-BFM ALL 2017: 2016-001935-12

Sponsor: Universitätsklinikum Schleswig-Holstein, Campus Kiel

Signature Page

Confidentiality
The information contained in this protocol has to be kept strictly confidential. Therefore the protocol is only provided confidentially to the investigators for review, to study staff, Independent Ethics Committee/Institutional Review Board, regulatory authorities and CROs (or KKS) and for obtaining written informed consent from patients.

Important information
The protocol was written by the trial steering committee to the best of their knowledge and belief. Nevertheless mistakes can never be completely excluded. Therefore every doctor is responsible for checking the treatment plans of the protocol before treating a patient.

Confirmation
The following persons accept the content of this protocol and confirm to conduct this study in compliance with Good Clinical Practice and applicable regulatory requirements.

Sponsor according to GCP:	**Universitätsklinikum Schleswig-Holstein, Campus Kiel,** Brunswiker Str. 10, 24105 Kiel, Germany
Coordinating Principal Investigator and representative of the sponsor	**Prof. Dr. Martin Schrappe** Universitätsklinikum Schleswig-Holstein, Campus Kiel Klinik für Kinder- und Jugendmedizin I Arnold-Heller-Str. 3, Haus 9, 24105 Kiel, Germany
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National coordinator	*Contact data of the respected national coordinator (to be completed)*
Date: _____	Signature: _____
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Date: _____	Signature: _____
Statistician (AIEOP)	**Prof. Dr. Maria Grazia Valsecchi** Centro Operativo Ricerca statistica (CORS) Via Donizetti 106, 20052 Monza, Italy
Date: _____	Signature: _____

Investigator:

Herewith I confirm that I have read the study protocol carefully and declare my consent to it. I will treat and examine the patients in accordance with the study protocol, the national applicable laws, the international guidelines of good clinical practice (ICH-GCP) and the Declaration of Helsinki.

Date: _____ Name: _____ Signature: _____

AIEOP-BFM ALL 2017

INTERNATIONAL COLLABORATIVE TREATMENT PROTOCOL FOR CHILDREN AND ADOLESCENTS WITH ACUTE LYMPHOBLASTIC LEUKEMIA

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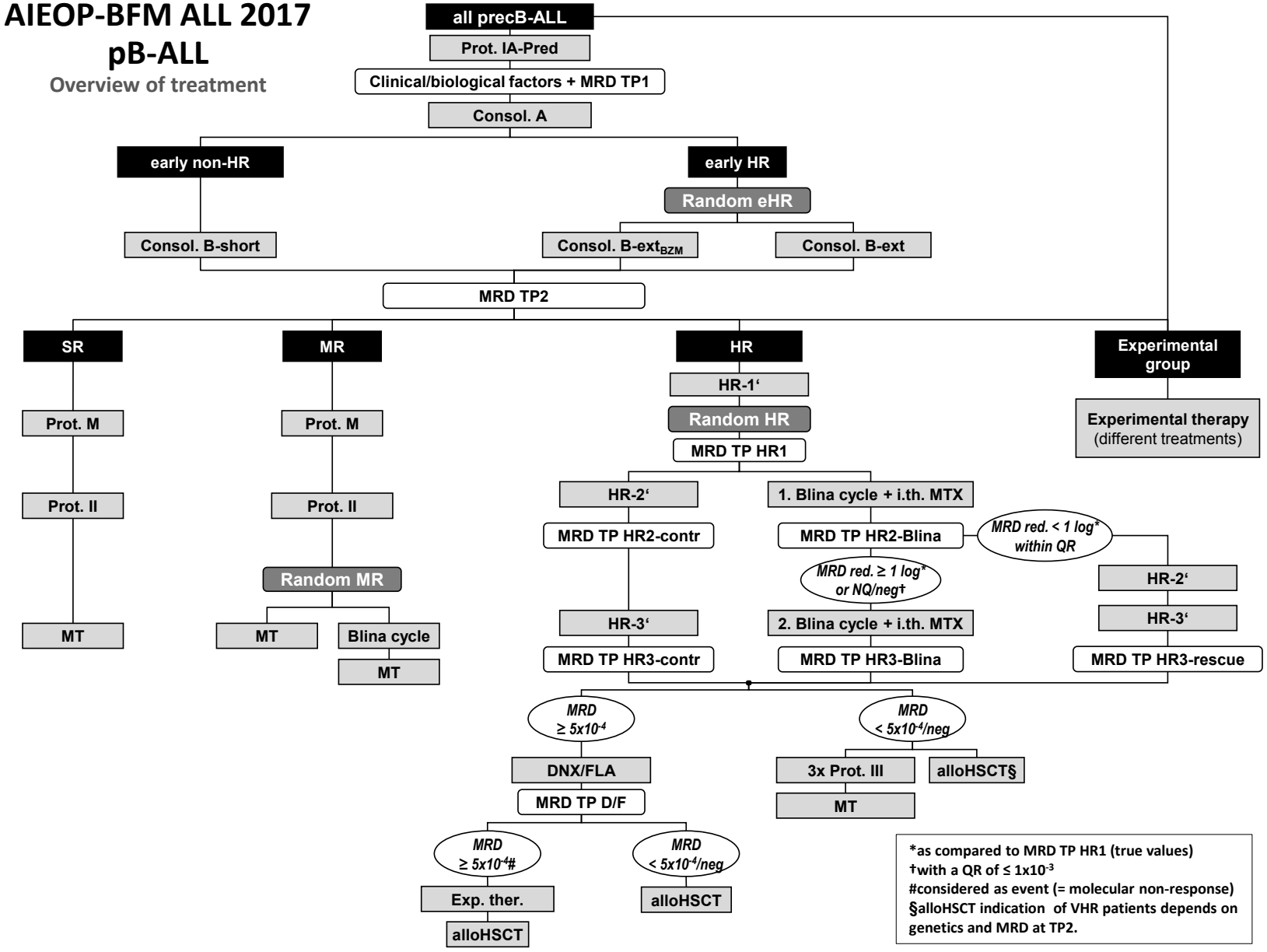
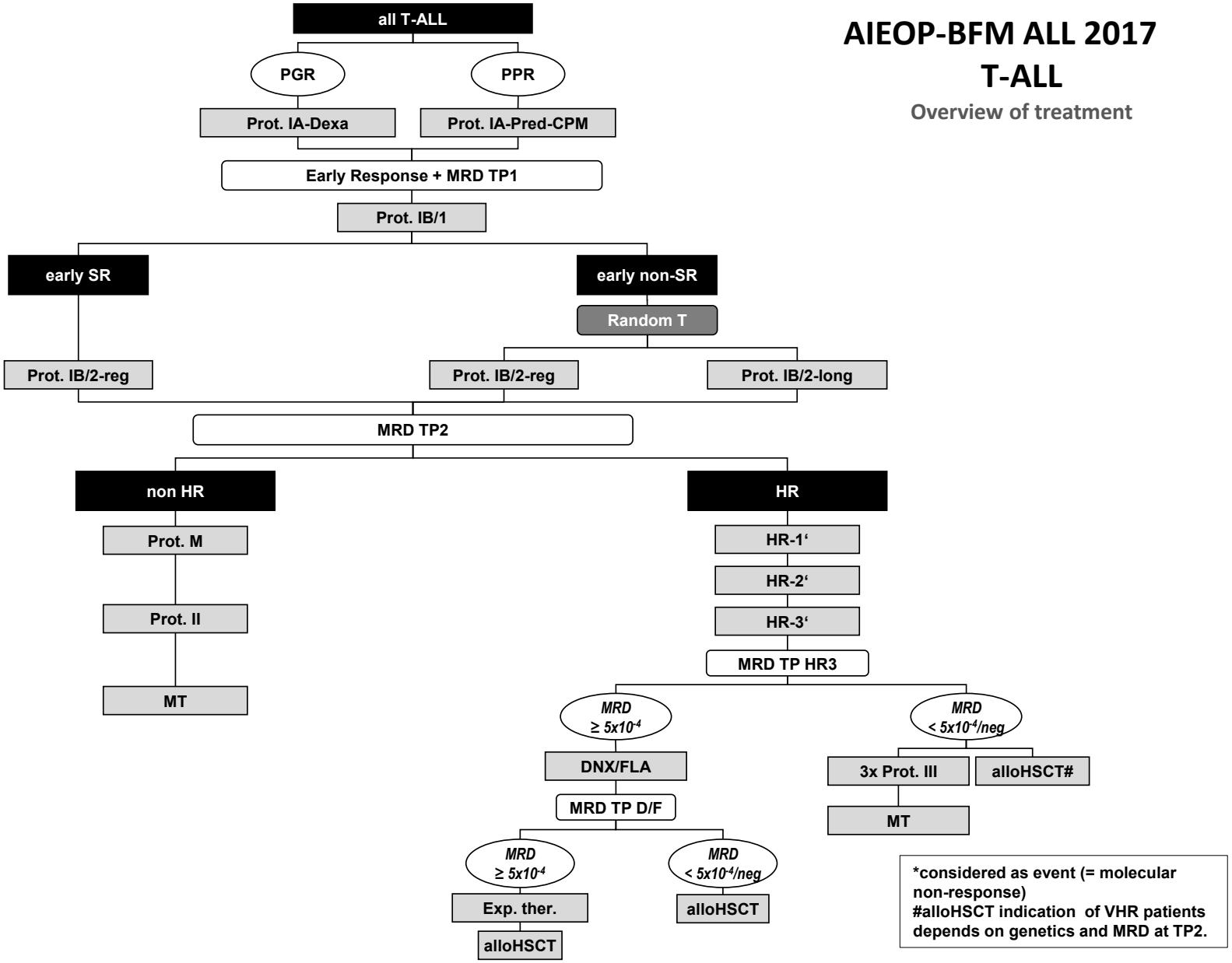
AIEOP-BFM ALL 2017
pB-ALL
Overview of treatment




AIEOP-BFM ALL 2017
T-ALL
Overview of treatment
all T-ALL
PGR
PPR
Prot. IA-Dexa
Prot. IA-Pred-CPM
Early Response + MRD TP1
Prot. IB/1
early SR
early non-SR
Random T
Prot. IB/2-reg
Prot. IB/2-reg
Prot. IB/2-long
MRD TP2
non HR
HR
Prot. M
Prot. II
MT
HR-1'
HR-2'
HR-3'
MRD TP HR3
MRD ≥ 5x10-4
MRD < 5x10-4/neg
DNX/FLA
MRD TP D/F
3x Prot. III
alloHSCT#
MT
MRD ≥ 5x10-4
MRD < 5x10-4/neg
Exp. ther.
alloHSCT
alloHSCT
*considered as event (= molecular non-response)
#alloHSCT indication of VHR patients depends on genetics and MRD at TP2.

AIEOP-BFM ALL 2017: Study Synopsis

Title	International collaborative treatment protocol for children and adolescents with acute lymphoblastic leukemia
Short title	AIEOP-BFM ALL 2017
National coordinators	Martin Schrappe, Germany (Coordinating Principle Investigator) Andrea Biondi, Italy Georg Mann, Austria Felix Niggli, Switzerland Draga Barbaric, Australia Jan Stary, Czech Republic Sarah Elitzur, Israel Alexandra Kolenova, Slovakia
Sponsor:	Universitätsklinikum Schleswig-Holstein, Kiel, Germany
Study timetable	Planned start: October 1, 2017 5 years recruitment until September 30, 2022 5 years of follow-up of each patient, until September 30, 2027
Patients	Children and adolescents < 18 years of age with acute lymphoblastic leukemia
Risk Stratification	**pB-ALL (or unknown immunophenotype)** - **early High Risk (early HR)** • no complete remission on day 33 *or* • positivity for *KMT2A-AFF or* • positivity for *TCF3-HLF*[1] *or* • hypodiploidy <45 chromosomes *or* • FCM-MRD in BM on day 15 ≥ 10% *and* ***not*** *ETV6-RUNX1* positive *or* • *IKZF1*[plus] *and* PCR-MRD at TP1 positive or inconclusive *and* ***not*** positive for *ETV6-RUNX1*, *TCF3-PBX1* or *KMT2A* rearr. other than *KMT2A-AFF1 or* • PCR-MRD at TP1 ≥ $5x10^{-4}$ • age < 1 year and any *KMT2A* rearrangement - **High Risk (HR)** • no complete remission on day 33 *or* • positivity for *KMT2A-AFF or* • positivity for *TCF3-HLF*[1] *or* • hypodiploidy <45 chromosomes *or* • FCM-MRD in BM on day 15 ≥ 10% *and* ***not*** *ETV6-RUNX1* positive *or* • *IKZF1*[plus] *and* PCR-MRD at TP1 positive or inconclusive *and* ***not*** positive for *ETV6-RUNX1*, *TCF3-PBX1* or *KMT2A* rearr. other than *KMT2A-AFF1 or* • PCR-MRD at TP1 ≥ $5x10^{-4}$ *and* positive < $5x10^{-4}$ at TP2 (PCR-MRD SER) • PCR-MRD at TP2 ≥ $5x10^{-4}$ • age < 1 year and any *KMT2A* rearrangement - **Standard Risk (SR)** • no HR criteria *and* • PCR-MRD at TP1 negative for all investigated markers with at least one marker with a quantifiable range of ≤ 10^{-4} *or* • inconclusive PCR-MRD result at TP1 *and* PCR-MRD ***not*** positive at TP2 *and* FCM-MRD in BM d15 < 0.1% - **Medium Risk (MR)** • no HR criteria *and* no SR criteria

[1] Patients with *TCF3-HLF* also qualify for experimental treatments (e.g. with *BCL2* inhibitors if those drugs are available).

	T-ALL - **early Standard Risk (early SR)** • complete remission on day 33 *and* • FCM-MRD in BM on day 15 <10% *and* • Prednisone Good-Response *and* • PCR-MRD at TP1 negative for all investigated markers with at least one marker with a quantifiable range of ≤ 10^{-4} *or* • inconclusive PCR-MRD result at TP1 *and* FCM-MRD in BM d15 < 0.1% - **High Risk (HR)** • No complete remission on day 33 *or* • FCM-MRD in BM on day 15 ≥ 10% *or* • Prednisone Poor-Response *or* • PCR-MRD at TP2 ≥ $5x10^{-4}$ - **non-High Risk (non-HR)** • No HR criteria **Experimental groups eligible for different treatments** - Positivity for *TCF3-HLF* - MRD non-response (≥ $5x10^{-4}$ after DNX/FLA)
Primary study questions	**Randomization R-eHR:** Early High-risk (early HR) pB-ALL defined by genetics and/or inadequate treatment response over the course of induction: Can the pEFS from time of randomization be improved by additional therapy with the proteasome inhibitor Bortezomib during an extended consolidation treatment phase compared with standard extended consolidation? **Randomization R-HR:** High-risk (HR) pB-ALL defined by genetics and/or inadequate treatment response by the end of consolidation: Can the pEFS from time of randomization be improved by a treatment concept including two cycles of post-consolidation immunotherapy with Blinatumomab (15 µg/m²/d for 2 x 28 days) plus 4 doses intrathecal Methotrexate replacing two conventional highly intensive chemotherapy courses? **Randomization R-MR:** Intermediate risk (MR) pB-ALL defined by genetics and intermediate MRD response: Can the probability of disease-free survival (pDFS) from time of randomization be improved by additional therapy with one cycle of post-reintensification immunotherapy with Blinatomomab (15 µg/m²/d for 28 days)? **Randomization R-T:** Early non-standard risk (early non-SR) T-ALL patients defined by treatment response over the course of induction: Can the pEFS from time of randomization be improved by the extension of the standard of care consolidation phase by 14 days with an increase of the consolidation cumulative doses of Cyclophosphamide, Cytarabine and 6-Mercaptopurine by 50%?

Secondary study questions	**All randomizations:** Can the overall survival be improved by the treatment in the experimental arm. **All randomizations:** What is the incidence of treatment-related toxicities and mortality in the experimental arm compared to the standard arm. **Randomization R-eHR:** Can the MRD load after consolidation treatment be reduced by the additional treatment with Bortezomib? **Randomization R-HR:** Can treatment-related life-threatening complications and mortality during the intensified consolidation phase of high-risk treatment be reduced when replacing two intensive chemotherapy courses by two cycles of immunotherapy with Blinatumomab? **Randomization R-HR:** What is the proportion of patients with insufficient MRD response to Blinatumomab as defined in the protocol (MRD reduction within the quantifiable range by less than 1 log over the first Blina cycle) as compared to the MRD response after the HR-2' block in the control arm? **Randomization R-HR:** Can the MRD load after the first treatment cycle (HR-2'/Blinatumomab) and the second cycle (HR-3'/Blinatumomab) be reduced in the experimental arm when compared with conventional intensive chemotherapy? **Randomization R-MR:** What is the proportion of patients with positive MRD after reintensification Protocol II who become MRD-negative over the Blina cycle compared to 4 weeks of standard maintenance therapy. **Randomization R-T:** Can the MRD load after consolidation treatment be reduced by extension of the consolidation phase?
Primary endpoints	For the randomized study questions, the primary endpoint will be the time from randomization until the first event defined as follows: **Randomization R-eHR, R-HR and R-T:** Cytomorphological or molecular non-response (resistance to protocol treatment, considered as event at day zero), relapse, second malignancy or death from any cause. This will be called EFS time. **Randomization R-MR:** Relapse, second malignancy or death from any cause. This will be called DFS time.
Secondary endpoints	- Survival starting at the same time point as the EFS/DFS - Frequency and incidence of treatment-related mortality in induction or CCR - Frequency and incidence of AE of interest and SAE in specific protocol phases, randomized arms and overall during follow-up - MRD load after the randomized treatment phases (R-eHR, R-HR and R-T) - MRD load after the first/second cycle of Blinatumomab or after the HR-2'/HR-3' block (R-HR) - Proportion of patients with poor MRD response to the first Blinatumomab cycle, i.e. MRD after Blinatumomab within the quantifiable range and reduction by less than 1 log compared to MRD after HR-1' ("Blinatumomab Poor-Response") (R-HR)
Study design	International inter-group multi-center open-label randomized clinical trial (Phase III)
Treatment groups	AIEOP (Italy) BFM-A (Austria) BFM-G (Germany) BFM-CH (Switzerland) ANZCHOG (Australia) CPH (Czech Republic) INS (Israel) SPHOS (Slovakia)

Inclusion criteria	- newly diagnosed acute lymphoblastic leukemia - age < 18 years (up to 17 years and 365 days) - patient enrolled in a participating center - written informed consent to trial participation and transfer and processing of data
Exclusion criteria	- Ph+ (*BCR-ABL1* or t(9;22)-positive) ALL[2] - pre-treatment with cytostatic drugs - steroid pre-treatment with ≥ 1 mg/kg/d for more than two weeks during the last month before diagnosis - treatment started according to another protocol - underlying diseases that does not allow treatment according to the protocol - ALL diagnosed as second malignancy - evidence of pregnancy or lactation period - Sexually active adolescents not willing to use highly effective contraceptive method (pearl index <1) until 12 months after end of anti-leukemic therapy - participation in another clinical trial that interferes with the protocol
Study size calculation	The participating groups are expected to recruit at least 1000 patients per year (850 with pB-ALL and 150 with T-ALL), resulting in the recruitment of 5000 patients during the recruitment period of 5 years. **Randomization R-eHR:** Patients who fulfill the criteria of early HR pB-ALL have an estimated 4-year pEFS of 68%. Taking into account 2 interim analyses at 2 and 3 years from randomization, 775 randomized patients (213 events) would be appropriate to detect a difference of 9% (HR=0.68), with α = 0.05 (two sided) and power 0.8, under the assumption of proportional hazards. Early HR pB-ALL account for about 20% of the patients, and the randomization rate is expected to be 80%. The number of patients who are expected to be randomized in R-eHR in 5 years will be 800 (5 x 1000 x 0.20 x 0.80). **Randomization R-HR:** Patients who fulfill the criteria of HR pB-ALL have an estimated 4-year pEFS of 68%. Taking into account 2 interim analyses at 2 and 3 years from randomization, 623 randomized patients (168 events) would be appropriate to detect a difference of 10% (HR=0.64), with α = 0.05 (two sided) and power 0.8, under the assumption of proportional hazards. HR pB-ALL account for about 16% of the patients, and the randomization rate is expected to be 80%. The number of patients who are expected to be randomized in R-HR in 5 years will be 640 (5 x 1000 x 0.16 x 0.80). **Randomization R-MR:** Patients who fulfill the criteria of MR pB-ALL have an estimated 4-year pEFS of 87%. Taking into account 2 interim analyses at 2.5 and 3.5 years from randomization, 1192 randomized patients (125 events) would be appropriate to detect a difference of 5% (HR=0.60), with α = 0.05 (two sided) and power 0.8, under the assumption of proportional hazards. MR pB-ALL account for about 35% of the patients, and the randomization rate is expected to be 80%. The number of patients who are expected to be randomized in R-MR in 5 years will be 1400 (5 x 1000 x 0.35 x 0.80). **Randomization R-T:** Patients who fulfill the criteria of early non-SR T-ALL have an estimated 4-year pEFS of 79%. Taking into account 2 interim analyses at 2 and 3 years from randomization, 430 randomized patients (69 events) would be appropriate to detect a difference of 10% (HR=0.54), with α = 0.05 (two sided) and power 0.8, under the assumption of proportional hazards. Early non-SR T-ALL account for about 12% of the patients and the randomization rate is expected to be 80%. The number of patients who are expected to be randomized in R-T in 5 years will be 480 (5 x 1000 x 0.12 x 0.80).

[2] Patients with unknown status regarding *BCR-ABL1* are eligible

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1 Introduction and Summary

Acute lymphoblastic leukemia (ALL) in children and adolescents is now considered a disease comprising very heterogeneous subentities which carry the same “look” - the leukemic phenotype - but harbor widely different activated pathways. Cytogenetics and molecular genetics reveal the wide variety in this disease (Moorman*, et al* 2010, Pui*, et al* 2011). High resolution sequencing has disclosed the enormous clonal heterogeneity of this disease (Fischer*, et al* 2015, Koren*, et al* 2014, Notta*, et al* 2011, Roberts*, et al* 2014a). It has previously also been noticed that the individual response to treatment (as assessed by minimal residual disease, MRD) is a very reliable parameter of outcome in nearly all of the subgroups of ALL (Borowitz*, et al* 2008, Cave*, et al* 1998, Schrappe*, et al* 2012, van Dongen*, et al* 1998). Recurrence of disease is most likely due to intrinsic resistance of disease subclones, and may emerge after short or extended periods of remission (Eckert*, et al* 2011, Ford*, et al* 2015, Ma*, et al* 2015), or through escape mechanisms (Alsadeq*, et al* 2015, Krause*, et al* 2015).

Progress in treatment outcome has been made in the last 10 to 15 years mainly through refinement of risk stratification and a more personalized risk-adaptation of treatment. Several clinical trials have demonstrated that it is feasible to monitor MRD in very large cohorts of ALL. These trials have succeeded to demonstrate the strong prognostic impact of quantified treatment response (Bruggemann*, et al* 2006, Conter*, et al* 2010, Schrappe*, et al* 2011, Vora*, et al* 2014).

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

Trial AIEOP-BFM ALL 2017 aims to take all of these novel circumstances into account for the treatment of patients at increased risk of failure. Risk of recurrence depends both on intrinsic genetic features as well as on individual treatment response, thus, all patients need risk-adapted genetic screening for prognostically relevant lesions, and response-oriented treatment adaptation. This integrated approach results in revised risk stratification and in alternative treatment modalities which have previously not been used in pediatric ALL:

Patients with “good risk” genetics and fast clearance of leukemic blasts receive standard post-induction therapy. By contrast, if response is delayed or inadequate as assessed by two contemporary techniques for detection of MRD, patients are considered “early high risk”, and are eligible for treatment escalation or alternative approaches. This depends on immunophenotype, and on the exact genetic subtype.

ALL patients with precursor-B-subtype (pB-ALL) and delayed early response (early high risk) may enter a prospective evaluation of proteasome inhibition which has been shown to be

effective in relapsed/refractory ALL (Messinger*, et al* 2012). If such patients have not cleared disease by the end of consolidation, they carry a significantly higher risk of relapse (Conter*, et al* 2010). Previous studies have shown that the benefit of intensive chemotherapy and hematopoetic stem cell transplantation (HSCT) in such patients confer a higher risk of treatment-related morbidity/mortality, and offer only limited efficacy (Conter*, et al* 2010). Therefore, these patients are eligible for a completely novel approach based on bispecific antibody therapy using a highly efficacious construct called Blinatumomab (Stackelberg*, et al* 2013, Topp*, et al* 2011). This will be the first upfront evaluation of targeted immunotherapy in childhood ALL. In addition, also patients in the medium-risk group with pB-ALL will be eligible for a randomized evaluation of Blinatumomab immediately after the end of intensive chemotherapy as the majority of relapses is observed in this large subgroup. If successful, these two immunotherapeutic interventions may form the basis for a completely modified approach to treat childhood ALL in the future.

Patients with T-cell ALL have previously been considered to carry a higher risk of relapse than patients with pB-ALL. While it appears that this has been overcome more recently (interim analysis in trial AIEOP-BFM ALL 2009), it is obvious that any disease recurrence in T-ALL is fatal in most cases (Tallen*, et al* 2010). Therefore, relapse prevention is essential, while reducing the risk of treatment-related morbidity and late effects. This will be achieved by two approaches: One of the most effective post-induction treatment phases in pediatric T-ALL appears to be the early consolidation as shown by MRD surveillance (Möricke*, et al* 2011, Schrappe*, et al* 2011). Therefore, this approach will be extended under controlled conditions. Only patients with poor late response will be eligible for allogeneic HSCT. To avoid secondary malignancies due to the late effects of radiotherapy, only patients with CNS involvement and/or very high white blood cell count (WBC) will be eligible for CNS-directed radiotherapy.

The new trial will provide all patients with reliable MRD-based risk stratification. This will be achieved through modified methodological requirements for PCR-based MRD analysis (Ladetto*, et al* 2014, Paganin*, et al* 2014, van Dongen*, et al* 2015), and through the use of MRD testing by flow cytometry (MRD by FCM) at day 15 of induction (Basso*, et al* 2009, Dworzak*, et al* 2002, Gaipa*, et al* 2012, Hrusak*, et al* 2014). This trial will be the largest quality-controlled study on MRD at three different time points with the most advanced state-of-the-art technologies. The approach guarantees long-term impact on optimized patient care.

Trial AIEOP-BFM ALL 2017 will also serve as platform to facilitate basic and applied leukemia research projects. Key objectives are focusing on the clinical questions raised in this trial. Therefore, there will be projects to decipher the genetic factors (pattern) which may be responsible for the heterogeneity of treatment response and outcome. In the context of immunotherapy, host-related predictors of response and resistance to this novel approach will be studied extensively. The diagnostic laboratories will use various genetic screening methods to identify the new subgroups in particular in pB-ALL. This is needed for stratification but also for modified treatment, e.g. by applying tyrosine-kinase inhibitors in patients with suitable genetic lesions. Thus, it offers the unique opportunity to run comparative studies to identify the most reliable, sensitive, and cost-effective methodology in a fast developing field. With regard to origin of disease, this trial will elucidate if predisposition syndromes can be uncovered in a larger proportion of patients. Such information may also be useful to describe treatment-related toxicity which is partly due to previously unknown germline predisposition. Thus, trial AIEOP-BFM ALL 2017 will contribute to better understanding of disease mechanisms, to future improvement of diagnostics, to better definition of response assessment as well as to individual risk adaptation of therapy.

2 Background

2.1 Important results of previous AIEOP and BFM ALL trials

2.1.1 ALL-AIEOP studies before 2000

AIEOP started using BFM-based protocols in the treatment of acute lymphoblastic leukemia in 1988.

AIEOP-LLA 88:

- BFM protocols were found “feasible” in AIEOP centers (Conter*, et al* 2000).
- Event-free survival (EFS) improved in respect to the previous AIEOP LLA 82 study (Conter*, et al* 2000).
- A low incidence of isolated central nervous system (CNS) relapses in medium-risk (MR) group was achieved by replacing cranial irradiation with protracted i.th. MTX. as CNS-preventive therapy (Conter*, et al* 2000).

AIEOP-LLA 91:

- In the standard-risk (SR) group, a reduction of therapy (omission of Protocol IB) was associated to inferior results; this disadvantage was however overcome by the administration (randomized study) of protracted asparaginase (Pession*, et al* 2005).
- In the MR patients, the administration of protracted asparaginase (randomized study) on top of conventional BFM therapy, was not associated with improved outcome; the absence of efficacy of this additional treatment might have been due to suboptimal pharmacokinetics of that therapy since the Erwinase product was used and given weekly at a dose of 25.000 IU/m^2 (Rizzari*, et al* 2001).
- Dismal results were obtained in T-ALL patients with prednisone good-response and WBC count ≥ 100 000/µl not receiving cranial radiotherapy (CRT) as preventive CNS therapy (Conter*, et al* 1997).
- The modified high-risk (HR) treatment with nine rotational high-dose chemotherapeutic pulses after induction phase achieved unsatisfying results which were worse than in the previous trial (Conter*, et al* 1998).

AIEOP-LLA 95:

- In the SR group, a reduction of treatment intensity (omission of anthracyclines and IB phase in Protocol I) in a small (9 %) cohort of highly selected patients (on the basis of DNA index, age, WBC count, no HR features) was associated with lower EFS than expected (Arico*, et al* 2005).
- In the MR patients the administration of vincristine (VCR) + dexamethasone (DEXA) pulses q10w during maintenance (randomized study) on top of conventional BFM therapy, was not associated with improved outcome; this was a collaborative intergroup prospective randomized study, conducted in the frame of the International BFM Study Group (I-BFM-SG) including also study ALL-BFM 95 (Conter*, et al* 2007).
- Consolidation/reintensification treatment in HR patients was modified by intensification with three HR blocks and administration of a double delayed intensification (Protocol II given twice) and led to considerable EFS improvement (Arico*, et al* 2002).
- A prospective intergroup study of the I-BFM-SG on the benefit of allogeneic hematopoetic stem cell transplantation (alloHSCT) in very high risk (VHR) ALL comprised HR patients from AIEOP-LLA 95 (see below in 2.1.2) (Balduzzi*, et al* 2005).

2.1.2 ALL-BFM studies before 2000

The most relevant results of the earlier ALL-BFM studies conducted since 1981 are summarized in the following:

ALL-BFM 81:

- The replacement of preventive cranial irradiation (18 Gy) with intermediate-dose methotrexate at a dose of 500 mg/m^2/24 h (4 times) in the standard- and medium-risk group resulted in a significant increase of relapses with CNS involvement (Schrappe*, et al* 1987, Schrappe*, et al* 1998). Additional intrathecal methotrexate had not been given during maintenance therapy.

ALL-BFM 83:

- The response to the 7-day prednisone prephase plus one intrathecal dose of methotrexate as measured with the absolute blast count in peripheral blood was prospectively evaluated and was identified as important prognostic factor (prednisone response) (Riehm*, et al* 1987b).
- Omission of the reinduction element Protocol III in standard-risk patients (BFM risk factor <0.8) led to a significant increase of the relapse rate (Henze*, et al* 1990, Riehm*, et al* 1987a).
- Shortening of maintenance therapy by 6 months (total treatment duration 18 months instead of 24 months) for patients of all risk groups resulted in a significantly higher event rate (Schrappe*, et al* 2000b).
- Preventive cranial irradiation with 12 Gy was as effective as 18 Gy in patients with intermediate relapse risk (BFM risk factor 0.8-1.2) (Buhrer*, et al* 1990, Riehm*, et al* 1987a, Schrappe*, et al* 1998).
- Prolongation of Protocol I meant to reduce toxicity may have had an additional unfavorable effect on the overall result in ALL-BFM 83, probably due to partly insufficient dose intensity in induction.

ALL-BFM 86:

- As in the previous trial, standard-risk patients received no reinduction treatment during the initial period of the study, which led to a significant increase of relapses.
- High-dose methotrexate (4 x 5000 mg/m^2/24 h) was introduced for all patients and allowed to safely omit the preventive cranial irradiation in all standard-risk patients (Reiter*, et al* 1994).
- The prednisone response was introduced as risk stratification criterion. The group of prednisone poor-responders achieved a 6y-EFS of 48±5 % with an intensified postinduction treatment with Protocol E and Protocol II (Reiter*, et al* 1994).

ALL-BFM 90:

- Despite reduction of the cumulative anthracycline dose during induction by 25 %, a significant overall improvement with a significantly lower relapse rate in the medium-risk group could be achieved in trial ALL-BFM 90, most likely due to a more condensed application of the induction therapy (Schrappe*, et al* 2000a).
- The modified HR treatment with nine rotational high-dose chemotherapeutic pulses after induction phase achieved unsatisfying results which were worse than in the previous trial (Schrappe*, et al* 2000a).
- The prognostic value of minimal residual disease (MRD) was prospectively studied in an international collaboration by the International BFM Study Group (I-BFM SG) (van Dongen*, et al* 1998) using PCR-based quantitative detection of clone-specific T-cell receptor and immunoglobulin gene rearrangements.

ALL-BFM 95:

- A new stratification strategy based on age and initial white blood cell count was introduced for non-HR patients.
- Despite further reduction of the cumulative antracycline dose in induction by 50 % for patients of the newly defined standard-risk group, a 6y-pEFS of 90 % could be achieved for this group (Möricke*, et al* 2008).
- Omission of preventive cranial irradiation in all non-T-ALL MR patients was possible without relevant loss of EFS despite a small but significant increase of CNS relapses.
- Consolidation/reintensification treatment in HR patients was modified by intensification of the HR blocks and reintroduction of Protocol II as late reintensification element. This led to considerable EFS improvement (Möricke*, et al* 2008).
- BFM and AIEOP initiated a prospective intergroup study of the I-BFM-SG to investigate the benefit of allogeneic HSCT from matched family donors (MFD) in very high risk (VHR) ALL. That study demonstrated the efficacy of allogeneic HSCT from MFD for event-free survival (Balduzzi*, et al* 2005).
- The specific benefit of allogeneic HSCT for HR-T-ALL patients was demonstrated in both ALL-BFM 90 and 95. Despite improvements through chemotherapy in trial ALL-BFM 95, allogeneic HSCT was highly efficacious in preventing relapses even in the small subset of patients transplanted by allografts from matched unrelated donors (Schrauder*, et al* 2006).
- The value of an intensification of the maintenance therapy phase with a schedule of six pulses of vincristine and dexamethasone on an intensive BFM-based chemotherapy backbone was prospectively investigated in a randomized intergroup study (including also AIEOP) in children with intermediate-risk ALL. The study showed that the patients did not benefit from the additional pulses (Conter*, et al* 2007).

2.1.3 Important results of AIEOP-BFM ALL 2000

The study AIEOP-BFM ALL 2000 was a collaborative trial of the AIEOP and the BFM study groups sharing the same risk stratification criteria and asking common randomized treatment questions on slightly different treatment backbones. These differences include the administration route of L-Asparaginase (i.m. in AIEOP vs. i.v. in BFM), the dose of HD-MTX in pB-ALL without CNS or testicular involvement at the diagnosis (2000 mg/m² vs. 5000 mg/m²), the dose of E. coli L-Asparaginase in the HR blocks (1 x 10,000 IU/m² in AIEOP vs. 2 x 25,000 IU/m² in BFM), the CNS therapy (protracted i.th. therapy during maintenance in all AIEOP patients not treated with CRT; preventive CRT (pCRT) in non-HR T-ALL limited to patients with >100,000 WBC count in AIEOP vs. pCRT for all T-ALL patients in BFM; irradiation dose 18 Gy/24 Gy in AIEOP vs. 12 Gy/18 Gy in BFM for preventive or therapeutic CRT, respectively), the HR control arm in the second random for the HR subgroup (3 HR blocks and 2 x Protocol II in AIEOP vs. 6 HR blocks and 1 x Protocol II in BFM) and HSCT indications (more restricted in AIEOP).

The main results of the trial are summarized in the following:

- Minimal residual disease (MRD): The feasibility of MRD-based risk stratification using the PCR-based quantitative detection of clone-specific T-cell receptor and immunoglobulin gene rearrangements could be proven in the setting of the large multicentric trial AIEOP-BFM ALL 2000 (Flohr*, et al* 2008). Final outcome analyses of the MRD risk groups confirmed the high prognostic value of MRD in pB-ALL as well as T-ALL (Conter*, et al* 2010, Schrappe*, et al* 2011). MRD levels on day +15 measured by flow cytometry (FCM-MRD) was shown to be highly predictive for

prognosis and was added in the stratification criteria in the subsequent AIEOP-BFM ALL 2009 Study (Basso*, et al* 2009).

- Randomized question of prednisone or dexamethasone in induction treatment: In induction phase Protocol IA, the randomized administration of dexamethasone (DEXA, 10 mg/m^2/day) vs. prednisone (PRED, 60 mg/m^2/day) for 21 days (plus tapering) was investigated in all study patients. The use of DEXA reduced the relapse rate (systemic and extramedullary relapses) significantly, but was also associated with a crucially higher risk of treatment-related serious toxicity leading to a higher incidence of induction-related fatalities in the DEXA arm. Subgroup analyses showed that patients with prednisone good-response (PGR) had a benefit through a significantly lower relapse risk and better pEFS in the DEXA arm, whereas patients with prednisone poor-response (PPR) apparently did not benefit from DEXA treatment. The largest benefit from DEXA regarding relapse reduction and pEFS could be shown for patients with T-ALL and PGR. Analyses of overall survival revealed no difference between the randomization arms in the total cohort. In subgroup analyses, a significant survival benefit from dexamethasone could only be shown for patients with T-ALL and PGR. The observation that patients with precursor B-ALL and PGR had a significantly worse survival after relapse if previously assigned to the dexamethasone arm, indicated that in this large subgroup, DEXA especially reduced the incidence of better salvageable relapses (Möricke*, et al* 2016). These findings formed the basis for the use of dexamethasone in induction only for T-ALL with PGR in study AIEOP-BFM ALL 2009.
- Randomizations during reintensification phase: The randomizations in the three risk groups aimed to reduce toxicity by the modified reintensification approaches while achieving better (risk groups MR and HR) or non-inferior (risk group SR) event-free survival. In risk group SR, patients were randomized to either receive the reduced intensity Protocol III or standard Protocol II as reintensification therapy. Disease-free survival was significantly inferior in patients assigned to Protocol III. Subgroup analyses revealed that disease-free survival of the randomization arms might be comparable only in patients with *ETV6-RUNX1*-rearranged ALL.Interim analyses of the randomizations in MR and HR do not indicate that the results could have relevance for the new study concept. The final analyses are ongoing
- HR group in AIEOP: Among HR AIEOP patients, those with HR MRD and/or no-CR at the end of Protocol IA and/or t(4;11) had a 5y-EFS <50% whereas those at HR only for PPR had a quite favorable outcome with a 5y-EFS >70%; no clear benefit was obtained with HSCT (Conter*, et al* 2014).
- Prognostic impact of MRD in the High Risk group: The MRD risk group criteria could be shown to also have a high prognostic value within the high-risk group as defined by the final risk group criteria (classical HR criteria and/or MRD-HR). The good prognosis of patients with prednisone poor-response (*BCR-ABL1*-negative, *MLL-AF4*-negative, CR on day 33) and MRD risk group MRD-SR or MRD-MR led to restrictions of the stem cell transplantation (SCT) indications for these patients in the BFM group. The prospective evaluation of the prognostic impact of the MRD response beyond week 12 in the HR group could show that in patients with high MRD load (≥ 10^{-3}) at week 12 the following MRD response to the HR blocks was closely correlated with the outcome. A particularly poor prognosis with 5y-pEFS of less than 20 % could be demonstrated for patients with MRD load of 10^{-3} or higher at the beginning of the 3rd HR block.
- Increased rate of life-threatening infections in the induction phase in patients treated with additional IT MTX: Retrospective analyses on the effect of the additional IT MTX doses on day 18 and 27 in induction Protocol IA in trial AIEOP-BFM ALL 2000 revealed more than twice as many life-threatening and fatal infections in patients with

CNS 2 or CNS 3 status that received the additional IT doses as compared to patients with CNS 1 status without these additional doses.

2.1.4 Important interim results of AIEOP-BFM ALL 2009

The study AIEOP-BFM ALL 2009 was conducted as collaborative international trial in seven countries (AIEOP [Italy], BFM-G [Germany], BFM-A [Austria], CPH [Czech Republic], INS [Israel] and ANZCHOG [Australia]). This study is recruiting approx. 1,000 patients per year.

The main interim results with relevance for the study concept of AIEOP-BFM ALL 2017 are summarized in the following. In general, follow-up time is too short to report data on relapse incidence or survival.

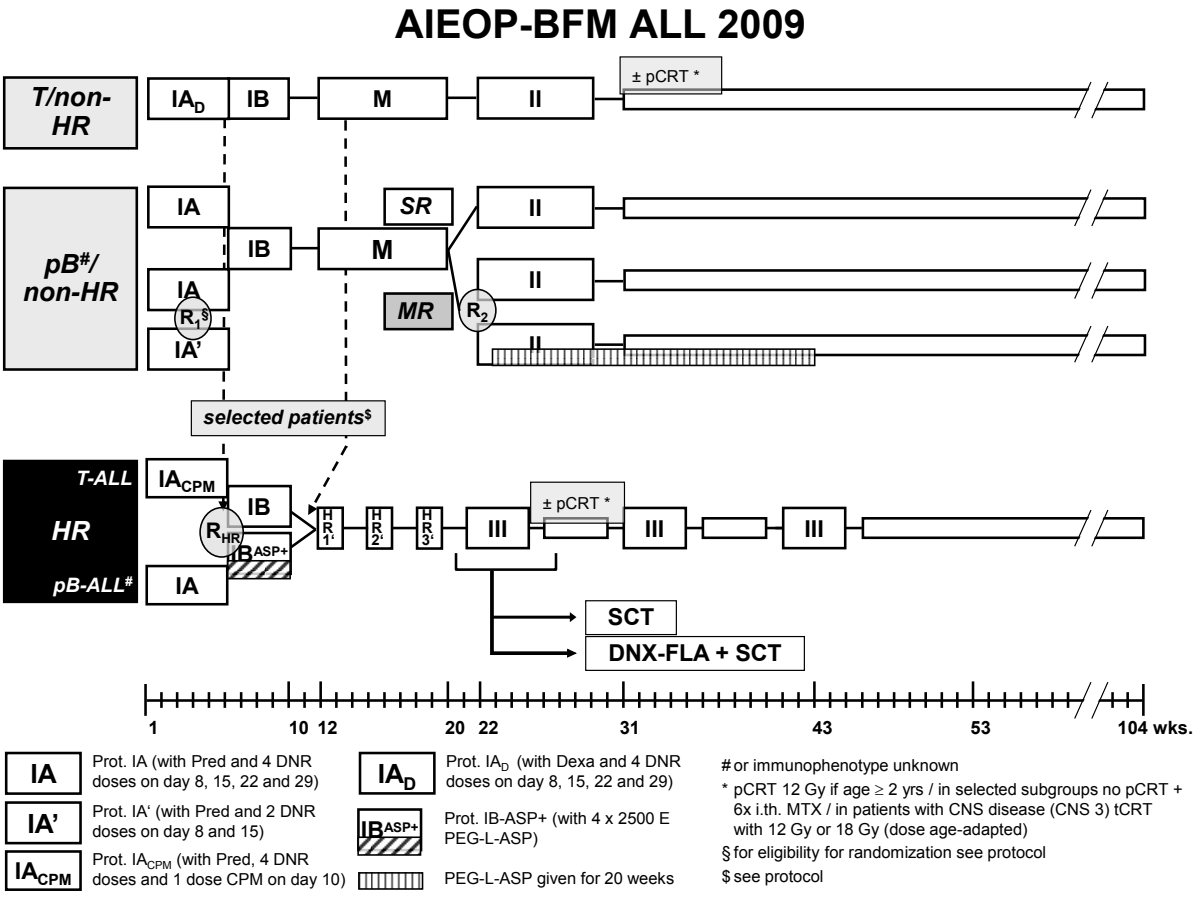


Figure 1 Treatment outline of protocol AIEOP-BFM ALL 2009

- Risk stratification:
    - Risk group definition by MRD: MRD measured by flow cytometry on day 15 (FCM-MRD d15) of induction treatment was implemented in AIEOP-BFM ALL 2009 and was used for MRD-based risk stratification of patients with inconclusive or missing PCR-MRD results and for allocation to the HR group in the case of > 10% blasts in FCM-MRD on d15. Furthermore, definition of MRD-SR by PCR-MRD was less restrictive in AIEOP-BFM ALL 2009 requiring only one highly sensitive marker with a quantitative range of at least 10^{-4} instead of two sensitive markers in AIEOP-BFM ALL 2000. Due to these modifications, risk stratification by PCR-MRD could be accomplished in 91.5% of the patients (interim analysis 03/2015) as compared to 76.5% in AIEOP-BFM ALL 2000 (Flohr*, et al* 2008). The majority of the patients not stratifiable

by PCR-MRD could be stratified by FCM-MRD enabling an MRD-based stratification in > 99% of patients.
Patients with pB-ALL and slow MRD response not meeting the MRD-HR criteria (i.e. ≥ $5x10^{-4}$ at TP1 and positive but <$5x10^{-4}$ at TP2, MRD-MR SER) had a poor EFS in trial AIEOP-BFM ALL 2000 in risk group MR and were therefore allocated to HR in AIEOP-BFM ALL 2009. Patients with MRD-MR-SER and/or FCM-MRD HR with no other HR criteria accounted for approx. 9% of the patients in AIEOP-BFM ALL 2009 (interim report 03/2015) resulting in a significantly larger HR group as compared to the trial AIEOP-BFM ALL 2000.

- Hypodiploidy as HR criterion: Hypodiploidy (defined as a modal chromosome number of <45 chromosomes in the leukemic clone) was implemented as high risk criterion in AIEOP-BFM ALL 2009. In 1.2% of patients a hypodiploid karyotype could be detected (interim report 03/2015); the majority of these patients would not have been stratified to HR according to the former risk criteria of AIEOP-BFM All 2000.

- PEG-L-asparaginase as first-line L-asparaginase product: While the toxicity profile appears acceptable, less allergies at time of reintensification are being observed. This is an extremely important clinical finding as allergies were previously found in one third of all patients, and prevented the further use of E. coli Asparaginase. The prospective analysis of pharmacodynamics in all treatment phases is still ongoing with the main focus on the randomized questions and correlation between enzyme activities in serum and clinical outcome.
- Intensified treatment with DNX-FLA before SCT in patients with poor MRD response over the HR blocks: Prospective MRD measurements during HR treatment in the study ALL-BFM 2000 could identify a small patient group with inadequate MRD response to the HR courses and very poor prognosis despite HSCT. Patients with an MRD load of ≥ $5x10^{-4}$ or higher after the third HR block therefore received an alternative chemotherapy element, DNX-FLA, with the aim to further reduce the MRD load before going into alloHSCT. In an interim analysis including BFM patients diagnosed until 31.10.2015, 35 patients had an MRD load of ≥ $5x10^{-4}$ after the third HR block and were therefore eligible for the treatment with DNX-FLA. Nine of them were not treated with DNX-FLA (early relapse/progression n=4, standard chemotherapy n=1, other experimental treatments n=4), and one had very short follow up. DNX-FLA was given to 25/35 patients, and in 23 of these patients, MRD results after DNX-FLA were available. An MRD reduction by at least one log-step could be achieved in 16/23 (70%) patients; in 12/23 patients, the MRD load could be reduced to <$5x10^{-4}$ (6/10 pB-ALL and 6/13 T-ALL). Two patients developed a life-threatening toxic event (infection) related to DNX-FLA; no patient died due to this treatment element.
- Addition of a Cyclophosphamide dose on day 10 of Protocol IA in T-ALL patients with prednisone poor-response (PPR): Preliminary interim analyses of patients with T-ALL/PPR who received an additional dose of Cyclophosphamide in induction Protocol IA did not indicate an increase of life-threatening and fatal toxic events in this treatment phase in comparison to pB-ALL patients treated with standard induction Protocol IA.
- ETP-ALL: Early T-precursor ALL is considered a high-risk subgroup of T-ALL by many investigators (Coustan-Smith*, et al* 2009). Preliminary results have shown that the treatment protocol AIEOP BFM-ALL 2009 is quite effective for ETP-ALL in AIEOP patients (Conter*, et al* 2016).
- Randomized questions in AIEOP-BFM ALL 2009: Sufficiently mature results of the randomized study questions of AIEOP-BFM ALL 2009 are currently not yet available. For the successor trial AIEOP-BFM ALL 2017, patients are therefore treated in the

former standard arms. In 2015, the DSMC recommended to extend recruitment by 1.5 years to allow comprehensive final analysis in randomized groups.

2.2 Rationale of the study concept of AIEOP-BFM ALL 2017

2.2.1 Inclusion of patients with infant ALL

ALL in infants younger than 1 year is biologically different from ALL in older children and is associated with particularly poor EFS rates. The two consecutive international trials INTERFANT 99 and INTERFANT 06 were specifically designed for patients with infant ALL and were available from 2000 to 2016 for the treatment of ALL patients at less than 1 year of age. Infants were therefore excluded from the trials AIEOP-BFM ALL 2000 and 2009. The end of recruitment into trial INTERFANT 06 was in August 2016, and it is not expected that a new ALL treatment protocol for infants will be available by the start of AIEOP-BFM ALL 2017.

Although the outcomes achieved for infant ALL in INTERFANT 99 were better than those with most previous protocols, they were not different from those of single group studies based on BFM regimens such as AIEOP-ALL 95 or ALL-BFM 95 (Pieters*, et al* 2007). This justifies the inclusion of infant patients in AIEOP-BFM ALL 2017 without expecting a disadvantage compared to the therapy with the standard-of-care treatment regimen according to INTERFANT. The inclusion of the infants in the randomizations as planned in AIEOP-BFM ALL 2017 will provide an additional chance for this prognostically poor patient group in particular for those patients with *KMT2A* (*MLL*) rearrangement where conventional chemotherapy approaches so far have failed in the majority of patients.

2.2.2 Stratification

The stratification in AIEOP-BFM ALL 2017 revises and extends the previous attempts to provide individual risk-adapted therapy. The wide range of biological features of the leukemia (immunophenotype, specific chromosomal aberrations) accounts for the different outcome of these subgroups. Consequently, very different requirements regarding treatment intensification and/or modification need to be met. Precise evaluation of treatment response (prednisone response, response after induction, MRD) will be retained for the identification of high-risk patients and for risk stratification (SR, MR) within the biological subgroups. Trial AIEOP-BFM ALL 2009 has shown that response-oriented stratification can now be accomplished in more than 99% of the patients. The stratification criteria are shown in detail in section 5.1.

2.2.2.1 PCR-MRD

The detection of clone-specific immunoglobulin (Ig) and T-cell receptor (TCR) gene rearrangements by PCR amplification is a technique widely used for MRD studies in ALL. Through analysis of rearrangements of immunoglobulin (heavy chain, IgH, and light chain, IgK) and T-cell receptors genes, it is possible to identify clone-specific sequences corresponding to N-junctional regions of different recombinations. These junctional regions can be regarded as fingerprint-like clone-specific sequences owing to deletion and random insertion of nucleotides. Oligonucleotide primers are designed at opposite sides of the junctional region. To discriminate between the leukemia-derived PCR products and PCR products of normal cells with comparable rearrangements, the amplification products are generally hybridized to an ALL clone-specific junctional region probe. PCR-based MRD detection by clone-specific junctional regions can generally reach sensitivities of 10^{-4} to 10^{-5} (one leukemic cell in 10^4 to 10^5 cells).

The I-BFM-SG MRD study (see section 2.1.2) showed the best discrimination of risk groups through the combined evaluation of MRD at two different time points during treatment, on day 33 (TP 1) and at week 12 (TP 2). MRD negativity at TP 1 had high specificity in

identifying patients with particularly low relapse risk, whereas high MRD load at late time points (in AIEOP-BFM ALL 2000 ≥ 10^{-3} at TP 2) revealed to be more specific for the identification of patients at high risk of relapse. The combined use of these two time points resulted in an excellent discrimination of risk groups in AIEOP-BFM ALL 2000 and 2009, and it is, thus, retained in AIEOP-BFM ALL 2017.

The PCR-MRD risk stratification criteria will basically be maintained in AIEOP-BFM ALL 2017. Thus, one sensitive marker with a sufficient quantifiable range (sensitivity and quantifiable range at least 10^{-4}) will be considered sufficiently reliable for stratification into SR if a second marker is not available. However, the establishment of at least two sensitive markers should still be aimed at. With this strategy, 91.5% of patients (92.7% in pB-ALL, 85.2% in T-ALL) could be stratified based on MRD analysis by PCR in AIEOP-BFM ALL 2009 (interim results 03/2015). As in the previous study, patients will basically be stratified in three MRD risk groups: MRD-SR, MRD-MR and MRD-HR. The so called MRD-MR Slow Early Response (SER, high PCR-MRD load [≥ $5x10^{-4}$] at TP 1 and still positive [< $5x10^{-4}$] at TP 2) which was introduced for stratification in AIEOP-BFM ALL 2009 will be retained as HR criterion for patients with pB-ALL also in AIEOP-BFM ALL 2017.

2.2.2.2 FCM-MRD

MRD measured on day 15 by the means of flow cytometry (FCM-MRD d15) will continue to be used in AIEOP-BFM 2017. High FCM-MRD load of ≥ 10% will qualify for treatment in the HR group also in AIEOP-BFM ALL 2017. Only the very small group of patients with *ETV6-RUNX1* (*TEL-AML1*) will not be stratified to HR solely on the basis of FCM-MRD d15 ≥ 10%, as data of the AIEOP-BFM ALL 2000 trial suggest an excellent overall survival of these patients if they show favorable MRD response during subsequent treatment.
Patients with missing or inconclusive PCR-MRD results and without any HR criteria (about 5% of the total group) will continue in AIEOP-BFM ALL 2017 to be stratified by the day 15 FCM-MRD result, i.e. as SR if residual blasts are less than 0.1%, MR if FCM-MRD 0.1 - < 10 % blasts.

2.2.2.3 Stratification in pB-ALL

Some modifications will be made in the risk stratification of pB-ALL in AIEOP-BFM ALL 2017 (for details see section 0):

2.2.2.3.1 Stratification according to response evaluation

Prednisone Poor-Response (PPR): After implementation of PCR-MRD and FCM-MRD d15 for risk stratification, the PPR has become less important as high-risk criterion in pB-ALL whereas it retained independent prognostic value in T-ALL. In AIEOP-BFM ALL 2009, only 2.3% of pB-ALL patients were stratified to HR because of PPR as only HR criterion. Preliminary analyses of this subgroup from trial AIEOP-BFM ALL 2000 suggest a cumulative relapse incidence of <10% for this subgroup. Although these excellent results were achieved with HR treatment, the high incidence of serious and fatal toxicity associated with the HR treatment seems not to justify to stratify these patients into the HR arm on this basis alone. Prednisone Response will therefore no longer be a stratification factor in pB-ALL patients except for the few cases in which both FCM-MRD d15 and PCR-based MRD detection are not available. Assessment and documentation of the Prednisone Response will nevertheless be continued in AIEOP-BFM ALL 2017 to allow comparative analyses with previous studies.

FCM-MRD HR in *ETV6-RUNX1*-positive ALL: Patients with *ETV6-RUNX1*-positive pB-ALL and FCM-MRD HR will not be stratified to HR in the absence of other HR criteria (see also 2.2.2.2).

Definition of an early HR group: The concept of implementing a randomized treatment question in poor-risk patients during consolidation and, thus, before final risk stratification at

MRD TP2 required the definition of a "preliminary" HR group on the basis of genetic and response criteria as available by MRD TP1. This led to the definition of the "early HR" group. Early HR patients will be eligible for randomization R-eHR and will consist of the patients that have been identified as HR by this time point in addition to those with MRD load of ≥ $5x10^{-4}$ at TP1, thus including also the majority of patients with later MRD-HR or MRD-MR SER as only HR criterion. For detailed risk group criteria in pB-ALL, see section 5.3.2.2.1.

Definition of PCR-MRD-SR by TP1 only: Unlike the former approach in AIEOP-BFM ALL 2009 when PCR-MRD samples of the two examination time points TP1 (d33) and TP2 (d78/96) were analyzed together after receiving the TP2 sample, the TP1 samples will be analyzed separately as soon as possible after d33 to enable early stratification of the patients to early HR and early non-HR in AIEOP-BFM ALL 2017. Patients with clear MRD negativity (at least one marker with sensitivity/quantitative range of $<10^{-4}$) at TP 1 will be classified as PCR-MRD-SR on the basis of TP1 only and will be stratified to SR in the absence of HR criteria. The analysis of the TP2 sample is not intended in these patients.

Other response-based stratification will remain unchanged in AIEOP-BFM ALL 2017. This includes morphological non-remission at the end of induction Protocol IA which qualifies the patients for HR treatment as well as MRD-based risk stratification by PCR and FCM.

2.2.2.3.2 Stratification according to genetic evaluation

***IKZF1*^{plus}:** Analyses on copy number alterations of selected genes that have been described as candidate markers potentially useful for risk stratification in childhood ALL were conducted in 986 patients enrolled in the BFM part of the AIEOP-BFM ALL 2000 trial. *IKZF1* deletions co-occurring with deletions in *CDKN2A*, *CDKN2B*, *PAX5*, or *PAR1* in the absence of *ERG* deletion conferred the worst outcome and were grouped as *IKZF1*^{plus}. In trial AIEOP-BFM ALL 2000, *IKZF1*^{plus} comprised 6% of precursor B-cell ALL patients with a 5-year pEFS of 53% compared to 77% in *IKZF1*-deleted but *IKZF1*^{plus}-negatives, or 87% in patients negative for any *IKZF1* aberration (*P*<0.0001); respective 5-year cumulative relapse incidences (CIR) were 44%, 12%, and 10% (p<0.0001). Results were confirmed in a replication cohort of 417 patients from AIEOP, and multivariate analyses demonstrated independence of *IKZF1*^{plus}. Dramatic differences of the prognostic impact of *IKZF1*^{plus} were detected in analyses stratified by MRD levels after induction treatment: 5-year EFS for MRD-negative *IKZF1*^{plus} patients was 94% compared to 40% in MRD-intermediate- and 30% in MRD-high-risk *IKZF1*^{plus} patients (p<0.0001); corresponding 5-year CIR: 6%, 60%, and 60% (p<0.0001) (Dagdan*, et al* 2014). These results led to the implementation of *IKZF1*^{plus} as risk stratification factor in patients who are <u>not</u> MRD-negative at the end of induction phase (MRD TP1) in AIEOP-BFM ALL 2017.

The single aberrations that define the *IKZF1*^{plus} pattern can be detected in combination with other recurrent genetic aberrations of known prognostic importance (such as *ETV6-RUNX1* (*TEL-AML1*), *TCF3-PBX1* (*E2A-PBX1*), *TCF3-HLF* (*E2A-HLF*) and *KMT2A* (*MLL*) rearrangements or hypodiploidy) (Moorman*, et al* 2010) but the prognostic importance is unknown. The incidence of the *IKZF1*^{plus} pattern in combination with the above-mentioned genetic aberrations that will in part be used for risk stratification in the AIEOP-BFM ALL 2017 trial is unknown. In the data presented above (Dagdan*, et al* 2014), such combinations could not be observed suggesting that it is at least a very rare constellation with completely uncertain prognostic relevance. *IKZF1*^{plus} will therefore be implemented for risk stratification in AIEOP-BFM ALL 2017, defining a hierarchy of predefined genetic aberrations. In summary, *IKZF1*^{plus} will qualify patients for HR treatment if they are <u>*not*</u> PCR-MRD-negative at TP1 <u>*and not*</u> detected positive for *ETV6-RUNX1* (*TEL-AML1*), *TCF3-PBX1* (*E2A-PBX1*) or *KMT2A* (*MLL*) rearrangement other than *KMT2A-AFF1* (*MLL-AF4*). Patients with *KMT2A-AFF1* (*MLL-AF4*) or *TCF3-HLF* (*E2A-HLF*) fusion or hypodiploidy are *per se* qualified for HR treatment.

***TCF3-HLF*:** Patients carrying the t(17;19)(q22;p13) translocation with the fusion gene *TCF3-HLF* (*E2A-HLF*) have almost invariably been reported with a fatal outcome (Hunger*, et al*

1992, Inukai, *et al* 2007). This subgroup of patients is therefore eligible for experimental treatment per se. Drug response profiling of *TCF3-HLF* positive patient-derived xenografts revealed a distinct profile for *TCF3-HLF* ALL with resistance to conventional chemotherapeutics, but sensitivity towards glucocorticoids, anthracyclines and other agents in clinical development, in particular towards the *BCL2*-specific inhibitor venetoclax (Fischer, *et al* 2015) which is, however, currently not available for the use in patients. As long as a specific therapeutic approach is not available for these patients, they are eligible for treatment in the HR group in the experimental arms of randomizations R-eHR and R-HR.

Other genetic HR criteria will remain unchanged in AIEOP-BFM ALL 2017. This includes the t(4;11)(q21;q23) translocation (or *KMT2A-AFF1* [*MLL-AF4]* gene fusion) and hypodiploidy (<45 chromosomes).

2.2.2.4 Stratification in T-ALL

Risk stratification of T-ALL will basically be unchanged in AIEOP-BFM ALL 2017 as compared to the trial AIEOP-BFM ALL 2009. In contrast to pB-ALL, PPR still has relevant prognostic value in T-ALL independent of PCR-MRD and FCM-MRD and will therefore remain as high-risk criterion. All other risk criteria are also response-oriented: No CR at end of induction Protocol IA, FCM-MRD d15 ≥10% leukemic blasts in the bone marrow and PCR-MRD at ≥ $5x10^{-4}$ will qualify for HR treatment. As in pB-ALL (see above) MRD negativity at TP1 is sufficient for allocation to PCR-MRD-SR.

2.2.2.5 Stratification of infants

The risk stratification of patients <1 year of age will basically be adopted from the stratification criteria of the infant ALL trial INTERFANT 06 that were based on the results of the previous trial INTERFANT 99 (Pieters, *et al* 2007). Patients with pB-ALL and evidence of any *KMT2A* rearrangement will be stratified into the early HR and final HR group and will be eligible for the randomizations R-eHR and R-HR. Risk stratification and randomization eligibility for other infant patients (pB-ALL without evidence of *KMT2A* rearrangement and all T-ALL) will be the same as for patients ≥ 1 year of age. Criteria for alloHSCT indication will also be based on the INTERFANT 06 protocol and will include age, initial WBC, prednisone response and MRD response.

2.2.3 Randomized questions

2.2.3.1 General remark

During the planning phase of trial AIEOP-BFM ALL 2017, the study group has extensively discussed available options to select the right therapeutic agents for a modified treatment approach. The group had to consider

- status of pediatric drug development,
- toxicity profile, in particular in combination therapy,
- data of efficacy in ALL, in particular in pediatric ALL (including relapsed ALL),
- proportion of patients at higher risk to relapse who may have a benefit from such an intervention,
- current outcome data with available therapies from ALL study groups worldwide.

While targeted therapies have received a lot of attention in recent years (e.g. (Annesley and Brown 2015, Roberts, *et al* 2014a, Schultz, *et al* 2009, Weston, *et al* 2013), it was not evident that in MRD-based risk-adapted treatment an alternative approach to these rather rare genetic lesions would succeed as current treatment schedules obviously overcome such adverse factors (Biondi, *et al* 2012, Dörge, *et al* 2013). Some other promising agents appeared to have interesting features in adult malignancies but had no or very little clinical

data in pediatric ALL (e.g. m-TOR inhibitors such as Rapamycin or Everolimus; JAK1/2 inhibitors such as Ruxolitinib; FLT3 inhibitors such as Lestaurtinib, Midostaurin, or Sorafenib; Histon-deacetylase inhibitors such as Vorinostat or Panobinostat). One rather new class of agents called proteasome inhibitors came into focus, and two agents have been considered: Carfilzomib is the latest proteasome inhibitor but the early clinical testing in pedatrics is not yet finalized. The first agent in this class has been Bortezomib which has been tested in pediatric ALL in US studies of the Childrens Oncology Group, and is currently being studied in a phase III trial in relapsed ALL in Europe. These data suggest not only an acceptable toxicity profile but also an interesting combinatorial efficacy in ALL (Messinger*, et al* 2012, Vora*, et al* 2016). In the class of immunotherapeutic agents, some interesting developments were taken into consideration: anti-CD22 mAb Epratuzomab, anti-CD22 mAb conjugate Inotuzumab, anti-CD20 mAb Rituximab, anti-CD19 CAR-T-cells (CTL-109), and the bispecific mAb-construct Blinatumomab. While Epratuzumab is being evaluated in a Phase III study in relapsed ALL, there is no early pediatric clinical data available for Inotuzumab. Rituximab has shown large efficacy in mature B-cell lymphoma and L3-(Burkitt) leukemia but has very limited data in pediatric pB-ALL. Thus, with the remaining anti-CD19 CAR-T-cells and the bispecific mAb-construct Blinatumomab two very different potentially promising approaches were looked at but finally the available data were in favor of Blinatumomab. Unfortunately, head-on comparison of both agents was not supported by either company. In T-ALL, no such agent is available.

2.2.3.2 Background for the use of Bortezomib

2.2.3.2.1 Mechanism of action of Bortezomib

The proteasome inhibitor Bortezomib has specifically been designed to inhibit the activity of the 26S proteasome in mammalian cells. The 26S proteasome is a large protein complex that degrades ubiquitinated proteins. The ubiquitin-proteasome system plays a crucial role in regulating the flow of proteins involved in regulatory cellular processes including cell cycle regulation, transcription factor activation, apoptosis, and cell trafficking. It is thereby essential for maintaining normal cellular functions. Bortezomib inhibits the 26S proteasome by reversible covalent binding to its catalytic site (Bonvini*, et al* 2007, Gelman*, et al* 2013).

The anticancer activity of Bortezomib is still not completely understood. Multiple mechanisms are involved in its action including intracellular pathways controlling apoptosis (Crawford*, et al* 2011, Frankland-Searby and Bhaumik 2012) e.g. NF-κB inhibition (Ciechanover 1994, Du and Chen 2013), inhibition of p53 and cell cycle regulatory proteins such as the cyclin-dependent kinase inhibitor p21 (Ciechanover 1994). Previous studies have shown that Bortezomib can act as chemosensitizers in malignant hematological cells (Landis-Piwowara*, et al* 2006, Martin-Lorenzo*, et al* 2015). It is assumed that proteasome inhibition effectively alters the ratio of pro-apoptotic and anti-apoptotic proteins within a cell, resulting in an increased sensitivity to apoptosis (Adams*, et al* 1999).

2.2.3.2.2 Pre-clinical studies

In several pre-clinical studies, Bortezomib potently inhibited cell proliferation in tumor cell lines(Adams*, et al* 1999) and showed anti-tumor activity in a wide variety of cancer cell lines (Annesley and Brown 2015, Horton*, et al* 2007, Roberts*, et al* 2014b, Szczepanek*, et al* 2010). *In vitro* and *ex vivo* experiments could demonstrate activity of Bortezomib against leukemic cells including pB-ALL and T-ALL as well as AML (Junk*, et al* 2015, Niewerth*, et al* 2013). Furthermore, it could be shown that Bortezomib has synergistic *in vitro* anti-leukemia activity when combined with Dexamethasone as well as additive effects in combination with Asparaginase, Vincristine, Doxorubicin, Cytarabine and Cyclophosphamide (Horton*, et al* 2006, Messinger*, et al* 2010).

Other *in vitro* studies (Martin-Lorenzo*, et al* 2015, Niewerth*, et al* 2013) could confirm the synergistic effects of glucocorticoids and Bortezomib and could moreover show that

glucocorticoid resistance could be overcome in ALL and AML cell lines by the combined treatment. This may be of clinical importance as a relevant proportion of the patients with high-risk ALL presents with *in vivo* glucocorticoid resistance (Prednisone Poor-Response), and may therefore benefit from glucocorticoid-sensitizing strategies. Bortezomib also seems to be able to overcome chemotherapy resistance to anthracyclines, alkylators, and glucocorticoids in a number of other malignancies (Chauhan*, et al* 2004, Ludwig*, et al* 2005, Ma*, et al* 2003, Mitsiades*, et al* 2003, Zheng*, et al* 2012).

2.2.3.2.3 Safety and efficacy of Bortezomib in children

As suggested by the findings in pre-clinical T-ALL mouse models in which Bortezomib showed only modest activity as single agent (Greco*, et al* 1990), almost no activity was observed in phase I studies in children and adults when Bortezomib was given as monotherapy to heavily pretreated patients with refractory or relapsed leukemia (Mujtaba and Dou 2011, Vora*, et al* 2016).

The first *in vivo* activity of bortezomib published was observed in combination with dexamethasone in a child with 5 relapses of ALL (Adams 2002). The American group Therapeutic Advances in Childhood Leukemia (TACL) has shown in phase I and phase II studies, that in patients (1-22 years of age) with refractory/relapsed ALL and combination with chemotherapy (Dexamethasone, Doxorubicine, Vincristine and PEG-asparaginase), Bortezomib showed promising results with better response rates in particular in pB-ALL (Kaspers*, et al* 2014, Messinger*, et al* 2012). Ten out of 22 patients developed ≥ grade 3 infection. After 3 patients had died from bacterial infections, the remaining patients received significant supportive care modifications (prophylactic vancomycin, levofloxacin, voriconazole or posaconazole) without further infections. Two patients developed grade 3 peripheral neuropathy after 4 doses of bortezomib with residual neuropathy, 3 patients had asymptomatic hypophosphatemia. No increase of bone marrow aplasia was observed.

Recent COG data from a phase II study adding bortezomib to reinduction chemotherapy in 61 children and young adults ≤ 21 years of age with 2nd or later relapse of ALL were presented at the ASCO and SIOP meetings 2015. Bortezomib (1.3 mg/m2) was administered together with prednisone, doxorubicine, vincristine, and PEG-asparaginase as the first reinduction therapy, followed by combination of bortezomib, cyclophosphamide, etoposide and methotrexate. In comparison with historical control patients who received standard reinduction therapy only, CR rates and minimal residual disease (MRD) response after the first reinduction block improved. Toxicity was similar to historical control with infections (20%) being the most common toxicity. Peripheral neuropathy occurred only in 3 patients, one of which was associated with herpes zoster.

A European phase II study in children (age 1.2 - 17.5 years) with 1st relapse after HSCT or ≥ 2nd relapse or refractory first relapse treated with bortezomib in combination with vincristine, dexamethasone and one intrathecal administration of MTX was conducted. Preliminary results were presented at the ASH meeting 2014 and showed similar results (Jagannath*, et al* 2005): Eleven out of 17 evaluable patients with ALL had a good initial response, 10 patients received a 2nd cycle of the combination therapy and half of them achieved a complete remission. After one cycle, 8 patients had a grade 3/l4 toxicity, pain was the most frequent toxicity (4/17 patients), peripheral neuropathy and fatigue were reported in 2 patients each. After 2 cycles 3/10 patients experienced 3/4 toxicity, two of them had peripheral neuropathy. Both, the COG and the European study have not reported increased toxicities, when bortezomib was given with vincristine.

DLTs in single agent trials included thrombocytopenia, confusion and febrile neutropenia associated with hypotension and an elevated creatinine (Shah*, et al* 2001, Vora*, et al* 2016). The Children's Oncology Group (COG) conducted a phase II study in children and young adults (age > 12 months - 29 years) with refractory or relapsed acute myeloid leukemia (AML), who received bortezomib in combination with chemotherapy (Houghton*, et al* 2008). It has been shown, that bortezomib was safely given together with combination chemotherapy

with either idarubicin/cytarabine or cytarabine/etoposide without increased toxicity compared to other pediatric trials in relapsed leukemia. Peripheral neuropathy occurred, but did not exceed grade 2. In this difficult patient population with refractory/relapsed AML, outcome was not improved, but the number of leukemia-initiating cells was depleted, which led to a phase III study currently conducted by the COG.

Another phase II study by the COG included 26 children and young adults until the age of 30 years with refractory or first relapse Hodgkin's lymphoma (Blaney*, et al* 2004). Bortezomib was added to combination chemotherapy with ifosfamide and vinorelbine for up to four cycles. Anatomic CR rates, assessed by CT scan after two cycles of therapy was low, but the overall response (OR) rate at the completion of therapy improved to 83% compared to historical controls with a OR rate of 72%. The most common toxicities were hematologic toxicities. Grade 3 neuralgia occurred in two patients, one of which was associated with varicella zoster infection.

2.2.3.2.4 Special pediatric consideration

Bortezomib might locally target the growth plate and permanently impair linear bone growth in young mice (increased apoptosis of resting/stem cell-like chondrocytes, decreased differentiation/hypertrophy of growth plate chondrocytes) *in vivo* and *in vitro* (Horton*, et al* 2014). The impact on children is still unknown. Vitamin D receptor in its cytosolic form is subject to ubiquitination and proteasomal degradation. Bortezomib leads to increase of markers of osteoblastic function in serum samples of patients (Horton*, et al* 2015, Lund*, et al* 2010) and in bone mineral density (Eriksson*, et al* 2012, Terpos*, et al* 2010). Bortezomib alone neither affects subcellular localization of vitamin D receptor nor induces signaling of vitamin D receptor, but together with vitamin D it leads to maximal stimulation of vitamin D receptor signaling activity and stimulatory effects on differentiation (Heider*, et al* 2006).

2.2.3.3 Background for the use of Blinatumomab

2.2.3.3.1 Mechanism of action of Blinatumomab

Blinatumomab is a murine, single-chain antibody belonging to a class of antibody constructs, called bi-specific T-cell engagers (BiTE) (Loffler*, et al* 2000, Mack*, et al* 1995). Blinatumomab has been designed to engage and tether cytotoxic T-cells (CTL) to CD19-expressing target B cells, irrespective of the T-cell receptor (TCR) specificity and of HLA-restriction by combining the binding specificity for the pan B-cell antigen CD19 and the epsilon chain of the T cell receptor/CD3 complex on one polypeptide chain. The anti-tumor activity of BiTE is measurable within a wide range of effector to target (E:T) ratios. $CD19^+$ lymphoma and leukemia cell lines were reported to be extremely sensitive to blinatumomab-mediated *in vitro* cytotoxicity when incubated with T cells (Loffler*, et al* 2000, Mack*, et al* 1995).

2.2.3.3.2 Trials on efficacy of Blinatumomab in adults

The German Multi-center Study Group on Adult ALL (GMALL) evaluated the safety and efficacy of a 28-day continuous intravenous infusion of blinatumomab at 15 $\mu g/m^2$/day in 21 adults with precursor B cell ALL and minimal residual disease (MRD) persistence or relapse after induction and consolidation therapy (Topp*, et al* 2011). According to this study protocol, patients received a cycle of 4 weeks' blinatumomab treatment followed by a treatment-free period of 2 weeks. Responders were permitted to receive three additional consolidation cycles of treatment with blinatumomab or could be given allogeneic hematopoietic stem cell transplantation (HSCT). Sixteen of these 21 patients became MRD negative, including subjects with either Philadelphia-positive (Ph^+) ALL or with t(4;11) ALL. Remarkably, 12 patients among the 16 responders had been molecularly refractory to previous chemotherapy (Topp*, et al* 2011). This study indicates that blinatumomab has the potential to induce durable remissions in molecularly refractory pB-ALL. In this study, 4 out of 20 evaluable MRD^+ ALL patients failed to respond. This was not correlated with either baseline

T-cell counts or *in vivo* measures of T-cell activation. Similarly, tumor load in either the bone marrow or the peripheral blood did not predict treatment response (Klinger*, et al* 2012) .

In a subsequent study, 36 adult patients with relapsed or refractory pB-ALL were treated with blinatumomab in cycles of 4-week continuous infusion followed by a 2-week treatment-free interval in a single-arm study with a dose-finding stage and an extension stage (Topp*, et al* 2014). The primary end point was complete remission (CR) or CR with partial hematologic recovery (CRh). The drug was administered by continuous intravenous infusion at 3 dose levels of 5-30 µg/m^2/day. Responding patients proceeded to allogeneic HSCT or received a total of up to 5 cycles of blinatumomab. Twenty-five of these 36 patients (69%) achieved a CR or CRh, with 88% of the responders achieving an MRD response (Topp*, et al* 2014). Median overall survival was 9.8 months (95% CI, 8.5 to 14.9), and median relapse-free survival was 7.6 months (95% CI, 4.5 to 9.5). Thirteen responders (52%) underwent HSCT after achieving a CR or CRh. This study indicates that the drug is also effective in ALL patients with either overt relapse or hematological resistance to conventional treatment.

A further multicentre, single-arm, open-label phase 2 study enrolled 189 adult patients with Philadelphia-chromosome-negative, primary refractory or relapsed (first relapse within 12 months of first remission, relapse within 12 months after allogeneic HSCT, or no response to or relapse after first salvage therapy or beyond) pB-ALL (Topp*, et al* 2015). Patients received blinatumomab (9 µg/day for the first 7 days and 28 µg/day thereafter) by continuous intravenous infusion over 4 weeks every 6 weeks (up to five cycles), per protocol. The primary endpoint was CR or CRh within the first two cycles. After two cycles, 81 (43%, 95% CI 36-50) patients had achieved a CR or CRh: 63 (33%) patients had a CR and 18 (10%) patients had a CRh. Thirty-two (40%) patients who achieved CR/CRh underwent subsequent allogeneic HSCT (Topp*, et al* 2015) Three deaths (due to sepsis, Escherichia coli sepsis, and Candida infection) occurring in this cohort were considered to be treatment-related by the investigators (Topp*, et al* 2015).

2.2.3.3.3 **Toxicity profile of Blinatumomab**

In trials conducted in adults with B-NHL or BCP-ALL, the majority of adverse events occurred during the first 72 hours of blinatumomab administration (Topp*, et al* 2015, Topp*, et al* 2014, Topp*, et al* 2011), and mainly consisted of pyrexia, chills, decrease in blood immunoglobulin, hypokalemia, cytokine-release syndrome (CRS), disseminated intravascular coagulation (DIC), and CNS events, such as seizures, disorientation, headache, dizziness, aphasia, and encephalopathy. Since CRS can be a life-threatening or even a fatal event, it must be timely diagnosed and promptly treated with either corticosteroids or with the anti-IL6 monoclonal antibody Tocilizumab. The neurological adverse events were fully reversible within 72 hours after treatment discontinuation (Topp*, et al* 2015, Topp*, et al* 2014, Topp*, et al* 2011). B-cell deficiency, a consequence of chronic depletion of CD19-positive cells induced by blinatumomab, can be managed by immunoglobulin infusions.

2.2.3.3.4 **Safety and efficacy of Blinatumomab in children**

Available data on the safety and efficacy of blinatumomab in children are more limited. Handgretinger and colleagues reported on 3 pediatric patients with ALL that relapsed after allogeneic HSCT (Handgretinger*, et al* 2011). Children were given the drug in continuous intravenous infusion at a dosage of 15 µg/m²/day for at least four weeks. In all patients, CD3$^+$ T lymphocytes initially declined and subsequently expanded, and were donor-derived, as suggested by chimerism studies. However, no signs of graft-versus-host disease (GVHD) were noted. All patients showed molecular complete response at 4 weeks' treatment, having an MRD level of $<10^{-4}$, which is below the quantitative detection limit. Safety was described as acceptable and there were no treatment interruptions or discontinuations due to adverse events (Handgretinger*, et al* 2011). These data were the first showing that blinatumomab is well tolerated and represents a promising option also in pediatric patients with relapsed BCP-ALL, when the aim is at inducing a second remission and clearing MRD before an allograft is performed.

In the pediatric setting, blinatumomab was also administered as a 4-week continuous intravenous infusion at a dosage of 5 or 15 µg/m^2/day in 9 children with either overt morphological or molecular relapse after allogeneic HSCT (Schlegel*, et al* 2014). Four patients achieved CR after the first cycle of treatment; 2 patients showed a CR from the second cycle after previous reduction of blast load by chemotherapy. Three patients did not respond, of whom one patient proceeded to a second cycle without additional chemotherapy and again did not respond. Four patients were successfully retransplanted in molecular remission from haploidentical donors (Schlegel*, et al* 2014). After a median follow up of 398 days, the probability of hematologic event-free survival is 30%. Major toxicities were grade 3 seizures in one patient and grade 3 cytokine release syndrome in 2 patients (Schlegel*, et al* 2014).

A recently conducted phase I-II multicenter international study evaluated safety, pharmacokinetics, recommended dose, and potential for efficacy of blinatumomab in children with relapsed/refractory BCP-ALL. Children <18 years with relapsed/refractory BCP-ALL were enrolled in the trial which consisted of a phase 1 dose-escalation part and a phase 2 part, using 6-week treatment cycles. Primary endpoints were maximum-tolerated dose (MTD; phase 1) and CR rate within the first two cycles of treatment (phase 2). Blinatumomab pharmacokinetics was linear across dose levels and consistent among age groups. Based on the phase 1 data, the recommended blinatumomab dose for children with relapsed/refractory ALL was 5 µg/m2/day for the first 7 days followed by 15 µg/m^2/day. Among the 70 patients who received the recommended dose, 27 (39%, 95%-CI 27-51%) achieved CR within the first two cycles, 14 (52%) of whom achieved complete MRD response. Blinatumomab at 5/15-µg/m²/day showed anti-leukemia activity across all age groups, including patients <2 years and those with unfavorable cytogenetics. The study in adults with MRD-positive disease reported higher incidences of neurologic events than that seen in this study.

So far, no data on the use of blinatumomab in children in morphological CR but with persistent MRD are available. However, considering that blinatumomab-related toxicities and CRS in particular are correlated with the tumor burden, it is not expected that the use of the drug in children with molecularly refractory disease will be associated with undue and not manageable toxicity.

At this time (01-2017), two randomized clinical trials with blinatumomab are ongoing in pediatric patients with relapsed ALL (one in the COG and one in the IntReALL group).

2.2.3.4 Randomization R-eHR: Bortezomid in extended consolidation for early high-risk pB-ALL

Most of the randomized approaches to intensify therapy during late (i.e. post-consolidation) treatment phases that have been made in the last decades of AIEOP-BFM ALL treatment did not prove successful (Conter*, et al* 2007, Möricke*, et al* 2008, Schrappe*, et al* 2000a). For high-risk patients, a further issue is the high toxicity observed in the post-consolidation/reintensification phase in the current AIEOP-BFM HR concept which does not allow further chemotherapy intensification indicating that the means of conventional chemotherapy are virtually exhausted for this patient group.

Recent outcome data of patients with the so-called *IKZF1*^{plus} signature (see section 2.2.2.3) showed a strikingly different relapse risk depending on the MRD load at the end of induction with almost no relapses if the patients were MRD-negative but a 5-year cumulative relapse incidence of 60% in patients with positive MRD. These data were derived from BFM patients of the AIEOP-BFM ALL 2000 study (Dagdan*, et al* 2014) and could be confirmed in a replication cohort of AIEOP patients of the same study as well as in a patients cohort of the successor trial AIEOP-BFM ALL 2009 (unpublished results). The most exciting aspect of these data is the finding that even low MRD positivity at the end of induction was associated with a dramatic increase of relapse risk without modulation of this risk by further MRD dynamics.

The predictive value of MRD response for the incidence of relapse is generally considered to be directly linked with the particular sensitivity of the leukemic cells to the chemotherapeutic agents. However, the non-gradual outcome differences between *IKZF1*^{plus} patients with MRD negativity, low or high positivity at the end of induction indicate that primary resistance of the leukemic cells does not fully explain the association between MRD and relapse risk. The data led to the hypothesis that early treatment of the patients with genotoxic agents like cyclophosphamide may induce genetic damage in the leukemic cells potentially increasing the risk of evolving resistant clones. This hypothesis would fit the striking MRD-related differences observed in *IKZF1*^{plus} patients assumed that only patients with residual disease would provide a sufficient target for the genotoxic substances. The postulated mechanism may not only be relevant for patients with the *IKZF1*^{plus} pattern but could also play a role in other biological subtypes of pB-ALL.

The considerations above led to the concept of the randomization R-eHR for poor-risk pB-ALL that should fulfill the following requirements:

1. The randomized treatment intensification should be implemented in an early post-induction phase: Randomization R-eHR will therefore be scheduled during the consolidation phase. To enable the use of the TP1 MRD results in order to exclude patients with rapid MRD response from the randomization, some time is required for MRD analyses before starting the randomized treatment phase.
2. The randomized treatment should include a “new” drug with a novel mechanism of action as this seemed to be more promising than a new attempt of intensification with conventional chemotherapeutic substances: The rational for the use of the proteasome inhibitor Bortezomib in ALL is described in section 2.2.3.2. Bortezomib will be used on top of an extended consolidation phase which has been derived from the conventional BFM consolidation phase Protocol IB and has been modified in some aspects (now called “Extended Consolidation”). As discussed above, Bortezomib has a synergistic effect with chemotherapeutic agents including glucocorticoids, vincristine and L-asparaginase. These three drugs will therefore be given during a 2-week phase, interrupting Protocol IB after 2 weeks. The principle of inserting a treatment phase with only little myelotoxic effect into Protocol IB was accomplished in the “augmented BFM” regimen in pB-ALL HR COG protocols (Nachman*, et al* 1997) though no glucocorticoids were included in consolidation in these protocols. Bortezomib will be given at the same dosing and schedule (1.3 mg/m²/dose for 4 doses every three days) as published by Messinger *et al.* (Messinger*, et al* 2012). Patients randomized into the control arm will also receive the Extended Consolidation phase but without Bortezomib.
3. Cyclophosphamide as a potentially genotoxic drug should be postponed in order to achieve a better molecular remission before exposure to this drug: The first Cyclophosphamide dose, which was scheduled on day 1 in the original Protocol IB, will be postponed by four weeks. As eligibility for this randomization is not yet known at start of consolidation, the first Cyclophosphamide dose will be postponed in all patients with pB-ALL.

Patients with “early HR“ pB-ALL are eligible for randomization R-eHR. For the definition of this risk group, see section 5.3.2.2.1. Patients with Down Syndrome as well as patients with translocation *TCF3-HLF* are excluded from the randomization. Down Syndrome patients should be treated with the Short Consolidation ($Consol_{short}$). Patients with *TCF3-HLF* should receive the experimental arm *with* Bortezomib (see section 5.4.1).

2.2.3.5 Randomization R-HR: Blinatumomab in intensified consolidation phase for HR pB-ALL

The pB-ALL HR group will account for 16% of pB-ALL patients. Although this group consists of patients that are considered to be insufficiently treated with standard chemotherapy regimens, it represents a rather heterogeneous group and comprises patient groups with various outcomes. A more accurate stratification of these patients, however, can only be

done in retrospect when late MRD response to the intensified consolidation is taken into account. AIEOP-BFM ALL 2009 data on treatment-related mortality in this group demonstrate the particularly high toxicity of the intensified HR treatment with an incidence of death due to chemotherapy of 2.5%. Adolescent patients were at particular risk. The highest risk emanates from the highly intensive HR blocks for which a rate of about 2% fatal treatment complications can be expected.

On the other hand, the HR blocks are highly effective. Sixty-five percent of pB-ALL HR patients have positive MRD when entering the first HR block. In 80% of these patients, an MRD reduction by at least one log step can be achieved over the first HR block and more than half of them attain MRD negativity. Patients with positive MRD after the first or second HR block still reduce their MRD load in 58% or 52% over the second or third HR block, respectively. This also means, however, that 40 to 50% of the patients not yet MRD-negative after the first HR block may have no further benefit from the following blocks with respect to treatment response, and 18% of pB-ALL HR patients are still MRD-positive after the third HR block associated with particularly unfavorable outcome. For the high proportion of patients with negative MRD after the first HR block (~70%), however, the actual need for the following HR blocks is not clear and it can be critically discussed if such an intensive treatment is justified for these patients in the light of the associated severe toxic effects.

The background data on the use of the bi-specific T-cell engager (BiTE) Blinatumomab in ALL are described in detail in section 2.2.3.3. Replacing conventional HR chemotherapy by Blinatumomab makes it possible to combine the two effects desired for a novel HR post-consolidation treatment in AIEOP-BFM ALL 2017: the significant reduction of toxicity and the more effective therapy for patients with insufficient response to the HR blocks. Reduction of toxic fatalities is an urgent need in particular for the HR patients with fairly low relapse risk, and considering the safety data on Blinatumomab, it can be expected that life-threatening or fatal complications will be exceptional in patients with residual leukemia at MRD level. Furthermore, a better general condition status is expected when continuing the therapy with either alloHSCT or the reintensification phase with 3 x Protocol III potentially resulting in less toxicity and mortality associated with these treatment procedures. We moreover hypothesize that the implementation of Blinatumomab as a drug that acts via a novel mechanism which is completely different to that of conventional chemotherapy may overcome resistance in patients with inadequate response to chemotherapy and improve MRD negativity with the result of the reduction of subsequent relapses.

Patients with pB-ALL and risk group HR are eligible for randomization R-HR. For definition of the HR group, see section 5.3.2.2.

Patients randomized into the R-HR experimental arm will receive 2 cycles of Blinatumomab given at 15 µg/m²/day as continuous i.v. infusion for 28 days per cycle with a 2-week treatment-free interval between the 2 cycles. These cycles will replace the second and third HR block (HR-2' and HR-3') of the standard-of-care HR therapy. In order to make up for the CNS-effective treatment included in HR-2' and HR-3' (two intrathecal MTX doses, HD-MTX, HD-Ara-C) 4 doses of i.th. MTX will be included in the Blinatumomab cycles (one dose each at start and end of each Blinatumomab cycle; dose age-adapted). The control arm consists of the standard-of-care HR chemotherapy including the blocks HR-2' and HR-3'.

It can be assumed that patients that do not respond to Blinatumomab eventually receive no effective treatment for 12 weeks. To minimize the risk that these patients suffer a disadvantage, patients who have positive MRD within the quantifiable range after the first Blinatumomab cycle and had no MRD reduction by at least one log step over the first Blinatumomab cycle discontinue the experimental treatment and receive the blocks HR-2' and HR-3' instead.

Patients with Down Syndrome are at particular high risk of fatal toxicity when treated with the HR blocks, and patients with translocation *TCF3-HLF* have been shown to have no reasonable chance for cure with conventional chemotherapy regimens. These patient groups

will therefore be excluded from randomization R-HR and should receive the experimental arm with Blinatumomab.

2.2.3.6 Randomization R-MR: Blinatumomab after re-intenisification phase for MR pB-ALL

Accounting for 37% of the patient population, the pB-ALL/MR group represents a large subset with a favorable pEFS of 85% at 4 years. Nevertheless, more than one third of relapses occur in these patients which means that improving the outcome in this group would benefit a large proportion of patients. A number of different randomized approaches has been made in the last decades of the AIEOP-BFM ALL trials with the intention to improve the outcome in the intermediate risk group by the means of modified or intensified use of conventional chemotherapy, and almost all attempts did not prove successful (Conter*, et al* 2007, Möricke*, et al* 2008, Schrappe*, et al* 2000a) (see also section 2.1). Each intensification of chemotherapy moreover bears the risk of unacceptable toxicity which is all the more an issue in patients with low relapse risk. As the vast majority of MR patients is already MRD negative before the reintensification phase Protocol II, the relapses emerging in these patients may primarily not be a matter of primary chemotherapy resistance, but other mechanisms of resistance such as cell dormancy may play a crucial role in this patient subgroup.

We hypothesize that through its specific mechanism of action, Blinatumomab is able to complement the chemotherapeutic effects by consolidating MRD negativity and attacking leukemic cells that escaped the chemotherapy and normal immunological effects with the result of reducing relapses.

Patients with pB-ALL and risk group MR are eligible for randomization R-MR. For definition of the MR group, see section 5.3.2.2.

Patients randomized into the R-MR experimental arm will receive 1 cycle of Blinatumomab given at 15 µg/m²/day as continuous i.v. infusion for 28 days, starting approx. 2 weeks after the end of reintensification Protocol II after hematological recovery. Start of Maintenance therapy is postponed by approx. 6 weeks. The control arm consists of the standard-of-care Maintenance phase starting approx. 2 weeks after end of reintensification phase Protocol II after hematological recovery.

2.2.3.7 Randomization R-T: Protocol IB_{long} for early non-SR T-ALL

The consolidation phase Protocol IB (28 days 6-Mercaptopurine, 4 weekly low-dose Cytarabine blocks of 4 days each, two doses Cyclophosphamide and 2 doses i.th. MTX) has been shown to be very effective in the BFM treatment strategy. The addition of Protocol IB in the HR treatment branch in ALL-BFM 2000 enabled the evaluation of the effectiveness of Protocol IB by historical comparison with the otherwise comparable HR treatment of the previous trial ALL-BFM 95. Patients who met the criteria for HR in both trials had a significantly better EFS when treated in trial ALL-BFM 2000 (with Protocol IB) compared with ALL-BFM 95 (without Protocol IB) (Möricke*, et al* 2011). This could in particular be shown for T-ALL patients (5y-pEFS ALL-BFM 2000 67% [SE 4%], ALL-BFM 95 50% [SE 5%], p=0.0097) and was an independent prognostic factor in multivariate Cox analysis including common risk factors and alloHSCT as covariates (Hazard ratio of 0.64; p=0.002). These findings were substantiated by the reduction of the MRD load over Protocol IB in patients with positive MRD at TP1: An MRD reduction by at least 2 log-steps could be seen in 34% of T-ALL and 17% of pB-ALL. The MRD load at TP2 was at the same level or higher than at TP1 in 11% of T-ALL and 20% of pB-ALL patients (unpublished data from AIEOP-BFM ALL 2000). The effectiveness of Protocol IB could also be demonstrated for T-ALL patients with ETP subtype who also had a marked reduction of MRD over Protocol IB (MRD $\geq 5x10^{-4}$:

17/20 pts. at end of induction, 4/20 pts. after Protocol IB) and good eventual CR rate in patients with induction failure (7/7) (Conter*, et al* 2016).

On the basis of these data, we hypothesize that the extension of Protocol IB with an increase of the cumulative chemotherapy dose by 50% could reduce the relapse incidence in T-ALL.

Although Protocol IB causes relevant bone marrow aplasia, it is rather well tolerated with a relatively low incidence of life-threatening or fatal events. It is thus reasonable to expect that an extension of this treatment phase will be tolerated with acceptable toxicity. T-ALL patients with negative MRD load at TP1 (d33, end of induction) and without early HR criteria such as prednisone poor-response, FCM-MRD d15 HR, or non-remission on day 33 have an excellent EFS with an expected relapse rate of < 10% and will therefore not be eligible for randomization R-T. Among the patients with positive MRD at TP1 without early HR criteria, the MRD load at TP2 (negative or positive) is crucial for the eventual relapse rate. TP2 MRD results, however, cannot be taken into account for randomization R-T eligibility as randomization R-T is scheduled earlier. Considering the relapse rate of approximately 20% of patients with low MRD at TP2 (i.e. < $5x10^{-4}$) and the generally poor rescue chances for relapsed T-ALL, it seems to be justified that all patients with positive MRD at TP1 will be eligible for this randomization even though this will include some patients with negative MRD at TP2.

Patients randomized into the R-T experimental arm will receive two weeks of therapy with 6-MP at 60 mg/m²/day, 2 blocks of 4 daily doses of Ara-C at 75 mg/m²/dose and one dose of cyclophosphamide at 1000 mg/m² in addition to the standard-of-care Protocol IB ("Protocol IB_{long}"). Patients randomized into the control arm will receive the regular standard-of-care consolidation phase Protocol IB ("Protocol IB_{reg}").

2.2.4 Intrathecal therapy in Protocol IA for patients detection of blast cells in CSF at diagnosis

The cytological assessment of CSF cytospin preparations for the identification of leukemic blast cells has many pre-analytical and analytical uncertainties. The so-called CNS2 status (≤ 5 nucleated cells/µl and detection of leukemic blasts) has the therapeutical consequence of the administration of two additional doses of intrathecal (IT) MTX in induction Protocol IA. This has been the standard procedure for more than 25 years in our and many other ALL treatment protocols. The prognostic relevance of the CNS2 status, however, and the importance of the additional IT doses are uncertain. Interim analyses of data of the AIEOP-BFM ALL 2009 trial revealed relevant differences in the proportion of patients with CNS2 status between the participating groups. The reason for these differences is not entirely clear. Different approaches regarding centralized or local cytospin assessment and various technical reasons may contribute to the differences observed. Retrospective analyses of toxic effects due to the two additional IT MTX doses in induction in trial AIEOP-BFM ALL 2000 revealed more than twice as many life-threatening and fatal infections in patients with CNS 2 status (treated with additional IT doses) as compared to patients with CNS 1 status (without these additional doses). This result could be confirmed in multivariate analyses and also in study AIEOP-BFM ALL 2009.

Due to the uncertain therapeutical benefit of CNS 2 patients through the additional IT MTX doses and to save the patients from the higher risk of serious infections associated with these additional doses, patients with CNS 2 status will no longer receive the additional IT MTX doses in Protocol IA but will be treated in the same way as those with CNS 1 status. Patients with true leukemic CNS involvement (CNS 3 status), however, will still receive the additional IT MTX doses on day 19 and 26 of Protocol IA in AIEOP-BFM ALL 2017.

The uniform CNS-directed treatment of CNS 1 and CNS 2 patients in AIEOP-BFM ALL 2017 will form the basis for prospective evaluation of the prognostic importance of blasts in CSF indicating minimal CNS involvement by using flow cytometric or molecular genetic

techniques with higher sensitivity, specificity and reliability than conventional cytological CSF cytospin assessments.

2.2.5 Intensified treatment before SCT in patients with insufficient MRD response

Patients with inadequate MRD response to the HR courses (MRD ≥ $5x10^{-4}$ after the third HR block) had a very poor prognosis despite alloHSCT in ALL-BFM 2000. In AIEOP-BFM ALL 2009, treatment of these patients should therefore be intensified with the alternative chemotherapy element DNX-FLA before alloHSCT. According to interim results of this trial, DNX-FLA showed to be rather effective inducing an MRD reduction by at least one log step in 70% of the patients. In 52% of the patients, MRD could be reduced to < $5x10^{-4}$ with this therapy block. It was well tolerated in most of the patients with an acceptable rate of life-threatening toxicity (see section 2.1.4).

This strategy will therefore be continued in AIEOP-BFM ALL 2017. pB-ALL patients who had been exposed to Blinatumomab before, as they had received the experimental therapy in randomization R-HR, will also be eligible for DNX-FLA if they present with high MRD (≥ $5x10^{-4}$) after the second Blinatumomab cycle.

A still high MRD load of ≥ $5x10^{-4}$ after the DNX-FLA block will be counted as statistical event in the EFS analyses in AIEOP-BFM ALL 2017 ("Molecular non-response", see section 3.1.8). These patients are eligible for experimental therapy, which may consist of Blinatumomab cycles for pB-ALL patients who have previously not yet been exposed to this drug (i.e. treated in the control arm in R-HR). For pB-ALL patients who had already been treated with Blinatumomab, experimental therapy could consist of CAR-T therapy (Grupp*, et al* 2013). Patients with pB-ALL and "MRD non-response" would also qualify for an experimental treatment with suitable tyrosine kinase inhibitors, if the leukemic cells carry drug-targetable kinase-activating lesions (Annesley and Brown 2015, Roberts*, et al* 2014a). A systematic screening for the most frequent of these aberrations will be done in all pB-ALL patients with positive MRD at end of induction (MRD-TP1) who do not present with *ETV6-RUNX1* rearranged ALL, hypodiploidy (< 45 chromosomes), a *KMT2A* (*MLL*) rearrangement, or a *TCF3* rearrangement.

2.2.6 Cranial irradiation

Irradiation-induced brain tumors and other late effects such as endocrinological and neuro-psychological impairments are still a major issue after cranial radiotherapy (CRT). This led to a reduction in the use of preventive CRT in AIEOP-BFM ALL 2009.

A meta-analysis including >16,000 patients evaluating the benefit or need of CRT in pediatric ALL could show that CRT was associated with a reduced risk of relapse only in the small subgroup of patients with overt CNS disease at diagnosis and had no prognostic impact when preventively given to patients without CNS involvement (Horton*, et al* 2007).

This encouraged the further reduction in the use of preventive CRT in AIEOP-BFM ALL 2017. Due to the high rate of potential irradiation-associated late effects in the young age, patients younger than 4 years of age (at start of irradiation) do no longer receive CRT in AIEOP-BFM ALL 2017. CRT will be used at the same dose of 12 Gy when given for therapeutic or preventive purposes and will be limited to patients ≥ 4 years old with CNS involvement at diagnosis (CNS3, therapeutic CRT, tCRT) and CNS-negative (CNS1 or CNS2) patients with T-ALL and initial WBC of >100 000/µl (preventive CRT, pCRT). pCRT has been retained in this patient group because of the generally higher risk of CNS and systemic relapses in T-ALL patients with hyperleukocytosis. Patients with T-ALL and HR patients who are not irradiated will receive intensified intrathecal therapy during maintenance.

2.2.7 Indications for allogeneic stem cell transplantation

The procedure of alloHSCT is not part of the AIEOP-BFM ALL 2017 study.

The indications for alloHSCT of study AIEOP-BFM ALL 2009 will basically be maintained in trial AIEOP-BFM ALL 2017.

The following changes will be implemented in AIEOP-BFM ALL 2017:

- Non-remission at end of induction (no CR d33): These patients formerly had an indication for alloHSCT from a mismatched donor (MMD) in the case of any MRD positivity at TP1 or TP2. In AIEOP-BFM ALL 2017, a MMD alloHSCT for patients with no CR d33 will only be indicated in the case of MRD ≥ $5x10^{-4}$ at TP2 or missing MRD results.
- *TCF3-HLF*: These patients will in any case have an indication for alloHSCT from matched donor (MD) or MMD independent of MRD response.
- *IKZF1*^{plus} + MRD not negative at TP1: In order to intensify the treatment for all these poor-risk patients as compared to the former trial, patients with FCM-MRD d15 ≥10% and/or MRD ≥ $5x10^{-4}$ at TP2 (or missing MRD) will have an indication for alloHSCT.
- Infants: alloHSCT indication for infants will basically be adopted from the trial INTERFANT 06.

All other indications will not change as compared with the previous trial and are presented in section 5.3.3 (Table 1).

3 Definitions

3.1 Definitions of Response and Remission Status

3.1.1 Prednisone response

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

- **Prednisone Good Response (PGR):** absolute blast count in peripheral blood < 1 000/µl
- **Prednisone Poor Response (PPR):** absolute blast count in peripheral blood ≥ 1 000/µl

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

3.1.2 Complete remission

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

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

- < 5 % blast cells (M1) in representative bone marrow with sufficient cellularity and signs of regeneration of normal myelopoiesis
- ≤ 5 nucleated cells/µl in CSF, or > 5 nucleated cells/µl and no evidence of blasts in cytospin
- no evidence of leukemic infiltrates as evaluated clinically and by imaging; a preexisting mediastinal mass must have decreased at least to 1/3 of the initial tumor volume

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

3.1.3 Late-Response

A patient, who is not in CR on day 33 but achieves CR in the regenerating bone marrow with Protocol IB or any of the three HR blocks (or with the Blinatumomab cycles if randomized to the R-HR experimental arm), is classified as having Late-Response.

3.1.4 Morphological Non-Response (resistance to protocol treatment)

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

3.1.5 Response by PCR-MRD and resulting PCR-MRD risk groups

Risk stratification by PCR-MRD uses the MRD load in bone marrow on day 33 (TP1) and at week 12 (before Protocol M or 1st HR block, TP2).

According to PCR-MRD analysis, it is possible to define three PCR-MRD risk groups:

- **PCR-MRD-SR:** MRD negative at TP1 and TP2 with at least one, if possible two markers with sensitivity and quantifiable range of at least 10^{-4}.
- **PCR-MRD-MR:** MRD positive at TP1 and/or TP2, and MRD load < $5x10^{-4}$ at TP2.

- **PCR-MRD MR Slow Early Responders (SER):** MRD ≥ $5x10^{-4}$ at TP1 and MRD positive at a level of < $5x10^{-4}$ at TP2. (Note: PCR-MRD MR SER patients with pB-ALL (or unknown immunophenotype) have to be treated in the HR arm)
- **PCR-MRD-HR:** MRD ≥ $5x10^{-4}$ at TP2.

For integration of PCR-MRD results in the final risk group assignment see section 5.3.2.

3.1.6 Response by FCM-MRD

The following MRD levels in bone marrow on day 15 measured by flow cytometry (FCM-MRD) are relevant for treatment and risk group assignment:

FCM-MRD on day 15 < 0.1 % lymphoblasts in bone marrow (FCM-MRD LR)

FCM-MRD on day 15 ≥ 0.1 % and < 10 % lymphoblasts in bone marrow (FCM-MRD IR)

FCM-MRD on day 15 ≥ 10 % lymphoblasts in bone marrow (FCM-MRD HR)

For final risk group assignment, FCM-MRD becomes relevant if PCR-based MRD classification is inconclusive or not feasible (see section 5.3.2) and in the case of FCM-MRD HR (patient not positive for *ETV6-RUNX1* (*TEL-AML1*) are allocated to HR regardless of PCR-MRD status; see 5.3.2.2).

3.1.7 Blinatumomab Poor-Response

A patient treated in the R.HR experimental arm who after the first Blina cycle has an MRD load within the quantitative range and no MRD reduction by at least 1 log (i.e. by factor 10) as compared to the MRD load before the first Blina cycle is classified as having a Blinatumomab Poor-Response. Blinatumomab Poor-Response is an indication for discontinuation of the experimental treatment in the respective patient and for a switch to the standard of care HR blocks (see sections 5.4.2 and 7.10.1.2.7).

3.1.8 Molecular Non-Response (resistance to protocol treatment)

A patient who has an MRD load of ≥ $5x10^{-4}$ after DNX-FLA and has received the high-risk treatment according to the protocol (either treatment arms of randomizations R-eHR or R-HR) is classified as having molecular Non-Response (resistance to protocol). This is considered an event for statistical analysis.

3.1.9 Relapse

The diagnosis of relapse can only be made if complete remission has been achieved before.

Definitions:

Isolated bone marrow relapse:

- ≥ 25 % lymphoblasts in bone marrow without extramedullary involvement.
- ≥ 5 % and <25 % lymphoblasts in bone marrow and confirmation of the prior clonal abnormality by flow cytometry and/or cytogenetics/FISH and/or PCR

  Level of detection considered confirmatory (two methods at least)

  - > 5 % by flow cytometry
  - > detection limit for FISH
  - at least 2 aberrant metaphases for cytogenetics
  - MRD increase by at least one log (true value) to ≥ 1% (≥ 1 x 10^{-2}) by ASO RQ-PCR)

If only one confirmatory test is available, two consecutive time points are needed (typically at least one week apart)

Combined bone marrow relapse:

- ≥5% lymphoblasts in bone marrow and at least one extramedullary site localization

CNS relapse:

- >5/µl nucleated cells in CSF and morphologically unequivocal evidence of lymphoblasts-

  If an intracranial mass is detected by imaging without evidence of blasts in CSF and this tumor is the only site of the suspected relapse, a biopsy is required.

Testicular relapse:

- Unilateral or bilateral painless hard testicular tumor

  If the testis is the only site of the suspected relapse, a biopsy of the tumor is mandatory.

Other extramedullary relapse:

- Diagnosis needs imaging and/or biopsy.

3.2 Definitions of stratification-relevant genetic aberrations

3.2.1 *IKZF1*^{plus}

The *IKZF1*^{plus} type is defined as *IKZF1* deletion co-occurring with deletion in *CDKN2A or CDKN2B* (only homozygous deletions) or *PAX5* or *PAR1 (P2RY8-CRLF2*) in the absence of *ERG* deletion.

Taking into consideration the potential co-occurrence of *IKZF1*^{plus} with other genetic aberrations, a hierarchy is defined for predefined recurrent genetic aberrations with well-defined prognosis. Furthermore, *IKZF1*^{plus} will have no consequence for risk stratification in patients with negative PCR-MRD at the end of induction phase (MRD TP1).

In summary, *IKZF1*^{plus} will qualify patients for HR treatment if they are ***not*** PCR-MRD-negative[3] at TP1 ***and not*** detected positive for *ETV6-RUNX1* (*TEL-AML1*), *TCF3-PBX1* (*E2A-PBX1*) or *KMT2A* (*MLL*) rearrangement other than *KMT2A-AFF1* (*MLL-AF4*).

3.2.2 Hypodiploidy

Hypodiploidy as high-risk factor in AIEOP-BFM ALL 2017 is defined by a modal chromosome number of less than 45 chromosomes. Patients with less than 44 chromosomes will in addition have an indication for alloHSCT except those PCR-MRD-negative at TP1.

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

Regarding diagnostics of hypodiploidy using the DNA index, see section 0.

[3] i.e. positive or missing/inconclusive result

3.2.3 Other genetic aberrations

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

3.3 Definition of organ involvement

3.3.1 CNS status and CNS disease

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

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

3.3.1.1 CNS status

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

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

In AIEOP-BFM ALL 2017, the CNS status is defined as follows:

- **CNS 1:** no clinical or imaging findings of CNS disease and absence of blasts on cytospin preparation in CSF, regardless of the number of white blood cells (WBCs) and regardless of red blood cells (RBCs) or bloody contamination.
- **CNS 2:** no clinical or imaging findings of CNS disease ***and***:
    - **CNS 2a:** < 10/µl RBCs and no macroscopical contamination with blood; ≤ 5/µl WBCs and cytospin positive for blasts
    - **CNS 2b:** Macroscopical contamination with blood and/or ≥ 10/µl RBCs; ≤ 5/µl WBCs and cytospin positive for blasts
    - **CNS 2c:** Macroscopical contamination with blood and/or ≥ 10/µl RBCs; > 5/µl WBCs and cytospin positive for blasts but negative by algorithm as specified below (i.e. WBC/RBC in CSF < 2x WBC/RBC in blood).
- **CNS 3**:
    - **CNS 3a:** < 10/µl RBCs and no macroscopical contamination with blood; > 5/µl WBCs and cytospin positive for blasts
    - **CNS 3b:** ≥ 10/µl RBCs and/or macroscopical contamination with blood, > 5/µl WBCs and cytospin positive for blasts and positive by algorithm as specified below (i.e. WBC/RBC in CSF > 2x WBC/RBC in blood).
    - **CNS 3c:** Clinical or imaging findings of CNS disease:
        - CNS masses or clear leptomeningeal infiltration on Magnetic Resonance Imaging (MRI) and/or Computed Tomography (CT)

- Cranial nerve palsies if not caused by extracerebral manifestations

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

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

In the case of a lumbar puncture with high blood contamination (e.g. > 100 RBC/µl), the above-mentioned algorithm may not be applicable. Please, contact the study coordinator for individual discussion of such patients and in other cases of difficult interpretation of the findings.

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

3.3.1.2 CNS disease

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

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

Note: see also section 7.15.2 for indications of preventive and therapeutic cranial irradiation

3.3.2 Testicular involvement

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

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

3.3.3 Mediastinal mass

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

3.3.4 Other organ involvement

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

4 Study Objectives

4.1 Primary objectives

The primary aims of the AIEOP-BFM ALL 2017 study are to answer the following questions:

- **Randomization R-eHR:** Early High-risk (early HR) pB-ALL defined by genetics and/or inadequate treatment response over the course of induction: Can the pEFS from time of randomization be improved by additional therapy with the proteasome inhibitor Bortezomib during an extended consolidation treatment phase compared to standard extended consolidation?
- **Randomization R-HR:** High-risk (HR) pB-ALL defined by genetics and/or inadequate treatment response by the end of consolidation: Can the pEFS from time of randomization be improved by a treatment concept including two cycles of post-consolidation immunotherapy with Blinatumomab (15 µg/m²/d for 2 x 28 days) plus 4 doses intrathecal Methotrexate compared to two conventional highly intensive chemotherapy courses?
- **Randomization R-MR:** Intermediate risk (MR) pB-ALL defined by genetics and intermediate MRD response: Can the probability of disease-free survival (pDFS) from time of randomization be improved by additional therapy with one cycle of post-reintensification immunotherapy with Blinatomomab (15 µg/m²/d for 28 days)?
- **Randomization R-T:** Early non-standard risk (early non-SR) T-ALL patients defined by treatment response over the course of induction: Can the pEFS from time of randomization be improved by the extension of the standard of care consolidation phase by 14 days with an increase of the consolidation cumulative doses of Cyclophosphamide, Cytarabine and 6-Mercaptopurine by 50%?

4.2 Secondary objectives

The secondary goals of the AIEOP-BFM ALL 2009 study are to answer the following questions:

- **All randomizations:** Can the overall survival be improved by the treatment in the experimental arm.
- **All randomizations:** What is the incidence of treatment-related toxicities and mortality in the experimental arm compared to the standard arm.
- **Randomization R-eHR:** Can the MRD load after consolidation treatment be reduced by the additional treatment with Bortezomib?
- **Randomization R-HR:** Can treatment-related life-threatening complications and mortality during the intensified consolidation phase of high-risk treatment be reduced when replacing two intensive chemotherapy courses by two cycles of immunotherapy with Blinatumomab?
- **Randomization R-HR:** What is the proportion of patients with insufficient MRD response to Blinatumomab as defined in the protocol (MRD reduction within the quantifiable range by less than 1 log over the first Blina cycle) as compared to the MRD response after the HR-2' block in the control arm?
- **Randomization R-HR:** Can the MRD load after the first treatment cycle (HR-2'/Blinatumomab) and the second cycle (HR-3'/Blinatumomab) be reduced in the experimental arm when compared with conventional intensive chemotherapy?

- **Randomization R-MR:** What is the proportion of patients with positive MRD after reintensification Protocol II who become MRD-negative over the Blina cycle compared to 4 weeks of standard maintenance therapy.
- **Randomization R-T:** Can the MRD load after consolidation treatment be reduced by extension of the consolidation phase?

4.3 Add-on studies

For add-on research projects, see section 16.

5 Study Design

5.1 Type of study

AIEOP-BFM ALL 2017 is a collaborative prospective randomized clinical trial for the treatment of children and adolescents (age ≥ 1 and < 18 years) with newly diagnosed ALL. Therapy is conducted in a risk-adapted manner stratifying patients according to biological and response criteria. Four randomized studies are scheduled with the aim (1) to reduce treatment-related morbidity and mortality during intensified consolidation of the high-risk treatment arm (Randomization R-HR), (2) to improve the outcome of medium and high-risk patients by treatment intensification/modification (all randomizations)

5.2 Registration and eligibility

5.2.1 Registration

All patients who are diagnosed with ALL and have been admitted to one of the participating centers have to be registered if they meet the inclusion criteria. Registered patients who are not eligible for the study as they meet any exclusion criteria, but are treated according to the protocol, might be analyzed separately for outcome.

5.2.2 Inclusion/exclusion criteria

Patients meeting all the following criteria should be registered in the study (inclusion criteria):

- newly diagnosed acute lymphoblastic leukemia
- age < 18 years (up to 17 years and 365 days)
- patient enrolled in a participating center
- written informed consent to trial participation and transfer and processing of data

A subsequent removal from the study is only allowed if the inclusion criteria turn out not to be fulfilled or in the case of pregnancy.

Patients meeting at least one of the following criteria are not eligible and will be analyzed separately:

- Ph+ (*BCR-ABL1* or t(9;22)-positive) ALL[4]
- pre-treatment with cytostatic drugs
- steroid pre-treatment with ≥ 1 mg/kg/d for more than two weeks during the last month before diagnosis
- treatment started according to another protocol
- underlying disease that does not allow treatment according to the protocol (e.g. severe congenital heart disease, Charcot-Marie Syndrome, Ataxia-teleangiectasia…)
- ALL diagnosed as second malignancy
- evidence of pregnancy or lactation period
- Sexually active adolescents not willing to use highly effective contraceptive method (pearl index <1) until 12 months after end of anti-leukemic therapy
- participation in another clinical trial that interferes with the protocol

[4] Patients with unknown status regarding *BCR-ABL1* are eligible

5.3 Stratification

5.3.1 Summary of risk group definitions

pB-ALL (or unknown immunophenotype)

- **early High Risk (early HR)**
  - no complete remission on day 33 *or*
  - positivity for *KMT2A-AFF1 (MLL-AF4) or*
  - positivity for *TCF3-HLF* (*E2A-HLF*)[5] *or*
  - hypodiploidy *or*
  - FCM-MRD in BM on day 15 ≥ 10% *and not ETV6-RUNX1* (*TEL-AML1*) positive *or*
  - *IKZF1*[plus] ***and*** PCR-MRD at TP1 positive or inconclusive[6] ***and not*** positive for *ETV6-RUNX1*, *TCF3-PBX1* or *KMT2A* rearrangement other than *KMT2A-AFF1 or*
  - PCR-MRD at TP1 ≥ $5x10^{-4}$ *or*
  - age <1 year ***and*** any *KMT2A* (*MLL*) rearrangement
- **High Risk (HR)**
  - no complete remission on day 33 *or*
  - positivity for *KMT2A-AFF1* (*MLL-AF4) or*
  - positivity for *TCF3-HLF (E2A-HLF)*[5] *or*
  - hypodiploidy *or*
  - FCM-MRD in BM on day 15 ≥ 10% *and not ETV6-RUNX1* (*TEL-AML1*) positive *or*
  - *IKZF1*[plus] ***and*** PCR-MRD at TP1 positive or inconclusive[6] ***and not*** positive for *ETV6-RUNX1*, *TCF3-PBX1* or *KMT2A* rearrangement other than *KMT2A-AFF1 or*
  - PCR-MRD at TP1 ≥ $5x10^{-4}$ *and* positive < $5x10^{-4}$ at TP2 (PCR-MRD SER) *or*
  - PCR-MRD at TP2 ≥ $5x10^{-4}$ *or*
  - age <1 year and any *KMT2A* (*MLL*) rearrangement
- **Standard Risk (SR)**
  - no HR criteria *and*
  - PCR-MRD at TP1 negative for all investigated markers with at least one marker with a quantifiable range of ≤ 10^{-4} *or*
  - inconclusive PCR-MRD result[6] at TP1 *and* PCR-MRD ***not*** positive at TP2 *and* FCM-MRD in BM on day 15 < 0.1%
- **Medium Risk (MR)**
  - no HR criteria *and*
  - no SR criteria

[5] Patients with *TCF3-HLF* (*E2A-HLF*) also qualify for experimental treatments (e.g. with BCL2 inhibitors if those drugs are available).
[6] i.e. PCR-MRD result missing or negative but no marker with a quantitative range of ≤ 10^{-4}

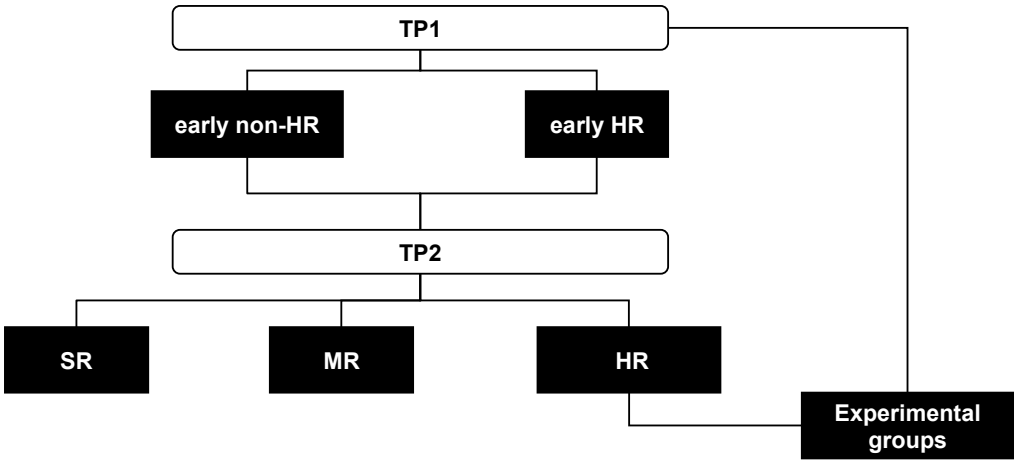


Figure 2 Basic schema of risk stratification of pB-ALL

T-ALL

- **early Standard Risk (early SR)**
  - complete remission on day 33 *and*
  - FCM-MRD in BM on day 15 <10% *and*
  - Prednisone Good-Response *and*
  - PCR-MRD at TP1 negative for all investigated markers with at least one marker with a quantifiable range of ≤ 10^{-4} *or*
  - inconclusive PCR-MRD result[7] at TP1 *and* FCM-MRD in BM on day 15 < 0.1%
- **High Risk (HR)**
  - No complete remission on day 33 *or*
  - FCM-MRD in BM on day 15 ≥ 10% *or*
  - Prednisone Poor-Response *or*
  - PCR-MRD at TP2 ≥ $5x10^{-4}$
- **non-High Risk (non-HR)**
  - No HR criteria

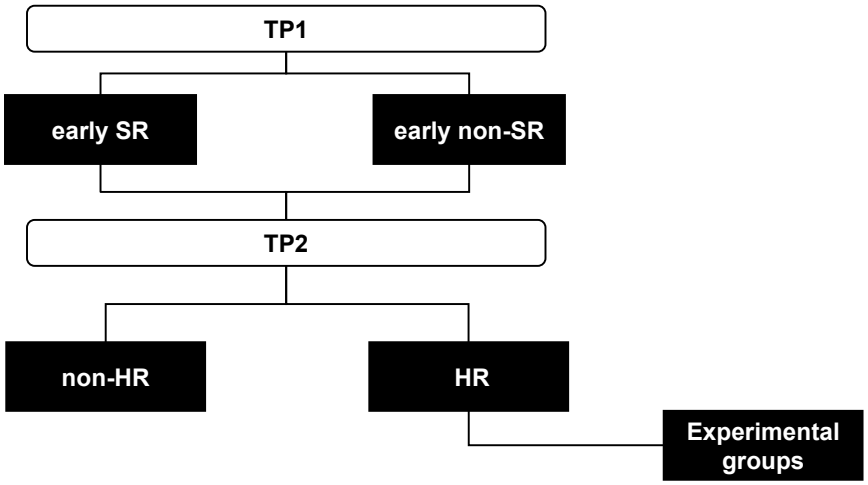


Figure 3 Basic schema of risk stratification of T-ALL

[7] i.e. PCR-MRD result missing or negative but no marker with a quantitative range of ≤ 10^{-4}

Experimental groups eligible for different treatments

- Positivity for TCF3-HLF(*E2A-HLF*)
- MRD non-response (≥ $5x10^{-4}$ after DNX/FLA)

5.3.2 Details of risk stratification procedure

5.3.2.1 Basics of stratification

Stratification is based on biological criteria, morphological treatment response and MRD findings. The stratification criteria are different for patients with pB-ALL (or unknown immunophenotype) and T-ALL.

There are two major time points (TP) of stratification:

1. TP1: at the end of induction Protocol IA on protocol day 33
2. TP2: after consolidation phase/Protocol IB on protocol day 78 or day 92 (depending on treatment arm).

At both time points, stratification can be done as soon as the PCR-MRD results are available.

5.3.2.2 Stratification of patients with pB-ALL (or unknown immunophenotype)

5.3.2.2.1 Definition of HR criteria in pB-ALL (or unknown immunophenotype)

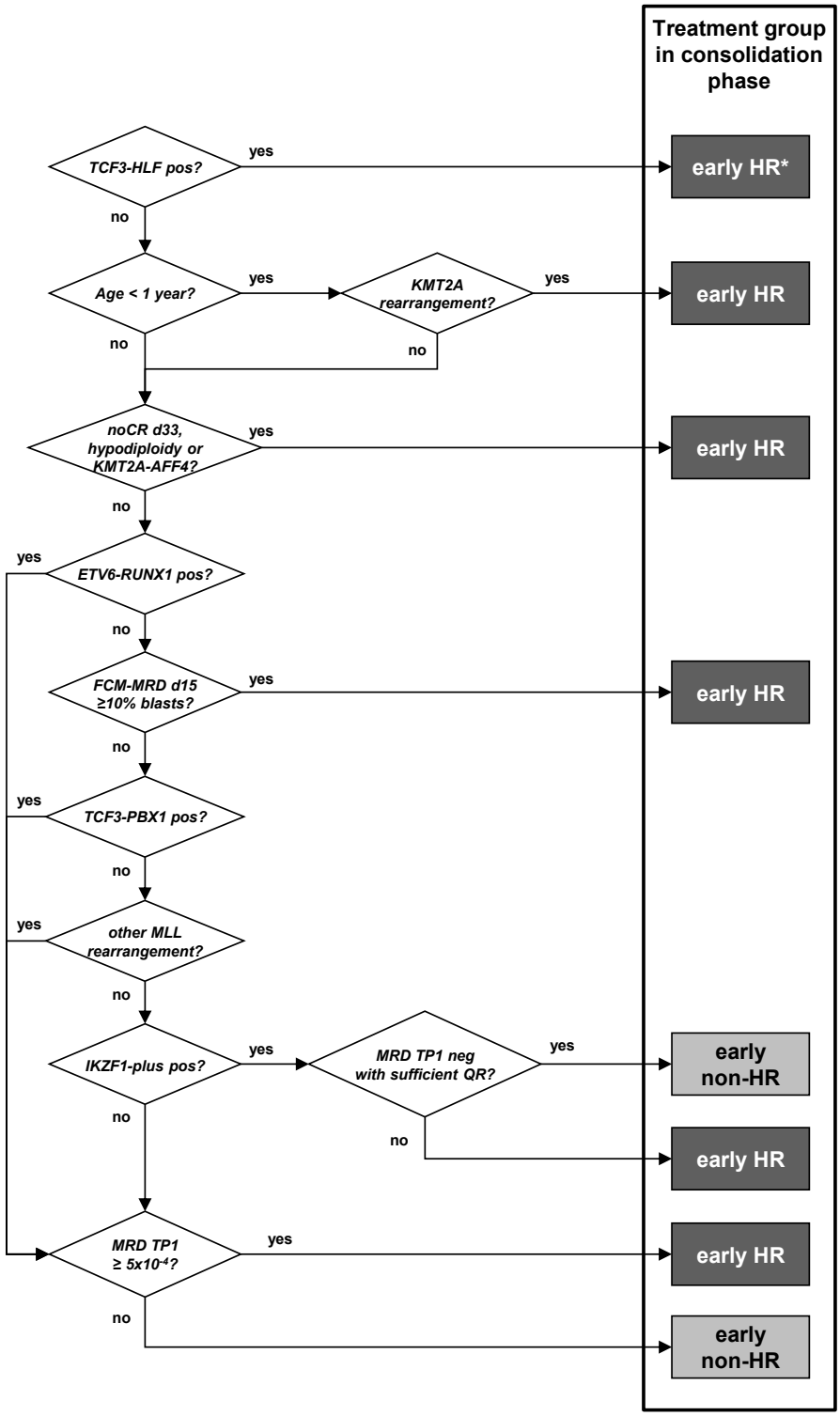
- **HR criteria by TP1:**
  - No complete remission on day 33 (see section 3.1.2) *or*
  - Positivity for *KMT2A-AFF1* (*MLL-AF4*) *or*
  - Positivity for *TCF3-HLF* (*E2A-HLF*) *or*
  - Hypodiploidy <45 chromosomes (see also section 6.1.1.3) *or*
  - FCM-MRD in BM on day 15 ≥ 10% and not *ETV6-RUNX1* (*TEL-AML1*) positive *or*
  - *IKZF1*^{plus} *and* PCR-MRD at TP1 positive or inconclusive[8] *and not* positive for *ETV6-RUNX1*, *TCF3-PBX1* or *KMT2A* rearrangement other than *KMT2A-AFF1 or*
  - age < 1 year of age *and* any *KMT2A* (MLL) rearrangement
- **MRD-HR at TP2:**
  - PCR-MRD at TP2 ≥ $5x10^{-4}$

[8] i.e. PCR-MRD result missing or negative but no marker with a quantitative range of ≤ 10^{-4}

5.3.2.2.2 Definition of the early High-risk group (early HR) at TP1 in pB-ALL

Patients with pB-ALL (or unknown immunophenotype) meeting *at least* one of the following criteria qualify for the consolidation treatment in the **early HR** group ($Consolidation_{ext}$):

- Any HR criteria at TP1 (see 5.3.2.2.1)
- PCR-MRD at TP1 ≥ 5 x 10^{-4}



***Patients with *TCF3-HLF* positive leukemia qualify for experimental treatment**

Figure 4 Risk stratification of pB-ALL at TP1 to determine the treatment group in consolidation phase

5.3.2.2.3 Final stratification of patients with pB-ALL (or unknown immunophenotype) at TP2

Patients are further stratified according to the MRD results of TP2 with additional consideration of TP1 PCR-MRD results. In patients without any HR criteria and with negative PCR-MRD at TP1 (with sufficient quantifiable range of ≤ 1 x 10^{-4}), no additional PCR-MRD analysis is performed at TP2. These patients can already at TP1 be assigned to risk group SR and remain in SR for post-consolidation treatment. Patients with any HR criteria at TP1 are in any case finally stratified to HR; in these patients, MRD at TP2 is only relevant for eligibility for alloHSCT (see section 5.3.3 for SCT eligibility criteria). For all other patients, PCR-MRD at TP2 is decisive for post-consolidation treatment.

Details of risk stratification at TP2 are specified in Tables A and B.

Table A: Final risk group assignment by PCR-MRD* in patients with pB-ALL (or unknown immunophenotype):

	PCR-MRD TP2			
	negative	**positive < 5x10^{-4}#**	**≥ 5x10^{-4}**	**missing/not investigated**
any HR criteria at TP1	HR	HR	HR	HR
no HR criteria at TP1 *and* PCR-MRD at TP1:				
- negative with sufficient QR§ of ≤ 1x10^{-4}	*n.a.*†	*n.a.*†	*n.a.*†	SR†
- negative with insufficient QR§	*See table B*	MR	HR	*See table B*
- pos < 5x10^{-4}#	MR	MR	HR	MR
- pos ≥ 5x10^{-4}	MR	HR	HR	MR
- missing	*See table B*	MR	HR	*See table B*

*Only if MRD markers or MRD material are not available to perform PCR-MRD, FCM-MRD can be used for MRD stratification provided that FCM-MRD analysis was done in one of the AIEOP-BFM reference laboratories.

#including cases MRD low positive/not quantifiable

§QR indicates quantifiable range (see section 3.1.5)

†PCR-MRD at TP2 is not investigated in patients with negative PCR-MRD with sufficient QR at TP1. These patients are stratified to SR.

Table B: Final risk group assignment of **non-HR patients with pB-ALL** (or unknown immunophenotype) by FCM-MRD on day 15 if the PCR-MRD results cannot discriminate between SR and MR risk group.

FCM-MRD d15	**Final risk group**
< 0.1 %	SR
0.1 - < 10 %	MR
missing	MR[9]

[9] *ETV6-RUNX1* (*TEL-AML1*) negative pB-ALL patients with Prednisone Poor-Response would be stratified to HR.

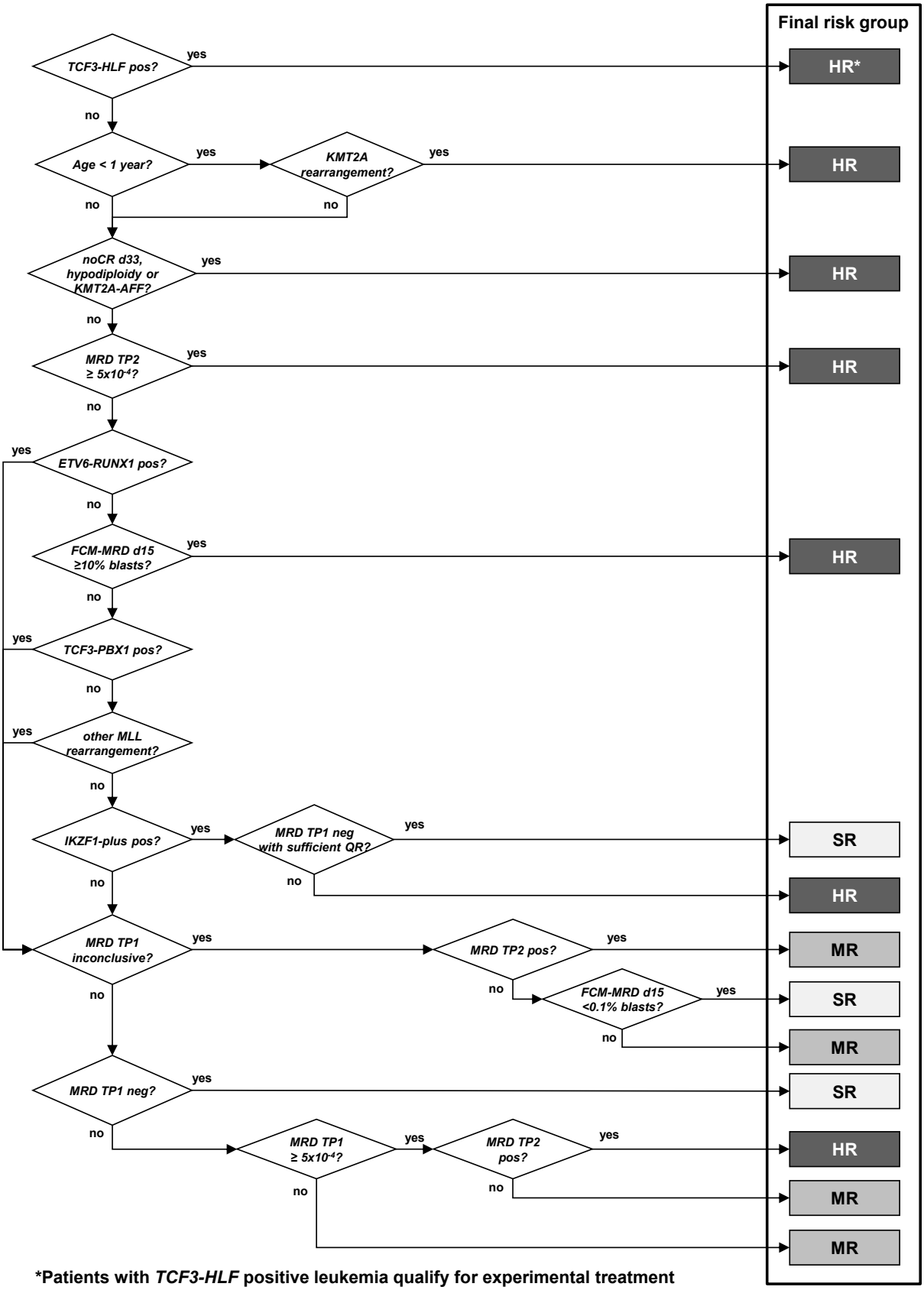


Figure 5 Final risk stratification of pB-ALL at TP2 to determine the treatment group in post-consolidation phase

5.3.2.3 Stratification of patients with T-ALL

5.3.2.3.1 Definition of HR criteria in T-ALL

- **HR criteria by TP1:**
  - Prednisone Poor-Response (see section 3.1.1)
  - FCM-MRD in BM on day 15 ≥ 10 *or*
  - No complete remission on day 33 (see section 3.1.2)
- **MRD-HR at TP2:**
  - PCR-MRD at TP2 ≥ $5x10^{-4}$

5.3.2.3.2 Definition of the early Standard Risk group (early SR) at TP1 in T-ALL

Patients with T-ALL meeting the following criteria qualify for consolidation treatment in risk group **early SR**:

- No evidence of HR criteria by TP1 (see section 5.3.2.3.1) *and*
- PCR-MRD at TP1 negative for all investigated markers with at least one marker with a quantifiable range of ≤ 10^{-4} *or*
- inconclusive PCR-MRD result[10] at TP1 *and* FCM-MRD in BM on day 15 < 0.1%

Patients with inconclusive or missing results on HR criteria by TP1 but meeting the TP1 MRD criteria for early SR, nevertheless qualify for risk group early SR.

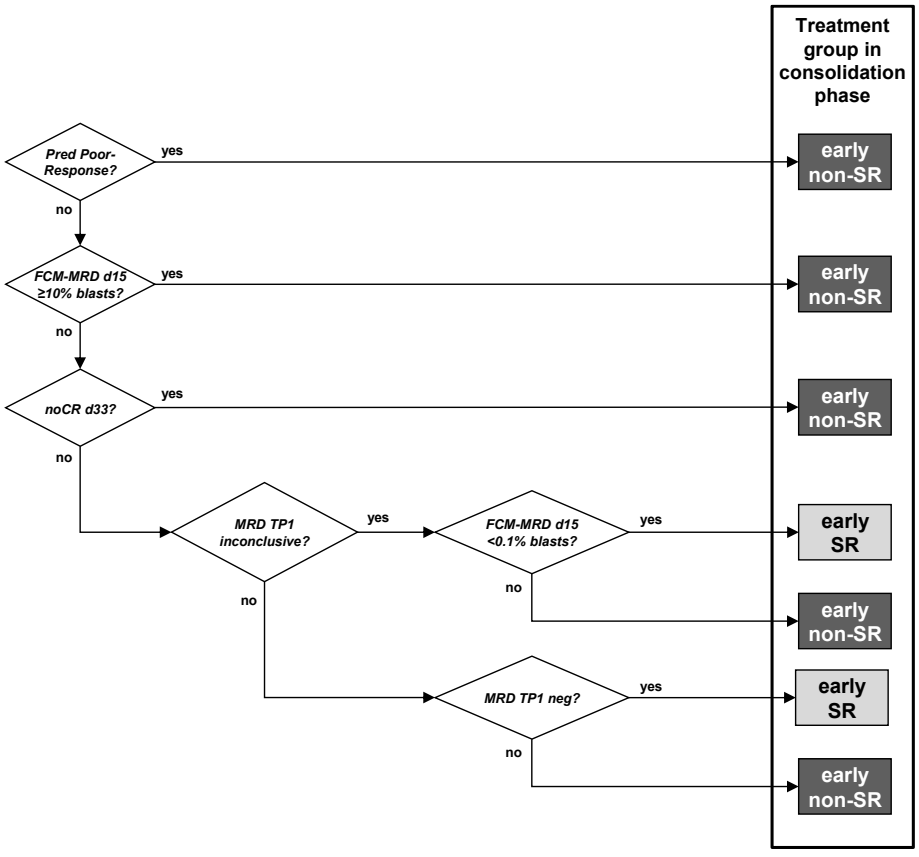


Figure 6 Risk stratification of T-ALL at TP1 to determine the treatment group in consolidation phase

[10] i.e. PCR-MRD result missing or negative but no marker with a quantitative range of ≤ 10^{-4}

5.3.2.3.3 Stratification of patients with T-ALL at TP2

Patients are further stratified according to the MRD results of TP2. In patients without any HR criteria and with negative PCR-MRD at TP1 (with sufficient quantifiable range), no additional PCR-MRD analysis is performed at TP2. These patient can already at TP1 be assigned to risk group non-HR and remain in non-HR for post-consolidation treatment. Patients with any HR criteria at TP1 are in any case finally stratified to HR; in these patients, MRD at TP2 is only relevant for eligibility for alloHSCT (see section 5.3.3 for SCT eligibility criteria). For all other patients, PCR-MRD at TP2 may be decisive for post-consolidation treatment.

Details of risk stratification at TP2 are specified in Table C.

Table C: Final risk group assignment of **T-ALL patients** according to PCR-MRD:

	PCR-MRD TP2			
	negative	**positive < $5x10^{-4}$§**	**≥ $5x10^{-4}$**	**missing/not investigated**
any HR criteria at TP1	HR	HR	HR	HR
no HR criteria at TP1 *and* PCR-MRD TP1:				
- negative with sufficient QR of ≤ $1x10^{-4}$	n.a.*	n.a.*	n.a.*	non-HR*
- negative with insufficient QR#	non-HR	non-HR	HR	non-HR
- positive (any positivity)§	non-HR	non-HR	HR	non-HR
- missing	non-HR	non-HR	HR	non-HR

* PCR-MRD at TP2 is not investigated in patients with negative PCR-MRD with sufficient QR at TP1. These patients are stratified to non-HR.

QR indicates quantifiable range (see section 3.1.5)

§ including cases MRD low positive/not quantifiable

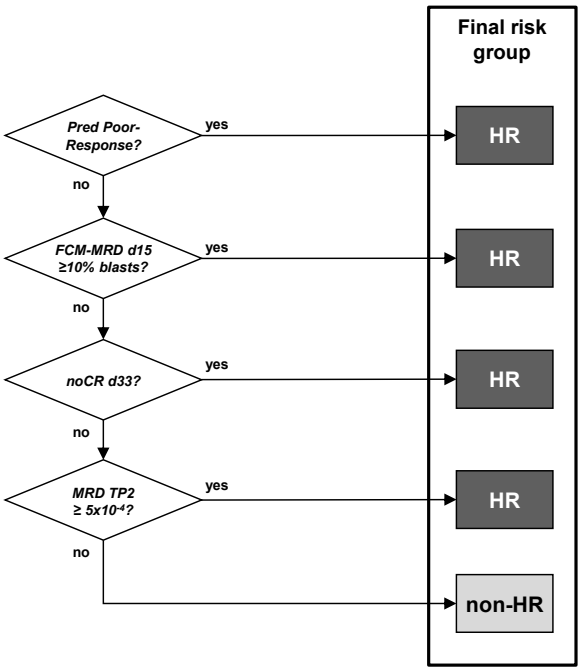


Figure 7 Risk stratification of T-ALL at TP2 to determine the final risk group

5.3.3 Indications for allogeneic stem cell transplantation

The indications for allogeneic hematopoietic stem cell transplantation (alloHSCT) are listed in the table below. For patients from countries which participate in the trial ALL SCTped FORUM 2012 enrolment in this trial is recommended. **The procedure of alloHSCT is not part of the AIEOP-BFM ALL 2017 study.**

Table 1 AlloHSCT indications according to study AIEOP-BFM ALL 2017 for all patients except for infants <1 year of age with pB-ALL and evidence of *KMT2A* rearrangement. The table includes HR as well as non-HR patients.

		PCR-MRD results				
				MRD-HR		
		TP1 neg	TP1 or TP2 pos and TP2 < $5x10^{-4}$	MRD TP2 ≥ $5x10^{-4}$- < $5x10^{-3}$	MRD TP2 ≥ $5x10^{-3}$	no MRD result
criteria hierarchical	*TCF3-HLF*	MMD	MMD	MMD	MMD	MMD
	no CR d33	no[b]	MD	MMD	MMD	MMD
	t(4;11)	no	MD	MD	MMD	MD
	hypodiploidy < 44 chr.[a]	no	MD	MD	MMD	MD
	IKZF1[plus] and FCM-MRD d15 ≥ 10%	no	MD	MD	MMD	MD
	IKZF1[plus] and FCM-MRD d15 < 10%	no	no	MD	MMD	MD
	T-ALL + PPR a/o FCM-MRD d15 ≥ 10%	no	no	MD	MMD	MD
	none of the above features	no	no	MD	MMD	no

no alloHSCT not indicated
MD permitted donor: HLA-matched sibling or non-sibling donor
MMD permitted donor: HLA-matched or HLA-mismatched donor

[a] the finding of exactly 44 chromosomes qualifies for HR treatment but has no impact on alloHSCT indication
[b] non-remission in patients with this rare combination should be due to extramedullary disease. AlloHSCT indication in these cases should be discussed with the national study coordinator.

Table 2 AlloHSCT indications according to study AIEOP-BFM ALL 2017 for infants <1 year of age with pB-ALL and evidence of *KMT2A* rearrangement.

	PCR-MRD results			
	MRD TP2 ≥ $5x10^{-4}$	MRD TP2 ≥ $5x10^{-4}$- < $5x10^{-3}$	MRD TP2 ≥ $5x10^{-3}$	no MRD result
no CR d33	MD	MMD	MMD	MD
age < 6 months and initial WBC > 300,000/µl	MD	MD	MMD	MD
age < 6 months and Prednisone Poor-Response	MD	MD	MMD	MD
none of the above features	no	MD	MMD	no

no alloHSCT not indicated
MD permitted donor: HLA-matched sibling or non-sibling donor
MMD permitted donor: HLA-matched or HLA-mismatched donor

5.4 Randomized study questions (see also sections 2.2.3 and 7)

5.4.1 Randomization R-eHR in consolidation phase for early High Risk (early HR) patients with pB-ALL (or unknown immunophenotype)

Control arm: Treatment with the extended standard-of-care consolidation phase "Extended Consolidation B" (Consolidation B_{ext}).

Experimental arm: Treatment with Consolidation B_{ext} plus Bortezomib (Consolidation B_{ext}+BZM). Bortezomib is given at 1.3 mg/m²/dose with 4 doses every third day on protocol days 50, 53, 56 and 59.

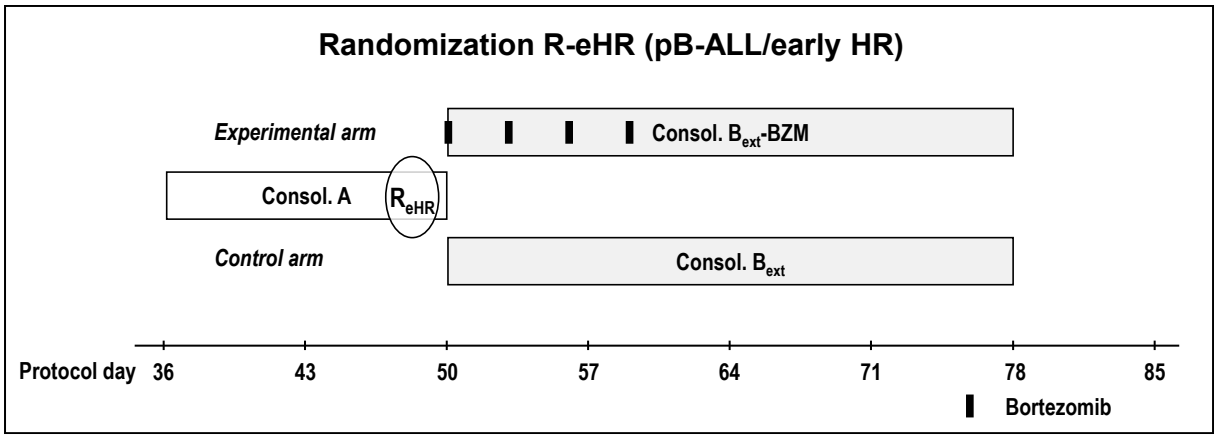


Figure 8 Schematic representation of randomization R-eHR

Inclusion criteria:

- pB-ALL or unknown immunophenotype
- Risk group early HR (see section 5.3.2.2.1)
- Written informed consent to randomization R-eHR from a legally acceptable representative. The assent of the child must be obtained, as appropriate to the age of the patient and/or based on local regulations.

Exclusion criteria:

- History of neuropathy CTCAE °IV in Prot. IA
- Neuropathy of CTCAE °III or higher at time of randomization
- Severe renal impairment (creatinine clearance < 20 ml/min/1.73 m^2)
- Hypersensitivity to the active substance of Bortezomib or to boron
- Acute diffuse infiltrative pulmonary or pericardial disease
- Patients with Down Syndrome should not be randomized and should be treated with Consolidation B_{short}.
- Patients with documented *E2A-HLF* rearrangement. These patients should not be randomized and should be treated in the arm with Bortezomib.

Individual stopping criteria:

- Grade IV° neuropathy and/or severe autonomic neuropathy
- Severe renal impairment (creatinine clearance < 20 ml/min/1.73 m^2)

Timing:

Randomization R-eHR should take place after stratification to early HR but not before protocol day 36 (start of Consolidation A).

5.4.2 Randomization R-HR in intensified consolidation phase for HR pB-ALL patients

Control arm: Treatment with the standard of care intensified consolidation blocks HR-2' and HR-3'.

Experimental arm: Intensified consolidation treatment with 2 cycles Blinatumomab given at a dose of 15 µg/m^2/day as continuous intravenous infusion for 28 days per cycle with a 2-week treatment-free interval between the cycles plus 4 doses of intrathecal Methotrexate (dose age-adapted), one dose each at the start and at the end of each Blinatumomab cycle.

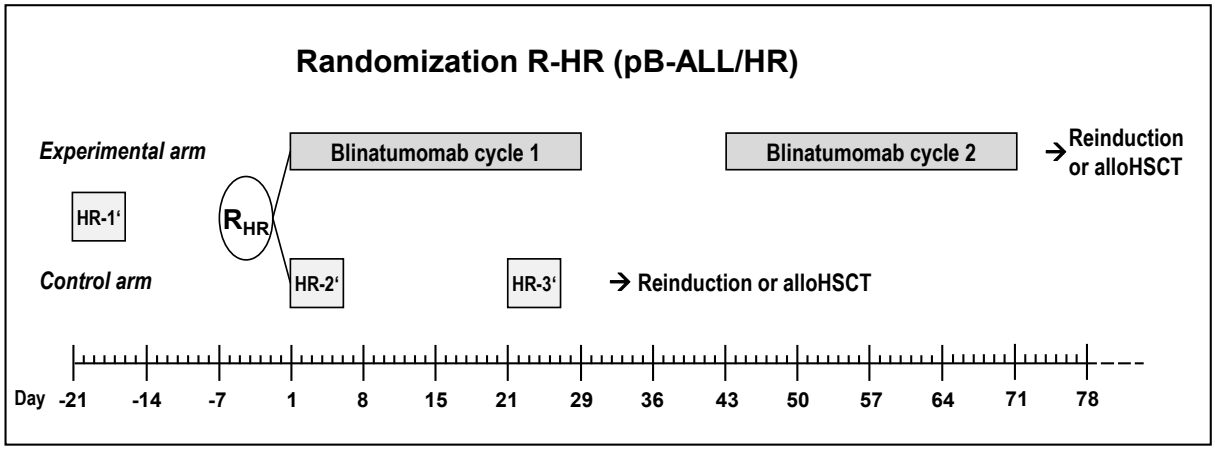


Figure 9 Schematic representation of randomization R-HR

Inclusion criteria:

- pB-ALL
- Allocation to the HR group (see section 5.3.2.2.3)
- Written informed consent to randomization R-HR from a legally acceptable representative. The assent of the child must be obtained, as appropriate to the age of the patient and/or based on local regulations.

Exclusion criteria:

- Prerequisite for adequate assessment of PCR-MRD response to the first Blinatumomab cycle as defined in the protocol (see below: Individual stopping criteria) are not fulfilled, i.e. no availability of at least one PCR-MRD marker with a quantitative range of ≤ 1 x 10^{-3}.
- Clinically relevant CNS pathology requiring treatment (e.g., unstable epilepsy).
- Evidence of current CNS (CNS 2, CNS 3) involvement by ALL. Patients with CNS involvement at initial diagnosis are included if CNS is successfully treated prior to randomization.
- Patients with Down Syndrome otherwise meeting the eligibility criteria: These patients should not be randomized and should be treated in the arm with Blinatumomab.
- Patients with documented *E2A-HLF* rearrangement These patients should not be randomized and should be treated in the arm with Blinatumomab.
- Symptoms and/or clinical signs and/or radiological and/or sonographic signs that indicate an acute or uncontrolled chronic infection, any other concurrent disease or medical condition that could be exacerbated by the treatment or would seriously complicate compliance with the protocol.
- Known infection with human immunodeficiency virus (HIV)
- Known hypersensitivity to immunoglobulins or any of the products or components to be administered during dosing

- History or evidence of any other clinically significant disorder, condition or disease (with the exception of those outlined above) that, in the opinion of the investigator or the national coordinator, if consulted, would pose a risk to subject safety or interfere with the study evaluation, procedures, or completion.

Individual stopping criteria[11]:

- Measurable PCR-MRD load after the first Blinatumomab cycle within the quantifiable range and no decrease of at least one log step (true MRD values) as compared to the PCR-MRD result before start of the first Blinatumomab cycle ("Blina Poor-Response").
- Adverse event(s) requiring dose interruption at the 5 µg/m^2/day dose
- Clinically relevant toxicities that by investigator's view impose an unacceptable safety risk to the subject
- Grade 4 cytokine release syndrome or neurologic event
- More than one cerebral seizure/convulsion
- Grade 3 neurologic event that needs more than one week to resolve to grade ≤ 1
- An infusion stop or delay of more than 14 days due to an adverse event or more than 2 discontinuations per cycle due to an adverse event

Timing:

Randomization R-HR should normally take place not earlier than 2 weeks from start of the preceding HR-1' course but not later than 3 days before scheduled start of randomized treatment in order to have sufficient time to ship the study drug Blinatumomab if applicable.

5.4.3 Randomization R-MR after reintensification phase for MR pB-ALL patients

Control arm: Treatment with the standard-of-care Maintenance phase starting approx. 2 weeks after end of reintensification phase Protocol II after hematological regeneration of blood counts.

Experimental arm: Treatment with 1 cycle Blinatumomab given at 15 µg/m^2/day as continuous intravenous infusion for 28 days starting approx. 2 weeks after end of reintensification phase Protocol II after hematological regeneration of blood counts before continuing with the standard of care treatment with Maintenance phase.

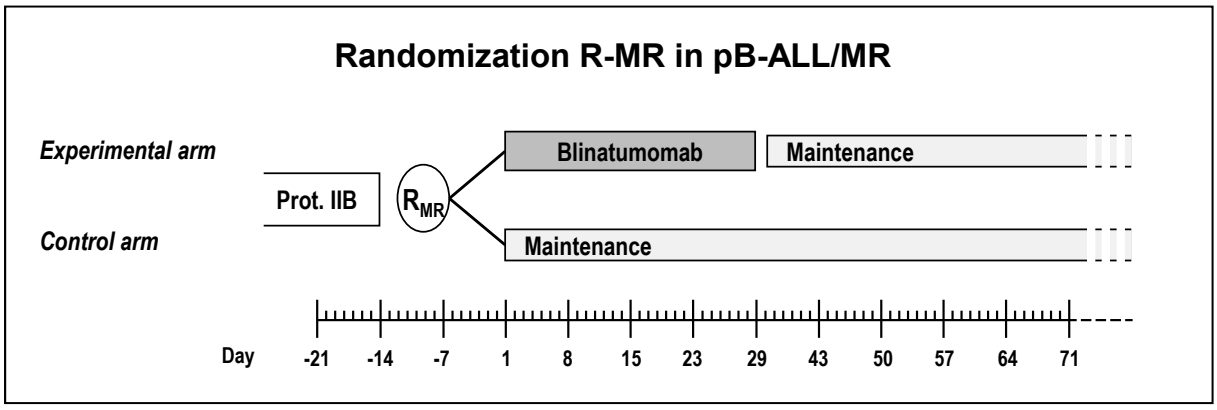


Figure 10 Schematic representation of randomization R-MR

[11] Patients of the R-HR experimental arm who either discontinued the Blinatumomab treatment after less than 14 days Blinatumomab or due to poor response to the first Blina cycle (see section 3.1.7) receive the blocks HR-2' and HR-3' of the control arm before continuation of the treatment with reintensification or alloHSCT.

Inclusion criteria:

- pB-ALL
- Allocation to the MR group (see section 5.3.2.2.3).
- Written informed consent to randomization R-MR from a legally acceptable representative. The assent of the child must be obtained, as appropriate to the age of the patient and/or based on local regulations.

Exclusion criteria:

- Clinically relevant CNS pathology requiring treatment (eg, unstable epilepsy).
- Symptoms and/or clinical signs and/or radiological and/or sonographic signs that indicate an acute or uncontrolled chronic infection, any other concurrent disease or medical condition that could be exacerbated by the treatment or would seriously complicate compliance with the protocol.
- Known infection with human immunodeficiency virus (HIV)
- Known hypersensitivity to immunoglobulins or any of the products or components to be administered during dosing
- History or evidence of any other clinically significant disorder, condition or disease (with the exception of those outlined above) that, in the opinion of the investigator or the national coordinator, if consulted, would pose a risk to subject safety or interfere with the study evaluation, procedures, or completion.

Individual stopping criteria:

- Adverse event(s) requiring dose interruption at the 5 µg/m^2/day dose
- Clinically relevant toxicities that by investigator‘s view impose an unacceptable safety risk to the subject
- Grade 4 cytokine release syndrome or neurologic event
- More than one cerebral seizure/convulsion
- Grade 3 neurologic event that needs more than one week to resolve to grade ≤ 1
- An infusion stop or delay of more than 14 days due to an adverse event or more than 2 discontinuations per cycle due to an adverse event

Timing:

Randomization R-MR should be prepared during Protocol II, and randomization procedure should normally take place not earlier than 1 week from end of Protocol IIB but not later than 3 days before scheduled start of randomized treatment in order to have sufficient time to ship the study drug Blinatumomab if applicable.

5.4.4 Randomization R-T in consolidation phase for non-SR T-ALL patients

Control arm: Treatment with the standard of care consolidation phase for T-ALL “Regular Protocol IB/Part 2” (Protocol IB/2$_{reg}$).

Experimental arm: Treatment with the consolidation phase “Long Protocol IB/Part 2” (Protocol IB/2$_{long}$) which in addition to the therapy with Protocol IB/2$_{reg}$ includes 1 extra dose of cyclophosphamide (1000 mg/m²), 2 extra blocks with 4 doses of Ara-C (75 mg/m²/dose) and 14 extra days of 6-mercaptopurine (60 mg/m²/day).

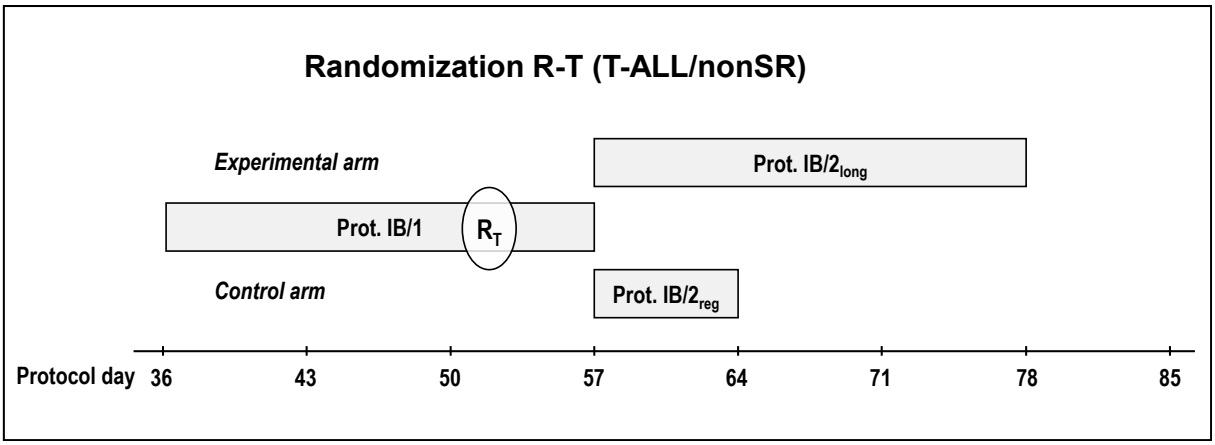


Figure 11 Schematic representation of randomization R-T

Inclusion criteria:

- T-ALL
- Allocation to the early non-SR group (see section 5.3.2.3.1).
- Written informed consent to randomization R-T from a legally acceptable representative. The assent of the child must be obtained, as appropriate to the age of the patient and/or based on local regulations.

Exclusion criteria:

- History or evidence of a clinically significant disorder, condition or disease that, in the opinion of the investigator or the national coordinator, if consulted, would pose a risk to subject safety or interfere with the study evaluation, procedures, or completion.

Timing:

Randomization R-T should take place after stratification to early non-SR but not before protocol day 36 (start of Protocol IB).

6 Diagnostics

6.1 Initial diagnostics

6.1.1 Diagnosis and biological characterization of ALL

The basic diagnostic program regarding diagnosis and biological characterization of the ALL is outlined in Table 3. Details on the required amount of material, shipment and addresses of the laboratories are given in in the national appendices.

Cytomorphology *(from native material without additives (e.g. EDTA))*	- Bone marrow: o Myelogram - Peripheral blood: o Complete blood count o Differential hemogram - CSF: o Cell count (counting chamber) o Cytospin preparation
Flow Cytometry	- Bone marrow (and/or peripheral blood): o Immunophenotyping o DNA index (optional) o Identification of suitable FCM-MRD targets
Molecular genetics and cytogenetics	- Bone marrow (and/or peripheral blood): o Identification of suitable PCR-MRD targets o Comprehensive genetic characterization of the leukemia (see Table 4)

Table 3 Obligatory diagnostics for the biological characterization of ALL

6.1.1.1 Cytomorphology

The diagnosis of ALL is primarily based on the cytomorphological examination of the bone marrow (BM), peripheral blood (PB) and cerebrospinal fluid (CSF). Diagnosis of ALL can be made if ≥25 % of the nucleated cells in the bone marrow are lymphoblasts. Peripheral blood count including differential hemogram is obligatory and has preferably to be determined before red cell or platelet transfusion. Cell count of CSF at diagnosis, analyzed by counting chamber, and cytospin preparations are mandatory for diagnosis of CNS involvement. A myelogram and FAB score should be done from well-spread BM smears preferably stained with May-Grünwald-Giemsa. Panoptic staining is also required for the cytomorphological evaluation of native PB smears and of CSF cytospin preparations. Conventional cytochemistry is not routinely required, but may be helpful in otherwise uncertain cases. However, examination of myeloperoxidase is mandatory by cytochemistry or by immunophenotyping. In every case, the diagnosis is to be confirmed by the national reference laboratory.

6.1.1.2 Immunophenotyping

Immunophenotyping by flow cytometry is an integral part of the initial work-up of every ALL patient due to its direct consequences regarding stratification and therapy. Flow cytometric analysis and interpretation/reporting of the findings should be done according to the AIEOP-BFM ALL Immunophenotyping Consensus Guidelines.

6.1.1.3 Genetic classification

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

6.1.1.3.1 Translocations/gene fusions

The preferred method for identifying all relevant categories of gene fusions is the hierarchical FISH screening with specific double-color split-apart probe sets to ascertain whether or not a particular (hub) gene is involved. The first set covers the most common fusions and the second the rare ones. Screening for rare fusion genes can be restricted to cases in which the presence of all other primary lesions (*ETV6, ABL1, KMT2A or TCF3* fusions and hyper-/haplo-/hypodiploidy) have been excluded.

The respective fusion partners should be identified subsequently in all hub gene-positive cases with either translocation-specific dual-color/dual-fusion FISH probe sets or, alternatively, with any other specific single or multiplex RT-PCR or any form of RNA- or DNA sequencing approach. The determination of the nature of the fusion partner and more specifically, eventually also the fusion breakpoint sequence is highly desirable because it can eventually be used for treatment decisions and MRD surveillance. This matter also forms the topic in an accompanying research project.

6.1.1.3.2 Quantitative changes

The preferred method for capturing all relevant small- and large-scale quantitative changes simultaneously in a comprehensive manner is DNA array analysis. The study-wide use of standardized high-density arrays that should be composed of SNP and oligo probes allows the joint collection, exchange and evaluation of the derived data on a sequence-based level. It thereby facilitates not only data validation and quality control programs but makes the obtained data also future-proof because they can easily be merged with all other sequence-derived (RNA, DNA and methylation) data sets. Finally, any appropriately sized copy number aberrations can in principle serve as a template for custom-made FISH probes and therefore in principle be utilized as a FISH-traceable marker for monitoring treatment response or disease progression on a single cell level, which may be especially pertinent for the analyses of particular tissues (e.g. cerebral fluid, testes).

Given the many advantages CGH analyses provides, all other alternative methods that were used previously for defining clinically relevant quantitative changes, such as DNA-index, cytogenetic, FISH, MLPA and specific DNA-based PCR analyses, are of subordinate interest. None of these methods can either alone or even in combination deliver the overall necessary information with a nearly complete and comparable standardized precision as DNA array analysis can.

6.1.1.3.3 Cytogenetics and metaphase FISH

The combined FISH and array approach can be viewed as an extended and refined form of standard cytogenetics. Metaphase spreads may still be required to resolve the topologic composition of the genome, to decipher potential rearrangements and to map particular lesions with FISH on chromosomes.

6.1.1.3.4 Basic genetic diagnostic requirements and workflow

All patients have to be investigated for the presence of *BCR-ABL1* until day 6 at the latest as *BCR-ABL1*-positive patients are not eligible for the trial.

All patients (pB-ALL and T-ALL) should further be screened for *KMT2A* (*MLL*) rearrangements, and the fusion partner should be identified in *KMT2A*-positive cases. The absence or presence of *TCF3-HLF* (or *E2A-HLF*), *TCF3-PBX1* (or *E2A-PBX1)*, *ETV6-RUNX1* (or *TEL-AML1*) and hypodiploidy should be ascertained in all patients with pB-ALL. Depending on the individual workflow in the lab, it is not necessarily required to investigate all pB-ALL for all these aberrations if a hierarchical screening approach is followed, assuming that these genetic aberrations usually are mutually exclusive. In order to identify patients with an *IKZF1*^{plus} pattern (for definition see 3.2.1), pB-ALL patients not presenting one of the specific aberrations *ETV6-RUNX1*, rearranged *KMT2A,* rearranged *TCF3,* or hypodiploidy are investigated for deletions of *IKZF1*, *PAX5*, *CDKN2A*, *CDKN2B*, *CRLF2* or *ERG*. The information on the stratification-relevant aberrations *KMT2A-AFF1* (or *MLL-AF4*), *TCF3-HLF, ETV6-RUNX1,* hypodiploidy and *IKZF1*^{plus} has to be available by the end of induction (d33, TP1) at the latest.

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

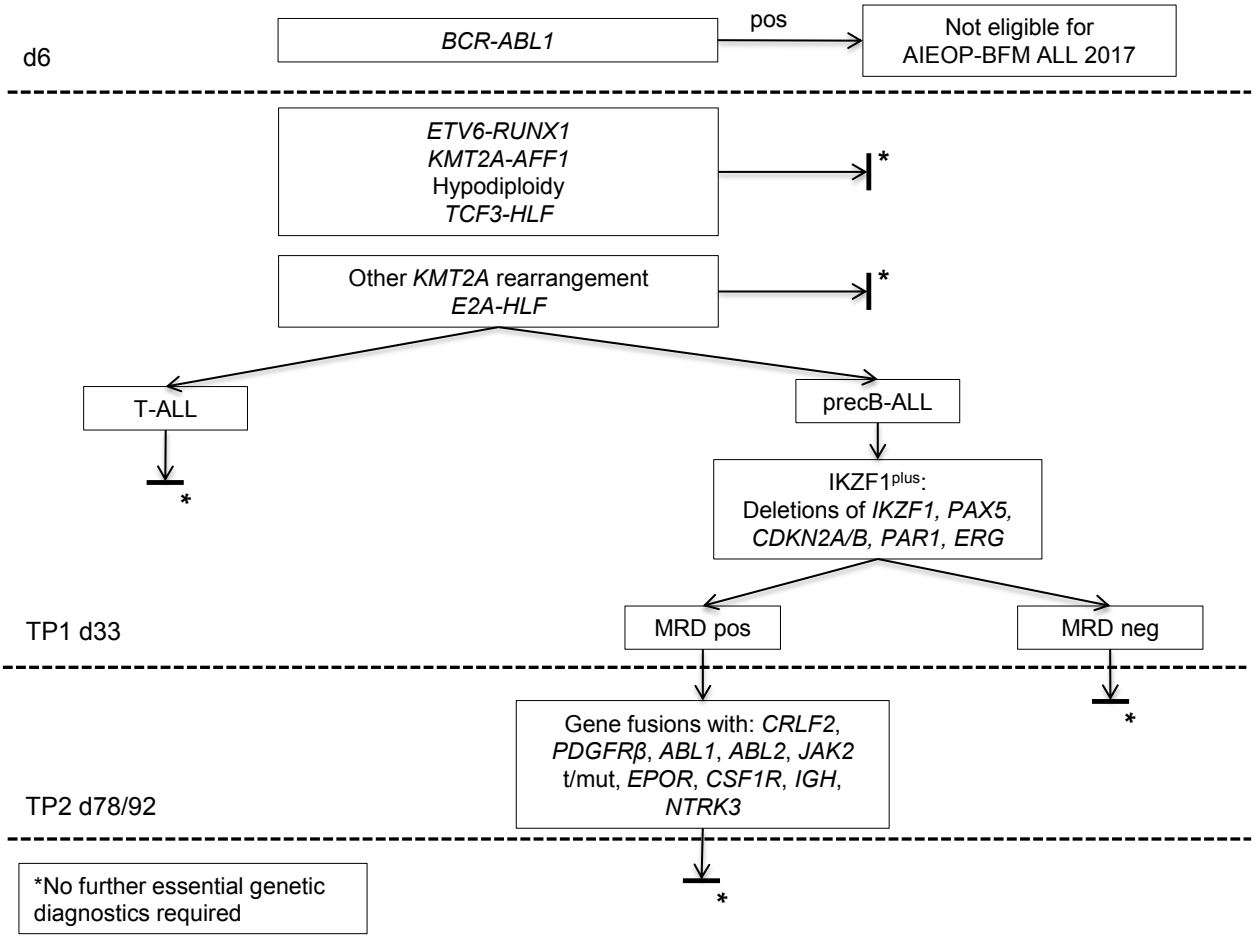


Figure 12 Algorithm of basic genetic diagnostics

Table 4 Minimal genetic diagnostics of stratification-relevant and other important prognostic aberrations

Genetic aberration	Relevant for stratification	Result required until day	Patient group to be investigated	% of pts to be investigated[12]
BCR-ABL1	yes	6	All	100%
KMT2A (MLL) rearrangement	(yes)[13]	33	All	100%
KMT2A-AFF1 (MLL-AF4)	yes	33	all pts positive for *KMT2A* split	3-4%
Identification of other *KMT2A* partner gene	no	n.a.	all positive for *KMT2A* split and negative for *KMT2A-AFF1*	2-3%
ETV6-RUNX1 (TEL-AML1)	yes	33	all pB-ALL[14]	85%
TCF3 (*E2A*) rearrangement	(yes)[15]	33	all pB-ALL[14]	85%
TCF3-PBX1 (E2A-PBX1)	no	n.a.	all pts positive for *TCF3* split	5%
TCF3-HLF (E2A-HLF)	yes	33	all pts positive for *TCF3* split and negative for *TCF3-PBX1*	<1%
Hypodiploidy	yes	33	all pB-ALL[14] except pts positive for *BCR-ABL1*, *ETV6-RUNX1*, *KMT2A* rearr. or *TCF3* rearr.	50%
Hyperdiploidy	no	n.a.	all pB-ALL[14] except pts positive for *BCR-ABL1*, *ETV6-RUNX1*, *KMT2A* rearr. or *TCF3* rearr.	50%
IKZF1 deletion	yes[16]	33	all pB-ALL[14] except pts positive for *BCR-ABL1*, *ETV6-RUNX1*, *KMT2A* rearr., *TCF3* rearr., or hypodiploidy	50%
PAX5 deletion	yes[16]	33		50%
CDKN2A deletion	yes[16]	33		50%
CDKN2B deletion	yes[16]	33		50%

[12] Pecentage of patients to be investigated may vary depending on local differences in the diagnostic workflow

[13] only *AFF1-KMT2A* (*MLL-AF4*)

[14] or unknown immunophenotype

[15] only *TCF3-HLF*

[16] only in combination with other genetical aberrations defining IKZF1^{plus}

Genetic aberration	Relevant for stratification	Result required until day	Patient group to be investigated	% of pts to be investigated[12]
P2RY8-CRLF2 (*PAR1* deletion)	yes[16]	33		50%
ERG deletion	yes[16]	33	all pts meeting other criteria for IKZF1^{plus}	6%
ABL1 rearr.	(yes)[17]	78/92	all pB-ALL[14] with positive MRD on day 33 except pts positive for *BCR-ABL1*, *ETV6-RUNX1*, *KMT2A* rearr., *TCF3* rearr, or hypodiploidy	35%
ABL2 rearr.	(yes)[17]	78/92		35%
CSF1R rearr.	(yes)[17]	78/92		35%
PDGFRβ rearr.	(yes)[17]	78/92		35%
IGH rearr.	(yes)[17]	78/92		35%
CRLF2 rearr	(yes)[17]	78/92		35%
EPOR rearr.	(yes)[17]	78/92		35%
NTRK3 rearr.	(yes)[17]	78/92		35%
JAK2 mutations	(yes)[17]	78/92		35%

[17] only in the case of MRD non-response

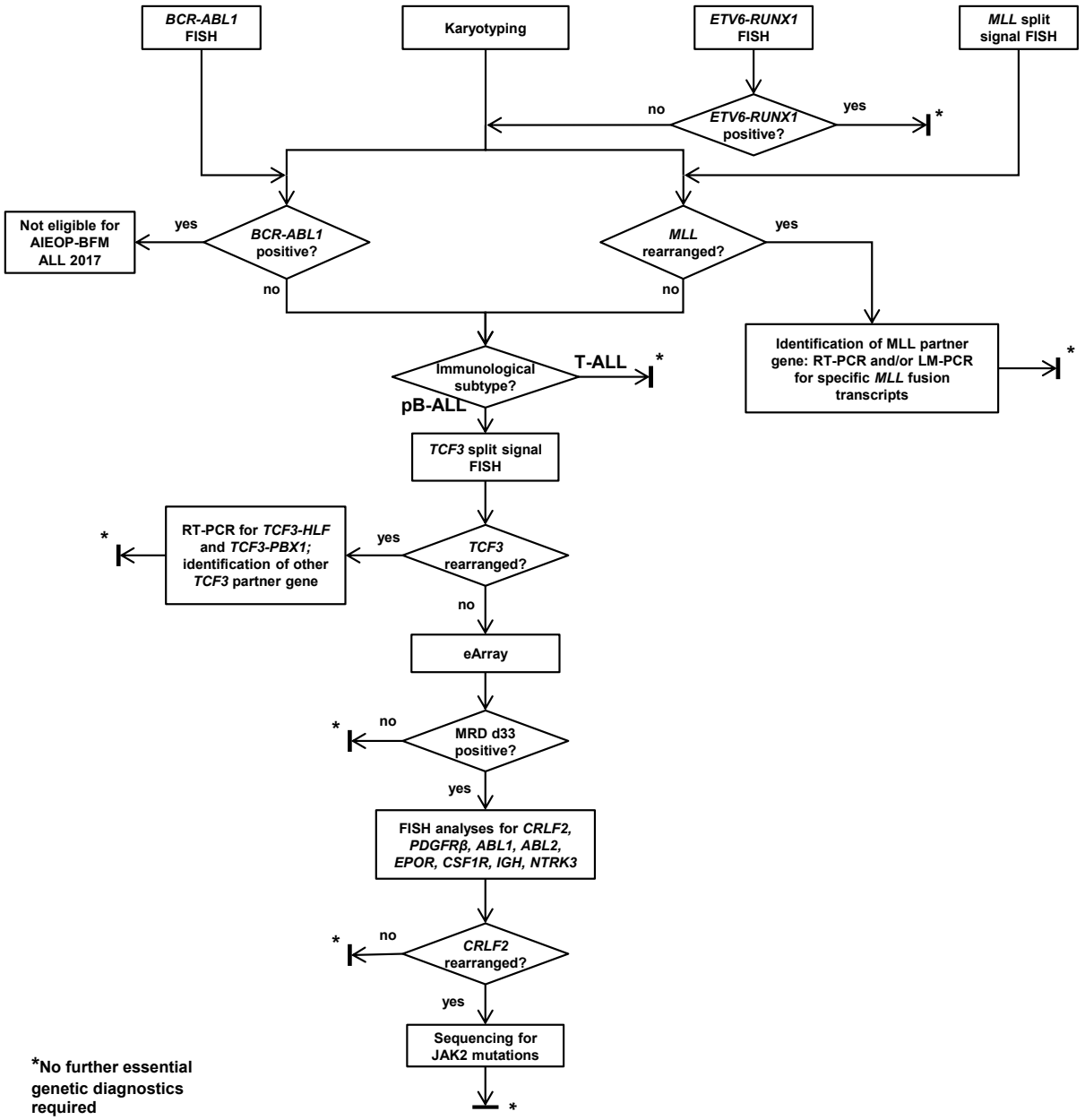


Figure 13 Workflow of genetic diagnostics in BFM-Germany

6.1.1.4 Evaluation of hypodiploidy by flow-cytometric analysis of the DNA index

Ploidy can be determined by genetic methods such as metaphase karyotyping or DNA array analyses or by measuring the DNA content by flow cytometry. Flow cytometry expresses the DNA content as DNA index (DI), a ratio between the normal amount of fluorescence seen in a diploid cell and the fluorescent content of the bone marrow blasts (in G_0/G_1) at diagnosis. However, hypodiploidy cannot be definitely excluded by a normal DI. On the contrary, in case of a clear “hypodiploid” DI, this finding can be considered reliable. The analysis of DI is not mandatory in the trial but can be helpful in cases with inconclusive genetic findings.

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

- If DI is < 0.8 or the conventional cytogenetics clearly shows less than 45 chromosomes no further confirmation by another method is required.
- If DI is ≥ 0.8 and < 1.00, confirmation by another method is mandatory, e.g.:

- Metaphase cytogenetics: a normal karyotype is only reliable, if at least 20 metaphases could be analyzed. Otherwise, a third method is required.
- DNA array
- MLPA (Multiplex Ligation-dependent Probe Amplification)
- Centromer FISH

Hypodiploidy, defined by less than 45 chromosomes and/or a DI of < 0.8, qualifies the patient for treatment in the high-risk group.

6.1.1.5 MRD marker establishment

Sampling of initial material (BM or PB with sufficient percentage of blast cells) for PCR-MRD diagnostics (establishment and testing of suitable PCR targets, standard dilution series) is required. The DNA amount (and the corresponding number of mononucleated cells) required for a successful MRD analysis depends on the number of established markers, DNA quality and the need for repetition of experiments. As a general guideline 1×10^7 cells (corresponding to about 20 µg DNA) are the minimally required amount.

6.1.2 General diagnostics and diagnostics of extramedullary disease

A careful clinical work-up documentating the patient's baseline status and potential extramedullary manifestations of the disease, is required including history and physical examination of the patient as well as extensive laboratory and instrumental diagnostic procedures. For details of the recommended diagnostic work-up see the national appendix.

For definition of organ involvement including CNS involvement see section 3.3.1.

6.1.2.1 Diagnostics of CNS status

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

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

For definition of the CNS status see section 3.3.1.

Technique of cytospin preparation:

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

Traumatic lumbar puncture:

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

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

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

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

6.2 Response Evaluation

6.2.1 Cytomorphological response and remission evaluation in BM and PB

Cytomorphological response evaluation should be reviewed by the study center.

Details on required material and shipment are given in the national appendix.

6.2.1.1 Peripheral blood on day 8 (prednisone response)

The prednisone response is evaluated on day 8 (before application of vincristine/daunorubicin) after 7 days of prednisone and one intrathecal application of MTX. Day 1 is the day of the first prednisone application. An isolated prednisone poor-response (i.e. in the absence of other HR criteria) does not anymore qualify for high-risk treatment in pB-ALL as long as MRD results (PCR and/or FCM) are available. In T-ALL, the prednisone poor-response still stratifies the patients to HR.

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

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

6.2.1.2 Bone marrow on protocol day 15 and day 33

Investigation of cytomorphological response in bone marrow on protocol days 15 and 33 of Protocol IA is mandatory in all patients. Protocol days 15 and 33 do not necessarily take place 15 or 33 days after start of therapy, but are defined by the treatment schedule, i.e. protocol day 15 is the day of the second VCR/DNR dose and protocol day 33 is the fourth day after the 4th VCR/DNR. The bone marrow puncture at protocol day 15 has to be performed <u>before</u> application of the 2nd VCR/DNR.

Morphologic and minimal residual disease (MRD) based investigation of BM day 33 is crucial for further risk-adapted stratification. In any case, a puncture, if clinically possible, should be performed on protocol day 33 (i.e. 4 days after 4th VCR/DNR). A re-puncture must be performed before start of Consolidation/Protocol IB in the case of insufficient cellularity or DNA content. The MRD finding (see below) will be communicated within 2 weeks for stratification into early risk groups.

6.2.1.3 Later time points

For all patients, cytomorphological evaluation of remission in bone marrow is recommended at the start of the subsequent treatment elements (Protocol M, Protocol II, all HR blocks, all Protocol III, and at specific time points during the randomized treatment phases when PCR-MRD investigation is scheduled (see 6.2.3.1).

6.2.2 Response evaluation of extramedullary manifestations

Definitions of remission of extramedullary manifestations and the consequences for risk group classification are described in section 3.1.2.

6.2.2.1 CSF

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

6.2.2.2 Mediastinal tumor

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

6.2.2.3 Other manifestations

In general, initial findings of extramedullary leukemic infiltrations should be reevaluated on day 33. In the case of incomplete regression, evaluation should be repeated after completion of Consolidation/Protocol IB. In the case of ambiguous findings, contact the study coordination center to discuss procedure and risk classification.

6.2.3 Minimal Residual Disease (MRD)

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

To assess the treatment response in the bone marrow, the presence and dynamics of minimal residual disease (MRD) is evaluated by molecular genetic analysis of clone-specific T-cell receptor and immunoglobulin gene rearrangements by real-time quantitative PCR.

6.2.3.1.1 PCR-MRD time points

The PCR-MRD response at distinct protocol time points is essential for risk group and treatment stratification (see section 5.3) and is prospectively evaluated in randomized treatment phases. MRD findings at time points that are only evaluated prospectively ("prospective evaluation").

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

All patients:

- **TP1:** at end of Protocol IA (d33). This time point is decisive for stratification to early HR/early non-HR in pB-ALL or early SR/early non-SR in T-ALL in the absence of any HR criteria at TP1 (see sections 5.3.2.2.1 and 5.3.2.3.1). In patients without TP1 HR criteria and PCR-MRD negativity at TP1 with quantifiable range of $<10^{-4}$, MRD-TP1 is sufficient for stratification to the SR group.
- **TP2 (d78/d92, after Consolidation or Protocol IB):** This time point is decisive for final stratification in patients without TP1 HR criteria except for those who could

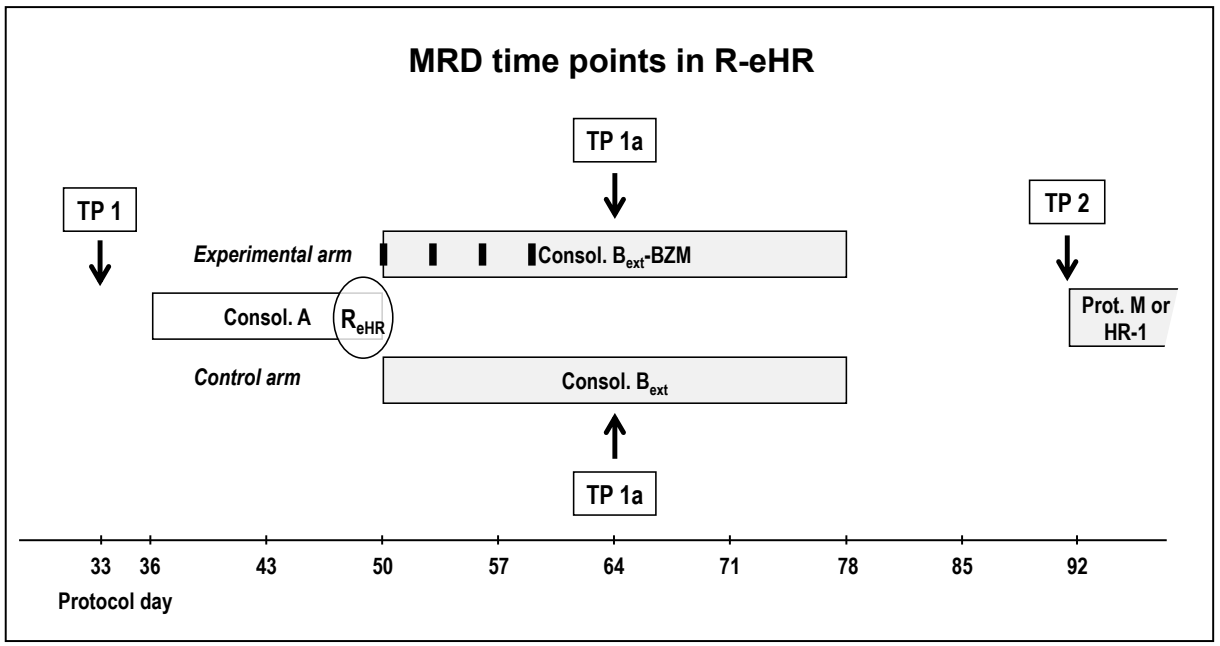
already be stratified to SR at TP1. For patients with HR crtieria by TP1, TP2 is decisive for SCT indication (see sections 5.3.2.2.3, 5.3.2.3.3 and 5.3.3)

HR patients:

- **TP HR1:** After HR-1' when hematopoesis has recovered
- **TP HR2:** After HR-2' when hematopoesis has recovered.
- **TP HR3:** After HR-3' when hematopoesis has recovered.
- **TP D/F:** After DNX-FLA when hematopoesis has recovered.
- **Other time points:**
  - Before start of the second and third Protocol III
  - Before alloHSCT in the case of long delay after TP HR3 or TP D/F or if another treatment element was given in between.

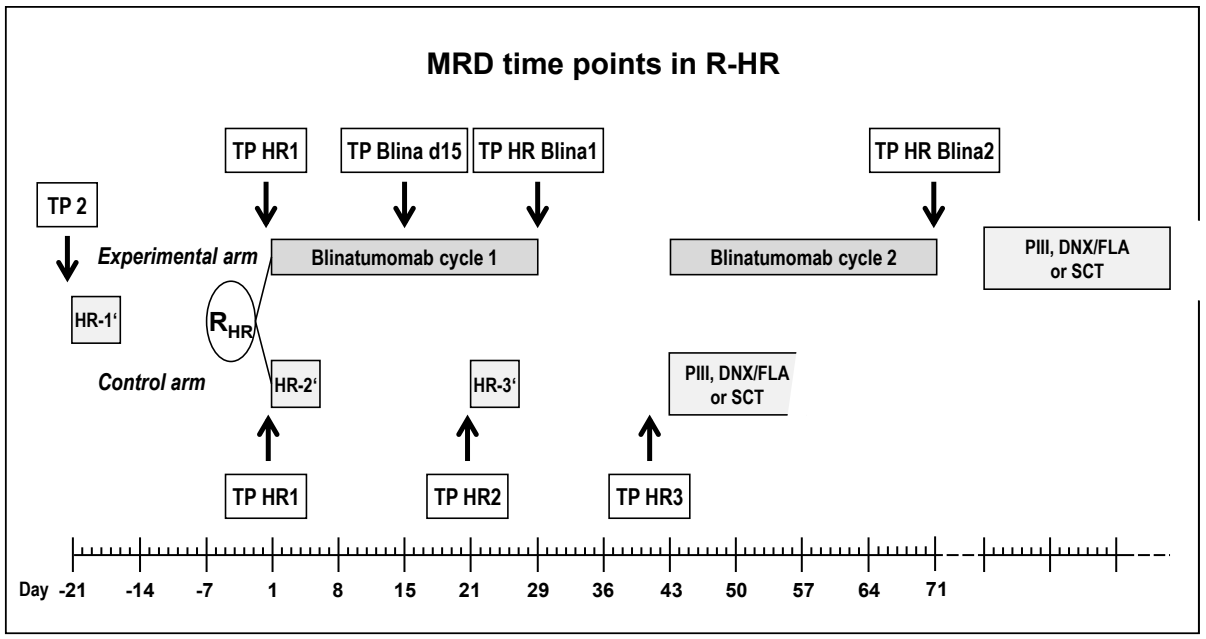
Randomization R-eHR:

- **TP1a:** on the day of first CPM in Consolidation B_{ext}, i.e. protocol day 64 (prospective evaluation)



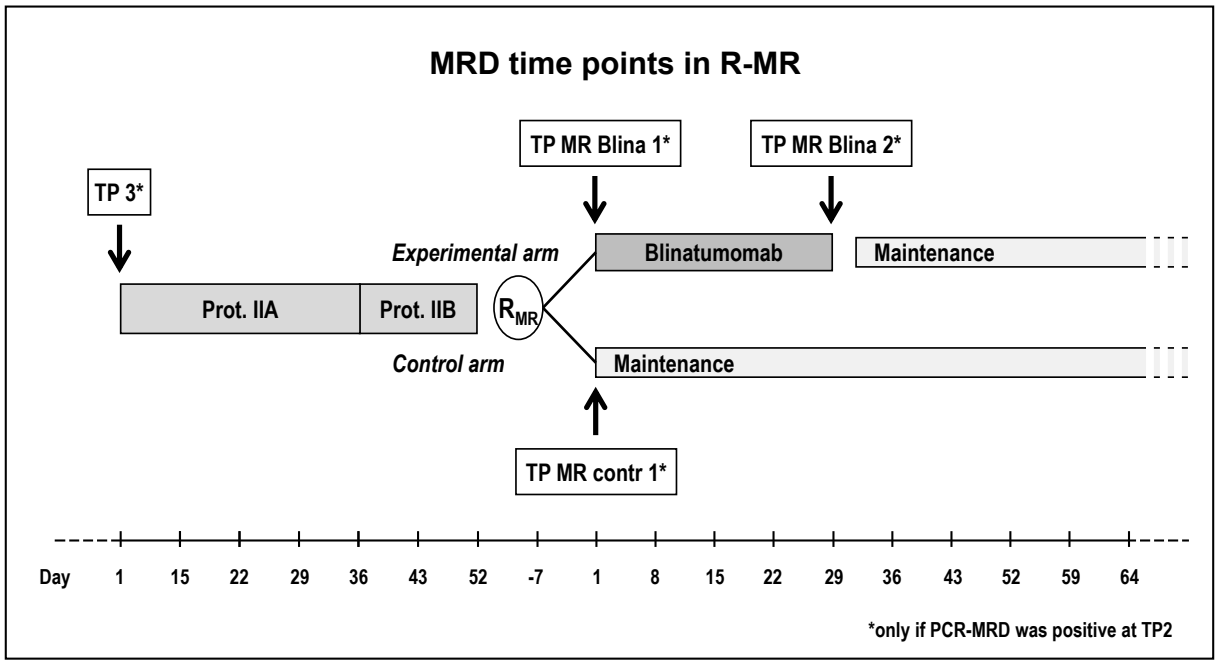
Randomization R-HR:

- **TP HR1:** After HR-1' when hematopoesis has recovered. MRD at this time point is essential for assessment of MRD reduction over the first Blina cycle.
- **TP HR2:** After HR-2' arm when hematopoesis has recovered.
- **TP HR3:** After HR-3' when hematopoesis has recovered. Patients with poor MRD response (≥ $5x10^{-4}$) at this time point qualify for the treatment with DNX/FLA before alloHSCT. BMP will usually be scheduled some days before planned start of the subsequent treatment element that the MRD result can be taken into account for the decision on following treatment (Protocol III/alloHSCT or DNX/FLA).
- **TP Blina d15:** On day 15 of the first Blina cycle (prospective evaluation).
- **TP HR Blina 1:** On day 29 of the first Blina cycle. Patients with insufficient MRD reduction over the first cycle (Blina Poor-Reponse, for definition see section 3.1.7) will discontinue the Blinatumomab treatment and will receive blocks HR-2' and HR-3' instead.
- **TP HR Blina 2:** On day 29 of the second Blina cycle.
- Later time points are scheduled as in non-randomized HR patients (see above)



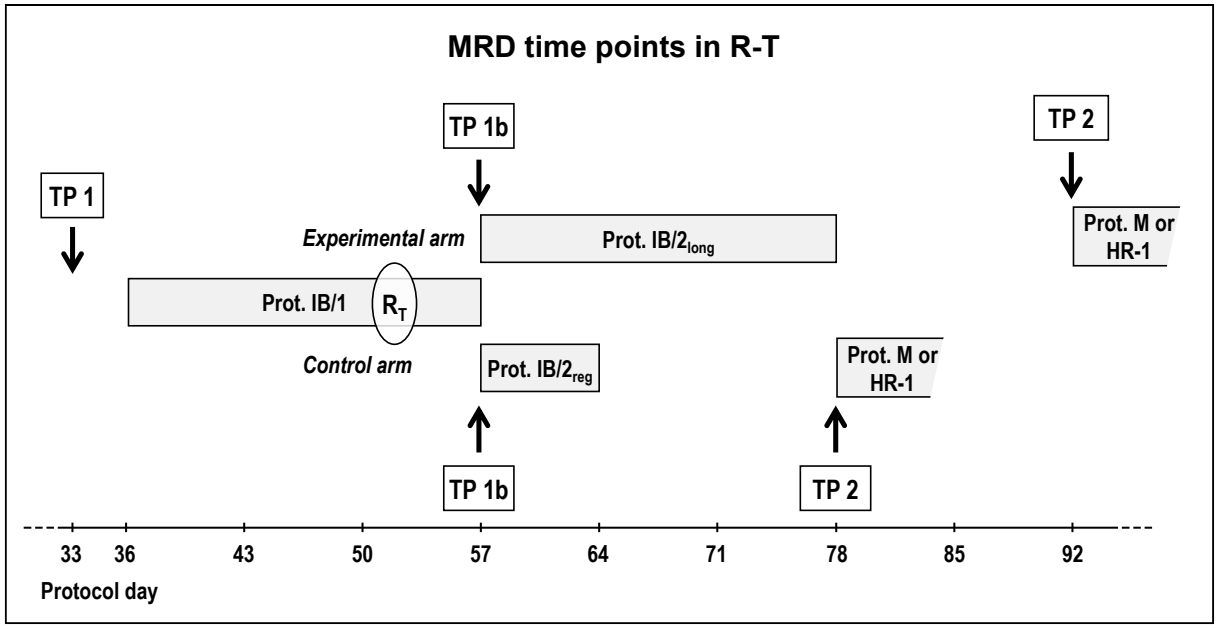
Randomization R-MR (prospective MRD evaluation only for patients with positive MRD at TP2):

- **TP3:** at start of Protocol II (prospective evaluation)
- **TP MR contr. 1:** at start of Maintenance (prospective evaluation)
- **TP MR Blina 1:** at start of the Blinatumomab cycle (prospective evaluation)
- **TP MR Blina 2:** on day 29 from start of the Blinatumomab cycle (prospective evaluation)



Randomization R-T:

- **MRD TP1b:** at protocol day 57, i.e. start of Prot. IB/2_{long} in the experimental arm and of Prot. IB/2_{lreg} in the control arm (prospective evaluation)



6.2.3.1.2 Logistical aspects of PCR-MRD analyses

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

At **TP1** (end of induction) the MRD load is required to stratify the patients into early HR/early non-HR in pB-ALL or in early SR/early non-SR in T-ALL patients and is an essential information for eligibilty to the randomization R-eHR and R-T. The result must therefore be available within 2 weeks from sampling on day 33.

Response over the first Blina cycle in R-HR is assessed by MRD response at **TP HR Blina 1** (at the end of the first Blina cycle). The result is crucial for the decision on continuation with the second Blina cycle and must therefore be available within 10 days.

Patients with MRD of ≥ $5x10^{-4}$ after hematological regeneration at **TP HR3** or **TP HR Blina 2**, i.e. after HR-3' or the second Blina cycle, are eligible to receive DNX-FLA before alloHSCT. The MRD analysis at this time point will be done as soon as possible but some days have to be taken in account when planning the following therapy procedure.

High MRD of ≥ $5x10^{-4}$ at **TP D/F**, i.e. after DNX/FLA after hematological recovery, ("MRD non-response) is a statistical event and qualifies the patient for off-protocol experimental treatment.

6.2.3.2 MRD by flow cytometry

6.2.3.2.1 FCM-MRD time points relevant for stratification

MRD by flow cytometry (FCM) on day 15 is relevant for risk stratification in certain situations; investigation of this time point is therefore obligatory for all patients:

- Patients with detection of ≥ 10 % blast cells by FCM in bone marrow on day 15 except patients positive for the *ETV6-RUNX1* (*TEL-AML1*) are eligible for treatment in the early HR and final HR group regardless of PCR-MRD status (see section 5.3.2.2).

- Patients with missing or inconclusive PCR-MRD results (see section 5.3.2), are eligible for treatment in SR if < 0.1 % blast cells are detectable by FCM in bone marrow on day 15.

6.3 Monitoring of toxicity

6.3.1 Toxicity during treatment

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

6.3.2 Study-related diagnostics in the randomized treatment phases

Apart from the continuous documentation of adverse events in the form of spontaneous reporting throughout the entire treatment phase as described in detail in section 12, a systematic monitoring of specific laboratory parameters is required during the randomized treatment phases covering the most relevant organ functions. In addition, an extensive immune monitoring program is intended in the randomizations involving Blinatumomab (R-HR and R-MR) as described in detail in the appendix “Add-on research projects”.

The schedule of these diagnostic samplings is given in Table 5 on page 78. For details of material and shipping modalities, please refer to the national appendix of your country “Logistics of diagnostics and sample shipment”.

Table 5 Schedule of blood and bone marrow sampling in the randomized treatment phases

Protocol day	Sampling time point specified	Blood counts incl. differential*	Serum chemistry*	Quantitative immuno-globulins*	MRD in bone marrow#	Immune monitoring#§		Cytokine profile§
						B-cell studies (bone marrow)	T-cell studies (blood)	
	Randomisation R-eHR: control arm and experimental arm							
Consolidation B_{ext} +/- BZM:								
Day 50	Start of consolidation B_{ext}	X	X					
Day 57	Before 2nd VCR dose	X	X					
Day 64	Before 1st CPM dose	X	X		X (TP1a)			
Day 73	Before 2nd ARA-C block of Consol. B_{ext}	X	X					
	Randomisation R-HR: control arm							
HR-2':								
Day 1	Start of HR-2'	X	X	X	X (TP HR1)			
Day 8	Day 8 (± 1 day) from start of HR-2'	X	X					
Day 15	day 15 (± 1 day) from start of HR-2'	X	X					
HR-3':								
Day 1	Start of HR-3'	X	X	X	X (TP HR2)			
Day 8	Day 8 (± 1 day) from start of HR-3'	X	X					
Day 15	Day 15 (± 1 day) from start of HR-3'	X	X					
	Randomisation R-HR: experimental arm							
Blina cycle 1:								
Day 1	Prior to infusion start of Blina cycle 1	X	X	X	X (TP HR1)	X	X	X
Day 2	24 hours after start of Blinatumomab infusion	X	X				X	X
Day 15	Day 15 (± 1 day) from start of Blina cycle 1	X	X		X (TP Blina 15)	X	X	X
Day 29	Day 29 (± 1 day) from start of Blina cycle 1	X	X			X	X	X
Blina cycle 2:								
Day 1	Prior to infusion start of Blina cycle 2	X	X	X	X (TP HR Blina 1)		X	X
Day 2	24 hours after start of Blinatumomab infusion	X	X				X	X
Day 15	Day 15 (± 1 day) from start of Blina cycle 2	X	X				X	X
Day 29	Day 29 (± 1 day) from start of Blina cycle 2	X	X		X (TP HR Blina 2)	X	X	X

Protocol day	Sampling time point specified	Blood counts incl. differential*	Serum chemistry*	Quantitative immuno-globulins*	MRD in bone marrow#	Immune monitoring#§		Cytokine profile§
						B-cell studies (bone marrow)	T-cell studies (blood)	
	Randomisation R-MR: control arm							
Maintenance (MT):								
Day 1	Start of MT	X	X	X	X (TP MR contr. 1)			
Day 15	Day 15 (± 1 day) from start of MT	X	X					
Day 29	Day 29 (± 1 day) from start of MT	X	X					
Day 43	Day 43 (± 1 day) from start of MT	X	X	X				
	Randomisation R-MR: experimental arm							
Blina cycle (followed by Maintenance):								
Day 1	Prior to infusion start of Blina	X	X	X	X (TP MR Blina 1)		X	X
Day 2	24 hours after start of Blinatumomab infusion	X	X				X	X
Day 15	Day 15 (± 1 day) from start of Blina	X	X				X	X
Day 29	Day 29 (± 1 day) from start of Blina	X	X		X (TP MR Blina 2)		X	X
Day 43	Day 43 (± 1 day) from start of Blina	X	X	X				
	Randomisation R-T: control arm							
Protocol IB/2_{reg}								
Day 57	Start of Prot. IB/2_{reg}	X	X		X (TP1b)			
Day 73	Day 9 (± 1 day) after last CPM in Prot. IB/2_{reg}	X	X					
	Randomisation R-T: experimental arm							
Protocol IB/2_{long}								
Day 57	Start of Prot. IB/2_{long}	X	X		X (TP1b)			
Day 73	Prior to 3rd ARA-C block of Prot. IB/2_{long}	X	X					
Day 87	Day 9 (± 1 day) after last CPM in Prot. IB/2_{long}	X	X					

* Local laboratory diagnostics:
Blood counts incl. differential: WBC, Platelets, Hb; Neutrophils, Lymphocytes
Serum chemistry: Creatinine, GOT, GPT, Bilirubine, Lipase, CRP
Quantitative Immunoglobulins: IgG, IgM

Central laboratory diagnostics (for sampling and shipment modalities, refer to the national appendix “Logistics of diagnostics and sample shipment”)

§ Immune monitoring and investigation of cytokine profile only in BFM-Germany

6.3.3 Follow-up, late side effects

The investigations recommended at the end of maintenance and follow-up diagnostics are specified in the appendix “Recommendations for management of toxicity and supportive care”.

7 Treatment

7.1 New naming of treatment phases

Phase	Stratification group	Treatment arm	Name of chemotherapy phase	Short name of phase	Description
Induction phase	pB-ALL	all	Protocol IA-Pred	Prot. IA_{Pred}	Protocol IA with 21 days Prednisone plus tapering
	T-ALL, PGR	all	Protocol IA-Dexa	Prot. IA_{D}	Protocol IA with 21 days Dexamethasone plus tapering
	T-ALL, PPR	all	Protocol IA-CPM	Prot. IA_{CPM}	Protocol IA with 21 days Prednisone plus tapering and one dose Cyclophosph.
Consolidation phase	all pB-ALL	all	Consolidation A	Consol. A	First part of consolidation phase
	pB-ALL, early non-HR	all	Short Consolidation B	Consol. B_{short}	Second part of short (standard) consolidation
	pB-ALL, early HR	standard arm R-eHR control arm	Extended Consolidation B	Consol. B_{ext}	Second part of extended (standard) consolidation
	pB-ALL, early HR	R-eHR experimental arm	Extended Consolidation B + Bortezomib	Consol. B_{ext}-BZM	Second part of extended (experimental) consolidation with Bortezomib
	T-ALL	all	Protocol IB/Part 1	Prot. IB/1	First part of Protocol IB
	T-ALL, SR T-ALL, non-SR	all standard arm R-T control arm	Regular Protocol IB/Part 2	Prot. $IB/2_{reg}$	Second part of regular (standard) Protocol IB
	T-ALL, non-SR	R-T experimental arm	Long Protocol IB/Part 2	Prot. $IB/2_{long}$	Second part of long (experimental) Protocol IB
Non-HR extra-compartment phase	pB-ALL, SR/MR T-ALL, non-HR	all	Protocol M	Prot. M	HD-MTX phase

Phase	Stratification group	Treatment arm	Name of chemotherapy phase	Short name of phase	Description
HR intensified consolidation phase	pB-/T-ALL, HR	all	HR-1' block	HR-1'	High-risk consolidation block 1
	T-ALL, HR pB-ALL, HR	all standard arm R-HR control arm	HR-2' block	HR-2'	High-risk consolidation block 2
	T-ALL, HR pB-ALL, HR	all standard arm R-HR control arm	HR-3' block	HR-3'	High-risk consolidation block 3
	pB-ALL, HR	R-HR experimental arm	Blinatumomab cycle with intrathecal MTX	Blina cycle + i.th. MTX	Experimental high-risk consolidation treatment with Blinatumomab plus i.th. MTX
Reintensification phase	pB-ALL, SR/MR T-ALL, non-HR	all	Protocol II	Prot. II	non-HR reintensification treatment
	pB-ALL, MR	R-MR experimental arm	Blinatumomab cycle	Blina cycle	experimental MR reintensification treatment
	HR	all	Protocol III	Prot. III	HR reintensification treatment

7.2 General remarks concerning guidelines for treatment modulation

In this chapter, guidelines are given concerning starting criteria of the treatment elements and modulation of therapy due to toxic effects. In particular in the standard chemotherapy parts of the protocol, these guidelines are not in every case binding. They have carefully to be considered in the light of the actual clinical situation (e.g., status of ALL disease) and may be adapted if clinically duly substantiated. Should a certain clinical situation require a deviation from the guidelines for interruption or discontinuation of the experimental treatments with Bortezomib or Blinatumomab in the experimental randomized arms of R-eHR, R-HR or R-MR, please consult the national study coordination centre.

7.3 Cytoreductive prephase

7.3.1 Precautions and therapy regulation

- In case of large initial leukemic cell mass (WBC ≥ 100 000/µl and/or marked liver and/or spleen enlargement and/or large mediastinal mass) the prednisone starting dose should be lower than the target dose (e.g. 1/3 of the calculated target dose). Adapted to the treatment response, laboratory values and renal excretion, the drug dose should be increased as quickly as possible. The target dose should regularly be reached not later than on day 3. For management of hyperleukocytosis and initial complications see the protocol appendix.
- In case of excessive increase of the peripheral blast count during the prephase, the additional administration of cyclophosphamide or the early start with vincristine and daunorubicin (day 8) can be considered after consulting the study center. These cases are to be classified as Prednisone Poor-Responders (PPR) if a Prednisone Poor-Response could reasonably be expected with great certainty and the modification of the pre-phase was justified in this regard. An initial increase of WBC during the first days of the prephase is *per se no* indication for chemotherapy modifications unless the patient is clinically threatened.

7.3.2 Treatment schedule of cytoreductive prephase

PRED: Prednisone (alternatively prednisolone) 60 mg/m^2/day p.o. or i.v.[18] divided into 3 doses per day, days 1 to 7 (i.e. 7 days)

MTX i.th.: Methotrexate intrathecally on day 1

Age-adjusted dose:

< 1 year:	6 mg
1 to < 2 years:	8 mg
2 to < 3 years:	10 mg
≥ 3 years:	12 mg

Lowered-head position for at least two hours after intrathecal methotrexate application.

7.4 Induction phase

The induction phase Protocol IA exists in three different variants that differ in the type of glucocorticoid and whether it includes an additional dose of Cyclophosphamide:

Protocol IA-Pred (IA_P): for pB-ALL

[18] In the case of i.v. application of prednisolone, attention should be payed to the dosage which is declared for the salt formulation (prednisolone-21-hydrogen succinate) in most products: A dose of 10 mg prednisolone-21-hydrogen succinate equates to 7.5 mg prednisolone).

Protocol IA-Dexa (IA_D): for T-ALL with Prednisone Good-Response

Protocol IA-CPM (IA_{CPM}): for T-ALL with Prednisone Poor-Response

7.4.1 Precautions and therapy regulation

This treatment phase carries a particularly high risk of treatment complications, above all the risk of severe infections. Therefore, a very close monitoring of the patients and the early detection and treatment of adverse medical conditions are essential. For detailed recommendations regarding careful monitoring and supportive care, it is referred to the protocol appendix.

On the other hand, treatment schedule in the induction phase should be adhered to as closely as possible. Treatment should only be delayed in exceptional conditions. Severe neutropenia in the absence of infection is not *per se* a reason for treatment delay or dose reduction in this treatment phase.

7.4.2 Protocol IA-Pred (Prot. IA_P)

Patients with **pB-ALL (or unknown immunophenotype)** receive Protocol IA-Pred (IA_P). Starting on day 8 after the cytoreductive pre-phase, Prot. IA_P includes three weeks of prednisone plus 9 days tapering.

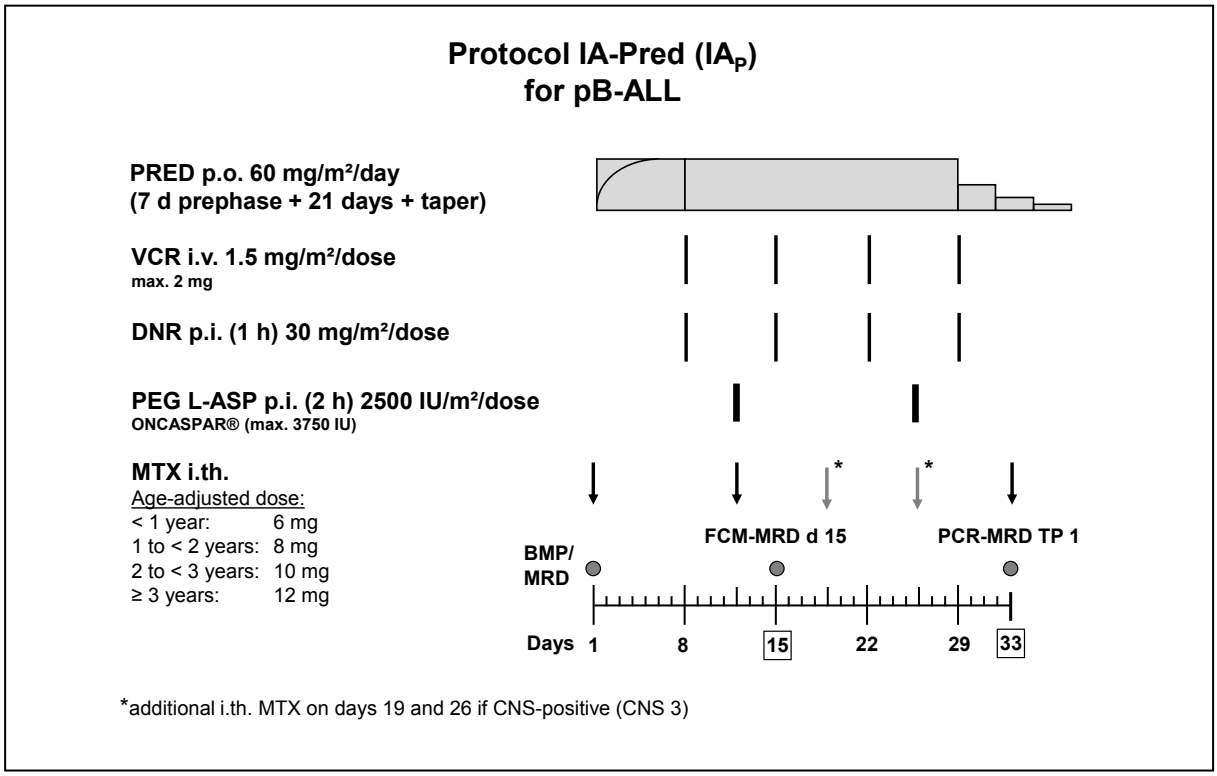


Figure 14 Treatment outline of Protocol IA_P. The element is applicable to patients with pB-ALL.

7.4.2.1 Treatment schedule of Prot. IA-Pred

PRED: **Prednisone (alternatively prednisolone)** 60 mg/m^2/day p.o. or i.v.[19] divided into 3 doses per day, days 8 to 28 (21 days); from day 29, tapering over 9 days with halving the dose on every third day.

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

The WHO recommend that "Vincristine should where possible be prepared by dilution in small volume intravenous bags (the 'minibag' technique), rather than in a syringe, to protect against accidental administration via a spinal route. The labeling of vincristine should include a clear warning label that reads: 'FOR INTRAVENOUS USE ONLY - FATAL IF GIVEN BY OTHER ROUTES'."

DNR: **Daunorubicin** 30 mg/m^2/dose p.i. (1 h) on days 8, 15, 22 and 29 (4 doses).

PEG-L-ASP: **PEG-L-Asparaginase (Oncaspar®)** 2 500 IU/m^2/dose p.i. (2 h) on days 12 and 26 (2 doses) (maximal single dose 3 750 IU).

In case of hypersensitivity to PEG-L-asparaginase, Erwinia asparaginase should be given at a dosage of 20 000 IU/m^2/dose p.i. (1 h) or i.m. every second day for the remaining days of scheduled asparaginase treatment, i.e. until two weeks after the last scheduled PEG-L-ASP dose in this element.

MTX i.th.: **Methotrexate intrathecally** on days 12[20] and 33.

In case of initial CNS status CNS 3 (see chapter 3.3.1) additional intrathecal methotrexate is given on days 19 and 26.

Age-adjusted dose:		
	< 1 year:	6 mg
	1 to < 2 years:	8 mg
	2 to < 3 years:	10 mg
	≥ 3 years:	12 mg

Lowered-head position for at least two hours after intrathecal methotrexate injection.

[19] In the case of i.v. application of prednisolone, attention should be payed to the dosage which is declared for the salt formulation (prednisolone-21-hydrogen succinate) in most products: A dose of 10 mg prednisolone-21-hydrogen succinate equates to 7.5 mg prednisolone).

[20] Note: The schedule for i.th. MTX is designed to avoid risk of i.th. injection of other drugs. In the case treating physicians for practical reasons would prefer to administer i.th. MTX on day 15 (i.e. to use the same anesthesia for BM aspiration and i.th. therapy), it is their responsibility to make sure that such mistakes cannot happen.

7.4.3 Protocol IA-Dexa (Prot. IA_D)

Protocol IA_D with dexamethasone for 3 weeks plus tapering is given to all patients with T-ALL and Prednisone Good-Response (see section 3.1.1).

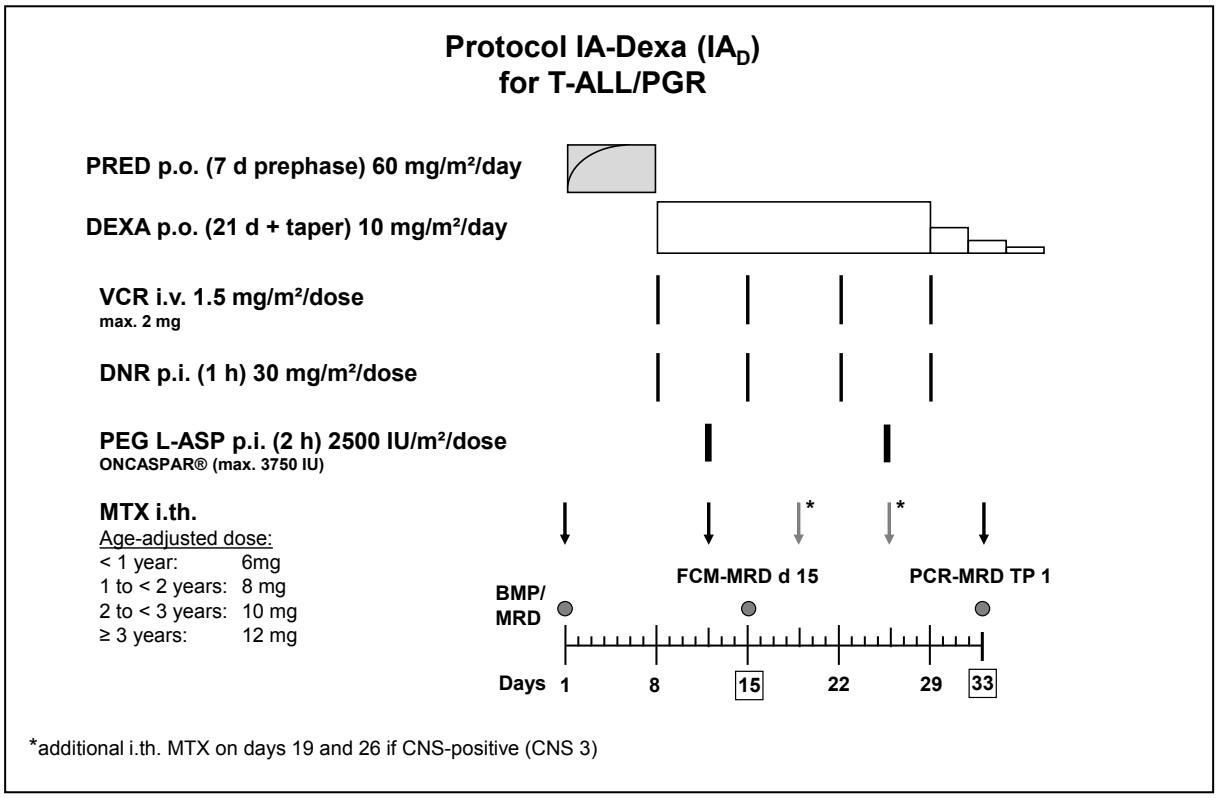


Figure 15 Treatment outline of Protocol IAD. The element is applicable to all patients with T-ALL/PGR, the glucocorticoid preparation is dexamethasone.

7.4.3.1 Treatment schedule of Protocol IA-Dexa

DEXA: **Dexamethasone** 10 mg/m^2/day p.o. or i.v. divided into 3 doses per day, days 8 to 28 (21 days); from day 29, tapering over 9 days, with halving the dose on every third day.

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

⚠ ***The WHO recommend that "Vincristine should where possible be prepared by dilution in small volume intravenous bags (the 'minibag' technique), rather than in a syringe, to protect against accidental administration via a spinal route. The labeling of vincristine should include a clear warning label that reads: 'FOR INTRAVENOUS USE ONLY - FATAL IF GIVEN BY OTHER ROUTES'."***

DNR: **Daunorubicin** 30 mg/m^2/dose p.i. (1 h) on days 8, 15, 22 and 29 (4 doses).

PEG-L-ASP: **PEG-L-Asparaginase (Oncaspar®)** 2 500 IU/m^2/dose p.i. (2 h) on days 12 and 26 (2 doses) (maximal single dose 3 750 IU).

In case of hypersensitivity to PEG-L-asparaginase, Erwinia asparaginase should be given at a dosage of 20 000 IU/m^2/dose p.i. (1 h) or i.m. every second day for the remaining days of scheduled asparaginase treatment, i.e. until two weeks after the last scheduled PEG-L-ASP dose in this element.

MTX i.th.: **Methotrexate intrathecally** on days 12[21] and 33.

In case of initial CNS status CNS 3 (see chapter 3.3.1) additional intrathecal methotrexate is given on days 19 and 26.

Age-adjusted dose:

< 1 year:	6 mg
1 to < 2 years:	8 mg
2 to < 3 years:	10 mg
≥ 3 years:	12 mg

Lowered-head position for at least two hours after intrathecal methotrexate injection.

7.4.4 Protocol IA-CPM (Prot. IA_{CPM})

Patients with **T-ALL and Prednisone Poor-Response** receive **Protocol IA-CPM** with one additional dose of Cyclophosphamide.

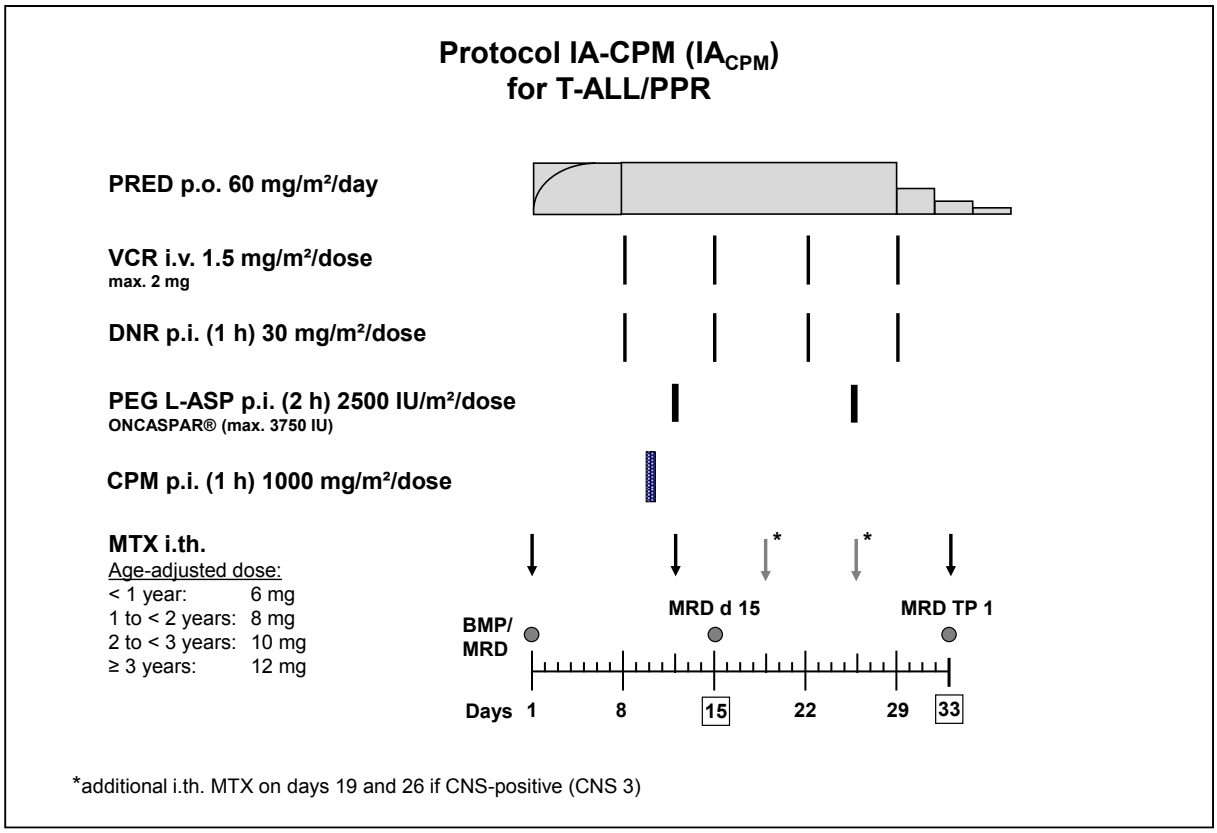


Figure 16 Treatment outline of Protocol IA_{CPM} with one additional dose of cyclophosphamide. The element is applicable to all patients with T-ALL/PPR, the steroid preparation is prednisone.

7.4.4.1 Treatment schedule of Prot. IA-CPM

PRED: **Prednisone (alternatively prednisolone)** 60 mg/m²/day p.o. or i.v.[22] divided into 3 doses per day, days 8 to 28 (21 days); from day 29 tapering over 9 days, with halving the dose on every third day.

[21] Note: The schedule for i.th. MTX is designed to avoid risk of i.th. injection of other drugs. In the case treating physicians for practical reasons would prefer to administer i.th. MTX on day 15 (i.e. to use the same anesthesia for BM aspiration and i.th. therapy), it is their responsibility to make sure that such mistakes can not happen.

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

⚠ ***The WHO recommend that "Vincristine should where possible be prepared by dilution in small volume intravenous bags (the 'minibag' technique), rather than in a syringe, to protect against accidental administration via a spinal route. The labeling of vincristine should include a clear warning label that reads: 'FOR INTRAVENOUS USE ONLY - FATAL IF GIVEN BY OTHER ROUTES'."***

DNR: **Daunorubicin** 30 mg/m^2/dose p.i. (1 h) on days 8, 15, 22 and 29 (4 doses).

CPM: **Cyclophosphamide** 1000 mg/m^2/dose p.i. (1 h) on day 10.
Cave: only for T-ALL/PPR patients

- Give **Mesna** (400 mg/m^2/dose) before and at hours 4 and 8 after start of cyclophosphamide infusion.
- For hydration and cystitis prophylaxis see protocol appendix.

PEG-L-ASP: **PEG-L-Asparaginase (Oncaspar®)** 2 500 IU/m^2/dose p.i. (2 h) on days 12 and 26 (2 doses) (maximal single dose 3 750 IU).

In case of hypersensitivity to PEG-L-asparaginase, Erwinia asparaginase should be given at a dosage of 20 000 IU/m^2/dose p.i. (1 h) or i.m. every second day for the remaining days of scheduled asparaginase treatment, i.e. until two weeks after the last scheduled PEG-L-ASP dose in this element.

MTX i.th.: **Methotrexate intrathecally** on days 12[23] and 33.

In case of initial CNS status CNS 3 (see chapter 3.3.1) additional intrathecal methotrexate is given on days 19 and 26.

Age-adjusted dose:		
	< 1 year:	6 mg
	1 to < 2 years:	8 mg
	2 to < 3 years:	10 mg
	≥ 3 years:	12 mg

Lowered-head position for at least two hours after intrathecal methotrexate injection.

7.5 Consolidation phase for pB-ALL

The consolidation phase for patients with pB-ALL consists of two parts (Consolidation A and Consolidation B). All patients with pB-ALL receive Consolidation A. There are three different variants of Consolidation B (short Consolidation B [Consol. B_{short}], extended Consolidation B [Consol. B_{ext}] and extended Consolidation B + Bortezomib [Consol. B_{ext}+BZM]). Which variant of Consolidation B is given to the patients depends on the risk group according to TP1 (early non-HR or early HR) and on the randomization group if the patient is included in the randomization R-eHR for early HR pB-ALL patients.

[22] In the case of i.v. application of prednisolone, attention should be payed to the dosage which is declared for the salt formulation (prednisolone-21-hydrogen succinate) in most products: A dose of 10 mg prednisolone-21-hydrogen succinate equates to 7.5 mg prednisolone).

[23] Note: The schedule for i.th. MTX is designed to avoid risk of i.th. injection of other drugs. In the case treating physicians for practical reasons would prefer to administer i.th. MTX on day 15 (i.e. to use the same anesthesia for BM aspiration and i.th. therapy), it is their responsibility to make sure that such mistakes cannot happen.

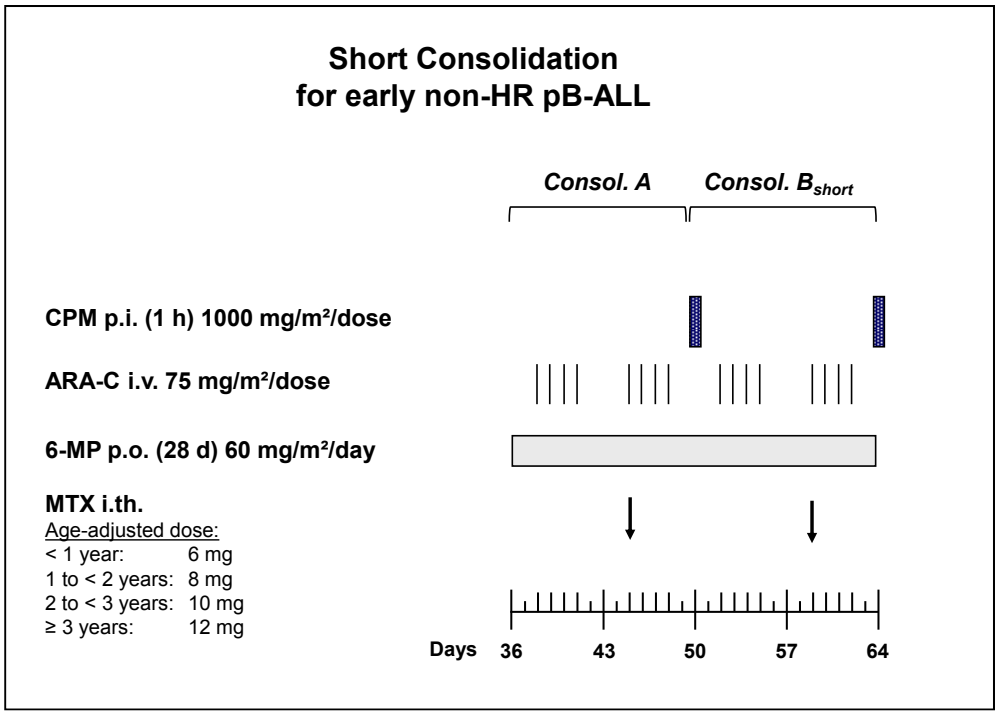


Figure 17 Short Consolidation ($Consol_{short}$) Applicable to all patients with pB-ALL of risk group early non-HR

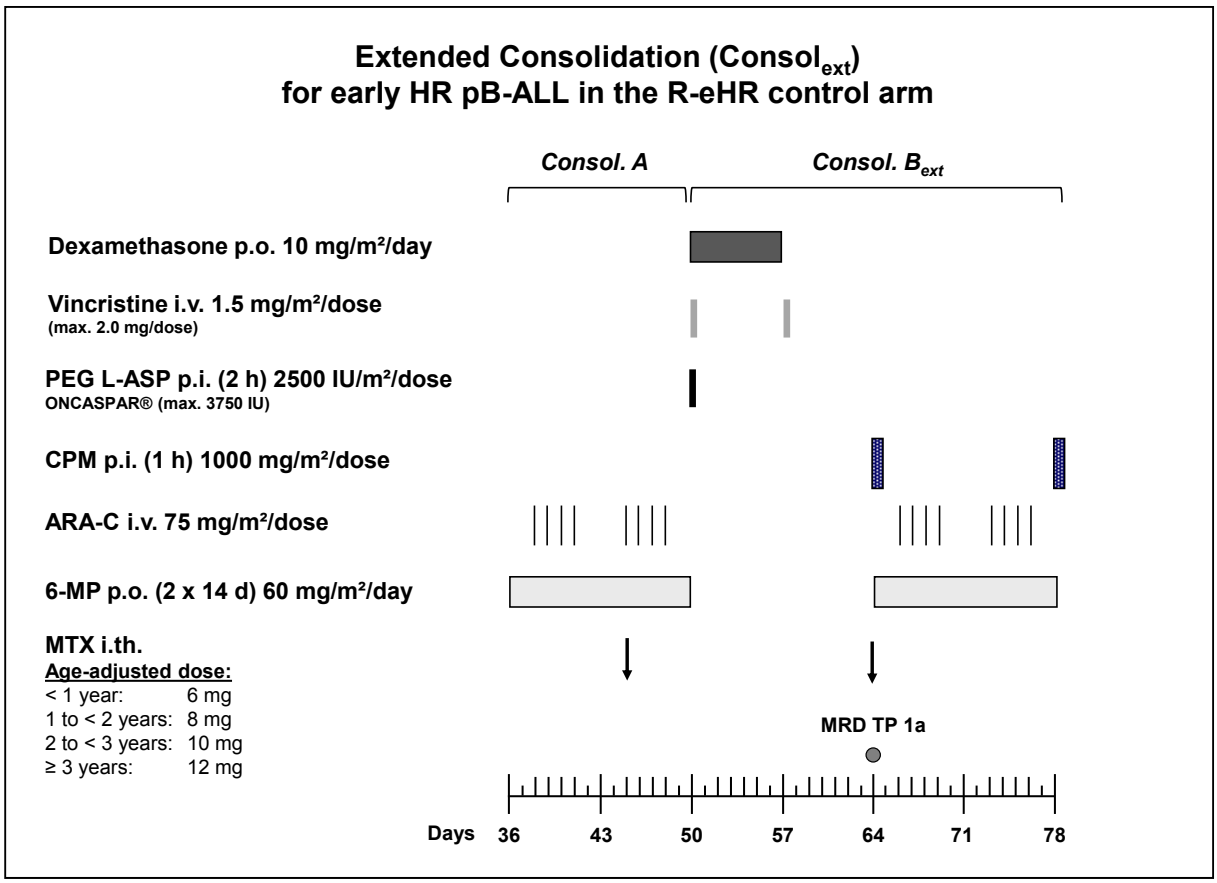


Figure 18 Extended Consolidation ($Consol_{ext}$) Applicable to all patients with pB-ALL of risk group early HR either not randomized or treated in the control arm of randomization R-eHR.

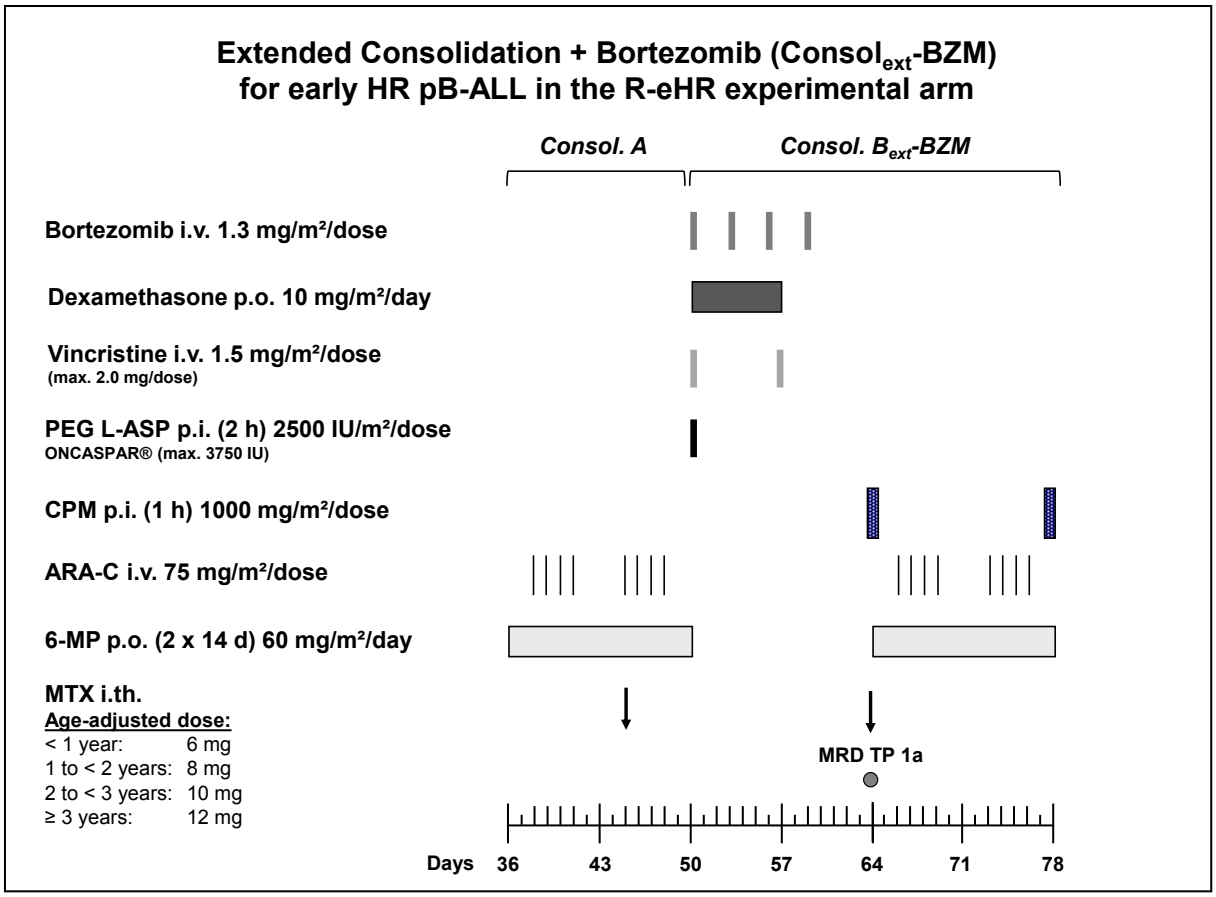


Figure 19 Extended Consolidation with Bortezomib (Consol$_{ext}$+BZM) Applicable to all patients with pB-ALL of risk group early HR assigned to the experimental arm of randomization R-eHR.

7.5.1 Consolidation A (Consol. A)

Consolidation A (Consol. A) is given to all patients with pB-ALL. It normally starts on protocol day 36 after the patient has reached the blood counts specified below.

7.5.1.1 Requirements for starting Consol. A

- Adequate clinical condition and no serious infection according to the assessment of the investigator
- Recovering (increasing) blood counts
  - Granulocytes ≥ 500/µl
  - Platelets ≥ 50 000/µl

7.5.1.2 Therapy modulation during Consol. A

As far as possible, Consol. A should not be interrupted. If nevertheless a cytarabine block has to be interrupted or postponed, 6-mercaptopurine should also be withheld for the same period. Omitted 6-mercaptopurine doses should be made up until the scheduled cumulative dose of 840 mg/m^2 (14 x 60 mg/m^2) has been reached.

7.5.1.3 Treatment schedule of Consol. A

6-MP: **6-Mercaptopurine** 60 mg/m^2/day p.o., days 36 to 49 (14 days).

Tablets should be spread evenly over the 14 days so that a cumulative dose of 840 mg/m^2 is reached within the 14 days, or use 6-MP as suspension. The drug should be taken in one single dose in the evening on an empty stomach (at least 30 min before or 60 min after the evening meal) without milk.

ARA-C: **Cytarabine** 75 mg/m^2/dose i.v. or s.c., two blocks of four days each; days 38-41, 45-48.

MTX i.th.: **Methotrexate intrathecally** on day 45.

Age-adjusted dose:

< 1 year:	6 mg
1 to < 2 years:	8 mg
2 to < 3 years:	10 mg
≥ 3 years:	12 mg

Lowered-head position for at least two hours after intrathecal methotrexate injection.

7.5.2 Short Consolidation B (Consol. B_{short})

Consolidation B short (Consol. B_{short}) is administered to all patients of risk group early non-HR (see section 5.3.2.2). The phase directly follows Consolidation A, starting on day 50 after the patient has reached the blood counts specified below.

7.5.2.1 Requirements for starting Consol. B_{short}

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

7.5.2.2 Therapy modulation during Consol. B_{short}

As far as possible, Consol. B_{short} should not be interrupted. If nevertheless a cytarabine block has to be interrupted or postponed, 6-mercaptopurine should also be withheld for the same period. Omitted 6-mercaptopurine doses should be made up until the scheduled cumulative dose of 840 mg/m^2 (14 x 60 mg/m^2) has been reached. There are no specific blood count requirements for the administration of the last Cyclophosphamide dose on day 64.

7.5.2.3 Treatment schedule of Consol. B_{short}

CPM: **Cyclophosphamide** 1 000 mg/m^2/dose p.i. (1 h) on days 50 and 64.

- Give **Mesna** (400 mg/m^2/dose) before and at hours 4 and 8 after start of cyclophosphamide infusion.
- For hydration and cystitis prophylaxis see protocol appendix.

ARA-C: **Cytarabine** 75 mg/m^2/dose i.v. or s.c., two blocks of four days each; days 52-55 and 59-62.

6-MP: **6-Mercaptopurine** 60 mg/m^2/day p.o., days 50 to 63 (14 days).

Tablets should be spread evenly over the 14 days so that a cumulative dose of 1 680 mg/m^2 is reached within the 14 days, or use 6-MP as suspension. The drug should be taken in one single dose in the evening on

an empty stomach (at least 30 min before or 60 min after the evening meal) without milk.

MTX i.th.: **Methotrexate intrathecally** on day 59.

Age-adjusted dose:
< 1 year: 6 mg
1 to < 2 years: 8 mg
2 to < 3 years: 10 mg
≥ 3 years: 12 mg

Lowered-head position for at least two hours after intrathecal methotrexate injection.

7.5.3 Extended Consolidation B (Consol. Bext ; control arm of R-eHR) and Extended Consolidation B <u>with</u> Bortezomib (Consol. B_{ext}-BZM; experimental arm of R-eHR)

Extended Consolidation B with or without Bortezomib is administered to the patients of risk group early HR (see section 5.3.2.2). Patients who do either not participate in randomization R-eHR or were assigned to the R-eHR control arm receive Consol. B_{ext}. Patients randomized in R-eHR and assigned to the R-eHR experimental arm receive Consol. B_{ext}-BZM. The phase directly follows Consolidation A, starting on day 50 after the patient has reached the blood counts specified below.

7.5.3.1 Requirements for starting Consol. B_{ext} or Consol. B_{ext}-BZM

- Adequate clinical condition and no serious infection according to the assessment of the investigator
- Creatinine clearance ≥ 20 ml/min/1.73 m^2 (only Consol. B_{ext} +BZM)
- Bilirubin < 1.5 x upper normal limit
- Blood counts
  - Granulocytes ≥ 500/µl
  - Platelets ≥ 50 000/µl

7.5.3.2 Therapy modulation during Consol. B_{ext} or Consol. B_{ext}-BZM

As far as possible, days 50 to 63 and days 64 to 78 of Consol. B_{ext} (+BZM) should not be interrupted. If nevertheless a cytarabine block has to be interrupted or postponed, 6-mercaptopurine should also be withheld for the same period. Omitted 6-mercaptopurine doses should be made up until the scheduled cumulative dose of 840 mg/m^2 (14 x 60 mg/m^2) has been reached.

7.5.3.2.1 Dose modulation of Bortezomib

If a patient experiences Bortezomib-related grade 3 neuropathy, Bortezomib should be stopped until symptoms of toxicity have improved to < grade 3. Permanent discontinuation of Bortezomib is required in case of grade 4 neuropathy and/or severe autonomic neuropathy.

In the case of moderate/severe (bilirubin level > 1.5 x ULN) hepatic impairment, the Bortezomib dose should be reduced to 0.7 mg/m^2.

For patients with mild to moderate renal impairment (creatinine clearance > 20 ml/min/1.73 m^2), dose adjustments of Bortezomib are not necessary. In the case of severe renal impairment (creatinine clearance < 20 ml/min/1.73 m^2), Bortezomib treatment should be discontinued.

Platelet count prior to each dose of Bortezomib should be ≥ 25,000/µl, since gastrointestinal and intracerebral hemorrhage have been reported in adult studies. Platelets should be substituted if required.

Potential immunocomplex-mediated reactions such as serum-sickness-type reaction, polyarthritis with rash and proliferative glomerulonephritis have been reported uncommonly. Therefore, Bortezomib should be discontinued, if serious reactions occur.

Contraindications for Bortezomib administration are hypersensitivity to the active substance or boron and acute diffuse infiltrative pulmonary or pericardial disease.

7.5.3.2.2 Requirements for starting with cyclophosphamide on day 64

- Creatinine clearance ≥ 25 ml/min/1.73 m^2. Dose adjustments of Cyclophosphamide are recommended in the case of severe renal dysfuction (Creatinine clearance < 25 ml/min/1.73 m^2)
- Recovering (increasing) blood counts
  - Granulocytes ≥ 500/µl
  - Platelets ≥ 50 000/µl

There are no specific blood count requirements for the administration of the last Cyclophosphamide dose on day 78.

7.5.3.3 Treatment schedule of Consol. B_{ext}/Consol. B_{ext}-BZM

DEXA: **Dexamethasone** 10 mg/m^2/day p.o. or i.v. divided into 3 doses per day, days 50 to 56 (7 days).

VCR: **Vincristine** 1.5 mg/m^2/dose i.v. (maximal single dose 2 mg) on days 50 and 57 (2 doses).

The WHO recommend that "Vincristine should where possible be prepared by dilution in small volume intravenous bags (the 'minibag' technique), rather than in a syringe, to protect against accidental administration via a spinal route. The labeling of vincristine should include a clear warning label that reads: 'FOR INTRAVENOUS USE ONLY - FATAL IF GIVEN BY OTHER ROUTES'."

PEG-L-ASP: **PEG-L-Asparaginase (Oncaspar®)** 2 500 IU/m^2/dose p.i. (2 h) on day 50 (maximal single dose 3 750 IU).

In case of hypersensitivity to PEG-L-asparaginase, Erwinia asparaginase should be given at a dosage of 20 000 IU/m^2/dose p.i. (1 h) or i.m. every second day for the remaining days of scheduled asparaginase treatment, i.e. until two weeks after the last scheduled PEG-L-ASP dose in this element.

Only for patients assigned to the R-eHR experimental arm to receive Consol. B_{ext}-BZM:

BZM: **Bortezomib** 1.3 mg/m^2/dose i.v., days 50, 53, 56, 59 (4 doses).

For potential drug interaction with cytochrome P450 inhibitors, please refer to the Summary of Product Characteristics.

CPM: **Cyclophosphamide** 1 000 mg/m^2/dose p.i. (1 h) on days 64 and 78.

- Give **Mesna** (400 mg/m^2/dose) before and at hours 4 and 8 after start of cyclophosphamide infusion.
- For hydration and cystitis prophylaxis see the protocol appendix.

ARA-C: **Cytarabine** 75 mg/m^2/dose i.v. or s.c., two blocks of four days each; days 66-69 and 73-76.

6-MP: **6-Mercaptopurine** 60 mg/m^2/day p.o., days 64 to 77 (14 days).

Tablets should be spread evenly over the 14 days so that a cumulative dose of 1 680 mg/m^2 is reached within the 14 days, or use 6-MP as

suspension. The drug should to taken in one single dose in the evening on an empty stomach (at least 30 min before or 60 min after the evening meal) without milk.

MTX i.th.: **Methotrexate intrathecally** on day 64.

Age-adjusted dose:
< 1 year: 6 mg
1 to < 2 years: 8 mg
2 to < 3 years: 10 mg
≥ 3 years: 12 mg

Lowered-head position for at least two hours after intrathecal methotrexate injection.

7.6 Protocol IB for T-ALL

Protocol IB (Prot. IB) for patients with T-ALL consists of two parts (Protocol IB/Part 1 [Prot. IB/1] and Protocol IB/Part 2 [Prot. IB/2]). All patients with T-ALL receive Prot. IB/1. There are two different variants of Protocol IB/Part 2 (Protocol IB/Part 2-regular [Prot. IB/2_{reg}] and Protocol IB/Part 2-long [Prot. IB/2_{long}]). Which variant of Protocol IB/2 is given to the patients, depends on the risk group according to TP1 (SR or non-SR) and on the randomization group if the patient is included in the randomization R_T for non-SR T-ALL patients.

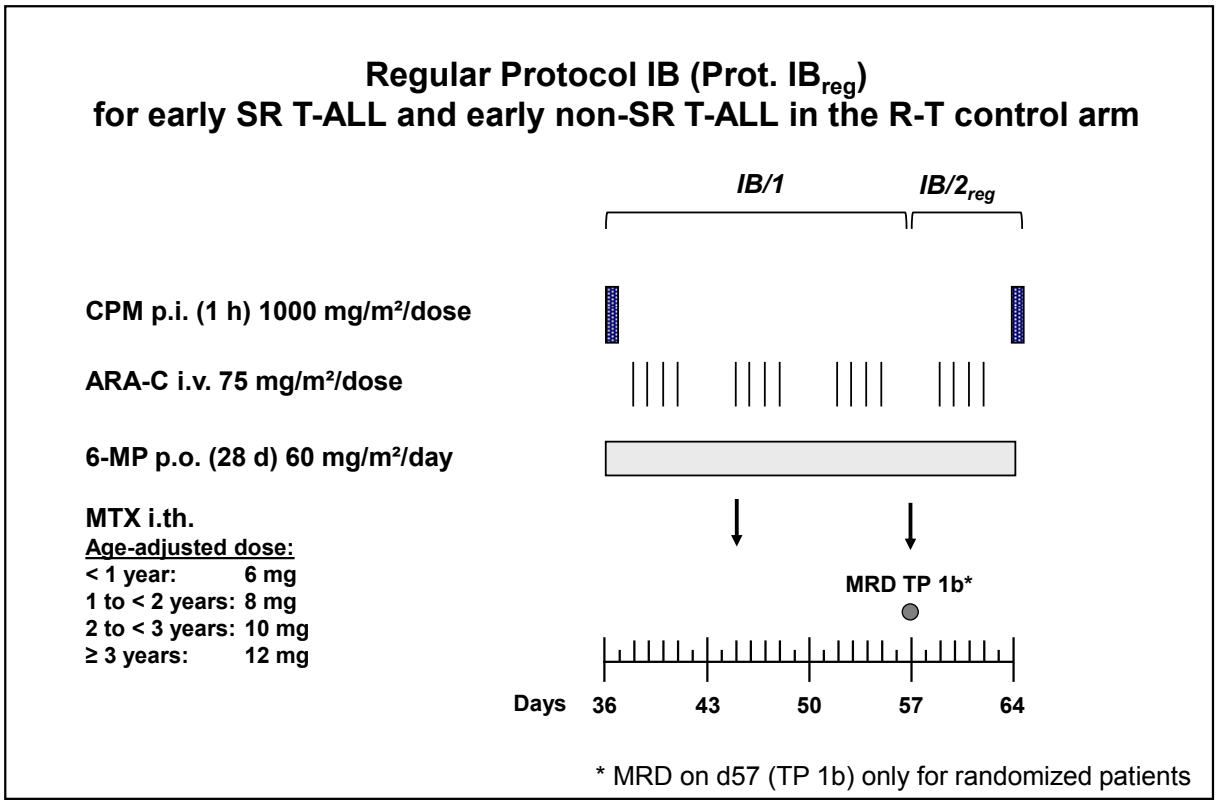


Figure 20 Treatment outline of regular Protocol IB. The element is applicable to all patients with SR/T-ALL or non-SR/T-ALL treated in the R-T control arm.

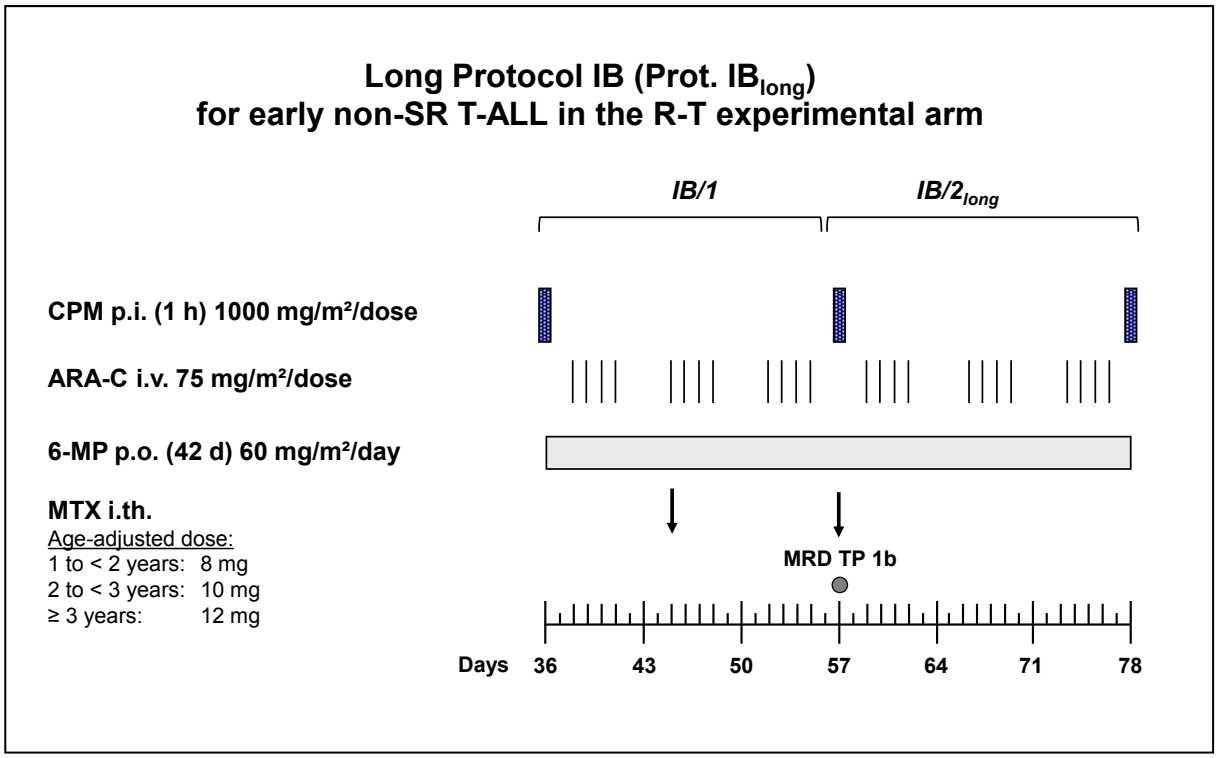


Figure 21 Treatment outline of long Protocol IB. The element is applicable to all non-SR T-ALL patients assigned to the R-T experimental arm.

7.6.1 Protocol IB/Part 1 (Prot. IB/1)

Protocol IB/1 is given to all patients with T-ALL. It normally starts on protocol day 36 after the patient has reached the blood counts specified below.

7.6.1.1 Requirements for starting Prot. IB/1

- Adequate clinical condition and no serious infection according to the assessment of the investigator
- Creatinine clearance ≥ 25 ml/min/1.73 m^2. Dose adjustments of Cyclophosphamide are recommended in the case of severe renal dysfuction (Creatinine clearance < 25 ml/min/1.73 m^2)
- Recovering (increasing) blood counts
  - Granulocytes ≥ 500/µl
  - Platelets ≥ 50 000/µl

7.6.1.2 Therapy modulation during Prot. IB/1

As far as possible, Prot. IB/1 should not be interrupted. If nevertheless a cytarabine block has to be interrupted or postponed, 6-mercaptopurine should also be withheld for the same period. Omitted 6-mercaptopurine doses should be made up until the scheduled cumulative dose of 1260 mg/m^2 (21 x 60 mg/m^2) has been reached.

7.6.1.3 Treatment schedule of Prot. IB/1

CPM: **Cyclophosphamide** 1 000 mg/m^2/dose p.i. (1 h) on day 36.

- Give **Mesna** (400 mg/m^2/dose) before and at hours 4 and 8 after start of cyclophosphamide infusion.

- For hydration and cystitis prophylaxis see the protocol appendix.

6-MP: **6-Mercaptopurine** 60 mg/m^2/day p.o., days 36 to 56 (21 days).

Tablets should be spread evenly over the 14 days so that a cumulative dose of 840 mg/m^2 is reached within the 14 days, or use 6-MP as suspension. The drug should be taken in one single dose in the evening on an empty stomach (at least 30 min before or 60 min after the evening meal) without milk.

ARA-C: **Cytarabine** 75 mg/m^2/dose i.v. or s.c., three blocks of four days each; days 38-41, 45-48, 52-55.

MTX i.th.: **Methotrexate intrathecally** on day 45.

Age-adjusted dose:		
	< 1 year:	6 mg
	1 to < 2 years:	8 mg
	2 to < 3 years:	10 mg
	≥ 3 years:	12 mg

Lowered-head position for at least two hours after intrathecal methotrexate injection.

7.6.2 Regular Protocol IB/Part 2 (Prot. IB/2$_{reg}$)

Regular Protocol IB/Part 2 (Prot. IB/2$_{reg}$) is administered to T-ALL patients with risk group SR or non-SR (see 5.3.2.3.1) if assigned to the control arm in randomization R-T. The phase directly follows Prot. IB/1, starting on protocol day 57.

7.6.2.1 Requirements for starting Prot. IB/2$_{reg}$

- Adequate clinical condition and no serious infection according to the assessment of the investigator
- Creatinine clearance ≥ 25 ml/min/1.73 m^2. Dose adjustments of Cyclophosphamide are recommended in the case of severe renal dysfuction (Creatinine clearance < 25 ml/min/1.73 m^2)
- Recovering (increasing) blood counts
  - Granulocytes ≥ 500/µl
  - Platelets ≥ 50 000/µl

7.6.2.2 Therapy modulation during Prot. IB/2$_{reg}$

As far as possible, Prot. IB/2$_{reg}$ should not be interrupted. If interruption is nevertheless required, 6-mercaptopurine should also be withheld for the same period. Omitted 6-mercaptopurine doses should be made up until the scheduled cumulative dose of 420 mg/m^2 (7x 60 mg/m^2) has been reached. There are no specific blood count requirements for the administration of the last Cyclophosphamide dose on day 64.

7.6.2.3 Treatment schedule of Prot. IB/2$_{reg}$

ARA-C: **Cytarabine** 75 mg/m^2/dose i.v. or s.c., one blocks of four days each; day 59-62.

CPM: **Cyclophosphamide** 1 000 mg/m^2/dose p.i. (1 h) on day 64.

- Give **Mesna** (400 mg/m^2/dose) before and at hours 4 and 8 after start of cyclophosphamide infusion.
- For hydration and cystitis prophylaxis see the protocol appendix

6-MP: **6-Mercaptopurine** 60 mg/m^2/day p.o., days 57 to 63 (7 days).

Tablets should be spread evenly over the 7 days so that a cumulative dose of 4200 mg/m^2 is reached within the 7 days, or use 6-MP as suspension. The drug should be taken in one single dose in the evening on an empty stomach (at least 30 min before or 60 min after the evening meal) without milk.

MTX i.th.: **Methotrexate intrathecally** on day 59.

Age-adjusted dose:
< 1 year: 6 mg
1 to < 2 years: 8 mg
2 to < 3 years: 10 mg
≥ 3 years: 12 mg

Lowered-head position for at least two hours after intrathecal methotrexate injection.

7.6.3 Long Protocol IB/Part 2 (Prot. IB/2$_{long}$)

Long Protocol IB/Part 2 (Prot. IB/2$_{long}$) is administered to T-ALL patients with risk group non-SR SR (see 5.3.2.3.1) assigned to the experimental arm in randomization R-T. The phase directly follows Prot. IB/1, starting on protocol day 57 after the patient has reached the blood counts specified below.

7.6.3.1 Requirements for starting Prot. IB/2$_{long}$

- Adequate clinical condition and no serious infection according to the assessment of the investigator
- Creatinine clearance ≥ 25 ml/min/1.73 m^2. Dose adjustments of Cyclophosphamide are recommended in the case of severe renal dysfuction (Creatinine clearance < 25 ml/min/1.73 m^2)
- Recovering (increasing) blood counts
  - Granulocytes ≥ 500/µl
  - Platelets ≥ 50 000/µl

1.1.1.1 Therapy modulation during Prot. IB/2$_{long}$

As far as possible, Prot. IB/2-long should not be interrupted. If nevertheless a cytarabine block has to be interrupted or postponed, 6-mercaptopurine should also be withheld for the same period. Omitted 6-mercaptopurine doses should be made up until the scheduled cumulative dose of 1260 mg/m^2 (21 x 60 mg/m^2) has been reached. There are no specific blood count requirements for the administration of the last Cyclophosphamide dose on day 78.

7.6.3.2 Treatment schedule of Prot. IB/2$_{long}$

CPM: **Cyclophosphamide** 1 000 mg/m^2/dose p.i. (1 h) on days 57 and 78 (2 doses).

- Give **Mesna** (400 mg/m^2/dose) before and at hours 4 and 8 after start of cyclophosphamide infusion.
- For hydration and cystitis prophylaxis see the protocol appendix

ARA-C: **Cytarabine** 75 mg/m^2/dose i.v. or s.c., three blocks of four days each; days 59-62, 66-69, 73-76.

6-MP: **6-Mercaptopurine** 60 mg/m^2/day p.o., days 57 to 77 (21 days).

Tablets should be spread evenly over the 21 days so that a cumulative dose of 1260 mg/m^2 is reached within the 21 days, or use 6-MP as suspension. The drug should to be taken in one single dose in the evening on an empty stomach (at least 30 min before or 60 min after the evening meal) without milk.

MTX i.th.: **Methotrexate intrathecally** on day 59.

Age-adjusted dose:
< 1 year: 6 mg
1 to < 2 years: 8 mg
2 to < 3 years: 10 mg
≥ 3 years: 12 mg

Lowered-head position for at least two hours after intrathecal methotrexate injection.

7.7 Protocol M

Protocol M is given to all patients without known HR criteria at the scheduled start of the treatment phase. It begins approximately 2 weeks after completion of Consolidation phase or Protocol IB when the patient has reached the blood counts specified below.

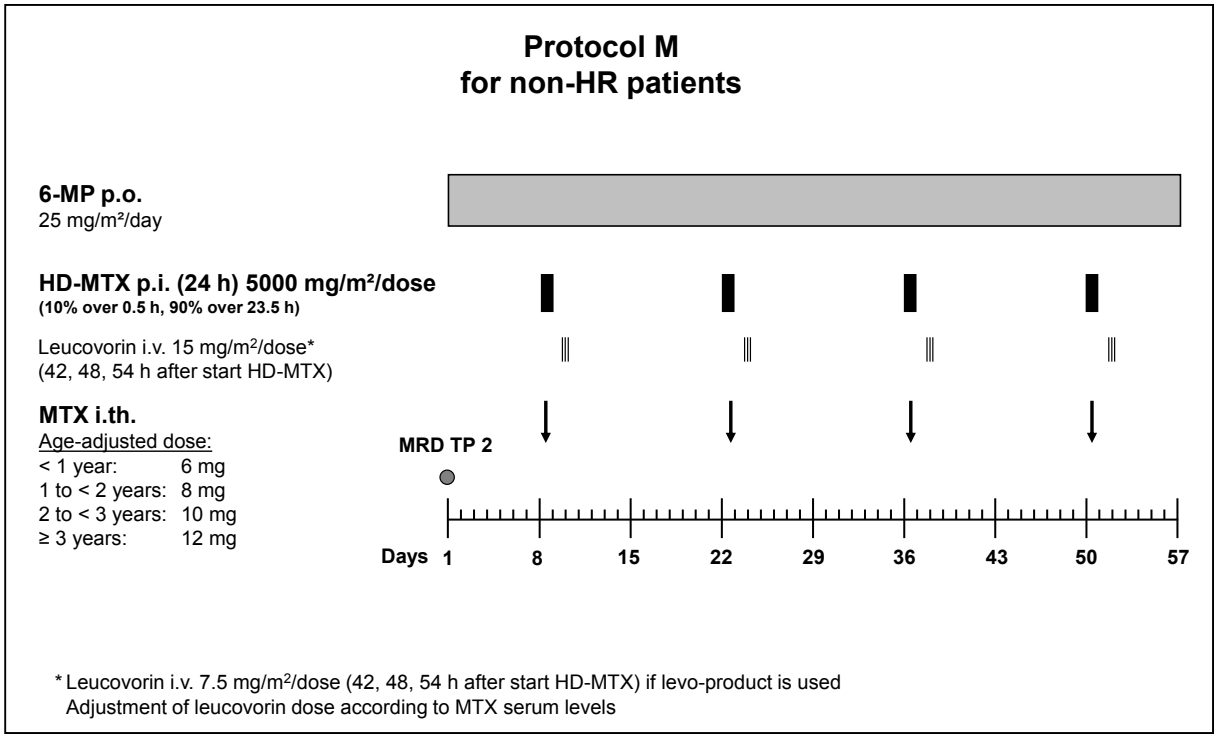


Figure 22 Treatment outline of Protocol M. Applicable to patients of all risk groups except HR patients as identified by week 12. The dose of leucovorin is halved if the levo-product is used.

7.7.1.1 Requirements for starting Protocol M

- Adequate clinical condition and no serious infection according to the assessment of the investigator
- Normal renal function. Dose adjustments of HD-MTX are recommended in the case of reduced creatinine clearance.
- No urinary obstruction
- Recovering (increasing) blood counts
    - Granulocytes ≥ 500/µl

  - Platelets ≥ 50 000/µl
- GOT/GPT < 10 x upper normal limit
- Bilirubin < 3 x upper normal limit with normal direct bilirubin

7.7.1.2 Requirements for start of a HD-MTX block

- Adequate clinical condition and no serious infection according to the assessment of the investigator
- Normal renal function. Dose adjustments of HD-MTX are recommended in the case of reduced creatinine clearance.
- No urinary obstruction
- Stable blood counts
  - Granulocytes ≥ 500/µl
  - Platelets ≥ 50 000/µl
- GOT/GPT < 10 x upper normal limit
- Bilirubin < 3 x upper normal limit with normal direct bilirubin

If transaminases are between 10 and 20 times of the upper normal limit, wait 36 to 48 hours and check to ensure that the levels are decreasing. If GOT and/or GPT are ≥ 20 times of normal upper limits, contact the national study coordinator for further recommendations.

7.7.1.3 Drug interactions

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

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

7.7.1.4 Treatment schedule

6-MP: **6-Mercaptopurine** 25 mg/m^2/day p.o., days 1 to 56.

Tablets should be spread evenly over the 56 days so that a cumulative dose of 1 400 mg/m^2 is reached within the 56 days, or use 6-MP as suspension. The drug is to be taken in one single dose in the evening on an empty stomach (at least 30 min before or 60 min after the evening meal) without milk.

HD-MTX: **High-Dose Methotrexate** 5 000 mg/m^2/dose p.i. (24 h) on days 8, 22, 36 and 50. 1/10 (500 mg/m^2) of the total methotrexate dose should be infused over 30 min as loading dose, immediately followed by the remaining 9/10 of the dose (4 500 mg/m^2) given by continuous intravenous infusion over 23.5 h.

- For management of urine alkalinization, (pre-)hydration and fluid balancing see protocol appendix.
- Leucovorin rescue: 15 mg/m^2 i.v. of the racemic product or 7.5 mg/m^2 of the levo-product 42, 48 and 54 hrs after the start of the methotrexate infusion. The leucovorin dose depends on the methotrexate plasma level. For details of methotrexate level monitoring and regulation of leucovorin rescue see section protocol appendix.
- For detailed guidelines of the management of impaired methotrexate excretion see the protocol appendix.

MTX i.th.: **Methotrexate intrathecally** on days 8, 22, 36 and 50, during HD-MTX infusion.

Age-adjusted dose: < 1 year: 6 mg
1 to < 2 years: 8 mg
2 to < 3 years: 10 mg
≥ 3 years: 12 mg

Lowered-head position for at least two hours after intrathecal methotrexate application.

7.8 Protocol II

Protocol II is given as reintensification phase for all non-HR T-ALL and SR/MR pB-ALL patients.

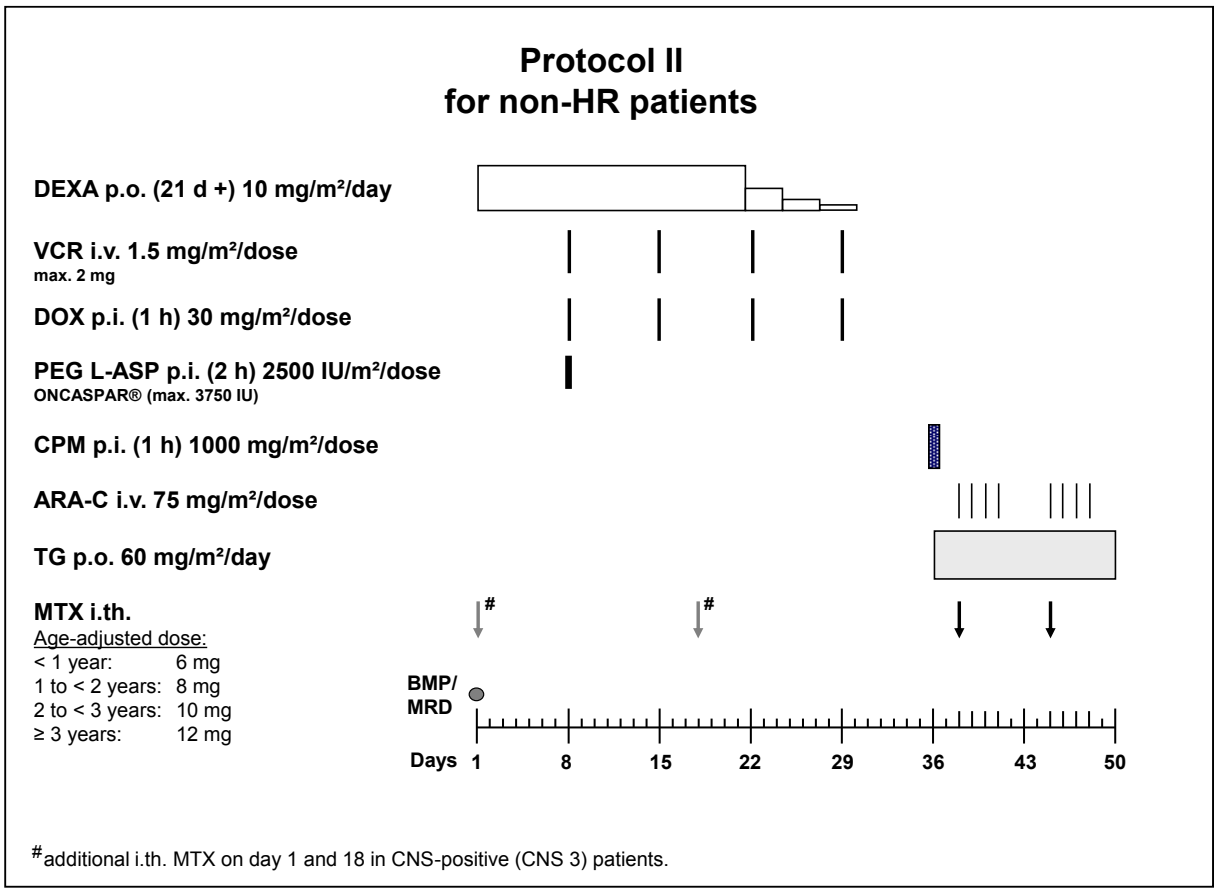


Figure 23 Treatment outline of Protocol II. Applicable to non-HR

7.8.1 Protocol IIA

Protocol IIA begins 2 weeks after the end of Protocol M.

7.8.1.1 Requirements for starting Protocol IIA

- Adequate clinical condition and no serious infection according to the assessment of the investigator
- Recovering (increasing) blood counts
  - Granulocytes ≥ 500/µl
  - Platelets ≥ 50 000/µl

- Echo-CG is mandatory prior to the 1st doxorubicin. If symptoms/signs of cardiac insufficiency are present and/or if the LV-SF is repeatedly below 30 % or the EF below 50 %, contact the national study coordinator for consideration of procedure.

7.8.1.2 Therapy modulation during Protocol IIA

In the case of severe neuropathy, it is permissible to omit vincristine. In the case of insufficient WBC recovery (WBC < 500/µl or granulocytes < 200/µl), the doxorubicin/vincristine doses may be postponed until blood count recovery.

7.8.1.3 Treatment schedule

DEXA: **Dexamethasone** 10 mg/m^2/day p.o. or i.v. divided into 3 doses per day, days 1 to 21 (21 days);

From day 22 tapering over 9 days, with halving the dose on every third day.

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

⚠ ***The WHO recommend that "Vincristine should where possible be prepared by dilution in small volume intravenous bags (the 'minibag' technique), rather than in a syringe, to protect against accidental administration via a spinal route. The labeling of vincristine should include a clear warning label that reads: 'FOR INTRAVENOUS USE ONLY - FATAL IF GIVEN BY OTHER ROUTES'."***

DOX: **Doxorubicin** 30 mg/m^2/dose p.i. (1 h) on days 8, 15, 22 and 29 (4 doses).

PEG-L-ASP: **PEG-L-Asparaginase (Oncaspar®)** 2 500 IU/m^2/dose p.i. (2 h) on day 8 (1 dose) (maximal single dose 3 750 IU).

In case of hypersensitivity to PEG-L-ASP, Erwinia asparaginase should be given at a dosage of 20 000 IU/m^2/dose p.i. (1 h) or i.m. every second day for 2 weeks (7 doses).

MTX i.th.: **Methotrexate intrathecally** on days 1 and 18 only for patients with initial CNS disease (CNS 3).

Age-adjusted dose:		
	< 1 year:	6 mg
	1 to < 2 years:	8 mg
	2 to < 3 years:	10 mg
	≥ 3 years:	12 mg

Lowered-head position for at least two hours after intrathecal methotrexate application.

7.8.2 Protocol IIB

7.8.2.1 Requirements for starting Protocol IIB

- Adequate clinical condition and no serious infection according to the assessment of the investigator
- Creatinine clearance ≥ 25 ml/min/1.73 m^2. Dose adjustments of Cyclophosphamide are recommended in the case of severe renal dysfuction (Creatinine clearance < 25 ml/min/1.73 m^2)
- Recovering (increasing) blood counts
  - Granulocytes ≥ 500/µl
  - Platelets ≥ 50 000/µl

7.8.2.2 Therapy modulation during Protocol IIB

As far as possible, an already started cytarabine block should not be withheld. If nevertheless a cytarabine block has to be interrupted or postponed, thioguanine should also be withheld for the same period of time. Omitted thioguanine doses should be made up until the scheduled cumulative dose of 840 mg/m^2 (14 x 60 mg/m^2) has been reached.

7.8.2.3 Treatment schedule

CPM: **Cyclophosphamide** 1 000 mg/m^2/dose p.i. (1 h) on day 36.

- Give **Mesna** (400 mg/m^2/dose) before and at hours 4 and 8 after start of cyclophosphamide infusion.
- For hydration and cystitis prophylaxis see protocol appendix.

ARA-C: **Cytarabine** 75 mg/m^2/dose i.v. or s.c. 2 blocks of 4 days each days 38-41, 45-48.

TG: **Thioguanine** 60 mg/m^2/day p.o., days 36 to 49 (14 days).

Tablets should be spread evenly over the 14 days so that a cumulative dose of 840 mg/m^2 is reached within the 14 days, or a TG suspension should be used. The drug should be taken in one single dose in the evening on an empty stomach (at least 30 min before or 60 min after the evening meal) without milk.

MTX i.th.: **Methotrexate intrathecally** on days 38 and 45.

Age-adjusted dose:		
	< 1 year:	6 mg
	1 to < 2 years:	8 mg
	2 to < 3 years:	10 mg
	≥ 3 years:	12 mg

Lowered-head position for at least two hours after intrathecal methotrexate application.

7.9 High Risk courses (HR')

7.9.1 HR-1'

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

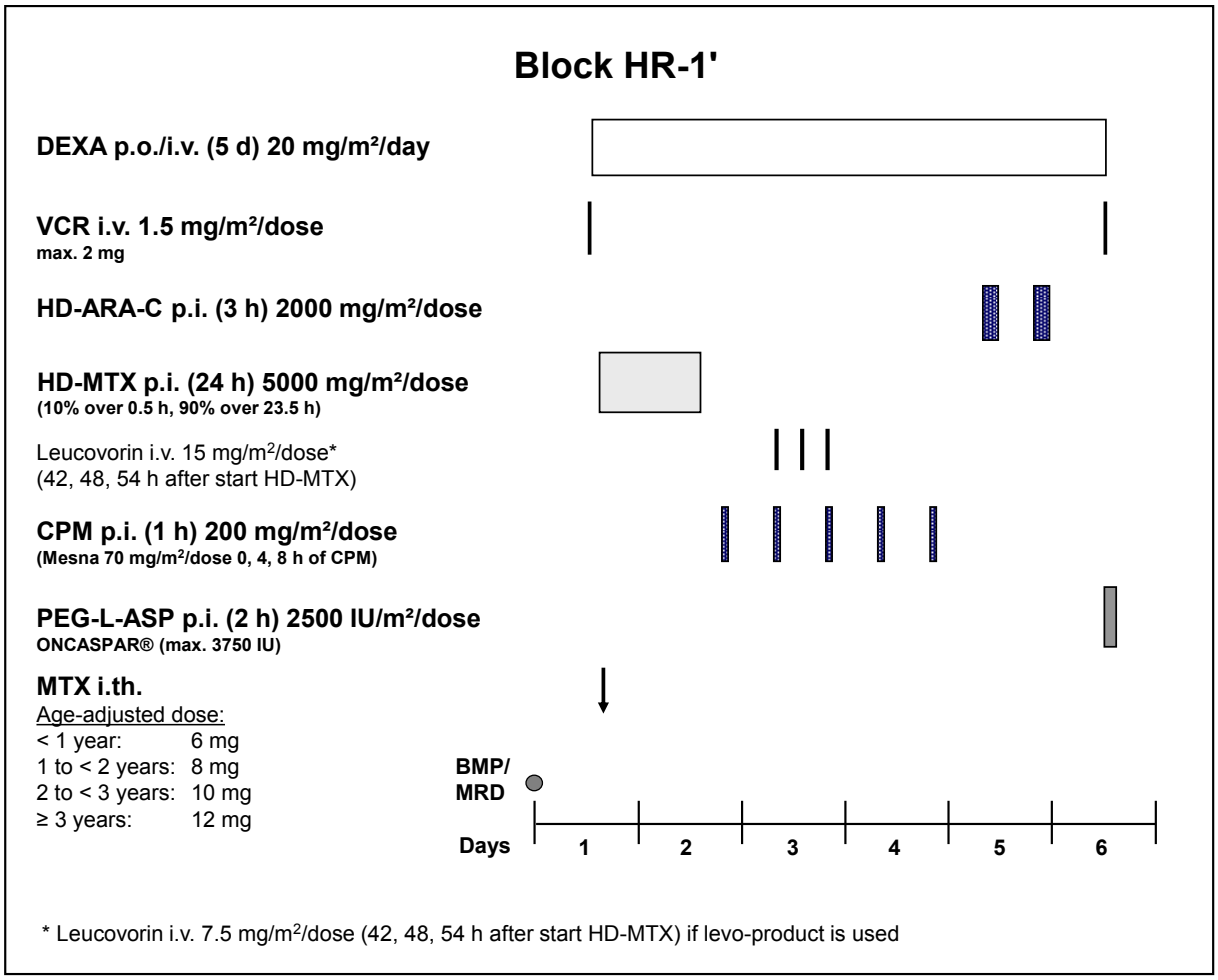


Figure 24 Treatment outline of Block HR-1'. Applicable to patients of the HR group. The dose of leucovorin is halved if the levo-product is used.

7.9.1.1 Requirements for starting HR-1'

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

7.9.1.2 Treatment schedule

DEXA: **Dexamethasone** 20 mg/m^2/day p.o. or i.v. divided into 3 doses per day, days 1 to 5 (5 days);

VCR: **Vincristine** 1.5 mg/m^2/dose i.v. (maximal single dose 2 mg) on days 1 and 6 (2 doses).

The WHO recommend that "Vincristine should where possible be prepared by dilution in small volume intravenous bags (the 'minibag' technique), rather than in a syringe, to protect against accidental administration via a spinal route. The labeling of vincristine should include a clear warning label that reads: 'FOR INTRAVENOUS USE ONLY - FATAL IF GIVEN BY OTHER ROUTES'."

HD-MTX: **High-Dose Methotrexate** 5 000 mg/m^2/dose p.i. (24 h) on day 1. 1/10 (500 mg/m^2) of the total methotrexate dose should be infused over 30 min as loading dose, immediately followed by the remaining 9/10 of the dose (4 500 mg/m^2) given by continuous intravenous infusion over 23.5 h.

- For management of urine alkalinization, (pre-)hydration and fluid balancing see protocol appendix.
- Leucovorin rescue: 15 mg/m^2 i.v. of the racemic product or 7.5 mg/m^2 of the levo-product 42, 48 and 54 hrs after the start of the methotrexate infusion. The leucovorin dose depends on the 6 hours before determined methotrexate plasma level. For details of methotrexate level monitoring and regulation of leucovorin rescue see protocol appendix.
- For detailed guidelines of the management of impaired methotrexate excretion see the protocol appendix.

CPM: **Cyclophosphamide** 200 mg/m^2/dose p.i. (1 h) every 12 hours on days 2 to 4 (5 doses).

- Give **Mesna** (70 mg/m^2/dose) before and at hours 4 and 8 after start of cyclophosphamide infusion.
- For hydration and cystitis prophylaxis see protocol appendix.

HD-ARA-C: **High-dose Cytarabine** 2 000 mg/m^2/dose p.i. (3 h) every 12 hours on day 5 (2 doses).

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

PEG-L-ASP: **PEG-L-Asparaginase (Oncaspar®)** 2 500 IU/m^2/dose p.i. (2 h) on day 6 (1 dose) (maximal single dose 3 750 IU).

In case of hypersensitivity to PEG-L-ASP, Erwinia asparaginase should be given at a dosage of 20 000 IU/m^2/dose p.i. (1 h) or i.m. every second day starting on day 6 for a total of 7 doses.

MTX i.th.: **Methotrexate intrathecally** during HD-MTX infusion on day 1.

Age-adjusted dose:		
	< 1 year:	6 mg
	1 to < 2 years:	8 mg
	2 to < 3 years:	10 mg
	≥ 3 years:	12 mg

Lowered-head position for at least two hours after intrathecal methotrexate application.

G-CSF: **Granulocyte colony stimulating factor** 5 µg/kg/dose s.c. once a day from day 11 until the neutrophil count exceeds 5 000/µl.

7.9.2 HR-2'

The HR-2' block is given to patients of risk group HR who do not participate in randomization R-HR or were assigned to the R-HR control arm. The course begins after regeneration of bone marrow as early as possible after the preceding HR-1' course. After cessation of G-CSF, a two-day interval should be maintained before start of the subsequent element.

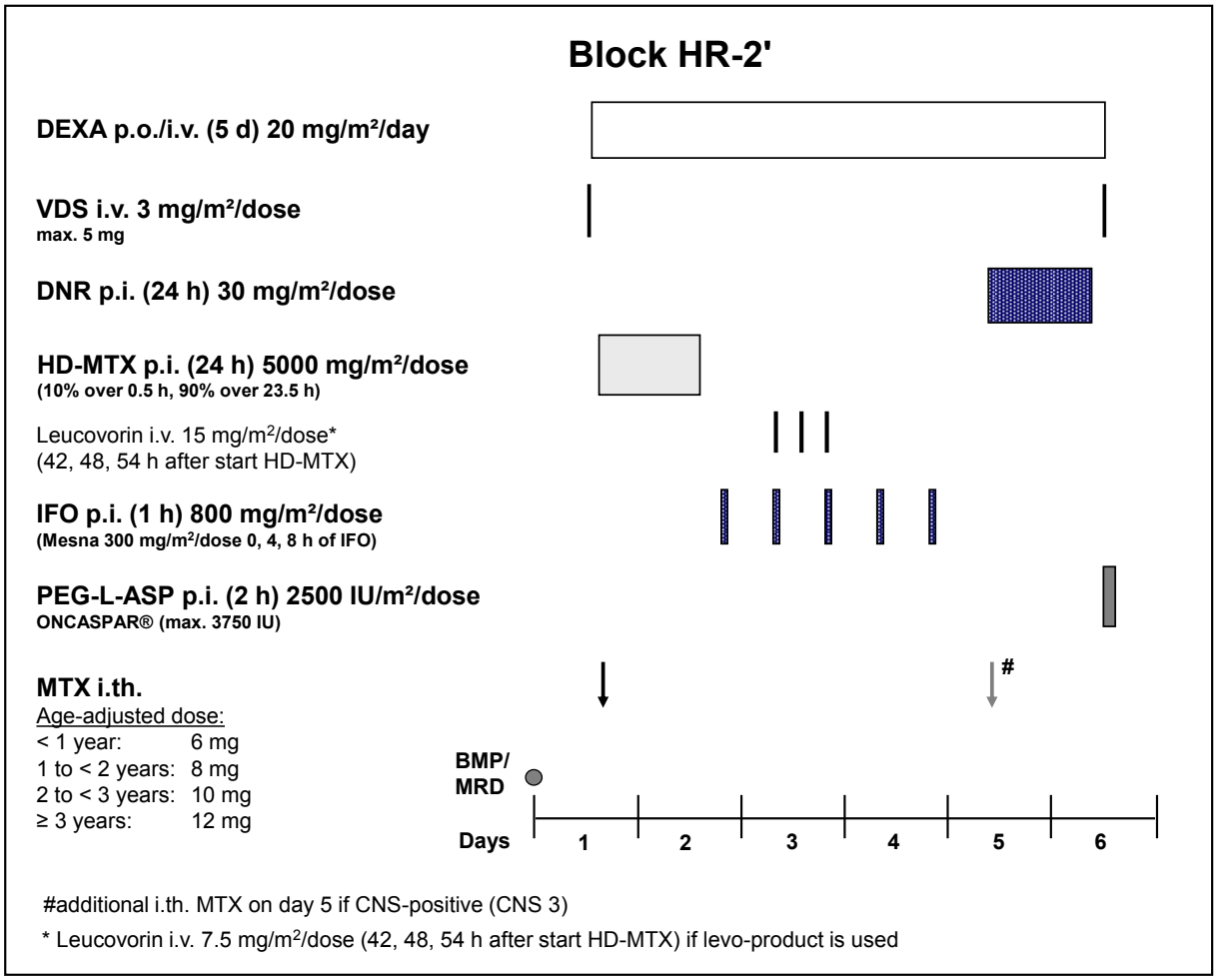


Figure 25 Treatment outline of Block HR-2'. Applicable to patients of risk group HR if not treated in the R-HR experimental arm. The dose of leucovorin is halved if the levo-product is used.

7.9.2.1 Requirements for starting HR-2'

- Adequate clinical condition and no serious infection according to the assessment of the investigator
- No urinary obstruction
- Intact mucous membranes
- Recovering (increasing) blood counts
    - Granulocytes ≥ 500/µl
    - Platelets ≥ 50 000/µl
- No essential organ dysfunction:
    - Normal renal function. Dose adjustments of HD-Methotrexate and Ifosfamid are recommended in the case of reduced creatinine clearance.
    - GOT/GPT < 10 x upper normal limit
    - Bilirubin < 3 x upper normal limit with normal direct bilirubin

- Echo-CG is mandatory prior to daunorubicin. If symptoms/signs of cardiac insufficiency are present and/or if the LV-SF is repeatedly below 30 % or the EF below 50 %, contact the national study coordinator for consideration of further procedure.

7.9.2.2 Treatment schedule

DEXA: **Dexamethasone** 20 mg/m^2/day p.o. or i.v. divided into 3 doses per day, days 1 to 5 (5 days);

VDS: **Vindesine** 3 mg/m^2/dose i.v. (maximal single dose 5 mg) on days 1 and 6 (2 doses).

HD-MTX: **High-Dose Methotrexate** 5 000 mg/m^2/dose p.i. (24 h) on day 1. 1/10 (500 mg/m^2) of the total methotrexate dose should be infused over 30 min as loading dose, immediately followed by the remaining 9/10 of the dose (4 500 mg/m^2) given by continuous intravenous infusion over 23.5 h.

- For management of urine alkalinization, (pre-)hydration and fluid balancing see protocol appendix.
- Leucovorin rescue: 15 mg/m^2 i.v. of the racemic product or 7.5 mg/m^2 of the levo-product 42, 48 and 54 hrs after the start of the methotrexate infusion. The leucovorin dose depends on the 6 hours before determined methotrexate plasma level. For details of methotrexate level monitoring and regulation of leucovorin rescue see protocol appendix.
- For detailed guidelines of the management of impaired methotrexate excretion see protocol appendix.

IFO: **Ifosfamide** 800 mg/m^2/dose p.i. (1 h) every 12 hours on days 2 to 4 (5 doses).

- Give **Mesna** (300 mg/m^2/dose) before and at hours 4 and 8 after start of cyclophosphamide infusion.
- For hydration and cystitis prophylaxis see protocol appendix.

DNR: **Daunorubicin** 30 mg/m^2/dose p.i. (24 h) on day 5 (1 dose).

PEG-L-ASP: **PEG-L-Asparaginase (Oncaspar®)** 2 500 IU/m^2/dose p.i. (2 h) on day 6 (1 dose) (maximal single dose 3 750 IU).

In case of hypersensitivity to PEG-L-ASP, Erwinia asparaginase should be given at a dosage of 20 000 IU/m^2/dose p.i. (1 h) or i.m. every second day starting on day 6 for a total of 7 doses.

MTX i.th.: **Methotrexate intrathecally** during HD-MTX infusion on day 1. Only in case of initial CNS involvement another dose is given on day 5.

Age-adjusted dose:		
	< 1 year:	6 mg
	1 to < 2 years:	8 mg
	2 to < 3 years:	10 mg
	≥ 3 years:	12 mg

Lowered-head position for at least two hours after intrathecal methotrexate application.

G-CSF: **Granulocyte colony stimulating factor** 5 µg/kg/dose s.c. once a day from day 11 until the neutrophil count exceeds 5 000/µl.

7.9.3 HR-3'

The HR-3' block is given to patients of risk group HR who do not participate in randomization R-HR or were assigned to the R-HR control arm. The course begins after regeneration of

bone marrow as early as possible after the preceding HR-2' course. After cessation of G-CSF, a two-day interval should be maintained before start of the subsequent element.

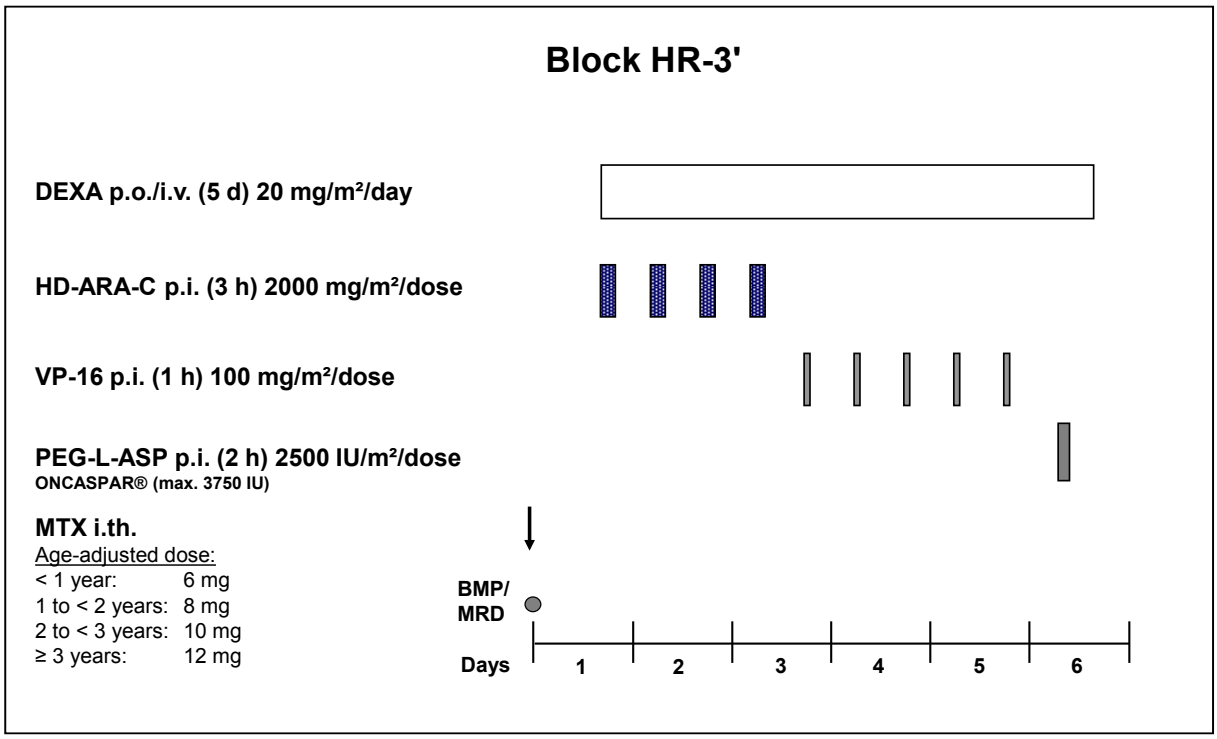


Figure 26 Treatment outline of Block HR-3'. Applicable to patients of the HR group if not treated in the R-HR experimental arm.

7.9.3.1 Requirements for starting HR-3'

- Adequate clinical condition and no serious infection according to the assessment of the investigator
- No urinary obstruction
- Intact mucous membranes
- Recovering (increasing) blood counts
    - Granulocytes ≥ 500/µl
    - Platelets ≥ 50 000/µl
- No essential organ dysfunction:
    - Normal renal function. Dose adjustments of High-dose Cytarabine and Etoposide should be considered in the case of reduced creatinine clearance.
    - GOT/GPT < 10 x upper normal limit
    - Bilirubin < 3 x upper normal limit with normal direct bilirubin

7.9.3.2 Treatment schedule

DEXA: **Dexamethasone** 20 mg/m^2/day p.o. or i.v. divided into 3 doses per day, days 1 to 5 (5 days);

HD-ARA-C: **High-dose Cytarabine** 2 000 mg/m^2/dose p.i. (3 h) every 12 hours on days 1 and 2 (4 doses).

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

VP-16: **Etoposide** 100 mg/m^2/dose p.i. (1 h) every 12 hours on days 3 to 5 (5 doses).

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

PEG-L-ASP: **PEG-L-Asparaginase (Oncaspar®)** 2 500 IU/dose p.i. (2 h) on day 6 (1 dose).(maximal single dose 3 750 IU).

In case of hypersensitivity to PEG-L-ASP, Erwinia asparaginase should be given at a dosage of 20 000 IU/m^2/dose p.i. (1 h) or i.m. every second day starting on day 6 for a total of 7 doses.

MTX i.th.: **Methotrexate intrathecally** on day 1.

Age-adjusted dose:		
	< 1 year:	6 mg
	1 to < 2 years:	8 mg
	2 to < 3 years:	10 mg
	≥ 3 years:	12 mg

Lowered-head position for at least two hours after intrathecal methotrexate application.

G-CSF: **Granulocyte colony stimulating factor** 5 µg/kg/dose s.c. once a day from day 11 until the neutrophil count exceeds 5 000/µl.

7.10 Blinatumomab in Randomizations R-HR and R-MR

Blinatumomab is given to pB-ALL patients assigned to the experimental arms of randomization R-HR (HR) and R-MR (MR).

In randomization R-HR, patients receive 2 cycles Blinatumomab plus i.th. MTX instead of the second and third HR course (HR-2' and HR-3').

In randomization R-MR, one cycle Blinatumomab is given after Protocol II before going on with Maintenance treatment.

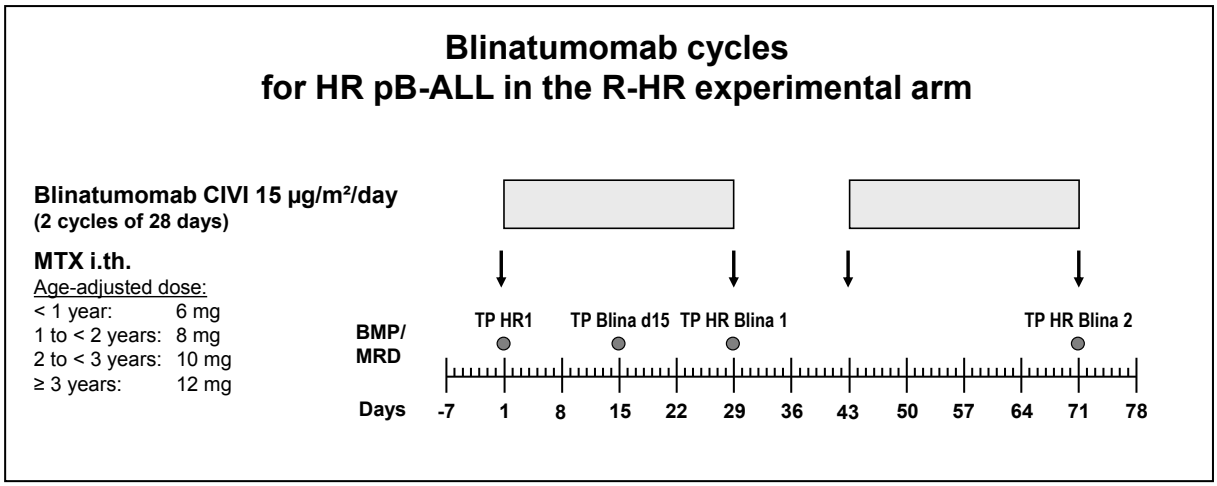


Figure 27 Blinatumomab cycles with i.th. MTX. This treatment is applicable to pB-ALL/HR patients assigned to the experimental arm of randomization R-HR.

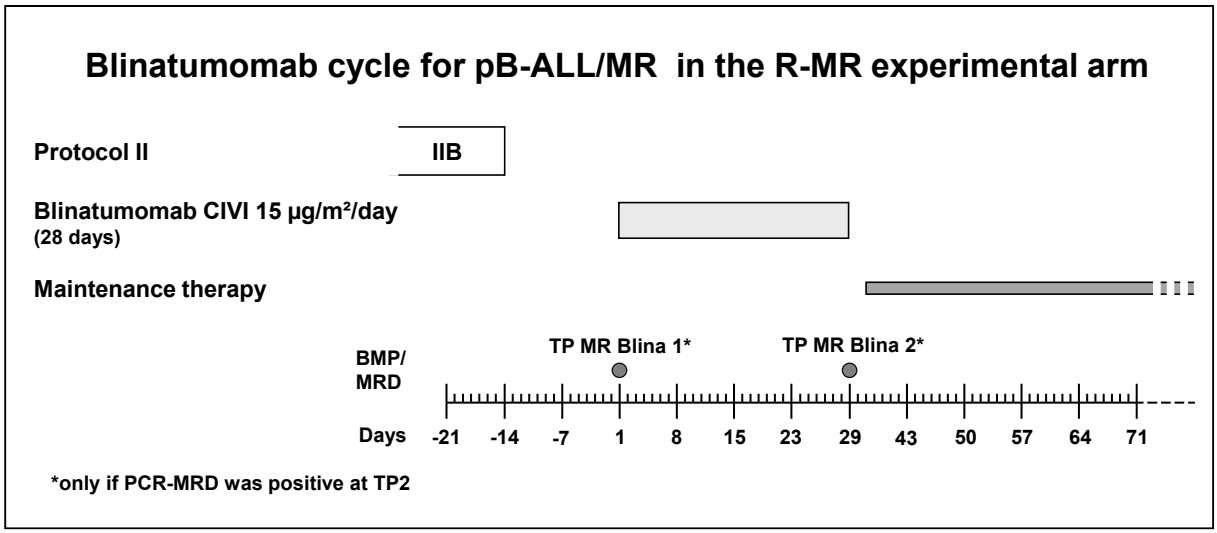


Figure 28 Blinatumomab cycle before start of Maintenance therapy. This treatment is applicable to pB-ALL/MR patients assigned to the experimental arm of randomization R-MR.

7.10.1.1 Requirements for starting a Blinatumomab cycle

- Adequate clinical condition and no serious infection according to the assessment of the investigator
- Intact mucous membranes
- Recovering (increasing) blood counts
    - Granulocytes ≥ 500/µl
    - Platelets ≥ 50 000/µl
- No essential organ dysfunction:
    - No severe renal dysfunction: Cratinine clearance >30 ml/min/1.73 m²
    - GOT/GPT < 10 x upper normal limit
    - Bilirubin < 3 x upper normal limit with normal direct bilirubin

7.10.1.2 Management of Blinatumomab treatment

7.10.1.2.1 Practical handling of the Blinatumomab administration

The infusion bags should be changed by site nursing personnel trained on the protocol and on the proper administration of blinatumomab.

After discharging the patient to continue the Blinatumomab treatment in an outpatient setting, the patient will return to the study site for changes of infusion bags. As an alternative, the patient can be visited by an ambulant/home care service provider at specific intervals to change the infusion bag. The Blinatumomab infusion bags should be changed in accordance with local pharmacy standards for infusion of compounded sterile products but at least every 4 days.

The daily blinatumomab dose may be up to 10% lower or higher in order to account for possible pump inaccuracies. For dose modifications in case of adverse events, see section 7.10.1.2.5.

The dose, start and stop date/time and lot number is to be recorded on the patient's CRF for the documentation of the Binatumomab therapy. The date and time of infusion bag changes, all infusion start and stop times, and any dose modifications should also be recorded accurately.

7.10.1.2.2 Premedication

Administration of dexamethasone premedication is required and is described in section 7.10.1.3.

7.10.1.2.3 Infusion interruptions

As far as possible, the drug administration of Blinatumomab should not be interrupted. If nevertheless an infusion interruption is required due to technical or logistical reasons (eg, diagnostic measurement), the interruption should be as short as possible and the infusion continued at the earliest time possible. An interruption may also be required due to adverse side effects of Blinatumomab (see section 7.10.1.2.5). Every interruption longer than 1 hour should be documented. If the interruption is longer than 4 hours, re-start of the infusion should be performed in the hospital, under the supervision of the investigator. The patient should possibly be observed overnight for possible side effects after the re-start, either in the hospital or in the outpatient setting as applicable. Administration of dexamethasone premedication as described in section 7.10.1.2.2 is recommended in these cases. If possible, the infusion duration per treatment cycle should be 28 days in total counting the infusion days before and after an interruption.

7.10.1.2.4 Clinical monitoring during Blinatumomab administration

Patients should be treated as inpatients for the first 3 days of the Blinatumomab treatment in each cycle. Close monitoring during the first 72 hours of treatment will be indicated because of the potential adverse events associated with T-cell redistribution and potential cytokine release effects triggered by the administration of Blinatumomab. Nurses/physicians trained in emergency medicine should be available for immediate intervention in case of complications. Particular attention should be paid to subject's mental status and neurologic function.

If after the first 72 hours of Blinatumomab infusion a patient is deemed stable by the investigator, the treatment may be continued in an outpatient setting provided that a 24-hour emergency on-call service is ensured.

During the outpatient phase, the patient will visit the study site if clinically indicated and for the examinations according to the diagnostic schedule specified in section 6.3.2 (Table 5).

7.10.1.2.5 Infusion interruption/dose modification of Blinatumomab due to adverse events

Temporary interruption or permanent discontinuation of Blinatumomab should be considered in the case of the following grade 3 or grade 4 toxicities related to Blinatumomab:

- Clinically relevant neurologic event (see list below)
- Cytokine release syndrome (CRS)
- Elevated liver enzymes if clinically relevant
- Any other clinically relevant adverse event

If the interruption of treatment after an adverse event is no longer than 7 days, continue the same cycle to a total of 28 days of infusion inclusive of days before and after the interruption in that cycle. If an interruption due to an adverse event is longer than 7 days, start a new cycle. If the toxicity takes more than 14 days to resolve, discontinue Blinatumomab permanently, except if described differently in

Table 6. The re-start of the infusion should be performed in the hospital under supervision of the investigator.

Table 6 Recommendation for infusion interruption or dose modification of Blinatumomab in the case of Blinatumomab-related toxicity

Toxicity	Grade*	Action
Cytokine release syndrome	Grade 3	Interrupt Blinatumomab until resolved, then restart Blinatumomab at 5 µg/m²/day. Escalate to 15 µg/m²/day after 7 days if the toxicity does not recur.
	Grade 4	Discontinue Blinatumomab permanently.
Neurological toxicity	Convulsion	In the case of convulsion of any grade, interrupt Blinatumomab and proceed as for other neurologic grade 3 toxicity. Discontinue Blinatumomab permanently if more than one convulsion occurs.
	Grade 3	Interrupt Blinatumomab until no more than grade 1 (mild) and for at least 3 days, then restart Blinatumomab at 5 µg/m²/day. Escalate to 15 µg/m²/day after 7 days if the toxicity does not recur. For re-initiation, premedicate with dexamethasone with 10 mg/m². Then reduce dexamethasone step-wise (d2: 2 x 5 mg/m², d3: 2 x 2.5 mg/m², d3: 2 x 1.25 mg/m²). If the toxicity occurred at 5 µg/m²/day, or if the toxicity takes more than 7 days to resolve, discontinue Blinatumomab permanently.
	Grade 4	Discontinue Blinatumomab permanently.
Elevated liver enzymes	Grade 3	If clinically relevant, interrupt Blinatumomab until no more than grade 1 (mild), then restart Blinatumomab at 5 µg/m²/day. Escalate to 15 µg/m²/day after 7 days if the toxicity does not recur.
	Grade 4	Grade 4 liver enzyme elevations are quite common during the first days of Blinatumomab treatment. Consider discontinuing Blinatumomab if enzymes have not returned to < grade 2 after one week of treatment or if grade 4 elevations emerge later during the course of Blinatumomab infusion.
Other clinically relevant (as determined by treating physician) adverse reactions	Grade 3	Interrupt Blinatumomab until no more than grade 1 (mild), then restart Blinatumomab at 5 µg/m²/day. Escalate to 15 µg/m²/day after 7 days if the toxicity does not recur.
	Grade 4	Consider discontinuing Blinatumomab permanently.

*Based on the NCI Common Terminology Criteria for Adverse Events (CTCAE) version 4.0.

7.10.1.2.6 **Criteria for permanent discontinuation of Blinatumomab**

- The patient experiences adverse event(s) requiring dose interruption at the 5 µg/m^2/day dose
- Clinically relevant toxicities that by investigator‘s view impose an unacceptable safety risk to the subject
- Grade 4 cytokine release syndrome or neurologic event
- More than one cerebral seizure/convulsion
- Grade 3 neurologic event
  - that needs more than one week to resolve to grade ≤ 1,
  - that recur after re-start of Blinatumomab treatment at 5 µg/m²/day..
- An infusion stop or delay of more than 14 days due to an adverse event or more than 2 discontinuations per cycle due to an adverse event

7.10.1.2.7 Additional criteria for discontinuing Blinatumomab in randomization R-HR

- PCR-MRD load after the first Blinatumomab cycle within the quantifiable range and no decrease of at least one log step (true MRD values) as compared to the PCR-MRD result before start of the first Blinatumomab cycle ("Blina Poor-Response").

⚠ ***Patients of the R-HR experimental arm who either discontinued the Blinatumomab treatment after less than 14 days Blinatumomab or due to poor response to the first Blina cycle (see section 3.1.7) receive the blocks HR-2' and HR-3' of the control arm before continuation of the treatment with reintensification or alloHSCT.***

7.10.1.2.8 Supportive care guidelines during Blinatumomab treatment

Recommended management of Blinatumomab-induced **drug fever** in patients who do not have signs of infection, based on regional differences in approved medication is provided in Table 7. Nonsteroidal anti-inflammatory drugs (NSAIDs) are restricted because they are a potential cause of endothelial stress.

Table 7 Recommended supportive measures in the case of fever

Symptom	Recommended measures
Fever ≥ 38.5°C	15 mg/kg paracetamol as short term infusion and/or 10 mg/kg metamizole as short term infusion
Fever persistent (≥ 2 hrs) and/or Fever ≥ 39.0°C	40-50 mg/kg metamizole over 24 hrs and/or up to 3 x 0.1 mg/kg Dexamethasone as short term infusion
Chills	up to 0.25 - 1 mg/kg pethidine i.v.

In the case of Blinatumomab-related **clinically relevant neurologic events**, diagnostic measures should be conducted to exclude potential infectious causes. Dexamethasone should be administered at a total daily dose of at least 0.2 to 0.4 mg/kg/day (maximum 24 mg per day), administered as 3 preferably intravenous applications per day for up to 3 days. The dose will then be step-wise reduced by at least 25% per day over up to 4 days. Most neurologic events start with a prodromal phase of kinetic tremor. A daily writing test or finger-nose test is recommended. In case of a pathological test, it is recommended to start dexamethasone. If the neurologic event was a seizure, appropriate prophylactic anticonvulsant treatment with a therapeutic dose of e.g., phenytoin or levetiracetam will be administered during the new treatment cycle.

Because Blinatumomab is an anti-CD19 antibody, **decreases in immunoglobulin levels** have been observed. IV immunoglobulin may be administered at the investigator's discretion. Immunoglobulins must not be administered through the line through which Blinatumomab is administered.

In patients treated with Blinatumomab, **transient neutropenia** has been observed. The administration of G-CSF can be done by investigator's discretion.

In the first days of treatment with Blinatumomab a rapid **transient drop in platelets and/or hemoglobin** may be observed. These effects are not necessarily cytokine mediated. Platelets and hemoglobin recover to baseline during treatment. The investigator has to take appropriate measures by hospital standards.

In the first days of treatment with Blinatumomab, **transient increases of transaminases** up to over 1000 U/L may develop. These have generally returned to baseline in the 1st week of treatment.

7.10.1.3 Treatment schedule of two Blina cycles + i.th. MTX in R-HR

Blina: **Blinatumomab** 15 µg/m^2/day as continuous intravenous infusion (CIVI) at a constant daily flow rate on days 1 to 28 and 43 to 70 (2 cycles of 28 days with a treatment-free interval of 14 days).

The final solution for infusion should be administered through a sterile 0.2 µm in-line filter.

Dexamethasone 5 mg/m^2 should be administered as **premedication** either orally or i.v. immediately before start of the Blinatumomab infusion (not earlier than 30 minutes before start of infusion).

MTX i.th.: **Methotrexate intrathecally** on days 1, 29, 43 and 71.

Age-adjusted dose:

< 1 year:	6 mg
1 to < 2 years:	8 mg
2 to < 3 years:	10 mg
≥ 3 years:	12 mg

Lowered-head position for at least two hours after intrathecal methotrexate application.

7.10.1.4 Treatment schedule of one Blina cycle in R-MR

Blina: **Blinatumomab** 15 µg/m^2/day as continuous intravenous infusion (CIVI) at a constant daily flow rate on days 1 to 28 (1 cycle of 28 days).

The final solution for infusion should be administered through a sterile 0.2 µm in-line filter.

Dexamethasone 5 mg/m^2 should be administered as **premedication** either orally or i.v. immediately before start of the Blinatumomab infusion (not earlier than 30 minutes before start of infusion).

7.11 Protocol III

- Protocol III is intended as reintensification treatment for all HR patients who do not undergo alloHSCT and is given three times with a 4-week Interim Maintenance phase between the elements. Each Protocol III and Interim Maintenance phase is followed by a 1-week treatment break.

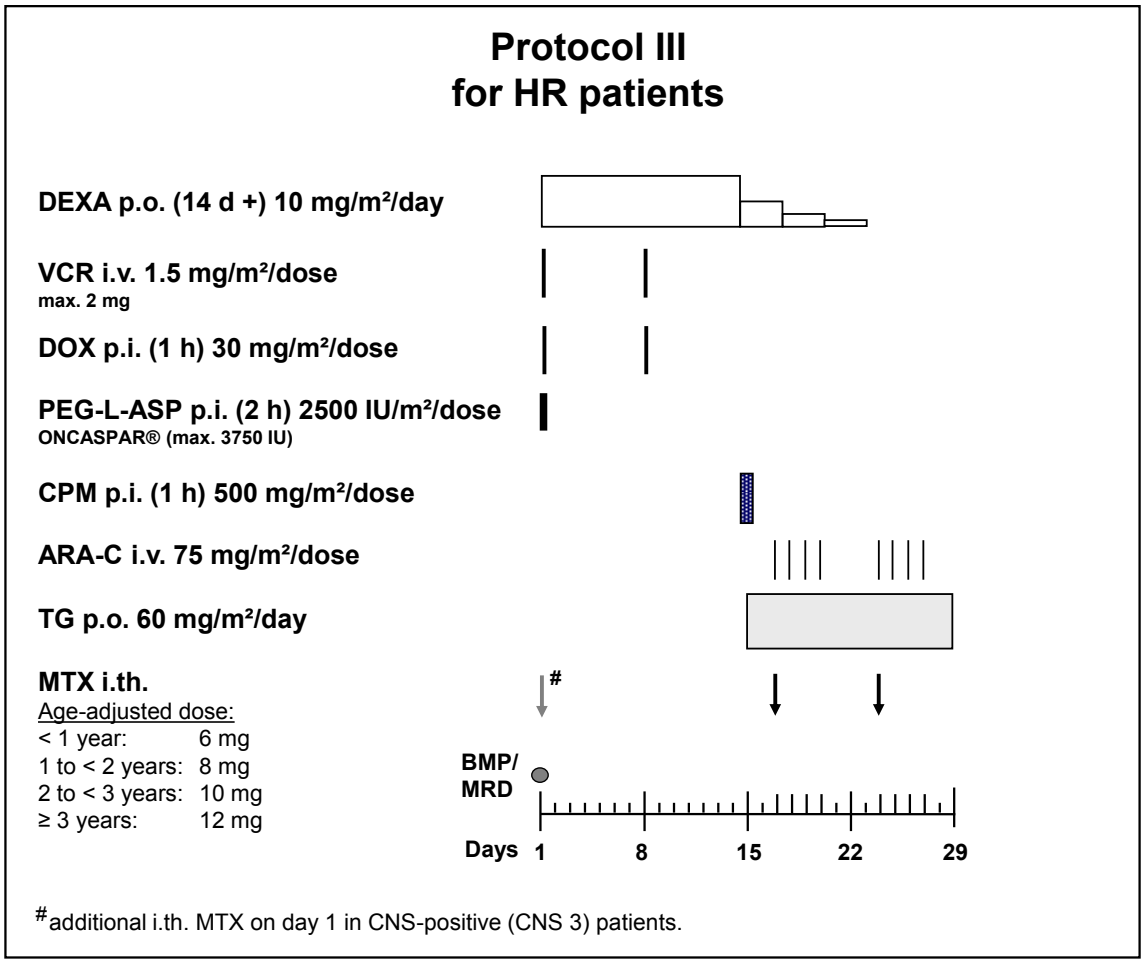


Figure 29 Treatment outline of Protocol III. Applicable to HR patients who do not undergo alloHSCT

7.11.1 Protocol IIIA

First Protocol IIIA starts after the end of third HR block (HR-3') or the second Blina cycle as soon as the start criteria are met (see below). Second and third Protocol IIIA begins one week after a 4-week Interim maintenance phase.

7.11.1.1 Requirements for starting Protocol IIIA

- Adequate clinical condition and no serious infection according to the assessment of the investigator
- Recovering (increasing) blood counts
  - Granulocytes ≥ 500/µl
  - Platelets ≥ 50 000/µl
- Echo-CG is mandatory prior to the 1st doxorubicin of the second Protocol III. If symptoms/signs of cardiac insufficiency are present and/or if the LV-SF is repeatedly below 30 % or the EF below 50 %, contact the national study coordinator for consideration of procedure.

7.11.1.2 Therapy modulation during Protocol IIIA

In the case of severe neuropathy it is permissible to omit vincristine. In the case of insufficient WBC recovery (WBC < 500/µl or granulocytes < 200/µl), the doxorubicin/vincristine doses may be postponed until blood count recovery.

7.11.1.3 Treatment schedule

DEXA: **Dexamethasone** 10 mg/m^2/day p.o. or i.v. divided into 3 doses per day, days 1 to 14 (14 days);

From day 15 tapering over 9 days, with halving the dose on every third day.

VCR: **Vincristine** 1.5 mg/m^2/dose i.v. (maximal single dose 2 mg) on days 1 and 8 (2 doses).

⚠ ***The WHO recommend that "Vincristine should where possible be prepared by dilution in small volume intravenous bags (the 'minibag' technique), rather than in a syringe, to protect against accidental administration via a spinal route. The labeling of vincristine should include a clear warning label that reads: 'FOR INTRAVENOUS USE ONLY - FATAL IF GIVEN BY OTHER ROUTES'."***

DOX: **Doxorubicin** 30 mg/m^2/dose p.i. (1 h) on days 1 and 8 (2 doses).

PEG-L-ASP: **PEG-L-Asparaginase (Oncaspar®)** 2 500 IU/m^2/dose p.i. (2 h) on day 1 (1 dose) (maximal single dose 3 750 IU).

In case of hypersensitivity to PEG-L-ASP, Erwinia asparaginase should be given at a dosage of 20 000 IU/m^2/dose p.i. (1 h) or i.m. every second day for 2 weeks (7 doses).

MTX i.th.: **Methotrexate intrathecally** on day 1 of each Protocol IIIA only <u>for patients with initial CNS disease (CNS 3)</u>

Age-adjusted dose:		
	< 1 year:	6 mg
	1 to < 2 years:	8 mg
	2 to < 3 years:	10 mg
	≥ 3 years:	12 mg

Lowered-head position for at least two hours after intrathecal methotrexate application.

7.11.2 Protocol IIIB

7.11.2.1 Requirements for starting Protocol IIIB

- Adequate clinical condition and no serious infection according to the assessment of the investigator
- Creatinine clearance ≥ 25 ml/min/1.73 m^2. Dose adjustments of Cyclophosphamide are recommended in the case of severe renal dysfuction (Creatinine clearance < 25 ml/min/1.73 m^2)
- Recovering (increasing) blood counts
    - Granulocytes ≥ 500/µl
    - Platelets ≥ 50 000/µl

7.11.2.2 Therapy modulation during Protocol IIIB

As far as possible, an already started cytarabine block should not be withheld. If nevertheless a cytarabine block has to be interrupted or postponed, thioguanine should also be withheld for the same period of time. Omitted thioguanine doses should be made up until the scheduled cumulative dose of 840 mg/m^2 (14 x 60 mg/m^2) has been reached.

7.11.2.3 Treatment schedule

CPM: **Cyclophosphamide** 500 mg/m^2/dose p.i. (1 h) on day 15.

- Give **Mesna** (200 mg/m^2/dose) before and at hours 4 and 8 after start of cyclophosphamide infusion.
- For hydration and cystitis prophylaxis see protocol appendix.

ARA-C: **Cytarabine** 75 mg/m^2/dose i.v. or s.c. 2 blocks of 4 days each days 17-20, 24-27.

TG: **Thioguanine** 60 mg/m^2/day p.o., days 15 to 28 (14 days).

Tablets should be spread evenly over the 14 days so that a cumulative dose of 840 mg/m^2 is reached within the 14 days, or a TG suspension should be used. The drug should be taken in one single dose in the evening on an empty stomach (at least 30 min before or 60 min after the evening meal) without milk.

MTX i.th.: **Methotrexate intrathecally** on days 17 and 24.

Age-adjusted dose:		
	< 1 year:	6 mg
	1 to < 2 years:	8 mg
	2 to < 3 years:	10 mg
	≥ 3 years:	12 mg

Lowered-head position for at least two hours after intrathecal methotrexate application.

7.12 DNX-FLA

Treatment element DNX-FLA is intended to be administered to patients of the HR group with insufficient MRD response (i.e. a MRD ≥ 5 x 10^{-4} in the regenerating bone marrow) to the HR blocks or the Blinatumomab cycles if treated in the R-HR experimental arm. The block can be started as soon as the patient meets the starting criteria of DNX-FLA (see below).

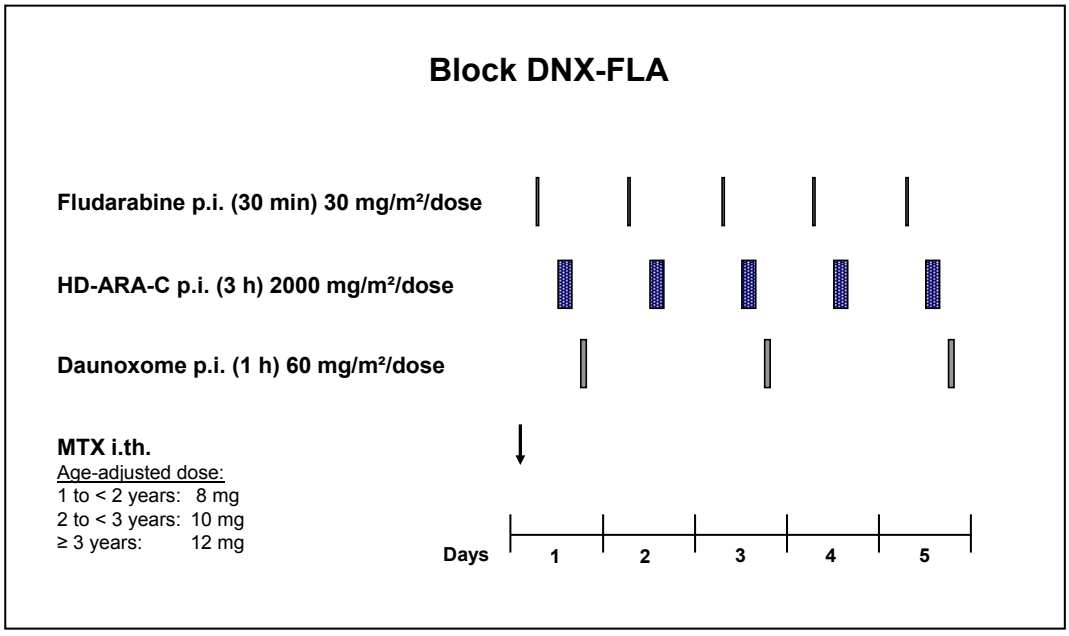


Figure 30 Treatment outline of block DNX-FLA. Applicable to HR patients with insufficient MRD response after the 3rd HR block or the 2nd Blinatumomab cycle.

7.12.1 Requirements for the start of DNX-FLA

- Adequate clinical condition and no serious infection according to the assessment of the investigator
- Recovering (increasing) blood counts
  - Granulocytes ≥ 500/µl
  - Platelets ≥ 50 000/µl
- No essential organ dysfunction:
  - Normal renal function. Dose adjustment of High-dose Cytarabine and Fludarabine should be considered in the case of reduced creatinine clearance.
  - GOT/GPT < 10 x upper normal limit
  - Bilirubin < 3 x upper normal limit with normal direct bilirubin

7.12.2 Treatment schedule

FLU: **Fludarabine** 30 mg/m^2/dose p.i. (30 min) every 24 hours on days 1 to 5 (5 doses).

HD-ARA-C: **High-dose Cytarabine** 2 000 mg/m^2/dose p.i. (3 h) every 24 hours starting 4 hours after end of Fludarabine infusion on days 1 to 5 (5 doses).

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

DNX: **Daunoxome** 60 mg/m^2/dose p.i. (1 h) on days 1, 3 and 5 (3 doses).

MTX i.th.: **Methotrexate intrathecally** on day 1.

Age-adjusted dose:

< 1 year:	6 mg
1 to < 2 years:	8 mg
2 to < 3 years:	10 mg
≥ 3 years:	12 mg

Lowered-head position for at least two hours after intrathecal methotrexate application.

7.13 Interim Maintenance (only HR patients)

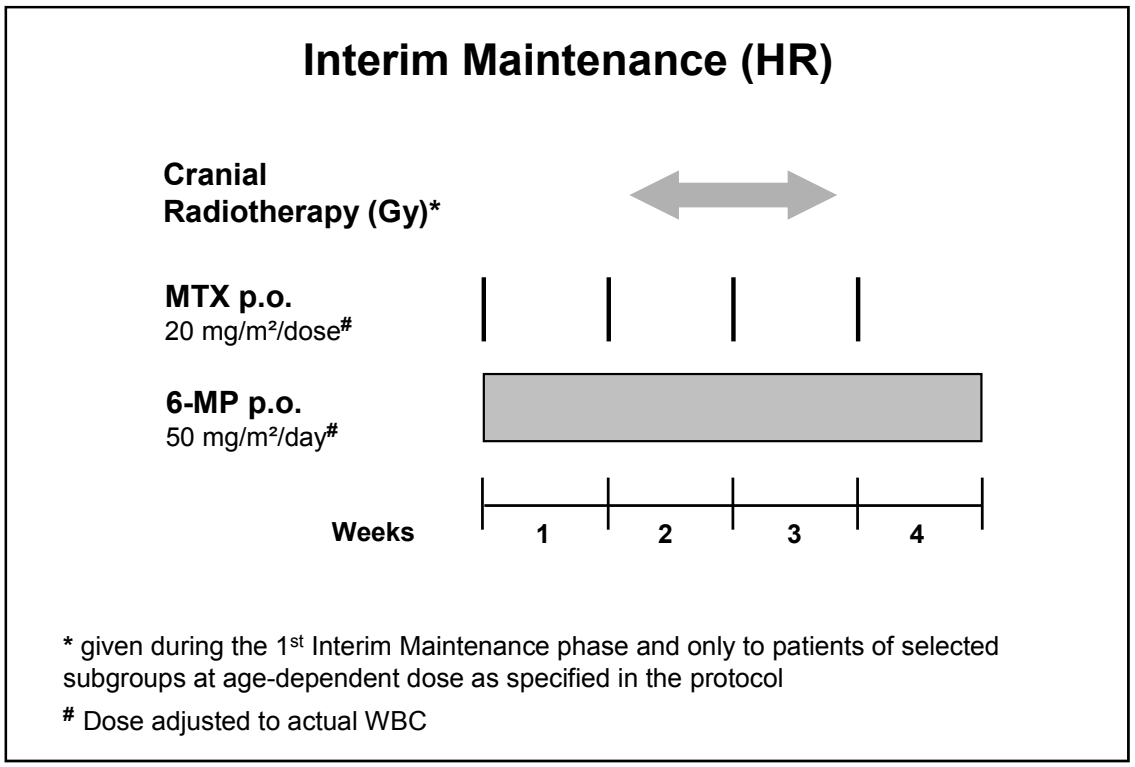


Figure 31 Treatment outline of Interim Maintenance phase

The Interim Maintenance phase starts one week after the end of the 1st and 2nd Protocol III, if blood counts have recovered as required, and lasts 4 weeks. Cranial radiotherapy is scheduled during the 1st Interim Maintenance phase, between the 1st and 2nd Protocol III and is given only to patients of selected subgroups (see section 7.15.2)

7.13.1 Requirements for the start of the Interim Maintenance

- Adequate clinical condition and no serious infection according to the assessment of the investigator
- Recovering (increasing) blood counts
  - Granulocytes ≥ 500/µl
  - Platelets ≥ 50 000/µl
- GOT/GPT < 10 x upper normal limit
- Bilirubin < 3 x upper normal limit with normal direct bilirubin

7.13.2 Therapy modulation during Interim Maintenance

For detailed guidelines of therapy regulation during Interim Maintenance phase see section 7.14.

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

7.13.3 Treatment schedule

The doses for 6-mercaptopurine and methotrexate as stated below are guide doses which have to be adjusted according to the blood cell count (see section 7.14).

6-MP: **6-Mercaptopurine** 50 mg/m^2/day p.o. daily.

Adjust dose using tablets at 50 mg or 10 mg and/or different doses on alternating days in order to attain the daily target dose on average, or use 6-MP as suspension. The drug should be taken in one single dose in the evening on an empty stomach (at least 30 min before or 60 min after the evening meal) without milk.

MTX: **Methotrexate** 20 mg/m^2/dose p.o. once a week.

CRT: **Cranial Radiotherapy** during the 1st Interim Maintenance phase only for selected subgroups of patients. See section 7.15.2 for dosage and administration.

7.14 Maintenance

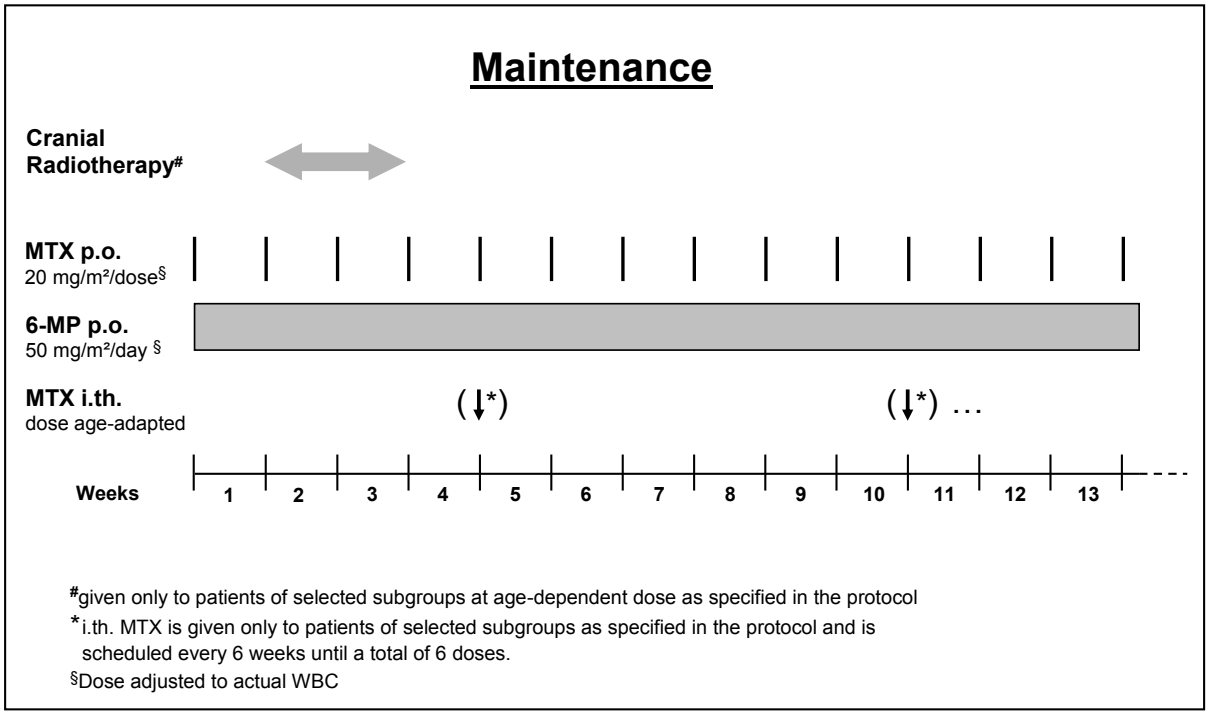


Figure 32 Treatment outline of Maintenance therapy for all patients (except pB-ALL MR patients randomized (R-MR) to the experimental arm). Maintenance therapy lasts until 104 weeks from initial diagnosis of ALL.

Maintenance treatment starts after completion of Protocol II, Protocol III or Blinatumomab (only R-MR experimental arm) as soon as blood counts have recovered as required. For all patients, the total duration of therapy including Maintenance is 24 months (104 weeks) from start of therapy at initial diagnosis.

Cranial radiotherapy is scheduled during the first weeks of Maintenance treatment and is given only to patients of selected subgroups (see section 7.15.2).

7.14.1 Requirements for the start of Maintenance

- Adequate clinical condition and no serious infection according to the assessment of the investigator
- Recovering (increasing) blood counts
  - Granulocytes ≥ 500/µl
  - Platelets ≥ 50 000/µl
- GOT/GPT < 10 x upper normal limit
- Bilirubin < 3 x upper normal limit with normal direct bilirubin

7.14.2 Therapy modulation during Maintenance

During Maintenance the doses of 6-MP and MTX should be adjusted upward (with no upper dose limit) to obtain a total white blood cell count below 3 000/µl. The drugs should be reduced in dosage or withdrawn if the white blood cell count falls below 1 500/µl, the absolute neutrophil count below 500/µl, the absolute lymphocyte count below 300/µl, or the platelet count below 50 000/µl.

In general, the doses of the two drugs 6-MP and MTX should be changed at the same ratio. The dose ratio 6-MP: MTX should usually be 2.5 :1.

Initially, blood count measurements are recommended once a week. If the blood counts are steady and the patient is clinically normal, intervals can be lengthened to 2 weeks. It is recommended to administer the weekly MTX dose at the evening of the day of regular blood count measurement in order to adjust the dose to the current blood count.

Routine measurements of liver parameters during Maintenance are usually not necessary in patients without symptoms, but should be considered in the case of high chemotherapy doses administered. Dose reductions should be based on a rise in bilirubin to more than 3 times the upper normal limit or aminotransferase levels more than 10-20 times the upper normal limit and rising. In such cases, other causes such as viral hepatitis or Gilbert syndrome/Morbus Meulengracht should be considered.

Long treatment breaks should be avoided if possible. A dose reduction or quick resumption of the chemotherapy at reduced dose is generally to be favored over longer intervals without therapy. An uncomplicated (viral) infection without fever and with stable blood counts, for example, is not in every case an indication for treatment interruption.

A dose reduction of chemotherapy (or treatment break, if necessary) is recommended under the following conditions:

- WBC < 1 500/µl
- Granulocytes < 500/µl
- Lymphocytes < 300/µl
- Platelets < 50 000/µl
- Febrile infections: If the blood counts are sufficient and the patient is in good general conditions, therapy should be resumed (possibly at reduced dose) when the patient has been free from fever for one day.
- Liver toxicity: GOT/GPT > 10 - 20 x UNL and rising (steadily high levels can be tolerated) and/or bilirubin > 3 x UNL.
- Mucositis: This is usually related to MTX and may mainly develop when the MTX dose is high or in patients with Down Syndrome. Reduce MTX dose while keeping 6-MP.
- MTX therapy should be interrupted in the case of well-founded suspicion of MTX pneumonitis (see section protocol appendix).

7.14.3 Treatment schedule

The doses for 6-mercaptopurine and methotrexate as stated below are guide doses which have to be adjusted according to the blood cell count.

6-MP: **6-Mercaptopurine** 50 mg/m^2/day p.o. daily.

Adjust dose using tablets at 50 mg or at 10 mg and/or different doses on alternating days in order to attain the daily target dose on average, or use 6-MP as suspension. The drug should be taken in one single dose in the evening on an empty stomach (at least 30 min before or 60 min after the evening meal) without milk.

For dose in patients with homozygous TPMT deficiency, see protocol appendix.

MTX: **Methotrexate** 20 mg/m^2/dose p.o. once a week.

MTX i.th.: **Methotrexate intrathecally,** starting at week 6 every 6 weeks up to a total of 6 doses is given to selected subgroups as specified in section 7.15.1.

Age-adjusted dose:

< 1 year:	6 mg
1 to < 2 years:	8 mg
2 to < 3 years:	10 mg
≥ 3 years:	12 mg

Lowered-head position for at least two hours after intrathecal methotrexate application.

CRT: **Cranial Radiotherapy** in selected subgroups of patients. See section 7.15.2 for dosage and administration.

7.15 CNS-directed treatment

7.15.1 Intrathecal therapy

Intrathecal therapy with MTX is given to all patients at distinct time points during treatment. Patients with CNS status 'CNS 2' receive two additional injections during induction treatment (Protocol IA / IA_{2wkD} / IA_D / IA_{CPM}); patients with initial CNS disease ('CNS 3') receive additional injections during Protocol IA / IA_{2wkD} / IA_D / IA_{CPM} (2 doses) as well as in Protocol IIA (2 doses) or during the HR2' block (1 dose) and each Protocol IIIA (1 dose each), if the patient is treated in the HR group.

The following patient groups receive 6 additional intrathecal injections in 6-week intervals during maintenance starting week 6 from start of maintenance:

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

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

Details on the intrathecal therapy are given in Table 8 and is also presented in Table 9 and Figure 33.

Table 8 Intrathecal methotrexate injection schedule

	Intrathecal injections for all patients	Additional injections in subgroups:	
		Selected CNS-negative (CNS 1 or CNS 2) subgroups*	CNS 3
Prot. IA	days 1, 12, 33		days 19, 26
Prot. IB_{reg}	days 45, 59		
Prot. IB_{long}	days 45, 59		
$Consol_{short}$	days 45, 59		
$Consol_{ext}$	days 45, 73		
Prot. M	days 8, 22, 36, 50		
Prot. II	days 38, 45		days 1, 18
Prot. III	days 17, 24		day 1
HR-1'	day 1		
HR-2'	day 1		day 5
HR-3'	day 1		
Blina cycles in R-HR	day 1 and 29 in both cycles		
Maintenance		6 additional doses in 6-week intervals	Patients < 4 yrs of age: 6 additional doses in 6-week intervals

* see above

7.15.2 Cranial irradiation

7.15.2.1 Indication for cranial radiotherapy (CRT)

Table 9 Cranial irradiation (not applicable to patients undergoing alloHSCT).

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

§ Six additional doses i.th. MTX (in six-week intervals) are given during maintenance

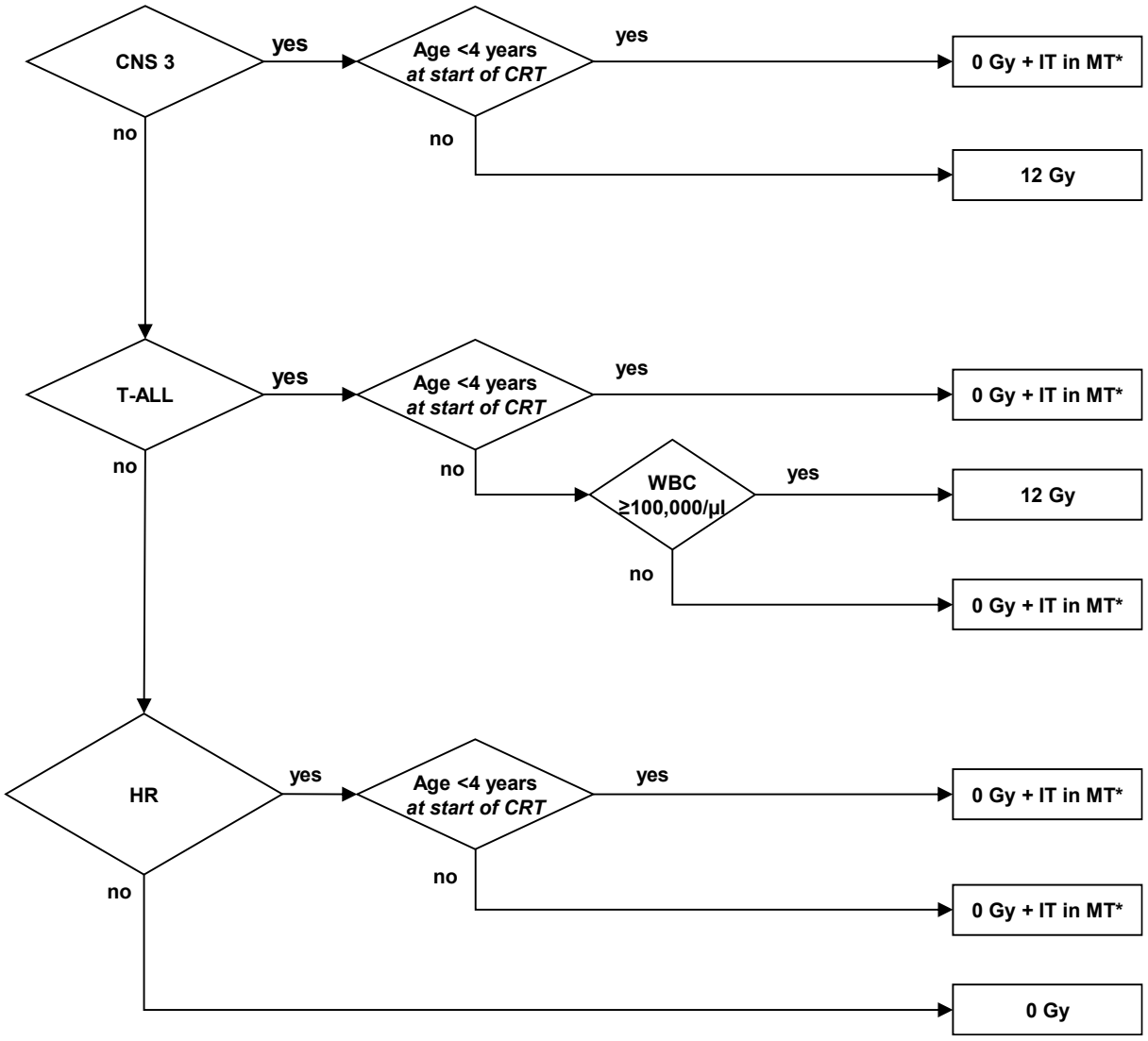


Figure 33 Flow chart for decision on cranial irradiation and additional i.th. MTX in Maintenance. *IT in MT: Six additional doses of i.th. MTX (in six-week intervals) are given during maintenance.

7.15.2.2 Timing of CRT

CRT is given from day 8 to day 22 of the first interim maintenance or maintenance phase in parallel with chemotherapy, about 2 weeks after conclusion of the preceding intensive chemotherapy element.

Requirements for start of CRT:

- Good general condition
- No serious infection
- Recovering (increasing) blood counts
  - WBC ≥1 000/µl
  - Granulocytes ≥200/µl
  - Platelets ≥50 000/µl
- No signs of cerebral disorder

7.15.2.3 Technique of CRT

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

7.16 Allogeneic stem cell transplantation

Indications for allogeneic stem cell transplantation (alloHSCT) are specified in section 5.3.3.

Conduction of alloHSCT 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 SCTped 2012 FORUM).

8 Drug Modification

8.1 Dose modification in specific patient groups

8.1.1 Infants <1 year of age

For infants, the dose calculated by body surface area (exceptions Bortezomib and Blinatumomab, see below) at the start of each treatment block should be adjusted by the actual current age:

< 6 months of age:	2/3 of the dose calculated by body surface area
6 - <12 months of age:	3/4 of the calculated dose
≥ 12 months of age:	full dose

These dose reductions are for all drugs including glucocorticoids, **but not for Bortezomib and Blinatumomab**. Dosage of **Blinatumomab** is 15 µg/m²/day without any reduction by age. Dosage of **Bortezomib** should be calculated by body weight for patients with a dosage of 0.043 mg/kg/dose without additional dose reduction by age.

8.1.2 Low-weight children

In small children ≥ 12 months of age with a body weight of < 10 kg, modification of dose should be considered by calculating the dose by body weight according to the following formula:

Dose = Scheduled dose per m^2 BSA x body weight [kg] / 30.

8.1.3 Obese patients

The problem of adjustment of drug dosage in obese patients remains controversial. There are published data available showing that obesity does not affect the toxicity of chemotherapy or outcome of children and adolescent ALL patients (Hijiya*, et al* 2006). Other data show an even inferior outcome of obese patients which was independent of changes in chemotherapy doses or treatment-related toxicity (Butturini*, et al* 2007). Thus, the need or benefit of drug dose adjustment in obese patients is ambiguous and a clear recommendation cannot be given. If nevertheless the drug dosages shall be adjusted in obese patients, the

following calculations are reasonable. The current definition of obesity according to World Health Organization (WHO) is a Body Mass Index (BMI) ≥ 30, calculated as follows:

BMI = Body Weight (kg) / HT (m) ^{2}

(e.g. patient with BW: 65 kg and HT: 1.4 m = 65 / 1.4 ^{2} = 65 / 1.96 = 33.16 kg/m^2)

For calculation of adjusted dosages, the following parameters should be considered:

BW: body weight
IBW: ideal body weight
ABW: adjusted body weight
HT: height
BS: body surface
ABS: adjusted body surface

8.1.3.1 Dose adjustment of drugs administered by body weight

Regarding drugs to be administered on the base of body weight, it is allowed to use empirically the average between dosages calculated by actual body weight and ABW. calculated as follows,

ABW = (BW – IBW) * 0.4 + IBW (Equation 1)

This equation implies that about 40 % of weight exceeding ideal weight is constituted by lean mass instead of fat mass

IBW values can be derived from tables dependent on frame size and height or according to the equations proposed by Devine in 1974 which is an empirical estimation widely used in pharmacokinetics literature:

IBW_{male} = 45.4 + 0.89 x (HT (cm) – 152.4) + 4.5 (Equation 2)

IBW_{female} = 45.4 + 0.89 x (HT (cm) – 152.4) (Equation 3) .

Example: For a male patient with a weight of 50 kg and HT of 125 cm, the IBW according to the formula is 25 kg (IBW = 45.4 + 0.89 x (125 – 152.4) + 4.5). The adjusted body weight is calculated as follows:

ABW = (50 – 25) * 0.4 + 25 = 35 kg.

8.1.3.2 Dose adjustment of drugs administered by body surface area

Regarding drugs to be administered basing on body surface, it is not agreed if dose adjustment is necessary; thus, it is allowed to use empirically the average between dosages calculated by BS and ABS.

BS = [√(BW * HT)] / 60

ABS = [√(ABW * HT)] / 60

Example: For the same patient mentioned above, BS and ABS are calculated as following:

BS = [√(50 * 125)] / 60 = 1.32

ABS = [√(35* 125)] / 60 = 1.10

Thus for this patient, a hypothetic dose of cyclophosphamide of 1000 mg/m^2 would be 1320 mg when calculated by ABS and 1110 mg when calculated by BS, with an “average” dose of 1215 mg, rounded to 1200 mg.

8.1.4 Down syndrome patients

Patients with Down syndrome are known to be at particularly high risk of severe toxicity under chemotherapy. However, data are available which demonstrate higher relapse rates in patients treated with less intensive treatment or significant treatment reduction suggesting the need of intensive treatment in this patient group (Dordelmann*, et al* 1998, Whitlock*, et al* 2005).

The clinical experience has shown that treatment tolerance is very variable in this patient group and a large proportion of the patients develop no extensive toxicity. Therefore, general dose reduction is not recommended in Down syndrome patients except for Protocol M (see below). Patients have to be monitored very carefully, and treatment reductions should be based on the actual toxicity.

Because of very high methotrexate sensitivity and hence severe toxicity in some Down syndrome patients, an *a priori* dose reduction of methotrexate is recommended in Protocol M. The first HD-MTX (Protocol M or HR-1') should be administered at a dose of 500 mg/m^2/24 h in all patients. If it has been tolerated without significant toxicity, dose increase to 2 000 mg/m^2/24 h and 5 000 mg/m^2/24 h should be considered for the following courses. In the case of relevant systemic toxicity (myelosuppression or mucositis) due to the intrathecal methotrexate, intrathecal dose should not be reduced, but leucovorin rescue is recommended (see the protocol appendix).

Patients with pB-ALL and Down Syndrome are not eligible for the randomisations R-eHR and R-HR. It is recommended to treat the patients in the consolidation phase with the stardard-of-care therapy Consolidation$_{short}$. To avoid severe toxicity from the HR blocks, post-consolidation treatment with the R-HR experimental arm (two Blinatumomab cycles instead of HR-2' and HR-3') is recommended.

8.2 Modification for toxicity

Guidelines for therapy modifications due to toxicity related to the experimental treatments with Bortezomib and Blinatumomab are given in Chapter 7 "Treatment". With regard to the standard chemotherapy, only basic recommendations for the modulation of treatment are given in this chapter, Treatment modification due to toxicity of the standard chemotherapy should follow local standards according to the common clinical pratice. In this contex, please refer also to the appendix "Recommendations for management of toxicity and supportive care".

9 Contraception

Adolescents have to be advised that there is a risk of an adverse effect on the fetus in the case of procreation of children during chemotherapy and one year afterwards. Patients who are sexually active have to be advised about the use of effective contraception (oral contraception, intrauterine devices, barrier method of contraception in conjunction with spermicidal jelly or surgical sterile). The occurrence of pregnancy in patients or their partners during this time period should be reported to the study office.

10 Chemotherapeutic Drugs

See Summaries of medicinal Product Characteristics included (SmPC) in the Investigator Site Files.

11 Guidelines for Supportive Care and Emergency Situations

Recommendations for supportive care and management of emergency situations are given the protocol appendix.

12 Safety

12.1 Serious Adverse Events (SAE)

12.1.1 Definition of SAE

A serious adverse event (SAE) is any untoward medical occurrence that

- results in death,
- is life threatening (defined as an event in which the patient was at risk of death at the time of the event; it does not refer to an event which hypothetically might have caused death if it were more severe),
- require or prolong hospitalization,
- results in persistent or significant disability/incapacity,
- is a congenital anomaly/birth defect in the offspring,

12.1.2 Other important medical events

Adverse events that do not meet one of the formal criteria of seriousness as defined above, but are considered medically important in the view of the investigator or the sponsor/national study coordinator (e.g., as they significantly jeopardize the patient and require medical or surgical intervention to prevent one of the outcomes listed in the SAE definition) are treated in the same way as SAE with regard to the reporting requirements.

12.1.3 Exceptions from the SAE definition

Hospitalization occurring under the following circumstances is not to be considered as SAE:

a) planned as per protocol medical/surgical procedure

b) routine health assessment requiring admission for baseline/trending of health status documentation

c) medical/surgical admission for purpose other than remedying ill health state (planned prior to entry into study trial)

d) admission encountered for other life circumstance that carries no bearing on health status and requires no medical/surgical intervention (i.e. lack of housing, economic inadequacy, care-giver respite, family circumstances, administrative)

12.1.4 SAE reporting timelines and exceptions from immediate reporting

The investigator has to report the SAEs and the other important medical events to the sponsor/national study coordinator within 24 hours or until the next working day following his knowledge of the event. For the details of SAE/AE reporting requirements see section 12.5. Specific individual SAEs or important medical events that are expected in relationship with the protocol treatment do not require immediate reporting. Such events should be reported within 4 weeks of the end (or discontinuation, if applicable) of the respective treatment phase. The SAEs or important medical events that are excepted from immediate reporting are listed in section 12.3 in Table 10.

12.1.5 Severity of AEs according to CTCAE v4.03

- Grade 1: mild; asymptomatic or mild symptoms; clinical or diagnostic observations only; intervention not indicated.
- Grade 2: moderate; minimal, local or noninvasive intervention indicated; limiting age-appropriate instrumental ADL*.
- Grade 3: severe or medically significant but not immediately life-threatening; hospitalization or prolongation of hospitalization indicated; disabling; limiting self care ADL*.
- Grade 4: Life-threatening consequences; urgent intervention indicated.
- Grade 5: Death related to AE.

*Activities of Daily Living (ADL)
Instrumental ADL refer to preparing meals, shopping for groceries or clothes, using the telephone, managing money, etc.
Self care ADL refer to bathing, dressing and undressing, feeding self, using the toilet, taking medications, and not bedridden.
A semi-colon indicates 'or' within the description of the grade.

12.1.6 Definition of life-threatening

An adverse event is considered **life-threatening** if it places the patient, in the view of the investigator, at ***immediate*** risk of death from the event as it occurred, i.e., it does not refer to an event which hypothetically might have caused death if it were more severe..

Life-threatening events therefore usually require an **emergency intervention** into the immediately life-threatening condition such as

- artificial ventilation with intubation due to respiratory failure
- urgent intervention (drug therapy, e.g. vasopressor support, or cardiac resuscitation) due to circulatory/cardiac failure
- emergency surgery or emergency endoscopic intervention due to other serious clinical situations, e.g.
    - gastrointestinal bleeding or perforation
    - cerebral abscess/bleeding

An event requiring an intervention for preventing a life-threatening situation (e.g. dialysis due to acute renal failure in a clinically stable patient) is not life-threatening *per definitionem*.

12.2 Medically important AEs of interest

The following list includes Adverse Events (AE) which are clinically important based upon medical judgment or are of clinical interest because they provide essential information to assess the toxicity profile of the treatment even if they do not meet the formal SAE criteria (Example: An osteonecrosis which requires no hospitalization and does not result in significant sequelae may not be a serious AE *per definitionem*, but is considered as an important medical event.. Most of the events listed below are expected throughout the protocol treatment phases and are therefore excepted from immediate reporting to the sponsor/national coordinator (see section 12.3).

Events are categorized by the MedDRA Primary System Organ Class (SOC).

1. Infections and Infestations:
    - Proven, probable or possible invasive fungal infection (according to the revised EORTC criteria (Cortes*, et al* 2004))
    - Infection of the central nervous system

- Severe extended tissue infections ≥ grade 3 (IV antibiotics/antifungals or operative intervention indicated)
- Infection along the central venous line (CVL) tunnel requiring explantation of CVL
- Neutropenic enterocolitis (typhlitis)
- Endocarditis

2. Blood and lymphatic system disorders

   - Symptomatic hemophagocytic lymphohistiocytosis (HLH)

3. Immune system disorders:

   - Hypersensitivity to asparaginase (definition and grading as in PdL consensus, see 12.2.1.6)
     - Allergy to asparaginase
     - Allergic-like reaction to asparaginase
   - Allergic reaction CTC ≥ grade 2 or anaphylactic reaction to other investigational products
   - Cytokine-release syndrome ≥ grade 2

4. Endocrine disorders:

   - Symptomatic SIADH (Na^+ < 120 mmol/l and neurological symptoms as lethargy, desorientation, seizures)

5. Metabolism and nutrition disorders:

   - Drug-induced diabetes mellitus (decreased or null insulin secretion or glucose intolerance with hyperinsulinism) with need of substitutive insulin therapy for more than 1 week
   - Symptomatic hypoglycemia
   - Severe hypertriglyceridemia: triglycerides and/or cholesterol > 20 times UNL

6. Psychiatric disorders:

   - Severe (≥ grade 3) psychiatric disorders such as depression, anxiety, mania, psychosis or suicidal ideation

7. Nervous system disorders:

   - MTX-related stroke-like syndrome (subacute MTX-related encephalopathy, for definition see 12.2.1.3)
   - Posterior reversible encephalopathy syndrome (PRES) (for definition see 12.2.1.4)
   - Seizures
   - Central paralysis or paresis of all grades
   - Peripheral paralysis or severe paresis with a muscle strength grade ≤ 2/5 (for definition of muscle strength grade see 12.2.1.6)
   - Paresthesia or neuropathic pain ≥ grade 3
   - Abnormal change of consciousness (for definition see 12.2.1.5)
   - Cerebral stroke
   - Cerebral hemorrhage
   - Cerebral venous thrombosis including sinus venous thrombosis

8. Cardiac disorders:

   - Symptomatic cardiac insufficiency and/or ejection fraction (EF) < 40% (≥ grade 3)
   - Symptomatic cardiac arrhythmias requiring treatment/medications (≥ grade 3)
   - Intracardiac thrombosis

9. Vascular disorders:
    - Venous and/or arterial thrombosis or embolism requiring *systemic anticoagulation or surgery* (cerebral and intracardiac thombosis are attributed to the SOC term nervous system disorders and cardiac disorders)
    - Severe Hypertension: defined as average systolic and/or diastolic blood pressure that is greater than or equal to the 95th percentile for sex, age, and height on three or more occasions (3 consecutive days, or separate clinic visits if outpatient).
10. Gastrointestinal disorders:
    - Gastrointestinal hemorrhages (≥ grade 3)
    - Gastrointestinal ulcer (≥ grade 3)
    - Gastrointestinal perforation
    - Other gastrointestinal complications requiring surgery
    - Acute Pancreatitis (for definition see 12.2.1.2)
11. Hepatobiliary disorders:
    - Severe hepatic failure: Signs of hepatic failure (asterixis, confusion, ascites, coma) accompanied by biochemical evidence of liver injury (raised transaminases and/or bilirubin, lactate, INR or ammonia) and no other apparent cause
    - Veno-occlusive disease (VOD) of the liver (sinosoidal obstruction syndrome, for definition see 12.2.1.1)
12. Skin and subcutaneous tissue disorders
    - Bullous dermatitis CTC ≥ grade 2 (blisters covering >10% BSA; limiting instrumental ADL)
13. Musculoskeletal and connective tissue disorders:
    - Symptomatic and asymptomatic osteonecrosis
    - Pathological fracture
14. Renal and urinary disorders:
    - Severe impaired MTX excretion (MTX level > 10 µmol/l at h 36 and/or > 5 µmol/l at h 42 and/or > 3 µmol/l at h 48)
    - Acute renal failure (requiring dialysis)
    - Chronic renal failure: reduction of glomerular filtration rate by ≥ 50 %

12.2.1 Definition of specific AEs according to the Ponte di Legno consensus definitions of acute toxicities during childhood ALL therapy (Schmiegelow*, et al* 2016)

12.2.1.1 Sinusoidal obstruction syndrome (veno-occlusive disease of the liver, VOD)

Fulfillment of at least three out of five criteria:

1) Hepatomegaly
2) Hyperbilirubinaemia
3) Ascites
4) Weight gain >5%, and
5) Thrombocytopenia, otherwise unexplained and transfusion-resistant.

Doppler ultrasound may document changes in hepatic portal venous flow and rule out alternative causes, but normal Doppler findings do not exclude a diagnosis of VOD.

12.2.1.2 Pancreatitis

At least two of three features must be fulfilled

1) Abdominal pain strongly suggestive of pancreatitis
2) Serum lipase or amylase ≥3 xUNL
3) Characteristic imaging findings of pancreatitis (ultrasound, computed tomography, magnetic resonance imaging).

12.2.1.3 MTX-related stroke like syndrome (also known as subacute MTX-related encephalopathy)

Patients must fulfil all 3 of the following criteria:

1) New onset of one or more of the following neurological symptoms/signs within 21 days of MTX therapy (intrathecal or intravenous):
   - Paresis/paralysis
   - Aphasia/dysarthria
   - Altered mental status, somnolence, confusion, disorientation, emotionally lability *etc*
   - Movement disorder
   - Loss of consciousness
   - Bilateral weakness
   - Seizures (isolated seizures without accompanying features from the list above and without criteria 2 and 3 below are excluded)
2) EITHER: Findings of characteristic white matter changes of leukoencephalopathy on MRI (see below)

   AND/OR: Characteristic clinical course with waxing and waning symptoms usually leading to complete resolution over 1-7 days (Note in severe cases only partial resolution may be seen in this time frame)

   Characteristic MRI findings (best seen on T2-weighted images or ideally with DWI) include: oval shaped lesions of the subcortical white matter, often in the frontal, or parietal areas and often not conforming to a vascular territory. Lesions are generally hyperintense on DWI and hypointense on ADC.
3) No other identifiable cause.

12.2.1.4 Posterior reversible encephalopathy syndrome (PRES)

Diagnosis of PRES is based on the essential and additional criteria below, but with no grading.

Essential criteria:

1) Clinical findings: any combination of headache, confusion, seizures and visual disturbances
2) Imaging findings: best imaging tool: contrast-enhanced MRI and DWI
   - Best diagnostic clue: patchy (and confluent) cortical (and subcortical) territory lesions
   - Most common location: cortex, subcortical white matter
   - Predilection: parietal and occipital lobes, cerebellum; less common: basal ganglia, brainstem, frontal lobes
   - Size: extent of abnormalities highly variable

- Mass effect: minimal or none
- Enhancement: minimal or none
- MRI findings:

  T1WI: hypointense cortical / subcortical lesions

  T2WI: hyperintense cortical / subcortical lesions

  On DWI images, PRES is ususally normal or hyperintense.

Additional supportive findings:

1) Timing: usually during the first 3-4 months of therapy (mostly during remission induction therapy)
2) Presence of arterial hypertension
3) Complete resolution of clinical/imaging findings, although MR findings may persist in some patients
4) EEG: usually non-specific alterations, sometimes epileptiform discharges in the posterior region of the brain may be visible
5) Other causes reasonably ruled out

12.2.1.5 Abnormal change of consciousness

Abnormal change in level of arousal or altered content of a patient's thought processes

Change in the level of arousal or alertness:

Grade A1: Lethargy - mild reduction in alertness

Grade A2: Obtundation - moderate reduction in alertness. Increased response time to stimuli.

Grade A3: Stupor - Deep sleep, patient can be aroused only by vigorous and repetitive stimulation. Returns to deep sleep when not continuously stimulated.

Grade A4: Coma (Unconscious) – Sleep-like appearance and behaviorally unresponsive to all external stimuli (Unarousable unresponsiveness, eyes closed).

Change in content (thought processes):

Grade B1: “Relatively simple” changes: e.g. speech, calculations, spelling.

Grade B2: More complex changes: emotions, behavior or personality, e.g. confusion, disorientation, hallucinations, poor comprehension, or verbal expression.

12.2.1.6 Hypersensitivity to Asparaginase

Definition of allergy to Asparaginase:

An adverse local or general response from exposure to Asparaginase characterised by flushing, rash, urticaria, drug fever, dyspnoea, symptomatic bronchospasm, oedema/angiooedema, hypotension and/or anaphylaxis

Severity

mild: transient flushing or rash or drug fever < 38o C; or

severe: drug fever >38°C; allergy-related oedema/angiooedema; dyspnoea and/or symptomatic bronchospasm with or without urticarial; and/or hypotension and anaphylaxis) with indication for Asparaginase infusion interruption and parenteral medication (e.g. antihistamines, glucocorticoids).

Definition of allergic-like reaction:

An intolerance (e.g. vomiting, stomach ache, rash etc) usually occurring later in the infusion than real Asparaginase allergy that in general occurs at the first drops. Note: Distinction between hypersensitivity and allergic-like reactions is critical but may be difficult, since clinical hypersensitivity (even mild) is closely associated with Asparaginase inactivation. Asparaginase activity measurements may distinguish and guide decision on switch to other Asparaginase preparation.

12.2.2 Muscle strength assessment according to Janda

0/5 = No visible and/or palpable muscle contraction

1/5 = Visible and/or palpable muscle contraction with no motor effect

2/5 = Distinct muscle tension, movement is possible if gravity effect is eliminated

3/5 = Movement against gravity possible

4/5 = Movement against low to medium resistance is possible

5/5 = Movement with normal strength

12.3 Exceptions from immediate reporting of expected SAE or other important medical events (non-life-threatening, non-fatal)

The occurrences of the following adverse events (either serious or other medically important events) are expected during the respective treatment phases as indicated in the table below with an 'X' (see also section 12.2). Please note that first occurrence during a specific treatment phase does not necessarily mean that the expected causal relationship is attributed to the drugs given in this treatment phase because some side effects become clinically apparent only after a certain latency period. For example, osteonecroses may be related to glucocorticoid treatment during induction and/or reintensification phase, or cardiac insufficiency can be attributed to anthracycline therapy, but it is nevertheless expected that these events occur for the first time during maintenance.

The occurrence of an event as indicated in the table below does not require immediate reporting to the sponsor/national study coordinator. Such events should be reported within 4 weeks from the end (or discontinuation if applicable) of the treatment phase in which the event occurred.

Please note: Life-threatening or fatal events are not included in the exceptions from immediate reporting and have to be reported to the sponsor/national study coordinator within 24 hours or until the next working day even if included in the table below.

Table 10 List of expected SAE or other important medical events that do not require immediate reporting if they are not life-threatening or fatal

	Prot. IA (all) Prot. IIA Prot. IIIA	Consol. A	Prot. IB (all) Consol. B_{short} Prot. IIB Prot. IIIB	Consol. B_{ext} (+/- BZM)	Prot. M	HR-1' HR-2'	HR-3'	Blina	Blina + i.th. MTX	DNX-FLA	Mainten.
Infections and Infestations											
Infections (all types)	X	X	X	X	X	X	X	X	X	X	X
Blood and lymphatic system disorders											
Symptomatic HLH	X	X	X	X	X	X	X			X	X
Immune system disorders											
Hypersensitivity to the respective drugs	X	X	X	X	X	X	X	X	X	X	X
Cytokine-release syndrome								X	X		
Endocrine disorders											
Symptomatic SIADH	X		X	X		X	X				
Metabolism and nutrition disorders											
Drug-induced diabetes mellitus	X			X	X	X	X				X
Symptomatic hypoglycaemia	X	X	X	X	X	X	X				X
Severe hypertriglyceridemia	X			X		X	X				
Psychiatric disorders											
Severe depression	X			X	X	X	X			X	X
Severe psychosis, anxiety, mania, suicidal ideation	X			X		X	X				

	Prot. IA (all) Prot. IIA Prot. IIIA	Consol. A	Prot. IB (all) Consol. B_{short} Prot. IIB Prot. IIIB	Consol. B_{ext} (+/- BZM)	Prot. M	HR-1' HR-2'	HR-3'	Blina	Blina + i.th. MTX	DNX-FLA	Mainten.
Nervous system disorders											
MTX-related stroke-like syndrome	X	X	X	X	X	X	X		X	X	X
Posterior reversible encephalopathy syndrome	X	X	X	X	X	X	X		X	X	X
Seizure	X	X	X	X	X	X	X	X	X	X	X
Central paralysis or paresis	X	X	X	X	X	X	X		X	X	X
Peripheral paralysis or paresis	X			X		X	X			X	
Paresthesia or neuropathic pain	X			X	X	X	X	X		X	X
Abnormal change of consciousness	X	X	X	X	X	X	X	X	X	X	X
Cerebral stroke	X	X	X	X	X	X	X			X	X
Cerebral venous thrombosis incl. SVT	X	X	X	X	X	X	X			X	X
Cerebral hemorrhage	X	X	X	X	X	X	X			X	X
Cardiac disorders											
Symptomatic cardiac insufficiency	X	X	X	X	X	X	X			X	X
Symptomatic cardiac arrhythmias	X	X	X	X		X	X			X	
Intracardiac thrombus	X	X	X	X	X	X	X			X	X
Vascular disorders											
Venous and/or arterial thrombosis or embolism	X	X	X	X	X	X	X			X	X
Severe Hypertension	X			X		X	X				

	Prot. IA (all) Prot. IIA Prot. IIIA	Consol. A	Prot. IB (all) Consol. B_{short} Prot. IIB Prot. IIIB	Consol. B_{ext} (+/- BZM)	Prot. M	HR-1' HR-2'	HR-3'	Blina	Blina + i.th. MTX	DNX-FLA	Mainten.
Gastrointestinal disorders											
Gastrointestinal hemorrhages	X	X	X	X	X	X	X			X	X
Gastrointestinal ulcers/perforation	X	X	X	X	X	X	X			X	X
Pancreatitis	X	X	X	X	X	X	X			X	X
Hepatobiliary disorders											
Severe hepatic failure	X	X	X	X	X	X	X				X
VOD	X	X	X	X		X	X				
Skin and subcutaneous tissue disorders											
Bullous dermatitis	X		X	X	X	X	X			X	X
Musculoskeletal and connective tissue disorders											
Osteonecrosis	X	X	X	X	X	X	X	X	X	X	X
Pathological fracture	X	X	X	X	X	X	X	X	X	X	X
Renal and urinary disorders											
Severe impaired MTX excretion					X	X					
Acute/chronic renal failure	X	X	X	X	X	X	X			X	X

12.4 Non-serious adverse events in the randomized treatment phases

Other adverse events which do not meet the serious criteria and are not considered medically important should be reported for the randomized treatment phases if they meet CTCAE criteria ≥ grade 3 (see 12.1.5) using the respective eCRF. The adverse event grading scale used will be the NCI Common Terminology Criteria for Adverse Events (CTCAE) version 4.03**.**

This relates to the following treatment phases:

Randomization R-eHR:

Control arm: Start of Consolidation B_{ext} until start of the following treatment course

Experimental arm: Start of Consolidation B_{ext}+BZM until start of the following treatment course

Randomization R-HR:

Control arm: Start of HR-2' until start of the treatment course that follows HR-3'

Experimental arm: Start of Blina cycle 1 until start of the treatment course that follows Blina cycle 2

Randomization R-MR:

Control arm: 8 weeks from start of Maintenance

Experimental arm: 8 weeks from start of the Blina cycle

Randomization R-T:

Control arm: Start of Protocol IB/2_{reg} until start of the following treatment course

Experimental arm: Start of Protocol IB/2_{long} until start of the following treatment course

Aside from laboratory tests that are indicated to assess the clinical condition of the patient and should be performed at the discretion of the investigator, the following laboratory parameters should be investigated on a regular basis during the treatment phases specified above and will be recorded in the electronic case report form (eCRF):

- Blood count including differential blood count
- Creatinine
- GOT
- GPT
- Bilirubine
- Lipase
- CRP
- Only R-HR and R-MR: quantitative immunoglobulins

If these laboratory parameters reveal a ≥ grade 3 toxicity, the documentation of those supersedes additional reporting of these findings as non-serious AE.

The schedule of the blood tests can be found in Table 5 on page 78

Additional investigations are scheduled in an immune monitoring program for the experimental arms of randomization R-eHR and R-MR (Blinatumomab treatment). For the respective instructions, please refer to section 6.

12.5 Reporting of SAE and other toxicity

The investigator is responsible for ensuring that the SAE as defined above (taken into account the exceptions specified) and observed by the investigator or reported by the

patient/parents are recorded in the patient's medical record and are submitted to the national study coordination center. The investigator has to report the SAE within 24 hours following his knowledge of the event using the respective SAE form; if the event occurs at a weekend or legal holiday, reporting has to be done until the next working day at the latest. The specific toxic events listed in Table 10 may meet the criteria of serious or are medically important but are considered as expected with respect to their relatedness to the chemotherapy. If these events are not life-threatening or fatal, they are excepted from immediate reporting. These events and other non-serious AE have to be reported to the national study coordinator within 4 weeks of the end (or discontinuation) of the respective treatment phase in which the event occurred using the applicable eCRF. The reporting period for SAE and non-serious AE starts with diagnosis of ALL and is restricted to events occurring within 1 month after the end of the study treatment except for osteonecrosis which should be reported during the entire study period within 4 weeks of learning of its occurrence. Events that occur after ALL diagnosis but before the patient/guardians gave their informed consent to the trial or the data transfer should be reported in retrospect as soon as the consent is available.

The investigator should follow reported serious adverse events until stabilization or reversibility. New information relating to a previously reported serious adverse event must be submitted to the national study coordination center via the applicable eCRF. Depending on the type of AE, the investigator will be asked for specific additional information regarding grading, laboratory or clinical parameters via the applicable eCRF. The investigator may also be asked to provide additional follow-up information, which may include a discharge summary or extracts from the medical record.
Relapse of ALL, death from ALL progression and secondary neoplasms are endpoints of the study and are therefore not considered as (S)AE as stated above. They should be reported within 4 weeks of the investigator's knowledge of the event using the applicable eCRF.
For patients enrollen in one of the randomizations R-eHR, R-HR, R-MR or R-T, additional information on CTCAE grade 3 or 4 non-serious adverse events is captured. This information should be provided for each treatment phase and should be submitted to the national study coordination center within 4 week after the end of the respective treatment phase via the applicable eCRF.

12.6 SUSAR assessment

The investigator must assess whether the serious adverse event is possibly causally related to the study medication or a trial-specific procedure, if applicable. This relationship is indicated on the applicable eCRF by a "yes" or "no" response to the question: "Is there a reasonable possibility that the event may have been caused by the study medication or a trial-specific procedure?"
The assessment of causality (suspected/not suspected relationship with the study medication) and expectedness (expected/not expected) is needed to define the SUSARs ("Suspected Unexpected Serious Adverse Reactions"). The National Coordinator gives his re-assessment of causality and his assessment of expectedness according to a pre-specified drug information document (Reference Safety Information). The Sponsor has access to all SAE data of the national study groups and is responsible for evaluation of the SAE with respect to other safety issues. SUSARs have to be reported to the Sponsor immediately. The Sponsor distributes the SUSAR reports to the national study centers, which circulate them to their respective national competent authorities, national leading ethics committees and the investigators of the respective national group.

12.7 Other safety issues requiring expedited reporting

The sponsor will immediately, at the latest 15 days after it becomes known, report all circumstances that require a revision of the risk-benefit analysis to the respective Ethics Committee, the relevant regulatory authorities and the relevant regulatory authorities of other

European member states and other contracting states of the European Economic Area (EEA) agreement, if the study is run in their territory. This includes:

- Singular cases of expected serious adverse events with an unexpected outcome
- Increased incidence of expected serious adverse events that are judged as being clinically relevant.
- SUSARs which occur after termination of the clinical trial
- Events related to study procedures or development of the study medication, which could affect a subject's safety
- All person-related data will always be transmitted pseudonymised.

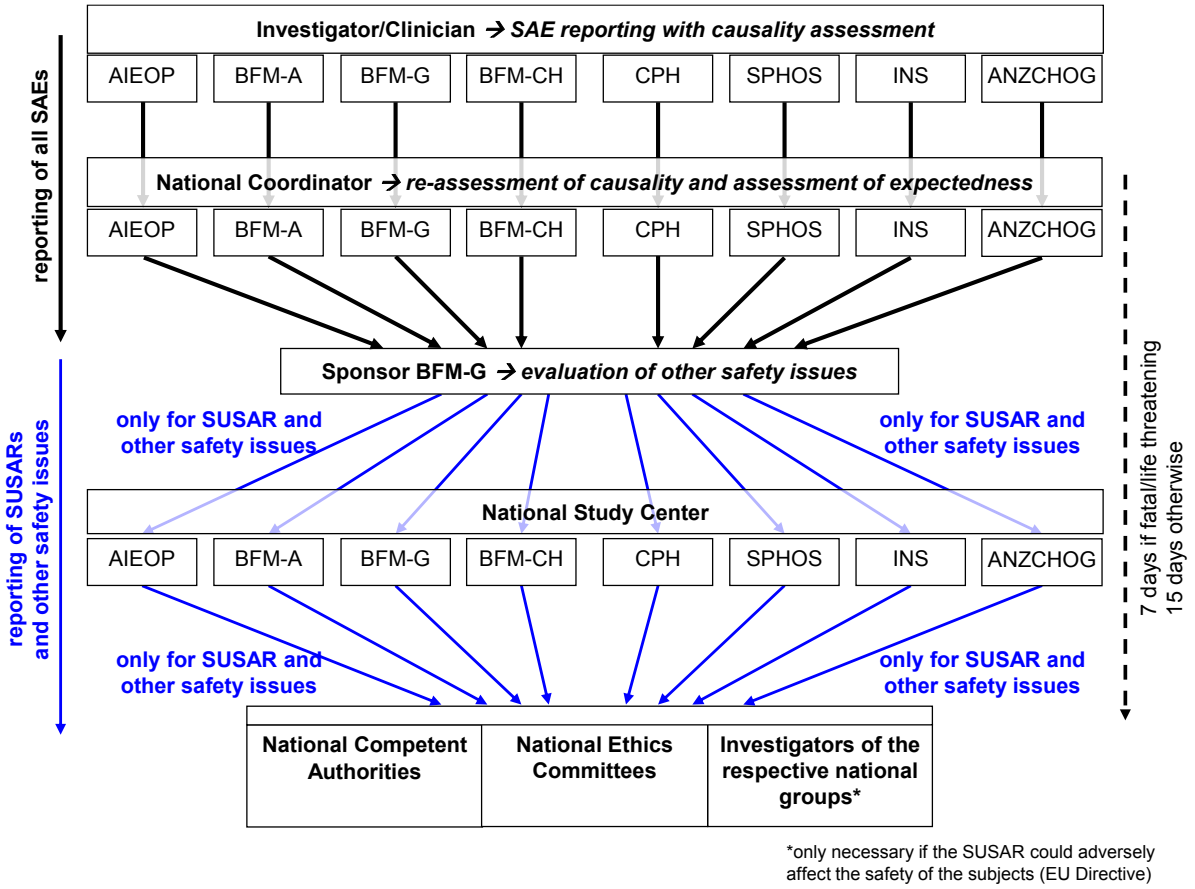


Figure 34 Expedited SAE reporting and SUSAR management in the international trial

12.8 Development Safety Update Report

All Serious Adverse Reactions (SAR), i.e. all SAE with suspected causality to the trial drugs whether expected or not, are annually reported to the national competent authority and the national ethics committee.

An annual safety report of all study groups will be included in the annual report to the common Data and Safety Monitoring Committee (DSMC).

12.9 Data and Safety Monitoring Committee (DSMC)

For tasks and members of the DSMC see section 14.5.5.

13 Statistical Considerations

13.1 Study Questions

There are four randomized questions (see section 4.1):

- **Randomization R-eHR:** Early High-risk (early HR) pB-ALL defined by genetics and/or inadequate treatment response over the course of induction: Can the pEFS from time of randomization be improved by additional therapy with the proteasome inhibitor Bortezomib during an extended consolidation treatment phase compared to standard extended consolidation?
- **Randomization R-HR:** High-risk (HR) pB-ALL defined by genetics and/or inadequate treatment response by the end of consolidation: Can the pEFS from time of randomization be improved by a treatment concept including two cycles of post-consolidation immunotherapy with Blinatumomab (15 µg/m²/d for 2 x 28 days) plus 4 doses intrathecal Methotrexate compared to two conventional highly intensive chemotherapy courses?
- **Randomization R-MR:** Intermediate risk (MR) pB-ALL defined by genetics and intermediate MRD response: Can the probability of disease-free survival (pDFS) from time of randomization be improved by additional therapy with one cycle of post-reintensification immunotherapy with Blinatomomab (15 µg/m²/d for 28 days)?
- **Randomization R-T:** Early non-standard risk (early non-SR) T-ALL patients defined by treatment response over the course of induction: Can the pEFS from time of randomization be improved by the extension of the standard of care consolidation phase by 14 days with an increase of the consolidation cumulative doses of Cyclophosphamide, Cytarabine and 6-Mercaptopurine by 50%?

13.2 Endpoints

13.2.1 Primary Endpoints

For the randomized study questions, the primary endpoint will be the time from randomization until the first event defined as follows:

- Cytomorphological or molecular non-response (resistance to protocol treatment, considered as event at day zero, see definition in section 3.1.4 and 3.1.8), relapse, second malignancy or death from any cause for question R-eHR, R-HR and R-T This will be called EFS time.
- Relapse, second malignancy or death from any cause for question R-MR. This will be called DFS time.

13.2.2 Secondary Endpoints

Secondary endpoints will be:

- Survival starting at the same time point as the EFS/DFS
- Frequency and incidence of treatment-related mortality in induction or CCR
- Frequency and incidence of AE of interest and SAE in specific protocol phases, randomized arms and overall during follow-up
- MRD load after the randomized treatment phases (R-eHR, RHR, R-T)
- MRD load after the first/second cycle of Blinatumomab or after the HR-2'/HR-3' block (R-HR)

- Proportion of patients with poor MRD response to the first Blinatumomab cycle, i.e. MRD after Blinatumomab within the quantifiable range and reduction by less than 1 log compared to MRD after HR-1' ("Blinatumomab Poor-Response") (R-HR)

13.3 Recruitment

The number of patients newly diagnosed each year is expected to be at least 1000, with the following distribution by country:

Austria	50
Australia	70
Czechia	60
Germany	370
Israel	70
Italy	340
Slovakia	30
Switzerland	30
TOTAL	1020

As indicated by calculations in section 13.5, 5 years of full recruitment would be sufficient to answer the study questions.

13.4 Analysis

13.4.1 Study question of all randomizations

The EFS or DFS in the two treatment arms will be compared with the log-rank test stratified by the participating group. A combined estimate of treatment effect in terms of hazard ratio and confidence interval will be given, adjusting by participating group in a Cox model stratified by participating groups, if no significant heterogeneity of the effects will be detected. Treatment effect will be evaluated according to the "intention-to treat" principle for all randomized patients in order to ensure an unbiased estimation. A per-protocol analysis will be performed as a secondary analysis. Prior to starting the randomizations a statistical analysis plan (SAP) will be written. The description of the criteria of eligibility for the per-protocol analysis of the different randomizations will be included in the SAP. Also the safety populations will be specified in the SAP.

13.4.2 General considerations

EFS, DFS and survival curves will be estimated according to Kaplan-Meier (standard errors by Greenwood formula). In the HR group, curves will be done regardless of whether the patient received a transplant. For the comparison of the study with results from other study groups and with previous AIEOP-BFM studies the EFS and survival curve from diagnosis for the entire study cohort will be calculated. The Cox model stratified by group will be applied (after assessment of proportional hazards) to adjust for the impact of prognostic factors not used for stratification purposes such as sex, age and WBC count, overall and within risk/biological subgroups.

The prognostic impact of relevant characteristics (sex, age, WBC, genetics, MRD) and their possible interaction with randomized treatment will be explored by means of the Cox model. The interaction between subsequent randomizations will also be explored, although the study is powered under the assumption of lack of interaction.

Cumulative incidence curves of relapse and toxic deaths (or specific serious adverse events) will be estimated and compared accounting for competing risks.

13.5 Sample size

The group of patients with IKZF1^{plus} (Stanulla et al, 2016, submitted) has an adverse outcome and will be included in this study in the early and the final HR-group. They comprised about 6% of the patients of study ALL-BFM 2000 which have been analyzed. A smaller series of patients from study ALL-BFM 2009 has been analyzed for IKZF1^{plus} (N=735). About 2% of all patients were IKZF1^{plus} but not SR by MRD at time point 1 or HR by other criteria. The 4-years pEFS of patients from study AIEOP-BFM ALL 2000 who have been investigated for IKZF1 and were classified as MR and IKZF1^{plus} was 50%.

13.5.1 Study question R-eHR

According to preliminary results of study AIEOP-BFM ALL 2009 (database frozen in 3/2016), patients who fulfill the criteria of early HR pB-ALL had a 4-year pEFS of 71%. The patients account for about 18% of the patients. IKZF1^{plus} was not included in this calculation since this has only been investigated in a small proportion of the patients. The pEFS of early HR pB-ALL is estimated to be:

(18 x 70% + 2 x 50%) / 20 = 68%

Target sample size: Taking into account 2 interim analyses at 2 and 3 years from randomization, 775 randomized patients (213 events) would be appropriate to detect a difference of 9% (target 4-year pEFS 77%, HR=0.68), with $\alpha = 0.05$ (two sided) and power 0.8, under the assumption of proportional hazards.

Expected sample size: early HR pB-ALL account for 20% of all patients. The percentage of patients who will be randomized is expected to be 80 %. The number of patients who are expected to be randomized in 5 years will be:

5 x 1000 x 0.20 x 0.80 = 800.

13.5.2 Study question R-HR

According to preliminary results of study AIEOP-BFM ALL 2009 (database frozen in 3/2016), patients who fulfill the criteria of HR pB-ALL had a 4-year pEFS of 70%. The patients account for about 14% of the patients. IKZF1^{plus} was not included in this calculation since this has only been investigated in a small proportion of the patients. The pEFS of HR pB-ALL is estimated to be:

(14 x 70% + 2 x 50%) / 16 = 68%

Target sample size: Taking into account 2 interim analyses at 2 and 3 years from randomization, 623 randomized patients (168 events) would be appropriate to detect a difference of 10% (target 4-year pEFS 78%, HR=0.64), with $\alpha = 0.05$ (two sided) and power 0.8, under the assumption of proportional hazards.

Expected sample size: HR pB-ALL patients account for 16% of all patients. The percentage of patients who will be randomized is expected to be 80%. The number of patients who are expected to be randomized in 5 years will be:

5 x 1000 x 0.16 x 0.80 = 640.

13.5.3 Study question R-MR

According to preliminary results of study AIEOP-BFM ALL 2009 (database frozen in 3/2016), patients who fulfill the criteria of MR pB-ALL had a 4-year pDFS of 85%. The patients account for about 37% of the patients. IKZF1+ was not taken into account in this calculation since this has only been investigated in a small proportion of the patients. These patients have to be taken out of MR pB-ALL for this study. The DFS of MR pB-ALL is estimated to be:

(37 x 85% - 2 x 50%) / 35 = 87%

Target sample size: Taking into account 2 interim analyses at 2.5 and 3.5 years from randomization, 1192 randomized patients (125 events) would be appropriate to detect a difference of 5% (target 4-year pDFS 92%, HR=0.60), with $\alpha = 0.05$ (two sided) and power 0.8, under the assumption of proportional hazards.

Expected sample size: MR pB ALL patients account for 35% of all patients. The percentage of patients who will be randomized is expected to be 80 %. The number of patients who are expected to be randomized in 5 years will be:

5 x 1000 x 0.35 x 0.80 = 1400.

13.5.4 Study question R-T

According to preliminary results of study AIEOP-BFM ALL 2009 (database frozen in 3/2016), patients who fulfill the criteria of early non-SR T-ALL had a 4-years pEFS of 79%. The patients account for about 12% of the patients.

Target sample size: Taking into account 2 interim analyses at 2 and 3 years from randomization, 430 randomized patients (69 events) would be appropriate to detect a difference of 10% (target 4-year pEFS 89%, HR=0.54), with $\alpha = 0.05$ (two sided) and power 0.8, under the assumption of proportional hazards.

Expected sample size: early non-SR T-ALL patients account for 12% of all patients. The percentage of patients who will be randomized is expected to be 80 %. The number of patients who are expected to be randomized in 5 years will be:

5 x 1000 x 0.12 x 0.80 = 480.

13.6 Interim Analysis

13.6.1 Study question R-eHR, R-HR and R-T

Two interim analyses are planned for the randomized questions R-eHR, R-HR and R-T on EFS at 2 and 3 years from randomization. If any of the boundaries are reached, further continuation or modification of the trial will have to be discussed by the DSMC and study committee. Calculation of sample size for study question R-eHR, R-HR and R-T accounts for the 2 planned interim analyses (nominal p-values for overall type I error of 0.05 (O'Brien-Fleming boundaries)).

	p value	Years from start of randomization
First analysis	0.00006	2
Second analysis	0.001	3

13.6.2 Study question R-MR

Two interim analyses are planned for the randomized question R-MR on DFS at 2.5 and 3.5 years from randomization. If any of the boundaries are reached, further continuation or modification of the trial will have to be discussed by the DSMC and study committee. Calculation of sample size for study question R-MR accounts for the 2 planned interim analyses (nominal p-values for overall type I error of 0.05 (O'Brien-Fleming boundaries)).

R-MR	p value	Years from start of randomization
First analysis	0.0004	2.5
Second analysis	0.003	3.5

13.7 Safety rules on mortality

Guidelines are designed to ensure that the trial will be stopped as early as possible if its application is associated with a treatment-related mortality higher than acceptable in standard treatment of ALL. Treatment-related mortality has been accounted for in terms of deaths in CCR (after chemo or HSCT) separately.

The method applied in the case of death in CCR during therapy follows an approach based on the sequential probability ratio test (SPRT). The procedure considers the following hypothesis based on the rate of death in CCR expressed as number of cases per 1000 person/years of observation:

$$H_0: \{ \lambda \geq \lambda_0 \}$$

$$H_1: \{ \lambda \leq \lambda_1 \}$$

We wish that the failure rate does not go beyond the alarm level as expressed in H_0 (given that we expect, based on historical data, the rate expressed in H_1). The stopping bounds reported in the following tables (lower boundary) are to be interpreted as follows: if, given the number of observed death in CCR at a given time, the corresponding observed cumulative follow-up time (in years) is inferior to the boundary then the alarm level is reached because the evidence from accumulating data suggests that the rate is higher than an acceptable level.

The boundaries calculations are based on the following choices: exponential model for the time to failure, with constant failure rate λ, type I error of 0.05 and type II error of 0.20.

For mortality in CCR during therapy, analysis will be run separately for patients treated in each arm of R-eHR. Given that in similar patients recruited in AIEOP-BFM ALL 2009, the observed mortality rate in CCR (λ_1) was 0.032 over 1000 persons/years (42 deaths over 1313), the boundaries were approximately set to a rate of 0.05 (λ_0).

Guidelines for early stopping rules due to mortality in CCR – R-eHR

N. of deaths in CCR	Cumulative years of follow-up
10	**159**
11	**183**
12	**208**
13	**233**
14	**258**
15	**283**
16	**307**
17	**332**

N. of deaths in CCR	Cumulative years of follow-up
18	**357**
19	**382**
20	**407**
21	**431**
22	**456**
23	**481**
24	**506**
25	**530**

For mortality in CCR during therapy, analysis will be run separately for patients treated in each arm of R-HR. Given that in similar patients recruited in AIEOP-BFM ALL 2009, the observed mortality rate in CCR (λ_1) was 0.04 over 1000 persons/years (42 deaths over 1054), the boundaries were approximately set to a rate of 0.06 (λ_0).

Guidelines for early stopping rules due to mortality in CCR – R-HR

N. of deaths in CCR	Cumulative years of follow-up	N. of deaths in CCR	Cumulative years of follow-up
10	122	18	284
11	143	19	305
12	163	20	325
13	183	21	345
14	203	22	366
15	224	23	386
16	244	24	406
17	264	25	426

For mortality in CCR during therapy, analysis will be run separately for patients treated in each arm of R-MR. Given that in similar patients recruited in AIEOP-BFM ALL 2009, the observed mortality rate in CCR (λ_1) was 0.009 over 1000 persons/years (25 deaths over 2725), the boundaries were approximately set to a rate of 0.015 (λ_0).

Guidelines for early stopping rules due to mortality in CCR – R-MR

N. of deaths in CCR	Cumulative years of follow-up	N. of deaths in CCR	Cumulative years of follow-up
10	584	18	1265
11	669	19	1351
12	755	20	1436
13	840	21	1521
14	925	22	1606
15	1010	23	1691
16	1095	24	1776
17	1180	25	1861

For mortality in CCR during therapy, analysis will be run separately for patients treated in each arm of R-T. Given that in similar patients recruited in AIEOP-BFM ALL 2009, the observed mortality rate in CCR (λ_1) was 0.028 over 1000 persons/years (24 deaths over 848), the boundaries were approximately set to a rate of 0.05 (λ_0).

Guidelines for early stopping rules due to mortality in CCR – R-T

N. of deaths in CCR	Cumulative years of follow-up
10	190
11	217
12	243
13	269
14	296
15	322
16	348
17	375

N. of deaths in CCR	Cumulative years of follow-up
18	401
19	428
20	454
21	480
22	507
23	533
24	559
25	586

Tables are limited to the first 25 deaths observed in randomized patients during recruitment, but extended calculations for the full recruitment are available for the DSMC.

13.8 Safety rules on toxicity

Reporting on serious adverse events (SAE) and on adverse events (AE) of clinical interest will take place periodically in the form of tables and rates by treatment arm (per-protocol analysis) and will be revised by the Data Monitoring Committee (once a year or more often if required by the DSMC).

14 Organizational Aspects and Quality Assurance

14.1 Study design

This is an international collaborative multicenter open-label randomized clinical trial (phase III) conducted in 8 study groups in 8 EU and non-EU countries. For regulatory purposes, the sponsorship is taken over by the University Hospital Schleswig-Holstein, Germany.

14.2 Registration

All patients who fulfill the eligibility criteria to the study (see section 5.2) and have been admitted to one of the participating centers are registered as study patients if the informed consent has been obtained.

14.3 Patient Education/Written Consent

Before enrolment, every patient will receive full oral and written information about the nature, purpose, expected advantages and possible risks of the trial. The patient and his parents/legal guardians will agree to participation in the trial by signing the informed consent form. The parent(s) or a legal guardian must read, sign, and date the consent form before his or her child enters the trial, takes study treatment or undergoes any study-specific procedures. If the child is able to comprehend the study, he/she will also sign an informed consent form.

Informed consent for participation in the randomized parts of the study is given separately from the consent to the enrollment into the study. The patients and parents (or persons entitled to custody) have the choice to participate in the randomization or not to be

randomized and to be treated in the control arm. After consent has been given and the patient has been randomized, consent can be withdrawn in any moment. Randomizations can be performed when the diagnostic findings, required for stratification into the respective treatment group, are complete and the informed consent for randomization has been obtained.

Patients will be informed that their disease-related data will be saved for scientific purpose (publication, etc.) using a pseudonym. Consenting patients have got the right to be informed about the data recorded. Patients will also be informed that their pseudonymized data will be transferred to the responsible competent authorities and to the ethics committees, in accordance with legal notification obligation for drug safety. Patients, who disagree with the process of data transfer, are not allowed to participate in this study.

14.4 Data collection and processing

All patient related data will be recorded in the electronic database. Every patient will receive a pseudonym which will be unique for this individual patient. The database provides a confidential list, which relates these patient numbers to the patients' full name. All data will be recorded in an electronic Case Report Form. The trial software used is based on a system provided by the CINECA consortium in the AIEOP group and on the MARVIN system provided by XClinical Inc. in the other participating countries. The CRFs will be completed by an authorized person (defined in the study team log). They will be checked, dated and signed electronically by the investigator. All data will be recorded online. Data will be transferred between the workstation computer at the study site and the study server via a secure connection (secure socket layer /SSL), so that the data cannot be manipulated.

Each group will be responsible for ensuring the quality of its own data. The biometrical center in Hannover and Monza will file all data electronically. To verify accuracy of the data, range, validity and consistency checks will be performed automatically by the database and additionally by the biometrical center. A set of variables, necessary for the analysis of the trial aims, is harmonized between both systems in order to guarantee the common core data set. The data will be pooled annually in the common database which will be used for the trial aims only.

14.5 Organizational structure of the trial

Each group will appoint a National Coordinator, who is per contract responsible for the sites in his country and takes on the sponsor responsibility of fulfilling all country-specific regulatory requirements of competent authorities and ethics committees concerned, particularly concerning approval, authorization and pharmacovigilance. Each group will keep its own internal organization for study conduct, data collection and randomization; regularly the groups will pool data for a combined evaluation of results. To this aim, a shared data file will be generated for each of the randomized questions and it will be evaluated by centers for statistical analysis in Monza (supervised by M.G. Valsecchi) and Hannover (supervised by M. Zimmermann).
The Trial Steering Committee will supervise the study conduct in terms of entry of patients and adhesion to protocol on the national level and will collaborate with the Trial Data Analysis Committee for data collection and review.
Furthermore, each group will maintain its own Scientific Committee (National Study Committee) for national study coordination (Figure 35).

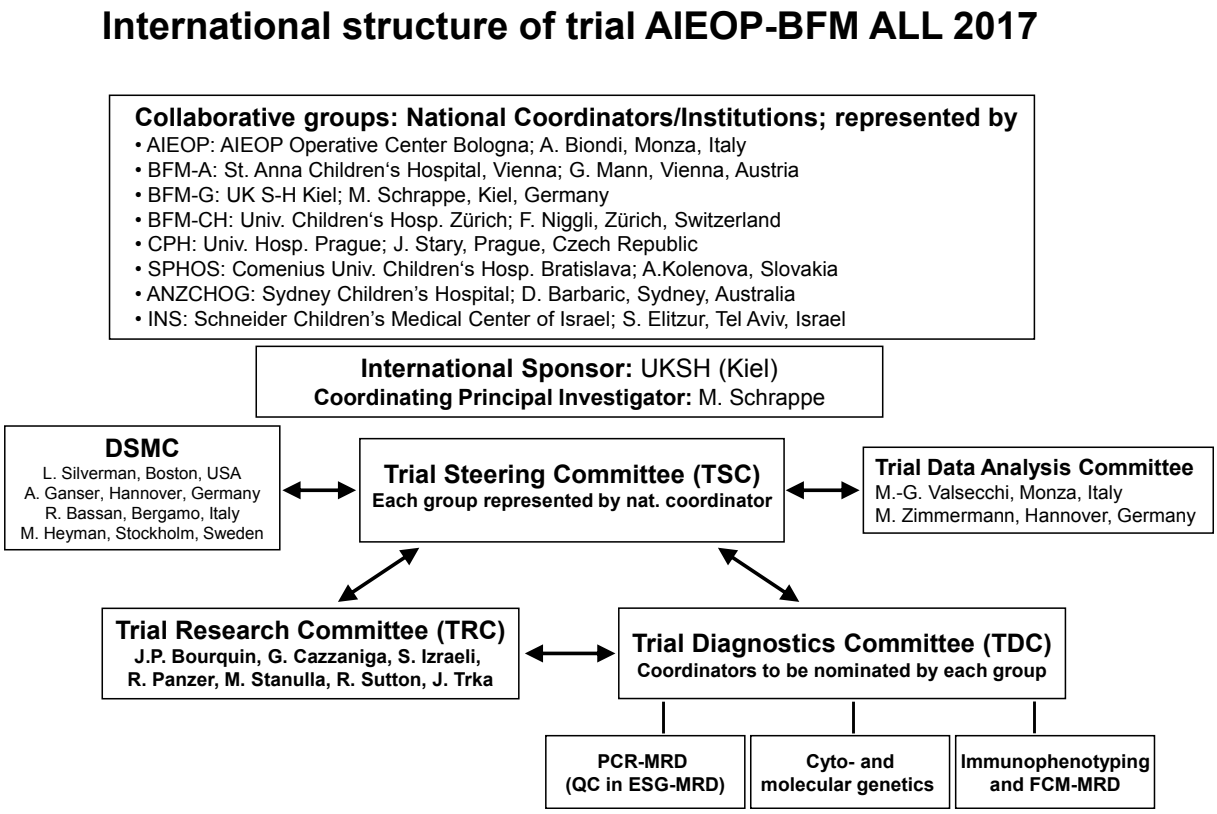


Figure 35 International structure of trial AIEOP-BFM ALL 2017

14.5.1 Trial Steering Committee (TSC)

The National Coordinators form the Trial Steering Committee (TSC). TSC will be responsible for every final decision regarding the study, as well as publication of results.

The TSC will consider the recommendations by Data and Safety Monitoring Committee (see below), concerning safety of patients enrolled in the study, and the possible proposal on protocol modifications.

TSC will periodically assemble (every year or more frequently if necessary).

14.5.2 Trial Research Committee (TRC)

The TRC has been set up to coordinate all research activities developed by members of the participating study groups and to support the TSC in study conduct. It is formed by one coordinator, each, assigned by the national groups (see Figure 35). The TRC will work on the basis of mutual agreements regarding listing of projects, responsibilities in intergroup projects, and publications. The TRC will also collaborate very closely with the TDC.

14.5.3 Trial Diagnostics Committee (TDC)

The TDC has been established to coordinate the leukemia specific diagnostics. The main aims will be standardization in all steps of diagnostics and to implement a robust quality assurance system. Due to the complexity of diagnostics, subgroups for the various diagnostic fields have been formed (see Figure 35).

14.5.4 Trial Data Analysis Committee (TDAC)

The Trial Data Analysis Committee (TDAC) is formed by the statisticians of the core groups AIEOP and BFM-Germany, who are responsible for the evaluation of the common data. The partner groups provide data to the TDAC for the common analysis. The TDAC will produce the yearly study progress reports (recruitment, toxicity etc.), and will perform the interim analyses when planned. Reports will be submitted (blinded) to the trial steering committee and DSMC. Interim analyses will be submitted (blinded) to the steering committee and (unblinded, if specifically required) to the DSMC.

14.5.5 Data and Safety Monitoring Committee (DSMC)

The TSC will appoint 4 persons not involved in the study and known as experienced in statistics, biology, pediatric onco-hematology and pediatrics to form the DSMC. This committee will be responsible

- to evaluate the “ad interim” results, with particular attention to adverse events and/or to possible higher efficacy of one treatment.,
- to minimize the possible risks for patients,
- to recommend possible modifications of protocol necessary to assure patients safety and a correct study conduction.

DSMC will periodically (every year or more frequently if necessary) revise study conduction, safety reports and planned interim analyses. Following each data revision, DSMC will suggest to TSC specific recommendations concerning the opportunity to continue with the patient recruitment or the possible modifications to protocol or the necessity to interrupt specific items of protocol. These recommendations could be also based on results produced by other important studies.

DSMC members will be:

Prof. Dr. L. B. Silverman, Boston, USA (MD)
Prof. Dr. A. Ganser, Hannover, Germany (MD)
Dr. Renato Bassan, Bergamo, Italy (MD)
Prof. Dr. M. Heyman, Stockholm, Sweden (MD)

14.6 Monitoring of Trial Activity and Data Quality

The purpose of the monitoring is to systematically examine all the study activities and documents to screen, if these activities were conducted, and data recorded, and accurately reported according to the protocol, to Good Clinical Practice (GCP), to guidelines of the International Conference on Harmonization (ICH), and any applicable regulatory requirements. General quality requirement of this study is a combination of a centralized and an on-site monitoring in the trial centers to be able to guarantee a highest level of patient safety in accordance to the ICH and a high level of data quality. Recurrent international and national investigator meetings will be held before and throughout the study conduct for the general information flow and for training purposes.

The sponsor authorises in a signed agreement the National Chairperson of each participating country to perform the sponsor duties through to third parties on behalf of the University Hospital Schleswig-Holstein. This authority is limited to those sponsor duties, which arise in the context of the performance of this clinical trial and include also the monitoring concept and implementation in the respective country. The Sponsor provides a Monitoring Manual template including the general scheme of the study Monitoring. This should be adopted in each country according to the individual legal requirements of the respective country.

With the investigator agreements the investigators allow the monitor to have access to all the study material needed for source data verification and proper review of the study process. At

all times, the sponsor/investigators/monitors will maintain the confidentiality of the study documents.

14.6.1 Sample Selection

A minimum scope of subject data to be monitored at each visit will be defined in the Monitoring Manual. In case of frequent protocol violations, incomplete documentation, unanswered queries or other problems, additional patients will be monitored.

14.6.2 Scope of Monitoring

In the course of preparations for the monitoring visit part of the documents will be centrally monitored in the study coordination centre.

The recruitment status in the study centre and the patient consents to participate at the study, add-on studies, randomisations and the data transmission will be checked. The actually randomised patients will be compared to the eligible patients for each randomisation in advance. The main emphasis of on-site monitoring should lie on the following topics which will be processed in form of a standard monitoring-check-list:

- patient identity (name, date of birth, sex)
- inclusion/exclusion criteria
- informed consent (signatures, version)
- patient status (alive, deceased, relapse, second neoplasm(s)

For a predefined number/percentage of patients in every trial site the following topics will be monitored:
- pre-selected data of the baseline assessment relevant for the study objectives
- therapy toxicity in randomised therapy elements
- therapy documentation in randomised therapy elements
- administered therapy elements
- basic data of the reported SAEs
- screening of the patient file for not reported SAEs

In addition, on-site monitoring visits ensure that the study is performed according to ICH-GCP, and that the protocol is adhered to. The Investigator Site File will be checked for completeness and timeliness at every monitoring visit. On-site monitoring plays an important role in the support and training of participating trial sites.

14.6.3 Frequency of Monitoring Visits

Frequency of on-site monitoring visits will be based on the pre-defined monitoring strategy taking into consideration: enrollment status, data quality, protocol compliance, and the prescribed amount of data to be monitored according to the Monitoring Manual. A minimum scope of subject data to be monitored at each visit will be defined in the Monitoring Manual. In case of frequent protocol violations, incomplete documentation, unanswered queries or other problems, additional monitoring visits should be performed.

14.6.4 Monitoring Report

Monitoring visit findings and resulting action items will be documented in reports based on the standard monitoring-check-list. The report will be read and acknowledged by a representative of the study coordination team or the national coordinator. In any case the principal investigator of the trial site will be addressed with the visit report including actions to be taken (e.g. corrections/File Notes/Training).
Once the visit documents are finalized, these documents should be printed, and stored in the TMF of the respective country.

14.7 Audits/Inspections

According to the European legislation, inspections of the trial sites may be performed by the competent authorities at any time during or after completion of the trial. By signing the informed consent form the participants allow access to their medical records. Auditors and inspectors are bound by professional confidentiality and may not pass on any personal information that comes to their knowledge.

15 Ethical, Regulatory and Administrative Principles

15.1 GCP

The trial is conducted in accordance with the principles of Good Clinical Practice and the Declaration of Helsinki, last revised by 64th WMA General Assembly, Fortaleza, October 2013).

15.2 Statutory Rules

Before entering into the trial and prior to registering the first patient, clinicians must ensure that they have ethical and regulatory approval to participate in the trial according to their national guidelines.

The national coordinators are responsible for the compliance with national regulations concerning:

- approval of Ethics Committee,
- approval of competent authority,
- notification to regional authorities,
- Informed consent,
- data privacy and confidentiality,
- insurance of the patients,
- pharmacovigilance,
- notification of substantial amendments,
- notification after end or on an early termination of the clinical trial.

15.3 Patient Insurance

Patient insurances are contracted by the national coordinators. The details and the policy are attached in the national appendix.

15.4 Financing

The national coordinators are responsible for an adequate financing of the trial in their country.

15.5 Final Report

The composition of a final integrated report will be conducted in accordance with ICH E3: Structure and Contents of Clinical Study Reports. After closure of the biometrical analysis the trial manager, the sponsor’s representative and/or members of the trial steering committee will compose an integrated report. This report contains a clinical record, a statistical record, single value tables and conclusions.

15.6 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 (Altman*, et al* 2001, Zangari*, et al* 2011) and include a thorough safety analysis. Data relating to the study must not be reported or published without prior consultation with the Trial Steering Committee.

16 Add-on Studies

Recently, an enhanced understanding of the molecular pathology of ALL gained from genomic studies has facilitated the development of better refined treatment stratification strategies for discrete molecular subclasses of ALL (e.g. Ph+, Ph-like, IKZF1^{plus}). It remains a major challenge to translate such findings into better risk stratification, and ultimately into individualized and truly targeted treatment strategies in the form of precision medicine.

Trial AIEOP-BFM ALL 2017 will be the first upfront evaluation of targeted immunotherapy in childhood ALL. Based on treatment with a bispecific antibody using a highly efficacious construct called Blinatumonab, this trial provides the opportunity to understand the basic mechanisms of response and resistance to this completely novel approach. In addition, this trial aims at another goal of precision medicine which is to deliver the most appropriate therapy to the patient on the basis of clinical and molecular features of the individual disease. Thus, targeted inhibitors of tyrosine kinases will be applied in case of refractory or resistant disease in cases with targetable lesions. These novel therapeutic strategies also pave the way for a better understanding of molecular mechanisms likely to trigger innovation in clinical-trial strategies.

The AIEOP-BFM ALL study group has a long history of successful collaborative research into basic mechanisms of leukemia development, translational projects to improve diagnosis, outcome prediction and therapeutic approaches. Trial AIEOP-BFM ALL 2017 will also serve as an intermediate platform in the transition phase from stratified medicine to precision medicine. This transition phase will be brought to life by stimulating, coordinating and integrating research activities. Thus, trial AIEOP-BFM ALL 2017 will be the research platform to pave the way to precision medicine in childhood ALL.

Priority research activities in AIEOP-BFM ALL 2017 will focus on and integrate the following areas:

1. Molecular characterization of mechanisms of response and resistance
2. Pre-clinical models to investigate novel therapeutic strategies
3. Germline genetic variants predisposing to ALL and influencing treatment response and toxicity
4. Influence of host immune system for outcome of targeted immunotherapy
5. Effect of host commensal microorganisms for leukemia development and toxicity
6. Integrated genomic platform – merging clinical, diagnostic and research information for better outcomes
7. Novel approaches for diagnostics and treatment of CNS-involvement
8. Improved response diagnostics.

Aims of add-on studies in AIEOP-BFM ALL 2017 are:

1) An enhanced understanding of mechanisms responsible for response and resistance to standard treatment and to targeted immunotherapy is essential for optimized stratification and improved outcome. A major focus of these research projects will be to decipher the portfolio of clinically relevant druggable targets for implementation in future rational individualized treatment strategies for childhood ALL.

2) Different groups in AIEOP-BFM ALL have generated NSG mice to expand the leukemic cell population and to investigate disease mechanisms. Most importantly, these disease models that require transplantation of primary ALL blasts enable *in vivo* evaluation of new targeted treatment approaches. Using this approach, novel targeted treatment strategies like ABT-199 for the treatment of t(17;19)/*TCF3-HLF* positive ALL have been developed (Fischer*, et al* 2015).

3) During the last few years, it has been becoming clear that in a significant proportion of childhood ALL there is a leukemia predisposition caused by germline mutations. Prominent examples are *TP53* mutations in hypodiploid ALL, mutations in genes relevant for lymphocytic development like *PAX5* in pB-ALL, and mutations in DNA repair genes

like *ATM* in T-ALL. The identification of germline variants in these genes may be relevant for treatment strategies, i.e. avoidance of radiation. Further studies are needed to define how to implement underlying ALL-predisposing genetic constellations into ALL treatment strategies and into the management of the diseased child and his/her family. Moreover, germline variations were shown to influence leukemia development as well as treatment response and toxicity (e.g. TPMT gene). Further systematic analysis of germline variations with regard to treatment response and toxicity will be performed.

4) Host immune systems may have a major impact on response to targeted immunotherapy in childhood ALL. Deciphering the interaction between host immune system and modifications induced by the bispecific antibody Blinatumonab will be of particular importance since immunotherapy is integrated in AIEOP-BFM ALL 2017 protocol for the first time as part of frontline treatment.
5) Infection exposure seems to be a causal factor in pB-ALL development. As demonstrated by a transgenic mouse model (*Pax5* heterozygous mice), pB-ALL was initiated only when the mice were exposed to common pathogens (Martin-Lorenzo*, et al* 2015). To gain further insight into the role of the microbiota for pathomechanisms of ALL and clinical endpoints (e.g., leukemia cell kinetics, treatment toxicity), these investigations have to be extended to humanized mouse models and clinical samples, for example, through delineation of microbiota crosstalk with tumor and immune cells.
6) For the future, we envisage that increasing numbers of genetic markers are going to be identified in childhood ALL. Therefore, we aim to develop an integrated DNA/RNA sequencing platform allowing to detect all relevant genetic markers in one investigational step. Before these methods can be implemented into clinical diagnostics, the diagnostic sensitivity and specificity has to be determined in samples with defined genetic markers. Moreover, cross-laboratory validation and prospective comparison of established diagnostic procedures with the newly developed integrated genomic platform have to be performed. To make use of these large data sets, integrated data management infrastructure is urgently needed and will be implemented during the first 2 years of the AIEOP-BFM ALL 2017 trial.
7) Detection of CNS-involvement is still a diagnostic challenge with unknown clinical implications. Current definitions and diagnostics rely on techniques which were introduced several decades ago. Considering the knowledge about CNS-involvement in ALL and the possible interactions to the environment and immune system require prospective validation and development of novel approaches for diagnostics and treatment (Kaiser*, et al* 2013, Krause*, et al* 2015).
8) Detection of MRD has been a major step forward in refining treatment in ALL. This development both in PCR- and FCM-based technologies succeeded due to robust quality assessment. The consortium is planning to introduce novel technologies (e.g. next generation sequencing) for faster target identification and higher sensitivity. Flow cytometry will utilize integrated information technology to improve sensitivity of this approach. Both areas will be explored extensively to pave the way for improved response diagnostics in the future.

Many additional developments need to be addressed in similar ways and demonstrate the need for coordinated integrated approaches beyond consideration of druggable aberrant pathways of the tumor cell.

Research workshops organized by the AIEOP-BFM Study Offices will be conducted on a regular basis to allow coordinated access and to secure timely integration of potentially relevant novel findings into the trial research infrastructure. A workshop one year before official start of the clinical trial will provide a detailed research agenda related to the topics and aims described above and will be laid down in a trial-associated research protocol.

Beyond significance for childhood ALL, insights into mechanisms of response and resistance will be enhance our understanding of other childhood and adult cancer, ultimately leading to cure with individualized and truly targeted treatment strategies.

17 List of Abbreviations

6-MP	6-mercaptopurine
AE	adverse event
AIEOP	Associazione Italiana Ematologia ed Oncologia Pediatrica
ALL	acute lymphoblastic leukemia
alloHSCT	allogeneic hematopoetic stem cell transplantation
ANZCHOG	Australian and New Zealand Children's Haematology/Oncology Group
ARA-C	Cytarabine
ASP	Asparaginase
BFM	Berlin-Frankfurt-Münster
BM	bone marrow
BSA	body surface area
CCR	continuous complete remission
CIVI	continuous intravenous infusion
COG	Children's Oncology Group
CORS	Centro Operativo Ricerca Statistica
CNS	central nervous system
CPH	Czech Pediatric Hematology Working Group
CPM	Cyclophosphamide
CR	complete remission
CRO	Clinical Research Organisation
CRT	cranial radiotherapy
CSF	cerebrospinal fluid
CVL	central venous line
DEXA	Dexamethasone
DFS	disease-free survival
DI	DNA index
DIC	disseminated intravascular coagulation
DNR	Daunorubicin
DNX	Daunoxome
DOX	Doxorubicin
DSMC	Data Monitoring and Safety Committee
EF	ejection fraction
EFS	event-free survival
FCM	flow cytometry
FLU	Fludarabine
GCP	Good Clinical Practice
G-CSF	Granulocyte colony stimulating factor
HD-ARA-C	high-dose ARA-C
HD-MTX	high-dose MTX
HR	high risk
i.m.	intramuscular
i.th.	intrathecal
i.v.	intravenous
I-BFM-SG	International BFM study group
ICH	International Conference on Harmonization
IFO	Ifosfamide
Ig	immunoglobulin
INS	Israel National Studies
KKS	Koordinierungszentrum für Klinische Studien
LP	lumbar puncture
LV-SF	left-ventricular shortening fraction
mAB	monoclonal antibody
MD	matched donor

MFD	matched family donor
MMD	mismatched donor
MR	medium risk
MRD	minimal residual disease
MSD	matched sibling donor
MTX	Methotrexate
NCI	National Cancer Institute
p.i.	per infusionem
p.o.	per os
pB-ALL	precursor B cell ALL
PCR	polymerase chain reaction
pCRT	preventive cranial radiotherapy
pDFS	probability of disease-free survival
pEFS	probability of event-free survival
PEG	Polyethylenglykol
PEG-L-ASP	PEG-L-asparaginase
PGR	Prednisone Good-Response
Ph+	Philadelphia chromosome-positive
PPR	Prednisone Poor-Response
PRED	prednisone/prednisolone
pts	patients
R	randomization
SAE	serious adverse event
SAR	serious adverse reaction
SCT	stem cell transplantation
SE	standard error
SER	Slow Early Responder/Response
SIADH	Syndrome of inadequate ADH secretion
SmPC	summary of medicinal product characteristics
SR	standard risk
SUSAR	suspected unexpected serious adverse reaction
TCR	T-cell receptor
tCRT	therapeutic cranial radiotherapy
TDAC	Trial Data Analysis Committee
TDC	Trial Diagnostics Committee
TG	thioguanine
TP	time point
TPMT	Thiopurine methyltransferase
TRC	Trial Research Committee
TSC	Trial Steering Committee
UNL	upper normal limit
VCR	Vincristine
VDS	Vindesine
VOD	veno-occlusive disease
VP-16	Etoposide
WBC	white blood cell count
WHO	World Health Organization

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