

IntReALL SR 2010

**International Study for Treatment of
Standard Risk Childhood Relapsed ALL 2010**

**A randomized Phase III Study Conducted by the
Resistant Disease Committee of the International BFM Study Group**

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Sponsor: Charité - Universitätsmedizin Berlin
Campus Virchow Klinikum
Augustenburger Platz 1
13353 Berlin

Confidentiality

The information contained in this protocol has to be kept strictly confidential. Therefore the protocol is only provided to Investigators in confidence for review, to study staff, Independent Ethics Committee/Institutional Review Board, regulatory authorities and CRO's (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

Charité - Universitätsmedizin Berlin

Augustenburger Platz 1, 13353 Berlin,
Germany

**Coordinating Principal Investigator
and representative of the sponsor in
Germany**

Dr. med. Arend von Stackelberg

Paediatric Oncology/Haematology, Charité –
Universitätsmedizin Berlin, Augustenburger
Platz 1, 13353 Berlin, Germany

Date: 08.11.2012

Signature: 

Study Co-chair

Prof. Vaskar Saha

Paediatric and Adolescent Oncology
Manchester Academic Health Sciences Centre
Central Manchester and Manchester
Children's NHS Trust, Christie Hospital,
Wilmslow Road, Manchester, M20 4BX, UK

Date: _____

Signature: _____

Statistician

Sharon Love

Centre for Statistics in Medicine
Wolfson College, Linton Road
Oxford, OX2 6UD, UK

Date: _____

Signature: _____

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1 SYNOPSIS

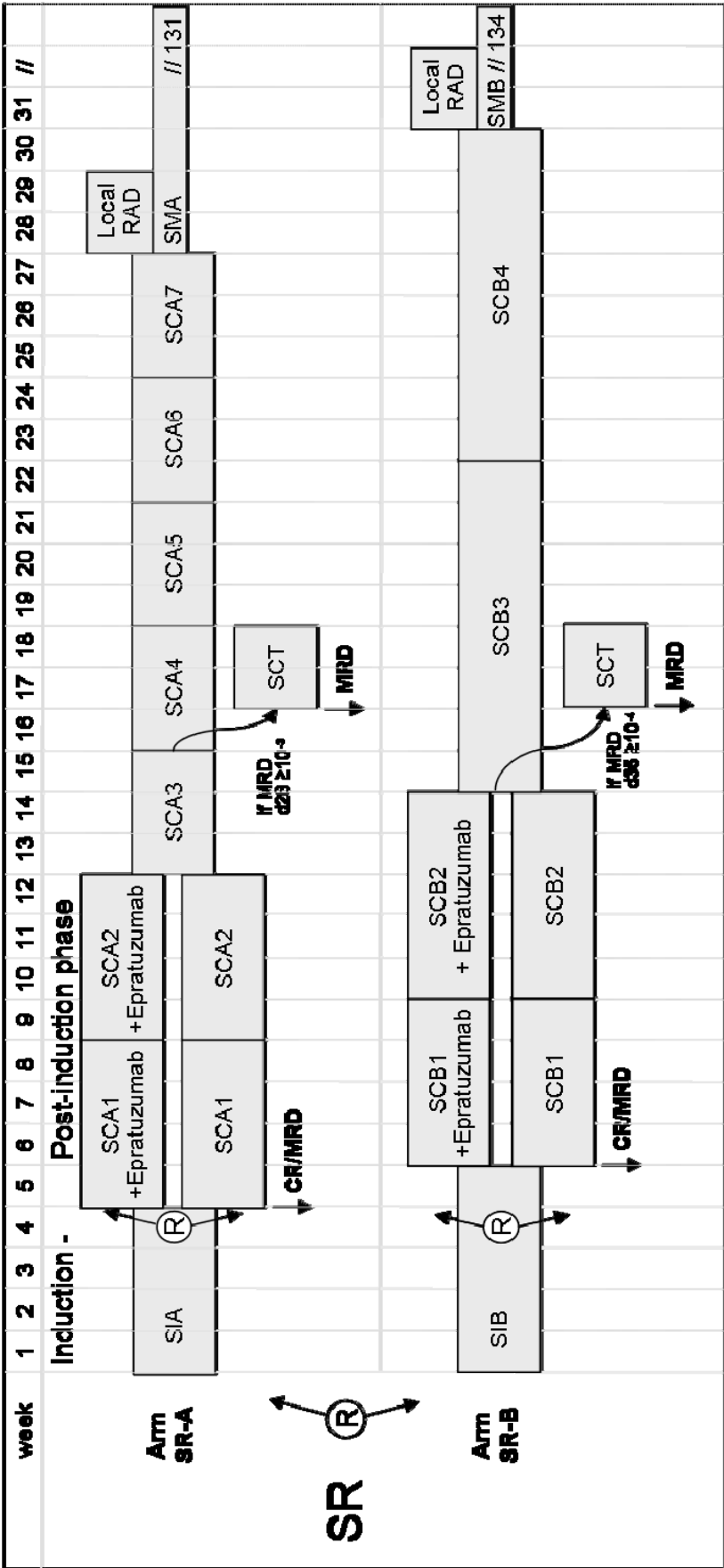
Title:	IntReALL SR 2010 International Study for Treatment of Standard Risk Childhood Relapsed ALL 2010
Principal Investigators:	Coordinating PI: A von Stackelberg, Berlin / Germany; Co-chair: V Saha, Manchester / UK T Revesz, Australia G Mann, Vienna / Austria A. Ferster, Brussels / Belgium L Sramkova, Prague / Czechia T Frandsen, Copenhagen / Denmark P Lähteenmäki, Turku / Finland P Rohrlach, Besancon / France O Smith, Dublin / Ireland R. Elhasid, Tel Aviv / Israel F Locatelli, Rome / Italy C Ogawa, Tokyo / Japan P Hoogerbrugge, Nijmegen / Netherlands M Hellebostad, Oslo / Norway E Gorczynska, Wroclaw/ Poland J Duarte, Lisbon / Portugal S Soderhall, Stockholm / Sweden JP Bourquin, Zurich / Switzerland D Bonney, Manchester / UK
Sponsor:	Charité – Universitätsmedizin Berlin / Germany
Statistician:	S Love, Centre for Statistics in Medicine, Oxford / UK
Data management:	A Barth, Heidelberg / Germany; Marvin RDE data base
Study centres:	See list Appendix
Study timetable:	Planned start: August 1, 2012 4 years recruitment until July 31, 2016 3 years of follow up after inclusion of last patient until July 31, 2019
Background	Though survival of children with acute lymphoblastic leukemia (ALL) has considerably improved over the past few decades, relapsed ALL remains a leading cause of mortality in children with cancer. Given the rarity of the disease, prospective clinical trials need to be coordinated within an international cooperative group such as the International BFM Study Group (I-BFM-SG). Within the group, over the last few years two different treatment protocols, ALL-REZ BFM 2002 and ALL R3 have been used by most study groups for treatment of relapsed ALL. Both trials have produced comparable results. The trials risk stratified patients based on duration of first remission, immunophenotype, site of relapse and post induction minimal residual disease (MRD) levels to identify patients who should be transplanted. For non-HR or standard risk (SR) patients both ALL-REZ BFM 2002 and ALL R3 have achieved better results than previous trials. Both protocols have however been primarily used in patients relapsing off different frontline protocols. Thus there is need for a prospective randomized controlled comparison across the study groups (randomization 1), before a uniform backbone for further trials can be developed. In SR patients, survival may be improved by modifying the consolidation therapy using targeted non-myelotoxic drugs. As ideal candidate, epratuzumab (humanised chimeric anti CD22 antibody) will be randomly tested in combination with conventional chemotherapy (randomization 2). CD22 is well expressed in all B-cell precursor ALL cells. Epratuzumab has been developed in combination phase I and II trials in childhood relapsed ALL and has shown a favourable toxicity profile and moderate antileukemic activity.

IntReALL SR 2010

Objectives:	<p><u>Primary objectives:</u></p> <ul style="list-style-type: none"> - Overall: Improvement of event-free survival (EFS) probabilities in childhood relapsed ALL - Randomization 1: EFS of Arm A (ALL-REZ BFM 2002) versus B (ALLR3) in SR patients - Randomization 2: Influence of epratuzumab on EFS in consolidation of SR patients <p><u>Secondary objectives:</u></p> <ul style="list-style-type: none"> - OS of Arm A (ALL-REZ BFM 2002) versus B (ALLR3) in SR patients - Influence of epratuzumab on OS in consolidation of SR patients - Rate of second complete remission (CR2) of Arm A versus Arm B - Rate of SCT performed in Arm A versus Arm B - Toxicity of randomized SR arms A versus B - Toxicity of consolidation with versus without epratuzumab - Improvement of MRD reduction during consolidation with versus without epratuzumab - Rate of MRD negativity prior to SCT with Arm A vs. Arm B - Rate of MRD negativity prior to SCT after consolidation with versus without epratuzumab - Pharmacokinetic of epratuzumab in context with arm A and arm B
Risk group stratification:	<p><u>Definition of standard risk group:</u></p> <p>Late isolated B-cell precursor (BCP) bone marrow (BM) relapse, late/early combined BCP BM relapse, any late/early isolated extramedullary (EM) relapse</p>
Study design:	<p>The IntReALL SR 2010 trial is an inter-group, international multi-centre, treatment optimization trial. It contains the followings branches:</p> <ul style="list-style-type: none"> - SR induction/consolidation arm A (ALL-REZ BFM 2002, arm protocol II-IDA) versus B (UK-ALL-R3, arm MITOX): prospective, randomized, open label, phase III trial - SR consolidation +/- epratuzumab: prospective, randomized, open label, phase III trial
Primary endpoints:	<ul style="list-style-type: none"> - SR induction/consolidation ALL-REZ BFM 2002 versus UK-ALL-R3 (randomisation 1): 10% pEFS superiority of arm B above a 65% pEFS at 4 years of arm A - SR consolidation +/- epratuzumab (randomisation 2): 10% pEFS superiority of the arm with epratuzumab above an expected 74% pEFS at 4 years of the standard arm
Statistical analysis:	<p><u>Analysis of primary outcomes:</u></p> <ul style="list-style-type: none"> - SR induction/consolidation: a cox analysis of treatment effect on EFS adjusting for the factors used in the randomisation stratification - SR consolidation +/- epratuzumab: a cox analysis of treatment effect on EFS adjusting for the factors used in the randomisation stratification <p><u>Analysis of secondary outcomes:</u></p> <ul style="list-style-type: none"> - SR induction/consolidation: comparison of OS, toxicity, rate of CR2, and rate of MRD between treatment groups - SR consolidation +/- epratuzumab: comparison of OS, toxicity, MRD levels, rate of MRD and evaluation of pharmacokinetic parameters of Epratuzumab
Sample size:	<p>Number of SR patients expected per year: 200</p> <p>Number for SR induction/consolidation: 306/arm; recruitment 4 years</p> <p>Number for SR consolidation: 286/arm; recruitment 4 years</p>
Diagnosis and criteria for inclusion/ exclusion:	<p><u>Inclusion criteria:</u></p> <ul style="list-style-type: none"> • Morphologically confirmed diagnosis of 1st relapsed precursor B-cell or T-cell ALL • Children less than 18 years of age at inclusion • Meeting SR criteria: late isolated or late/early combined BCP BM relapse, any late/early isolated extramedullary relapse • Patient enrolled in a participating centre • Written informed consent • Start of treatment falling into the study period • No participation in other clinical trials 30 days prior to study enrolment that interfere with this protocol, except trials for primary ALL <p><u>Inclusion criteria specific for the epratuzumab randomization:</u></p> <ul style="list-style-type: none"> • Precursor B-cell Immunophenotype of ALL • M1 or M2 bone marrow status after induction <p><u>Exclusion criteria:</u></p> <ul style="list-style-type: none"> • BCR-ABL / t(9;22) positive ALL • Pregnancy or positive pregnancy test (urine sample positive for β-HCG > 10 U/l)

	<ul style="list-style-type: none"> • Sexually active adolescents not willing to use highly effective contraceptive method (pearl index <1) until 2 years after end of antileukemic therapy • Breast feeding • Relapse post allogeneic stem-cell transplantation • The whole protocol or essential parts are declined either by patient himself/herself or the respective legal guardian • No consent is given for saving and propagation of pseudonymized medical data for study reasons • Severe concomitant disease that does not allow treatment according to the protocol at the investigator's discretion (e.g. malformation syndromes, cardiac malformations, metabolic disorders) • Karnovsky / Lansky score < 50% • Subjects unwilling or unable to comply with the study procedures • Subjects who are legally detained in an official institute
Test drug/treatment, dose and mode of administration	<p>- SR arm A (ALL-REZ BFM 2002 arm Prot II-IDA): Induction: SIA (F1, F2); Post induction: SCA1 and SCA2 ± epratuzumab (8x360mg/m²/ 1 hrs IV weekly, week 5-12), 5 courses SCA3-7 (R1/2/1/2/1), 24 months maintenance (6MP, MTX) with 6 x TIT / 4 weeks. Cranial irradiation 18Gy for CNS relapse.</p> <p>- SR arm B (UK-R3, arm mitoxantrone): Induction: SIB (phase I); Post induction: SCB1 and SCB2 (R3-consolidation and intensification) ± epratuzumab (8x360mg/m²/ 1hrs IV weekly, week 6-13), 2 courses SCB3-4 (R3-interim maintenance 1 and 2), 24 months maintenance (6MP, MTX, 4-weekly VCR/DEX/IT reinduction pulses). Cranial irradiation 18 for CNS disease.</p> <p>- SCT indications: Any donor Arm A with MRD ≥ 10⁻³ after SIA, arm B with ≥ 10⁻⁴ after SIB. Matched donor any early combined, isolated extramedullary relapse or patients without MRD results. SCT is scheduled at week 16</p>

2 TREATMENT SCHEDULE INTREALL SR 2010



.Arrow down (↓), bone marrow puncture with CR/MRD assessment; CNS-RAD, irradiation of the central nervous system, if indicated; CR, cytological remission; MRD, minimal residual disease; SIA ALL-REZ BFM induction course; SCA 1-7 ALL-REZ BFM consolidation courses; Ⓡ, randomization; SIB, UK-R3 induction courses; SCB 1-4 UK-R3 consolidation / intensification courses; SCT, stem-cell transplantation; SR, standard risk group.

3 RESPONSIBILITIES

3.1 International coordinating functions

Function	Name	Contact
Sponsor	Charité - Universitätsmedizin Berlin	Augustenburger Platz 1, 13353 Berlin, Germany
Principle Investigator and sponsor representative in Germany	Dr. med. Arend von Stackelberg	Paediatric Oncology/Haematology, Charité Universitätsmedizin Berlin, Augustenburger Platz 1, 13353 Berlin, Germany Tel: +49 30 450 666833 Fax: +49 30 450 566901 e-mail: arend.stackelberg@charite.de
Study Co-chair	Prof. Vaskar Saha	Paediatric and Adolescent Oncology, Manchester Academic Health Sciences Centre, Central Manchester & Manchester Children's Hospitals NHS Trust, Christie Hospital, Wilmslow Road, Manchester, M20 4BX Tel: +44 161 446 3023 Fax: +44 1 446 3092 e-mail: vaskar.saha@manchester.ac.uk
Clinical trial management	Corinna Meier-Windhorst	Corinna Meier-Windhorst Coordination Centre for Clinical Trials (KKS) Charité Augustenburger Platz 1, 13353 Berlin, Germany Tel: +49 30 450 553544 Fax: +49 30 450 553937 e-mail: corinna.meier- windhorst@charite.de
Data management	Dr. Andrea Barth	Andrea Barth Universitätsklinikum Heidelberg Paediatric Oncology/Haematology Marvin Database of the GPOH Im Neuenheimer Feld 430 60210 Heidelberg Tel: +49 6221 568354 Support Tel: +49 511 5326717 e-mail: andrea.barth@med.uni- heidelberg.de
Biometry	Sharon Love	Centre for Statistics in Medicine Wolfson College, Linton Road Oxford, OX2 6UD, UK Tel: +44 1865 284414 Fax: +44 1865 284424 e-mail: sharon.love@csm.ox.ac.uk
Data Safety Monitoring Committee 1. Statistician	Maria-Grazia Valsecchi	Prof Maria Grazia Valsecchi, Centre of Biostatistics for Clinical Epidemiology, Department of Clinical Medicine and Prevention, University of Milano-Bicocca, 20052

Data Safety
Monitoring
Committee
2. Adult oncologist

Nikola Goekbuget

Monza, Milan, Italy
Tel: +39 0392333074
e-mail: grazia.valsecchi@unimib.it

Klinikum Joh.Wolfg.Goethe-UNI
Medizinische Klinik II
Hämatologie/Onkologie
Theodor-Stern-Kai 7
60596 Frankfurt
Tel: 069/6301-5194
e-mail: goekbuget@em.uni-frankfurt.de

Data Safety
Monitoring
Committee
3. Paediatric
oncologist

James A. Whitlock

The Hospital for Sick Children
Division Head
Haematology/Oncology
555 University Avenue
Toronto, Ontario
Canada, M5G 1X8
Tel: +1 416-813-8885
e-mail: jim.whitlock@sickkids.ca

3.2 National coordinating functions

Country	National coordination investigator	National co-sponsor
Australia and New Zealand	Prof. Tamas Revesz SA Pathology @ Women's & Children's Hospital 72 King William Rd, Adelaide, SA 5006	Australian & New Zealand Childhood Hematology & Oncology Group PO Box 2158 RMH VIC 3050
Austria	Dr. Georg Mann Dr. Andishe Atterbash St. Anna Kinderspital Kinderspitalgasse 6 A-1090 Vienna	St. Anna Kinderkrebsforschung, CCRI Zimmermannplatz 10 A -1090 Vienna
Belgium	Dr. Alina Ferster Paediatric Hematology/Oncology Unit, Hôpital Universitaire des Enfants Reine Fabiola. Avenue J.J. Crocq, 15, 1020 Bruxelles	Hôpital Universitaire des Enfants Reine Fabiola Avenue J.J. Crocq, 15, 1020 Bruxelles, Belgium
Czech Republic	Dr. Lucie Sramkova Dept. Pediatric Hematology/Oncology University Hospital Motol V uvalu 84, Prague 5 – Motol 150 06	University Hospital Motol V uvalu 84, Prague 5 – Motol 150 06
Denmark	Dr. Thomas Frandsen Copenhagen University Hospital (Rigshospitalet) Rigshospitalet, Blegdamsvej 9, 2100 Copenhagen.	Copenhagen University Hospital (Rigshospitalet) Rigshospitalet, Blegdamsvej 9, 2100 Copenhagen.
Finland	Dr. Päivi Lähteenmäki Turku University Central Hospital Kiinamyllykatu 4-8 SF-20520 Turku	Turku University Central Hospital Kiinamyllykatu 4-8 SF-20520 Turku
France	Prof. Pierre Rohrlich CHU Besançon Place St Jacques 25030 Besançon Cedex	CHU Besançon Mr Barberousse CHU Besançon
Germany	Dr. med. Arend von Stackelberg Pediatric Oncology/ Hematology, Charité - Universitätsmedizin Berlin, Augustenburger Platz 1, 13353 Berlin	Charité - Universitätsmedizin Berlin Augustenburger Platz 1, 13353 Berlin
Ireland	Prof. Owen Smith Our Lady's Children's Hospital Crumlin, Dublin12	Our Lady's Children's Hospital Crumlin, Dublin12
Israel	Dr. Ronit Elhasid Tel Aviv Sourasky Medical Centre Dana-Dwek Children's Hospital 6 Weizmann Street Tel Aviv 64239	Tel Aviv Sourasky Medical Centre 6 Weizmann Street Tel Aviv 64239
Italy	Prof. Franco Locatelli Ospedale Pediatrico Bambino Gesù Piazza S. Onofrio 4, 00165 Roma	Ospedale Pediatrico Bambino Gesù Piazza S. Onofrio 4, 00165 Roma

Japan	Prof. Chitose Ogawa St.Lukes International Hospital 9-1,Akashi-cho,Chuo-ku,Tokyo	Japanese Pediatric Leukemia/Lymphoma Study Group (JPLSG)
Netherlands	Prof. Peter Hoogerbrugge Radboud University Nijmegen Medical Centre The Netherlands	Dutch Childhood Oncology Group (DCOG) PO Box 43515, 2504 AM The Hague The Netherlands
Norway	Dr. Marit Hellebostad Oslo University Hospital, Sognsvannsveien 20, N-0027 Oslo	Oslo University Hospital, Sognsvannsveien 20, N-0027 Oslo
Poland	Dr. med. Ewa Goczynska Dpt. SCT and Hematology/Oncology University Wroclaw Bujwida 44 50 354 Wroclaw	Medical University of Wroclaw Bujwida 44 50 354 Wroclaw
Portugal	Dr. Joaquin Duarte Instituto Português de Oncologia de Lisboa Rua Professor Lima Basto, Lisbon	Instituto Português de Oncologia de Lisboa Rua Professor Lima Basto
Sweden	Dr. Stefan Soderhall University Hospital Stockholm, SE 171 76 Stockholm	University Hospital Stockholm, SE 171 76 Stockholm
Switzerland	PD Dr. Jean-Pierre Bourquin University Children's Hospital Zurich Steinwiesstrasse 75, 8032 Zürich	University Children's Hospital Zürich Steinwiesstrasse 75, 8032 Zürich
United Kingdom	Dr. Denise Bonney Consultant Paediatric Haematologist Dept of Paediatric Haematology/Oncology Royal Manchester Children's Hospital Oxford Road, Manchester, M13 9WL	Central Manchester University Hospitals NHS Foundation Trust Trust Headquarters, Corbett house, Manchester Royal Infirmary, Oxford Road, Manchester, M13 9WL

3.3 Reference facilities

The reference facilities are specific for the different national groups and are thus listed in the national appendices.

4 BACKGROUND AND RATIONALE

4.1 Introduction

Acute lymphoblastic leukemia (ALL) is the most frequent malignant disease in childhood with an incidence of about 4/100.000 children per year. The currently achieved rates of event-free survival (EFS) in 1st complete remission (CR) are 70-80% with multi-drug chemotherapy. About 15-20% of the patients suffer a relapse. Results of frontline therapy have increasingly improved over the past 2 decades (Figure 1).

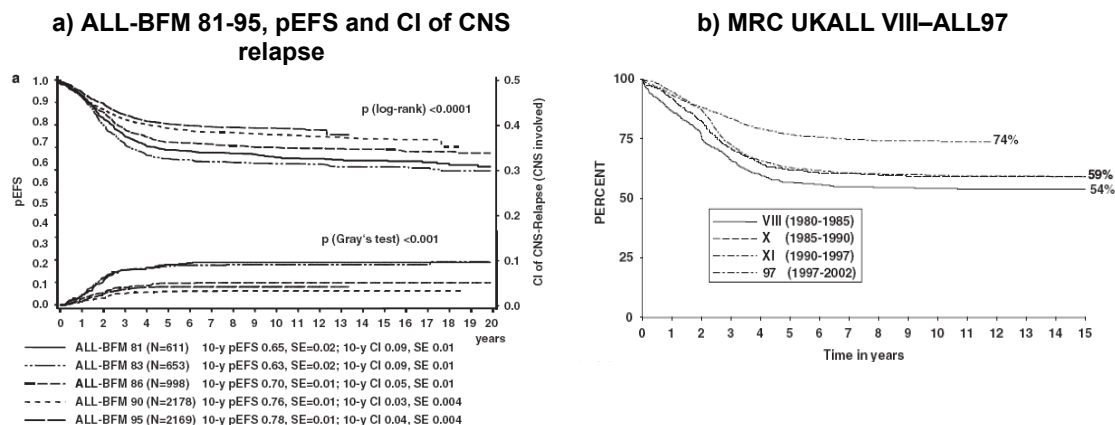


Figure 1 Event-free survival of children with ALL treated according to (a) ALL-BFM or (b) UKALL protocols by trials since 1980 until 2001^{1,2}

At relapse, about 40% of the children can be salvaged with intensified multi- and high-dose chemotherapy regimen and allogeneic stem-cell transplantation (allo-SCT) in most cases. Since 15% of children die from the disease, ALL is still the most frequent cause of death in childhood malignancies.³

Various prospective phase III studies have been performed over the last 3 decades all over Europe in order to improve outcome of children with relapsed ALL (e.g. AIEOP, ALL-REZ BFM, COPRALL, UKALLR).⁴⁻⁹ Representatives of the different national study groups for treatment of childhood relapsed ALL have formed the Resistant Disease Committee under the umbrella of the International BFM Study Group (I-BFM SG).¹⁰ This intergroup of experts has developed a homogeneous definition of diagnostic parameters, methods, and risk groups allowing for comparing results and strategies. Due to the improvement of frontline therapy results, patient numbers in national trials for relapsed ALL have dropped. Furthermore, new targeted compounds become increasingly available which are effective in biologic subgroups with comparably small patient numbers. Therefore, the group has decided to organize an international study for treatment of childhood relapsed ALL, IntReALL 2010. The study allows developing a platform for randomized optimization of standard therapy, and accruing enough patients for controlled integration of interesting new drugs in a curative salvage concept.

4.2 Results from ALL relapse trials

The rationale for the IntReALL 2010 treatment strategy is based on results of the various ALL relapse trials conducted all over the world in the past decades. The main strategy of salvage treatment after ALL relapse was to induce a 2nd CR with conventional intensive chemotherapy, to apply consolidation, re-intensification and maintenance therapy, or allogeneic stem-cell transplantation (SCT) as further intensification of treatment.

4.2.1 Chemotherapy

Among chemotherapy strategies two different approaches have been successfully followed: The application of multidrug intensive and comparably short courses with treatment-free intervals until regeneration of the bone marrow function has been developed and promoted by the Italian AIEOP, the German/Austrian Berlin-Frankfurt-Münster (BFM) Study Group, and the French/Belgium/Luxembourg Cooperative Group for relapsed ALL (COPRALL) for high risk patients or those with ALL relapse.^{2,4,5,8,11,12} Within the treatment courses F1, F2 and R1, R2, intermediate-dose MTX (1g/m^2 over 36 hrs) has been established as feasible, tolerable and effective way to apply this drug.⁹ A more continuous approach with repetitive application of comparably less intensive chemotherapy has been developed and used by the Children's Oncology Group (COG), and the United-Kingdom ALL Relapse Study Group (MRC UKALL-R).^{1,6,13,14} These approaches have been developed in context with the respective frontline treatment strategies. The COPRALL group has developed an intensive multi-drug treatment element called VANDA including Dex, ARA-C, mitoxantrone, VP16, etoposide and IT therapy, showing substantial activity in childhood relapsed ALL and which is used as established standard for HR patients.^{5,15}

4.2.2 Risk factors

One important goal of the trials was to determine risk factors which allow identifying those patients, who would have an acceptable event-free survival rate with chemotherapy alone and those who would need to receive allogeneic bone marrow transplants to have an acceptable cure rate. Finally, those groups needed to be identified, which do not benefit from standard chemotherapy and SCT at all and should be eligible for palliative treatment or phase I/II trials with the chance to benefit from new agents.

The time to relapse (very early, early and late), the site of relapse (isolated bone marrow-combined bone marrow- and isolated extramedullary relapse), and the immunological lineage of the disease (B-precursor versus T-cell ALL) have been identified as the most important clinical risk factors in childhood relapsed ALL.¹⁶ Since 1995, the ALL-REZ BFM used the strategy groups S1-4 on the basis of these risk factors to stratify to risk adapted treatment groups.¹⁷ Retrospectively, S1 patients (late isolated extramedullary relapse) had acceptable event-free survival (EFS) rates with chemo/radiotherapy of 70%, whereas S3 (early isolated BM relapse of BCP immunophenotype) and S4 (very early BM relapse and any T-lineage BM relapse) patients achieved EFS rates below 5% with chemotherapy alone and where obligatorily eligible for SCT since then. The large and heterogeneous S2 groups (very early/early isolated extramedullary relapse, early/late combined BM - and late isolated BM relapse of BCP immunophenotype) achieved intermediate EFS rates of about 40%.¹⁸ The S-group classification has also been applied in the ongoing or recently closed study ALL-REZ BFM 2002.^{6,19} The MRC UKALL-R group used a modified version combining the S3 and S4 patients to a high risk (HR) group including to this as well patients with very early isolated extramedullary relapse.²⁰

4.2.3 Genetic alterations

Various genetic alterations have been identified as clinically and prognostically relevant biologic entities in childhood ALL.²¹ At relapse, the translocation t(12;21) with its fusion gene TEL/AML1 has been identified in about 20% of patients and is associated with a favourable prognosis.²² However, this revealed no independency in context of multivariate or matched pair analysis.^{23,24} Therefore, TEL/AML1 is not used as risk factor for patient stratification. The translocation t(9;22) with its fusion transcript BCR/ABL has been shown to be associated with poor prognosis at relapse with independent significance.^{10,25,26} However, since children with BCR/ABL positive ALL are treated within specific protocols including tyrosine kinase inhibitors and are nearly all transplanted in 1st remission, nowadays no relevant subgroup of patients is registered in ALL relapse trials and patients are treated according to individual recommendations.²⁷ Other genetic lesions such as hyper- or hypodiploidy, MLL involving translocations, translocation t(1;19) have been identified in children with relapsed ALL but have not been established as clinically relevant risk factors so far.²⁸

4.2.4 Minimal residual disease (MRD)

The detection of residual leukemic cells (minimal residual disease, MRD) in the BM after achievement of a cytological CR with clone specific and highly sensitive methods has emerged in the last decades.^{29,30} MRD can be assessed by PCR based detection/quantification of clone specific T-cell receptor gamma/delta or immunoglobulin heavy chain rearrangements, or by the detection/quantification of aberrant antigen combinations on the cell surface of leukemic cells using flow cytometry. In Europe, the molecular genetic approach has been best established and validated as important risk factor.³¹⁻³³ In the USA and increasingly in Europe as well, flow cytometry has been established as easily accessible method with reliable results in most situations.^{28,34} Various studies have shown that MRD quantified by molecular genetic methods or by flow cytometry shows high correlation. Therefore, MRD quantified by flow cytometry can be applied as stratification parameter in patients, in whom MRD quantified by molecular genetic methods is not available.

MRD has been shown to be a highly predictive risk factor in ALL and has been successfully used for treatment stratification in frontline protocols for childhood ALL.³⁵ At relapse, intermediate risk (IR, S2) patients with bone marrow involvement and good MRD reduction after induction therapy achieved good disease-free survival (DFS) rates of above 70%, whereas those with poor MRD response ($> 10^{-3}$ after ALL-REZ BFM F1/2 induction) had DFS rates below 20%.¹⁷ Based on these results, European study groups on treatment of childhood relapsed ALL have allocated IR patients with MRD poor response to allogeneic SCT post consolidation.¹⁹ The MRD cut-off has been adapted in that context to the intensity of the induction regimen and was set to a lower level of 10^{-4} in the internationalized MRC UKALL-R protocol ALLR3.³⁶ Within the trial ALL-REZ BFM 2002 it could be shown, that by allocation of MRD poor responding patients to allogeneic SCT, the prognosis of this subgroup could be improved to above 60% DFS (Figure 2). Allogeneic SCT can thus be regarded as proven indication for IR patients with MRD poor response.

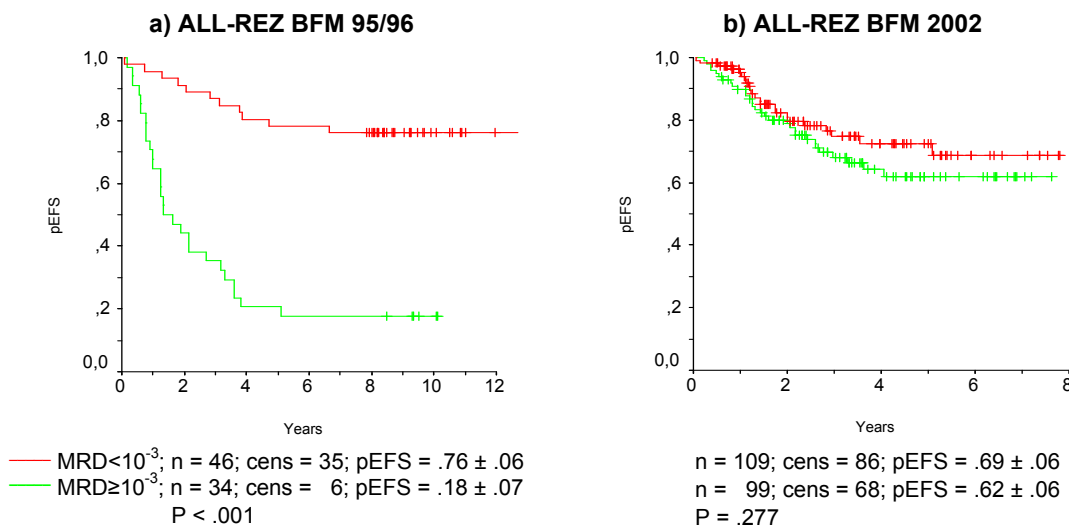


Figure 2 Disease-free survival of children with intermediate risk BM relapse of ALL relapse by MRD response after induction therapy treated according to (a) trial ALL-REZ BFM 95/96 (MRD results blinded) and (b) ALL-REZ BFM 2002 (MRD $\geq 10^{-3}$ indication for allogeneic SCT). Stackelberg, unpublished data.

Preliminary results of the ALL-R3 trial showed that in patients with MRD levels below 10^{-4} post induction DFS rates of 80% at 5 years could be achieved with idarubicin $2 \times 10 \text{ mg/m}^2$ or mitoxantrone $2 \times 10 \text{ mg/m}^2$ given at day 1 and 2 of induction. However, in patients with higher MRD levels and SCT indication, DFS rates after idarubicin were significantly inferior compared to the MRD good response group, whereas after mitoxantrone results of patients with MRD poor response were favourable and at an identical level of those patients with

MRD good response. This finding lead to the conclusion that the MRD cut off of 10^{-4} was applicable in the context of the ALLR3 protocol and that the mitoxantrone arm should be followed as standard arm of IR patients in future.⁸

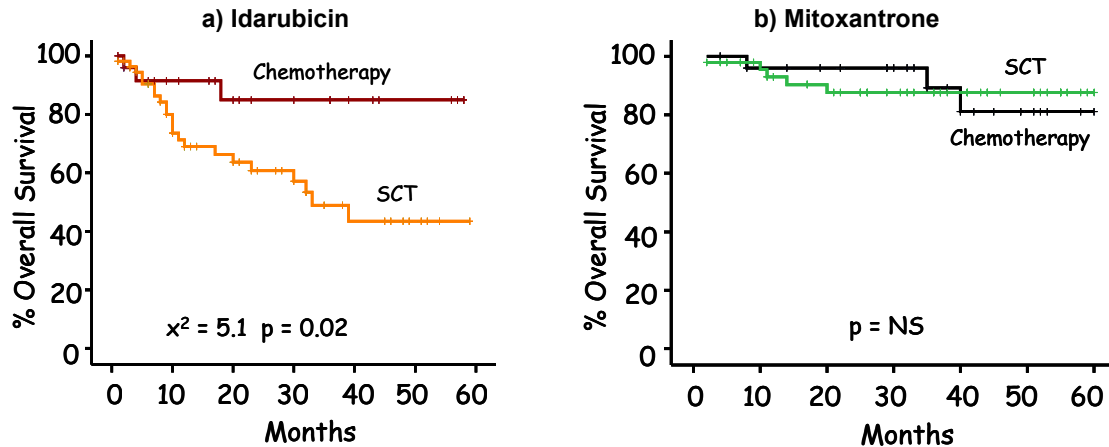


Figure 3 Event-free (a) and overall survival (b) of children with ALL relapse treated according to trial ALLR3 by randomized induction with idarubicin versus mitoxantrone.

Whereas the AIEOP ALL relapse group could find comparable prognostic relevance of MRD post induction in HR patients,^{37,38} this could not be confirmed by other groups so far.

While MRD post induction can be regarded as a very sensitive response parameter, MRD quantification to later time-point of treatment indicates refractory disease. Thus MRD before allogeneic stem cell transplantation (SCT) has been shown to be the only independent predictor for subsequent relapse after SCT.³⁹ A goal of any pre-transplant therapy must be to reduce the disease burden below 10^{-4} . If this can not be achieved with conventional therapy, experimental interventional strategies may be indicated to improve the remission quality prior to SCT.

In isolated extramedullary relapse, submicroscopic bone marrow involvement at relapse diagnosis at different levels has been detected within a collaborative initiative of the I-BFM SG Resistant Disease Committee. In patients with cytologically isolated CNS relapse, detectable submicroscopic bone marrow involvement at relapse diagnosis could be associated with a higher subsequent relapse rate.²⁹ This finding needs to be confirmed in a larger prospective study, which is currently performed by the group, before being considered as parameter for treatment stratification.

MRD measured sequentially during therapy allows assessing the dynamics of response to treatment and can be considered as early efficacy endpoint in randomized trials.⁴⁰

Taken together, quantification of MRD at diagnosis of ALL relapse and regularly during therapy is an essential tool to characterize the responsiveness of the disease and to allocate the patients to a risk adapted treatment. MRD quantification at validated time-point within controlled treatment strategies has in the meantime achieved the status of standard of care and is in Germany financed by health insurance system.

4.2.5 Stem cell transplantation

Allogeneic SCT has been shown to improve disease-free and overall survival in children with relapsed ALL with poor prognosis after chemo/radiotherapy alone. The antileukemic effect has been related to the high-dose chemotherapy and/or total body irradiation (TBI) given as myeloablative conditioning regimen but above all to the allo-immune effect of the transplanted immune system against residual leukemia.^{41,42} In patients with relapsed ALL, a proven benefit of allogeneic SCT has been shown for patients with high risk features (HR or S3/4 group).¹⁴ In patients with standard risk profile (SR, S1/2), only those with BM involvement and MRD poor response after salvage induction clearly benefit from SCT (Figure 2, Figure 3). In SR patients with BM involvement, in whom MRD results by molecular

genetic methods and by flow cytometry are not available, an allo-SCT may be considered only, if a well matched stem-cell donor is available.

Patients with late isolated extramedullary relapse (SR/S1) and those with late bone marrow relapse and MRD good response as defined by the respective relapse protocols have acceptable DFS rates with chemo/radiotherapy and are not eligible for SCT. In patients with early isolated or combined extramedullary relapse, the prognosis with chemo/radiotherapy is intermediate. Whether these patients may benefit from a well HLA-matched donor SCT needs to be proven.^{43,44}

Whereas the indication to SCT is defined by relapse protocols, the procedure itself is not part of these. In the past, the SCT procedure has been heterogeneous and was dependent on the preferences of the single centres. Since 2003, under the umbrella of the I-BFM SG a standardized SCT protocol ALL SZT BFM 2003 and an international version ALL-SCT-BFM-International have been implemented.⁴⁵ With this platform prospective controlled studies on SCT related issues can be performed independently from the pre-SCT treatment protocols.

4.2.6 Radiation therapy

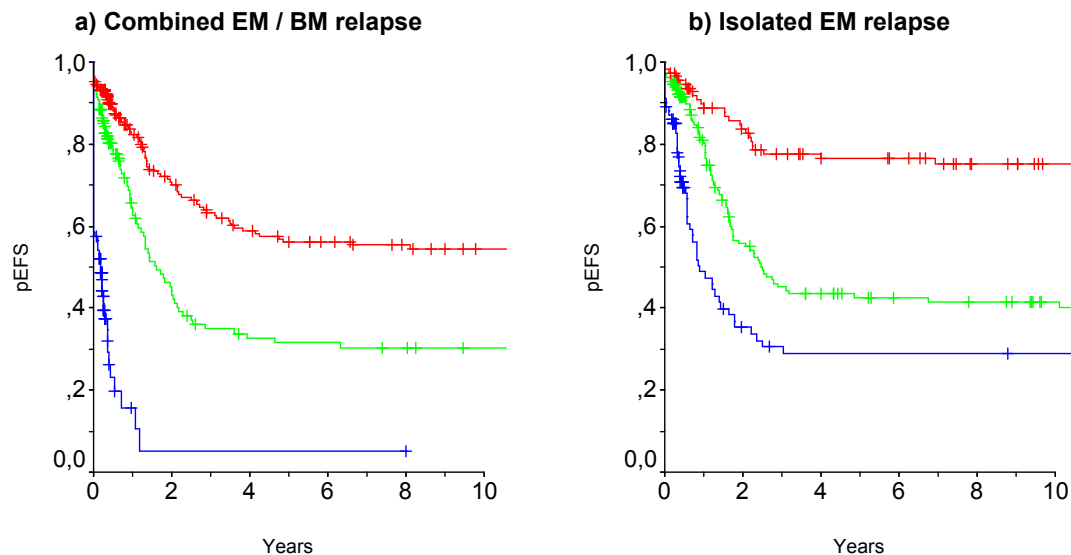
Radiation therapy is an important tool for local disease control in particular in blood-barrier protected sanctuary sites such as the CNS and the testes. Therapeutic irradiation of manifest extramedullary leukemia can be regarded as standard of care as discussed in the respective chapters on CNS- and testicular relapse (chapter 4.2.7.1 and 4.2.7.2). Whether protective CNS irradiation is necessary in patients with isolated bone marrow relapse, remains controversial. In a retrospective analysis the BFM group has clearly shown, that the rate of subsequent medullary and CNS relapses can be significantly reduced by preventive CNS irradiation at 12 Gy.^{46,47} With the ALLR3 trial, outstanding results in IR patients with BM relapse and MRD good response have been achieved without preventive cranial irradiation (Figure 3, page 18). The omittance of cranial irradiation in childhood ALL has been propagated by various groups due to the documented radiation associated late effects such as neuro-behavioural changes and secondary malignancies.^{4,14,48} The group has decided to omit preventive cranial irradiation for all patients with isolated bone marrow relapse and to replace it with 6 triple intrathecal chemotherapy applications during maintenance therapy of the ALL-REZ BFM 2002 arm.⁴⁹ The risk to loose efficacy in patients with MRD good response was presumed to be low and counterbalanced by the reduction of late sequelae associated with irradiation. Furthermore, it is hypothesized that those patients who had a benefit from preventive cranial irradiation in the past, now belong to the group of MRD poor responders who receive an allogeneic SCT with TBI containing conditioning regimens. A comparable approach has already been realized in patients with T-ALL within the ALL-BFM 2000 protocol and in patients with AML within the protocol AML BFM 2004.

4.2.7 Extramedullary Disease

Involvement of extramedullary sites in children with ALL relapse has been registered in about 35-40% of patients depending on the frontline protocol.^{1,2} However, in the last decade due to more effective frontline protocols, the rate of all but also of extramedullary relapses has been decreased. Since the disease is protected from chemotherapy by biologic blood barriers in extramedullary sanctuary sites such as the CNS and the testes, specific local therapy in addition to systemic chemotherapy is generally recommended. Involvement of an extramedullary site in patients with BM relapse has been identified as favourable prognostic feature compared to patients without extramedullary involvement (Figure 4a).⁵ This finding lead to the stratification of patients with early combined BM relapse to the intermediate risk group S2, whereas patients with early isolated BM relapse can not be cured with chemo/radiotherapy alone and are stratified to the high risk group S3. In patients with early or very early isolated extramedullary relapse, prognosis has been unsatisfying with chemotherapy plus even specific local treatment (Figure 4b).⁵⁰ It remains controversial, whether allogeneic SCT can improve survival of children with high risk isolated extramedullary relapse.⁵¹ Several reports mostly on small patient numbers however seem to support the effectiveness of allogeneic SCT in isolated extramedullary (IEM) relapses and encourage to investigate the SCT indication in this subgroup prospectively.^{52,53}

4.2.7.1 CNS relapse of ALL

In CNS relapse, the accepted local treatment is intensified intrathecal therapy and cranial irradiation. The adequate dose (18 versus 24 Gray[Gy]) and mode of CNS irradiation (cranial versus craniospinal) remains controversial.^{5,54,55}



Time-point of relapse

— late: n = 225; cens = 144; pEFS = .55 ± .04
 — early: n = 155; cens = 77; pEFS = .30 ± .05
 — very early: n = 94; cens = 30; pEFS = .05 ± .05
 P < .001

n = 113; cens = 88; pEFS = .75 ± .04
 n = 166; cens = 88; pEFS = .42 ± .04
 n = 100; cens = 45; pEFS = .29 ± .06
 P < .001

Figure 4 Event-free survival of children with (a) combined bone marrow (BM) and extramedullary (EM) relapse or with (b) isolated extramedullary (IEM) relapse of ALL treated with ALL-REZ BFM 83-2002 protocols by time-point of relapse. Stem-cell transplantation is considered as censored event. Stackelberg, unpublished data.

4.2.7.2 Testicular relapse of ALL

The testes are the second most frequent sites of extramedullary involvement. Local therapy is not uniform in different study groups. Most study groups treating childhood relapsed ALL including the COG, the UKALL-R and the COPRALL recommend local irradiation of both testes at 24 Gy regardless whether only one or both testes are involved and causing a complete loss of hormonal function and atrophy.⁵⁶⁻⁵⁸ BFM relapse strategies have recommended to remove a clinically involved testis and to irradiate a contralateral clinically not involved and bioptically negative testis with 15 Gy, or with 18 Gy if bioptically positive. It has been shown that this strategy offers the chance for spontaneous puberty without hormonal substitution in a substantial part of patients.⁵⁹

4.2.7.3 Other extramedullary sites of ALL relapse

A variety of other extramedullary sites may be involved at ALL relapse. Little data is available on the prognostic impact of these manifestations and on the necessity of local therapy. Since a blood barrier is not present in these sites, systemic chemotherapy is supposed to be effective. Only in case of local persistence of the disease after induction/consolidation chemotherapy it is recommended to take a biopsy and to apply local irradiation therapy if vital leukemic cells are still present.¹⁸

4.2.8 Results of the Trial ALL-REZ BFM 2002

The trial ALL-REZ BFM 2002 was based on experiences of preceding BFM relapse trials using the same risk stratification S1-4 and a standard chemotherapy for all risk groups with induction courses F1/2, consolidation courses R2/1, and irradiation/maintenance therapy or allogeneic SCT depending on the individual risk profile. In all strategy groups a 6-week more continuous and less intensive chemotherapy regimen Prot II-IDA was randomized against the conventional early consolidation with intensive R-courses.^{6,60} Whereas the EFS probability with Prot II-IDA was not significantly superior compared to R-courses, the cumulative incidence of subsequent relapses was significantly lower and the toxicity of both regimens was comparable in an early analysis (Figure 5, ASH 2011). Therefore, the ALL-REZ BFM Study Committee decided to take Prot II-IDA arm forward as standard for early consolidation in future trials. As 2nd important question, the impact of allocation of IR patients to allogeneic SCT or conventional chemo/radiotherapy according to the MRD response after induction on event-free and overall survival was investigated comparing results with historical controls. The strategy turned out to be feasible and lead to a highly significant improvement of pEFS/OS in IR patients with MRD poor response and of the total IR group (Figure 2, Figure 6). Overall, the results of the trial ALL-REZ BFM 2002 turned out to be superior to all preceding trials despite the continuously increasing selection pressure from effective frontline protocols (Figure 6). The improvement can be attributed in particular to the correct selection of IR patients eligible for allogeneic SCT, and to the standardization of SCT procedures due to the SCT protocol leading to lower rates of treatment related mortality. Whereas the S1/S2 strategy can be regarded as best available standard and should not be substantially changed, the results of S3/4 (HR) patients remain poor and unsatisfactory.

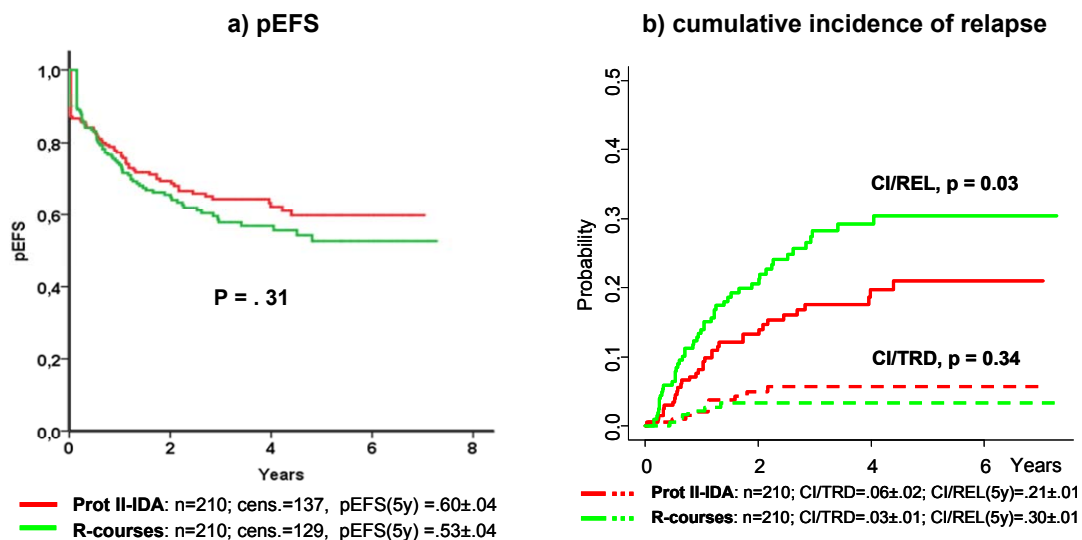


Figure 5 Event-free survival (a) and cumulative incidence of subsequent events (b) of children with relapse of ALL treated according to trial ALL-REZ BFM 2002 by randomized consolidation arms Protocol II IDA versus R-courses. Stackelberg, unpublished data.

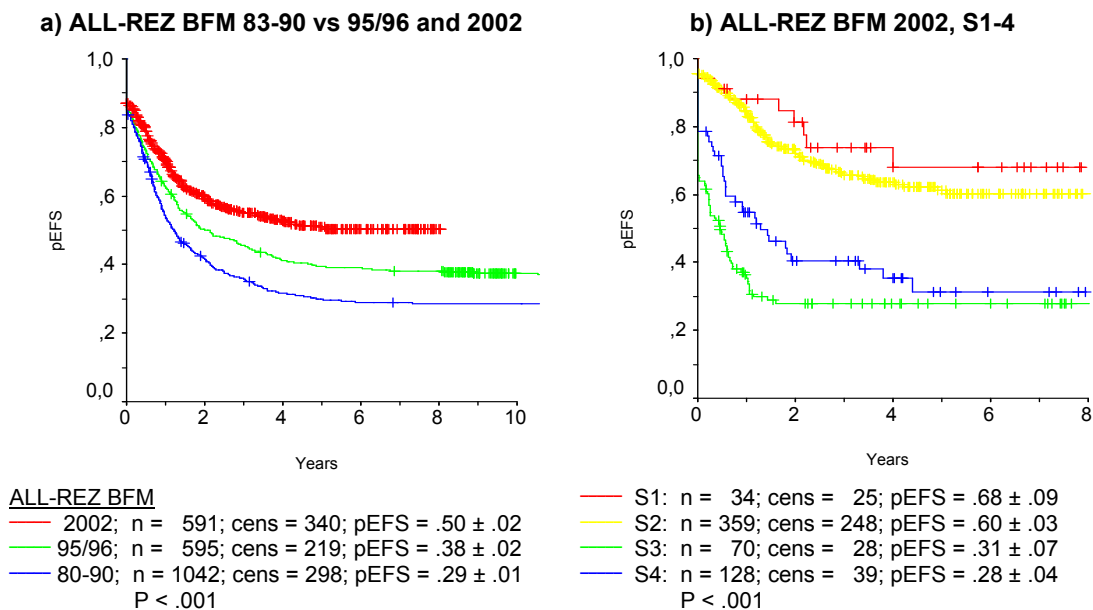


Figure 6 Event-free survival of children with relapse of ALL (a) treated with ALL-REZ BFM protocols by protocol period and (b) treated according to Trial ALL-REZ BFM P02/2002 by risk group (S1-4). Stackelberg, unpublished data.

4.2.9 Results of the Trial ALLR3

The design of trial ALLR3 was based on the experience with preceding ALL relapse trials in the UK, MRC UKALL R1 and R2.⁶ Chemotherapy was scheduled as a rather continuous repetitive treatment avoiding longer treatment-free intervals and thus different from the rotational intensive short course chemotherapy strategy used by the BFM group. The risk stratification corresponded largely to the BFM S-groups except that patients with very early extramedullary relapse have been stratified to the HR group due to their poor 5-years EFS rate (20%) registered within the MRC UKALL R2 protocol.³⁶ The main question of ALLR3 was whether EFS can be improved by replacing idarubicin $2 \times 10 \text{ mg/m}^2$ with mitoxantrone $2 \times 10 \text{ mg/m}^2$ given the 1st 2 days of induction. Interim analyses showed a significantly superior EFS probability with mitoxantrone leading to interruption of the randomization and continuation with the mitoxantrone arm (Figure 7).³⁶ In subgroup analysis the improvement could be confirmed in SR and IR patients but not in HR patients (Figure 8). Furthermore, the difference was only seen in patients having received allogeneic SCT. In particular in transplanted patients, the arm with idarubicin turned out to be dramatically inferior. In contrast, in patients with MRD good response ($< 10^{-4}$ post induction) receiving chemotherapy only, EFS probabilities were equally favourable in both groups (Figure 3).

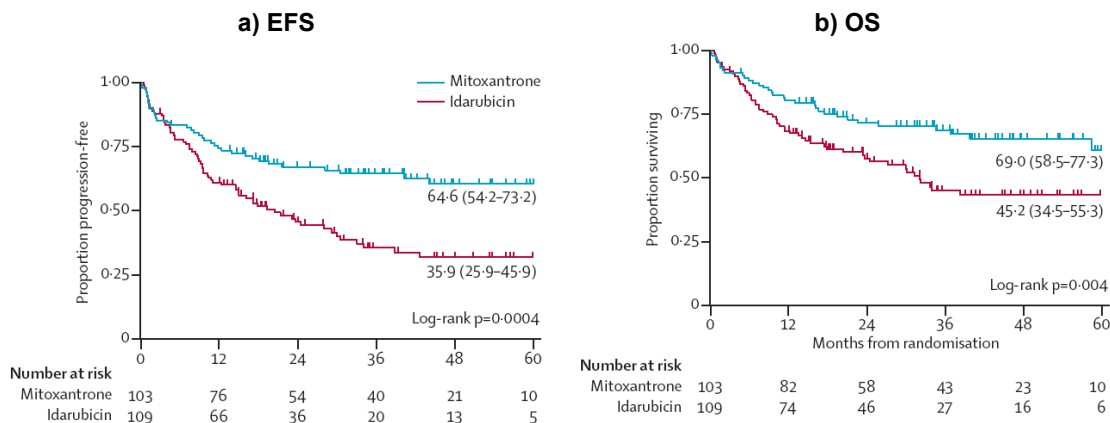


Figure 7 Event-free survival (a) and overall survival (b) of children with relapse of ALL treated within the ALL-R3 trial by randomization of induction with idarubicin versus mitoxantrone.³⁶

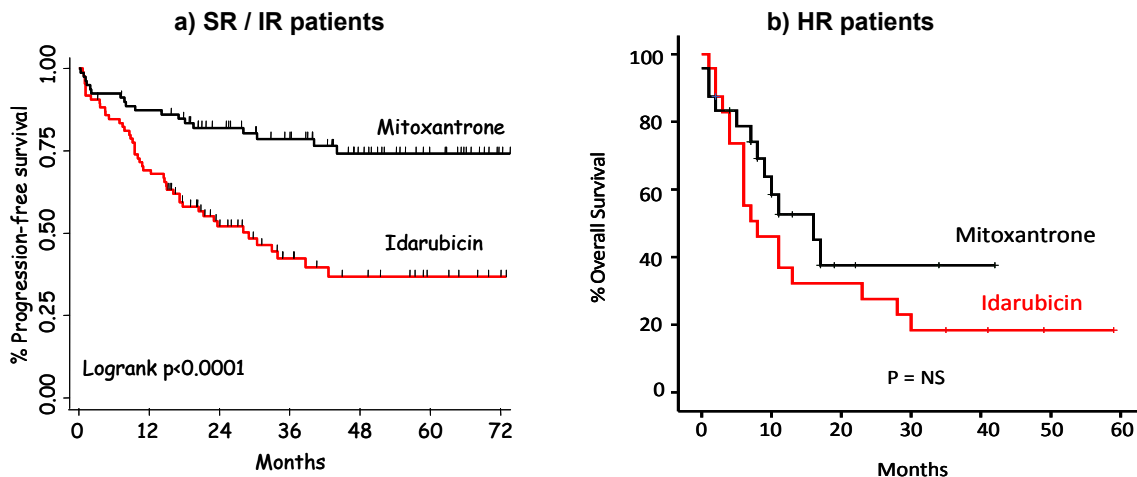


Figure 8 (a) Disease-free survival of children with SR/IR relapse of ALL or (b) overall survival of patients with HR relapse of ALL by randomization IDA versus MITOX in induction therapy of trial ALLR3. Saha, unpublished data.

In IR patients with BM relapse, MRD response post induction was used as criterion for allocation to allogeneic SCT or chemotherapy. Compared to BFM strategy, a lower cut-off of 10^{-4} has been chosen extrapolating the potentially stronger antileukemic effect of the anthracycline based induction compared to the MTX- and ARA-C based ALL-REZ BFM induction. HR patients received the same induction/consolidation chemotherapy but were all eligible for allogeneic SCT. In those patients with MRD persistence of $>10^{-3}$ at week 13, an interventional intensification with a FLAD (fludarabine, ARA-C, liposomal daunorubicin) was given with the aim to reduce MRD prior the SCT. The treatment strategy of ALLR3 for SR/IR patients resulted in the best overall outcome ever achieved and can be regarded as best standard of care in the context of UK frontline protocols. Results of HR patients remained to be unsatisfactory with poor remission and EFS rates.

4.2.10 Results of other study groups

European national study groups on treatment of childhood relapsed ALL have participated at the international trials ALL-REZ BFM 2002 (Austria, Czech Republic, selected Swiss centres, Toronto/Canada),^{27,61} ALLR3 (The Netherlands, Australia), COPRALL (Belgium, selected centres of Portugal),⁵ have followed own national conceptions such as the Italian AIEOP REC 2003 protocol,⁸ or have treated patients on individual basis.^{62,63} Whereas European

groups focused on phase III treatment optimization studies with standard chemotherapy, the COG has established a strategy for childhood relapsed ALL allowing for integrating a variety of new and mostly targeted drugs applicable for selected subgroups.^{13,64-66} In such phase II strategies, activities of new agents can be documented. However, a superiority of new drugs in context with a curative treatment strategy compared to standard therapy in phase III trials can not be investigated with this platform.

4.2.11 Use of asparaginase

In all participating countries both, PEG-asparaginase and Erwinia asparaginase are used as standard therapy for ALL and relapsed ALL if unpegulated coli asparaginase is not tolerated although marketing authorisation is not achieved in all of them.^{36,67-70} In most countries, PEG-asparaginase is used as 1st line drug in the current frontline therapy trials treating primary ALL.^{71,72} Since no clinical data are available that unpegulated coli asparaginase can be used after treatment with PEG asparaginase, and PEG asparaginase has clear pharmacokinetic advantages, it is considered as 1st line asparaginase compound also in relapse trials. Nevertheless, patients having received only and tolerated unpegulated coli asparaginase, may continue to receive this drug as well at relapse, as long as tolerated.

4.2.12 Comparison of ALL-REZ BFM 2002 and ALLR3

The Resistant Disease Committee of the I-BFM SG decided to develop a common chemotherapy platform for optimization of standard therapy and integration of new agents. As most attractive and best developed protocols, the ALL-REZ BFM and the mitoxantrone arm of the ALLR3 protocol have been retrospectively compared.

The interim analysis of the two studies was performed by an independent statistician in May 2009. In both studies, patients in the high-risk group (S3, S4) performed poorly. The bulk of the patients analysed therefore fall in the now defined standard-risk group (S1, S2). At the time of analysis, the randomization in ALLR3 comparing mitoxantrone with idarubicin was closed, but ALL-REZ BFM 2002 randomization was still open. This analysis has therefore been done comparing the best performance of ALLR3 (mitoxantrone) with ALL-REZ BFM 2002. In total, 93 patients with idarubicin and 112 with mitoxantrone were compared with 320 patients on REZ-2002. Progression free survival (PFS) is significantly higher with mitoxantrone than idarubicin (hazard ratio: 3.19, 95% confidence intervals 1.84-5.53, $p < 0.0001$). The PFS (95% confidence intervals) were 37% (25, 49) for idarubicin, 74% (62, 83) for mitoxantrone and 60% (53, 67) for ALL-REZ BFM 2002. Further analysis showed that the populations in the two study groups had different characteristics. The most significant difference was the median time to relapse which was 48 months in the mitoxantrone cohort and 37 months in ALL-REZ BFM 2002 ($p = 0.0001$). As time to relapse is one of the most significant predictors of outcome, when adjusted for this factor there is no significant difference in PFS between patients who were treated on ALL-REZ BFM 2002 or on the mitoxantrone arm of ALLR3. This suggested that the outcome for relapsed protocol was dependent on the results of the frontline protocol. Changes in pattern and distribution of relapsed patients would therefore greatly influence the outcome of any relapsed protocol. Thus we could not predict if ALL-REZ BFM 2002 would have same survival rates as use of treatment for patients relapsing off UK protocols or vice versa.

Furthermore, the MRD levels at time point 1 (week 5) used as cut off for bone marrow transplantation were analyzed and compared. Surprisingly given the differences between idarubicin and mitoxantrone there was no significant difference between the levels of MRD at week 5 between the two drugs. MRD level achieved after induction with the ALL-R3 mitoxantrone arm were significantly lower as compared to ALL-REZ BFM 2002. This is most likely an effect of the intensive use of anthracycline during induction in ALLR3. Clearly, the MRD cut off would be dependent on the induction therapy used and could not be extrapolated across protocols.

On the basis of this comparison the group came to the conclusion that both trials gave similar results with different approaches and in context with different frontline protocols. This has provided the rational design of IntReALL SR 2010, where the two approaches will be randomized.

4.3 New agents in childhood ALL

Most drugs for treatment of childhood ALL have been used since decades. With the increasing molecular genetic and immunologic knowledge and technology, a variety of new drugs with a more specific antileukemic activity have been detected and are in preclinical or clinical development. The most important groups among a large variety of developments are novel nucleoside analogues, tyrosine kinase-, NFkappaB-, m-TOR-, and FLT3 - inhibitors, unconjugated and conjugated monoclonal antibodies.⁷³ The new EU directive for drug development in children obliges companies to submit a comprehensive paediatric drug development plan (PIP), when a new compound shall be licensed in Europe. The adequate dose, the tolerability and the efficacy of these drugs as single agents and in combination with conventional chemotherapy are investigated in phase I/II trials in patients with advanced disease without curative treatment options. The most promising new agents have to be taken forward and investigated within curative strategies. For this purpose, the protocols for treatment of childhood relapsed ALL are most applicable because patients have poor or intermediate prognosis and have a much higher necessity for new agents with new mechanisms of action than patients with primary disease. It is most important, that integration of new drugs in curative strategies is guided by the academic experts in the field in close cooperation with the responsible authorities and the drug companies. Academic consortiums such as the I-BFM SG and the ITCC (Innovative Therapies in Children with Cancer) are dedicated to establish this important link and to warrant the academic lead and thus a development in the best interests of the paediatric patients.^{10,74,75}

4.4 Epratuzumab

4.4.1 CD22 characterization

CD22 is a 140 kDa transmembrane immunoglobulin-like lectin, which specifically binds sialic acid at its N-terminus (SICLEC). The presence of immunoglobulin domains makes CD22 a member of the immunoglobulin superfamily. CD22 acts as an accessory co-receptor that modulates B-cell receptor (BCR) signalling upon ligation. Independently from ligation, the BCR associated kinase Lyn phosphorylates tyrosines of the cytoplasmatic CD22 tail leading to immunoreceptor tyrosine-based inhibitory motifs (ITIMs). These inhibit BCR signaling, enhance calcium efflux and lead to endocytosis.⁷⁶ Furthermore, it is involved in the CD19/21 and CD40 signaling regulation, peripheral B-cell homeostasis and survival, and the promotion of BCR-induced cell cycle progression.⁷⁷ CD22 is expressed on immature and maturing B-cells, however not on stem-, pro B cells and plasma cells. As antigen restricted to B cells and being expressed on the majority of precursor B-cell lymphoblastic leukemias, CD22 is a suitable antigen for immunotherapy. Its rapid endocytosis upon ligand binding qualifies it as ideal target for immunoconjugated toxins that can exhibit their cytotoxicity against the target cell intracellularly.⁷⁸ CD22, a B-cell surface antigen, is highly expressed in more than 90% of cases of childhood B-precursor ALL.

4.4.2 CD22 expression on BCP ALL blasts at relapse and during early phase of treatment

CD22 is typically expressed on blasts at lower levels compared to non-malignant mature B cells. Physiologically expression of CD22 is lower on B cell precursors compared to mature B cells, expression on leukemic cells is comparable to expression we observe on B cell precursors. Part of patients with pro-B ALL is completely CD22 negative. As preliminary observation based on 19 cases we can state that expression of CD22 remain stable during the early phase of relapse therapy (day 15 and day 28). In 1 case we observed increased expression of CD22 at day 28 compared to diagnosis. This confirms that CD22 is a suitable target for therapeutic strategies in consolidation treatment of childhood relapsed ALL (unpublished data, personal communication with Dr. Ester Mejstrikova, Prague).

4.4.3 Epratuzumab, mechanisms of action

Epratuzumab is a humanized IgG1 anti-CD22 antibody directed against the 3rd extracellular domain (epitope B) of CD22. Whereas epratuzumab displays no direct apoptosis and no complement dependent cytotoxicity (CDC) against lymphoma cells in vitro, a (compared to rituximab) moderate antibody dependent cellular cytotoxicity (ADCC) and biologic activity through B-cell receptor modulation has been registered.⁷⁹ Although epratuzumab induces a rapid internalization of the antigen antibody complex, Fc receptor bearing effector cells get enough opportunity to bind to the complex and exhibit cytotoxic activity.

4.4.4 Clinical data in adults

Epratuzumab showed promising activity in rheumatologic diseases.⁸⁰ The U.S. Food and Drug Administration (FDA) has granted epratuzumab fast track product designation for the treatment of patients with lupus. In clinical phase I/II trials on CD22 positive malignancies, epratuzumab has shown objective responses in 15-20% of patients with relapsed and refractory CD22 positive diffuse large B-cell or indolent non-hodgkin lymphoma and 40% in patients with recurrent follicular lymphoma. The single weekly dose has been escalated up to 1000 mg/m² without achieving dose limiting toxicities. However, the dose of 360 mg/m² has been determined as sufficiently effective.^{81,82} Furthermore, a combination of epratuzumab and rituximab revealed improved responses and CR rates compared to rituximab alone.⁸³ Given in four weekly intravenous infusions, epratuzumab exhibited a mean serum half-life (t_{1/2}) increasing from 6.9 to 26.5 days between the first and the fourth infusion, peak serum levels also increased with every infusion likely due to saturation of antibody binding sites on CD22. Epratuzumab serum levels increase with each infusion and remain detectable in serum 12 weeks later.

Several clinical trials with epratuzumab are ongoing in rheumatologic and malignant indications. The National Cancer Institute (NCI) performs a phase II trial combining epratuzumab with clofarabine and cytarabine in adults with relapsed refractory ALL.⁸⁴ The combination of epratuzumab, rituximab and conventional chemotherapy (ER-CHOP) has shown promising results in a phase II trial for adults with diffuse large B-cell lymphoma.⁸⁵

4.4.5 Clinical data in childhood relapsed ALL

In children with relapsed ALL, epratuzumab has been investigated within a 14 days single drug window (360mg/m², 2 times per week), followed by a 4-week combination with multidrug chemotherapy (360 mg/m², once a week). Toxicity was mostly limited to mild infusion related symptoms (grade 1-2). Among 15 patients receiving the 2 week single drug window, 1 had a reduction of leukemic blasts in the blood, 3 had progressive disease, and the others stable disease. Among 12 evaluable patients receiving the combination cycle, 9 achieved a CR, 7 of them with a negative MRD status post induction. Furthermore, one patient with partial response, one with stable disease and one with progressive disease was registered after the combination cycle. Although substantial antileukemic activity of the single drug has not been observed, the response in combination with chemotherapy and the high rate of MRD negativity in the high risk patient population and the throughout favourable toxicity profile lead to the conclusion, that epratuzumab plus chemotherapy has interesting antileukemic activity and should be further investigated in prospective trials.⁶⁴ Subsequently, an extended combination phase II trial has been performed within the COG to confirm the data in a larger cohort of patients. Patients received the COG 3-block platform chemotherapy for childhood relapsed ALL plus epratuzumab (360 mg/m²/dose) during block 1. Initially during part B, epratuzumab was administered weekly for 4 doses starting on day 1 based on pharmacokinetic (PK) data from adults with lymphoma (B1). Since PK data showed that the half-life of epratuzumab was much shorter in children with ALL, the trial was amended to give epratuzumab twice weekly for 8 doses, starting on day 1 (B2). Between 1/2007 and 1/2011, 116 patients (114 eligible) were enrolled; 54 on B1 and 60 on B2, including 23 (B1) and 19 (B2) very early relapses. Median age at relapse was 10.2 years for the B1 cohort and 8.4 years for the B2 cohort. Concomitant extramedullary disease was present in 3 and 9 of the B1 and B2 patients, respectively. At the end of block 1, 48 B1 patients and 50 B2 patients

were evaluable for response with CR2 achieved in (31/48) 65% of B1 and (33/50) 66% of B2 patients. Minimal residual disease (MRD) was measured by flow cytometry in a COG reference laboratory at the end of block 1. Among the 62 pooled B1 and B2 patients who achieved CR2 and had MRD data available at the end of block 1, 26 (42%) (14/31 B1 and 12/31 B2) were MRD negative ($< 0.01\%$), which was significantly higher than the 25% with chemotherapy alone on the conventional COG 3-block platform (one-sided $p=0.001$). The addition of epratuzumab to reinduction chemotherapy was well tolerated with no significant increase in the baseline toxicity observed with the platform regimen alone with either schedule. Toxic deaths occurred in 3 patients (2.6%) during block 1 (2 in B1, 1 in B2), compared to 2.4% with block 1 chemotherapy alone. PK analyses in a cohort of B2 patients showed that the epratuzumab trough serum concentration steadily increased to 501 ± 149 mcg/mL before the final dose on day 25 ($n=26$). The mean serum half-life of epratuzumab was 17.0 ± 4.9 days ($n=17$) and was shorter than the value of 23 days observed in adults treated with epratuzumab for indolent non-Hodgkin lymphoma. No patient developed human anti-human antibodies. Epratuzumab, as given on the B1 and B2 schedules was tolerable in combination with chemotherapy in this paediatric and young adult patient cohort with early relapsed CD22-positive BCP ALL, but did not improve CR2 rates when compared to historical controls treated with chemotherapy alone. However, among patients who attained a complete remission, those treated with epratuzumab were significantly more likely to become MRD negative as compared to those treated without epratuzumab.⁸⁶ In summary, epratuzumab is the 1st monoclonal antibody targeting an antigen well expressed on lymphoblastic leukemic cells having passed phase I and II trials in paediatric relapsed ALL and being ready for phase III investigation for its efficacy on event-free and overall survival. Although the drug has not improved CR rates, it has induced improved MRD negativity as compared to historical controls. Furthermore, it has not shown any relevant toxicity and seems to be safe in combination with intensive standard chemotherapy. Therefore, it seems to be the ideal drug to be added to standard consolidation therapy to improve the remission quality and reduce persistent minimal residual disease in children with standard/intermediate risk relapsed ALL.

4.4.6 Rationale for the dosing of epratuzumab

In phase I/II dose finding studies in adult non hodgkin lymphoma weekly doses of epratuzumab between 120 and 1000 mg/m² have found to be safe without reaching dose limiting toxicities. Since a dose escalation beyond 360mg/m² did not lead to increased activity, this dose was taken forward as recommended dose for further evaluation. The mean serum half life in the adult NHL trial was 23 days corresponding to the half life of human IgG1.⁸¹ The COG has used the recommended dose twice weekly within a single drug window and weekly in combination with standard reinduction chemotherapy in children with relapsed ALL.⁶⁴ The median serum concentrations of epratuzumab ranged from 72µg/ml to 163 µg/ml. Pharmacodynamic studies showed a blocking of the specific binding sites of epratuzumab suggesting a saturation of the target with the given serum level in most patients. In an extended phase II study patients received epratuzumab weekly or biweekly in combination with standard reinduction chemotherapy. The serum half life was shorter with 17 days, possibly related to the higher leukemia burden of initial reinduction treatment. Mean serum concentrations were 501µg/ml.⁸⁶ The dosing in the IntReALL SR 2010 trial relates on the experience of the COG. 360mg/m² has been shown to be safe as single agent and in combination with multi drug chemotherapy and exhibiting full saturation of CD22 in the presence of cytologically evident ALL. Since in IntReALL SR 2010, epratuzumab is used in patients with a cytological remission in most patients, a full saturation of the remaining target antigen is expected. Considering the reported half life in the serum, drug levels are expected to accumulate even if the drug is applied at a weekly schedule for a total of 8 courses. Thus, an application twice weekly as investigated in a subgroup of patients in the extended phase II combination trial by the COG does not seem to be advantageous.³ Pharmacokinetic and dynamic investigations are planned to confirm the rationale for dosing and scheduling in a representative cohort of patients in each backbone arm.

4.5 Conclusions for the trial IntReALL SR 2010

Low patient numbers and distinct biologic subgroups require large international trials to recruit enough patients for randomized studies. The study allows for developing a platform of randomized optimization of standard phase III therapy for the SR group.

4.5.1 Stratification

The risk factors time to and site of relapse, the immunophenotype, and the MRD response in IR patients with BM involvement allow a clear stratification into a patient group with poor outcome with conventional chemotherapy and with clear indication for allogeneic SCT. S1 and S2 patients have now with the current protocols and the MRD based SCT stratification EFS rates in a comparable range and can be grouped together to a new SR strategy group (Figure 6b, page 22).

4.5.2 SR strategy

With the ALL-REZ BFM 2002 and the ALLR3 protocols better results for IR/S2 patients have been achieved than ever before. Both protocols are effective in context with the respective frontline protocols and should not be adopted without a controlled approach. The strategies of both protocols are fundamentally different: The ALL-REZ BFM 2002 protocol with short course intensive regimen, with an induction without anthracyclines, with a MRD cut-off of 10^{-3} , with preventive CNS irradiation in patients with BM relapse, and with a conventional and un-toxic maintenance therapy, versus the ALLR3 with a more continuous less intensive strategy, an anthracycline based induction, a MRD cut-off of 10^{-4} , no preventive CNS irradiation and an intensified CNS directed and more toxic maintenance therapy. Since the retrospective comparison of both protocols showed a even though not significantly better DFS probability of the ALLR3 mitoxantrone arm compared to the ALL-REZ BFM 2002 protocol, the statistical hypothesis of the randomization may well be an 10 % improvement of EFS probability with the ALLR3 protocol. Although results of SR patients have been dramatically improved still one fourth to one third of the patients will fail to be cured. Thus, there is still potential for improvement of EFS and OS rates. Epratuzumab is an ideal drug for this purpose, because it targets an antigen highly expressed on nearly all leukemias of the SR group, it has shown promising activity in combination with conventional intensive chemotherapy in children with relapsed ALL, and it has an absolute favourable toxicity profile allowing for combining with the SR consolidation chemotherapy without restrictions. Finally, at the stage of the current development of the drug, only a classical randomized phase III study can determine the antileukemic potential of the drug within a curative setting.

4.5.3 HR strategy

Patients with HR features (S3/4 and very early IEM relapse) have poor remission rates with all regimens applied so far. They are eligible for new and intensified induction regimens with the aim to improve remission rates. They are furthermore eligible for intensified consolidation regimens to achieve the best possible remission quality as prerequisite for successful allogeneic SCT. A standard control arm is required by members of the group to get reliable prospectively proven data, before a new potentially more toxic regimen is chosen as new standard. A study design should allow a rather rapid conclusion on efficacy/toxicity of applied regimens to switch to a next promising option, if no substantial improvement could be achieved. HR patients are treated in a separate study with phase II endpoints such as CR rates and with short recruitment periods of 2 to maximum 3 years allowing for screening of promising agents or combinations with a substantial better potency to overcome resistance than the currently used regimens.

4.5.4 Strategy for the use of asparaginase

PEG asparaginase is considered as 1st line drug in the IntReALL SR 2010 trial. If PEG asparaginase cannot be given due to allergic reaction or in case of silent inactivation, Erwinia asparaginase is currently the only alternative to induce asparagine depletion as essential part of the protocol. Both drugs are part of a treatment concept and not object of a clinical

study in the sense on an IMP. In the concept the biologic effect of asparaginase is intended, namely the asparagine depletion. Therefore, the drugs are exchangeable as long as the pharmacokinetic specificities are taken into account. In countries without marketing authorisation for PEG asparaginase and patients having received only and tolerated unpegualted coli asparaginase, this drug may be continued to be given in the IntReALL SR 2010 trial. This deviation can be implemented into the national appendix of the IntReALL SR 2010 protocol, if required.

4.5.5 Diagnostics and biological scientific programmes

The essential routine diagnostics for patients with relapsed ALL treated with intensive chemotherapy, monoclonal antibodies and stem cell transplantation include conventional cytology, flow cytometry to determine the immunophenotype and potential targets for monoclonal antibodies, and to perform MRD analysis including the potentially targeted antigen, furthermore cytogenetics and molecular genetic to characterize the genetic features of the leukemias which can be potentially targeted by specific drugs and which allow an additional prognostic assessment, and finally MRD analyses on with molecular biologic methods, to stratify patients to the adequate post induction therapy, to monitor the treatment response after achievement of a CR, to provide an adequate short term efficacy entrance and endpoint for individual interventions and to provide an information on the remission quality prior to SCT. All these methods have to be performed according to a controlled standard. All reference laboratories participate at pan-European initiatives to harmonize methods and interpretations of data. A large international trial for childhood relapsed ALL provides the unique opportunity to investigate characteristics on rare subgroups, to characterize mechanisms of resistance and to identify biologic features associated with treatment response or applicable for targeted therapy. Therefore, a structured tumour banking of the patient material is required.

5 STUDY OBJECTIVES

5.1 Main goals of the study

The main goal of this study is to improve the outcome of children and adolescents with standard risk first relapsed acute lymphoblastic leukemia. Furthermore, goal is to set up a large international study group platform allowing for optimization of standard treatment strategies and integration of new agents.

5.2 Primary objectives

- Improvement of EFS with arm B (ALLR3) compared to arm A (ALL-REZ BFM 2002) in SR patients
- Improvement of EFS after consolidation with versus without epratuzumab in SR patients

5.3 Secondary objectives

- Improvement of OS with arm B (ALLR3) compared to arm A (ALL-REZ BFM 2002)
- Improvement of OS after consolidation with versus without epratuzumab in SR patients
- Rate of CR2 of arm B (ALLR3) compared to arm A (ALL-REZ BFM 2002)
- Rate of SCT performed in Arm A versus Arm B
- Toxicity of Arm B (ALLR3) versus Arm A (ALL-REZ BFM 2002) in SR patients
- Toxicity of consolidation with versus without epratuzumab in SR patients
- Improvement of MRD reduction during consolidation with versus without epratuzumab in SR patients
- Rate of MRD negativity prior to SCT with Arm B (ALLR3) versus Arm A (ALL-REZ BFM 2002) in SR patients
- Rate of MRD negativity prior to SCT after consolidation with versus without epratuzumab in SR patients

- Pharmacokinetic of epratuzumab in context with Arm A and B in SR patients

5.4 Add-on studies

Add-on studies include investigations which do not have direct influence on the treatment of the patients and which are not communicated to the treating centres. They contribute to the improvement of knowledge on the biology of ALL and offer the chance to detect new risk factors or targets for new specific drugs. Whereas the individual patient will not benefit from these analyses, the future group of patients with ALL may benefit from the results. For the add-on studies, particular consent forms are provided according to the national requirements of the participating groups.

5.4.1 MRD in isolated extramedullary relapse

The rate and extent of sub-microscopic bone marrow involvement of by cytology isolated extramedullary leukemia will be investigated prospectively. The feasibility of this technically difficult project has been shown within a pilot study of the Resistant Disease Committee if the I-BFM SG (chapter 4.2.7, page 19).³⁹ Leukemia burden will be quantified using the established molecular biologic methods. The clone specific markers will be detected from extramedullary material as far as possible or adopted from primary diagnosis or from subsequent relapse. The dynamics of MRD reduction and elimination with the initial treatment courses will be investigated.

The extent of sub-microscopic leukemia burden and the rapidity of MRD elimination with induction therapy will be correlated with prognosis to support the preliminary finding of the pilot study, that high initial tumour burden is associated with increased rates of subsequent relapse.

Extramedullary tissue will be investigated with respect to its contribution to leukaemogenesis and interaction with the leukaemic cells.

5.4.2 MRD by flow cytometry including CD22 in patients receiving epratuzumab

MRD will be quantified in parallel to molecular biologic methods with flow cytometry. In patients without clonal markers detected by PCR methods, MRD after induction quantified by flow cytometry will be used for patient stratification. The feasibility of this method is investigated using CD22 as additional marker. Since CD22 is targeted with epratuzumab, the study will allow investigating, how MRD quantified by flow cytometry is influenced, if one of the MRD markers is targeted by a monoclonal antibody.

5.4.3 Extended genetic characterization of childhood relapsed ALL

In addition to routine genetic diagnostics such as conventional cytogenetics, FISH and identification of defined translocations by PCR, MLPA (Multiplex ligation-dependent probe amplification) and SNP arrays will be applied to identify numerical abnormalities of chromosomes such as deletions or amplifications in key target genes relevant for leukaemogenesis, proliferation and resistance. In an selected number of candidate genes, small genomic mutations are detected by conventional or next generation sequencing methods. With bio-informational technology the genetic data will be associated with clinical data, thus providing a hypothesis driven genotype-phenotype association. The identification of variants for those candidate genes supporting the hypothesis allows the development of DNA-based biomarkers for diagnosis of ALL disease types either for pre-disposition genes before chemotherapy or monitoring in the course of chemotherapy to identify early appearance of new mutations associated with therapy-resistant leukaemia. In selected leukemias representative for relevant biologic or clinical subgroups, whole genome sequencing of disease and remission samples will be performed to screen for new relevant pathogenetic and prognostic factors and possible structures for targeted drugs.

5.4.4 Primograft

A foreseeable problem is even with a well structured bank, that not enough primary tissue will be available to perform all the required analyses. Often only small amounts of cells can be obtained in the patient population and in some patients the marrow aspirate is only partially infiltrated. Biomarker research has therefore often used cultured cell lines as a surrogate for primary leukaemic cells. The study group wishes to take advantage of new technology which permits the expansion of primary samples, using NSG mice to create primografts. It is intended to use a network of laboratories to create a highly characterised primograft bank of > 400 patients. These samples will be analysed using high throughput techniques and checked periodically. They will form the basis of research pathway identification, drug discovery and drug testing which will be done in a collaborative fashion with industry. This will be a unique standardised resource for the European Union and will benefit patient's world wide.

6 TRIAL DESIGN AND DESCRIPTION

6.1 Trial design

The IntReALL SR 2010 trial is an inter-group, international multi-centre, treatment optimization trial. It contains the followings branches:

Randomization 1: Prospective, randomized, open label phase III trial comparing the efficacy and toxicity of standard protocols ALL-REZ BFM 2002 (Arm SR-A) and ALLR3 (Arm SR-B).

Randomization 2: Prospective, randomized, open label phase III trial comparing the efficacy and toxicity of the respective consolidation therapy plus epratuzumab versus standard consolidation alone.

6.2 Requirements for participating investigators and trial sites

The principal investigators have to be specialized in paediatric haematology/oncology and need to have documented experience with GCP and clinical trials.

Requirements for trial sites:

- Access to intensive care unit
- Access to hematologic laboratory facilities for cytological diagnosis
- Pediatric haematologist/oncologist on call 24 hours/day 7 days/week
- Access to diagnostic facilities: CT, MRI
- Access to radiotherapy facilities
- Fulfilling the national criteria for a paediatric haematology/oncology centre (specified in the national appendices).

6.3 Expected duration of the trial

Start of the IntReALL 2010 trial	01.10.2012
End of patient recruitment	30.09.2016 (4 years)
End of the trial (3 years follow-up after recruiting the last patient)	30.09.2019
Further follow up by national cancer registries	

6.4 Premature termination

6.4.1 Premature termination of the trial for individual patients

Individual patients are excluded from the study in case of one of the following situations:

- Withdrawal of consent
- Pregnancy

- Retroactive failure to fulfil inclusion/exclusion criteria
- Significant noncompliance
- New medical conditions not allowing for continuation of the protocol conform treatment
- Patients excluded from the study are further observed and considered for intention to treat analyses unless they withdraw their consent for registration within the study.

6.4.2 Premature closure of a trial site

Premature closure of a trial site is to be considered if:

- The conduct of the study is not compliant with the protocol
- Data quality does not meet required standards
- Data return is insufficient for trial purposes
- Insufficient recruitment (less than 1 patient within 3 years)

The premature closure of a site will be decided by the national coordinating investigator and the international coordinating investigator and sponsor representative. The DSMC and trial statistician should be consulted. Investigators and trial sites deciding not to take part in the trial any longer have to inform the national coordinating and the international coordinating investigators immediately. Details on further treatment and follow-up of patients already on study have to be discussed with the co-ordinating national and international investigator.

6.4.3 Premature termination of the trial or of trial arms

In case of the following situations, a premature termination of the trial or of trial arms has to be considered:

- Serious adverse drug reactions leading to substantial changes in risk-benefit considerations
- Unacceptable toxicity (e.g. cumulative occurrence of deaths conditional on therapy)
- Insufficient efficacy
- Superiority of one therapy arm
- New insights from other trials
- Insufficient recruitment rate
- Unsustainable trial organization
- New scientific evidence provided during the study that could affect the patient's safety (benefit-risk analysis no longer positive)

If only one arm or one risk group is affected by premature termination, the not affected arms may be continued.

The Data Monitoring Committee will monitor the study conduct and the safety aspects of the trial on a regular basis, and will give recommendations to the Inter-Group Trial Steering Committee whether to stop the trial or to change the trial protocol. The Inter-Group Trial Steering Committee will then decide on the actions to be taken.

7 RISK-BENEFIT CONSIDERATIONS

The trial is designed to provide at least the established standard therapy for children with relapsed ALL resulting in the best results ever achieved or to provide a potential additional benefit from new and promising strategies.

7.1 New stratification

For ALLR3 no changes in stratification are foreseen. For BFM patients, former S1 patients are now grouped together with S2 patients into the SR group. They do now receive a longer treatment with 2 more consolidation-courses and a prolongation of maintenance therapy from 12 to 24 months. Since EFS rates of S1 patients with the trial ALL-REZ BFM 2002 were

below 70% and not different from S2 patients, the prolongation of treatment for former S1 patients seems to be justified and the increased toxicity is counterbalanced by a potential improvement of EFS and OS rates. As S2 patients, S1 patients may benefit from the epratuzumab randomization. Patients with very early isolated extramedullary relapse are now stratified to the HR group.

7.2 SR strategy

7.2.1 Randomization arm A (ALL-REZ BFM 2002) versus arm B (ALLR3)

For each group with an established effective relapse strategy it may be a risk to switch to different strategy, which has been proven to be effective within the original context, but not necessarily within the new context. Therefore an introduction of both protocols into this new context requires a prospectively controlled design. Both protocols can be regarded as the currently best available standard. Both protocols provide potential benefits for the patients: The ALL-REZ BFM 2002 strategy a lower induction toxicity, a slightly lower SCT rate, a less toxic maintenance therapy; the ALLR3 a more effective induction, a less intensive late consolidation (interim maintenance), and as statistical hypothesis of the trial the EFS improvement compared to the ALL-REZ BFM 2002 arm.

7.2.2 Randomization of epratuzumab

The expected EFS probability of SR patients ranges from 70-80%. The use of epratuzumab offers a chance to achieve even higher cure rates. The documented toxicity of epratuzumab is mild, infusion related toxicity grade 1-2, and so far no long-term toxicities have been reported so far. Since leukencephalopathy has been reported after rituximab therapy, a comparable CD20 directed monoclonal antibody, such effects should be considered for epratuzumab as well. Although a clear association has not yet been established, enhanced risk management and pharmacovigilance have been initiated to monitor this potential effect.⁸⁷ Since in patients with ALL relapse the risk for development of leukencephalopathy is present due to other treatment elements such as high dose methotrexate, intrathecal therapy, cranial irradiation and allogeneic SCT, a monitoring of CNS alterations with MRI at start and at the end of the protocol treatment is recommended regardless if epratuzumab is given or not. Taken together the potential benefit of epratuzumab far outweighs the risk of side effects.

8 STUDY POPULATION

Eligible for the study are principally children with relapsed acute lymphoblastic leukemia.

8.1 Inclusion criteria

- Morphologically confirmed diagnosis of 1st relapsed precursor B-cell or T-cell ALL
- Children less than 18 years of age at inclusion
- Meeting SR criteria: late isolated or late/early combined BCP BM relapse, any late/early isolated extramedullary relapse
- Patient enrolled in a participating centre
- Written informed consent
- Start of treatment falling into the study period
- No participation in other clinical trials 30 days prior to study enrolment that interfere with this protocol, except trials for primary ALL

8.2 Inclusion criteria specific for the epratuzumab randomization

- Precursor B-cell immunophenotype. A specific CD22 expression level is not required
- M1 or M2 status of the bone marrow after induction

8.3 Exclusion criteria

- BCR-ABL / t(9;22) positive ALL
- Pregnancy or positive pregnancy test (urine sample positive for β -HCG > 10 U/l)
- Sexually active adolescents not willing to use highly effective contraceptive method (pearl index <1) until 2 years after end of antileukemic therapy
- Breast feeding
- Relapse post allogeneic stem-cell transplantation
- The whole protocol or essential parts are declined either by patient himself/herself or the respective legal guardian
- No consent is given for saving and propagation of pseudonymized medical data for study reasons
- Severe concomitant disease that does not allow treatment according to the protocol at the investigator's discretion (e.g. malformation syndromes, cardiac malformations, metabolic disorders)
- Karnovsky / Lansky score < 50%
- Subjects unwilling or unable to comply with the study procedures
- Subjects who are legally detained in an official institute

8.3.1 Allowed systemic diseases and concomitant medication

- Patients with systemic diseases such as Down syndrome, cystic fibrosis or diabetes mellitus are eligible for enrolment in this study. Due to the anticipated increase of toxicity dose reductions are suggested after discussion with the national study centre.
- Any kind of concomitant medication given due to medical reasons is allowed except antileukemic therapy, investigational drugs other than scheduled in the protocol, and attenuated live vaccines which are strictly prohibited during and until 6 months after end of chemotherapy or 18 months after allogeneic HSCT. Incompatibilities and drug interactions with the study medications are listed in the appendix. In case of expected adverse interactions, concomitant therapies should be changed to alternative less problematic agents if possible.

8.4 Duration of study participation

The standard duration of participation in this study includes the intensive phase of treatment, a maintenance phase and a follow-up period of 4 years after the diagnosis of relapse. Stem cell transplantation is not a part of this protocol. For patients who receive SCT study participation will end immediately before SCT. However, EFS and OS data will be recorded after SCT and are relevant as study endpoints. After termination of the study, patients will be followed up by national children's cancer registries to capture safety relevant late effects, secondary malignancies and to have the opportunity to give the feedback to the patients, if necessary. Patients will be taken off study if there is no response to treatment, if there is a subsequent relapse, in case of treatment-induced death and in cases of significant protocol violations for non-medical reasons. Whether a patient will be taken off study will be decided after consultation with the Berlin study centre. A medically indicated deviation from protocol therapy is not a reason to come off study. Rather, such a deviation will be documented and used in the assessment of the feasibility of the study. The national study centres offer consultation on further therapy for patients without a response to treatment. Patients who are off study continue to be observed including a report of death in order to allow the assessment of overall survival.

8.5 Inclusion of children / minors

ALL differs clearly in its biological and genetic features as well as in its outcome regarding the different age groups of adult and childhood ALL. Adult patients with ALL have a considerably poorer outcome (between 30-40% EFS) with Philadelphia chromosome being the most frequent chromosomal aberration in this group. Therefore the IntReALL 2010 trial

only recruits children and adolescents until the age of 18 years to explore their biologic features as well as their response to treatment and to improve event-free and disease-free survival for these patients.

8.5.1 Justification of the SR strategy in children/ minors

The first randomization of SR patients between Arm A and B compares 2 standard regimen well established as treatment of childhood relapsed ALL (chapter 4.2.8, page 21; chapter 4.2.9, page 22). Only within this particular patients and age group it can be determined, which of the best available treatment regimen would be the most appropriate for the whole study group. In contrast, an uncontrolled introduction of in each case the external regimen might result in un-interpretable results (chapter 7.2.1, page 33).

With epratuzumab, a drug is studied, which is not licensed so far in adults and children. However, the development in adults is far advanced and extensive safety and efficacy data are available in patients with precursor B-cell malignancies. In children with relapsed ALL, a phase I/II trial with the use of a single drug window and of a combination with chemotherapy has been performed and published. Only in a randomized phase III combination study, the benefit of the drug in context with intensive consolidation therapy can be clarified. Since the safety profile of the compound has been favourable both as single drug and in combination with chemotherapy, the use in children at this stage of the development is justified and may result in a substantial benefit for the patients (chapter 7.2.2, page 33). Due to the different biology and clinical behaviour of adult ALL, these data can not be generated in other age groups.

8.6 Enrolment procedure of a patient

If an ALL relapse is confirmed in a participating centre, all inclusion- and exclusion criteria have to be checked. If these criteria are fulfilled, the informed consent should be acquired. According to the clinical finding the patient should be allocated by randomization to one of the strategy groups, and informed accordingly. The information has to be given by one of the registered investigators of the centres. The patient has to be informed in an age-adapted way and should document his consent as far as possible. Children from the age of 14 years have to give their written consent as prerequisite for study participation. The informed consent should be obtained as soon as possible since the 1st randomization in both risk groups decides the induction therapy from the beginning on. The confirmation of inclusion and exclusion criteria as well as the achievement of the written informed consent of the guardians and the patients, if applicable, should be immediately documented within the electronic CRF. The result for the 1st randomization is then obtained directly from the system. During the process of getting written informed consent and obtaining the randomization results, the patients can receive a dexamethasone pre-phase for 3 up to a maximum of 10 days. The written informed consent for the 2nd randomization in SR patients should be obtained during the induction phase. However, randomisation will be performed only directly after receiving the result of the bone marrow puncture after induction (i.e. SR arm A week 5, arm B week 6) as this is a mandatory inclusion criterion.

9 RISK GROUP STRATIFICATION AND SCT INDICATION

9.1 Stratification

The risk stratification is based on the clinical parameters time point, site and immunophenotype of relapse which are defined as follows:

9.1.1 Definition of time-point and site of relapse

The time-point of relapse is defined in relation to the date of primary diagnosis and the date of completion of primary therapy

Table 1). Completion of primary therapy is defined as the end of the antileukemic therapy of the frontline protocol. This is in most cases the end of the maintenance therapy, but may also

be the last treatment after interruption of the intensive treatment, or of an inadequately short primary therapy. Since the duration of maintenance therapy varies between different protocols and individual patients (in most patients and protocols total treatment duration of 24 months), completion of primary therapy is a flexible time point in contrast to time point definitions referring to the date of primary diagnosis. Data from the ALL-REZ BFM Study Group clearly show, that the end of frontline therapy is more important for the prognosis than the absolute duration of 1st CR (unpublished). “Good risk” i.e. late relapses can be withheld by a prolongation of maintenance therapy, and ALL relapses have a good prognosis if they occur more than 66 months after the end of an inadequately short primary therapy.

The site of relapse is determined on the basis of conventional light microscopy using the FAB criteria. In morphologically unclear situations such as bone marrow involvement of extramedullary relapse around 5%, minimal residual disease quantification may be regarded as secondary criteria to determine the extent of bone marrow involvement.

Table 1 Definition of time point of relapse

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

Table 2 Definition of site of relapse

Bone marrow		M1 (< 5% blasts)	M2 (≥ 5% and < 25% blasts)	M3 (≥ 25% blasts)
extramedullary relapse	No	No ALL relapse	Requires follow up control	Isolated bone marrow relapse
	Yes	Isolated extramedullary relapse	Combined bone marrow / extramedullary relapse	

The immunophenotype is defined according to EGIL criteria.

9.1.2 Definition of the SR and HR group

Two risk groups are defined: standard risk (SR) and high risk (HR).

Standard Risk (SR): Late or early isolated extramedullary relapse of BCP or T-ALL; late or early combined bone marrow / extramedullary relapse, late isolated bone marrow relapse of BCP ALL.

High Risk (HR): Very early isolated extramedullary relapse of BCP or T-ALL, early isolated or any very early bone marrow relapse of BCP-ALL, any bone marrow relapse of T-ALL.

Table 3 Definition of IntReALL SR/HR 2010 risk groups

\ Site Time-point \	Immunophenotype: B-cell precursor			Immunophenotype: (pre) T		
	Extramed. Isolated	Bone marrow combined	Bone marrow isolated	Extramed. isolated	Bone marrow combined	Bone marrow isolated
Very early	HR	HR	HR	HR	HR	HR
Early	SR	SR	HR	SR	HR	HR
Late	SR	SR	SR	SR	HR	HR

9.2 Indication for allogeneic stem cell transplantation

The eligibility for allogeneic SCT has been developed on the basis of results with chemo/radiotherapy only. A proven indication is given in patient subgroups with poor EFS/OS rates with chemo/radiotherapy alone if a significant and clinically relevant improvement of EFS and OS rates could be achieved with allogeneic SCT. The SCT procedure is not part of this protocol. It is recommended to include patients with SCT indication into the national SCT studies and protocols as far as available to warrant a quality-controlled and homogeneous treatment.

Relevant for the SCT indication are the parameters IntReALL risk group, site of relapse, and MRD response in SR patients. MRD is quantified by molecular genetic methods. The cut off for SCT indication depends on the induction intensity of the respective treatment arm: Patients of arm A (ALL-REZ BFM 2002) are eligible for allogeneic SCT if MRD after induction is $\geq 10^{-3}$; patients of arm B (ALLR3) are eligible for allogeneic SCT if MRD after induction is $\geq 10^{-4}$. If MRD quantified by molecular genetic methods is not available, results of MRD quantified by flow cytometry can be applied with the same cut off values. If MRD can not be quantified after induction at all, patients with late BM relapse are eligible for MD-SCT but not for MMD-SCT and patients with early combined BM relapse are eligible for both, MD and MMD-SCT.

Table 4 Indication for allogeneic stem cell transplantation, IntReALL SR 2010 protocol

SCT	SR							
	Late isolated or combined BM relapse			Early combined BM relapse			Isolated EM relapse	
	MRD GR	MRD PR	MRD ND	MRD GR	MRD PR	MRD ND	Late	Early
MD*	No	Yes	Yes	Yes	Yes	Yes	No	Yes
MMD**	No	Yes	No	No	Yes	Yes	No	No

* Matched donor is defined as at least 9 out of 10 HLA allele identical with high-resolution typing of HLA A, B, C, and Dq, Dr. ** Mismatched donor is defined as less than 9 out of 10 HLA allele identical (= more than 1 antigen mismatch). Abbreviations: BM, bone marrow; GR, good response as defined by the specific SR-arm; HR, high risk; IEM, isolated extramedullary; MD, matched donor; MMD, mismatched donor; MRD, minimal residual disease after induction; PR, poor response, as defined by the specific SR-arm; SCT, stem-cell transplantation; SR, standard risk.

The indication of matched donor SCT for SR patients with BM relapse and MRD poor response can be regarded as proven. The indication of matched donor SCT in patients with early combined BM relapse and MRD good response, the indication of matched donor SCT in patients with early isolated extramedullary relapse and of mismatched donor SCT in any SR patients need to be prospectively confirmed using historical controls.

10 DIAGNOSTICS

The diagnostic procedures before start of the study treatment (day 1 of week 1) are not part of the study. They are essential to establish the correct diagnosis as prerequisite for assessment of the in- and exclusion criteria.

10.1 Bone marrow relapse

Bone-marrow involvement is assessed by light microscopy applying the French-American-British (FAB) criteria. To minimize non-representative material, BM aspirations from 2 sides (usually both posterior iliac spines) are required. If the aspiration is insufficient (sicca), a

bone marrow biopsy should be taken instead and referred to pathology. In this case, the aspiration is repeated after a few days of dexamethasone prephase, which usually leads to mobilisation of the blast cells. The extent of BM involvement is classified as listed in Table 2, page 36. If the extent of bone marrow involvement quantified by cytomorphology is inconclusive, MRD techniques may be considered in addition.

10.2 CNS relapse

A CNS relapse is diagnosed if morphologically unequivocal leukemic lymphoblasts are detected in the CSF and there is a pleocytosis of $>5/\mu\text{l}$ nucleated cells. If the CSF is contaminated with blood the following procedure is recommended after discussion with the national study centre: If blasts are present in the CSF and the peripheral blood shows no blasts, a CNS relapse is assumed. If the proportion of blasts in the CSF is equivalent to the proportion of blasts in the peripheral blood the Steinherz/Bleyer algorithm is applied: if the ratio of WBC/RBC in the CSF exceeds the same ratio in the peripheral blood for > 2 times, a CNS involvement is assumed, otherwise a contamination is assumed. In unclear situations a case-by-case decision may be necessary. If blasts are present the patient receives the intensified intrathecal chemotherapy similarly to patients with CNS involvement but not the increased dose of cranial irradiation. If clinical signs of CNS involvement are present such as visual disturbances, polyphagia, cranial nerve palsies in the absence of CSF pleocytosis, the presence of a CNS relapse has to be confirmed or ruled out with all available diagnostic methods (cranial CT, MRI). If evidence of meningeal infiltration is found by imaging, a biopsy may have to be performed.

10.3 Testicular relapse

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

10.4 Other extramedullary sites of relapse

Any site, organ or tissue may be infiltrated by leukemia. To detect clinically not overt organ involvement, an explorative ultrasound examination of the abdomen and the lymph node regions has to be performed. Additionally other radiological measures such as MRI or CT may be necessary to determine organ involvement. Usually, no specific local treatment has to be applied since systemic chemotherapy is not impaired by blood barriers. However, bradytrophic sites such as the anterior chamber of the eye may be involved requiring specific diagnostics and local therapy. If a local leukemic infiltration seems to be persistent during induction and early consolidation, a biopsy should rule out persistence of vital leukemic cells, otherwise, a local irradiation would be recommendable. This may be in particular an issue in case of mediastinal tumour of a T-ALL relapse and should be discussed individually with the national study centre.

10.5 Leukemia specific diagnostics

The diagnosis of relapsed ALL relies on cytological, immunological, cytogenetic and molecular genetic investigations used for optimal treatment stratification and comprehensive characterisation of leukemia at relapse diagnosis and during relapse treatment. An additional aim is a better understanding of the pathophysiology of relapsed ALL to define novel parameters for specific, efficacious and risk-adapted therapeutic strategies.

10.5.1 Cytological examination

The cytological examination of bone marrow and/or blood smears is performed in order to diagnose a relapse. The classical May Grunewald Giemsa staining allows for identifying morphologically abnormal immature cells in varying proportions. The morphological

characterisation of leukemic cells can be used to identify the lineage involved applying FAB classification system.⁸⁸

10.5.1.1 Schedule of cytological examinations

Cytology of bone marrow aspirate is done at diagnosis in all groups. Follow up controls are performed in SR arm A at weeks 5, 9, 13, 16 or prior to SCT respectively. Optional bone marrow aspirates can be done at week 3, at week 27 before start and at week 132 after the end of maintenance therapy to confirm cytoreduction or CCR2, respectively. In SR arm B, follow up controls are performed at weeks 6, 10, 13, 15 or prior to SCT respectively. Optional bone marrow aspirates can be done at week 3, at week 31 before start and at week 135 after the end of maintenance therapy to confirm cytoreduction or CCR2, respectively.

Cytology of the CSF is done at diagnosis in all groups. In patients with CNS involvement, CSF cytology is performed until 2 consecutive samples free of leukemia have been documented.

Table 5 Overview of diagnostics

Type	Methods	Usage
Mandatory diagnostics used for treatment stratification		
Cytological examination	May Gruenwald Giemsa staining	Morphologic French-American-British classification; cytological quantification of leukemia in the blood count, the bone marrow aspirate or cytospin preparation of the CSF.
Immunophenotype	Multicolour flow cytometry	Classification of lineage origin (BCP- or T-cell), classification of maturity stadium within the lineage according to EGIL criteria.
Quantification of MRD	PCR using clonal TCR/IG rearrangements and/or multicolour flow cytometry	Quantification of sub-microscopic disease in the bone marrow; <u>SR patients:</u> quantification of MRD response post induction in as parameter for SCT indication; determination of MRD reduction kinetics during post induction randomization. <u>HR patients:</u> Detection of MRD-persistence post induction. <u>Isolated extramedullary relapses:</u> sub-microscopic bone marrow involvement at diagnosis and its reduction during treatment
Important diagnostics used for genetic characterisation of leukemic relapse		
Fusion genes: ETV6-RUNX1, BCR-ABL, E2A-PBX1, MLL-AF4, MLL-ENL, MLL-Rearr	PCR on gene expression level and/or FISH	confirmation of fusion genes, identified at initial diagnosis, and identification of new fusion genes at relapse
DNA-Index / analysis of chromosomes in inter- or metaphase. Ploidy: Hyper-/Hypodiploidy	flow cytometry, FISH and/or assessment of chromosomes in metaphase by Giemsa staining	basic cyto-/molecular genetic characterisation of leukemia as gold standard for new molecular genetic screening methods
Analysis of chromosomes in metaphase Karyotype	assessment of the chromosomes in metaphase by Giemsa staining	identification of possible new structural and numerical chromosomal changes

10.5.2 Immunophenotype

The immunophenotyping is an essential method for diagnosis of a relapse by lineage assignment based on the assessment of surface and cytosolic markers. The classification in B-cell precursor (BCP)- or T-cell immunophenotype at relapse diagnosis is necessary for stratification in standard or high risk group and is done according to the EGIL classification.⁸⁹ Moreover, in context of the current protocol, the assessment of the immunophenotype will provide information on the expression of antigens like CD22 which is used for antibody treatment in the current protocol. Standardisation and quality control for immunophenotyping will be organised by defined national or international groups in the different countries.

10.5.2.1 Schedule of immunophenotyping

Immunophenotyping is performed with bone marrow aspirate at diagnosis for all groups. Immunophenotyping of the CSF in patients with CNS relapse is not obligatory but can be performed.

10.5.3 Quantification of minimal residual disease

Minimal residual disease (MRD) is quantified in bone marrow taken at defined time points during treatment of all patients.

In standard risk patients, MRD used for treatment stratification is quantified immediately after bone marrow aspiration on day 28 in Arm A and day 35 in Arm B. These results will be communicated to the treating centre. Bone marrow taken after first induction course and at later time points during and after consolidation will be used to compare the antileukemic effect of the randomized arms with and without epratuzumab. These MRD results will not be communicated to the treating centres.

In patients with an isolated extramedullary relapse the submicroscopic bone marrow involvement at relapse diagnosis and its reduction during treatment is measured. After first promising retrospective data, the prognostic impact of submicroscopic bone marrow involvement in patients with isolated extramedullary relapse has been evaluated in an international prospective study. The results of quantification of submicroscopic bone marrow involvement will not be communicated to the treating centres.

MRD-quantification on genomic level with Polymerase Chain Reaction (PCR) using clonal T-cell receptor (TCR) / immunoglobulin (IG) gene rearrangements is currently considered as the gold standard for this protocol. Flow cytometry is used instead of PCR if PCR based MRD-quantification can not be performed because criteria for a reliable and reproducible sensitive quantification are not fulfilled. A prospective comparison of MRD results measured with PCR and flow cytometry has been currently performed in several countries applying the ALL-REZ BFM 2002 or ALL R3 protocol.

Multicolour flow cytometry will be used in parallel with PCR in order to gain additional important information on antigen expression on residual leukemic cells during different treatment phases and information of lymphoid and myeloid regeneration.

10.5.3.1 Schedule for MRD quantification

MRD by PCR and flow cytometry is always done in parallel. If material is limited, MRD by PCR is the preferential method. MRD with both methods is performed at diagnosis to establish the clonal markers for PCR and the clone specific antigen profile for flow cytometry. Follow up analyses are performed at the same time points as the bone marrow cytology (see chapter 10.5.2.1).

10.5.4 PCR

TCR/IG gene rearrangements can be used as a 'fingerprint' to identify clonal lymphoblastic populations at a highly sensitive (MRD) level at diagnosis and during treatment of ALL. In the context of relapsed ALL it is strongly recommended to perform a new screening for clonal TCR/IG gene rearrangements at relapse diagnosis in order to identify markers specific for clonal relapsed populations. These markers will be selected according to their sensitivity for MRD-quantification. It is recommended to use at least two markers for quantification of a bone marrow taken at a time point relevant for treatment stratification. If only one marker is

available, the results are correlated with the flow cytometry results. If they are consistent, the results are accepted for stratification. If they are contradictory, the MRD of this time point may be set to "not done/available". All MRD-data measured in the protocol have been assessed according the guidelines of the Euro-MRD group (former name: European Study Group on MRD detection in ALL).^{29,30}

10.5.5 Multicolour flow cytometry

Detection of MRD by flow cytometry is based on the identification of aberrant or specific expression of antigens on leukemic cells compared to normal hematopoietic cells. Beside the sensitive detection of MRD, the method is applied to assess the expression antigens like CD22 for standard risk patients and additional interesting proteins which might be important for a targeted treatment. Further, the method is important to describe and find a quantitative dimension for regeneration during treatment.

The standardisation and quality control for flow cytometry based MRD-quantification have been established by several international and national groups.³¹

10.5.6 Structural and numerical chromosomal changes

The information of structural and numerical chromosomal changes is essential for a comprehensive biological characterisation of an ALL relapse. Therefore, the assessment of common fusion genes, the ploidy status and the cytogenetic karyotype are included in the routine cytogenetic and molecular genetic analyses for all patients in the protocol.

10.5.6.1 Schedule for genetic diagnostics

Assessments of fusion genes, ploidy status and karyotype are performed with bone marrow aspirate at diagnosis of all groups.

10.5.6.2 Fusion genes

Common fusion genes in childhood ALL as ETV6-RUNX1, BCR-ABL, E2A-PBX1, MLL-AF4, MLL-ENL are assessed at relapse diagnosis in order to confirm the initially identified fusion gene or to identify a fusion gene gained at relapse. In most groups of the study both methods, the molecular genetic identification of the fusion gene transcript by PCR and the cytogenetic analysis by fluorescence in situ hybridisation (FISH) using often interphase nuclei are applied. Both technologies are standardised and included in national or international quality control rounds.

10.5.6.3 Ploidy status

The assessment of the ploidy status in order to identify ALL relapses with a hypo- or a high hyperdiploidy is done using the karyotype, FISH with centromeric and other probes or the chromoprobe of Multiprobe-I system and additionally in some groups by the measurement of the DNA-Index. The assessment of DNA-Index is performed using propidium iodide for DNA staining measured by flow cytometry. It is a very fast and robust method. For the identification of whole chromosome gains or losses it is necessary to assess the classic karyotype or newer molecular genetic methods as multiplex ligation dependent probe amplification or single nucleotide polymorphism/comparative genomic hybridisation arrays.

10.5.6.4 Karyotype

The cytogenetic karyotype of leukemic blast cells can be used to identify chromosomal anomalies as aneuploidy, chromosomal translocations excluding cryptic translocation and deletions. Mitotic cells are chemically treated to obtain G (Giemsa) or Q (quinacrine)-banding. Conventional karyotyping is a method to obtain a global genomic analysis. However, it is often difficult to get metaphases from ALL samples (failure rate 10-20%). There are national and international groups responsible for standardisation and quality control concerning cytogenetic methods as karyotyping and FISH.

10.6 Pharmacokinetic and pharmacodynamic diagnostics

Pharmacokinetic and dynamic analyses are performed for asparaginase and epratuzumab.

10.6.1 Pharmacokinetic analyses for asparaginase

All patients receive PEG-asparaginase from the beginning on. PEG-asparaginase is known to be associated with silent inactivation in some patients.⁹⁰ The clinical significance of this is not yet determined. A change of the asparaginase preparation to Erwinia-asparaginase is only done in case of overt allergic reaction. Therefore, the trial steering committee recommended to analyse asparaginase activity in selected centres but to blind the data.

10.6.1.1 Schedule for analysis of PEG-asparaginase activity in selected centres

Asparaginase activity is analyzed on day 7 and day 14 after every PEG-asparaginase application.

10.6.2 Pharmacokinetic and pharmacodynamic analyses of epratuzumab

Although the serum levels and half live of epratuzumab in children with relapsed ALL has been determined by the COG,⁶⁴ these data should be collected in the context of the IntReALL 2010 trial as well in a defined number of patients in selected centres. In particular, in arm A versus B, different background schedules are given, one with and one without dexamethasone, which may have an influence on pharmacokinetics and -dynamics. Since mean serum half life has been reported to range around 4 weeks after the last application, serum levels should be measured until 6 weeks after the 8th dose. Pharmacodynamic of epratuzumab is determined by quantifying the CD22 positive cells in the blood and the bone marrow by flow cytometry and analyzing them in context with the clone specific antigen panel determined for MRD monitoring.

10.6.2.1 Schedule for Pharmacokinetic and pharmacodynamic analyses of epratuzumab

The epratuzumab serum level is quantified before each application of the drug and once a week until 6 weeks after the last application (SR arm A day 1 of weeks 5-18, arm B of weeks 6-19). Quantification of CD22 positive cells in peripheral blood will be performed before the 1st dose of epratuzumab, then every 2nd week until 6 weeks after the last dose, then every 6 weeks until 6 months after start of epratuzumab, if possible also after allogeneic SCT (SR arm A day 1 of weeks 5, 7, 9, 11, 13, 15, 17, 23, 29; SR arm B day 1 of weeks 6, 8, 10, 12, 14, 16, 18, 24, 30). At the same time-points, immunoglobulin classes are quantified as parameter for B-cell function.

10.7 General medical diagnostics, monitoring of infections and organ function

At diagnosis, a complete work up of infection parameters and organ function is performed as basic values for further monitoring. This includes biochemistry, coagulation parameters, virology, HLA-typing, ultrasound of abdomen, testes, lymph nodes and venous vessels for central line implantation, echocardiogram, chest X-ray, electroencephalography, and MRI of the head. A flow chart for all diagnostic measures is given in Table 5 and Table 9, page 39 and 76, respectively.

10.7.1 Physical examinations

Physical examination needs to be performed and documented in the medical records at any application of chemotherapy, at every presentation during maintenance therapy, and at any occurrence of toxicity or clinical problems. If relevant signs of toxicity or clinical problems become evident, they need to be documented as AE's or SAE's according to the guideline within the IntReALL SR 2010 protocol (chapter 14, page 81).

10.7.2 Biochemistry analyses

Biochemistry parameters (electrolytes, creatinine, Alat/Asat, LDH, bilirubine, protein, glucose) are analysed at start and end of every treatment element. During maintenance therapy bilirubine is controlled every 12 weeks in both arms. If it is elevated 1.5 times beyond the upper normal level, Alat/Asat is analyzed. Maintenance therapy is adapted in case of elevated liver enzymes (chapter 11.6.7, page 55; chapter 11.7.6, page 63). Furthermore extended biochemistry analyses are performed upon clinical requirements in case of toxicities of clinical problems.

The results of biochemistry analyses are documented in the medical records and in the study data base, if required (see definition of toxicity, AE, and SAE reporting, chapter 14, page 81).

Table 6 Disease- and drug specific diagnostic procedures by protocol week and by risk group and treatment arm

Parameters \ Week	Arm	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	
BM Cytology	SR-A	+		(+)		+				+				+			+											(+)				
	SR-B	+		(+)			+				+			+																		
BM Immunophenotype		+																														
BM Genetics		+																														
BM MRD	A	+		(+)		+				+				+			+											(+)				
	B	+		(+)			+				+			+																		(+)
CSF cytology		+	(+)	(+)																												
Asparaginase PK	SR-A		+	+	+	+	+	+	+						+			+	+		+				+	+						
	SR-B		+	+	+	+	+	+	+		+			+																		
Epratuzumab PK (serum levels)	SR-A					+	+	+	+	+	+	+	+	+	+	+	+	+	+													
	SR-B					+	+	+	+	+	+	+	+	+	+	+	+	+	+	+												
Epratuzumab PD (CD22 & Ig levels)	SR-A					+		+		+		+		+		+	+	+						+							+	
	SR-B					+	+		+		+		+		+			+	+	+											+	+

Abbreviations: BM, bone marrow; CSF, cerebro-spinal fluid; MRD, minimal residual disease analysis (PCR and flow); PD, pharmacodynamic analysis; PK, pharmacokinetic analysis; SR, standard risk.

10.7.3 Blood volumes for diagnostic analyses

Blood volumes for diagnostic analyses should be restricted to a necessary minimum. In infants, special tubes with reduced volumes as provided by the local laboratories should be used.

For clinical routine at relapse diagnosis (1st visit), extended analysis of biochemistry, coagulation function and viral serology are performed and total maximal blood volume is expected to be 10-15ml (Table 9, page 76). Blood counts and biochemistry parameters are analyzed regularly during protocol therapy and expected maximal blood volumes per visit are 5-7ml (1-2ml for blood counts, 2-3ml for biochemistry analysis, 2-3ml for coagulation parameters).

For leukemia diagnosis and monitoring, at diagnosis 20 ml BM and 5 - max. 10 ml PB are required for immunophenotyping and genetic analysis at diagnosis. For MRD follow up analysis, 10-15 ml BM and 5-10 ml PB are taken according to planned schedules. For PK/PD analysis, 2ml blood (Asparaginase PK) and 2 ml blood (1ml serum, Epratuzumab PK/PD each) are required at each planned time point (Table 6 page 43).

Taken these analyses together, at diagnosis a maximum of 45 ml BM/PB is required whereas for follow up visits with MRD monitoring and PK/PD a maximum of 36 ml BM/PB is required.

11 PLAN FOR MEDICAL TREATMENT

11.1 Diagnostic and therapeutic lumbar puncture

At diagnosis involvement of the CNS is investigated by a lumbar puncture. If the diagnosis is unequivocal due to leukemic cells in the blood count or due to a preceding diagnostic bone marrow aspirate, intrathecal therapy can be given during the 1st diagnostic lumbar puncture. Methotrexate or triple intrathecal therapy can be given at the discretion of the treating physician. In that case, intrathecal therapy according to the treatment arm at start of multidrug chemotherapy can be omitted.

11.2 Cyto-reductive pre-phase

The cyto-reductive pre-phase is not part of the study. The aim of the cyto-reductive pre-phase is to achieve a well controlled reduction of the initial leukemic cell mass. The acute tumour lysis syndrome is to be avoided by close monitoring of biochemical parameters (LDH, uric acid, phosphate, potassium, calcium), treatment with allopurinol and alkalization of the urine or with rasburicase. Patients typically receive dexamethasone at a dose of 6 mg/ m²/day for five days. In children with a large leukemic cell mass a reduced dose should be used initially. If necessary the phase can be extended to 10 days. If a cyto-reductive effect is not observed or if the disease progresses this phase may also be shortened. The time of the pre-phase can be used to place a permanent central venous line (e.g. Broviac catheter or port-a-cath system), to complete the initial diagnostic tests, to achieve written informed consent before starting the study treatment on day 1 of week 1 and the result of the induction randomizations (see chapter 8.6, page 35).

11.3 Asparaginase treatment

The treatment elements are designed for the use of PEG-asparaginase since this is the standard of care in most participating countries. Nevertheless, in patients having received before only and tolerated unpegualted coli asparaginase, this drug may continued to be given in the IntReALL SR 2010 trial. One dose of PEG asparaginase 1.000 U/m² is replaced by 4 doses of native coli asparaginase 10.000 U/m² every third day (day 1, 4, 7, 10) to achieve a therapeutic asparaginase activity for 14 days.^{91,92}

11.4 New Naming

In order to facilitate the comparison of the treatment arms A and B, naming have been standardized as shown in Table 7

Table 7 Naming of the IntReALL SR 2010 protocol elements

RG	Arm	Old	New	New Long
SR	A	F1/F2	SIA	SR Induction Arm A
		Prot II-IDA part a ±Epra	SCA1±E	SR Consolidation Arm A 1 ± Epra
		Prot II-IDA Part b ±Epra	SCA2±E	SR Consolidation Arm A 2 ± Epra
		R1	SCA 3,5,7	SR Consolidation Arm A 3, 5, 7
		R2	SCA 4,6	SR Consolidation Arm A 4, 6
		Maintenance	SMA	SR Maintenance Arm A
	B	R3I1/2+VCR/DEX (VD)	SIB	SR Induction Arm B
		R3 Consolidation±Epra	SCB1±E	SR Consolidation Arm B 1 ± Epra
		R3 Intensification±Epra	SCB2±E	SR Consolidation Arm B 2 ± Epra
		R3 Interim maint Cycle I	SCB3	SR Consolidation Arm B 3
		R3 Interim Maint Cycle II	SCB4	SR Consolidation Arm B 4
		R3 Maintenance	SMB	SR Maintenance Arm B

11.5 Standard Risk Group

Definition: All patients with early and late isolated extramedullary relapse
All patients with late isolated bone marrow relapse of a precursor B-cell ALL
All patients with early or late combined medullary and extramedullary relapse of a precursor B-cell ALL

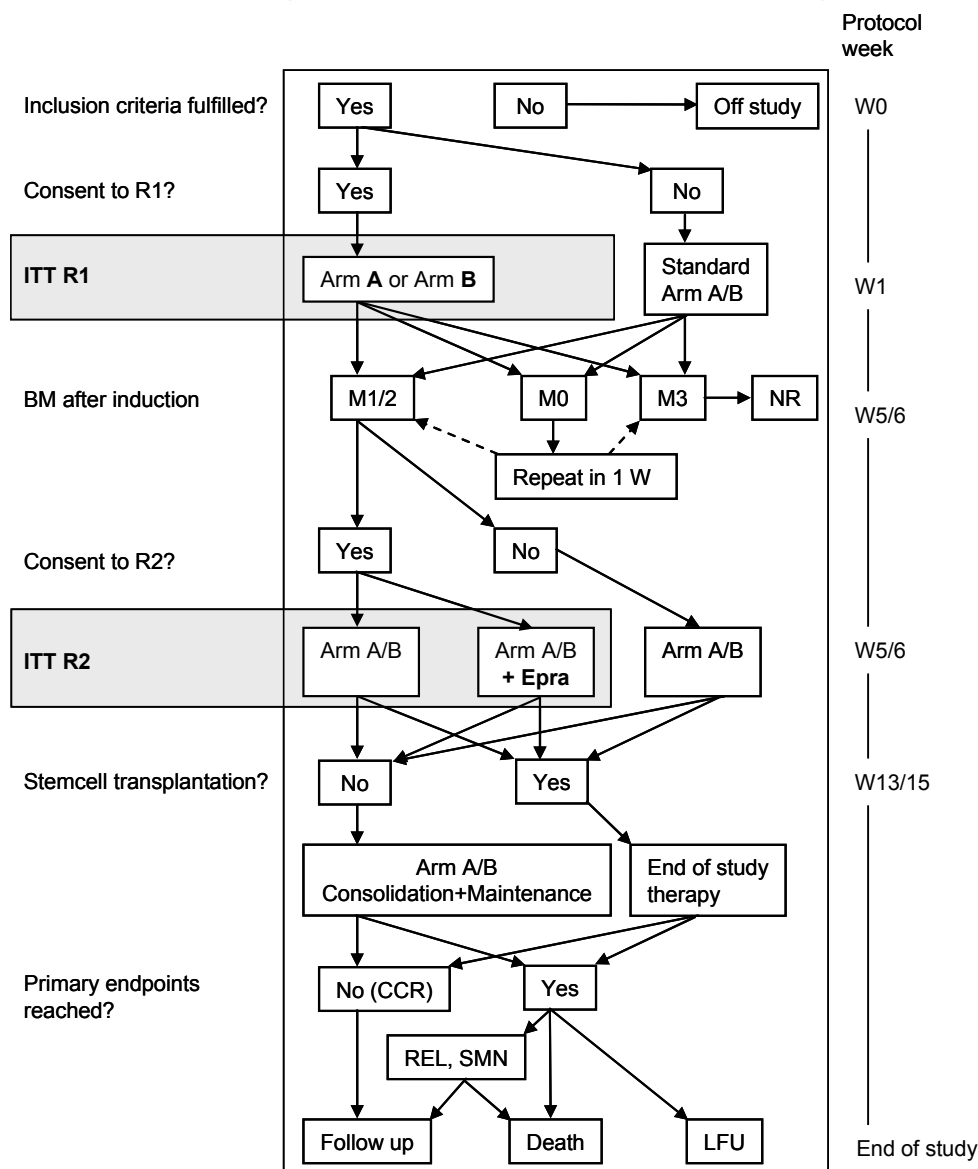
The definition of the SR groups corresponds to group S1 + S2, except very early isolated extramedullary relapses, which are stratified into the HR group.

11.5.1 Randomization in SR patients

Randomization 1 in SR patients: SR patients are randomized to receive the total treatment according to the ALL-REZ BFM 2002 arm Protocol II-IDA (Arm SR-A) or the ALL R3 Trial Arm Mitoxantrone (Arm SR-B). The diagnostic procedures, the MRD cut off and the SCT indications are specific for each arm.

Randomization 2 in SR patients: SR patients are randomized to receive or not to receive epratuzumab during early consolidation within the arm selected with randomization 1. The randomization is performed directly after the response assessment at week 5/6. Patients with M3 marrow have reached their primary endpoint of randomization 1 as early nonresponders, are off study and not included into the randomization 2. In patients with M0 according to the definitions (12.2, page 73) a control analysis needs to be done within one week until an M1 (or in case an M2-) status has been achieved before the patients are randomized. Patients with M2 status are immediately randomized and continue with treatment without delay, as long as the general status allows and as soon as the starting criteria for week 5/6 are fulfilled.

Figure 9 Flow chart for patient inclusion, randomization and follow up

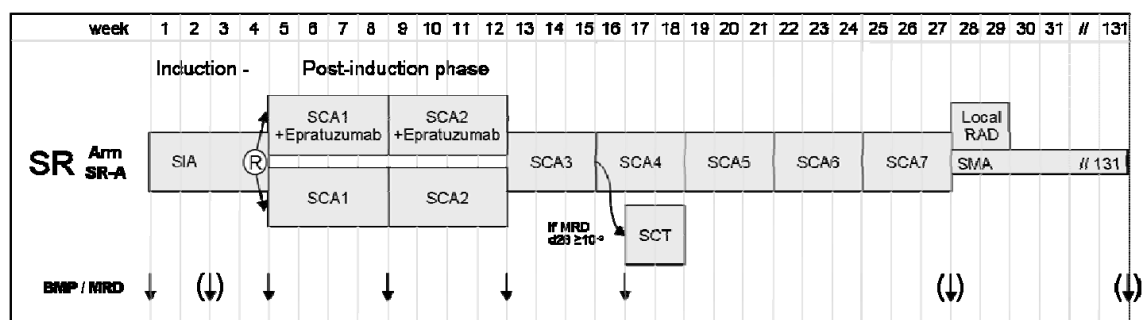


Legend to Figure 9: Patients fulfilling the inclusion criteria for the IntReALL SR 2010 trial with written informed consent to the 1st randomization are randomly allocated to Arm A or B, contributing to the ITT analysis of R1. Patients without consent to R1 receive the treatment arm declared as local standard by their study group. After induction the response is evaluated by assessing the bone marrow cytology. Patients with M3 are classified as early non-responders, have reached their primary endpoint and are off study. Those with M1 or 2 continue with the study therapy and are, if the inclusion criteria for the 2nd randomization are fulfilled and written informed consent is given randomly allocated to the respective consolidation therapy with versus without epratuzumab, thus contributing to the ITT analysis of R2. Patients with M0 (defined as aplastic bone marrow with < 5% leukemic cells) will receive a control bone marrow puncture after 1 week and are further treated according to the marrow status M1, 2 or 3. Patients not fulfilling the inclusion criteria for R2 or without consent are treated with the respective consolidation arm A or B without epratuzumab. Patients fulfilling the criteria for allogeneic haematopoietic stem-cell transplantation (see chapter 9.2, page 37) with an adequate donor will stop the study treatment and receive SCT at week 17 whereas the others continue with the respective consolidation and maintenance therapy of arm A or B. All patients are followed up until death or the end of the study (year 7 after start of the trial) for all primary endpoints (ID, NR, REL, TRD, SMN, LFU). **Abbreviations:** BM, bone marrow; CCR, complete continuous remission; Epra, epratuzumab; ID, induction death; ITT, intention to treat; LFU, lost to follow up; M0-3, cytological bone marrow status 0 – 3 (definition see chapter 12.3, page 74); NR, nonresponse; R1, first randomization (Arm A versus B); R2, second randomization (consolidation +/- epratuzumab); REL, subsequent relapse; TRD, treatment relapsed death; SCT, stem-cell transplantation; SMN, secondary malignancy; W, week.

11.6 Arm SR-A (ALL-REZ BFM)

Patients randomized to the SR-A Arm receive induction, consolidation and maintenance therapy according to a modified protocol ALL-REZ BFM 2002 with Protocol II-IDA as 1st consolidation element. Induction consists of the course SIA (Standard Risk Induction Arm A), and at day 29 the cytological remission and the MRD status are quantified. Post induction, the SCA1 course (Standard Risk Consolidation Arm A1) is given as first consolidation. Patients are randomized to receive or not to receive epratuzumab in addition to SCA1 and SCA2. Patients with MRD $\geq 10^{-3}$ (more than 1 leukemic cell out of 1000 mononucleated cells) at week 5 and those with early isolated extramedullary relapse are allocated to allogeneic stem-cell transplantation (SCT) after the SCA3 course, whereas the other patients continue with the study protocol until SCA7 course followed by a maintenance therapy of 24 months duration and a total treatment duration of 131 weeks. At the beginning of maintenance therapy, all patients without CNS irradiation receive triple intrathecal therapy once every 4 weeks for a total of 6 times. Patients with CNS involvement receive cranial irradiation at a dose of 18 Gray (Gy) and not intrathecal therapy during maintenance treatment. Boys with testicular relapse receive local treatment according to the national guidelines and as described in chapter 11.9.2, page 67, and chapter 11.10.2, page 69. MRD is monitored before every treatment element until SCT or until the SCA4 course (week 16) as additional response parameter. However, this will not be communicated to the treating centres.



















11.6.1 Treatment schedule for SR-A arm



Arrow down ↓, bone marrow puncture with CR/MRD assessment (in brackets optional); BMP, bone marrow puncture; CR, cytological remission; MRD, minimal residual disease; ®, randomization; RAD, irradiation; SCA 1-7, SR consolidation 1-7 arm A; SCT, stem-cell transplantation; SIA, SR induction arm A; SMA, SR maintenance arm A; SR, standard risk group

11.6.2 Course SIA

(SR, arm A / ALL-REZ BFM, induction, week 1-4)

Agent	Dosage	Application	Week 1	Week 2	Week 3	Week 4
Dexamethasone	20 mg/m ² /d	PO				
Vincristine	1.5 mg/m ²	IV	 			
Methotrexate	1 g/m ²	IV 36 h				
Cytarabine	2x3 g/m ² /d	IV 3 h				
PEG-Asp.	1000 U/m ²	IM / IV 2 h				
Methotrexate	Age dep.	IT	 			
Cytarabine	Age dep.	IT	 			
Prednisolone	Age dep.	IT	 			
		Day	1 2 3 4 5 6 7	1 2 3 4 5 6 7	1 2 3 4 5 6 7	1 2 3 4 5 6 7

Criteria to start and guide the course

No specific blood counts or clinical conditions are mandatory to start week 1 and 3. In case of renal insufficiency (elevated creatinine) MTX 1g/m² should be avoided and induction should be started with week 3.

Diagnostic measures

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

Dexamethasone

20 mg/m² (max. 40 mg/day) orally divided in two daily doses on day 1-5 of week 1 and 3.

Vincristine

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

MHD IV Methotrexate

1 g/m² IV over 36 hours starting on day 1 of week 1. 10% is given as a 30 min bolus and the remaining 90% as a continuous infusion for 35.5 hours. Concomitant alkaline hydration with 3000 ml/m²/24 hours is given on day 1 and 2. Serum methotrexate levels are measured at 36h and 48h after the start of the MTX infusion.

Folinic acid rescue after methotrexate

Rescue with folinic acid of 15 mg/m² is given at 48 and 54 hours after start of MTX. The dose is adapted to elevated MTX serum levels.

PEG-asparaginase

1000 units/m² as 2-hour infusion or intramuscularly on day 4 of week 1 and day 4 of week 3. The infusion of L-asparaginase should be started at a reduced rate and increased stepwise, if applicable. It is recommended to quantify L-asparaginase activity in the serum 7 and 14 days after each administration of PEG-asparaginase. Results of asparaginase activity are not communicated to the treating centres and are not considered for changing of the preparation. In case of overt allergic reaction, Erwinia-asparaginase will be used instead at a dose of 20000 units/m² IV or IM every 2nd day for a total of 6 doses replacing one dose of PEG-asparaginase.

Cytarabine

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

Intrathecal chemotherapy

Age adapted doses (see table) of methotrexate, cytarabine, and prednisolone are administered on day 1 of week 1, and day 5 of week 3, directly before or until 1 hour after start of the methotrexate infusion. Patients with CNS involvement receive an additional intrathecal injection on day 6 of week 1 and weekly doses until two clear CSF samples are obtained.

Doses of triple intrathecal chemotherapy

Age [years]	methotrexate [mg]	cytarabine [mg]	prednisolone [mg]	0.9% NaCl [ml]
< 1	6	16	4	1.5
1	8	20	6	2.0
2	10	26	8	2.5
≥ 3	12	30	10	3.0

11.6.3 Course SCA1 with or without epratuzumab

(SR, arm A / ALL-REZ BFM, consolidation 1, week 5-8)

Agent	Dosage	Application	Week 5							Week 6							Week 7							Week 8													
Dexamethasone	6 mg/m ² /d	PO																																			
Vincristine	1.5 mg/m ²	IV																																			
Idarubicin	6 mg/m ²	IV 2 h																																			
PEG-Asp.	1000 U/m ²	IM / IV 2 h																																			
Methotrexate	Age dep.	IT																																			
Cytarabine	Age dep.	IT																																			
Prednisolone	Age dep.	IT																																			
± Epratuzumab	360 mg/m ²	IV 1 h																																			
		Day	1	2	3	4	5	6	7	1	2	3	4	5	6	7	1	2	3	4	5	6	7	1	2	3	4	5	6	7	1	2	3	4	5	6	7

Criteria to start and guide the course

Start of week 5 and application of vincristine / idarubicin ± epratuzumab at weeks 6-8 requires neutrophils > 0.5 x 10⁹/L and a clinical status allowing for treatment continuation each time. Dexamethasone is given without discontinuation.

Diagnostic measures

Bone marrow aspirate at day 1 of week 5 is obligatory to assess cytological remission and MRD response. Results should reveal a representative marrow. In case of a non representative or an aplastic marrow the analysis should be repeated and the start of therapy postponed accordingly. In case of persistent ALL with up to 25% leukaemic cells in the BM, the treatment should be continued irrespectively of the cellularity. Patients with M3 are off study.

Dexamethasone:

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

Vincristine

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

Idarubicin

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

PEG-asparaginase

1000 units/m² as 2-hour infusion or intramuscularly on day 1 of week 5 and day 4 of week 6. The infusion of PEG-asparaginase is started at a reduced rate and increased stepwise, if applicable. It is recommended to quantify L-asparaginase activity in the serum 7 and 14 days after each administration of PEG-asparaginase or before the 2nd dose of PEG asparaginase, respectively. Results of asparaginase activity are not communicated to the treating centres and are not considered for changing of the preparation. If an overt allergic reaction is observed, Erwinia-asparaginase will be used instead at a dose of 20000 units/m² IV or IM every 2nd day for a total of 5 doses replacing the 1st dose of PEG-asparaginase (day1/week5), and 6 doses replacing the 2nd dose of PEG asparaginase (day 4/week 7).

Intrathecal chemotherapy

Age adapted doses (see table) of methotrexate, cytarabine, and prednisolone are administered on day 1 of week 5 and 7. Patients with CNS involvement receive an additional triple intrathecal therapy on day 1 of week 6.

Doses of triple intrathecal chemotherapy

Age [years]	methotrexate [mg]	cytarabine [mg]	prednisolone [mg]	0.9% NaCl [ml]
< 1	6	16	4	1.5
1	8	20	6	2.0
2	10	26	8	2.5
≥ 3	12	30	10	3.0















11.6.3.1 For the treatment arm randomized to get epratuzumab (SCA1 + Epra)

Epratuzumab

360 mg/m² as 1-hour infusion on day 1 of week 5-8. The infusion of epratuzumab is started at a reduced rate and increased stepwise (see appendix for premedication and details). Approximately 10% of the total Epratuzumab dose will be given over the first 5-10 minutes. If the vital signs remain stable, the remaining dose can be given within the next 50 minutes. Epratuzumab serum levels are quantified weekly before every application of the drug (day 1 of weeks 5-8). CD22 expression in peripheral blood leucocytes is quantified on day 1 of weeks 5, 7.

11.6.4 Course SCA2 with or without epratuzumab

(SR, arm A / ALL-REZ BFM, consolidation 2, week 9-12)

Agent	Dosage	Application	Week 9	Week 10	Week 11	Week 12			
Cyclophosphamide	1g/m ²	IV 1 h							
Cytarabine	75 mg/m ²	IV							
Thioguanine	60 mg/m ² /d	PO							
Methotrexate	Age dep.	IT							
Cytarabine	Age dep.	IT							
Prednisolone	Age dep.	IT							
± Epratuzumab	360 mg/m ²	IV 1 h							
		Day	1 2 3 4 5 6 7	1 2 3 4 5 6 7	1 2 3 4 5 6 7	1 2 3 4 5 6 7			

Criteria to start and guide the course

Start of week 9 requires leukocytes > 1.5 x 10⁹/L, neutrophils > 0.5 x 10⁹/L, platelets > 80 x 10⁹/L, and a clinical status allowing for treatment continuation. Cytarabine courses on day 3 of weeks 9 and 10 and epratuzumab at weeks 10-12 are given irrespectively of the blood count as long as the clinical status allows for treatment continuation.

Diagnostic measures

Bone marrow aspirate at day 1 of week 9 is obligatory to assess cytological remission and MRD response. Results are not relevant for treatment stratification, the measure must not be repeated in case.

Cyclophosphamide

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

Cytarabine

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

Thioguanine

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

Intrathecal chemotherapy

Age adapted doses (see table) of methotrexate, cytarabine, and prednisolone are administered on day 3 of week 9 and 10.

Doses of triple intrathecal chemotherapy

Age [years]	methotrexate [mg]	cytarabine [mg]	prednisolone [mg]	0.9% NaCl [ml]
< 1	6	16	4	1.5
1	8	20	6	2.0
2	10	26	8	2.5
≥ 3	12	30	10	3.0

11.6.4.1 For the treatment arm randomized to get epratuzumab (SCA2 + Epra)

Epratuzumab

360 mg/m² as 1-hour infusion on day 1 of week 9-12. The infusion of epratuzumab is started at a reduced rate and increased stepwise (see appendix for premedication and details). Approximately 10% of the total Epratuzumab dose will be given over the first 5-10 minutes. If the vital signs remain stable, the remaining dose can be given within the next 50 minutes. Epratuzumab serum levels are quantified weekly before every application of the drug and until 5 weeks after the last application (day 1 of weeks 9-17). CD22 expression in peripheral blood leucocytes is quantified on day 1 of weeks 9, 11, 13, 19, and 25.

11.6.5 Course SCA3, 5, and 7

(SR, arm A / ALL-REZ BFM, consolidation 3, 5, and 7, week 13-15, 19-21, 25-27)

Agent	Dosage	Application	Week 13,19,25							Week 14,20,26							Week 15,21,27						
Dexamethasone	20 mg/m ² /d	PO																					
Mercaptopurine	100mg/m ² /d	PO																					
Vincristine	1.5mg/m ²	IV																					
Methotrexate	1g/m ²	IV 36 h																					
Cytarabine	2x2 g/m ² /d	IV 3 h																					
PEG-Asparaginase	1000 U/m ²	IM / IV 2 h																					
Methotrexate	Age dep.	IT																					
Cytarabine	Age dep.	IT																					
Prednisolone	Age dep.	IT																					
		Day	1	2	3	4	5	6	7	1	2	3	4	5	6	7	1	2	3	4	5	6	7

Criteria to start and guide the course

Start of the course (week 13, 19, or 25) requires leukocytes > 2.0 x 10⁹/L, neutrophils > 0.5 x 10⁹/L, platelets > 80 x 10⁹/L, and a clinical status allowing for treatment continuation. In case of prolonged treatment delays, dose reductions according to the specific guidelines outlined in chapter 11.8.5 (page 66) have to be considered. After Course SCA3 at week 17, allogeneic SCT is scheduled, if indicated.

Diagnostic measures

Bone marrow aspirate at day 1 of week 13 is obligatory to assess cytological remission and MRD response. Results are not relevant for treatment stratification, the measure must not be repeated in case. Prior to courses SCA 5 and 7 (week 19 and 25) no BM aspirate is foreseen.

Dexamethasone

20 mg/m² (maximum 40 mg/d) orally, divided in two daily doses on day 1-5, and 10 mg/m² on day 6 of each SCA3, 5, or 7 course, week 13, 19, and 25.

6-Mercaptopurine

100 mg/m² orally on day 1-5 of each SCA3, 5, or 7 course, weeks 13, 19, and 25.

Vincristine

1.5 mg/m² (**maximum single dose 2 mg**) as 15 min short infusion (or as IV bolus not on the same day as IT therapy) on day 1 and 6 of each SCA3, 5, 7 course, weeks 13, 19, and 25.

MHD IV Methotrexate

1 g/m² IV over 36 hours starting on day 1 of each SCA3, 5, or 7 course, weeks 13, 19, and 25. 10% is given as a 30 min bolus and the remaining 90% as a continuous infusion for 35.5 hours. Concomitant alkaline hydration with 3000 ml/m²/24 hours is given on day 1 and 2. Serum methotrexate levels are measured at 36h and 48h after the start of the MTX infusion. In case of levels elevated above the upper limits (> 10 µmol/l at 36h and/or > 0.5 µmol/l at 48h) serum methotrexate levels are measured every 6 hours until reaching a level below 0.25 µmol/l. The management is adapted according to recommendations in chapter 11.11.2, page 69.

Folinic acid rescue after methotrexate

Rescue with folinic acid of 15 mg/m² is given at 48 and 54 hours after start of MTX. The dose is adapted to elevated MTX serum levels according to recommendations in chapter 11.11.2.

Cytarabine

2 g/m² as a 3-hours infusion every 12 hours on day 5 (a total of 2 doses) of each SCA3, 5, or 7 course, weeks 13, 19, and 25. A prophylaxis of conjunctivitis with eye drops and of neuropathy with vitamin B6 at a dose of 100mg/m² IV is recommended prior to each cytarabine dose.

PEG-asparaginase

1000 units/m² as 2-hour infusion or intramuscularly on day 6 of each SCA3, 5, or 7 course, weeks 13, 19, and 25. The infusion of L-asparaginase should be started at a reduced rate and increased stepwise, if applicable. It is recommended to quantify L-asparaginase activity in the serum 7 and 14 days after each administration of PEG-asparaginase. Results of asparaginase activity are not communicated to the treating centres and are not considered for changing of the preparation. In case of overt allergic reaction, Erwinia-asparaginase will be used instead at a dose of 20000 units/m² IV or IM every 2nd day for a total of 6 doses replacing one dose of PEG-asparaginase.

Intrathecal chemotherapy

Age adapted doses (see table) of methotrexate, cytarabine, and prednisolone are administered on day 1 of each SCA3, 5 or 7 course, weeks 13, 19, and 25, directly before or until 1 hour after start of the methotrexate infusion.

Doses of triple intrathecal chemotherapy

Age [years]	methotrexate [mg]	cytarabine [mg]	prednisolone [mg]	0.9% NaCl [ml]
< 1	6	16	4	1.5
1	8	20	6	2.0
2	10	26	8	2.5
≥ 3	12	30	10	3.0

11.6.6 Course SCA 4, 6

(SR, arm A / ALL-REZ BFM, consolidation 4 and 6, week 16-18, 22-24)

Agent	Dosage	Application	Week 16,22	Week 17, 23	Week 18, 24
Dexamethasone	20 mg/m ² /d	PO			
Thioguanine	100mg/m ² /d	PO			
Vindesine	3mg/m ²	IV			
Methotrexate	1g/m ²	IV 36 h			
Ifosfamide	400 mg/m ²	IV 1 h			
Daunorubicin	35 mg/m ²	IV 24 h			
PEG-Asparaginase	1000 U/m ²	IM / IV 2 h			
Methotrexate	Age dep.	IT			
Cytarabine	Age dep.	IT			
Prednisolone	Age dep.	IT			
		Day	1 2 3 4 5 6 7	1 2 3 4 5 6 7	1 2 3 4 5 6 7

Criteria to start and guide the course

Start of the course (week 16, or 22) requires leukocytes > 2.0 x 10⁹/L, neutrophils > 0.5 x 10⁹/L, platelets > 80 x 10⁹/L, and a clinical status allowing for treatment continuation. In case of prolonged treatment delays, dose reductions according to the specific guidelines outlined in chapter 11.8.5 (page 66) have to be considered.

Diagnostic measures

Bone marrow aspirate at day 1 of week 16 is obligatory to assess cytological remission and MRD response, if no allogeneic SCT is indicated or SCT is postponed. Results are not relevant for treatment stratification, the measure must not be repeated in case. Prior to courses SCA 6 (week 22) no BM aspirate is foreseen. If allogeneic SCT is planned for week 17, BM aspirate is done in context with the SCT preparation and can be postponed accordingly.

Dexamethasone

20 mg/m² (maximum 40 mg/d) orally divided in two daily doses on day 1-5, and 10 mg/m² on day 6 of each SCA4, or 6 course, week 16 and 22.

Thioguanine:

100 mg/m² orally on day 1-5 of each SCA4, or 6 course, week 16 and 22.

Vindesine

3 mg/m² as 15 min short infusion (or as IV bolus not on the same day as IT therapy) on day 1 of each SCA4 or 6 course, week 16 and 22.

MHD IV Methotrexate

1 g/m² IV over 36 hours starting on day 1 of each SCA4, or 6 course, week 16 and 22. 10% is given as a 30 min bolus and the remaining 90% as a continuous infusion for 35.5 hours. Concomitant alkaline hydration with 3000 ml/m²/24 hours is given on day 1 and 2. Serum methotrexate levels are measured at 36h and 48h after the start of the MTX infusion. In case of levels elevated above the upper limits (> 10 µmol/l at 36h and/or > 0.5 µmol/l at 48h) serum methotrexate levels are measured every 6 hours until reaching a level below 0.25

µmol/l. The management is adapted according to recommendations in chapter 11.11.2, page 69.

Folinic acid rescue after methotrexate

Rescue with folinic acid of 15 mg/m² is given at 48 and 54 hours after start of MTX. The dose is adapted to elevated MTX serum levels according to recommendations in chapter 11.11.2.

Ifosfamide

400 mg/m² as a 1-hour infusion day 1-5 of each SCA4, or 6 course, week 16 and 22. Mesna is administered at a dose of 200 mg/m² IV prior to as well as 4 and 8 hours after lphosphamide. Hydration with 1500 ml/m²/day is administered from start of Ifosfamide until day 5.

Daunorubicin

35 mg/m² as a 24-hour infusion on day 5 of each SCA4, or 6 course, week 16 and 22.

PEG-asparaginase

1000 units/m² as 2-hour infusion on day 6 of each SCA4, or 6 course, week 16 and 22. The infusion of L-asparaginase should be started at a reduced rate and increased stepwise. It is recommended to quantify L-asparaginase activity in the serum 7 and 14 days after each administration of PEG-asparaginase. Results of asparaginase activity are not communicated to the treating centres and are not considered for changing of the preparation. In case of overt allergic reaction, Erwinia-asparaginase will be used instead at a dose of 20000 units/m² IV or IM every 2nd day for a total of 6 doses replacing one dose of PEG-asparaginase.

Intrathecal chemotherapy

Age adapted doses (see table) of methotrexate, cytarabine, and prednisolone are administered on day 1 of each SCA4, 6 course, week 16 and 22, directly before or until 1 hour after start of the methotrexate infusion. Patients with CNS involvement receive an additional intrathecal injection on day 6 of each SCA4 or 6 course, week 16 and 22.

Doses of triple intrathecal chemotherapy

Age [years]	methotrexate [mg]	cytarabine [mg]	prednisolone [mg]	0.9% NaCl [ml]
< 1	6	16	4	1.5
1	8	20	6	2.0
2	10	26	8	2.5
≥ 3	12	30	10	3.0

11.6.7 Course SMA (maintenance therapy arm A)

(SR, arm A / ALL-REZ BFM, maintenance week 28– 131)

Agent	Dosage	Application	Week 28,40	Week 29,41	Week 30,42	Week 31,43	Week 32,44	Week 33,45	Week 34,46	Week 35,47	Week 36,48	Week 37,49	Week 38,50	Week 39,51
Mercaptopurine	50 mg/m ² /d	PO												
Methotrexate	20mg/m ²	PO												
Methotrexate	Age dep.	IT												
Cytarabine	Age dep.	IT												
Prednisolone	Age dep.	IT												
		Day	1234567	1234567	1234567	1234567	1234567	1234567	1234567	1234567	1234567	1234567	1234567	1234567

Agent	Dosage	Application	Week 52-131
Mercaptopurine	50 mg/m ² /d	PO	
Methotrexate	20mg/m ²	PO	
		Day	1 2 3 4 5 6 7

General structure

Maintenance therapy is given throughout from week 28 for a total of 104 weeks (= 2 years). During the first 24 weeks, a total of 6 applications of intrathecal chemotherapy are given only to those patients without prior CNS irradiation.

Criteria to start and guide the course

Start of maintenance (week 28) requires leukocytes $> 2.0 \times 10^9/L$, neutrophils $> 0.5 \times 10^9/L$, platelets $> 80 \times 10^9/L$, and a clinical status allowing for treatment continuation. The dosing of mercaptopurine and methotrexate is adapted to the leukocyte count which should range between 2.0 and $3.0 \times 10^9/L$ (150% of 6MP/MTX dose if WBC $> 3.0 \times 10^9/L$, 100% if WBC > 2.0 and $< 3.0 \times 10^9/L$, 50% if WBC > 1.0 and $< 2.0 \times 10^9/L$ or if the lymphocyte count drops below $0.3 \times 10^9/L$, 0% if WBC $< 1.0 \times 10^9/L$). Both drugs are started with reduced doses and weekly increased. In case of drop of leukocytes $< 1.0 \times 10^9/L$, neutrophils $< 0.5 \times 10^9/L$, and/or platelets $< 80 \times 10^9/L$ or in case of febrile episodes, the treatment is interrupted.

Diagnostic measures

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

Mercaptopurine

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

Methotrexate

20 mg/m² (adapted individually to leukocyte counts and toxicity) orally on day 1 of each week as a single dose.

Intrathecal chemotherapy

Age adapted doses (see table) of methotrexate, cytarabine, and prednisolone are administered on day 1 of week 28, 32, 36, 40, 44, 48 (6 application, every 4 weeks). Patients with CNS involvement and cranial irradiation at the start of maintenance therapy do not receive intrathecal therapy during maintenance therapy.

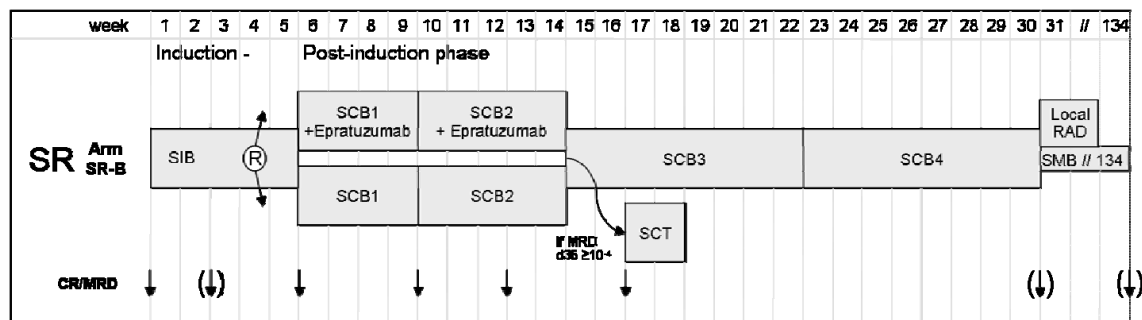
Doses of triple intrathecal chemotherapy

Age [years]	methotrexate [mg]	cytarabine [mg]	prednisolone [mg]	0.9% NaCl [ml]
< 1	6	16	4	1.5
1	8	20	6	2.0
2	10	26	8	2.5
≥ 3	12	30	10	3.0

11.7 Arm SR-B (R3)

Patients randomized to the SR-B Arm receive induction, post-induction and maintenance therapy according to the protocol ALL-R3. Induction consists of the courses SIB (former R3-Induction I, II, and III). After regeneration of blood count at day 35 (start of week 6) the cytological remission and the MRD status are quantified. Patients are randomized to receive or not to receive epratuzumab in addition to the R3-Consolidation (SCB). Patients with MRD $\geq 10^{-4}$ (more than 1 leukemic cell out of 10000 mononucleated cells) at day 35 and those with early isolated extramedullary relapse are allocated to allogeneic stem-cell transplantation (SCT) after the SCB 2 (former R3-Intensification) course whereas the other patients continue with 2 SCB cycles (SCB3, SCB4) and with a maintenance therapy of 24 months duration and a total treatment duration of 134 weeks. At the beginning of maintenance therapy (week 31), patients with CNS involvement receive cranial irradiation at a dose of 18 Gray (Gy). Boys with testicular relapse receive local treatment according to the national guidelines and as described in chapter 11.9.2, page 67, and chapter 11.10.2, page 69. MRD is closely monitored before every treatment element until SCT or the 1st interim maintenance cycle (week 15) as additional response parameter but not communicated to the treating centres.

11.7.1 Treatment schedule for SR-B arm



.Arrow down ↓, bone marrow puncture with CR/MRD assessment (in brackets optional); CR, cytological remission; MRD, minimal residual disease; ®, randomization; RAD, irradiation; SCB 1-4, SR consolidation 1-4 arm B; SCT, stem-cell transplantation; SIB, SR induction arm B; SMB, SR maintenance arm B; SR, standard risk group

11.7.2 Course SIB

(SR, arm B / R3 protocol, induction, week 1-5)

Agent	Dosage	Application	Week 1	Week 2	Week 3	Week 4	Week 5
Dexamethasone	20 mg/m ² /d	PO					
Vincristine	1.5 mg/m ²	IV					
Mitoxantrone	10 mg/m ²	IV 1 h					
PEG-Asp.	1000 U/m ²	IM / IV 2 h					
Methotrexate	Age dep.	IT					
		Day	1 2 3 4 5 6 7	1 2 3 4 5 6 7	1 2 3 4 5 6 7	1 2 3 4 5 6 7	1 2 3 4 5 6 7

Criteria to start and guide the course

No specific blood counts or clinical conditions are mandatory to start and continue the course until week 4. Week 5 starts as soon as the clinical status allows for treatment continuation.

Diagnostic measures

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

Dexamethasone

20 mg/m² (maximum 40 mg/d) orally divided in two daily doses on day 1-5 of week 1, 3, and 5.

Vincristine

1.5 mg/m² (**maximum single dose 2 mg**) as 15 min short infusion (or as IV bolus not on the same day as IT therapy) on day 3 of week 1-5

Mitoxantrone:

10mg/m² as 1 hour infusion on day 1 and 2 of week 1.

PEG-asparaginase

1000 units/m² as 2-hour infusion or intramuscularly on day 3 of week 1 and 3. The infusion of L-asparaginase should be started at a reduced rate and increased stepwise, if applicable. It is recommended to quantify L-asparaginase activity in the serum 7 and 14 days after each administration of PEG-asparaginase. Results of asparaginase activity are not communicated to the treating centres and are not considered for changing of the preparation. In case of overt allergic reaction, Erwinia-asparaginase will be used instead at a dose of 20000 units/m² IV or IM every 2nd day for a total of 6 doses replacing one dose of PEG-asparaginase.

Intrathecal chemotherapy



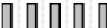
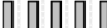





An age adapted dose (see table) of methotrexate is administered on day 1 of week 1 and 2. Patients who have CNS disease at presentation should receive weekly doses until two clear CSF samples are obtained.

Doses of intrathecal chemotherapy

Age [years]	methotrexate [mg]
< 1	6
1	8
2	10
=> 3	12

11.7.3 Course SCB1 with or without epratuzumab

(SR arm B / R3 protocol, consolidation 1, weeks 6-9)

Agent	Dosage	Application	Week 6							Week 7							Week 8							Week 9						
Methotrexate	1 g/m ²	IV 36 h																												
PEG-Asp.	1000 U/m ²	IM / IV 2 h																												
Cyclophosphamide	440 mg/m ²	IV 0.5 h																												
Etoposide	100 mg/m ²	IV 4 h																												
Methotrexate	Age dep.	IT																												
± Epratuzumab	360 mg/m ²	IV 1 h																												
		Day	1	2	3	4	5	6	7	1	2	3	4	5	6	7	1	2	3	4	5	6	7	1	2	3	4	5	6	7

Criteria to start and guide the course

Start of week 6 and of week 7 requires neutrophils $> 0.5 \times 10^9/L$, platelets $> 50 \times 10^9/L$ and a clinical status allowing for treatment continuation each time. Epratuzumab at weeks 8 and 9 is given irrespectively of the blood count.

Diagnostic measures

Bone marrow aspirate at day 1 of week 6 is obligatory to assess cytological remission and MRD response. Results should reveal a representative marrow. In case of a non representative or an aplastic marrow the analysis should be repeated and the start of therapy postponed accordingly. In case of persistent ALL with up to 25% leukemic in the BM, the treatment should be continued irrespectively of the cellularity. Patients with M3 are off study.

MHD IV Methotrexate

1 g/m² IV over 36 hours starting on day 1 of week 6. 10% is given as a 30 min bolus and the remaining 90% as a continuous infusion for 35.5 hours. Concomitant alkaline hydration with 3000 ml/m²/24 hours is given on day 1 and 2. Serum methotrexate levels are measured at 36h and 48h after the start of the MTX infusion.

Folinic acid rescue after methotrexate

Rescue with folinic acid of 15 mg/m² is given IV at 48 and 54 hours after start of MTX. The dose is adapted to elevated MTX serum levels.

PEG-asparaginase

1000 units/m² as 2-hour infusion or intramuscularly on day 2 of week 6. The infusion of L-asparaginase should be started at a reduced rate and increased stepwise, if applicable. It is recommended to quantify L-asparaginase activity in the serum 7 and 14 days after each administration of PEG-asparaginase. Results of asparaginase activity are not communicated to the treating centres and are not considered for changing of the preparation. In case of overt allergic reaction, Erwinia-asparaginase will be used instead at a dose of 20000 units/m² IV or IM every 2nd day for a total of 6 doses replacing one dose of PEG-asparaginase.

Cyclophosphamide

440 mg/m² as 30 minutes infusion on day 1-5 of week 7. Mesna is administered at a dose of 200 mg/m² IV prior to as well as 4 and 8 hours after cyclophosphamide. Hydration with 2000 ml/m²/day is administered from start of cyclophosphamide until day 5.

Etoposide

100 mg/m² as 4 hour infusion on day 1-5 of week 7.

Intrathecal chemotherapy

An age adapted dose (see table) of methotrexate is administered on day 1 of week 6 directly before or until 1 hour after start of the methotrexate infusion.

Doses of intrathecal chemotherapy

Age [years]	methotrexate [mg]
< 1	6
1	8
2	10
=> 3	12

11.7.3.1 For the treatment arm randomized to get epratuzumab

Epratuzumab

360 mg/m² as 1-hour infusion on day 1 of week 6-9. The infusion of epratuzumab is started at a reduced rate and increased stepwise (see appendix for premedication and details). Approximately 10% of the total Epratuzumab dose will be given over the first 5-10 minutes. If the vital signs remain stable, the remaining dose can be given within the next 50 minutes. Epratuzumab serum levels are quantified weekly before every application of the drug and until 5 weeks after the last application (day 1 of weeks 6-19). CD22 expression in peripheral blood leucocytes is quantified on day 1 of weeks 6, 8, 10, 12, 14, 20, and 26.

11.7.4 Course SCB2 with or without epratuzumab

(SR, Arm B / R3 protocol; consolidation 2, weeks 10-14)

Agent	Dosage	Application	Week 10	Week 11	Week 12	Week 13	Week 14
Dexamethasone	6 mg/m ² /d	PO					
Vincristine	1.5 mg/m ²	IV					
Cytarabine	2x3 g/m ² /d	IV 3 h					
PEG-Asp.	1000 U/m ²	IM / IV 2 h					
Methotrexate	1g/m ²	IV 36 h					
Methotrexate	Age dep.	IT					
± Epratuzumab	360 mg/m ²	IV 1 h					
		Day	1 2 3 4 5 6 7	1 2 3 4 5 6 7	1 2 3 4 5 6 7	1 2 3 4 5 6 7	1 2 3 4 5 6 7

Criteria to start and guide the course

Start of week 10 requires leukocytes > 2.0 x 10⁹/L, neutrophils > 0.5 x 10⁹/L, platelets > 80 x 10⁹/L, and a clinical status allowing for treatment continuation. Week 11 is given irrespectively of the blood count as long as the clinical status allows. Start of week 13 requires neutrophil counts > 0.5 x 10⁹/L, and platelets > 50 x 10⁹/L.

Diagnostic measures

Bone marrow aspirates at day 1 of week 10 and at day 1 of week 13 are obligatory to assess cytological remission and MRD response. Results are not relevant for treatment stratification, the measure must not be repeated in case.

Dexamethasone

6 mg/m² orally divided in two daily doses on day 1-5 of week 10.

Vincristine

1.5 mg/m² (**maximum single dose 2 mg**) as 15 min short infusion (or as IV bolus not on the same day as IT therapy) on day 3 of week 10.

Cytarabine

3 g/m² as 3 hour infusion every 12 hours on day 1 and 2 (a total of 4 doses) of week 10 and 11. A prophylaxis of conjunctivitis with eye drops and of neuropathy with vitamin B6 at a dose of 100 mg/m² IV is recommended prior to each cytarabine dose. Prednisolone eye drops are administered Q2-3 hourly from day 1 until 5 days after the last dose of cytarabine.

PEG-asparaginase

1000 units/m² as 2-hour infusion or intramuscularly on day 2 of week 10. The infusion of L-asparaginase should be started at a reduced rate and increased stepwise, if applicable. It is recommended to quantify L-asparaginase activity in the serum 7 and 14 days after each administration of PEG-asparaginase. Results of asparaginase activity are not communicated to the treating centres and are not considered for changing of the preparation. In case of overt allergic reaction, Erwinia-asparaginase will be used instead at a dose of 20000 units/m² IV or IM every 2nd day for a total of 6 doses replacing one dose of PEG-asparaginase.

MHD IV Methotrexate

1 g/m² infusion over 36 hours starting on day 1 of week 13. 10% is given as a 30 min bolus and the remaining 90% as a continuous infusion for 35.5 hours. Concomitant alkaline hydration with 3000 ml/m²/24 hours is given on day 1 and 2. Serum methotrexate levels are measured at 36h and 48h after the start of the MTX infusion. In case of levels elevated above the upper limits (> 10 µmol/l at 36h and/or > 0.5 µmol/l at 48h) serum methotrexate levels are measured every 6 hours until reaching a level below 0.25 µmol/l. The management is adapted according to recommendations in chapter 11.11.2, page 69.

Folinic acid rescue after methotrexate

Rescue with folinic acid of 15 mg/m² is given at 48 and 54 hours after start of MTX. The dose is adapted to elevated MTX serum levels according to recommendations in chapter 11.11.2.

Intrathecal chemotherapy

An age adapted dose of methotrexate is administered on day 1 of week 10, and day 1 of week 13, the latter directly before or until 1 hour after start of the methotrexate infusion.

Doses of intrathecal chemotherapy

Age [years]	methotrexate [mg]
< 1	6
1	8
2	10
=> 3	12

11.7.4.1 For the treatment arm randomized to get epratuzumab

Epratuzumab

360 mg/m² as 1-hour infusion on day 1 of week 10-13. The infusion of epratuzumab is started at a reduced rate and increased stepwise (see appendix for premedication and details). Approximately 10% of the total Epratuzumab dose will be given over the first 5-10 minutes. Epratuzumab serum levels are quantified weekly before every application of the drug and until 5 weeks after the last application (day 1 of weeks 6-19). CD22 expression in peripheral blood leucocytes is quantified on day 1 of weeks 6, 8, 10, 12, 14, 20, and 26.

11.7.5 Course SCB3 + SCB4

(SR, arm B / R3 protocol, consolidation 3 and 4, week 15-22 and 23-30)

Agent	Dosage	Application	Week 15/23	Week 16/24	Week 17/25	Week 18/26
Dexamethasone	6 mg/m ² /d	PO				
Mercaptopurine	75 mg/m ²	PO				
Vincristine	1.5 mg/m ²	IV 1 h				
Methotrexate	4x25 mg/m ²	PO				
Methotrexate	20 mg/m ²	PO				
Methotrexate	Age dep.	IT				
		Day	1 2 3 4 5 6 7	1 2 3 4 5 6 7	1 2 3 4 5 6 7	1 2 3 4 5 6 7

Agent	Dosage	Application	Week 19/27	Week 20/28	Week 21/29	Week 22/30
Mercaptopurine	75 mg/m ² /d	PO				
Thioguanine	40 mg/m ² /d	PO				
Methotrexate	20 mg/m ²	PO				
Etoposide	150 mg/m ²	IV 4 h				
Cyclophosphamide	300 mg/m ²	IV 0.5 h				
Cytarabine	50 mg/m ²	IV or SC				
Methotrexate	Age dep.	IT				
		Day	1 2 3 4 5 6 7	1 2 3 4 5 6 7	1 2 3 4 5 6 7	1 2 3 4 5 6 7

Criteria to start and guide the course

Start of week 15 (SCB3) and 23 (SCB4) requires leukocytes > 2.0 x 10⁹/L, neutrophils > 0.5 x 10⁹/L, platelets > 80 x 10⁹/L, and a clinical status allowing for treatment continuation. Drop of neutrophil counts < 0.5 x 10⁹/L, and/or platelets < 50 x 10⁹/L requires treatment interruption until recovery. Start of week 21 (SCB3) and 29 (SCB4) requires leukocytes > 2.0 x 10⁹/L, neutrophils > 0.5 x 10⁹/L, platelets > 80 x 10⁹/L. Week 22 (SCB3) and 30 (SCB4) is given irrespectively of the blood count as long as the clinical status allows.

Diagnostic measures

Bone marrow aspirates at day 1 of week 15 or prior to allogeneic SCT if indicated (usually week 16) are obligatory to assess cytological remission and MRD response. Results are not relevant for treatment stratification, the measure must not be repeated in case.

Dexamethasone

6 mg/m² orally divided in two daily doses on day 1-5 of week 15 (SCB3) and 23 (SCB4).

Mercaptopurine

75 mg/m² orally day 1-7 of week 15-20 (SCB3), and week 23-28 (SCB4). Doses should be taken at least one hour after the evening meal without milk products.

Vincristine

1.5 mg/m² i.v. (**maximum single dose 2 mg**) as 15 min short infusion (or as IV bolus not on the same day as IT therapy) on day 3 of week 15 (SCB3) and 23 (SCB4).

Oral methotrexate

20 mg/m² orally on day 1 of week 16, 17, 19 and 20 (SCB3), and week 24, 25, 27 and 28 (SCB4).

Intensified oral methotrexate

25 mg/m² orally every 6 hours for 4 doses on day 1 of week 18 (SCB3), and of week 26 (SCB4). To avoid waking up patients intervals can be stretched to 8 hours overnight.

Folinic acid

10 mg/m² orally every 6 hours for 2 doses on day 3 of week 18 (SCB3) and of week 26 (SCB4), starting 48 hours after first dose of HD oral methotrexate.

Intrathecal chemotherapy

An age adapted dose of methotrexate is administered on day 1 of week 15 and 21 (SCB3), and of week 23 and 29 (SCB4).

Doses of intrathecal chemotherapy

Age [years]	methotrexate [mg]
< 1	6
1	8
2	10
=> 3	12

Thioguanine

40 mg/m² orally on day 1-7 of week 21 (SCB3) and of week 29 (SCB4). Doses should be taken at least one hour after the evening meal without milk products.

Etoposide

150 mg/m² as 4 hour infusion on day 1 of week 21 and 22 (SCB3), and of week 29 and 30 (SCB4).

Cyclophosphamide

300 mg/m² as 30 minutes infusion on day 1 of week 21 and 22 (SCB3), and of week 29 and 30 (SCB4). Hydration with 2000 ml/m²/day is administered for at least 4 hours from the start of cyclophosphamide.

Cytarabine

50 mg/m² as IV bolus or SC on day 2-5 of week 21 and 22 (SCB3), and week 29 and 30 (SCB4).

11.7.6 Course SMB, maintenance therapy

(SR, arm B / R3, maintenance therapy, week 31 – 134, Cycles 1-7, 4 weeks of cycle 8)

Agent	Dosage	Application	C1-8 Week 1	C1-8 Week 2	C1-8 Week 3	C1-8 Week 4	C1-7 Week 5	C1-7 Week 6	C1-7 Week 7	C1-7 Week 8	C1-7 Week 9	C1-7 Week 10	C'-7 Week 11	C'-7 Week 12
Dexamethasone	6 mg/m ² /d	PO												
Vincristine	1.5 mg/m ²	IV												
Mercaptopurine	75 mg/m ²	PO												
Methotrexate	20 mg/m ²	PO												
Methotrexate	Age dep.	IT												
		Day	1234567	1234567	1234567	234567	234567	234567	234567	234567	234567	1234567	1234567	234567

General structure

Each cycle lasts 12 weeks and there are 7 complete cycles. The 8th cycle is of 4 weeks duration. Maintenance therapy is terminated after a total of 104 weeks (= 2 years).

Criteria to start and guide the course

Start of maintenance (week 31 = week 1 of cycle 1) requires leukocytes $> 2.0 \times 10^9/L$, neutrophils $> 0.5 \times 10^9/L$, platelets $> 80 \times 10^9/L$, and a clinical status allowing for treatment continuation. The dosing of mercaptopurine and methotrexate is adapted to the leukocyte count which should range between 2.0 and $3.0 \times 10^9/L$ (150% of 6MP/MTX dose if WBC $> 3.0 \times 10^9/L$, 100% if WBC > 2.0 and $< 3.0 \times 10^9/L$, 50% if WBC > 1.0 and $< 2.0 \times 10^9/L$ or if the lymphocyte count drops below $0.3 \times 10^9/L$, 0% if WBC $< 1.0 \times 10^9/L$). Both drugs are started with reduced doses and weekly increased. In case of drop of leukocytes $< 1.0 \times 10^9/L$, neutrophils $< 0.5 \times 10^9/L$, and/or platelets $< 80 \times 10^9/L$ or in case of febrile episodes, the treatment is interrupted.

Diagnostic measures

Bone marrow aspirate at day 1 of week 31 at start of maintenance therapy and at the end of maintenance therapy (after week 134) is not obligatory and can be done at the discretion of the treating investigator to confirm cytological remission and continuous MRD response.

Dexamethasone

6 mg/m² orally divided into 2 doses d1-5 of week 1, 5, and 9 of each cycle.

Vincristine

1.5 mg/m² (**maximum single dose 2 mg**) as 15 min short infusion (or as IV bolus not on the same day as IT therapy) on d1 of week 1, 5, and 9 of each cycle.

Mercaptopurine

75 mg/m² orally every day (day 1-7 of week 1-12 of each cycle). Doses should be taken at least one hour after the evening meal without milk products.

Oral methotrexate

20 mg/m² orally on day 4 of each week as a single dose. No oral methotrexate is given in the third week of each cycle as an intrathecal dose is given during that week except in cycle 8.

Intrathecal chemotherapy

An age adapted dose of methotrexate is administered on day 1 of week 3 of cycle 1-7.

Patients with CNS irradiation on week 1 of maintenance do not receive intrathecal methotrexate! They get oral methotrexate 20mg/m² on day 4 of that week instead.

Doses of intrathecal chemotherapy

Age [years]	methotrexate [mg]
< 1	6
1	8
2	10
≥ 3	12

11.8 Guidelines for the administration of chemotherapy**11.8.1 General principles**

The analysis of preceding studies suggests that treatment intensity is an essential parameter for the success of relapse therapy.⁹³ Consequently, a prolongation of treatment-free intervals particularly during the induction phase must only be accepted in case of life-threatening complications. If previous experience with individual patients suggests that a timely delivery of therapy is unlikely or associated with an undue risk because of insufficient tolerance, the protocol stipulates the possibility of a dose reduction. Furthermore, treatment may need to be

postponed to allow the patient to recover from severe toxicity. The need to delay therapy has to be reassessed at least every other day. Thus, the treating physician should not blindly follow the protocol schemas but take into account the individual treatment tolerance of his patients and adapt the protocol to individual requirements, if necessary. In these cases we suggest to contact the national study coordinator.

11.8.2 SR-A (Standard risk group arm A / ALL-REZ BFM)

11.8.2.1 SIA, (SR induction, arm A / F blocks)

The SR induction of arm A should be administered on time and regardless of the peripheral blood counts. The early achievement of a remission has priority and frequently is an essential prerequisite for the long term control of infections. Platelet counts are maintained above $15 \text{ to } 20,000 \times 10^9/\text{L}$ with platelet transfusions until a remission is achieved.

11.8.2.2 SCA1 + 2 (SR consolidation 1 + 2, arm A / Prot-II IDA \pm epratuzumab)

SCA1 starts when the daily neutrophil count reaches or exceeds $0.5 \times 10^9/\text{L}$, unless continuation of therapy would appear to result in a life-threatening situation. The result of the bone marrow aspirate on day 14 of SIA serves as an additional factor in this decision. If persistent leukemic metaplasia is detected, treatment is continued without delay since regeneration cannot be expected. Platelets have to be transfused as necessary. The weekly administration of vincristine/IDA \pm epratuzumab should be performed on time with a neutrophil count of at least $0.5 \times 10^9/\text{L}$.

Minimal requirements for the start of the cyclophosphamide infusion (day 1, week 9) are:

leukocytes $> 1.5 \times 10^9/\text{L}$

neutrophils $> 0.5 \times 10^9/\text{L}$

platelets $> 80 \times 10^9/\text{L}$.

Both courses of cytarabine (day 3 to 6, week 10 and 11) are administered regardless of blood counts. Only in case of overt infections should treatment be interrupted (including 6-TG).

11.8.2.3 SCA3 - 7 (SR consolidation 3 – 7, arm A / R-courses)

The subsequent blocks SCA3-7 are administered after a 21-day interval from the start of the preceding block. The SCA3 block starts 14 days after the last day of SCA2. Shorter intervals are possible but not required. The following minimal requirements for the start of a treatment block apply:

leukocytes $> 2.0 \times 10^9/\text{L}$

neutrophils $> 0.5 \times 10^9/\text{L}$

platelets $> 80 \times 10^9/\text{L}$

In case of prolonged treatment delays, dose reductions according to the specific guidelines outlined below have to be considered. In this case please contact the national study centre. The need to delay therapy has to be reassessed at least every other day.

11.8.3 SR-B (Standard risk group arm B / R3)

11.8.3.1 SIB, (SR induction, arm B / R3 induction)

The SR induction of arm B should be administered on time and regardless of the peripheral blood counts. The early achievement of a remission has priority and frequently is an essential prerequisite for the long term control of infections. Platelet counts are maintained above $15 \text{ to } 20,000 \times 10^9/\text{L}$ with platelet transfusions until a remission is achieved. Week 5 starts as soon as the child is able to tolerate it.

11.8.3.2 SCB1 (SR consolidation 1, arm B / R3 consolidation)

The day methotrexate is given will be counted as the beginning of week 6. Minimal requirements for the start of week 6 will be a recovering blood count with:

neutrophils $> 0.5 \times 10^9/\text{L}$

platelets $\geq 50 \times 10^9/\text{L}$

The treatment of consolidation weeks 7 can be started as long as the child is well and neutrophils $> 0.5 \times 10^9/\text{L}$ and platelets $> 50 \times 10^9/\text{L}$ and counts are recovering. Chemotherapy

should be continued in a well child with fever of unknown origin but no neutropenia. Any serious infection, such as varicella, *Pneumocystis pneumonia*, or neutropenia with fever, and presumed or proven infection, warrants chemotherapy interruption at any time.

11.8.3.3 SCB2 (SR consolidation 2, arm B / R3 intensification)

This phase starts as soon as the child is able to tolerate it. The neutrophils should be $>0.5 \times 10^9/L$ and platelets $>50 \times 10^9/L$, with evidence of count recovery. Vincristine and Dexamethasone may be given if the counts have not recovered. The day cytarabine is first given will be counted as the beginning of week 10.

Week 13 of SCB2 starts as soon as the child is able to tolerate it. The neutrophils should be $>0.5 \times 10^9/L$ and platelets $>50 \times 10^9/L$, with evidence of count recovery and the child should be clinically well.

11.8.4 SMA/B (Standard risk group maintenance therapy arm A or B)

In contrast to all other chemotherapy elements, the dosage of mercaptopurine and methotrexate in maintenance therapy is considered as guidance level, which should be adapted to the blood counts, and the hepatotoxicity. The drugs should be reduced in dosage or withdrawn if the white blood cell count falls below $1.5 / nl$, the absolute neutrophil count below $0.5 / nl$, the absolute lymphocyte count below $0.3 / nl$, or the platelet count below $50 / nl$. Transaminases and bilirubine should be analysed every 3 month. Drug doses should be reduced or temporarily omitted if transaminases exceed the 10 fold or bilirubine the 3 fold upper normal values.

11.8.5 Dose reduction recommendations

Dose reductions may be applied as a definite exception in case of unacceptable toxicity or substantial treatment delays due to impaired tolerance to treatment. The need for dose reduction should be carefully reassessed prior to every treatment element. In general, one or several cytotoxic drugs may be reduced to 2/3 of the scheduled protocol dosage. In case of severe methotrexate associated toxicity such as mucositis, renal insufficiency and elimination failure, high-dose methotrexate may be given at a shorter infusion duration of 24 hours, at a lower dose of 500 mg/m^2 , or with earlier given leucovorin rescue, eventually at higher (doubled) doses. In case of corticosteroid-associated diabetes, dexamethasone dose should be reduced and infusion therapy should be given without any glucose. In case of asparaginase-associated complications such as thrombosis of pancreatitis, the asparaginase application may be postponed or even cancelled.

11.8.5.1 Body weight below 10 kg

In the rare case of a body weight below 10 kg at relapse, the drug doses are calculated according to body weight instead of body surface according to the following formula:

$\text{Dose} = \text{Scheduled dose} / \text{m}^2 \text{ BSA} \times \text{body weight [kg]} / 30$

11.8.5.2 Obesity

For obese patients no general recommendations for dose reduction are made. In individual patients, dose adaptations may be made due to toxicity according to the recommendations made in chapter 11.8.5..

11.8.5.3 Down Syndrome patients

Patients with Down Syndrome and ALL-relapse have a worse tolerance to treatment as others and in particular a high induction death rate and mortality rate in CR2. Since the tolerance to HD methotrexate is in particular poor, the first application should be given at an infusion duration of 24 hours only and eventually at a reduced dose of 500 mg/m^2 . Only if this schedule is well tolerated, the next HD MTX may be adjusted to the ways as scheduled in the protocol. For all other drugs, no general reduction of the doses is recommended. The option of dose reduction should be however applied in patients with Down Syndrome more generously than in other patients.

11.8.6 Modalities of oral application of chemotherapeutics

Small children may not be able to take in tablets which are prescribed within the protocol such as Mercaptopurine, Thioguanine and Methotrexate. Mercaptopurine is available in most participating countries in liquid form (Xaloprine®). Thioguanine and Methotrexate can be dissolved in liquid form. This should be done by a pharmacy under biological-safety conditions (ventilated cabinet) and given to the patients in individually portioned syringes. Workers handling this procedure should wear gowns, gloves and masks. It should be avoided crushing tablets at home. Instead, oral chemotherapy should be prescribed in alternating schedules allowing for intake of complete tablets. Handling with chemotherapeutic drugs should be done according to the "Guidelines for the safe handling of hazardous drugs" as published by the American Society of Health-System Pharmacists.⁹⁴

11.8.7 Drug incompatibilities and interactions

In the summaries of product characteristics (SmPCs) of the involved chemotherapeutic and immunosuppressive drugs a variety of drugs are listed that may have adverse interactions and should be avoided or at least handled with care if combined with the respective drug. Among those there are drugs that are essential part of the treatment protocol or the supportive therapy and cannot be avoided. Others, such as cytochrome P450 activating antiepileptic agents may be replaced by other substances if applicable. The extractions from the SmPCs listing the drug interactions and incompatibilities are added to the appendix of the protocol. The decision, whether the drugs can be omitted or replaced need to be taken by the treating physician taking into account the clinical situation of the individual patient.

No drugs are listed that are strictly contraindicated and would result in an exclusion from the trial. Live attenuated vaccines are strictly prohibited during and for at least 6 months after stopping therapy for those not transplanted and 18 months for those transplanted.

11.9 Radiation Therapy

Radiation therapy is given to control disease in extra-compartments protected from effective systemic chemotherapy by biologic blood barriers. This concerns CNS- and testicular relapse. Furthermore, in case of persistence of leukemia in other extramedullary sites such as mediastinum, lymph nodes, bone, skin, or other organs, a local radiation therapy may be necessary. This should be discussed individually with the national coordinating centres.

Radiation therapy is scheduled at the beginning of maintenance therapy in SR patients not receiving SCT.

11.9.1 Radiation therapy in patients with CNS relapse

Patients with a CNS relapse not eligible for allogeneic SCT receive irradiation of the cranium and the upper three cervical segments at a dose depending on the treatment arm after completion of intensive chemotherapy. There is no clear evidence that craniospinal irradiation is superior to cranial irradiation. Particularly in isolated CNS relapse, however, there is a trend in favour of craniospinal irradiation. Craniospinal irradiation, therefore, is permitted. There is evidence to suggest that the use of thiopurines during cranial irradiation may predispose to the occurrence of brain tumours. Therefore, thiopurines are omitted during CNS irradiation. Children under 2 years of age with CNS disease at diagnosis are not eligible for cranial radiotherapy. They receive intrathecal therapy during maintenance therapy according to the respective treatment arm.

CNS irradiation is given at a dose of 18 Gy in daily fractions of 1.5 to maximum 2.0 Gy. If the previous exposure to irradiation exceeds 18 Gy the radiation dose is reduced to 15 Gy. If the interval to the first course of radiation therapy is shorter than 24 months and the previous radiation dose exceeds 15 Gy the radiation dose should be reduced to 15 Gy.

11.9.2 Testicular relapse

Local treatment of testicular relapse can be performed including orchiectomy and reduced irradiation of the contralateral non-involved testicle or including full dose irradiation of both

testicles at the discretion of the treating centre. Following a dose of 24 Gy, atrophy of the irradiated testis and absent endocrine function has to be expected. In case of allogeneic SCT, the orchiectomy should be performed at diagnosis or during early consolidation. In context of total body irradiation, the radiation dose given to the testes should be increased to 18 Gy giving a 6 Gy boost.

11.9.2.1 Option 1: Orchiectomy and reduced irradiation of the contralateral testicle

In case of a unilateral clinical involvement, the contralateral testis should be biopsied during the orchiectomy procedure. If the biopsy shows no involvement, local irradiation with 15Gy is given. After this dose sufficient residual endocrine function is expected to allow the spontaneous onset of puberty. If the biopsy is positive or not performed, the clinically not involved testis should be irradiated with 18Gy. If a clinically involved testis is not removed irradiation with 24Gy should be performed.

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

11.9.2.2 Option 2: Full dose irradiation of both testicles

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

11.9.3 Radiation technique and dose

Radiation therapy is principally performed using high-voltage technique (telecobalt or linear accelerator). The exact reproducibility of the daily positioning has to be ensured (for example using masks for immobilization).

During irradiation of the CNS individual attenuators have to be made to protect the visceral cranium and the anterior cervical soft tissues. The retroorbital spaces and the skull base have to be well included in the radiation field. If the entire neuroaxis is irradiated dosage gaps and overlaps of adjacent fields have to be avoided using divergence compensation.

Due to the low lying frontal skull base in children under two years of age the protection of the eye lenses is not always possible. During follow-up regular ophthalmologic assessments, therefore, are required to detect and treat radiation cataracts in a timely manner.

Emphasis is placed on a homogeneous distribution of the radiation dose. Principally, all fields are irradiated in each session. Single fraction should have a minimum dose of 1.5Gy and a maximum dose of 2.0Gy (1.8Gy in children under the age of 2 years) and should be administered 5 times per week.

To minimize the risk of leukoencephalopathy CNS irradiation is started only after the intensive phase of treatment is completed, i.e. after the last block (SCA7 or SCB4).

11.10 Other forms of local therapy

11.10.1 Intrathecal chemotherapy

Patients may receive intrathecal chemotherapy at the time of the diagnostic lumbar puncture (chapter 11.1, page 44) if the diagnosis of relapsed ALL is already confirmed at that time which then can be considered as the scheduled intrathecal therapy at the start of induction (day 1 of week 1). The schedule and dose of intrathecal chemotherapy is described in the chapters of the respective treatment arm.

11.10.1.1 Intensified intrathecal therapy in patients with CNS involvement

Patients with CNS disease at diagnosis should receive weekly intrathecal chemotherapy according to the individual treatment arm until two consecutive clear CSF's have been obtained. SR patients of arm A with CNS involvement receive additional intrathecal injections on day 1 of week 6 (SCA1), and on day 6 of week 16 and 22 (SCA 4 and 6). The interval between intrathecal injections should be at least five days. Triple intrathecal chemotherapy is dosed according to age (see table chapter 11.6.2).

SR patients of arm B with CNS disease at diagnosis should receive weekly intrathecal methotrexate until two consecutive clear CSF's have been obtained. After clearance of the CSF, no intrathecal injections additional to those scheduled for all patients are applied.

11.10.2 Orchiectomy

Orchiectomy is the most radical local therapy for a clinically involved testis. It can be performed as alternative (option 1, chapter 11.9.2.1) to a local irradiation with 24 Gy (option 2) at the discretion of the treating centre. The procedure is performed at the beginning of therapy if the clinical finding is unequivocal or during the course of therapy if histo-pathologic confirmation is required. In this case the decrease in size of the testis can be used as an indicator for the response to therapy. During orchiectomy or after termination of chemotherapy, a testicular prosthesis should be implanted. The hormonal dysfunction after orchiectomy or irradiation at 24Gy is identical. The cosmetic result may be better compared to the testicular atrophy following local irradiation with 24Gy. Subclinical involvement of the clinically not involved contralateral testis has to be excluded by biopsy, if a reduced local irradiation with 15 Gy in a non-involved and 18 Gy in a sub-clinically involved testis is intended. Depending on the result local irradiation is given according to the guidelines described in chapter 11.9.2.

11.11 Emergencies

The main problem of intensive multi-agent chemotherapy is the combination of marked immunosuppression, direct organ and mucosal toxicity and the resulting immunodeficiency toward potentially pathogenic microorganisms. A number of protective and supportive measures are urgently required to prevent potentially serious harm associated with therapy.

11.11.1 Acute tumour lysis syndrome

The acute tumour lysis syndrome is rare in children with relapsed ALL since this type of leukemia in general is comparatively resistant to therapy. During the lysis of leukemic cells the purine degradation products xanthine, hypoxanthine and uric acid as well potassium and phosphate are released. A rapid lysis of large cell numbers may result in precipitation within the renal tubules and collecting ducts and in life-threatening hyperkalemia.

To prevent the acute tumour lysis syndrome, forced diuresis with 3-6L/m²/d (the fluid balance is maintained as needed with furosemide), and allopurinol (at a dose of 10mg/kg/day, maximum single dose 300mg) are used.

In case of hyperuricemia, beginning renal insufficiency or marked hyperleukocytosis treatment with rasburicase (Fasturtec ®) may be indicated.

In case of marked hyperkalemia, hyperphosphatemia, hyperuricemia or renal insufficiency, haemodialysis may become necessary.

11.11.2 Impaired elimination of methotrexate

The serum methotrexate level 48 hours after the start of the methotrexate infusion is generally below 0.5 µmol/L. Otherwise, folinic acid is extended at six hourly intervals beyond the scheduled doses at 48 and 54 hours until the methotrexate level falls below 0.25µmol/L. The dose of folinic acid depends on the methotrexate level and is calculated as 15 mg/m² antagonizing up to 1 µmol/l serum methotrexate (diagram shown in the appendix). If the methotrexate level at 48 hours is greater than 2.0 µmol/L alkaline diuresis with 3 to 4.5 L/ m² is used in addition. If the methotrexate level at 48 hour is greater than 5 µmol/L or in cases of a marked intolerance with severe vomiting, diarrhea and neurological symptoms, the use of carboxypeptidase should be considered. Carboxypeptidase results in an enzymatic cleavage of methotrexate. In this case, contact the national or international study centre.

If a decreased elimination of methotrexate is apparent at 36 hours (MTX level > 10µmol/L) a methotrexate serum level at 42 hours is recommended. In this case the administration of leucovorin should be moved up at a dose equivalent to that recommended by the rescue

schema at 42 hours (15 mg/m² antagonizing up to 1 µmol/l serum methotrexate). If the value is greater than 5 µmol/L, the dose of folinic acid is calculated using the following formula:
 leucovorin (mg) = MTX 42h (µmol/L) x body weight (kg).

11.11.3 Extravasation of anthracyclines or vinca alkaloids

In case of an extravasation of an anthracycline, the extravasate should first be aspirated, tissue fluid and blood using the existing venous access and, if possible diluted by instilling normal saline before removing the vascular access. Topical application of the dimethylsulfoxide (DMSO 99%), four drops per 10cm² skin three times a day for several days may ameliorate the course (Bertelli et al., 1995). The local area of skin should be kept cool for several days.

In case of extravasation of a vinca alkaloid, the extravasate should first be aspirated, tissue fluid and blood using the existing venous access. Then hyaluronidase (150units/ mL normal saline) should be injected into the area of the extravasation using the existing venous access before removing it. Subsequently, the affected tissue can be infiltrated subcutaneously with several small injections of hyaluronidase (Bertelli, 1995). The local area should be kept warm (in contrast to the cooling recommended for anthracycline extravasations).

If a necrosis develops despite these local measures early surgical revision should be considered.

11.12 Guidelines for supportive care

The guidelines are recommendations and can be adapted to local schemas and requirements.

11.12.1 Anti-infectious prophylactic measures

11.12.1.1 Pneumocystis carinii pneumonia prophylaxis

cotrimoxazole, 2-3 mg/kg trimethoprim (10-15mg/kg sulfamethoxazole) BID on 2 days per week (e.g. Saturday and Sunday) throughout the intensive and in case the maintenance therapy. The drug should not be given at the same day as oral methotrexate. Cotrimoxazole may cause prolonged cytopenias. If this is suspected, the drug should be interrupted or discontinued. Alternative: pentamidine 300mg by inhalation per month or Dapsone 4mg/kg weekly. More details on PCP prophylaxis see appendix.

11.12.1.2 Antifungal prophylaxis

From the beginning until approx. 4 weeks after the end of the intensive phase of therapy the following measures are recommended

- Amphotericin B suspension for local candida prophylaxis:

age (years)	Amphotericin B suspension (ml/d)
= <1.5	4 x 1.0
1.5-2	4 x 1.5
=> 3	4 x 2.0

The amphotericin suspension is carefully spread over the entire oral mucosa and then swallowed. If prophylaxis with amphotericin solution is not feasible or if thrush becomes apparent despite prophylaxis, fluconazole (approx. 2mg/kg/d) can be used as an alternative.

- Inhalation of amphotericin B for prophylaxis of pulmonary aspergillosis

Inhalation of amphotericin B BID may be applied as prophylaxis of pulmonary aspergillosis. 2ml Amphotericin B stock solution (1 vial = 50mg, dissolved in 10 ml distilled water) is used for one inhalation. The inhalations proved useful in the prevention of infections with *Aspergillus fumigatus* in the bronchial system.

- General systemic antimycotic prophylaxis

In case of prolonged aplasia and in consideration of local preferences, systemic antifungal prophylaxis may be applied. Schemas such as daily oral voriconazole or intermittent ambisome 3 x / week have been applied.

11.12.1.3 Vaccination recommendations

Patients with allogeneic HSCT receive a full panel of vaccination according to guidelines published by the EBMT, independently from prior treatment with epratuzumab.⁹⁵ Patients with ALL may lose their immune protection after treatment with intensive and maintenance chemotherapy. Patients can be successfully revaccinated 3-6 months after termination of chemotherapy with killed vaccines. Although Hib immunity is mostly maintained, a revaccination is recommended since it leads to a more reliable immunity and protection against Hib colonisation.⁹⁶ Therefore, immunisation with DT, acellular pertussis, IPV and Hib (Pedicel) as killed vaccines is recommended independently from the application of epratuzumab and without measuring the respective immunisation titres. Furthermore, for live vaccines a booster dose of MMR is recommended not before 6 months after stopping therapy for those not transplanted and 18 months for those transplanted. For the live vaccines, immunisation titres against these agents may be measured 6 months after completion of maintenance chemotherapy and immunisation may be adapted to the results. Also Hepatitis B may be revaccinated depending on the anti-HbS titre quantified at the end of therapy. Whether these immunisation titres are indeed lower in patients having received epratuzumab in addition to intensive chemotherapy will be investigated by comparing the immunisation titres of both arms. Immunisation with varicella vaccines is controversial and may be considered in accordance to national guidelines.

The vaccination recommendations should be adapted to national guidelines as far as they are established.⁹⁵⁻⁹⁷

11.12.2 Anti-emetic treatment

Ondansetron (two doses of 5mg/m²/day) is used for highly emetogenic treatment elements such as high-dose Ara-C, ifosfamide and cyclophosphamide. Additional treatment with aprepitant or dimenhydrinate may be required if this agent is insufficient particularly in adolescents. Many treatment elements already include the administration of dexamethasone so that no further anti-emetic effect can be expected from this agent.

11.12.3 Interventional supportive therapy

11.12.3.1 Mucosal Lesions

Care for oral mucosal lesions: oral rinses at least 4 times a day, e.g. with chamomile solution; at least once daily local use of astringents on open sores, e.g. watery solutions of methylene blue.

Severe large ulcerations generally are not limited to the mouth. They require close monitoring and a consistent and early replacement of protein and electrolyte losses. In addition, sufficient analgesia should be ensured including opiates as needed.

The mucosal area under the tongue generally is representative of the status of the entire gastrointestinal tract. It remains almost always accessible to inspection and assessment even in cases with marked swelling and pain.

11.12.3.2 Febrile Neutropenia

In case of a neutrophil count below $0.5 \times 10^9/L$ and fever greater than 38.5 °C systematic antibiotic and possibly anti-fungal treatment has to be administered. Particularly patients with a high therapeutic risk (e.g. patients with a very early relapse during initial treatment or fever at the beginning of the critical cytopenia) require a rapid escalation of antibiotic protection to be able to control severe septic infections until regeneration occurs. The following table shows an example of such an escalation with proved i.v. antibiotic combinations.

Table 8 Escalation of antibiotic therapy

start with	Cefotaxime or ceftriaxone and gentamicin
if still febrile 48h later	add vancomycin or teicoplanin
if still febrile 48h later	Replace cephalosporin/gentamicin with meropenem
if still febrile 48h later	add liposomal amphotericin B or voriconazole

This approach is an example that has to be supplemented by clinical findings and microbiological results and requires modification according to the experience of the local treating physician. Delays in the change of antibiotic medications may provide an irretrievable advantage to problem organisms such as pseudomonas, coagulase negative staphylococci or aspergillus. If there is a clinical suggestion of an infection with pseudomonas medication with certain efficacy such as amikacin and ceftazidim should be added. If an atypical pneumonia is suspected the combination of antibiotics should include a macrolide antibiotic such as erythromycin.

In case of prolonged and/or repeated neutropenia G-CSF can be applied at the discretion of the treating physician.

11.12.3.3 Transfusion of blood products

For red cell and platelet transfusions only leukocyte-depleted, irradiated (30Gy) and filtered concentrates should be used. HLA-compatible platelet concentrates should be used particularly after a poor response to transfusion. Granulocyte concentrates are nowadays only used in rare and exceptional circumstances, e.g. uncontrollable fungal infections during periods of prolonged aplasia.

11.12.3.4 Pain management

Pain is however a side effect of the standard therapy backbones and will independently from the application of epratuzumab be treated according to established standards of the participating centres based on published supportive therapy guidelines.^{98,99}

12 DEFINITIONS

12.1 Site of Relapse

▪ Isolated bone marrow relapse

≥25% lymphoblasts in the bone marrow quantified by cytology in the absence of extramedullary involvement.

▪ Combined bone marrow relapse

≥5% lymphoblasts in the bone marrow contains quantified by cytology and at least one extramedullary manifestation of ALL.

▪ CNS relapse

Morphologically unequivocal leukemic lymphoblasts in the CSF and a pleocytosis of >5/μl nucleated cells. If the CSF is contaminated with blood the following procedure is recommended after discussion with the national study centre: If blasts are present in the CSF and the peripheral blood shows no blasts, a CNS relapse is assumed. If the proportion of blasts in the CSF is equivalent to the proportion of blasts in the peripheral blood and there is no additional morphologic evidence that the blasts persisted longer in the CSF, contamination is assumed. In unclear situations a case-by-case decision may be necessary. If blasts are present the patient receives the intensified intrathecal chemotherapy similar to patients with CNS involvement but not the increased dose of cranial irradiation. If clinical signs of CNS involvement are present such as visual disturbances, polyphagia, cranial nerve palsies in the absence of CSF pleocytosis, the presence of a CNS relapse has to be confirmed or ruled out with all available diagnostic methods (cranial CT, MRI). If evidence of meningeal infiltration is found by imaging, a biopsy may have to be performed.

▪ Testicular relapse

Uni- or bilateral painless testicular enlargement with infiltration of leukemic lymphoblasts confirmed by biopsy. In case of a clinically normal contralateral testis a subclinical involvement has to be ruled out by biopsy.

- **Relapse at other sites**

Detection of leukemic infiltration by appropriate imaging techniques with confirmation by biopsy.

12.2 Response to therapy

The assessment of the response to therapy in the bone marrow and CSF is solely based on cytological criteria.

- **Aplastic bone marrow (M0)**

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

- **Complete remission (CR)**

remission bone marrow and no further evidence of persistent leukemic lymphoblasts based on cytological, histopathologic, radiologic or clinical findings (the detection of leukemic cells below the threshold of cytological detection using molecular or flow cytometric methods is compatible with the definition of complete remission).

- regenerating bone marrow with less than 5% blasts (M1) and
- peripheral blood without blasts and with evidence of regeneration and
- absence of extramedullary leukemic involvement.

- **Minimal residual disease (MRD) good response**

Arm A: MRD at day 1 of week 5 of $< 10^{-3}$; arm B: MRD at day 1 of week 6 of $< 10^{-4}$.

- **Molecular remission**

MRD value of $< 10^{-4}$. This level is accepted as the lower quantifiable margin for PCR quantification of MRD.

- **MRD poor response**

Arm A: MRD at day 1 of week 5 of $\geq 10^{-3}$; arm B: MRD at day 1 of week 6 of $\geq 10^{-4}$.

- **MRD reappearance**

A reversion after MRD negativity to reproducible MRD positivity $\geq 10^{-4}$ (at least one sample, collected during post-consolidation) is called molecular reappearance. A reconfirmation is strongly recommended. This finding does not fulfil the conditions for the definition of subsequent relapse.

- **Non-representative bone marrow**

markedly reduced cellularity despite signs of regeneration in the peripheral blood and differential count of nucleated cells in the marrow largely corresponding to that in the peripheral blood. Such a bone marrow aspirate should be repeated particularly when therapeutic decisions are taken based on the result.

- **Partial response / marrow involvement (M2)**

Bone marrow with $\geq 5\%$ and $< 25\%$ of lymphoblastic leukemic blasts irrespectively of the cellular content.

- **Refractory marrow (M3)**

Bone marrow with $\geq 25\%$ of lymphoblastic leukemic blasts irrespectively of the cellular content.

- **Remission bone marrow (M1)**

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

12.3 Events relevant for the endpoints

The following events are relevant endpoints for the event-free survival analysis (DIR, ID, NR, SMN, TRD, REL as events and LFU, CCR as censored events), and for the overall survival analysis (any death as event and LFU, CCR as censored events). Stem-cell transplantation is not considered as event and is not censored in the EFS/OS analyses.

- **Complete continuous remission (CCR)**

Complete remission at the time point of last follow up (censored event)

- **Death in remission (DIR)**

A death in remission is death of any reason after achievement of a CR.

- **Induction death (ID)**

An induction death is a treatment- and/or disease-related death that occurs during induction prior to achievement of a CR.

- **Lost to follow up (LFU)**

Patient is not anymore available for follow up investigations (censored event).

- **Non-response (NR)**

Non-response is diagnosed in patients who have a persisting M3 marrow ($\geq 25\%$ ALL cells) after the induction phase (SR arm A week 5, arm B week 6) or who have not achieved a CR ($< 5\%$ ALL cells in the bone marrow, no evidence of extramedullary disease) after the first consolidation element (SR arm A week 9, arm B week 10).

- **Secondary malignancy (SMN)**

Any subsequent malignant disease occurring after diagnosis of the ALL relapse except a subsequent relapse of the underlying ALL itself.

- **Treatment-related death (TRD)**

A treatment-related death is a death with a temporal and/or causal relationship to treatment that occurs during continuous CR.

- **Subsequent relapse (REL)**

A subsequent relapse is defined as evidence of ALL after achievement of a 2nd CR as defined for the first ALL relapse.

13 TECHNIQUE AND LOGISTICS OF DIAGNOSTIC PROCEDURES

13.1 Diagnostic tests related to ALL

The diagnosis of an ALL relapse has to be established unequivocally using the criteria listed below before relapse therapy is begun. In difficult cases the national study centre should be contacted.

13.1.1 Bone Marrow

The bone marrow aspirate has to be performed at least at two different aspiration sites. After bone marrow smears are prepared from the first marrow aliquot using a cover glass (without addition of heparin or EDTA; touch preps are of inferior quality and should be avoided) at least two aspirates of 5mL are collected from each site into a heparinised syringe (total volume approximately 20mL). In case of a dry tap at both sites a bone marrow biopsy is performed. The *first heparinised* bone marrow aspirate collected from each site (e.g. left and right posterior iliac spine) is sent without delay to the national IntReALL 2010 study centre/national reference laboratory for immediate processing and molecular/cytogenetic studies. Immunophenotyping is performed on the second bone marrow syringe and sent to the national reference laboratory. In general, the entire diagnostic evaluation and classification of relapse is performed on the bone marrow sample collected at the time of relapse. This includes cytomorphology, molecular tests (MRD, fusion genes), immunophenotyping and cytogenetics.

13.1.2 CNS

Every time a relapse is diagnosed a diagnostic lumbar puncture is performed. This lumbar puncture can be used to administer the first dose of intrathecal chemotherapy. If a CNS relapse is suspected, a CSF volume of at least 10mL has to be collected since this sample is possibly the only material for the design of a clonal probe to monitor MRD. The CSF has to be promptly assessed by cytology or prepared for such an assessment.

If a CNS relapse is suspected and the CSF is unremarkable, a cranial MRI should be performed to detect a localized involvement. Such an involvement may have to be confirmed by biopsy.

13.1.3 Testis

In the interest of a precise diagnostic evaluation, the immunophenotyping of lymphoblasts and the detection of molecular markers should also be performed in cases of isolated testicular relapse. The biopsy sample should be sent in sterile saline solution to the local pathologist and to the national study centre for review.

13.1.4 Other forms of relapse

Any other suspicious manifestations such as an infiltration, swelling, effusion or space-occupying lesion should be imaged. Biopsy samples should be evaluated morphologically as well as by immunophenotyping and molecular methods.

13.1.5 Response to therapy

All manifestations of an ALL relapse have to be monitored until their complete resolution using the most appropriate diagnostic methods. These include bone marrow aspirates and lumbar punctures at the beginning of each treatment block, and imaging techniques for other sites. Complete blood and differential counts are performed daily until the disappearance of blasts, then 2 to 3 times per week.

Mandatory bone marrow aspirates are performed at the time of relapse, and further on as described in the respective chapter of the treatment arms. Additional bone marrow aspirates are performed prior to SCT and prior to start of maintenance therapy, to document a continuous cytological remission. Following SCT bone marrow aspirates are scheduled on day 30, 60, 100, 180 as well as during month 9, 12 and 18 after SCT.

Bone marrow aspirates are performed even after the achievement of a cytological remission to determine the level of MRD. This also applies to patients with an isolated extramedullary relapse.

Table 9 ALL-Relapse: diagnostics, shipment and asservation of material

<u>Time point</u>	<u>Laboratory / Sample</u>
Relapse diagnosis	<u>National reference laboratory for cytology:</u> <ul style="list-style-type: none"> • 5 BM smears, unstained (from the first native BM aspirate) • 5 PB smears, unstained • 2 CSF smears, unstained • Smears of -EM (if applicable) – Touch preparation <u>National reference laboratory for MRD (PCR, FLOW) and molecular genetics:</u> <ul style="list-style-type: none"> • 2-3 x 5ml heparin. BM • 5-10ml heparin. PB • Biopsy sample of testis or other EM in medium and snap frozen if possible • 1-2 additional CSF smears if possible • 1-2 additional smears of EM (if applicable) – Touch preparation, if possible <u>National reference laboratory for Cytogenetics:</u> 1 x 5ml heparin. <u>National reference laboratory for immunophenotyping:</u> <ul style="list-style-type: none"> • 2ml heparin. BM • 2 BM smears, unstained
During relapse treatment <ul style="list-style-type: none"> • Start of week 3 (arms A/B) • start of week 5 (A) or 6 (B) • start of week 9 (A) or 10 (B) • start of week 13 (A) or 15 (B) • start of week 17 (A/B) (immediately before SCT) 	<u>National reference laboratory for cytology:</u> <ul style="list-style-type: none"> • 2 BM smears, unstained (from the first native BM aspirate) • 2 PB smears, unstained • 2 CSF smears, unstained <u>National reference laboratory for MRD:</u> <ul style="list-style-type: none"> • 2-3 x 5ml heparin. BM • 5-10ml heparin. PB
after SCT	<u>National reference laboratory for cytology:</u> <ul style="list-style-type: none"> • 2 BM smears, unstained (from the first native BM aspirate) • 2 PB smears, unstained • 2 CSF smears, unstained <u>National reference laboratory for MRD:</u> <ul style="list-style-type: none"> • 2-3 x 5ml heparin. BM • 5-10ml heparin. PB

13.2 Diagnostic tests to monitor organ toxicity

13.2.1 Biochemistry, coagulation, virus serology

At diagnosis, an extended analysis of blood biochemistry, coagulation function and virus serology are performed to identify any organ damage before start of therapy. A blood biochemistry of the essential values (ALAT, ASAT, bilirubine, protein, creatinine, glucose, C-reactive protein, electrolytes) is analysed at the start and the end of every treatment element. Coagulation parameters are measured also at start of every treatment element and before

any surgical measure. Virus serology is repeated at the end of intensive therapy and after the end of maintenance therapy (see Table 10).

13.2.2 HLA Typing

If the family has not been HLA-typed at the time of relapse diagnosis, this should be done immediately for SR patients as soon as a MRD-poor-response becomes evident. If a compatible family donor is not available the search for an unrelated donor should be initiated. HLA typing may also be used to identify HLA compatible platelet concentrates, if applicable.

13.2.3 Diagnostic tests during continuation therapy

During this phase of treatment peripheral blood counts are initially performed once a week then every 14 days provided counts are stable and drug doses remain unchanged. Basic biochemical parameters are monitored approx. every 3 months. Regular physical examinations are required.

A bone marrow aspirate is recommended at the beginning of continuation therapy then every 6 months and at the end of continuation therapy. In addition to the morphological evaluation a molecular assessment of MRD is performed.

Additional bone marrow aspirates and lumbar punctures are only performed if clinical symptoms or changes of the peripheral blood counts suggest the suspicion of relapse.

13.2.4 Diagnostic tests at the end of therapy

Continuing complete remission is documented six to eight weeks after completion of therapy by lumbar puncture and bone marrow aspirate including molecular tests. In addition a chest x-ray, echocardiogram as well as biochemical and serological follow-up investigations are obtained.

13.2.5 Follow-up investigations, detections of late effects

The follow-up assessment should include at a minimum a physical assessment and complete blood count. The interval between follow-up visits after the completion of therapy should be 4 weeks during the first year, 6-8 weeks during the second and third year, 3 months during the fourth year and 6 months during the fifth year. Thereafter a yearly assessment is sufficient. An evaluation of the bone marrow as part of the MRD study is offered for the time point of one year after completion of therapy. The adequate development of growth and sexual development are documented. In case, specific diagnostics to clarify delays should be undertaken.

Table 10 Diagnostic tests recommended for monitoring of organ toxicity

	at diagnosis	during the intensive phase of therapy	after the intensive phase of therapy
CBC and differential BC	+	at the start and end of each block, q2-3d in case of myelosuppression	+
Biochemistry (electrolytes, glucose, protein, albumin, crea, urea, AST, ALT, LDH, AP, CPK)	+	at the start and end of each course	+
amylase (serum)	+	if clinically indicated	+
uric acid	+	daily during the cytoreductive prephase	+
Coagulation (aPTT, INR, fibrinogen, thrombin time, ATIII)	+	at the start of each course	+
Immunoglobulins	+	every 2 nd week during epratuzumab treatment, + week 19/25 arm A and 20/26 arm B	+
virology (HBV, HCV, EBV, CMV, VZV, HSV, HIV)	+		+
Ferritine	+		+
Methotrexate level (potentially folate level) according to protocol			
Urine analysis (glucostix)	+	daily during chemotherapy courses	+
EKG, echocardiogram	+		+
EEG	+		+
X ray	Chest		if indicated
Abdominal ultrasound	+		+
Brain MRI	+		+

13.3 Shipment of samples

Shipment to reference laboratories is described in the national appendices. Samples should be received within 24h and sent with a courier service.

13.4 Reference Institutions

The morphologic diagnosis is generally made at the treating centre. The national study centre performs a central review to ensure a uniform diagnostic evaluation. In case of unclear results, an expedited review can be performed, the result of which is typically communicated within one business day. If the result of the central review is different from that of the treating centre, the responsible study physician at the treating centre will be contacted immediately by phone. Immunophenotyping results are also reviewed by the study centre. In case of discrepant results the treating centre will be contacted by phone.

The MRD results of the reference lab being relevant for the stratification of patients will be communicated by the study centre to the treating centre. This notification includes a statement whether two clonal markers with the required sensitivity could be established and the result of the MRD test after induction in SR patients. At the same time a statement will be made regarding the indication for SCT based on MRD. MRD results of all other time points will not be communicated to the treating centres.

14 SAFETY

14.1 Definitions (according to guideline 2001/20/EG)

14.1.1 Adverse Event (AE)

An adverse event is any untoward medical occurrence in a patient or clinical trial subject administered a medicinal product and which does not necessarily have a causal relationship with this treatment. An AE can therefore be any unfavourable and unintended sign (including abnormal laboratory findings for example), symptom, or disease temporally associated with the use of a medicinal (investigational) or medical product, whether or not it is considered to be related to the medicinal (investigational) product. Adverse events encompass illness, signs of illness (including pathological laboratory findings) and symptoms that initiate during the trial or previous conditions that become worse.

AE's could be diseases, signs or symptoms which occur or worsen after enrolment of the patient in the clinical trial.

Adverse Events are to be assessed as described in section 14.3.1:

14.1.2 Serious Adverse Event (SAE)

A **serious adverse event (SAE)** or serious adverse reaction is any untoward medical occurrence or effect that at any dose

- results in death,
- is life-threatening,
- requires hospitalization or prolongation of existing hospitalization,
- results in persistent or significant disability or incapacity,
- is a congenital anomaly or birth defect,
- is medically significant, may jeopardize the subject and may require medical or surgical intervention to prevent one of the outcomes listed above.

Life-threatening events are defined as:

- circulatory/cardiac insufficiency requiring catecholamines/positive inotropes
- respiratory failure requiring intubation/ventilation
- other clinical situations requiring *immediate* intervention, e.g. (and not restricted to)
 - gastrointestinal bleeding or perforation requiring surgery
 - cerebral abscess/bleeding requiring immediate neurosurgical intervention.

14.1.3 Adverse Reactions

Adverse reactions are all untoward and unintended responses to an investigational medicinal product related to any dose administered.

14.1.4 Suspected Unexpected Serious Adverse Reactions (SUSAR)

A Suspected Unexpected Serious Adverse Reaction (SUSAR) is any suspected adverse reaction related to the study treatment that is both serious and unexpected.

"Unexpected" means that the nature and severity of the adverse reaction are not consistent with the information about the study medication in question set out in the respective reference documents (investigator's brochure and/or available summary of product characteristics concerning all other applied drugs in this protocol)..

14.2 Treatment of AE's

All AE's should be treated appropriately. Such treatment may include changes in study drug treatment including possible interruption or discontinuation, starting or stopping concomitant treatments, changes in the frequency or nature of assessments, hospitalization or any other medically required intervention.

14.3 Assessment of AE's

As far as possible, each AE should be evaluated to determine:

- the severity grade (mild, moderate, severe)
- its relationship to the study drug (assessment of causality)
- its duration (start and end dates or if continuing at final exam)
- action taken (no action taken; study drug dosage adjusted/temporarily interrupted; study drug permanently discontinued due to this AE; hospitalization)
- whether it constitutes a serious adverse event (SAE)
- outcome

14.3.1 Severity

- Grade 1: Mild: The Adverse Event is transient and can be tolerated easily.
- Grade 2: Moderate: The Adverse Event causes discomfort and impedes normal activities
- Grade 3: Severe: The Adverse Event causes severe impairment of normal activities
- Grade 4: Life-threatening or disabling
- Grade 5: Death related to AE

14.3.2 Causal relationship

To assess causality between administration of the investigational product and the Adverse Event the following definitions apply:

- Sure: The reaction comprehensively follows the administration of the investigational product in the right timeframe or can be measured in body tissues or fluids or represents a known or expected response to the study medication or disappears after discontinuation or dose reduction and reoccurs after re-exposition.
- Probable: The reaction comprehensively follows the application of the investigational product the right timeframe or represents a known or expected response to the study medication or disappears after discontinuation or dose reduction and cannot be explained by known characteristics of the patient's disease.
- Possible: The reaction comprehensively follows the application of the investigational product in the right timeframe or represents a known or expected response to the study medication, but could easily be caused by other factors.
- Not related: Adequate Information supporting the assumption that there is no causality
- Cannot be evaluated: The causality cannot be determined.

14.3.3 Expected/Unexpected

An Unexpected AE is an AE, the nature or severity of which is not consistent with the applicable product information (SmPC). The distinction expected/unexpected only depends on whether the untoward reactions have been previously described. The evaluation of expectedness will be done by the pharmacovigilance centre and the sponsor representative. The following events (among others) related to multi drug chemotherapy therapy are adverse but expected:

- allergic reactions
- alopecia
- cardio myopathy
- coagulation disorder
- cushing syndrome
- cystitis
- dermatopathy
- diabetes mellitus
- diarrhea
- fatigue

- febrile neutropenia
- haemorrhagia
- hepatopathy
- infections
- mucositis
- Myelosuppression
- nausea / vomiting
- necroses in paravascular injection
- constipation
- pain
- pancreatopathy
- paralytic subileus
- peripheral polyneuropathy,
- renal insufficiency
- thrombosis

14.4 Investigator's and sponsor's responsibilities

The sponsor's responsibilities entail:

- recording of adverse events
- reporting of suspected unexpected serious adverse reactions ('SUSARs') to the national competent authority and the Ethics Committee
- informing the investigators
- annual safety reporting to the national competent authority and the Ethics Committee

The investigator's responsibilities entail:

- recording of adverse events
- reporting of serious adverse events to the sponsor
- reporting of certain non-serious adverse events and/or laboratory abnormalities to the sponsor

14.5 Documentation and reporting of AE's

All Serious Adverse Events (SAE's) and all Adverse Events (AE's) need to be documented in the patients clinical file by the investigator, no matter if the Investigator suspects a causal relationship to the investigational product or not. The documentation needs ideally to include the type of event (term), start, duration, severity and causality. Related signs symptoms and laboratory changes should be summarized to a specific disease.

14.5.1 Documentation of typical and expected AE's/toxicity

Typical and expected toxicity of the treatment is not documented as separate event but is systematically documented for all treatment elements in the electronic case report form (eCRF) covering defined periods of the protocol. In this case the worst value/clinical status of the respective category will be documented and graded according to CTCAE criteria. AE's that are covered by this definition are not documented separately. Typical and expected toxicity is defined as blood value changes, alteration of clinical chemistry parameters, infection, fever, nausea/vomiting, mucositis, diarrhea, skin alterations, peripheral neuropathy, and thrombosis.

14.5.2 Documentation of relevant AE's

All AE's not meeting the criteria of typical and expected toxicity will be documented in the eCRF if they meet CTCAE criteria \geq °III. They are classified according to CTCAE and described in own words (verbatim) if applicable. AE's meeting the SAE criteria are separately documented using the SAE forms (see 14.6).

All adverse events need to be followed until they subside or stabilize. On request the documentation will be sent by the sponsor to the concerned competent authorities and to concerned competent authorities of other European member states and other contracting states of the EWR agreement, if the study is run in their territory and if they so request.

14.6 Documentation and reporting of SAE's

First, every SAE must be documented by the investigator in the patients file. The occurrence of a serious adverse event (including death, irrespective of the reason) has to be reported **immediately (within 24 h)** at latest on the next working day using the provided SAE reporting fax form signed by the investigator to the international pharmacovigilance safety desk (address see section 14.10), as long as it does not meet the exclusion criteria for SAE reporting.

The initial report shall be promptly (within 24 h) followed by detailed, written reports within the eCRF. The initial and follow-up reports shall identify the trial subjects by unique code numbers assigned to the latter. Personal data of patients must be passed on only after they were pseudonymized. The investigator shall supply the international pharmacovigilance safety desk with any additionally requested information.

14.6.1 Exception rules for SAE reporting

Exceptions to immediate SAE reporting are applied if hospitalization is the only criterion for SAE classification and the below listed criteria are met. Since hospitalization in context with intensive multidrug chemotherapy is required in most of the patients, reporting of any hospitalization would overload the pharmacovigilance system and would impair the handling of clinically relevant events and the detection of SUSARS. SAE's are not reported (but are documented as AE's if they meet the AE documentation criteria) if the only reason of SAE classification is:

- Hospitalizations for treatment of expected adverse events (see chapter 14.3.3). These are in particular:
 - Hospitalization for i.v. antibiotic treatment due to uncomplicated infections (fever with neutropenia after chemotherapy).
 - Hospitalization for parenteral nutrition or i.v.-rehydration and analgetic therapy due to mucositis, inappetence/anorexia or vomiting/diarrhea.
 - hospitalizations planned before entry into the clinical study
 - elective treatment of a pre-existing condition
 - hospitalizations on an outpatient basis that do not result in overnight hospitalization
 - routine treatment not associated with any deterioration in condition.

14.7 Reporting of Suspected Unexpected Serious Adverse Reactions (SUSARs)

The Sponsor will report all suspicious cases of Suspected Unexpected Serious Adverse Reactions (SUSARs) to the concerned Ethics Committee, the concerned competent authorities and to concerned competent authorities of other European member states and other contracting states of the EWR agreement, if the study is run in their territory immediately, at the latest 15 days after it becomes known. He will also inform all Investigators involved in the trial.

In case of a fatal or life threatening SUSAR the Sponsor will report all information relevant for the evaluation of the event immediately, at the latest 7 days after the event becomes known to the concerned Ethics Committee, the concerned regulatory authorities and to concerned competent authorities of other European member states and other contracting states of the EWR agreement, if the study is run in their territory as well as to all investigators involved in the trial. After a further 8 days all further relevant information must be available.

14.8 Development Safety Update Report

The sponsor submits yearly or on request a list of all SUSAR / SAR's documented, together with an extensive safety report on the investigational products to the concerned competent authorities, the concerned ethics committees, the national study sites

14.9 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 concerned Ethics Committee, the relevant regulatory authorities and to 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 especially includes:

- Singular cases of expected severe adverse events with an unexpected outcome.
- Increased incidence of expected severe 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 pseudonymized.

14.10 Pharmacovigilance safety desk / contact person responsible for reporting (international)

Rita Pilger
Koordinierungszentrum für Klinische Studien
(KKS) Charité
Augustenburger Platz 1
D-13353 Berlin
Tel: +49 30 450 553 016
Fax: +49 30 450 553 937

14.11 Follow-up of adverse events

Once an (S)AE is detected, it should be followed until its resolution or stabilisation, and assessment should be made at each visit of any changes in severity, the suspected relationship to the study, the interventions required to treat it and the outcome.

Follow-up information is given via the eCRF system referring to the respective SAE. Each re-occurrence, complication, or progression of the original event should be reported as a follow-up to that event regardless of when it occurs. The follow-up information should describe whether the event has resolved or continues, if and how it was treated, whether the patient continued or withdrew from study participation.

For a follow-up report to authorities, the investigator may be required to collect further information for a detailed description and a final evaluation of the case, including copies of hospital reports, autopsy reports, or other relevant documents.

14.12 Pregnancy

Pregnancies must be reported to the Sponsor immediately. Every pregnant female will be excluded from the trial immediately.

All pregnant subjects will be followed at least until delivery.

This includes abortions, miscarriages, details of labour and birth, congenital abnormalities and complications in mother or child.

This data will be documented on the SAE form, which will be faxed to the international safety desk. The follow up of the pregnancy will be documented on the same form and will be sent 7 days after termination of the pregnancy or 4 weeks after childbirth.

In case of a pregnancy in the partner of a study patient, consent needs to be obtained which allows reporting information about the pregnancy outcome.

15 STATISTICAL ANALYSIS

15.1 Statistical design and sample size calculation

After opening of all participating national groups, a recruitment of about 300 patients with relapsed ALL per year can be expected. About 2/3 (n = 200) are expected to belong to the SR and 1/3 (n = 100) to the HR group. It is expected to take up to 2 years to open all centres, so in the first and second year recruitment will be assumed to be half that given above. Follow-up will continue until at least 3 years after the last patient is recruited to give a median follow-up of at least 4 years.

15.1.1 SR induction, randomization SR1

SR induction follows an open label 2-arm randomized design (SR1). The primary endpoint is event-free survival (EFS) of arm A (ALL-REZ BFM 2002) versus arm B (ALL-R3). The expected 4 year EFS of arm A (ALL-REZ BFM 2002 arm) is 65%. The hypothesis of the study is that EFS can be improved by at least 10% with arm B (ALL-R3). To detect this difference at a power of 82% and a p-value of 0.05, with 100 recruited in the first and second year and 200 per year thereafter, allowing for 3 years follow-up from the last patient recruitment and 5% loss to follow-up at 7 years, 306 patients per arm is required (207 events in total). The expected recruitment time for the total of 612 patients is 4 years. The power is calculated at 82% rather than the more usual 80% in order to give enough patients from SR1 for a power of 80% to be achieved in SR2 (see section 15.1.2).

Note that it is possible that another country may take part in only the SR1 randomization increasing the recruitment for SR1 in the first 2 years to 130 each year and to 260 per year thereafter. If this happens, then it is proposed to keep 4 years recruitment and 3 years follow-up and realise that there would be 80% power to show an 8.6% difference in 4 years EFS to be significant using a p-value of 0.05 and allowing for a 5% loss to follow-up at 7 years with a total of 786 patients (271 events in total).

15.1.2 SR post-induction/ consolidation, Epratuzumab, randomization SR2

Within open label randomization SR2 the prognostic effect of Epratuzumab combined with the conventional intensive consolidation therapy of arm A or B is investigated. It is expected that the effect of Epratuzumab will be independent from the respective treatment arm A or B but randomization will be stratified by the induction arm to give balanced groups. Since patients with M2 bone marrow status after induction are included and thus nonresponse and induction death are possible events, event-free survival is applied as outcome parameter.

The expected event-free survival (EFS) after both SR arms A and B is 74% at 4 years (induction failures are off study and not randomized). The hypothesis of the study is that EFS can be improved by at least 10% with Epratuzumab. To detect this difference at a power of 80% and a p-value of 0.05, with 75 recruited in each of the first two years and 150 per year thereafter, allowing for 3 years follow-up from the last patient recruitment and 5% loss to follow-up at 7 years a number of 228 patients per arm is required (109 events in total). The expected recruitment time for the total of 456 patients is 4 years.

15.2 Criteria for the evaluation of the study results

Event free survival (EFS) for SR1 and SR2 is defined as the time from randomization to the first of induction failure, relapse, death from any cause or second malignancy or is censored at the date of last follow-up.

Overall Survival (OS) is defined as the time from randomization to death from any cause.

Stem-cell transplantation is not considered as event and is also not censored in the EFS/OS analyses.

15.3 Randomization

Informed consent for participation in the randomized parts of the study is given separately from the consent to the enrolment into the study. This enables the patients and parents (or persons entitled to custody) to have the choice to participate in the randomization, or not to be randomized. Non-randomized patients will be treated as specified for the control arm but their data will not be used for the main analysis. After consent has been given and the patient has been randomized, consent can be withdrawn at any time. Randomization occurs when the diagnostic findings required for inclusion/exclusion criteria and randomization stratification are complete and the informed consent for randomization has been obtained.

Randomization is performed centrally by the internet-based data entry system with stratification according to the clinical parameters relevant for each randomization. Randomization will use variable block size.

The first randomization of the SR group (SR1), arm A versus B, is performed immediately after relapse diagnosis during the cytoreductive prophase and is stratified by country-group and site of relapse (IBM,CBM,IEM).

The second randomization of the SR group (SR2), addition of epratuzumab with conventional consolidation is performed directly after the bone marrow assessment after induction (week 5/6). Patient with M3 marrow have reached their study endpoint as nonresponders and are not included into the 2nd randomization. Patients with M0 marrow will be reassessed after 1 week and are randomized only after proven M1 or M2 marrow, otherwise excluded. Drug labelling and shipment of epratuzumab to the local treating centre will be provided by a central institution in Europe for the 1st 2 doses independently of the randomization result, such that the drug will be immediately available after randomization. The randomization will be stratified by the induction regimen (arm A or B), country-group and site of relapse (IBM, CBM, IEM).

15.4 Analysis

A detailed statistical analysis plan will be available from the time the first patient is recruited. This will be reviewed and signed off by the DSMC before the interim and before the final analysis. Wherever possible, the trial is analysed as intention to treat with all patients randomized included.

15.4.1 Analysis of safety

The safety sample is those patients who received at least one dose of at least one drug. At each DSMC meeting safety and toxicity data will be summarised between the patient groups and also by stratification factors within each randomization. The SAE will be investigated by

the DSMC and treatment related deaths corroborated. Any further safety or toxicity data will be provided to the DSMC upon request.

15.4.2 Interim Analysis

During the course of the study one interim analysis after 4 years of recruitment is planned for SR patients to exclude significant results of the randomized questions that would require a preterm termination of the trial. The details of this analysis will be included in the analysis plan. The results of this analysis will be discussed with the DSMC and are not planned for general release.

15.4.3 Early stopping guidelines for treatment related mortality

Based on I-BFM and the R3 trial, the best estimate of expected mortality is 6% treatment related mortality (TRM) in SR patients.

The stopping guideline for each randomization is: Stop if the difference in number of deaths exceeds 3 standard deviations between the two randomized arms. This will be shown by a p-value of 0.001 or less on a two-sided Fisher's exact test. All deaths in randomized patients regardless of cause and timing will be included. This rule will be agreed by the DSMC and the DSMC will take this guideline into account when assessing the trial.

15.4.4 Final analyses

This expected to take place 3 years after final patient recruitment for both SR randomizations.

For the SR1 randomization, a Cox analysis of treatment effect on EFS adjusting for the factors used in the randomization stratification will be the main analysis.

For the SR2 randomization, the main analysis will be a Cox analysis of treatment effect on EFS adjusting for the factors used in the randomization stratification. An alternative to the Cox model will be used if the model assumptions are not met.

Full details are given in the statistical analysis plan.

15.5 Data and Safety Monitoring Committee

The study will be monitored by an independent Data and Safety Monitoring Committee (DSMC) that is specifically chosen to include an expert in paediatric oncology, in biostatistics and in clinical trials to ensure utmost competence and vigilance. The DSMC will meet before the start of the trial, then the first DSMC meeting at 1 year recruitment or 100 patients recruited whichever first, and every 6 months thereafter during first 4 years recruitment and annually thereafter in order to review the trial's progress, safety data (SAE's) and adherence to protocol.

16 DOCUMENTATION AND DATA MANAGEMENT

16.1 Documentation

16.1.1 Case Report Forms (CRF)

Data collected on each subject will be recorded on an eCRF.

16.1.2 Investigator Site File (ISF)

All essential documents will be kept in the Investigator Site File which will be stored at the study site in accordance with ICH GCP chapter 8.

16.2 Data entry und data management

All patient related data will be recorded in the electronic database under a pseudonym. Every patient will receive a pseudonym which will be unique for this individual patient. Original patient files may be viewed by monitors, auditors and inspectors.

16.2.1 Data collection/Case Report Form (CRF)

All data will be recorded in an electronic Case Report Form. The study software used is based on the MARVIN system, Xclinical inc. The CRFs will be filled in by an authorized person (defined in the study team log) as soon as possible. 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.

All patient data will be saved under a pseudonym. The study software will automatically generate a pseudonym for every new patient. A document for clear patient allocation will be printed and will be kept in the ISF.

16.2.2 Source Data and Patient Files

The information in original documents and records (e. g. patient files, laboratory notes) are defined as Source Data and will be reviewed by the Monitor for Source Data Verification. All data that will be recorded directly in the eCRF without prior written or electronic record will be described in the protocol and considered to be Source Data.

16.2.3 Data processing

The biometrical centre 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 centre. Implausible or missing data can be corrected or added after consulting the Investigator. Documentation for these corrections will be stored with the eCRFs.

All validated data will be stored in the database MARVIN. After termination of the study and after completion of all entries, the database will be closed for further entries. This process will be documented.

17 QUALITY MANAGEMENT

17.1 Direct access to source data

According to ICH-GCP and to the European laws (if applicable), the principal investigator must permit all authorized third parties access to the trial site and insight into the medical records of the trial subjects (source data). This permission includes the clinical trial monitors, auditors and other authorised employees of the sponsor, as well as members of the competent authorities. All these persons are sworn to secrecy.

17.2 Monitoring

The national cosponsors are responsible for the organisation of an adequate monitoring process in the respective country. An on-site monitoring in all participating centres and including all patients is planned. In case of frequent protocol violations, incomplete documentation, unanswered queries or other problems, for cause monitoring visits may be performed. The main emphasis of on-site monitoring should lie on the check of the informed consent forms and of the inclusion and exclusion criteria, as well as on the main efficacy and safety endpoints. In addition on-site monitoring visits make sure that the study is performed according to ICHGCP, and that the protocol is adhered to. Thus, on-site monitoring plays an important role in the support and training of participating trial sites.

A detailed monitor plan will be provided within a monitor manual by the sponsor.

The investigators allow the monitor to have access to all of the study materials needed for source data verification and proper review of the study process. All times, the sponsor/investigators/monitors will maintain the confidentiality of the study documents. Furthermore, problems with inconsistent and incomplete data will be discussed. By signing the declaration of informed consent the participants allow access to their documents. With the signature in the protocol, the investigators confirm that auditors and health authority inspectors may have access to the study documentation and accordant medical records. Auditors and inspectors are bound by professional confidentiality and may not pass on any personal information that comes to their knowledge. In the course of audits or inspections, data in the case report forms will be compared with the data for medical record. All the documentation held by the investigators within the scope of the clinical trial, as well as the drug logs of the study medications will be verified.

17.3 Audits / Inspections

Authorized representatives of the Sponsor, a regulatory authority, or an Independent Ethics Committee (IEC) may visit the centre to perform audits or inspections, including source data verification. The purpose of a Sponsor audit or inspections is to systematically and independently examine all the study activities and documents to determine whether these activities were conducted, and data were recorded, analysed, and accurately reported according to the protocol, Good Clinical Practice (GCP), guidelines of the International Conference on Harmonization (ICH), and any applicable regulatory requirements.

According to the pertinent European legislation, inspections of the trial sites may be performed by the competent authorities at any time during or after completion of the trial.

18 REPORTING

18.1 Statistical report

The statistical analysis and composition of a biometrical report will be performed by the statistics and data centre in Oxford (Centre for Statistics in Medicine, Oxford University, Oxford, UK) in cooperation with the Sponsor and the Principal Investigator. All data in this report is confidential.

18.2 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 termination of the biometrical analysis the trial manager, the sponsor 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.

18.3 Publication (policy)

The study results will be published irrespective of the study outcome. The authorships are nominated by the trial steering committee.

19 ETHICAL, LEGAL AND REGULATORY ASPECTS AND AGREEMENTS

19.1 ICH-GCP-guidelines

This trial will be conducted in accordance with the current ICH-GCP-guidelines (CPMP/ICH/135/95, January 1997). Good Clinical Practice (GCP) is an international ethical and scientific quality standard for designing, conducting, recording and reporting trials that involve the participation of human subjects. Compliance with this standard provides public assurance that the rights, safety and well-being of trial subjects are protected, consistent with

the principles that have their origin in the Declaration of Helsinki, and that the clinical trial data are credible.

19.2 Sponsorship

Responsible for the trial is one international sponsor (Charité – Universitätsmedizin Berlin). The sponsor delegates duties and responsibilities to the sponsor representative (Dr. A. v. Stackelberg), and to the national co-sponsors. Responsibilities and duties are agreed on within a contract signed by both sides.

19.3 Legal requirements of the study

- Approval of Ethics Committee
- Approval of competent authority
- Notification to regional authorities
- Informed Consent
- Insurance
- Data privacy and confidentiality

19.3.1 Approval of Independent Ethics Committee (according to AMG § 42 (1) and ICH-GCP guideline §3)

Study protocol, patient information and consent form will be presented to the relevant Ethics Committee (IEC) for survey. The study will only start after ethics approval has been granted. The IEC will immediately be informed (by the Sponsor) of all changes to the protocol (according to GCP-V § 10) and of all events that could affect a patient's safety according to ICH-GCP guideline §3. The IEC will also be informed of all SUSARs (Suspected Unexpected Serious Adverse Reactions) and of regular or premature termination of the study.

The Investigators have to be approved by the IEC (enter proof of qualification) before they enrol any patients.

19.3.2 Approval of competent authority (according to AMG § 42 (2) and ICH-GCP guideline § 5.10)

The trial will be submitted to the relevant federal authorities for approval. The trial in a given country will only start after approval has been granted.

19.3.3 Notification to local authorities (according to AMG §67 and if applicable in a given participating country)

The trial will be submitted to the local authorities. The Sponsor and all Investigators will be reported by full name.

19.3.4 Patient information and informed 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 or his legal guardians will agree to participation in the trial by signing the informed consent form. Patients and their parents must be given an opportunity to enquire about details of the study. After a sufficient period of time for the individual's consideration and decision, comprehension and consent shall be documented on the consent form by the dated signature of the patient and the Investigator/ treating doctor.

The parent(s) or a legally acceptable representative of minors must read, sign, and date a consent form before his or her child enters the study, takes study treatment, or undergoes any study-specific procedures. If the child is able to comprehend the study he will also sign an informed consent form.

An example of the patient information and the patient consent form is attached to this master protocol in the respective national appendices. Design and language will be adjusted to the study site's needs. The final versions of patient information and consent will be presented to the Ethics Committee. Both the patient information and the patient consent form are

prepared in duplicate. One of each form for the Investigator, a duplicate will be handed to the patient.

19.3.5 Patient insurance

Patient insurances are contracted by the national co-sponsors. The details and the policy are attached in the national appendix.

19.3.6 Data Privacy and confidentiality

The participants' data will be saved in a pseudonymous form. All regulative requirements applying to data protection will be met. Re-identification of a participant subject's name is possible from the patient identification log, which is kept in a locked research office at the trial site where access is only possible by the principal investigator or persons authorized by the principal investigator.

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 forwarded to the competent authorities and to the IEC responsible, in accordance with legal notification obligation for drug safety. Patients, who disagree with this process of data transfer, are not allowed to participate in this study.

19.4 Labelling

The investigational medicinal product Epratuzumab will be centrally labelled in accordance to international and national laws and shipped to the participating centres upon request. All other drugs used in the protocol are considered as standard chemotherapy with approval for the indication or with proven/published evidence of being used as standard treatment for the indication since many years. These drugs are implemented as generics and are not specifically labelled for the trial.

19.5 Archiving of data / access to records

Originals of all study-related report forms will be stored in the study headquarters at the trial site for at least 10 years after completion of the trial (according to the respective national laws).

The Investigator/principle Investigator stores all administrative documents (correspondence with the Ethics Committee, the Supervising Authority, trial centre, study site), patient identification log, the signed patient consent forms, copies of the data documentation form and common study documentation (protocol, amendments) for the duration mentioned above. Original data of study patients (medical records) will be stored for at least 10 years. A list allowing patient identification will be kept for 15 years (directive 2001/83/EG).

19.6 Financing

The full financing of the trial is warranted. The national cosponsors are responsible for an adequate financing of the trial in their country. The international coordinative aspects and the national performance of the trials in European participating countries will be financed by the FP7 project IntReALL 2010 the European Commission for the first 5 years. For the follow up period a financing by national funding sources is warranted. The international coordination of the trial in this period will be funded by the Deutsche Kinderkrebsstiftung (DKKS), Bonn. The drug epratuzumab, the labelling, shipment, and drug accountability is financed by the company Immunomedics, Inc.. Furthermore, additional monitoring necessary exclusively for licensing purposes are covered by Immunomedics, Inc.

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21 ABBREVIATIONS

ADCC	Antibody dependent cellular toxicity
AE	Adverse event
ALL	Acute lymphoblastic leukemia
ALL R3	UK R3 protocol for childhood relapsed ALL
ALL-REZ BFM	ALL Relapse BFM
AR	Adverse reaction
ARA-C	cytarabine
BCP	Precursor B-cell
BCR	B-cell receptor
BID	Twice daily
BM	Bone marrow
BSA	Body surface area
CBM	Combined bone marrow (relapse)
CDC	Complement dependent cytotoxicity
CI	Cumulative Incidence
(C)CR	(Continuous) complete remission
CNS	Central nervous system
COG	Childrens oncology group
COPRALL	Cooperative Group for relapsed ALL
CR	Complete remission
CRF	Case report form
CSF	Cerebro spinal fluid
CT	Computer tomography
CTCAE	Common Terminology Criteria for Adverse Events
Dex	Dexamethasone
eCRF	Electronic case report form
DFS	Disease-free survival
D(S)MC	Data (Safety) Monitoring Committee
EEA	European Economic Area
EFS	Event-free survival
EM	Extramedullary
FAB	French-American-British
FISH	Fluorescence in situ hybridisation
GCP	Good clinical practice
GvHD	Graft versus host disease
GvL	Graft versus leukemia
Gy	Gray
HLA	Human leukocyte antigen
HR	High risk
hrs	Hours
I-BFM SG	International BFM Study Group
IBM	Isolated bone marrow (relapse)
ICH	International Conference of Harmonization
IEC	Institutional Ethical Committee
IG	Immunoglobulin
ID	Induction death
IDA	idarubicin
IEC	Independent Ethics Committee
IEM	Isolated extramedullary
IR	Intermediate risk
ISF	Investigator site file

ITCC	Innovative Therapies for Children with Cancer
ITT	Intent to treat population
LFU	Lost to follow up
M0-3	Cytological bone marrow status 0-3
MD	HLA Matched donor
MHD	Medium high dose
MITOX	Mitoxantrone
MLPA	Multiplex ligation – dependent probe amplification
MMD	HLA Mismatched donor
MP	Mercapto purine
MRD	Minimal residual disease
MRI	Magnetic resonance imaging
MTX	Methotrexate
NR	Nonresponse
NSG	NOD-scid gamma
OS	Overall survival
p	Probability
PEG	Polyethylene glycol
PCR	Polymerase chain reaction
PIP	Pediatric investigational plan
PPP	Per protocol population
R1/2	Randomization 1/2
R courses	ALL relapse courses of the BFM group
RCT	Randomized clinical trial
REL	Relapse
S1-4	Strategy group 1-4 (ALL-REZ BFM criteria)
SAE	Serious adverse event
SAR	Serious adverse reaction
SCT	Stem-cell transplantation
SMN	Secondary malignancy
SmPC	Summary of Product Characteristics
SR	Standard risk
SUSAR	Suspected unexpected severe adverse reaction
TBI	Total body irradiation
TCR	T-cell receptor
(T)IT	(T)riple intrathecal therapy
TRD	Treatment related death
TRM	Treatment related mortality
UKALL	United Kingdom ALL trial
VCR	Vincristine
VP16	Etoposide
WBC	White blood cell count

22 APPENDICES