Pediatric Relapsed AML 2009/01

International randomized phase III study on the treatment of children and adolescents with refractory or relapsed acute myeloid leukemia

To be performed within the International BFM Study Group (I-BFM-SG) and the International Pediatric AML Group

International Sponsor:

Hannover Medical School
Hannover
Germany

International Study Coordinators (steering committee):

- **G.J.L. Kaspers (chair, NL and I-BFM-SG)**
- **D. Reinhardt (vice-chair, Germany)**
- **A. Baruchel (France)**
- **C. Rizzari (Italy)**
- **D. Webb (UK and I-BFM-SG)**

International data manager and statistician (also member of the steering committee):

- **M. Zimmermann**
- **AML-BFM Group**
- **Hannover**
- **Germany**

Planned period of study:	**5 years**
Begin of study:	**Q2, 2010**
End of accrual and end of study:	**Q2 2015/end of 2020**
EudraCT number:	
ISRCTN:	
Draft version number:	**7**
Date of draft:	**24.11.2009**

Addresses

Gertjan J.L. Kaspers
Chairman AML Relapse Working Group/I-BFM-SG
DCOG representative
Pediatric Oncology/Hematology
VU University Medical Center
De Boelelaan 1117
NL-1081 HV Amsterdam
The Netherlands.
Phone: +31 (0) 20 444 2420
Fax: +31 (0) 20 444 2422
E-mail: gjl.kaspers@vumc.nl

David Webb
Chairman AML committee/I-BFM-SG
UK-CCLG representative
Pediatric Hematology
Great Ormond Street Hospital
Great Ormond Street
London WC1N 3JH
United Kingdom
Phone: +44 (0) 207 813 8410/8189
Fax: +44 (0) 207 813 8410
E-mail: webbd@gosh.nhs.uk

Dirk Reinhardt
BFM-AML representative
Pediatric Hematology and Oncology
Hannover Medical School
Carl-Neuberg-Str. 1
D-30625 Hannover
Germany
Phone:+49 (0) 511 532 6720
Fax: +49 (0) 511 532 9029
E-mail:
Reinhardt.dirk@mh-hannover.de

Andre Baruchel
FRALLE/LAME representative
Hopital St. Louis
1, Avenue Claude Vellefaux
75475 Paris Cedex 10
France
Phone: +33 (0) 142 49 9721
Fax: +33 (0) 142 49 9823
E-mail:
andre.baruchel@sls.aphp.fr

Carmelo Rizzari
AIEOP representative
Pediatric Oncology/Hematology
Clinica Pediatrica, Ospedale S. Gerardo
Via Donizetti, 106
20052 Monza
Italy
Phone: +39 039 233 3513
Fax: +39 039 230 1646
E-mail:
Carmelo.Rizzari@pediatriamonza.it

Martin Zimmermann (Statistician)
AML-BFM Trial Center
Hannover Medical School,
Children's Hospital
Carl-Neuberg-Str. 1
D-30625 Hannover
Germany
Phone: +49 (0) 511 532 3764
+49 (0) 511 532 9123
Fax: +49 (0) 511 532 9029
E-mail: zimmermann.martin@mh-hannover.de

International Statistician and Central Data Office:

Martin Zimmermann, PhD (Statistician)
Jans-Enno Müller, Ursula Bernsmann (Documentation)
AML-BFM Trial Center
Hannover Medical School,
Children's Hospital
Department of Paediatric Haematology and Oncology
Carl-Neuberg-Str. 1
D-30625 Hannover
Germany
Phone: +49 (0) 511 532 3764
+49 (0) 511 532 9123
Fax: +49 –511 532 9029
E-mail: AML-BFM@MH-Hannover.de
zimmermann.martin@mh-hannover.de

I-BFM-SG/AML Relapse Working Group: Study Pediatric Relapsed AML 2009/01

1. Protocol synopsis

Protocol Title	**Pediatric Relapsed AML 2009/01:** **International randomized phase III study on the treatment of children and adolescents with refractory or relapsed acute myeloid leukemia**
International study coordinators (steering committee, together with the international statistician)	**Gertjan J.L. Kaspers (chair)** **Chairman AML relapse working group/International BFM Study Group** **DCOG representative** **Head and Professor of Pediatric Oncology/Hematology** **VU University Medical Center** **De Boelelaan 1117** **NL-1081 HV Amsterdam, the Netherlands** **Phone: +31 (0) 20 444 2420; fax: +31 (0) 20 444 2422** **E-mail: gjl.kaspers@vumc.nl** **Dirk Reinhardt (vice-chair)** **BFM-AML Group representative** **Professor of Pediatric Hematology/Oncology** **Hannover Medical School** **Carl-Neuberg-Strasse 1** **30625 Hannover, Germany** **Phone: +49 (0) 511 532 6720; fax: +49 (0) 511 532 9120** **E-mail: reinhardt.dirk@mh-hannover.de** **David Webb** **Chairman AML committee/International BFM Study Group** **UK-CCLG representative** **Pediatric Hematology** **Great Ormond Street Hospital** **Great Ormond Street** **London WC1N 3JH, UK** **Phone: +44 (0) 207 813 8410/8189; fax: +44 (0) 207 813 8410** **E-mail: webbd@gosh.nhs.uk** **Andre Baruchel** **FRALLE/LAME representative** **Hopital St. Louis** **1, Avenue Claude Vellefaux** **75475 Paris Cedex 10, France** **Phone: +33 (0) 14 249 9721; fax: +33 (0) 14 249 9823** **E-mail: andre.baruchel@sls.aphp.fr** **Carmelo Rizzari** **AIEOP representative** **Pediatric Oncology/Hematology** **Clinica Pediatrica, Ospedale S. Gerardo** **Via Donizetti, 106** **20052 Monza** **Italy** **Phone: +39 039 233 3513** **Fax: +39 039 230 1646** **E-mail: Carmelo.Rizzari@pediatriamonza.it**

National clinical and data-management representatives, respectively	**To be determined/appointed, potential groups and names provided:** **AIEOP, Italy: C. Rizzari/R. Rondelli** **BFM-A, Austria: M. Dworzak/I. Hirsch** **BFM-G, Germany: U. Creutzig/M. Zimmermann** **Brasil:** **BSPHO (Belgium): B. De Moerloose/** **COG, USA:** **CPH, Czech Republique:** **Croatia:** **DCOG, the Netherlands: G.J.L. Kaspers/K. van der Pal** **GATLA, Argentina:** **Greece:** **Hong Kong:** **Hungary:** **Israel:** **JPLSG, Japan: A. Tawa** **LAME (France): A. Baruchel/** **Moscow:** **Minsk: O. Aleinikova** **NOPHO, Nordic countries: J. Abrahamsson/G. Gustafsson** **PINDA, Chile:** **PPLLSG, Poland: J. Wachowiak/M. Witak** **Serbia:** **Slovakia:** **Slovenia:** **SHOP, Spain:** **Turkey:** **UK CCLG, United Kingdom: David Webb/** **Ukraine:** **Uruguay:**
International Sponsor	**Hannover Medical School, AML-BFM Group** **Hannover, Germany**
International Data-center	**AML-BFM Group** **Hannover, Germany**
International Statistician	**Martin Zimmermann** **Hannover, Germany** **Phone: +49 (0) 511 532 3764** **Fax: +49 (0) 511 532 9029** **E-mail: zimmermann.martin@mh-hannover.de**
Data Safety and Monitoring Committee	**Y. Ravindranath, USA** **P. Vyas, UK** **H. van Houwelingen, NL**
Trial centers	**To be appointed by all participating national/local groups**
Main study objective	**Determine the initial efficacy of gemtuzumab ozogamicin (GO, Mylotarg®) when added to liposomal daunorubicin (DaunoXome®)/fludarabine/cytarabine (DX-FLA) in the first course of reinduction chemotherapy compared to DX-FLA only. Efficacy will be measured by the percentage of patients having not more than 20% blasts in the bone marrow before the second induction course.**

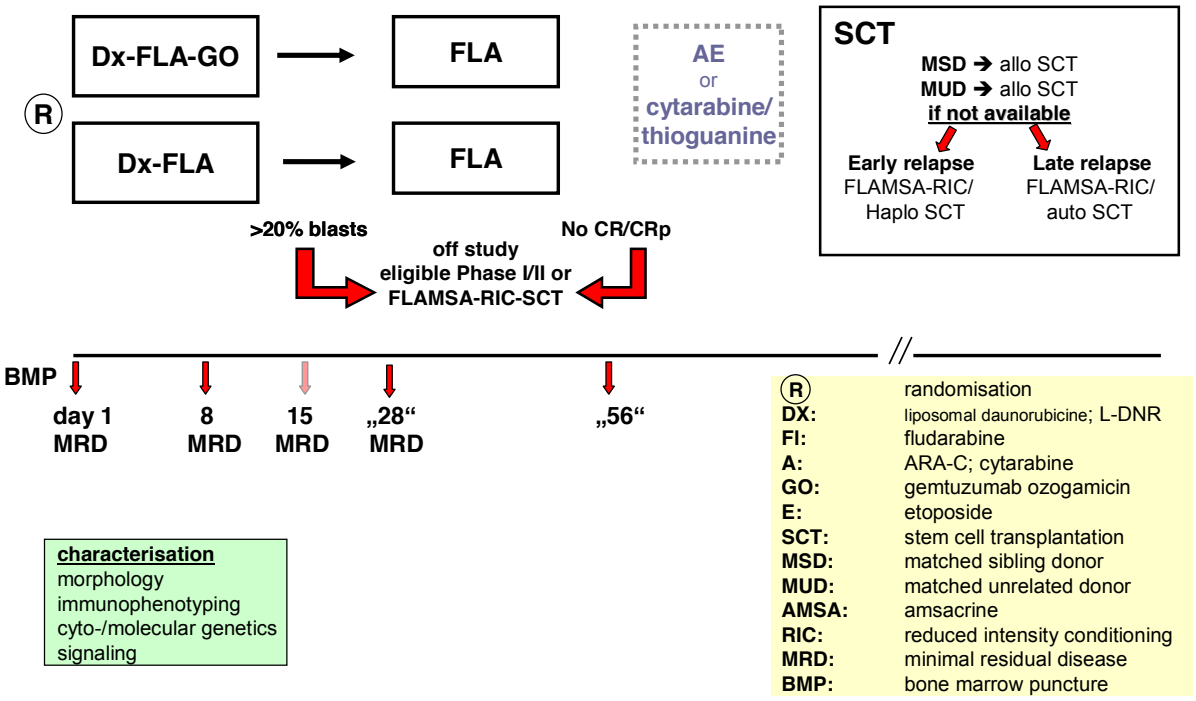
Secondary Objectives	**1. Determine the efficacy of dosing GO (Mylotarg®) at 4.5 mg/m^2/dose in combination chemotherapy in a randomised study** **2. Determine the toxicity of GO (Mylotarg®) when added to DX-FLA in terms of bone marrow aplasia, liver toxicity including VOD and other side effects according to CTC-Criteria when compared to treatment with DX-FLA only.** **3. Determine complete remission rates and long-term outcome in the different study arms.** **4. Identify additional prognostic factors in pediatric relapsed AML, other than early treatment response, cytogenetics and duration of CR1.** **5. Provide individual biological characterisation of leukemia (morphology, cytogenetics, molecular genetics and activated signalling pathways), for future individualized stratification to targeted therapy.**
Additional Objectives	**Add-on research studies (to be finalised separately).**
Study rationale	**Pediatric relapsed AML still has a poor prognosis[1,2]. The probability of survival at 4 years is 36% in the most recent study Relapsed AML 2001/01 (Kaspers 2007a), which is better than reported before, but not good enough. Further improvements of current treatment are thus required. Gemtuzumab ozogamicin (GO, Mylotarg®) consists of a calicheamicin conjugated to the monoclonal CD33 antibody and has proven to be effective in terms of CR achievement and better overall survival in adult AML studies and in pediatric AML relapse and salvage therapy studies with moderate toxicity. Since more than 90% of pediatric AML are CD33 positive, adding GO (Mylotarg®) to the standard regimen offers a great potential as targeted therapy for the majority of patients in our study [3,4]. MRC [5](Burnett et al. abstracts) and COG studies[19] have shown the feasibility of adding GO (Mylotarg®) at 3.0 mg/m² for one dose to either ADE or IDA-FLAG induction chemotherapy in adults and children (MRC) and to mitoxantrone and cytarabine in children (COG) with newly diagnosed AML. Preliminary data from MRC AML15 show that GO (Mylotarg®) at 3.0 mg/m² with combination chemotherapy improves outcome in adult AML, at least in subgroups (Burnett, ASH 2006 and EHA 2007). In addition, several groups have demonstrated the feasibility and efficacy of GO (Mylotarg®) at up to 6 mg/m² with combination chemotherapy (Tsimberidou 2003; Alvarado 2003; Eom 2007; Chevalier 2008). Data on saturation of CD33 in relation to the dose of GO show a dose-dependent effect, and suggest 4.5 to be better than 3.0 mg/m². Although GO-associated liver toxicity is clearly dose-dependent as well, higher toxicity occurs only at doses of 6.0 mg/m² and higher (McKoy 2007). The international phase III study Relapsed AML 2001/01 has proven the feasibility of large intergroup studies in pediatric AML and its results are among the best ever reported [6]. Therefore, the best arm of the current study), which is includes liposomal daunorubicin (DaunoXome®), fludarabine and cytarabine (DX-FLA) will constitute the standard first reinduction course of**

	chemotherapy. Adding DX to FLAG improved the early response rate by 11%, from 69 to 80% (p=0.017). This regimen will be compared with DX-FLA plus GO (Mylotarg®) at 4.5 mg/m^2 as single dose. In case of too much toxicity with GO at 4.5 mg/m^2, the dose will be reduced permanently to 3.0 mg/m^2. In the current study, G-CSF will be omitted because of a recent meta-analysis that showed no beneficial effect of it, also not as priming agent. Moreover, it has disadvantages such as possible lung toxicity and potential increase in WBC with its associated complications. Finally, novel drug combinations also do not include G-CSF, such as studies on Clofarabine/cytarabine and DaunoXome®, and clofarabine and DaunoXome®. **After reinduction chemotherapy, all patients are eligible for allo-SCT. Consolidation guidelines, both intensive and non-intensive, are provided for patients to bridge the time to transplant if required. In view of reports on children surviving relapsed AML while having been treated with chemotherapy or autologous SCT only (Kaspers 2007c, Goemans 2008), the use of very-high risk allo-SCT (such as major mismatched unrelated donor SCT) that will be associated with a high treatment-related mortality should be carefully balanced against the possibility of cure with chemotherapy only in patients in very good quality second CR.** **Study Relapsed AML 2001/01 showed very poor outcome for patients with a poor response to the first course of reinduction chemotherapy, for patients not achieving CR, and for patients with subsequent relapse (Kaspers 2007a). Therefore, these patients will be eligible for phase I/II studies with novel agents. These studies will be performed in the context of this phase III study, but will be run as separate studies with different P.I.'s. This effort will be run and coordinated by Dirk Reinhardt (AML-BFM Group, Hannover, Germany), and once successful will be followed by actual clinical studies of different tyrosine kinase inhibitors in different subgroups on top of the backbone of chemotherapy as described in this study Relapsed AML 2009/01.**
Study Population	**Inclusion criteria** **1.Children and adolescents <18 years of age at start of initial chemotherapy and <21 years of age at start of this relapsed AML treatment** **2. Patients with first relapsed or primary refractory AML** **3. Patients with a second or subsequent relapsed AML that were not previously treated according to this particular protocol** **4.Signed written informed consent from patients or from parents or legal guardians for minor patients, according to local law and regulations** **5. For patients with childbearing potential, a negative test for pregnancy is to be considered before entry on study** **Exclusion criteria** **1. FAB type M3 (please refer to your local group for the appropriate treatment protocol)**

	2. Myeloid Leukemia of Down syndrome (please refer to your local group for treatment alternatives) 3. Symptomatic cardiac dysfunction (CTC grade 3 or 4) and/or a Fractional Shortening at echocardiography below 29% 4. A Karnofsky performance status <40% (children ≥ 16 years) or an Lanksy performance status of <40% (children < 16 years) before start of chemotherapy 5. Any other organ dysfunction (CTC grade 4) that will interfere with the administration of the therapy according to this protocol 6. Impaired liver function defined as > than NCI-CTC grade 1 (max 2.0 x ULN for transaminases and bilirubin) 7. History of veno-occlusive disease (VOD) 8. Hypersensitivity to gemtuzumab ozogamicin 9. Inability to potentially complete the treatment protocol for any other reason
General Study Design	Intergroup, international, multicenter open label comparative and randomised phase III study on the efficacy of GO (Mylotarg®) added to standard reinduction chemotherapy in children and adolescents with refractory and relapsed AML.
Detailed Study Design	The overall design of the study is shown in the flowchart. There will be a randomisation in a 1:1 fashion for the addition or not of GO (Mylotarg®) at 4.5 mg/m^2 to FLA-DX in the first course of reinduction chemotherapy. Patients who do respond poorly to this first course of chemotherapy with >20% BM blasts on "day 28" (before the start of the second course) become eligible for phase I/II studies. All other patients proceed to the second reinduction course. In this course, patients are advised to get FLA. Patients who do not achieve a CR after these two courses become eligible for phase I/II studies. In CR, all patients are eligible for allo-SCT. If more time is needed to perform that SCT, guidelines for intensive and low-intensive consolidation are provided in the protocol. The choice for either the intensive or non-intensive regimen must be based on the anticipated time until SCT and on the condition of the patient.
Primary Endpoint	Bone marrow blasts on "day 28" (before the start of the second reinduction course) given as ≤ 20% or >20%.
Secondary Endpoints	1. overall survival 2. event-free survival 3. Percentage of patients that achieve CR after two courses of reinduction chemotherapy 4. Incidence of treatment related mortality and toxicity according to NCI criteria 5. Actual performance of allo-SCT 6. Percentage of patients successfully characterized for molecular abnormalities that can be targeted by novel agents
Statistical considerations	The randomisation will be done centrally by the BFM-AML group in Hannover, and will be balanced for study group and early and late relapses. A total of 252 (126 in each arm) eligible and fully evaluable patients must have been randomised in order to prove that adding GO (Mylotarg®) to DX-FLA increases the early response rate from 80 to 91%, with a power of 80% and a type 1 error of 5%. The study is planned to accrue patients from Q2,

	2010 onwards for 5 years; if at that time accrual is below the number of patients that is being aimed for, the study will continue if approved by the principal investigators and the DSMC until the planned size of the study has been reached. Interim-analyses on efficacy will be done after 100 and 200 randomised patients have been enrolled.
Toxicity Monitoring	**Frequent toxicity monitoring will be required throughout the trial, and will be evaluated using the National Cancer Institute Common Toxicity Criteria (NCI-CTC version 3.0). All Serious Adverse Events must be reported. The steering committee (international study coordinators) will evaluate toxicity reports on a regular basis and may stop the trial at the advice of the DSMC if an unacceptable rate of severe toxicity is recognized. Dose and treatment modifications due to toxicity are specified in the protocol. Late toxicity will be assessed by long term follow-up. Early toxicity of this treatment will be compared to that of study Relapsed AML 2001/01. Dose-reduction of GO (Mylotarg®) from 4.5 mg/m^2 to 3.0 mg/m^2 will be considered if mortality and/or grade III/IV toxicity exceed pre-set limits at several interim-analyses, after 50, 100 and 200 randomised patients. Basically, mortality before achieving CR should not exceed 10%. Toxicity will be analysed in the perspective of reversibility and the need to postpone treatment, and will focus on grade III/IV cardiotoxicity (which should not exceed 10%) and grade III/IV liver toxicity (which should not exceed 10%, except for raised transaminases).**
Trial Conduct	**The trial will be carried out according to the Declaration of Helsinki (1996), the principles of Good Clinical Practice and the Directive 2001/20/EC of the European Parliament and of the Council of 4 April 2001.**
Study period	**Recruitment Q2, 2010 – Q2, 2015; Study Q2, 2010 – Q2, 2020. The study is planned to accrue patients for 5 years.**

Relapsed AML 2009/01 I-BFM-SG



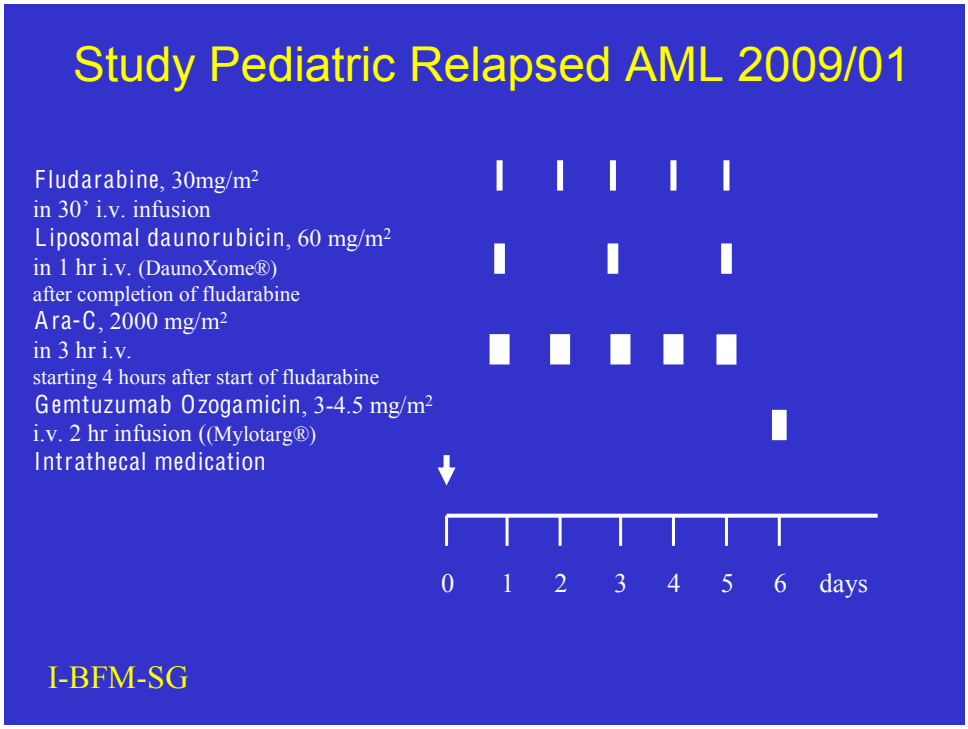
characterisation
morphology
immunophenotyping
cyto-/molecular genetics
signaling

R	randomisation
DX:	liposomal daunorubicine; L-DNR
FI:	fludarabine
A:	ARA-C; cytarabine
GO:	gemtuzumab ozogamicin
E:	etoposide
SCT:	stem cell transplantation
MSD:	matched sibling donor
MUD:	matched unrelated donor
AMSA:	amsacrine
RIC:	reduced intensity conditioning
MRD:	minimal residual disease
BMP:	bone marrow puncture

Relapsed AML2009/01 version4

I-BFM-SG/AML Relapse Working Group: Study Pediatric Relapsed AML 2009/01 Detailed flowchart.

First course of reinduction chemotherapy, experimental arm. The standard arm is exactly the same except no GO (Mylotarg®) will be given. Gemtuzumab ozogamicin (GO) will be dosed at 4.5 mg/m^2/dose with this combination chemotherapy.



I-BFM-SG/AML Relapse Working Group: Study Pediatric Relapsed AML 2009/01
TABLE OF CONTENTS

I-BFM-SG/AML Relapse Working Group: Study Pediatric Relapsed AML 2009/01

International addresses (partly copied from 2001/01 => needs to be checked!!)

Participating Groups:

	Clinical	Data management
AIEOP	**Carmelo Rizzari**	**Roberto Rondelli**
e-mail	**carmelo.rizzari@tiscalinet.it**	**rondelli@aosp.bo.it**
phone	**+39 – 39 233 3513**	**+39 – 51 636 4667**
fax	**+39 – 39 230 1646**	**+39 – 51 34 5759**
BFM-G	**Ursula Creutzig**	**J.E. Müller**
e-mail	**Ucreutzig@aol.com**	**mullerj@uni-muenster.de**
phone	**+49 – 251 835 6486**	**+49 – 251 835 6487**
fax	**+49 – 251 835 6489**	**+49 – 251 835 6489**
BFM-A:	**M. Dworzak**	**I. Hirsch**
e-mail	**michael.dworzak@ccri.at**	
phone	**+43 – 1 4017 0250**	**+43 – 1 4017 0478**
fax	**+43 – 1 4017 070**	**+43 – 1 4017 0430**
BSPHO	**B. de Moerloose**	
e-mail	**barbara.demoerloose@ugent.be**	
phone		
fax		
CPH	**Petr Smisek**	**Alena Vrzalova**
e-mail	**Petr.smisek@lfmotol.cuni.cz**	**alena.vrzalova@lfmotol.cuni.cz**
phone	**+420 – 2 2443 2201/2251/2232**	**+420 – 2 2443 2243**
fax	**+420 – 2 2443 2220**	**+420 – 2 2443 2220**
DCOG	**Gertjan J.L. Kaspers**	**Karin van der Pal**
e-mail	**gjl.kaspers@vumc.nl**	**info@skion.nl**
phone	**+31 – 20 444 2420**	**+31 – 70 367 4545**
fax	**+31 – 20 444 2422**	**+31 – 70 367 0868**
LAME	**Andre Baruchel**	
e-mail	**andre.baruchel@sls.aphp.fr**	
phone	**+33 – 4 7238 5780**	**+33 – 4 7238 5780**
fax	**+33 – 4 7238 5503**	**+33 – 4 7238 5503**
GATLA		**Francisco J. Lastiri**
e-mail		**flastiri@tutopia.com**
phone	**+54 – 221 451 4641**	**+54 – 11 4806 8457**
fax	**+54 – 221 457 5209**	**+54 – 11 4806 8457**
Hong Kong	**S.Y. Ha**	**S.Y. Ha**
e-mail	**syha@hkucc.hku.hk**	**syha@hkucc.hku.hk**
phone	**+852 - 2855 3453**	**+852 - 2855 3453**
fax	**+852 - 3005 7808**	**+852 - 3005 7808**
Hungary	**E. Magyarosy**	**E. Magyarosy**

I-BFM-SG/AML Relapse Working Group: Study Pediatric Relapsed AML 2009/01

e-mail	**tapolcai@sztaki.hu**	**tapolcai@sztaki.hu**
phone	**+36 – 1 459 9108**	**+36 – 1 459 9108**
fax	**+36 – 1 459 9154**	**+36 – 1 459 9154**
Israel	**Batia Stark**	**David Steinberg**
e-mail	**dnaomi@clalit.org.il**	**dms@post.tau.ac.il**
phone	**+972 – 3 925 3669**	**+972 – 3 640 8035/8036**
fax	**+972 – 3 925 3042**	**+972 – 3 640 9357**
Japan	**I. Tawa**	
Minsk	**O. Aleinikova**	
e-mail	**aleinikova2004@mail.ru**	
Moscow	**Alexei Maschan**	**Alexei Maschan**
e-mail	**Maschan@asvt.ru**	**Maschan@asvt.ru**
phone	**+7 – 095 936 9423/935 6656**	**+7 – 095 936 9423/935 6656**
fax	**+7 – 095 935 5510**	**+7 – 095 935 5510**
NOPHO	**J. Abrahamsson**	**Göran Gustafsson**
e-mail	**jonas.abrahamsson@vgregion.se**	**goran.gustafsson@kbh.ki.se**
phone	**+46 31 34 34610/46 707 69 5159 (mob)**	**+46 – 8 5177 2484**
fax	**+46 31 21 5486**	**+46 – 8 5177 3184**
PINDA	**J. Quintana**	**J. Quintana**
e-mail	**jquintan@entelchile.net**	**jquintan@entelchile.net**
phone	**+56 – 2 210 4073**	**+56 – 2 210 4073**
fax	**+56 – 2 210 4075**	**+56 – 2 210 4075**
PPLLSG	**J. Wachowiak**	**Marta Witak**
e-mail	**jacek.wachowiak@plusnet.pl**	**khod@xmail.sk5.am.poznan.pl**
phone	**+48 – 61 849 1446**	**+ 48 - 61 849 14 47**
fax	**+48 – 61 847 4356**	**+ 48 - 61 847 43 56**
SHOP	**Amparo Verdeguer**	**Dainora Jaloveckas**
e-mail	**Verdeguer_amp@gva.es**	**clever@recerca.com**
phone	**+34 - 96 398 7727**	**+34 - 93 458 3800**
fax	**+34 - 96 398 7727**	**+34 - 93 459 0997**
UK-CCLG	**David Webb**	**Rachael Clack**
e-mail	**WEBBD@gosh.nhs.uk**	**rachael.clack@ctsu.ox.ac.uk**
phone	**+44 207 813 8410/8189**	
fax	**+44 207 829 8831**	
UK-Ireland	**Owen Smith**	
e-mail	**osmith@stjames.ie**	
phone	**+35 3 – 1 416 2014/2953**	**+44 – 1865 40 4861**
fax	**+353 – 1 410 3475**	**+44 – 1865 40 4849**

3. Confidentiality Statement /Disclaimer

This document describes an open-label, randomized phase III clinical trial in children and adolescents with relapsed and refractory Acute Myeloid Leukemia. It provides information for entering patients into this trial. This protocol is not intended for use as a guide for the treatment of non-registered patients.

Hospitals with limited experience in AML treatment in children (less than two children with AML per year), should consider transferring the child to a more experienced center.

Responsibility for the diagnosis, administration of protocol treatments and other interventions in study patients lies with the participating clinician. Before entering patients into this protocol, clinicians must ensure that the protocol has received approval from both their ethical committee and national regulatory body.

This protocol has been written with greatest accuracy; however errors cannot be completely excluded. Amendments may be necessary. Amendments will be circulated to known participants in the trial, but institutions entering patients for the first time are advised to contact their appropriate study centers.

4. Patient insurance

In view of the complex international character of the study, there is no central patient insurance for risks related to participating in this study. Each group or center has to make sure that the patients included in this protocol are insured adequately for such risks, which is the responsibility of the clinical investigator in the center where the protocol is used.

Signature:

_____ _____

Principal Investigator **Date**

_____ _____

Sponsor representative **Date**

5. Summary

Relapsed and refractory acute myeloid leukemia (AML) in children is a rare problem, and has a poor prognosis. However, the consequent and intensive treatment of AML relapses according to the protocols AML-BFM REZ-93 and -97 as well as Relapsed AML 2001/01 have lead to improved outcome. Nevertheless, further efforts are needed in face of more than 60% of patients still dying of relapse. Improved knowledge about mechanisms and mutations involved in leukemogenesis and the consecutive development of new agents targeting these mechanisms led to the concept of targeted and tailored therapies which might further improve outcome when added individually to conventional chemotherapy. Data on the use of these specific substances in pediatric AML are still lacking. Gemtuzumab ozogamicin (GO, Mylotarg®) which links the cytotoxic calicheamicin to a conjugated monoclonal CD33 antibody has proven effective in terms of CR achievement and better overall survival in (subgroups of) adult AML patients and in pediatric AML relapse in a salvage study, with moderate toxicity. Since more than 90% of pediatric AML are CD33 positive, adding GO (Mylotarg®) to the standard regimen offers a great potential as targeted therapy for the majority of patients in our study.

Reinduction treatment will be done with 2 courses of combination chemotherapy, with DX-FLA (DaunoXome®, fludarabine, and ara-C) in the first course and FLA in the second course as standard treatment. In the first course there will be a randomisation for gemtuzumab ozogamicin (Mylotarg®) to be added or not. The second course should always be FLA. Mylotarg® will be dosed at 4.5 mg/m^2. In case of too much toxicity (as compared to the experience with study Relapsed AML 2001/01) with the 4.5 mg/m^2, the dose will be reduced to 3.0 mg/m^2 permanently. If patients have >20% of blasts in the bone marrow after the 1st course, or if they are not in complete remission (CR) after the 2nd course, they will go off protocol and will be eligible for phase I/II studies. Patients in CR after reinduction treatment can immediately proceed to stem cell transplantation. Consolidation chemotherapy should be given if SCT is delayed. A 3rd course of intensive consolidation chemotherapy (VP16 and continuous infusion with cytarabine) is only recommended if a SCT is not available immediately. In selected patients, a low intensity consolidation- as described in this protocol- may be preferred. This protocol provides guidelines only the type of SCT and the type of conditioning and graft-versus-host disease. In view of reports on children surviving relapsed AML while having been treated with chemotherapy or autologous SCT only (Kaspers 2007c), the use of very-high risk allo-SCT (such as major mismatched unrelated donor SCT) that will be associated with a high treatment-related mortality should be carefully balanced against the possibility of cure with chemotherapy only in patients in very good quality second CR.

Study Relapsed AML 2001/01 showed very poor outcome for patients with a poor response to the first course of reinduction chemotherapy, for patients not achieving CR, and for patients with subsequent relapse (Kaspers 2007a). Therefore, these patients will be eligible for phase I/II studies with novel agents. These studies will be performed in the context of this phase III study, but will be run as separate studies and chaired by different principal investigators. One such study will be on the combination of clofarabine with ara-C and liposomal daunorubicin (DaunoXome®, DX). Not only will these novel drug combinations potentially benefit the patients in whom they are being studied, but promising combinations will also be implemented in future treatment protocols for relapsed AML (and potentially in protocols for newly diagnosed AML). Phase I/II studies will also be done with tyrosine kinase inhibitors (TKI's, see below). TKI's in combination with chemotherapy in acute leukemias have promising efficacy, best illustrated by the improved CR rates with imatinib (Glivec®) added to chemotherapy in Philadelphia-positive acute lymphoblastic leukemia (Yanada et al.,

2006). Therefore, it is the aim to implement TKI's in the treatment of subgroups of pediatric relapsed AML patients, such as patients with activating mutations in Flt3 or c-kit. Immediate implementation of these new agents is not possible for two reasons. First, pediatric phase I/II studies still need to be done in Europe with most TKI candidate drugs. Second, TKI's are only useful in subgroups of patients with the appropriate drug targets, ie, patients with activating mutations in receptor tyrosine kinases in their AML cells. Thus, reliable and fast identification of these molecular abnormalities is required at relapse. Information on molecular characterisation at initial diagnosis is not reliable enough, because instability of these mutations has been reported, with both losses and gains at relapse (Cloos 2006, Bachas ASH 2008). Therefore, in addition to phase I/II studies with TKI's, molecular characterisation of relapsed AML cases must be established in all participating groups. A complete characterization of the individual AML features of a patient, including morphology, immunophenotype, molecular- and cytogenetics, treatment response and the identification of specific activated signalling pathways are crucial to improve our understanding of leukemogenesis and are required to direct subsequent therapeutic decisions on the use of innovative small molecules individually. Hence, we aim to establish a diagnostic workup providing a detailed individual biological characterization within 7 days from diagnosis thus enabling the future addition of specific inhibitors on an individual basis. Some of these features will additionally serve as markers in the monitoring of minimal residual disease (MRD). This effort will be run and coordinated by the AML-BFM Group (Hannover, Germany), and once successful will be followed by actual clinical studies of different TKI's in different subgroups on top of the backbone of chemotherapy as described in this study Relapsed AML 2009/01.

Main objective of the study is to determine the early efficacy of GO (Mylotarg®) when added to DX-FLA in children with relapsed and refractory AML. In addition, the study will prospectively determine the long-term clinical outcome of these patients stratified according to the different risk groups (refractory disease, early relapse, late relapse, multiple relapses) and the previous treatment protocol. Other secondary objectives are to determine the toxicity and long-term efficacy of dosing GO (Mylotarg®) at 4.5 mg/m^2/dose in combination chemotherapy in terms of toxicity, the identification of novel prognostic factors, and to characterise relapsed AML to enable future more leukemia-specific therapy. The study expects to accrue up to 85 patients annually, and will run about 5 years. This will allow the accrual of 252 eligible, randomised and fully evaluable patients, assuming a randomisation rate of 75% and the primary endpoint being available in 80% of randomised patients.

6. List of Abbreviations

A	Cytarabine, cytosine arabinoside, ARA-C	GO	Gemtuzumab ozogamicin
AE	adverse reaction	ha	Intermediate-dose cytarabine
ALL	acute lymphoblastic leukemia	HA	high-dose cytarabine
AML	acute myeloid leukemia	HD	high-dose
BM	bone marrow	I	idarubicin
BMP	Bone marrow puncture	ITH	intrathecally
CA	competent authorities	IV	intravenously
CNS	Central nervous system	LP	lumbar puncture
CR	complete remission	LV	left ventricle
CRp	complete remission with incomplete neutrophil or platelet recovery	LVET	left ventricle ejection time
CRP	C-reactive protein	M	mitoxantrone
CSF	cerebral spinal fluid	MDS	myelodysplastic syndrome
CT	Computer tomography	MRD	minimal residual disease
DFS	Disease free survival	MSD	matched sibling donor
DX	DaunoXome®	MUD	matched unrelated donor
DX-FLAG	DaunoXome®, Fludarabine, cytarabine and G-CSF	NR	non-response
E	Etoposide-phosphate	OS	overall survival
EC	ethical committee	p	probability, significance
ECHO	ECHO-cardiography	pEFS	probability of event-free survival
EFS	event-free survival	PO	orally
FAB	French-American-British classification	PR	partial response
FAB M0	acute myeloid leukemia with minimal differentiation	SAE	serious adverse event
FAB M1	acute myeloid leukemia without maturation	SC	subcutaneously
FAB M2	acute myeloid leukemia with maturation	SCT	stem cell transplantation
FAB M3	acute promyelocytic leukemia (APL)	SE	standard error
FAB M4	acute myelomonoblastic leukemia	SF	see FS
FAB M5	acute monoblastic leukemia	SUSAR	Suspected unexpected adverse reaction
FAB M6	acute erythroblastic leukemia	TRM	Treatment related mortality
FAB M7	acute megakaryoblastic leukemia	UNL	upper normal level
FLAG	Fludarabine, cytarabine and G-CSF	VP-16	etoposide
FS	fractional shortening	WBC	White Blood Count
GCP	Good clinical practice	µl	microlitre

7. Background and Introduction

Newly diagnosed acute myeloid leukemia (AML) is a rare disease in children. The prognosis has improved considerably using intensive chemotherapy with or without stem cell transplantation. However, 5-10% of patients do not achieve first complete remission (CR) due to resistant disease (primary refractory AML), and 30-50% of CR patients relapse. Primary refractory AML and relapsed AML have a poor prognosis with a long-term overall survival of less than 35%. For first relapse disease, the prognosis mainly depends on the time of relapse, cytogenetics, and the early response to reinduction therapy. For early relapses, defined as within 1 year from initial diagnosis, the second CR rate is about 50%, and overall survival about 20%. For late relapses, defined as after 1 year from diagnosis, the second CR rate is 70-75% and the overall survival up to 50%. Multiple relapsed AML has an even worse prognosis. Several treatment schedules have been studied recently, and improvement seems feasible using new drugs and new drug combinations. The currently available alternatives are described below.

7.1 Gemtuzumab ozogamicin

7.1.1 Pharmacology and pharmacodynamics

Gemtuzumab ozogamicin (GO, Mylotarg®) is a conjugated monoclonal CD33 antibody that selectively targets CD33 positive cells. CD33 is a glycoprotein belonging to the sialoadhesin family und is expressed on the surface of 90% of AML blasts as well as on myeloid precursors, and in lesser frequency on granulocytes and monocytes but not on CD34-positive normal hematopoietic stem cells, lymphocytes and non-hematopoietic tissues. Gemtuzumab ozogamicin is composed of a humanized IgG4 class monoclonal antibody linked to a derivate of the antineoplastic antibiotic calicheamicin[7]. Calicheamicin is an antineoplastic antibiotic which consists of two parts: an aryltetrasaccharid part responsible for the ligation with DNA and an enediyne part causing DNA ruptures. In the pharmacologic preparation of GO (Mylotarg®), 50% of the antibody is conjugated and 50% is unconjugated. GO (Mylotarg®) attaches via its antibody part to the CD33 molecule and is then internalized in the cytoplasma where the two components are hydrolized. Free calicheamicin enters the nucleus and intercalates with DNA producing double strand brakes and induced apoptosis[8].

7.1.2 Pharmacokinetics

Gemtuzumab ozogamicin binds with high specificity to myeloid blasts and monocytes with maximal binding being related to the level of CD33 expression[9]. As CD33 is downregulated with maturation of the myeloid lineage, binding to granulocytes has beens shown to be less, and virtually no binding to CD33-negative lymphocytes has been observed. As the antibody represents the targeting portion of GO (Mylotarg®) measurements of its pharmacokinetics are considered the primary surrogate of the overall pharmacokinetic of the drug[10]. The highest antibody concentrations were seen shortly after the end of infusion. CD33 saturation is dose- dependent with a near-complete saturation of CD33 antigenetic site 0.5-6 hours after infusion at dose levels at and above 4 mg/m^2, while 3 mg/m^2 has not been studied in that respect.

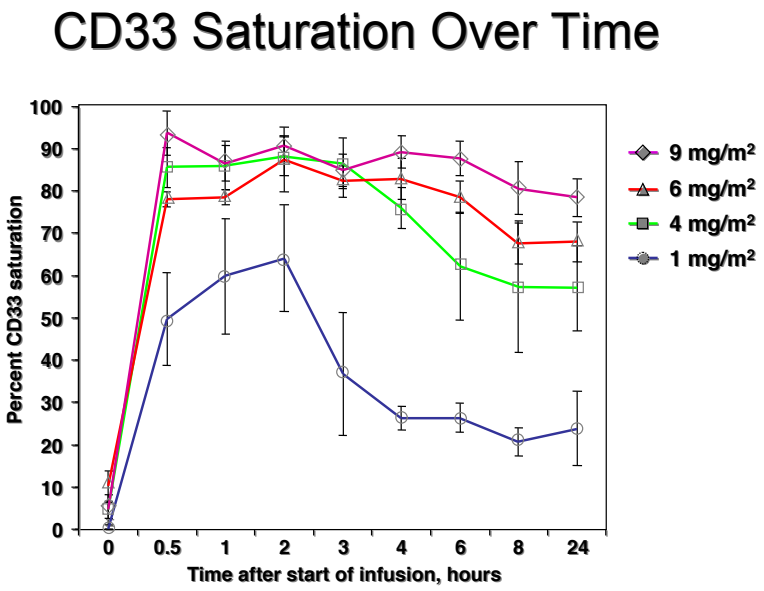


Figure. Wyeth personal communication (data on file)

Elimination half-life varied between mean 43.1 h at a 6 mg/m² dose level versus 63.6 h at a 9 mg/m² dose level for the first treatment course and 49.4 h versus 57.8 h respectively for the second treatment course[11]. With two-week dose intervals between GO (Mylotarg®) administrations, little accumulation of the antibody was observed with only 1% of the maximum concentration being found just before the next application. Antibody clearance did not significantly differ related to age or body weight.

Table 1

Dose (mg/m²)	Period	N	C_{max}(mg/l)	$t_{1/2}$	AUC (mg x h/l)	CL (l/h)
6	1	14	1,70 ± 22,7	43,1±22,7	48,8± 47,6	0,61±0,94
6	2	14	1,94 ± 1,09	49,4 ± 25,6	79,7 ± 66,2 *	0,32±0,49
7,5	1	2	3,11	40	112	0,12
7,5	2	1	3	33,4	153	0,09
9	1	14	3,47 ± 1,04	63,7 ± 44,3	136 ± 107	0,16 ± 0,23
9	2	9	4,68 ± 2,18	57,8 ± 33,4	241 ± 157 *	0,21 ± 0,45

Buckwalter et al: Summary of hP67.7 Pharmakokinetic parameters by dose group for dose periods 1 and 2
*** significant differences between periods 1 and 2**

For both age groups- pediatric and adult patients- an increase of maximum concentration levels, elimination half-life and AUC have been described between the first and second treatment course. These differences do not seem to reflect simple reduction of tumor burden. Decreased blast uptake, CD33 downregulation, increased plasma protein binding or resistance to GO (Mylotarg®) induced apoptosis have been discussed as mechanisms of drug resistance.
The concentration x time profiles of total calicheamicin showed similar shape to the profiles of the antibody. The intersubject variability within dose periods was large for most parameters.
Levels of potentially toxic unconjugated calicheamicin were low and only measurable shortly after the end of infusion. Pharmacokinetic parameters were consistent for all pediatric age groups.

The main route of drug elimination is hepatobiliary. Biodistribution studies with radiolabeled CD33 antibody revealed specific bone marrow uptake and distribution of antibody only in organs with large blood pool such as spleen and liver[12]. GO (Mylotarg®) does not penetrate the blood-brain barrier[13].

7.1.3 Clinical studies

In vitro and *in vivo* studies that demonstrated the ability of GO (Mylotarg®) to inhibit the proliferation of CD33-positive AML cell lines as well as in transplanted human AML blasts in a xenograft mouse models led to a first dose-escalating clinical phase I study[14]. Forty patients with relapsed or refractory AML were enrolled. 18 of 40 patients (45%) had previously undergone autologous or allogenic bone marrow transplantation. Patients were required to have good clinical performance (Karnofsky Index > 60%), good liver and kidney function and a WBC ≤30.000/µl. Three to eight patients were treated at each of eight dose levels of GO (Mylotarg®) ranging from 0.25 to 9 mg/m² i.v. at 14 days intervals. Infusion related toxicity with fever, chills and hypotension was common and occured 2-4 hours after infusion. Moreover, transient elevation of liver enzymes was observed in 20% of patients. Hematologic toxicity including prolonged neutropenia and thrombocytopenia were mainly seen at the 9mg/m² dose level. Application of more than two doses resulted in prolonged myelosuppression in two patients, and one patient died of sepsis in neutropenia within 30 days from application. CR rate was 7.5 %, and 12.5 % showed remission without full platelet recovery (CRp). According to the toxicity profile the maximal tolerated dose (MTD) was defined 9mg/m². CD33 saturation data further supported this choice.

Sievers and Larson[15-17] sequentially reported the results of three open label multicenter phase II studies including a total of 277 adult patients treated with GO (Mylotarg®) monotherapy in first relapse of AML. GO (Mylotarg®) was administered as 2hour infusion with two doses of 9mg/m² at 14 days intervals. 13% of patients achieved CR and 13% CRp. Remission duration was slightly higher in the CR group (median 6.4 months) than in the CRp group (median 4.5 months). A significant difference in remission duration was observed between patients younger than 60 years of age when compared to patients older than 60 years of age. This was possibly affected by postremission therapy options, especially hematopoetic stem cell transplantation. Achievement of remission was the most significant factor for patients at age >60 years who were not eligible for transplantation. At this age group, patients who achieved CR and did not get any further treatment displayed significantly longer overall survival (OS) with a median survival of 12.8 months than non-responders with a median of 2.5 months. Based on these results, GO (Mylotarg®) was approved by the FDA in May 2000 for the treatment of patients with AML relapse or refractory AML at >60 years of age who are not candidates for conventional chemotherapy.

In 2003 Zwaan et al[18] published first experiences with GO (Mylotarg®) monotherapy in children with relapsed and refractory AML. 15 children had been treated at dose levels between 4 and 9mg/m² with up to three courses of GO (Mylotarg®). The overall response rate including CR and CRp was 53%. Main toxicities were grade 3-4 myelotoxicity (15/15 pts), infusion related toxicity with 2 febrile reactions and one case of hypotension, and liver toxicity with one case of grade 3 hyperbilirubinemia without signs of VOD and another patient who developed VOD after a prior HSCT in first CR. Aside from liver toxicity, non-hematologic toxicity was considerably mild, no mucositis or severe infections were reported. Overall survival was 13% following subsequent HSCT (2/15pts).

A larger cohort of pediatric patients was reported by Arceci et al[22]. 29 children with refractory and relapsed AML were entered in an open-label dose escalation study and received 2 courses of GO (Mylotarg®) at 14 days intervals. Dose levels were 6 and 9 mg/m², respectively. 28% of patients achieved overall remission. Mean multidrug

resistance-protein-mediated efflux was significantly lower in leukemic blasts of patients who achieved CR. Elevation of liver enzymes (21%), grade 3-4 hyperbilrubinemia (7%) and VOD (3%) were the main non-hematologic toxicities reported and defined the dose limiting toxicity. The maximal tolerated dose (MTD) was determined to be 6 mg/m². Thirteen patients proceeded to HSCT < 3.5 months after the last GO (Mylotarg®) dose; 6 of which (40%) developed VOD subsequently.
Due to its potential to induce valuable remission rates in a selected patient cohort with extremely poor prognosis when treated in single agent studies, and because of its moderate toxicity profile apart from liver toxicity and prolonged myelosuppression, GO (Mylotarg®) is increasingly implemented in combination regimens. The results of these trials are heterogeneous.
In a feasibility study, Kell at al[20]. combined GO (Mylotarg®) at a 3 mg/m² dose level with different intensive chemotherapy regimens as first line treatment in 72 adolescent and adult AML patients. Chemotherapy consisted of either GO (Mylotarg®) with fludarabine, cytarabine and idarubicine, or GO (Mylotarg®) with daunorubicine, cytarabine and thioguanine, respectively. CR rates obtained with these regimens were 86% and the median overall survival was 78% at 8 months. However, incidence of VOD was 10.9 %, and combining GO at 6 mg/m^2 with conventional chemotherapy was not feasible, although this was significantly influenced by the use of thioguanine. Combining GO with conventional chemotherapy for two consecutive courses also was not feasible. In a subsequent study Burnett et al. compared conventional chemotherapy with the same schedules plus a single dose of Mylotarg® at 3 mg/m^2 in a randomised study in adult and pediatric AML. Preliminary data presented at international meetings suggest both improved disease-free and overall survival, at least in subgroups of patients, with GO added to chemotherapy (Burnett et al., ASH 2006 and EHA 2007). Similarly, COG very recently reported that Mylotarg® at 3.0 mg/m^2 could be combined safely with ADE, and with mitoxantrone and cytarabine (Aplenc 2008), while the group from Baruchel reported that Mylotarg® could be combined safely with cytarabine, when given at days 1, 4 and 7 at 3.0 mg/m^2 for a cumulative dose of 9.0 mg/m2 per course (Brethon 2008). Another study comparing the addition of low-dose GO (Mylotarg®, MY) to fludarabine, cytarabine and idarubicin followed by G-CSF (MY-FLAI) with historical controls (FLAI) revealed comparable CR rates but a lower relapse frequency at two years after addition of MY at 3.0 mg/m^2 (40% vs. 80%) compared to FLAI alone in *de novo* AML[21]. Patients with secondary AML had comparable outcome with or without GO (MY). The MY-FLAI regimen was well tolerated. Hematological toxicity was comparable to that of FLAI. Non-hematological toxicity was mild with no case of grade 3-4 liver toxicity but 4 patients experienced transient liver enzyme elevation. In addition, several groups have demonstrated the feasibility and efficacy of GO (Mylotarg®) at even 6 mg/m² or higher with combination chemotherapy (Tsimberidou 2003; Alvarado 2003; Eom 2007; Chevalier 2008). Chevalier et al. combined GO at 9.0 mg/m^2 (day 4) with Ara-C at 1 gr/m^2 twice daily for 5 days and mitoxantrone at 12 mg/m^2/day for 3 days, in adults with refractory or relapsed AML. The rate of CR/CRp was 63% and the regime was tolerable. Eom et al. reported that GO at 6.0 mg/m^2 could be safely combined with idarubicin at 12 mg/m^2/dose for 3 days plus ara-C at 300 mg/m^2/day for 5 days, and resulted in a CR/CRp rate of 78% in a series of adults of ≥55 years of age with de novo AML. Alvarado et al. studied GO at 6.0 mg/m^2 on days 1 and 15 together with idarubicin at 12 mg/m^2/dose for 3 consecutive days and Ara-C at 1.5 mg/m^2/day for 4 consecutive days, in adults with refractory or relapsed AML. The CR/CRp rate was 42% and the regime was tolerable, although liver toxicity did occur, with veno-occlusive disease in 14% of patients. Finally, Tsimberidou et al. combined GO at 6.0 mg/m^2 on day 1 with fludarabine and Ara-C at 15 mg/m^2 and 0.5 mg/m^2 respectively on days 2-6, plus cyclosporine A, in adults with de novo AML. The CR/CR rate was 48% and the regime was feasible, although the incidence of veno-occlusive disease was 7%. From these

studies it can be concluded that a single administration of GO at a dose of up to 6.0 mg/m^2 can be combined with intensive chemotherapy in adults with AML, although liver toxicity still seems significant at the latter dose level. Therefore, this treatment protocol will apply GO at 4.5 mg/m^2 for one dose.
In conclusion, there is considerable evidence that Gemtuzumab ozogamicin is an effective drug in the treatment of AML in children and adults. GO (Mylotarg®) induces reproducible CR/CRp rates in up to 30% of patients with second relapsed and refractory AML when administered in single agent studies. Combination studies have shown even better results with moderate toxicity profiles. However, the risk of developing VOD is increased when GO (Mylotarg®) is given prior to or less then 3-4 months after stem cell transplantation, indicating that GO (Mylotarg®) treatment should be weighted against the potential risk of VOD in patients with history of SCT, VOD and pre-existing impairment of liver function. However, this side-effect seems dose-dependent, since at doses of less than 6 mg/m² Mylotarg®, as single agent or in combination chemotherapy, gives a risk of VOD or sinusoidal obstructive syndrome (SOS) of only 3% (McKoy et al., 2007). Therefore, this treatment protocol will apply GO at a single dose of 4.5 mg/m^2.

7.1.4 Adverse effects

The most common side effects of GO (Mylotarg®) treatment concern neutropenia and thrombocytopenia due to the fact that CD33 is expressed not only on AML blasts but also in high frequency on myeloid precursors and, to a lesser extend, on granulocytes, monocytes and also on a small minority of megakaryocytes.
Infusion related symptoms occurred in a significant number of patients 2-6 hours after infusion and consisted of fever, chills, hypotension and dyspnoea and disappeared upon treatment with acetaminophen and occasionally steroids. Infusion related syndromes have previously been described after infusion of monoclonal antibodies (e.g. CD20 antibody Rituximab®) and tend to be more common after first infusion. The postmarketing surveillance survey [22] of GO (Mylotarg®) revealed 9 cases of severe hypersensitivity resulting in four deaths.
Tumorlysis syndrome and adult respiratory distress syndrome (ARDS) have been reported in patients with leukocytes > 30 000/µl treated with GO (Mylotarg®). Therefore reduction of WBC below 30.000/µl prior to the start of GO (Mylotarg®) treatment is recommended.
Hepatic toxicity with elevation of liver enzymes and bilirubin is encountered in up to 30% of patients after GO (Mylotarg®) treatment and is reversible in most cases, and is dose-dependent. Frequency of severe liver toxicity and veno-occlusive-disease (VOD) is estimated to occur in 4-5% of patients, with higher frequencies in patients after stem cell transplantation [23,24]. At doses beow 6 mg/m2, GO either as single agent or in combination chemotherapy, was associated with VOD/SOS in 3% of patients (McKoy 2007). Therefore patients treated with GO (Mylotarg®) should be monitored for clinical and laboratory signs of VOD closely even if they have not been treated with SCT.

7.2 FLA(G)

Since the favourable effect of combining fludarabine and cytarabine had first been described by Gandhi et al. (1993) and the sensitizing effect of G-CSF when added to this regimen had been reported FLAG has been routinely used in the treatment of AML. Recent data on the effect of G-CSF on the in vitro cytotoxicity of FLA (Hubeek Int.J.Oncology 2004) revealed a higher leukemic cell survival upon administration of G-CSF alone whereas combination treatment with FLAG led to additive cell killing. These findings supported the clinical use of this regimen. Numerous clinical studies in relapsed and refractory AML in children and adults including own data (Fleischhack, Reinhardt) outlined the efficacy of FLAG in achievement of second remission. Since

I-BFM-SG/AML Relapse Working Group: Study Pediatric Relapsed AML 2009/01

2001 FLAG has been implemented in the current international BFM treatment protocol for refractory and relapsed AML in childhood Relapsed AML 2001/01. G-CSF in this setting is being used as priming agent. However, recent studies raise doubt about the beneficial role of G-CSF and a recent meta-analysis of randomised trials did not reveal a favourable effect of adding G-CSF to chemotherapy in AML (Sung et al., Leukemia 2009). In addition, although with G-CSF used between chemotherapy courses and not as priming agent, a recent analysis of the pediatric study BFM-AML 98 identified an increased relapse risk in the subgroup of high-risk patients associated with the use of G-CSF which could be explained at least in part by G-CSF receptor variants (Reinhardt & Creutzig 2009). Finally, G-CSF as priming agent is started 1 day before chemotherapy and disadvantages are the possible increase in white blood cells with an increased risk of sludging and also the risk of unexplained lung toxicity as has been described in several patients in study Relapsed AML 2001/01 (unpublished data). Thus, like in several phase I/II studies, is has been decided to omit G-CSF as priming agent and also to discourage its routine use to decrease the duration of neutropenia.

7.3 FLA(G) + DaunoXome® (DX-FLA(G))

Apart from cytarabine and its derivates the anthracyclines play a key role in the treatment of AML. First-line induction regimen in AML includes high cumulative doses of anthracyclines. Therefore further use of conventional anthracyclin preparation in reinduction therapy is limited by the risk of cardiotoxicity. Liposomal daunorubicin (DaunoXome®, DX) has proven effective in terms of CR in a pilot study (Fleischhack et al. , Reinhardt et al.) Toxicity was tolerable and similar as with IDA-FLAG, but treatment in experienced centers was recommended because of high risk of infections during prolonged neutropenia. No clinical cardiotoxicity was reported. Based on this favorable data, the impact of adding DaunoXome® to FLAG in reinduction therapy has been subject of an open label randomized trial of the international BFM Study Group (relapsed AML 2001/01). This trial showed comparable grade 3-4 toxicity for DX-FLAG followed by FLAG as with two courses of FLAG. Adding DX to FLAG improved the early response rate by 11%, from 69 to 80% (p=0.017). In addition, CR rate was 8% higher with DX-FLAG, 68% versus 60% (p=0.10). Overall survival was not different between both arms, suggesting that additional measures are necessary to maintain the higher response rates (Kaspers 2007, personal communication). However, it should be stressed that study Relapsed AML 2001/01 did not intent and was not powered to demonstrate any beneficial effect of DX on long-term outcome, and treatment after the first course of reinduction chemotherapy was uncontrolled. The general conclusion from study Relapsed AML 2001/01 has been that adding DX to cytarabine-based treatment improves early treatment response, which is an important first step towards successful therapy.

7.4 Other reinduction chemotherapy regimens

Based on the known antileukemic efficacy of cytarabine and FLAG various studies have been performed with cytarabine derivatives such as Cladribine® (2-chloro-deoxyadenosine, 2-CDA) or clofarabine. Combination of 2-CDA with cytarabine and G-CSF (CLAG) showed a response rate of 50% among 20 patients with relapsed or refractory AML (Robak, Leuk. Lymphoma 2000) with a median CR duration of 22,5 weeks. Combination of mitoxantrone with FLAG as salvage therapy (Hänel Onkologie 2001) in 29 adult patients with relapsed and refractory AML resulted in a CR rate of 59%, a median EFS of 3,2 months and an median overall survival of 6,8 months. A multidrug reinduction protocol including topotecan, vinorelbine, thiotepa, dexamethason and gemcitabine has been developed by Kolb et al.. The study cohort comprised a total of 28 patients of whom fourteen had precursor B-cell ALL and nine had AML. Overall response rate with this regimen was 36% (55% for AML patients).

One patient died in remission because of infectious complication. Grade 4 neutropenia was seen in all patients and transient grade 2 liver toxicity was reported in 10 patients (36%). Studies with GO (Mylotarg®) were summarized above, and are very rare in pediatric relapsed AML. As single agent, it does not achieve subsequent complete remissions to such an extend as FLA(G)-DX does.
Clinical results on the use of small molecules and specific inhibitors such as the inhibitor of proteasome and NF-kappaB bortezumib in children have not been published yet.
These response rates, although difficult to compare, are not as good as with DX-FLA(G).

7.5 CNS prophylaxis and treatment

Overt CNS leukemia is relatively rare in AML. This protocol applies high-dose chemotherapy, with an anticipated CNS prophylactic effect as well. This is also true for the conditioning regimen (see later), that all children are supposed to get. There is virtually no literature available on CNS prophylaxis and treatment in this patient-group to further refine or substantiate the recommendations given in this protocol, which were however extensively discussed and agreed upon.

7.6 Consolidation chemotherapy

To consolidate an achieved CR, and to have time to prepare a transplantation if not available immediately, further chemotherapy is indicated. However, whether this is really necessary and if so, which drugs should be used, is essentially unknown. Yet, in these high-risk patients, further intensive treatment seems justified. Therefore, a schedule of VP16 and cytarabine given by continuous infusion has emerged. In case of a delayed SCT and the need to avoid further potentially severe side-effects, a low-intensive combination of orally thioguanine and cytarabine subcutaneously is included as well. Extensive experience with this regimen has been obtained within the AML-BFM protocols.

7.7 Stem cell transplantation (SCT)

It is generally assumed that allogeneic MSD SCT has a beneficial effect on the prognosis in AML (Woods 2001). However, major morbidity and mortality is associated with it. Therefore, allogeneic SCT should be considered in the context of the prognosis with chemotherapy only. In patients with primary refractory and relapsed AML, this prognosis is poor. Moreover, few long-term survivors have been described after chemotherapy only for this particular disease, in contrast to chemotherapy followed by SCT. Although not proven in properly designed randomized clinical trials, allogeneic SCT may have a bigger impact on the prognosis than autologous SCT, because: 1) a graft-versus-leukemia effect may be present in the allogeneic, but not in the autologous setting, and 2) the autologous transplant is likely to contain minimal residual leukemia cells, which may cause a relapse. On the other hand, allogeneic SCT may be complicated by graft-versus-host-disease. We do recommend allogeneic SCT for all patients who achieve CR, preferably using a matched sibling donor (MSD), or if not available, a matched unrelated (MUD) donor. Patients at higher risk of relapse, i.e. primary refractory disease, early relapse, and ≥2nd relapse, might be eligible for more experimental haplo-identical donor (HID) or mismatched unrelated donor (MMUD) SCT if both a MSD and a MUD are lacking. For patients with a late relapse such treatment seems too experimental in view of their better prognosis, and autologous SCT may be considered instead. There are several conditioning regimens, including a combination of busulfan, cyclophosphamide and melphalan. Experiences using this regimen in children with myelodysplastic syndrome were good (Locatelli 1994). Depending on the type of donor, additional immunosuppression may be required in conditioning.

Patients who were not irradiated previously and who will have a SCT for the 1st time may receive TBI/cyclophosphamide (also depending on age) as conditioning instead of busulfan, cyclophosphamide and melphalan. Detailed guidelines on conditioning and SCT are beyond the purpose of this treatment protocol. However, patients will be monitored for toxicity and events, also after SCT.

7.8 Salvage treatment

Patients who will not respond sufficiently to the reinduction treatment and are off study will be eligible for Phase I/II trials as salvage therapy. Currently available options are:

1. Clofarabine combined with ARA-C and liposomal daunorubicin (DaunoXome®)
2. FLAMSA-RIC-SCT
3. ITCC studies on small molecules, such as currently dasatinib and in the near future PKC412.

This is a rapidly evolving field and detailed guidelines for these patients are beyond the purpose of this protocol.

Conclusion

Conventional chemotherapy regimen based on FLAG in addition with anthracyclines did improve outcome of AML relapse and refractory AML while exerting tolerable toxicity. However in view of only 35-40% overall survival in this patient group the addition of new therapeutic concepts to current standard regimen seems mandatory. Gemtuzumab ozogamicin (GO, (Mylotarg®) has been shown effective in the setting of relapsed and refractory AML in adults and children with CR rates up to 30% in selected groups. We and others have shown that combining GO (Mylotarg®) with Cytarabine and with IDA-FLAG and ADE is feasible and effective with moderate toxicity. The addition of GO (Mylotarg®) to FLA-DX based standard reinduction chemotherapy may thus be the next step to further improvement of outcome.

This can only be achieved in the setting of large intergroup trials because of the rarity of the disease. This protocol describes such an international randomized phase III study which aims at improving the outcome of these children and to learn more about the cause(s) of the poor treatment response. The obtained knowledge may also be useful for the future treatment of newly diagnosed AML, especially with respect to the use of Mylotarg® in addition with conventional chemotherapy.

8. Study objectives

The Study Relapsed AML 2009/01 aims to improve prognosis in relapsed and refractory AML in childhood by addition of GO (Mylotarg®) to the standard FLA-DX based reinduction chemotherapy. The use of GO (Mylotarg®) at 4.5 mg/m^2/dose, a dose that results in CD33 saturation and that should have limited toxicity when combined with conventional chemotherapy, could improve outcome such has been suggested in studies in adult AML.

8.1 Primary objective

Improvement of prognosis in terms of early response (BM blasts ≤20% at d28) of relapsed and refractory AML in children through intensification of reinduction chemotherapy by randomised addition of the conjugated anti-CD33 antibody linked to calicheamicin, GO (Mylotarg®), as compared to standard reinduction therapy (DX-FLA) only.

8.2 Secondary objectives

Determine the feasibility of dosing GO (Mylotarg®) at 4.5 mg/m^2/dose in combination chemotherapy in terms of toxicity.

Determine the toxicity of GO (Mylotarg®) when added to DX-FLA, according to NCI-CTC criteria in terms of mucosal toxicity, bone marrow aplasia, liver toxicity with special respect to the development of Veno-Occlusive-Disease (VOD, also called SOS or sinusoidal obstruction syndrome), short- and long-term cardiotoxicity and other side-effects, as compared to patients treated with DX-FLA only.

Determine the long term clinical outcome in a large group of children with relapsed and refractory AML. Comparison of overall survival, event free survival and disease free survival in both groups.

Determine novel, additional prognostic factors.

Establish a diagnostic and logistic network to obtain an individual characterisation of AML based on morphology, immunophenotype, type I and type II mutations, signal pathway activation, and monitoring of minimal residual disease/ treatment response.

In phase I (2 to 3 years) the feasibility to obtain all relevant data in time and to enable a virtual stratification according to biological characteristics should be demonstrated. If specific subgroups can be identified which might benefit from an individualized treatment, the patients will go off study after the first course of reinduction chemotherapy to be eligible for studies with targeted and promising treatment options.

8.3 Additional objectives (add on research studies)

The add-on studies will be developed separately, but certainly are aimed for.

9. Study design

9.1 Therapy optimisation study

This study is an open label international multicenter therapy optimisation study with a single randomised question each with a test arm and a control arm. Many groups in Europe and in other continents have agreed to fulfil the conditions of participation. Regulations, legal requirements and guidelines not mentioned in this protocol may have to be made for individual countries.

9.2 Study time schedule

The study is intended to open in the second quarter of 2010, with end of patient recruitment in the first half of 2015. Final data analysis will be done on 01.07.2020. Interim analysis will be done for efficacy and toxicity after randomisation of 100 and 200 patients. In addition, an additional interim-analysis on toxicity will be done after 50 patients have been randomised.

9.3 Number of subjects

This is a multicenter, pan-European study with an estimated annual recruitment of 85 children with relapsed or refractory AML. This results in an estimated accrual of 420 patients over 5 years, which is expected to result in 252 randomised and fully evaluable patients with having the primary endpoint available.

9.4 Inclusion criteria

- Children and adolescents <18 years of age at diagnosis of de-novo AML
- First relapsed AML
- Primary refractory AML
- Patients with a second or subsequent relapsed AML that were not previously treated according to this particular protocol
- signed written informed consent from patients or from parents or legal guardians for minor patients, according to local law and regulations
- For patients with childbearing potential, a negative test for pregnancy is to be considered before entry on study

Patients with a combined relapse, or an isolated extramedullary relapse, or a bone marrow relapse (isolated or combined) with <20% blasts in the BM are eligible. However, the latter two groups should complete both reinduction courses, irrespective of the response after the first course. In case of progressive disease in these patients, please consult the study coordinators.

9.5 Exclusion criteria

- FAB type M3 (please refer to your local group for the appropriate treatment protocol)
- Myeloid Leukemia of Down syndrome (please refer to your local group for the appropriate treatment protocol)
- Symptomatic cardiac dysfunction (CTC grade 3 or 4) and/or a Fractional Shortening at echocardiography below 29%
- A Karnofsky performance status <40% (children ≥ 16 years) or an Lanksy performance status of <40% (children < 16 years) before start of chemotherapy
- Any other organ dysfunction (CTC grade 4) that will interfere with the administration of the therapy according to this protocol
- Impaired liver function defined as > than NCI-CTG grade 1 (max 2.0 x ULN for transaminases and bilirubin)
- History of veno-occlusive disease (VOD)
- Hypersensitivity to gemtuzumab ozogamicin

- Inability to potentially complete the treatment protocol for any other reason

Patients who fulfil all inclusion criteria and who do not meet any exclusion criteria can be enrolled on this trial.

If a patient fulfils the inclusion criteria but also one or more exclusion criteria or can not be treated according to protocol, he/she may be admitted as observation patient. Observation patients will not be included in evaluations of treatment effectiveness.
Please do register all patients with relapsed or refractory AML, independent of treatment according to this protocol, so that selection bias may be considered.

9.6 Patient registration and randomisation

There will be a continuous possibility (7 days/week, 24 hours/day) of computerized registration and randomization. The local/national representatives for data management will be instructed soon by the central data office (chaired by Martin Zimmermann) about the procedure. After that, these local data managers will distribute login names and passwords to all participating centers of their group. Then, each individual center will have the possibility to randomize a new patient using the internet. The two addresses (the second one being the relevant one for the individual centers) are:

http://www.mh-hannover.de/institute/biometrie/AML/LoginCoordinator.html
http://www.mh-hannover.de/institute/biometrie/AML/LoginInvestigator.html

There will be a back-up procedure if the internet system is not available. In these circumstances, please consult your local representative for data management (see appendix), or if not available the central data office for this study in Hannover (page 2).

10. Definitions

10.1 CNS disease

≥ 5 white blood cells/mm^3 (ie, ≥ 5/μl, or ≥ 15/3 μl) and unequivocal evidence of blasts on cytospin examination, and/or clinical (seizures, cranial nerve palsy and symptoms of increased cranial pressure or other signs/symptoms not readily explained by another disease) and/or radiological evidence of leukemic infiltration in the central nervous system. In all of the symptomatic cases, a MRI should be made. CSF not evaluable in case of >50/3 erythrocytes.

10.2 Complete remission (CR)/CR but with incomplete regeneration (CRi)

≤5% leukemic blasts in the bone marrow with signs of normal hematopoiesis in the BM and with clear signs of regeneration of normal blood cell production in the peripheral blood (platelets > 50x10^9/l without transfusions, neutrophils >1.0x10^9/l), and no leukemic cells in the PB or anywhere else.

CR but with incomplete regeneration (CRi) is defined as ≤5% leukemic blasts in the bone marrow with signs of normal hematopoiesis in the BM and with signs of regeneration of normal blood cell production in the peripheral blood (platelets > 20x10^9/l without transfusions, neutrophils >0.2x10^9/l), and no leukemic cells in the PB or anywhere else.

Do consider the possibility of normal, regenerating blasts.

10.3 Early death

Death during the first 2 months of treatment (i.e., before the time, that complete remission could have been documented). The cause of death (disease, therapy or both) should be recorded as well as the last known percentage of blasts in the BM. Only patients that were randomised will be included for this analysis.

10.4 Toxic death

Death due to treatment-related complications, and not caused by the leukemia itself.

10.5 Partial remission

No complete remission, BM leukemic blasts > 5 ≤ 20% and/or no regeneration as required for CR or CRi. Do consider the possibility of normal regenerating blasts.

10.6 Early treatment response

This will be determined by morphological examination of the bone marrow sampled at « day 28 » (in practice anytime between day 28 and 42 after start of first reinduction chemotherapy). If the bone marrow shows 20% of leukemic blasts or less, the response is good. If the bone marrow shows >20% leukemic blasts, the response is poor. Do consider the possibility of normal, regenerating blasts.

10.7 Refractory disease/non-response

More than 20% of leukemic blasts in the bone marrow after 1 or 2 complete blocks of chemotherapy, and/or elsewhere documented leukemic cells. Do consider the possibility of normal, regenerating blasts.

10.8 Relapse

After a documented complete remission, the recurrence of ≥10% unequivocal leukemic blasts in a representative bone marrow, and/or evidence of leukemic infiltration or recurrence at any site. CSF with <5 cells/mm^3 but with blasts on the cytospin

examination should be investigated repeatedly, as should the BM in case of >5<10% leukemic blasts.

10.9 Early relapse
Relapse occurring within 1 year from initial diagnosis.

10.10 Late relapse
Relapse occurring 1 year or later from initial diagnosis.

10.11 Serious adverse event (SAE)
Death, life-threatening or permanently disabling event, and/or need for unexpected hospitalization or prolongation of hospitalization.

11. Diagnostics

11.1 Diagnostics before start of treatment

Before the start of the treatment the following parameters need to be obtained:

- medical history and physical examination performance status (depending on age, either the Karnofsky or Lansky Performance scale)
- complete blood count (hemoglobin, platelets, white blood cell count and differentiation). This should be determined within 2 days prior to the first reinduction course.
- serum chemistry: creatinine, sodium, potassium, calcium, phosphate, ALAT, ASAT, bilirubin, uric acid, LDH, CK, glucose, total protein, albumin;
- clotting (APTT, PT, fibrinogen)
- HLA typing (if not done previously)
- viral serology and PCR (antibodies: EBV, HSV, CMV, PVB19, HAV, HBV, HCV, HIV; PCR: CMV, HCV)
- IgG and (if not done previously) IgA levels
- bone marrow aspiration (marrow cellularity and percentage of blasts). Perform a biopsy in case of a dry tap. This should be performed within 3 days prior to the first reinduction course. Bone marrow should also be sent to the reference laboratory for confirmation of the diagnosis, and should be studied for immunophenotype and cytogenetics. Please consider to participate in research studies (see information in Appendices).
- lumbar puncture (quantification of cells and protein, pathological examination), also to be done within 3 days from the start of reinduction chemotherapy.
- urine (protein, glucose, ery's)
- Chest X-Ray
- Abdominal ultrasound including liver perfusion status
- MRI (=NMR) of the brain in case of (the suspicion on) localized symptomatic CNS disease
- Electrocardiogram
- Echocardiography (please refer to the more comprehensive guidelines below)
- Lung function test (optional)

11.2 During the first treatment course

Disease monitoring during the first treatment course should include:

- complete blood count at least twice weekly
- serum chemistry as described above at least twice weekly
- physical examination twice weekly
- bone marrow aspiration at day +8 and day +15 after the start of reinduction treatment to determine early clinical response, which may be different between both treatment arms and which may have prognostic significance. Slides and heparin-bone marrow should also be send to the reference laboratory for morphology (and flowcytometry), together with peripheral blood slides.

11.3 Before the start of each treatment course

The remission status and toxicity should be determined:

- bone marrow and peripheral blood morphology (with central review by the reference laboratories)
- lumbar punction (quantification of cells and protein, pathological examination of the CSF)
- physical examination
- complete blood count
- serum chemistry (see above)

I-BFM-SG/AML Relapse Working Group: Study Pediatric Relapsed AML 2009/01
- echocardiography (see below)

11.4 Cardiotoxicity monitoring
To determine possible short- and long-term cardiotoxicity echocardiography must be performed within 1 week before start of each reinduction course, within 1 week prior to the conditioning regimen as part of SCT, and 6 months after SCT.
In case of a normal FS of >28%, then repeat echocardiography every 3 years (earlier if clinically indicated). In case of an abnormal FS, then repeat echocardiography at least every year. Additional echocardiography (and other monitoring) may be indicated in case of growth spurt, involvement in regular strenuous exercise, growth hormone and/or sex hormone replacement therapy and in case of pregnancy.

Technique: M-mode echo measurements according to the American Heart Association guidelines. Whenever possible, the patient should be afebrile and have a normal hemoglobin (at least 9 g/dl). If an ECG is not recorded simultaneously with M-mode echo, greatest and smallest LV dimensions should be used for diastolic and systolic measurements respectively. Please note the date of each scan, along with the height, body weight, and three blood pressure measurements (preferably obtained during the last part or immediately after the echo; ensure that the cuff is in place before starting the echocardiography), as well as initials, gender, race, and identification number.
If possible, please save all the information of one patient on one tape (VHS/S-VHS) to allow central review at a later stage. At least, save the recordings on tape, and note which type of echo-machine was used.
Attach ECG
Baseline echocardiogram:
apical 4 chamber view
apical 5 chamber view (with aorta)
short axis left ventricle (papillary muscle level)
short axis left ventricule (aortic valve/pulmonary artery level)
Baseline colour Doppler: apical 4 chamber (mitral, tricuspid, aortic flows)
Pulsed Doppler:
aortic flow (measurement of LVET)
mitral valve (tips of mitral valve leaflets)
M-mode: left ventricle – parasternal long axis
M-mode cursor perpendicular to interventricular septum in a plane where the mitral valve and aorta (aortic valve) are visible
M-mode cursor immediately below tips of mitral valve
Confirm on tape that the M-mode cursor is positioned correctly
M-mode recording of aortic valve opening (for LVET) if aortic Doppler was not obtained (see above)

Record a minimum of 10 good quality cardiac cycles.

Record blood pressure (see above).

Fractional Shortening will be used as main measurement of cardiac function.

12. Treatment schedule

12.1 General information

Before the start of treatment, each patient must be randomized for the addition of gemtuzumab ozogamicin or not to DX-FLA. The procedure for registration and randomization is explained in section 9.6 and in Appendix B. Please also register all patients who are not randomized for whatever reason. The second course will consist of FLA for all patients, except for non-responders (off-study). Patients not in CR after the 2nd reinduction course are also off-study. After the 2nd course, one can proceed immediately to SCT, if available. If not, we recommend an intensive consolidation with VP16 and continuous infusion cytarabine. In selected patients (e.g. concerns of cumulative toxicity or a short time to bridge to transplant) a low-intensive consolidation with thioguanine orally and cytarabine subcutaneously may be preferred. SCT is recommended for all patients who achieved CR, ideally using a MSD or a MUD. If not available, the use of a haplo-identical donor is proposed to be restricted to patients with a very high risk of relapse (see later), while autologous SCT is to be considered for patients with late relapsed AML.

12.2 Dose Reduction for Children < 12 months or <12 kg.

Because of differences in cytarabine metabolism in young children, and in line with the experiences of many other study groups, we recommend that children younger than 12 months of age, and/or weighing less than 12 kg, should be treated according to body weight rather than body surface area. For dose adjustment the prescribed doses should be corrected as follows: [weight (kg) x dose (per m^2)] divided by 30.

12.3 Special measures for reinduction

12.3.1 Hyperleukocytosis, high blast cell counts

Patients with high peripheral blast counts (>50-100x10^9/l) and significant organomegaly have increased problems related to metabolic abnormalities, bleeding, and hyperviscosity. Platelets should be transfused if < 15-30x10^9/l; in this phase, hemoglobin should not be raised above 6 mmol/l (= 9,6 g/dl); treatment of coagulation disorders is required. The use of leukapheresis, exchange transfusion, or hemodialysis may be necessary. To prevent tumor lysis syndrome, the patient should be well hydrated and get rasburicase before start of therapy:
Rasburicase (Uricozyme®): 0,2 mg/kg/dose once daily i.v. for up to 7 days;
Hydration: 2400-3000 ml/m²/day;
be careful with potassium containing solutions;
electrolyte substitions only based on laboratory results;
check fluid balance.

In cases without hyperleukocytosis, or in situations when leukocytes have decreased significantly and rasburicase could be stopped, allopurinol and alkalinisation can be initiated:
Allopurinol: 200-300 mg/m² p.o. divided q 8-12 hours; in case of cases without prior rasburicase, give at least 2 doses of allopurinol prior to initiation of chemotherapy
NaHCO3: 4 g/m²/day (= 50 mEq/m²/day) to maintain urine pH 7-8 (only required with allopurinol, not with rasburicase).

12.3.2 Cytarabine

During cytarabine and for 24-48 hours after completion, dexamethasone ophthalmic solution or isotears (q 6 hours) may prevent cytarabine induced conjunctivitis.

Because of the association of streptococcus viridans infection and high dose cytarabine, penicillin prophylaxis is recommended from completion of cytarabine administration until neutrophil recovery.
Nurses and doctors should note the relatively high (15%) incidence of neurotoxicity, which may necessitate to interrupt or stop high-dose cytarabine administration.
Pyridoxine (300 mg/m²/day in 2 doses) might ameliorate cytarabine-induced mucositis.

12.3.3 Gemtuzumab ozogamicin (GO, Mylotarg®)

As infusion related symptoms such as fever and chills are frequent and hypotension has as well been reported, monitoring of vital parameters of the patient is essential during and at least 4 hours after the end of infusion. Premedication with acetaminophen and clemastin may prevent fever and chills and should be prescribed in all patients.
Premedication:
Acetaminophen (Paracetamol®): 10mg/kg p.o. at least 2 doses every 4 hours; starting 1 hour before start of infusion
Clemastin (Tavegil®): 25µg/kg IV 10 minutes before start of GO (Mylotarg®) infusion
Start the IV infusion with the prescribed dose of GO (Mylotarg®) infusing 10ml over the first 15 minutes, then repeat check of vital signs. If no adverse effects are noted increase the infusion rate to finish the administration of the within 2 hours.
Check vital signs (blood pressure, heart frequency, body temperature and saturation) before start of infusion and repeatedly at least every 30 minutes during and 4-6 hours after the end of GO (Mylotarg®) infusion.
In case of any sign of dyspnea, hypotension or any unexpected adverse event stop infusion immediately. Fever, chills and rigor can be expected and do not require interruption of infusion.
In case of symptomatic hypotension fluid replacement, and/or administration of cardiostimulantia and steroids is recommended.
Any medication that might increase the GO-mediated liver toxicity should be avoided if possible. Especially azoles (itraconazole, fluconazole, posaconale, voriconazole) should be withhold from 3-5 days before, during and until 3 days after administration of GO (Mylotarg®).

12.3.4 Granulocyte colony stimulating factor (G-CSF)

G-CSF will not be used routinely (please refer to "background and introduction"). It is also recommended not to routinely initiate G-CSF after chemotherapy, unless very severe, uncontrolled infections in neutropenia develop. Alternatively, granulocyte transfusions should then be considered instead of G-CSF. The reason for this reluctancy to use G-CSF is that it was found that G-CSF when used between chemotherapy courses had an adverse impact on outcome in pediatric AML, which might be explained by the presence of an G-CSF receptor isoform which is expressed in 30% of pediatric AML patients.

12.3.5 Irradiation of blood products

Mainly due to fludarabine and body irradiation, profound and long term lymphocytopenia can occur. Therefore, cellular blood products should be irradiated with 25 Gy to prevent transfusion related graft versus host disease, starting at least two weeks before the first dose of fludarabine and at least up to 6 months after the last fludarabine administration. Of course, all blood products should be leukocyte-depleted as well. Similarly, irradiation and leukocyte-depletion of the blood products is required at least up to 6 months after SCT.

I-BFM-SG/AML Relapse Working Group: Study Pediatric Relapsed AML 2009/01
12.4 First Reinduction Course DX-FLA + Gemtuzumab ozogamicin (Mylotarg®)

Randomize to know if DX-FLA or DX-FLA + GO (Mylotarg®) should be given as 1st course!
Start induction chemotherapy at diagnosis or immediately following cytoreductive pre-phase (WBC< 50,000/µl). In case of hyperleucocytosis the initial lumbar puncture should be delayed until sufficient blast reduction has been achieved (WBC < 10,000/µl).
Perform BMP and LP first! BMP at day 8 and 15! (➔ MRD) Please note: record day of peripheral blood blast clearance!

DX-FLA-GO

Reinduction

Fludarabine 30 mg/m²/d **day 1 to 5**
30 min IV infusion

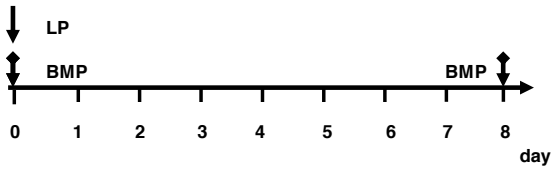
L-DNR 60 mg/m²/d **day 1, 3, 5**
60 min IV infusion, after Fludarabine

Cytarabine 2000 mg/m²/d **day 1 to 5**
3hr IV infusion, begin 4 h after start of Fludarabine

Gemtuzumab Ozogamicin 4.5 mg/m²/d, day 6
2hr IV infusion

Cytarabine/Pred/MTX **day 1**
IT bolus injection, **age related dosage**

	<1 year	1<2year	2<3year	≥ 3 year
AraC	16 mg	20 mg	26 mg	30 mg
MTX	6 mg	8 mg	10 mg	12 mg
Pred	4 mg	6 mg	8 mg	10 mg



In case of a body weight <12 kg, calculate dose according to weight as follows: [weight (kg) x dose (per m2)] /30.

Fludarabine	30 mg/m^2/day by 30 minutes IV infusion once every 24 hours on day 1, 2, 3, 4 and 5 (5 total doses)
DaunoXome®	60 mg/m²/day by 60 minutes IV infusion once every 24 hours on day 1,3 and 5 (3 total doses). Begin infusion after start of Fludarabine.
Cytarabine	2000 mg/m²/day by 3 hours IV infusion once every 24 hours on 1, 2, 3, 4 and 5 (5 total doses). Begin infusion 4h after start of Fludarabine.
GO (Mylotarg®)	4.5 mg/m²/day by 2h IV infusion on day 6 (see infusion guidelines below)

Triple IT administer in age-related dosage on day 1
in case of CNS involvement weekly until 1 week after complete blast clearance of CSF.

	<1 year	1<2 year	2<3 year	≥ 3 year
Cytarabine	16 mg	20 mg	26 mg	30 mg
MTX	6 mg	8 mg	10 mg	12 mg
Prednisone	4 mg	6 mg	8 mg	10 mg

Guidelines for infusion of Gemtuzumab ozogamicin:

As infusion related symptoms such as fever and chills are frequent and hypotension has as well been reported, monitoring of patient's vital parameter is essential during and at least 4 hours after end of infusion. Premedication with acetaminophen and clemastin may prevent fever and chills.
Check vital signs (blood pressure, heart frequency, body temperature and saturation) before start of infusion and repeatedly at least every 30 minutes during and 4-6 hours after end of GO (Mylotarg®) infusion
Premedication:
Acetaminophen (Paracetamol®): 10mg/kg p.o. at least 2 doses every 4 hours; starting 1 hour before start of infusion
Clemastin (Tavegil®): 25µg/kg IV 10 minutes before start of GO (Mylotarg®) infusion
Start the IV infusion with the prescribed dose of GO (Mylotarg®) infusing 10ml over the first 15 minutes, then repeat check of vital signs. If no adverse effects are noted increase the infusion rate to finish the administration of the within 2 hours.
In case of any sign of dyspnea, hypotension or any unexpected adverse event stop infusion immediately. Fever, chills and rigor can be expected and do not require interruption of infusion.
In case of symptomatic hypotension fluid replacement, and/or administration of cardiostimulantia and steroids is recommended.
Any medication that might increase the GO-mediated liver toxicity should be avoided if possible. Especially azoles (itraconazole, fluconazole, posaconale, voriconazole) should be withhold from 3-5 days before, during and until 3 days after administration of GO (Mylotarg®).

BMP after course 1 (apart from the days 8 and 15 BM's) should be performed not earlier than 28 days after the start of course1, but in case of delay, not later than day 42 after the start of course 1.

I-BFM-SG/AML Relapse Working Group: Study Pediatric Relapsed AML 2009/01
12.5 First Reinduction Course DX-FLA

Randomize to know if DX-FLA or DX-FLA + GO (Mylotarg®) should be given as 1st course!
Start induction chemotherapy at diagnosis or immediately following cytoreductive pre-phase (WBC< 50,000/µl). In case of hyperleucocytosis the initial lumbar puncture should be delayed until sufficient blast reduction has been achieved (WBC < 10,000/µl).
Perform BMP and LP first! BMP at day 8 and 15! (➔ MRD) Please note: record day of peripheral blood blast clearance!

DX-FLA

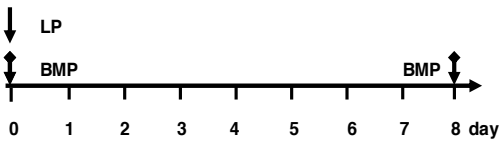
Reinduction

Fludarabine 30 mg/m²/d **day 1 to 5**
30 min IV infusion, after G-CSF

L-DNR 60 mg/m²/d **day 1, 3, 5**
60 min IV infusion, after Fludarabine

Cytarabine 2000 mg/m²/d **day 1 to 5**
3hr IV infusion, begin 4 h after start of Fludarabine

Cytarabine/Pred/MTX **day 1**
IT bolus injection, **age related dosage**



	<1 year	1<2year	2<3year	≥ 3 year
AraC	16 mg	20 mg	26 mg	30 mg
MTX	6 mg	8 mg	10 mg	12 mg
Pred	4 mg	6 mg	8 mg	10 mg

In case of a body weight <12 kg, calculate dose according to weight as follows: [weight (kg) x dose (per m2)] /30.

Fludarabine	30 mg/m^2/day by 30 minutes IV infusion once every 24 hours on day 1, 2, 3, 4 and 5 (5 total doses)
DaunoXome®	60 mg/m²/day by 60 minutes IV infusion once every 24 hours on day 1,3 and 5 (3 total doses). Begin infusion after start of Fludarabine.
Cytarabine	2000 mg/m²/day by 3 hours IV infusion once every 24 hours on 1, 2, 3, 4 and 5 (5 total doses). Begin infusion 4h after start of Fludarabine.
Triple IT	administer in age-related dosage on day 1
	in case of CNS involvement weekly until 1 week after complete blast clearance of CSF.

	<1 year	1<2 year	2<3 year	≥ 3 year
Cytarabine	16 mg	20 mg	26 mg	30 mg
MTX	6 mg	8 mg	10 mg	12 mg
Prednisone	4 mg	6 mg	8 mg	10 mg

BMP after course 1 (apart from the days 8 and 15 BM's) should be performed not earlier than 28 days after the start of course1, but in case of delay, not later than day 42 after the start of course 1.

I-BFM-SG/AML Relapse Working Group: Study Pediatric Relapsed AML 2009/01
12.6 Second Reinduction Course FLA

All patients will receive FLA only in 2nd reinduction.
The second reinduction course should be started not earlier than 28 days but not later than 42 days after the start of the first reinduction course.

To commence this block of treatment the patient must have a neutrophile count >1.0x10^9/l and platelets >50x10^9/l (without transfusions) and be in good clinical condition.
Perform a bone marrow (MRD and morphology) and lumbar puncture on day 1.

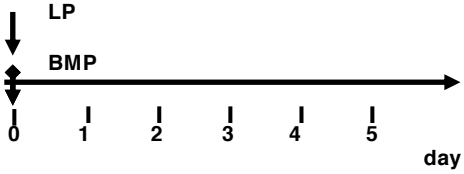
FLA

Reinduction

Fludarabine 30 mg/m²/d **day 1 to 5**
30 min IV infusion, after G-CSF

Cytarabine 2000 mg/m²/d **day 1 to 5**
3hr IV infusion, begin 4 h after start of Fludarabine

Cytarabine/Pred/MTX **day 1**
IT bolus injection, **age related dosage**



	<1 year	1<2year	2<3year	≥ 3 year
AraC	16 mg	20 mg	26 mg	30 mg
MTX	6 mg	8 mg	10 mg	12 mg
Pred	4 mg	6 mg	8 mg	10 mg

In case of a body weight <12 kg, calculate dose according to weight as follows: [weight (kg) x dose (per m2)] /30.

Fludarabine	30 mg/m^2/day by 30 minutes IV infusion once every 24 hours on day 1, 2, 3, 4 and 5 (5 total doses)			
Cytarabine	2000 mg/m²/day by 3 hours IV infusion once every 24 hours on 1, 2, 3, 4 and 5 (5 total doses). Begin infusion 4h after start of Fludarabine.			
Triple IT	administer in age-related dosage on day 1			
	in case of CNS involvement weekly until 1 week after complete blast clearance of CSF.			
	<1 year	1<2 year	2<3 year	≥ 3 year
Cytarabine	16 mg	20 mg	26 mg	30 mg
MTX	6 mg	8 mg	10 mg	12 mg
Prednisone	4 mg	6 mg	8 mg	10 mg

12.7 Consolidation -high intensity

To be given as consolidation to all patients, if a SCT is not available immediately but only to patients who will not tolerate a 3rd course of intensive chemotherapy before SCT.

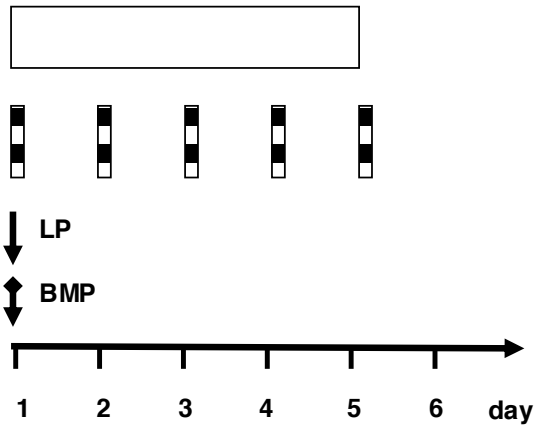
Perform BMP + LP first! BMP after the 2nd reinduction course not earlier than 28 days after the start of course 2, but in case of delay, not later than day 42 after that start. If no CR after course 2the patient is off study; please contact your national study coordinator for Phase I/II study options for salvage therapy.

To be started not earlier than 28 days after the start of course 2, and only in case of good clinical condition. The patient must have a neutrophile count >1.0x10^9/l and platelets >50x10^9/l (without transfusions).

Cytarabine 500 mg/m²/d **day 1-4**
96 hrs continous IV infusion

Etoposide 100 mg/m²/d **day 1-5**
3hr IV infusion

Cytarabine/Pred/MTX **day 1**
IT bolus injection, **age related dosage**



	<1 year	1<2year	2<3year	≥ 3 year
AraC	16 mg	20 mg	26 mg	30 mg
MTX	6 mg	8 mg	10 mg	12 mg
Pred	4 mg	6 mg	8 mg	10 mg

In case of a body weight <12 kg, calculate dose according to weight as follows: [weight (kg) x dose (per m2)] /30.

12.8 Consolidation – low intensity

Perform BMP and LP first! BMP after the 2nd reinduction course not earlier than 28 days after the start of course 2, but in case of delay, not later than day 42 after that start. If no CR is achieved after course 2, the patient is off study; please contact your national study coordinator for Phase I/II study options for salvage therapy.
To be started not earlier than 28 days after the start of course 2, and only in case of good clinical condition with platelets > 50x10^9/l (without transfusion) and neutrophils > 1.0x10^9/l.

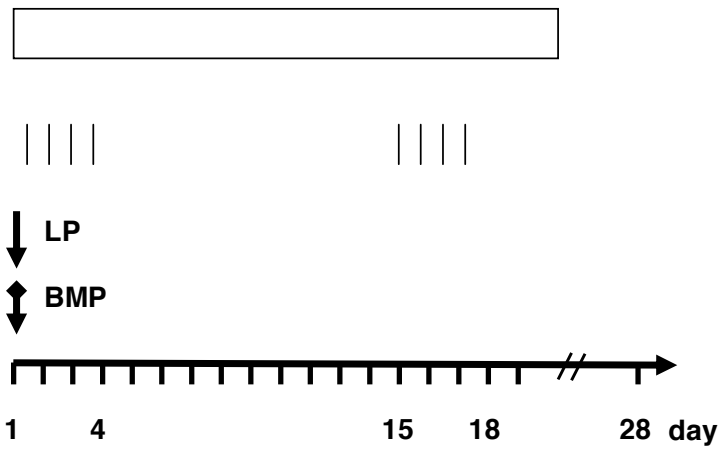
Consolidation

Thioguanine 100 mg/m²/d **day 1 to 28**
PO once daily (evenings)

Cytarabine 75 mg/m²/d **day 1 to 4**
SC bolus injection
day 15 to 18

Cytarabine/Pred/MTX **day 1**
IT bolus injection, age adjusted dosage

	<1 year	1<2year	2<3year	≥ 3 year
AraC	16 mg	20 mg	26 mg	30 mg
MTX	6 mg	8 mg	10 mg	12 mg
Pred	4 mg	6 mg	8 mg	10 mg



In case of a body weight <12 kg, calculate dose according to weight as follows: [weight (kg) x dose (per m2)] /30.

Criteria for reduction or discontinuation of consolidation therapy:

	Dose		%
6-Thioguanin (Thioguanin®)	Leukocytes	> $3.0x10^9$/l	150
	Leukocytes	> $2.0x10^9$/l; < $3.0x10^9$/l	100
	Leukocytes	> $1.0x10^9$/l; < $2.0x10^9$/l	50
	Leukocytes	< $1.0x10^9$/l	0
Cytarabine (Alexan®, Udicil®)	Leukocytes	> $2.0x10^9$/l, and	
	Platelets	> $80x10^9$/l	100
	Below these counts discontinuation for 1 week		

Note: all patients should get high-intensity consolidation, except selected patients who might tolerate only this low-intensity consolidation.

12.9 CNS Prophylaxis and treatment

CNS-Prophylaxis
Triple intrathecal medication at age-adjusted doses (table), by LP. The first dose should be given immediately before the start of reinduction course 1.

CNS treatment
CNS leukemia is defined in section 10.

Table: Intrathecal triple chemotherapie for CNS leukemia

Age	Cytarabine mg/dose	Methotrexate mg/dose	Prednisolone mg/dose
<1 year	**16**	**6**	**4**
1<2 year	**20**	**8**	**6**
2<3 year	**26**	**10**	**8**
≥ 3 years	**30**	**12**	**10**

The second and subsequent doses should be given every 7 days until 1 week after complete clearance of the CSF of leukemic blasts. Then, 2 more doses must be given, one immediately before the start of the second reinduction course, and the other at the start of consolidation treatment.
Intrathecal chemotherapy is to be given via lumbar puncture or reservoir.

Cranial irradiation is not recommended, but may be considered in those patients who do achieve complete remission but who will not undergo SCT, and who did not receive cranial irradiation in the past.

I-BFM-SG/AML Relapse Working Group: Study Pediatric Relapsed AML 2009/01
13. Stem cell transplantation

In CR, all patients are eligible for allo-SCT. If more time is needed to perform that SCT, intensive and low-intensity consolidation guidelines are provided in this protocol. The choice for either the intensive or non-intensive regimen must be based on the anticipated time until SCT and on the condition of the patient.
This protocol does not provide guidelines on conditioning regimens, this is left to each individual group. However, collaborative protocols in that setting are strongly encouraged.

Guidelines for indications for SCT:

Patients in complete remission after the 2 courses of reinduction chemotherapy are eligible for SCT. Therefore, the search for a donor should start as soon as the diagnosis of refractory or relapsed AML has been made. The preferred type of SCT depends on the risk group and the donor availability.

Low risk = late (≥1 year from initial diagnosis) first relapse
matched sibling donor SCT
matched unrelated donor SCT
autologous SCT

High risk = early (<1 year from initial diagnosis) first relapse, multiple relapse, refractory disease
matched sibling donor SCT
matched unrelated donor SCT
haplo-identical donor SCT /cord-blood SCT
autologous SCT

Whatever risk group, one should in general avoid a SCT within 6 months after a previous allogeneic SCT in view of the toxicity of such procedures. If necessary, consolidation treatment according to the low intensity regimen could be given after the 3rd course of intensive consolidation chemotherapy to achieve this time period.

In case of an autologous SCT, stem cells that were obtained in stable first CR are preferred. If not available (which usually will be the case), stem cells should be harvested upon regeneration after the 2nd reinduction course. Peripheral blood stem cells are preferred, but bone marrow can be harvested as alternative. Also if stem cells are available from CR1, it is recommended to harvest stem cells again as back-up. Purging is not generally recommended. The goal is to harvest a total of ≥2.0 x 10^{8} mononuclear cells/kg. The minimum number of CD34-positive cells required for reinfusion is 2.0 x 10^{6}/kg.

14. Drug information, expected toxicity and dose adjustments

14.1 Cytarabine

This nucleoside analogue (pyrimidine antagonist) is an antimetabolite that is mainly metabolised in the liver. It may cause myelosuppression, hepatotoxicity, exanthema, malaise, and gastro-intestinal toxicity such as nausea, vomiting, stomatitis, and ileus. At high doses, fever, severe diarrhea, central nervous system disturbances such as somnolence, cerebellar ataxia and nystagmus, keratitis, veno-occlusive disease, pneumonitis and adult respiratory distress syndrome with streptococcus viridans infection are possible. Intrathecal administration has been associated with headache, fever, vomiting, and pleiocytosis, rarely with aseptic meningitis or central nervous system disturbances. To prevent keratitis, indifferent or corticoid eye drops every 4-6 hours are recommended during and until 12 hours after stopping cytarabine. Prolonged corticoid eye drops have side-effects as well, such as infection. Conjunctivitis should be treated with corticoid eye drops. To prevent streptococcal infection, penicilline prophylaxis may be considered starting as soon as neutropenia develops after high-dose cytarabine until neutrophils > $0.5x10^9$/l.

Patients who developed grade 3 or 4 neurotoxicity following high-dose cytarabine should not receive further high-dose cytarabine, but are eligible for continuous lower-dose cytarabine.

14.2 Fludarabine

This nucleoside analogue (purine antagonist) is an antimetabolite that is excreted mainly in the urine. After phosphorylation, it is less susceptible to deamination than cytarabine. Main side-effects are myelosuppression, fever, chills, malaise, nausea, and vomiting. More rare are auto-immune phenomena (hemolytic anemia), pneumonitis, and CNS symptoms such as agitation. Since it causes profound and long-term lymphocytopenia, irradiation of blood products to prevent graft-versus-host disease and antifungal prophylaxis is strongly recommended, from the start of treatment until 6 months after SCT or if SCT is not being perfomed until 6 months after the last administration of fludarabine.

14.3 Gemtuzumab ozogamicin (GO, Mylotarg®)

Gemtuzumab ozogamicin (GO, Mylotarg®) is a conjugated monoclonal CD33 antibody that selectively targets CD33 positive cells. CD33 is a glycoprotein belonging to the sialoadhesin familiy und is on granulocytes and monocytes but not on CD34 positive hematopoetic stem cells, lymphocytes and non hematopoetic tissues. GO (Mylotarg®) is composed of a humanized IgG4 class monoclonal antibody linked to a derivate of the antineoplastic antibiotic calcheamicin. Calicheamicin is an antineoplastic antibiotic. The most common side effects of GO (Mylotarg®) treatment are neutropenia and thrombocytopenia as well as infusion related fever, chills, hypotension and dyspnea which mostly disappear upon treatment with acetaminophen and occasionally steroids. Rare cases of severe and fatal hypersensitivity have been reported. Regarding prevention of infusion related symptoms and treatment of hypersensitivity note guidelines for GO (Mylotarg®) infusion specified in section 12. Tumorlysis syndrome and adult respiratory distress syndrome (ARDS) have been reported in patients with leukocytes > 30 000/µl treated with GO (Mylotarg®). Therefore reduction of WBC below 30000/µl prior to the start of GO (Mylotarg®) treatment is recommended.
Hepatic toxicity with elevation of liver enzymes and bilirubin is encountered in about 30% of patients after GO (Mylotarg®) treatment and is reversible in most cases. Frequency of severe liver toxicity and veno-occlusive-disease (VOD) is estimated to

occur in 4-5% of patients, with higher frequencies in patients after stem cell transplantation, and a higher incidence in case of doses of >6.0 mg/m^2. Therefore patients are required to have normal liver function defined as transaminases and bilirubin < 2 x UNL. Patients with impaired liver function according to this criteria and/ or history of VOD are not eligible for randomisation and GO (Mylotarg®) treatment. Any medication that might increase the GO-mediated liver toxicity should be avoided if possible. Especially azoles (itraconazole, fluconazole, posaconale, voriconazole) should be withhold from 3-5 days before, during and until 3 days after administration of GO (Mylotarg®).

14.4 Liposomal daunorubicin (DaunoXome®)

This drug is the combination of an anthracycline with a unilamellar liposomal transport system. Daunorubicin is released slowly, and then mainly excreted in the bile, less so in the urine. Daunorubicin itself is metabolised to daunorubicinol, which is again mainly excreted in the urine and in the bile. The drug should be soluted in dextrose 5% only, because of agglutination in other solutions! Although not reported for DaunoXome®, the concomitant use of daunorubicin with heparin or dexamethasone i.v. is complicated by precipitation. Therefore, these drugs should not be used simultaneously i.v. with DaunoXome®. Side-effects that have been described were nausea, vomiting, mucositis, hepatotoxicity and the dose-limiting toxicity is myelosuppression.

In case of a fractional shortening at echocardiography below 29%, DaunoXome® should not be given, at least not as part of this randomised trial.

In case of hepatic disturbances before the first administration of DaunoXome®, a reduction for all three doses is recommended:

Transaminases >2.5 times upper normal limit (UNL):	reduction of 25%.
Transaminases >5-10 times UNL:	reduction of 50%
Transaminases >10 times UNL:	not eligible

All three doses of DaunoXome® should be similar, in other words, no further reductions after the first dose.

14.5 Thioguanine

This purine antagonist is an antimetabolite that is metabolised rapidly and then excreted renally. It may cause myelosuppression, nausea, vomiting, anorexia, mucositis, diarrhea, hepatotoxicity, and veno-occlusive disease.

14.6 VP-16 (Etoposide)

This inhibitor of DNA topoisomerase II should not be infused intravenously in less than 1 hour because of the risk of hypotension and anaphylaxis. The drug has several side-effects, including nausea, vomiting, alopecia, and myelosuppression. More rare side-effects are fever, headache, cholestasis, mucositis and peripheral neuropathy. Dose reduction is indicated in case of hepatic dysfunction.

15. Supportive care

The treatment to be given is aggressive and immunosuppressive. Therefore, meticulous care is required in the management of patients entering the study.

15.1 General measures

- **Venous access: placement of a double lumen central venous catheter for administration of chemotherapy, nutrients, antibiotics and blood products is strongly advised.**
- **Tumor lysis prevention: to prevent tumor lysis syndrome, the patient should be well hydrated, alkalinized and placed on allopurinol (or rasburicase = uricozyme) before initiation of therapy (see also page 35 and below)**
- **Nutrition: the combination of chemotherapy induced vomiting, mucositis, infection, and hemorrhage may result in significant weight loss. Progressive weight loss should be treated aggressively with supplemental enteral or parenteral nutrition; enteral feedings are preferred to parenteral; adjustment of the diet (p.e. no lactose or semi elementary) may be beneficial.**
- **Nill-by-mouth: when enteral feedings must be withheld, protection of the gastric mucosa should be initiated**
- **Antiemetics: prophylactic antiemetic therapy should be instituted to prevent chemotherapy induced nausea and vomiting. Steroids should not be used in case of (suspected) fungal infections, and ideally are avoided totally because of a potential proliferative effect on AML cells.**
- **Mucositis: meticulous oral hygiene is required; tooth brushing, hydrogen peroxide, saline and bicarbonate rinses; chlorhexidine solution; liberal use of pain medication for this condition is encouraged. Stomatitis due to herpes virus may be confused with drug induced mucositis; therefore, viral cultures should be obtained frequently. Anti-herpetic and anti-fungal therapy should be given as indicated.**
- **Conjunctivitis prophylaxis: during cytarabine and for 24-48 hours after completion, dexamethasone ophthalmic solution or isotears (q 6 hours) should be used to prevent cytarabine induced conjunctivitis.**
- **Suppression of menstruation: menstruating females should receive depo-provera or another suppressant during the entire course of the protocol until the platelet count is >40-50x10^9/l without transfusion support.**
- **Vaccinations: these should be postponed till after completion of treatment**
- **Physical therapy: timely exercises for preventing and maintaining optimal motor skills are recommended**
- **Psychosocioeconomic support: recommended**
- **Prevention and treatment of pain: according to local guidelines**

15.2 Hyperleukocytosis and metabolic derangement

Patients with high peripheral blast counts (>50-100x10^9/l) and significant organomegaly have increased problems related to metabolic abnormalities, bleeding, and hyperviscosity. Therefore, special measures for reinduction are indicated and are described in section 10.2.

15.3 Prevention and treatment of infections

15.3.1 Prevention of infections

- **mandatory hospitalization during and after treatment courses until the absolute neutrophil count is rising (and depending on the clinical condition)**
- **barrier nursing**
- **HEPA air filtration in the nursing room if available**
- **oral hygiene (see above)**

- treatment of infectious foci before initiating therapy: ENT, dental prior to initiating therapy; multiple dental extractions may be necessary.
- surveillance cultures may be considered, according to local guidelines; routine culture of throat to detect the presence of penicillin-resistant streptococcus
- test for toxoplasmosis, hepatitis viruses, CMV, varicella and HSV antibodies
bowel decontamination may be considered, according to local guidelines (Guiot 1983)
- prophylactic antibiotics in case of bacteriaemia causing operations
- pneumocystis carinii prophylaxis with trimethoprim-sulfamethoxazole, e.g. 150/750 mg/m^2/day for 3 consecutive days every week (Hughes 1987); alternatives: dapsone or aerosolized pentamidine
- because of the association of streptococcus viridans infection and high dose cytarabine, penicillin prophylaxis is recommended from completion of cytarabine administration until neutrophil recovery
- prophylactic treatment of fungal (including aspergillus) infections is recommended: e.g. with oral itraconazole (liquid!, 5-10 mg/kg/day in 1-2 doses, maximum 600 mg/day). T- hree to five days before, during and 3 days after administration of gemtuzumab ozogamicin, azoles should not be used because of potential increased risk for SOS/VOD.
- at the time of stem cell transplantation, prophylactic acyclovir for patients with positive HSV titers
- administration of zoster hyper immune globulin within 48 hours after a real varicella contact, and initiation of acyclovir or valaciclovir

15.3.2 Treatment in case of fever and neutropenia

Because of the high risk of serious infectious complications, patients developing fever should be treated for presumed sepsis with broad spectrum antibiotics. Because of the association of high dose cytarabine with streptococcus viridans infection and because of the possibility of a contaminated central venous line (staphylococci) the antibiotic regimen should include drugs specific for the treatment of an infection with gram-positive bacteria (vancomycin, teicoplanin). Antifungal therapy, such as amphotericin B or voriconazole should be administered when defervescence does not occur within 3-5 days.
AML treatment is associated with a high risk of pulmonary aspergillus; however, pulmonary infiltrates can also be caused by bacteria, virus (CMV), legionella and PCP; therefore, agressive diagnostic measures including (repeated) high resolution CT scans, bronchoscopy with biopsy or bronchoalveolar lavage, and open lung biopsy, should timely be contemplated.
G-CSF may be considered to reduce the duration of neutropenia, but only in patients with life-threatening, uncontrolled bacterial or fungal infections. Consider granulocyte transfusions in such situations as well.

15.3.3 Treatment in case of a documented infection

The empiric treatment may be adjusted when a specific cause for the infectious symptoms is found, although broad-spectrum antibiotics remain (at least initially) indicated in case of neutropenia.

15.4 G-CSF

It is not recommended to routinely initiate G-CSF after chemotherapy to reduce the period of neutropenia. This is because a pediatric study observed more relapses in high-risk patients that routinely obtained G-CSF in between chemotherapy courses, which might be associated with the occurrence of splice variants of the G-CSF-R in 30% of patients.

15.5 Transfusion support

Bleeding due to thrombocytopenia should be treated promptly; during the induction period and throughout admissions for fever and neutropenia, it is recommended that the platelet count be maintained >15-30x10^9/l. Higher thresholds may be indicated, for instance in case of lumbar punctures and surgery.

Packed red blood cell transfusions should be used to correct hypovolemia from blood loss or pre-existing anemia (Hb < 5 mmol/l = 8,0 g/dl). Higher threshold levels may be indicated in case of pneumonitis or (imminent) bleeding or other causes of a compromised clinical condition.

The blood products should be leukocyte-depleted to prevent HLA sensibilization; leukocyte-depleted blood products can be considered CMV-safe.

To prevent transfusion related graft versus host disease, cellular blood products should be irradiated (25 Gy) during and at least till 6 months after fludarabine, in case of (imminent) lymphocytopenia (< 500/ml), and from 2 weeks before till at least 6 months after stem cell transplantation.

In case of allo-immunization HLA-matched platelets may be required.

I-BFM-SG/AML Relapse Working Group: Study Pediatric Relapsed AML 2009/01
16. Statistical considerations

This concerns an international multicenter open label randomised phase III clinical study on the treatment of children with relapsed or refractory AML. As can be seen from the overview on page 2, a large number of groups from all over the world will collaborate.

The main end point is the percentage of BM blasts (yes or no >20%) after one block of Chemotherapy (DX-FLA or DX-FLA/gemtuzumab ozogamicin), which will be compared between both arms.

Secondary end points are:
Toxicity will be measured by duration of bone marrow aplasia and by CTC-NCI grading for mucosal toxicity, short- and long-term cardiotoxicity, liver toxicity and other NCI-CTC scales which are considered to be relevant in relapsed AML and the proposed therapy. Again, toxicity will be compared between both treatment arms.
Efficacy will also be determined by: percentage of blasts in the day 15 bone marrow, time to peripheral blood blast clearance, CR and CRi rates after 2 courses of (re-) induction, percentage of patients with performance of SCT, overall survival (event: death from any cause), event-free survival (events: death from any cause, non-response, relapse or second malignancy) and disease-free survival (DFS, events: death from any cause, relapse or second malignancy). Survival times will be calculated from date of entry on the protocol (randomisation) (or date of CR for DFS) to last follow up or next event. These parameters of clinical treatment response will be compared between both arms.
Percentage of patients who have successfully been characterized in terms of morphology, immunophenotype, type I and type II mutations and activation of signalling pathways and are thus eligible for further stratification to tailored therapy.

The analysis of the main end-point will be performed on all randomized patients according to the intent-to-treat principle. The difference in response rate (yes or no >20% blasts in the BM at d28) will be tested with a logistic regression model taking into consideration time from first diagnosis of AML to relapse and whether patients had primary refractory AML or relapsed disease.
The expected number of patients that will be included in this trial is 85/year, based on the participation of the above mentioned groups. The study duration will be 5 years, or less in case of a higher accrual rate. Assuming a randomisation rate of 75% about 315 patients will be randomised. Based on experience from the study Relapsed AML 2001/01, that will provide 252 patients with known data on the day "28" BM. The power will then be 80% to detect a decrease in the number of patients with ≥ 20% BM blasts at day "28" from 20% to 9% (one-sided test, alpha=5%). This is a similar improvement as was shown in study Relapsed AML 2001/01.

Final analysis will be performed 5 years after the inclusion of the last patient. Two interim analyses for efficacy and toxicity are planned after 100 and 200 patients. An additional interim-analysis will be done after 50 patients, in view of the combination of conventional chemotherapy with GO at 4.5 mg/m^2. The O'Brien and Fleming rules will be followed for alpha-correction.

Dose-reduction of GO (Mylotarg®) from 4.5 mg/m^2 to 3.0 mg/m^2 will be considered if mortality (see below) and/or grade III/IV toxicity exceed pre-set limits at several interim-analyses, i.e. after 50, 100 and 200 randomised patients. Basically, mortality before achieving CR should not exceed 10%. Toxicity will be analysed in the

perspective of reversibility and the need to postpone treatment, and will focus on grade III/IV cardiotoxicity (which should not exceed 10%) and grade III/IV liver toxicity (which should not exceed 10%, except for raised transaminases).

The absolute death rate observed in each arm (and globally in the whole population) will be compared to a reference rate (based on study Relapsed AML 2001/01) in order to detect an absolute excess of toxic deaths. All patients who die within the first two months of therapy or have a follow up of at least two months will be included to calculate the rate of early death (see table, left column). If with a given number of patients the number of deaths should reach the limit (see table, right column), the Data Safety and Monitoring Committee and the study steering committee have to decide about stopping or continuing the study. Based on previous experience, we choose the following parameters: p_0 = 10 % and p_1 = 20 % with α = 5% and β = 1%. This means that the risk to wrongly conclude that there is an excess of toxic deaths (whereas the real rate is equal to $p_0 \leq 10$) is equal to α = 5%. On the other hand, the power to detect an excess of toxic deaths (if the real rate is equal to p_1 = 20 is equal to 1-β = 99%. Major adverse events will be monitored continuously.

Wald sequential plan to detect an excess of deaths

N (death within/follow-up of at least two months)	N (death within 2 months of therapy)
-9	5
-15	6
-22	7
-29	8
-36	9
-43	10
-50	11
-57	12
-65	13
-72	14
-77	15
-84	16
-91	17
-98	18
-105	19
-112	20
-119	21
-126	22
-133	23
-139	24

17. Data management

Each participating group will refer to their clinical contact person and to the usual network of clinical centers, data center and experts (statistician, laboratories, etc) for the application of this protocol. Page 15 mentions the names and e-mail addresses of the contact persons (clinical and data management) of each participating group. The international study coordinator, vice coordinator and study statistician will act as a co-ordination unit for the monitoring and exchange of information and for the pooling of the data.

The set of data to be collected and pooled by the participants are listed on the website. Each group may:
design its own data collection forms for this protocol according to its own standards, but in such a way that the forms include the data items listed in the separate CRF's; Please note, however, that toxicity collection after each course should be done using the common toxicity forms.
centralize the forms for quality checks in its own data center, according to the approach routinely used in the group.
decide wether to input data in their own data base and send data as a file for pooling (at least every 6 months) or to send each checked and corrected forms to the operating center in Hannover.

In summary, the major requirements that each group will have to ask to each of its clinical oncology centers are:
To register at the data center each patient with relapsed or refractory AML, regardless of whether the patient will subsequently enter the protocol (Appendix B). This is necessary in order to know which percentage of eligible patients is treated according to the protocol. Registration should be done as soon as possible after diagnosis; entry on this study can only be done with informed consent and after completing the randomisation procedure.
To send at least every 6 months the forms on diagnosis, response, randomization, treatment, toxicity and events as soon as they can be completed, to the central data office (see addresses).

Please note that serious adverse events must be reported immediately to the central data office in Hannover (as well as to the own data center).

17.1 Data pooling

A common coding system and format has been made, for use by the data managers and/or statisticians, in order to minimize mistakes in data exchange. This coding system is defined for the purpose of data pooling only: it is required that a data set including each registered patient is prepared with this format by the data managers and/or statisticians of each group. The data have to be sent every 6 months for pooling to the operating center, in a file structured according to a file that will be forwarded separately to the data managers/statisticians, or on forms. The operating center, in collaboration with the clinical contact person and the data manager/statistician of each group, pools the data and circulates a report on these data.

17.2 Data Safety and Monitoring Committee (DSMC)

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

18. Safety evaluation

18.1 Definitions

18.1.1 Adverse Event

An Adverse Event (AE) is any untoward medical occurrence in a patient administered an investigation medicinal product (IMP) or procedure and which does not necessarily have a casual relationship with this treatment/procedure. An AE can be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom or disease temporally associated with the use of a medical treatment or procedure that may or may NOT be considered related to the medical treatment or procedure.

18.1.2 Serious Adverse Event (SAE)

An adverse event is defined as serious if it satisfies any of the following criteria:
Life threatening/Fatal:
Patient was at immediate risk of dying from the event. Includes deaths up to 30 days after the cessation of treatment. Death due to progression of disease is not considered an SAE.
Unexpected hospitalization or prolongation of hospitalization:
Patient was admitted to hospital for at least one overnight stay. Hospitalization for elective surgery planned prior to treatment, part of normal treatment or progressive disease is not a serious adverse event.
Causes persistent or significant disability or incapacity:
Considered a substantial disruption in a person's ability to conduct normal life functions. Does not have to be permanent.
Events that require medical intervention to prevent one of the outcomes listed above:
Usually a surgical procedure. The reason for the medical intervention will be reported as the adverse event, not the procedure. Elective surgery, stopping treatment, changing the dose of any concomitant medication and treatment with a prescribed drug are not adverse events.
Congenital anomaly/birth defect (of female patients descent)
Secondary malignancies
any new malignancy other than a relapse of the current disease, during protocol treatment or in the 30 days following end of treatment

18.1.3 Serious Unexpected Suspected Adverse Reaction (SUSAR)

A serious adverse event where a casual relationship to the investigational product cannot be excluded is a suspected SAR and when the nature or severity is not consistent with the product information it constitutes a serious unexpected suspected adverse reaction (SUSAR).

18.2 Documentation and evaluation of adverse events (AE)

The NCI Common Terminology Criteria for Adverse Events v3.0 (CTCAE) are applied for reporting AEs. NCI CTCEA v3.0 (as pfd) and instruction how to use NCI CTCAE v3.0 can be found on http://ctep.info.nih.gov/reporting/ctc.html. This will not be incorporated into the protocol.

The CTCAE, v3.0 includes 28 categories of adverse events with more than 900 individual adverse events.

Each AE can be graded according to its severity. CTCAE v3.0 categories the grades based on these general guideline:
Grade 1 mild AE
Grade 2 Moderate AE

Grade 3 Severe AE
Grade 4 Life Threatening or disabling AE
Grade 5 Death related to AE

Note:
Any treatment-related adverse event experienced by a patient is graded using the specific adverse event terms listed in the NCI CTCAE.
Grading is not modified based on a patient's condition at baseline
If a given adverse event is experience more than once during a cycle, only the grade associated with the most severe adverse event is reported.
Adverse events not included in the NCI CTCAE, v3.0 should be reported and graded as "Other" and graded 1-5.

18.3 Documentation and report of serious unexpected events (SAEs)
In compliance with EU-Directive 2001/20/EC and ICH Guidelines for Good clinical Practice (GCP), Serious Adverse Events should be reported immediately using the protocol specific SAE form.

All events have to be reported during the trial treatment and up to 30 days after the last dose of treatment.

Exemptions from reporting as SAEs
The following events are expected under protocol treatment, and if they resolve and do not require life saving intervention, are not considered as serious adverse events:
Neutropenia and neutropenic fever
Infection and fever
Haematological toxicity
Hospitalization due to severe myelosuppression, systemic infections, and/or feeding problems

18.4 SAE reporting after the end of trial treatment
During the follow-up phase (starting 30 days after treatment termination), the following events have to be reported:
Fatalities and severe events possibly, probably or definitely related to late effects of therapy
Disabling events
Secondary primary cancer

18.5 SAE Reporting Procedures and Time Limits
The center investigator or designee must fax any SAE occurrence defined by the protocol, using the protocol specific SAE form, within 24 hours of knowledge of the event to the data center to be reviewed by the international principal coordinator. This initial SAE form can be preceded with a follow-up form detailing any additional information. This follow-up SAE form should be faxed to the Data Center within 8 days.

18.6 SUSAR Reporting Procedures and Time Limits
The coordinating Principal investigator or designee of each country ensures the competent authorities (CA), main ethical committee (EC) and investigators [participating within his/her own country are informed of all serious unexpected suspected adverse reaction (SUSAR) and all other relevant safety information in accordance with definitions and lime limits set by the EU-directive 2001/20/EC as implemented into National Laws

All relevant information about SUSARs that are fatal or life-threatening must be reported as soon as possible to the CA, Main EC and to all investigators involved in the clinical trial and in any case no later than seven days after knowledge by the data center of such a case. Relevant follow-up information is subsequently communicated within an additional eight days.
All other SUSARs shall be reported to the CA, main EC and to all investigators as soon as possible but within a maximum of 15 days of first knowledge by the data center.

18.7 Annual Safety Reporting

An annual safety report with a line listing of all suspected serious adverse reactions (SAR) including SUSARs, an aggregate summary tabulation of suspected SARs which occurred in the trial and a report of the subject safety will be compiled by the data center. It is the responsibility of the coordinating Principal investigator or designee of each country to forward this annual report onto their national competent authorities (CA), main ethical committee (EC).

19. Publication and other policies

19.1 Authors on abstracts and manuscripts:

Final main publications to be written by the international study coordinators; the manuscript on the main study question (primary endpoint) will be written by the P.I..
Members of the steering committee of the protocol will be included.
Clinical contact persons of each of the participating groups, if a total of at least 10 patients has been included from that group in the Relapsed AML 2001/01 study, will be included.
International statistician.
Manuscripts concerning add-on studies only will include those who made a significant contribution to that particular study, including the P.I. and co-P.I. in case clinical data are being used, according to international guidelines for authorship.

19.2 Acknowledgements in manuscripts:

All of those who made a significant contribution to the preparation, execution and/or analysis of the study, not included as a co-author, will be mentioned in the acknowledgements. Examples are: data managers of each of the participating groups, and clinical representatives of groups who did not fulfil the requirements for co-authorship. In addition, the International BFM Study Group will be mentioned as the platform on which the study has been performed.

19.3 Other guidelines:

Any publication, abstract or presentation based on patients included in the international studies: approval is required by both international study coordinators.
Publications (including abstracts, presentations etc.) comparing the randomised treatment arms and/or concerning study end-points by individual groups are not allowed, until final results of the international studies have been published. An exception will be made if the international study coordinators fail to submit a manuscript fo publication within 3 years after closure of the patient enrollment.
Participating groups are obliged to send all required patient data every 6 months to the central data office in Hannover. Groups who fail to do so will risk to loose their status as collaborating group.

20. Ethical considerations

The appendices contain an example of patient/parent information and an informed consent form. This will be obligatory to have, however, local policies will determine final details about these procedures.

I-BFM-SG/AML Relapse Working Group: Study Pediatric Relapsed AML 2009/01

21. Translational research, add-on studies

These will be developed separately

22. Sponsors and monitoring

Hannover Medical School (Hannover, Germany) will be the international sponsor of this study. In addition, local co-sponsors will have to be identified, normally representing the national study group in question. Certain tasks will be delegated by the sponsor to the co-sponsors.

The co-sponsor in each country will provide insurance or indemnity in accordance with the applicable regulatory requirements for all patients within that country.

Any investigator or co-investigator who signed this protocol agrees to carry out this research in accordance with the protocol approved by the ethic committee, GCP and regulatory requirements.

Study personnel involved in conducting this trial will be qualified by education, training, and experience to perform their respective task(s).

Each local investigator should provide the co-sponsor with his CV. The co-sponsors assure the sponsor that this has been taken care off.

The Sponsor and the P.I. have the right to prematurely discontinue the study for significant efficacy or safety problems and will notify the co-sponsors and co-investigators in writing, as well as the ethics committees and the competent authorities according to local law and regulations.

Monitors will ensure that the clinical trial is conducted, recorded, and reported in accordance with the protocol, ICH-GCP, and the applicable regulatory requirement(s). The sponsor will delegate monitoring activities to national/local co-sponsors.

Representatives of the sponsor and/or co-sponsor must be allowed to visit all study site locations periodically to assess the data, quality and study integrity. On site they will review study records and directly compare them with source documents, and discuss the conduct of the study with the Investigator, and verify that the facilities remain acceptable. Source documents are defined as: patient files, letters, laboratory / histology records. The type and scope of monitoring will be defined in the Monitoring Manual and documented on study specific Source Data Verification Forms.

With his participation in the study the investigator is obligated to support the activities of the monitors, provide them with direct access to the files and give them the opportunity to inspect the laboratory facilities, storage of the investigational product, etc.

Reference List

(1) Abrahamsson J, Clausen N, Gustafsson G, Hovi L, Jonmundsson G, Zeller B, Forestier E, Heldrup J, Hasle H. Improved outcome after relapse in children with acute myeloid leukaemia. Br J Haematol 2007; 136(2):229-236.

(2) Aladjidi N, Auvrignon A, Leblanc T, Perel Y, Benard A, Bordigoni P, Gandemer V, Thuret I, Dalle JH, Piguet C, Pautard B, Baruchel A, Leverger G. Outcome in children with relapsed acute myeloid leukemia after initial treatment with the French Leucemie Aique Myeloide Enfant (LAME) 89/91 protocol of the French Society of Pediatric Hematology and Immunology. J Clin Oncol 2003; 21(23):4377-4385.

(3) Sievers EL, Larson RA, Stadtmauer EA, Estey E, Lowenberg B, Dombret H, Karanes C, Theobald M, Bennett JM, Sherman ML, Berger MS, Eten CB, Loken MR, van Dongen JJ, Bernstein ID, Appelbaum FR. Efficacy and safety of gemtuzumab ozogamicin in patients with CD33- positive acute myeloid leukemia in first relapse. J Clin Oncol 2001; 19:3244-3254.

(4) Zwaan ChM, Reinhardt D, Corbacioglu S, van Wering ER, Bokkerink JP, Tissing WJ, Samuelsson U, Feingold J, Creutzig U, Kaspers GJ. Gemtuzumab ozogamicin: first clinical experiences in children with relapsed/refractory acute myeloid leukemia treated on compassionate-use basis. Blood 2003; 101(10):3868-3871.

(5) Kell WJ, Burnett AK, Chopra R, Yin JAL, Clark RE, Rohatiner A, Culligan D, Hunter A, Prentice AG, Milligan DW. A feasibility study of simultaneous administration of gemtuzumab ozogamicin with intensive chemotherapy in induction and consolidation in younger patients with acute myeloid leukemia. Blood 2003; 102(13):4277-4283.

(6) Kaspers GJ, Zimmermann M, Fleischhack G, Tamminga R, Gibson B, Armendariz H, Dworzak M, Ha S, Hovi L, Maschan A, Philippe N, Razzouk B, Rizzari C, Smisek P, Smith O, Stark B, Will A, Creutzig U. Relapsed acute myeloid leukemia in children and adolescents: Interim report of the international randomised phase III study relapsed AML 2001/01. Blood 2006; 108(11):570A.

(7) Hinman LM, Hamann PR, Wallace R, Menendez AT, Durr FE, Upeslacis J. Preparation and Characterization of Monoclonal-Antibody Conjugates of the Calicheamicins - A Novel and Potent Family of Antitumor Antibiotics. Cancer Research 1993; 53(14):3336-3342.

(8) Voutsadakis IA. Gemtuzumab Ozogamicin (CMA-676, Mylotarg) for the treatment of CD33(+) acute myeloid leukemia. Anti-Cancer Drugs 2002; 13(7):685-692.

(9) van der Velden VHJ, te Marvelde JG, Hoogeveen PG, Bernstein ID, Houtsmuller AB, Berger MS, van Dongen JJM. Targeting of the CD33-calicheamicin immunoconjugate Mylotarg (CMA-676) in acute myeloid leukemia: in vivo and in vitro saturation and internalization by leukemic and normal myeloid cells. Blood 2001; 97(10):3197-3204.

(10) Dowell JA, Korth-Bradley J, Liu H, King SP, Berger MS. Pharmacokinetics of gemtuzumab ozogamicin, an antibody-targeted chemotherapy agent for the treatment of patients with acute myeloid leukemia in first relapse. J Clin Pharmacol 2001; 41(11):1206-1214.

(11) Buckwalter M, Dowell JA, Korth-Bradley J, Gorovits B, Mayer PR. Pharmacokinetics of Gemtuzumab Ozogamicin as a Single-Agent Treatment of Pediatric Patients With Refractory or Relapsed Acute Myeloid Leukemia. J Clin Pharmacol 2004; 44(8):873-880.

(12) Appelbaum FR, Matthews DC, Eary JF, Badger CC, Kellogg M, Press OW, Martin PJ, Fisher DR, Nelp WB, Thomas ED, Bernstein ID. The Use of Radiolabeled Anti-Cd33 Antibody to Augment Marrow Irradiation Prior to Marrow Transplantation for Acute Myelogenous Leukemia. Transplantation 1992; 54(5):829-833.

I-BFM-SG/AML Relapse Working Group: Study Pediatric Relapsed AML 2009/01

(13) Weinthal JA, Lenarsky C, Goldman S. Central nervous system and plasma Gemtuzumab Ozogamicin (Mylotarg™, CMA-676) levels in a patient with medullary and extramedullary relapsed Acute Myeloid Leukemia: a case report. Blood 96. 2000. Ref Type: Abstract

(14) Sievers EL, Appelbaum FR, Spielberger RT, Forman SJ, Flowers D, Smith FO, Shannon-Dorcy K, Berger MS, Bernstein ID. Selective Ablation of Acute Myeloid Leukemia Using Antibody-Targeted Chemotherapy: A Phase I Study of an Anti-CD33 Calicheamicin Immunoconjugate. Blood 1999; 93(11):3678-3684.

(15) Sievers EL, Larson RA, Stadtmauer EA, Estey E, Lowenberg B, Dombret H, Karanes C, Theobald M, Bennett JM, Sherman ML, Berger MS, Eten CB, Loken MR, van Dongen JJM, Bernstein ID, Appelbaum FR. Efficacy and Safety of Gemtuzumab Ozogamicin in Patients With CD33-Positive Acute Myeloid Leukemia in First Relapse. J Clin Oncol 2001; 19(13):3244-3254.

(16) Larson RA, Boogaerts M, Estey E, Karanes C, Stadtmauer EA, Sievers EL, Mineur P, Bennett JM, Berger MS, Eten CB, Munteanu M, Loken MR, van Dongen JJM, Bernstein ID, Appelbaum FR. Antibody-targeted chemotherapy of older patients with acute myeloid leukemia in first relapse using Mylotarg (gemtuzumab ozogamicin). Leukemia 2002; 16(9):1627-1636.

(17) Larson RA, Sievers EL, Stadtmauer EA, Lowenberg B, Estey EH, Dombret H, Theobald M, Voliotis D, Bennett JM, Richie M, Leopold LH, Berger MS, Sherman ML, Loken MR, van Dongen JJM, Bernstein ID, Appelbaum FR. Final report of the efficacy and safety of gemtuzumab ozogamicin (Mylotarg) in patients with CD33-positive acute myeloid leukemia in first recurrence. Cancer 2005; 104(7):1442-1452.

(18) Zwaan CM, Reinhardt D, Corbacioglu S, van Wering ER, Bokkerink JPM, Tissing WJE, Samuelsson U, Feingold J, Creutzig U, Kaspers GJL. Gemtuzumab ozogamicin: first clinical experiences in children with relapsed/refractory acute myeloid leukemia treated on compassionate-use basis. Blood 2003; 101(10):3868-3871.

(19) Aplenc R, Alonzo TA, Gerbing RB, Lange BJ, Hurwitz CA, Wells RJ, Bernstein I, Buckley P, Krimmel K, Smith FO, Sievers EL, Arceci RJ; Children's Oncology Group. Safety and efficacy of gemtuzumab ozogamicin in combination with chemotherapy for pediatric acute myeloid leukemia: a report from the Children's Oncology Group. J Clin Oncol. 2008;26(14):2390-3295.

(20) Clavio M, Vignolo L, Albarello A, Varaldo R, Pierri I, Catania G, Balocco M, Michelis G, Miglino M, Manna A, Balleari E, Carella AM, Sessarego M, Van Lint MT, Bacigalupo A, Gobbi M. Adding low-dose gemtuzumab ozogamicin to fludarabine, Ara-C and idarubicin (MY-FLAI) may improve disease-free and overall survival in elderly patients with non-M3 acute myeloid leukaemia: results of a prospective, pilot, multi-centre trial and comparison with a historical cohort of patients. British Journal of Haematology 2007; 138(2):186-195.

(21) Bross PF, Beitz J, Chen G, Chen XH, Duffy E, Kieffer L, Roy S, Sridhara R, Rahman A, Williams G, Pazdur R. Approval summary: Gemtuzumab ozogamicin in relapsed acute myeloid leukemia. Clinical Cancer Research 2001; 7(6):1490-1496.

(22) Arceci RJ, Sande J, Lange B, Shannon K, Franklin J, Hutchinson R, Vik TA, Flowers D, Aplenc R, Berger MS, Sherman ML, Smith FO, Bernstein I, Sievers EL. Safety and efficacy of gemtuzumab ozogamicin in pediatric patients with advanced CD33(+) acute myeloid leukemia. Blood 2005; 106(4):1183-1188.

(23) Roman E, Cooney E, Harrison L, Militano O, Wolownik K, Hawks R, Foley S, Satwani P, Unal E, Bhatia M, Bradley B, Del Toro G, George D, Garvin J, van de Ven C, Cairo MS. Preliminary Results of the Safety of Immunotherapy with Gemtuzumab Ozogamicin following Reduced Intensity Allogeneic Stem Cell Transplant in Children with CD33+ Acute Myeloid Leukemia. Clin Cancer Res 2005; 11(19):7164s-77170.

(24) Brethon B, Yakouben K, Oudot C, Boutard P, Bruno B, Jérome C, Nelken B, de Lumley L, Bertrand Y, Dalle JH, Chevret S, Leblanc T, Baruchel A. Efficacy of

I-BFM-SG/AML Relapse Working Group: Study Pediatric Relapsed AML 2009/01 fractionated gemtuzumab ozogamicin combined with cytarabine in advanced childhood myeloid leukaemia. Br J Haematol. 2008;143(4):541-7.

Reference List

(1) Abrahamsson J, Clausen N, Gustafsson G et al. Improved outcome after relapse in children with acute myeloid leukaemia. Br J Haematol. 2007;136:229-236.

(2) Aladjidi N, Auvrignon A, Leblanc T et al. Outcome in children with relapsed acute myeloid leukemia after initial treatment with the French Leucemie Aique Myeloide Enfant (LAME) 89/91 protocol of the French Society of Pediatric Hematology and Immunology. J Clin Oncol. 2003;21:4377-4385.

(3) Sievers EL, Larson RA, Stadtmauer EA et al. Efficacy and safety of gemtuzumab ozogamicin in patients with CD33- positive acute myeloid leukemia in first relapse. J Clin Oncol. 2001;19:3244-3254.

(4) Zwaan ChM, Reinhardt D, Corbacioglu S et al. Gemtuzumab ozogamicin: first clinical experiences in children with relapsed/refractory acute myeloid leukemia treated on compassionate-use basis. Blood. 2003;101:3868-3871.

(5) Kell WJ, Burnett AK, Chopra R et al. A feasibility study of simultaneous administration of gemtuzumab ozogamicin with intensive chemotherapy in induction and consolidation in younger patients with acute myeloid leukemia. Blood. 2003;102:4277-4283.

(6) Kaspers GJ, Zimmermann M, Fleischhack G et al. Relapsed acute myeloid leukemia in children and adolescents: Interim report of the international randomised phase III study relapsed AML 2001/01. Blood. 2006;108:570A.

(7) Hinman LM, Hamann PR, Wallace R et al. Preparation and Characterization of Monoclonal-Antibody Conjugates of the Calicheamicins - A Novel and Potent Family of Antitumor Antibiotics. Cancer Research. 1993;53:3336-3342.

(8) Voutsadakis IA. Gemtuzumab Ozogamicin (CMA-676, Mylotarg) for the treatment of CD33(+) acute myeloid leukemia. Anti-Cancer Drugs. 2002;13:685-692.

(9) van der Velden VHJ, te Marvelde JG, Hoogeveen PG et al. Targeting of the CD33-calicheamicin immunoconjugate Mylotarg (CMA-676) in acute myeloid leukemia: in vivo and in vitro saturation and internalization by leukemic and normal myeloid cells. Blood. 2001;97:3197-3204.

(10) Dowell JA, Korth-Bradley J, Liu H, King SP, Berger MS. Pharmacokinetics of gemtuzumab ozogamicin, an antibody-targeted chemotherapy agent for the treatment of patients with acute myeloid leukemia in first relapse. J Clin Pharmacol. 2001;41:1206-1214.

(11) Buckwalter M, Dowell JA, Korth-Bradley J, Gorovits B, Mayer PR. Pharmacokinetics of Gemtuzumab Ozogamicin as a Single-Agent Treatment of Pediatric Patients With Refractory or Relapsed Acute Myeloid Leukemia. J Clin Pharmacol. 2004;44:873-880.

(12) Appelbaum FR, Matthews DC, Eary JF et al. The Use of Radiolabeled Anti-Cd33 Antibody to Augment Marrow Irradiation Prior to Marrow Transplantation for Acute Myelogenous Leukemia. Transplantation. 1992;54:829-833.

(13) Weinthal JA, Lenarsky C, and Goldman S. Central nervous system and plasma Gemtuzumab Ozogamicin (Mylotarg™, CMA-676) levels in a patient with medullary and extramedullary relapsed Acute Myeloid Leukemia: a case report. [abstract]. Blood. 2000;96.

(14) Sievers EL, Appelbaum FR, Spielberger RT et al. Selective Ablation of Acute Myeloid Leukemia Using Antibody-Targeted Chemotherapy: A Phase I Study of an Anti-CD33 Calicheamicin Immunoconjugate. Blood. 1999;93:3678-3684.

(15) Sievers EL, Larson RA, Stadtmauer EA et al. Efficacy and Safety of Gemtuzumab Ozogamicin in Patients With CD33-Positive Acute Myeloid Leukemia in First Relapse. J Clin Oncol. 2001;19:3244-3254.

(16) Larson RA, Boogaerts M, Estey E et al. Antibody-targeted chemotherapy of older patients with acute myeloid leukemia in first relapse using Mylotarg (gemtuzumab ozogamicin). Leukemia. 2002;16:1627-1636.

(17) Larson RA, Sievers EL, Stadtmauer EA et al. Final report of the efficacy and safety of gemtuzumab ozogamicin (Mylotarg) in patients with CD33-positive acute myeloid leukemia in first recurrence. Cancer. 2005;104:1442-1452.

(18) Zwaan CM, Reinhardt D, Corbacioglu S et al. Gemtuzumab ozogamicin: first clinical experiences in children with relapsed/refractory acute myeloid leukemia treated on compassionate-use basis. Blood. 2003;101:3868-3871.

(19) Aplenc R, Alonzo TA, Gerbing RB, Lange BJ, Hurwitz CA, Wells RJ, Bernstein I, Buckley P, Krimmel K, Smith FO, Sievers EL, Arceci RJ; Children's Oncology Group. Safety and efficacy of gemtuzumab ozogamicin in combination with chemotherapy for pediatric acute myeloid leukemia: a report from the Children's Oncology Group. J Clin Oncol. 2008;26(14):2390-3295.

(20) Kell WJ, Burnett AK, Chopra R et al. A feasibility study of simultaneous administration of gemtuzumab ozogamicin with intensive chemotherapy in induction and consolidation in younger patients with acute myeloid leukemia. Blood. 2003;102:4277-4283.

(21) Clavio M, Vignolo L, Albarello A et al. Adding low-dose gemtuzumab ozogamicin to fludarabine, Ara-C and idarubicin (MY-FLAI) may improve disease-free and overall survival in elderly patients with non-M3 acute myeloid leukaemia: results of a prospective, pilot, multi-centre trial and comparison with a historical cohort of patients. British Journal of Haematology. 2007;138:186-195.

(22) Bross PF, Beitz J, Chen G et al. Approval summary: Gemtuzumab ozogamicin in relapsed acute myeloid leukemia. Clinical Cancer Research. 2001;7:1490-1496.

(23) Arceci RJ, Sande J, Lange B et al. Safety and efficacy of gemtuzumab ozogamicin in pediatric patients with advanced CD33(+) acute myeloid leukemia. Blood. 2005;106:1183-1188.

(24) Roman E, Cooney E, Harrison L et al. Preliminary Results of the Safety of Immunotherapy with Gemtuzumab Ozogamicin following Reduced Intensity Allogeneic Stem Cell Transplant in Children with CD33+ Acute Myeloid Leukemia. Clin Cancer Res. 2005;11:7164s-77170.

Tsimberidou 2003

Alvarado 2003

Eom 2007

Chevalier 2008

McKoy et al., 2007

Robak 2000

Woods 2001

Locatelli 1994

I-BFM-SG/AML Relapse Working Group: Study Pediatric Relapsed AML 2009/01

24. Appendices

A. **Patient/Parent information and informed consent**
B. **Registration form and Randomisation Procedure**
C. **Serious adverse event form**
D. **Karnofsky and Lansky performance status scales**
E. **Flowchart of the protocol**

I-BFM-SG/AML Relapse Working Group: Study Pediatric Relapsed AML 2009/01
Appendix A Patient/parent information and informed consent

"A randomised phase III study on the treatment of children and adolescents with refractory or relapsed acute myeloid leukemia – TRIAL Relapsed AML 2009/01"

This is an example and has to be re-written according to local institution format and policies.

This form informs you on a clinical trial. Clinical trials include only those patients who choose to take part. Please take your time to decide if you want to participate in this study or not.

You/your child has unfortunately been diagnosed to have refractory or relapsed acute myeloid leukemia (AML). The chance of cure now is lower than it was at the moment of the initial diagnosis. The optimal treatment of refractory and relapsed AML in children is not available as yet. However, in a previous study, the chemotherapeutic combination of fludarabine and cytarabine (at high doses; FLA) with liposomal daunorubicin (DaunoXome®) was effective in a significant part of the patients. This combination is called DX-FLA. A relatively new drug, gemtuzumab ozogamicin or Mylotarg® also has antileukemic activity in AML. However, it is unknown if adding Mylotarg® to FLA-DX will lead to better treatment response and to an increased chance of cure in pediatric relapsed AML. Therefore, we want to compare both regimes, ie, DX-FLA and DX-FLA plus Mylotarg® in the first course of the treatment of your/your child's current disease. In order to be able to make a fair and unbiased comparison, fate should decide whether you/your child will be treated with DX-FLA or DX-FLA plus Mylotarg®. We call this a randomised study. Since about 420 patients should be included in this study, and because refractory/relapsed AML is rare in children, the study will be done in collaboration with most European countries and even some non-European centers. After the first course of treatment, all children that responded well will receive a second course consisting of FLA. If your child does not respond well to the first course of so-called reinduction treatment, or if she/he is not in so-called complete remission after the 2 courses, further treatment according to this protocol is considered useless, and you should talk with your doctor about further treatment options. After the 2 reinduction courses, patients in so-called complete remission should proceed to a stem cell transplantation from a donor if that is possible. If this takes time to organize, a 3rd course of treatment will be given, followed by the stem cell transplantation. The type of donor that will be used depends on the availability of a donor and the type of AML (relapsed or refractory, early or late relapse) that you/your child has. You will receive additional information on the stem cell transplantation later on. The possible side-effects of this chemotherapeutic treatment will essentially not be different from the side-effects associated with the treatment of the initial AML. However, Mylotarg® can cause liver toxicity in a few percent of children. The efficacy and toxicity of the treatment will therefore be carefully monitored.

In order to make progress in the treatment of AML, additional scientific research is necessary. Therefore, we ask for your permission to use left-over bone marrow and/or blood that was taken for necessary clinical tests. Sometimes cells are left-over, and sometimes not. The use of material that is left-over does not require additional procedures. We would like to study e.g. the presence of so-called minimal residual disease (AML cells that are not visible using the normal light microscope) in the course of the treatment, the detailed characteristics of your/your child's AML cells, and the cytotoxicity of old and new drugs on your/your child's AML cells as measured in the laboratory. Scientific studies that would require additional procedures will be

I-BFM-SG/AML Relapse Working Group: Study Pediatric Relapsed AML 2009/01 discussed with you separately, and information will also be given separately. All these scientific studies do not affect the treatment of you/your child. You should feel free to decide not to participate in these additional scientific studies, at any time. Such a decision will in no way affect the treatment of you/your child. Finally, all information that will be obtained in this study (including the additional scientific studies) will be analysed and reported anonymously, in order to protect your/your child's privacy.

I-BFM-SG/AML Relapse Working Group: Study Pediatric Relapsed AML 2009/01

Informed consent form with respect to:
"A randomised phase III study on the treatment of children and adolescents with refractory or relapsed acute myeloid leukemia – TRIAL Relapsed AML 2009/01"

☐ **Yes, I have been properly informed on treatment with this protocol, and I have been explained that the study aims at investigating the efficacy and toxicity of gemtuzumab ozogamicin (Mylotarg®) in relapsed/refractory childhood AML, and aims to offer a comprehensive treatment of this disease. I have received a copy of the patient information sheet.**

☐ **Yes, I agree with treatment according to the protocol, entitled: "A randomised phase III study on the treatment of children and adolescents with refractory or relapsed acute myeloid leukemia – TRIAL Relapsed AML 2009/01"**

☐ **Yes, I agree with randomisation for the 1st course of reinduction chemotherapy, either "FLA-DX" or "FLA-DX plus Mylotarg®"**

☐ **Yes, I agree to have the above mentioned additional scientific studies done on extra cells taken from my bone marrow and peripheral blood, as explained in the patient/parent information. It concerns cells that were taken at necessary routine tests, but that were left-over (which sometimes does and sometimes does not occur)**

☐ **Yes, I agree to have the required information, both on my disease as well as on the results of the treatment, transferred to the investigators, to allow an evaluation of the results of the treatment, and for scientific research. However, my (my child's) privacy is guaranteed and data will be transferred and reported anonymously.**

☐ **Yes, I know that I may withdraw my cooperation at any time without having to provide an explanation and without experiencing any disadvantage from it.**

Place and date

Hospital

Signature parents

Signature patient **(if applicable)**

Signature pediatric oncologist

I-BFM-SG/AML Relapse Working Group: Study Pediatric Relapsed AML 2009/01
Appendix B: Registration form and Randomisation Procedure

ONLY TO BE USED IF THE INTERNET PROCEDURE IS NOT AVAILABLE
Whenever possible, please use the internet for registration and randomisation:
http://www.mh-hannover.de/institute/biometrie/AML/LoginCoordinator.html
http://www.mh-hannover.de/institute/biometrie/AML/LoginInvestigator.html

To: Local Data Office page 2 for the Relapsed AML 2009/01 Study

FAX nr: E-mail:

Θ This is to inform our local data office for this study in relapsed/refractory AML, that we will include a patient (please fill in the details below) in this randomised phase III study, entitled:

"A randomised phase III study on the treatment of children and adolescents with refractory or relapsed acute myeloid leukemia – TRIAL Relapsed AML 2009/01"

I declare that the patient I will include is eligible for the Relapsed AML 2009/01 protocol for relapsed or refractory AML. I have obtained formal written informed consent, which I will forward soon.

This is to inform the principal investigators of this study in relapsed/refractory AML, that we will not include a patient (please fill in the details below) with relapsed/refractory AML in this randomised phase III study, because: a) randomisation was refused by the patient/parents, b) randomisation was refused by the doctor, c) the patient was not eligible, d) for other reasons (please indicate the reason)
(please encircle)

Treating Pediatric Oncologist:

Name:
Center:
Phone:
Fax:
E-mail:

Patient data: **a) primary refractory disease**
(please encircle) **b) refractory to other reinduction protocol after relapse**
b) first relapse **1) early 2) late**
c) subsequent relapse

Initials:
Date of birth:

PLEASE CONTACT YOUR LOCAL OR THE INTERNATIONAL DATA OFFICE FOR RANDOMISATION IN CASE THE INTERNET PROCEDURE IS NOT AVAILABLE

I-BFM-SG/AML Relapse Working Group: Study Pediatric Relapsed AML 2009/01
Appendix C: Serious adverse event form

To: International Data Office study Relapsed AML 2009/01
FAX: +49 – 251 835 6489
E-mail: zimmermann.martin@mh-hannover.de

And to: Local Data Office of the Own Group

FAX:

E-mail:

Date:

This is to inform you that we have observed a serious adverse event in a patient with relapsed or refractory AML, who was treated according to the international multicenter phase III study, entitled:

"A randomised phase III study on the treatment of children and adolescents with

refractory or relapsed acute myeloid leukemia – TRIAL Relapsed AML 2009/01"

Reporting pediatric oncologist/treating physician:

Name:

Center:

Phone:

Fax:

E-mail:

Please indicate:

- death	**Y/N**
- life-threatening	**Y/N**
- permanently disabling	**Y/N**
- need for hospitalization or prolongation of hospitalization	**Y/N**

Comments:

Lansky score: 0-16 years	
Fully active	100
Minor restriction in normal physical activity	90
Active, but tires more quickly	80
Both greater restriction and less time spent in active play	70
Minimal active play, busy with quieter activities	60
Gets dressed, but no active play, able to participate in all quiet play and activities	50
Mostly in bed, participates in quiet activities	40
In bed, needs assistance even for quiet play	30
Often sleeping, play limited to passive activity	20
No play, does not get out of bed	10
Unresponsive	0

Karnofsky score: 16 years and older	
Normal, no complaints, no evidence of disease	100
Able to carry on normal activities	90
Normal activity with effort	80
Cares for self, unable to carry on normal activity or to do active work	70
Requires occasional assistance, is able to care for most of own needs	60
Requires considerable assistance, frequent medical care	50
Disabled, requires special care/assistance	40
Severely disabled, hospitalization	30
Hospitalization, very sick, active treatment	20
Moribund, fatal processes in progression	10
Dead	0

Appendix E. Flowchart of the entire protocol.