

# **Myeloid Leukemia Down Syndrome 2006**

## **(ML DS 2006)**

**for the treatment of Myeloid Leukemia in children with Down Syndrome.**

International Cooperative Pediatric AML Study Group

Version 12, 2006

Start date: 01.01.2007

EUDRACT number: pending

**Name and address of sponsor:**

National sponsor

**International Principal Investigator:**

D Reinhardt, AML-BFM trials

Pediatric Hematology/Oncology

Hannover Medical School

Carl-Neuberg-Straße 1

D-30625 Hannover, Germany

+49 (0) 511 532 6720 / -9123

+49 (0) 511 532 9029

[reinhardt.dirk@mh-hannover.de](mailto:reinhardt.dirk@mh-hannover.de)

**Participating countries**

<b>Country</b>	<b>Cooperative group</b>	<b>National coordinator</b>
Austria	AML-BFM	M. Dworzak
Czech Republic	AML-BFM	J. Sary
Denmark	NOPHO	H. Hasle
Finland	NOPHO	H. Hasle
France	SFCE	A. Baruchel
Germany	AML-BFM	D. Reinhardt
Iceland	NOPHO	H. Hasle
Ireland	MRC	A. O'Marcaigh
Norway	NOPHO	H. Hasle
Sweden	NOPHO	H. Hasle
Switzerland	AML-BFM	JP. Bourquin
The Netherlands	DCOG	CM Zwaan
United Kingdom	MRC	D. Webb

**Associated members**

<b>Country</b>	<b>Cooperative group</b>	<b>National coordinator</b>
Poland		
Russia	Moscow-Minsk	
Israel		B. Stark
Japan		
Brazil		
Turkey		
Argentina	GATLA	

## 1 Confidentiality Statement / Disclaimer

This document describes a non-randomized, open, phase III clinical trial in children with Down Syndrome and 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 centre. This recommendation applies especially for patients with Down Syndrome because of their high risk for toxicity.

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 ethics 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.

**Signature:**

---

**Principle Investigator**

---

**Date**

## 2 Table of Contents

<b><u>1</u></b>	<b><u>Confidentiality Statement / Disclaimer .....</u></b>	<b><u>3</u></b>
<b><u>2</u></b>	<b><u>Table of Contents .....</u></b>	<b><u>4</u></b>
<b><u>3</u></b>	<b><u>Key contact names &amp; addresses .....</u></b>	<b><u>6</u></b>
<b><u>4</u></b>	<b><u>List of Abbreviations .....</u></b>	<b><u>9</u></b>
<b><u>5</u></b>	<b><u>Synopsis .....</u></b>	<b><u>10</u></b>
<b><u>6</u></b>	<b><u>Introduction.....</u></b>	<b><u>13</u></b>
<b><u>7</u></b>	<b><u>Rational for treatment elements in ML DS 2006 .....</u></b>	<b><u>16</u></b>
7.1	Dose Reduction of Anthracyclines .....	16
7.2	Dose Reduction for Children < 12 months or <12 kg .....	16
7.3	CNS Therapy .....	16
7.4	Maintenance Therapy .....	16
7.5	Continuation of Therapy / Dose Intensity .....	17
<b><u>8</u></b>	<b><u>Children with Down Syndrome and myeloid leukemia older than 4 years: the role of GATA1 mutations .....</u></b>	<b><u>18</u></b>
<b><u>9</u></b>	<b><u>Study Objectives .....</u></b>	<b><u>19</u></b>
9.1	Primary study objectives .....	19
9.2	Secondary study objectives .....	19
<b><u>10</u></b>	<b><u>Study Population.....</u></b>	<b><u>20</u></b>
10.1	Number of Subjects .....	20
10.2	Inclusion & Exclusion Criteria .....	20
10.3	Transient myeloproliferative disorder (TMD).....	21
<b><u>11</u></b>	<b><u>Patient Registration .....</u></b>	<b><u>22</u></b>
<b><u>12</u></b>	<b><u>Treatment Schedule.....</u></b>	<b><u>23</u></b>
12.1	Assessment prior to starting Treatment .....	23
12.2	Assessment during Treatment.....	23
12.3	CNS-prophylaxis.....	24
12.3.1	Primary CNS involvement.....	24
12.4	Cytoreductive pre-phase.....	24
12.5	Dosages for infants and children < 12 kg of bodyweight .....	25
12.6	Induction course 1 - AIE - .....	26

12.7	Course 2 - AI - .....	28
12.8	Course 3 – haM - .....	29
12.9	Course 4 – HA - .....	30
12.10	Duration of participation and follow-up .....	30
12.11	Relapse therapy .....	31
12.12	Early termination of the study, withdrawal of subjects.....	31
<b>13</b>	<b><u>Evaluation of Efficacy .....</u></b>	<b><u>32</u></b>
13.1	Definitions of efficacy parameters.....	32
13.2	Assessing treatment response .....	32
<b>14</b>	<b><u>Safety evaluation.....</u></b>	<b><u>33</u></b>
14.1	Definitions.....	33
14.2	Documentation and evaluation of adverse events (AE).....	33
14.3	Documentation and report of serious unexpected events (SAEs) .....	34
14.4	SAE reporting after the end of trial treatment .....	34
14.5	SAE Reporting Procedures and Time Limits .....	35
14.6	SUSAR Reporting Procedures and Time Limits .....	35
14.7	Annual Safety Reporting.....	35
<b>15</b>	<b><u>Statistics.....</u></b>	<b><u>36</u></b>
15.1	Principles and design .....	36
15.2	Target variables and statistical methods.....	36
15.3	Interim and final evaluations, immediate discontinuation of the whole trial 36	
15.4	Recruitment of patients.....	36
<b>16</b>	<b><u>Trial Monitoring &amp; Auditing.....</u></b>	<b><u>37</u></b>
<b>17</b>	<b><u>Publication Policy .....</u></b>	<b><u>37</u></b>
<b>18</b>	<b><u>Financing .....</u></b>	<b><u>37</u></b>
<b>19</b>	<b><u>Data Handling &amp; Record Keeping .....</u></b>	<b><u>37</u></b>
<b>20</b>	<b><u>Data monitoring and safety board .....</u></b>	<b><u>37</u></b>
<b>21</b>	<b><u>Bibliography .....</u></b>	<b><u>38</u></b>

### 3 Key contact names & addresses

<b>International Principal Investigator:</b> D Reinhardt AML-BFM trials Pediatric Hematology/Oncology Medical School Hannover Carl-Neuberg-Straße 1 D-30625 Hannover, Germany Tel: +49 (0) 511 532 6720 / -9123 Fax: +49 (0) 511 532 9029 Email: <a href="mailto:reinhardt.dirk@mh-hannover.de">reinhardt.dirk@mh-hannover.de</a>	
<b>National Principal investigators</b>	
<b>Dutch Childhood Leukemia Study Group (DCLSG)</b> CM Zwaan, MD, PhD Erasmus MC/Sophia Children's Hospital Dr Molewaterplein 60 3015 GJ Rotterdam Netherlands Tel: +31-10-463.6691 Fax: +31-20-463.6801 (fax) E-mail: <a href="mailto:c.m.zwaan@erasmusmc.nl">c.m.zwaan@erasmusmc.nl</a>	<b>Nordic Society of Pediatric Hematology and Oncology (NOPHO)</b> H Hasle, MD, PhD, Consultant Pediatric Hematologist, Department of Pediatrics Skejby Hospital, Aarhus University 8200 Aarhus N, Denmark Tel: +45 8949 6716 Fax: +45 8949 6023 E-mail: <a href="mailto:hasle@dadlnet.dk">hasle@dadlnet.dk</a>
<b>United Kingdom Medical Research Council</b> A.O'Marcaigh, MD Department of Haematology Our Lady's Hospital for Sick Children Crumlin, Dublin 12, Ireland Tel: +353-1-4096908 Fax: +353-1-4563041 Email: <a href="mailto:aengus.omarcaigh@olhsc.ie">aengus.omarcaigh@olhsc.ie</a>	<b>United Kingdom Medical Research Council</b> D.K. Webb Department of Haematology, Great Ormond Street Hospital for Children, London, UK. Tel: Fax: Email: <a href="mailto:david.webb@gosh.nhs.uk">david.webb@gosh.nhs.uk</a>
<b>Berlin-Frankfurt-Münster Study Group (BFM)</b> U Creutzig Department of Pediatric Hematology and Oncology, University Children's Hospital Muenster, Albert-Schweitzer-Str 33, D-48129 Muenster, Germany. Tel: +33 -1-42-49-9639 Fax: +33-1-42-49-9634 Email: <a href="mailto:ursula@creutzig.de">ursula@creutzig.de</a>	<b>Societe Francaise des Cancers de l'Enfant (SFCE)</b> A Baruchel Hospital St Louis, Dept. of Hematology Avenue Claude Vellefaux 75475 Paris Cedex 10, France Tel: +33 -1-42-49-9639 Fax: +33-1-42-49-9634 E-mail: <a href="mailto:a.baruchel@chu-stlouis.fr">a.baruchel@chu-stlouis.fr</a>

<b>Data safety and monitoring board</b>	
<p>Prof. Dr. Robert Arceci  ☎ 0251 / 83 52671  e-mail: <a href="mailto:arcecro@jhmi.edu">arcecro@jhmi.edu</a></p> <p>AOR Dr. Achim Heinecke  Institut für medizinische Informatik und Biomathematik  Universitätsklinikum Münster  Von-Esmarch-Str. 62  48129 Münster  ☎ 0251 / 83 - 5 52 64  e-mail: <a href="mailto:heineck@uni-muenster.de">heineck@uni-muenster.de</a></p> <p>Prof. Alan Gamis  Children's Mercy Hospital and Clinics,  Kansas City, Missouri, USA.  <a href="mailto:agamis@cmh.edu">agamis@cmh.edu</a></p>	
<b>GATA 1 mutation analysis</b>	<b>Statistician</b>
<p>Paresh Vyas FRCP, FRCPATH, DPhil,  The Weatherall Institute of Molecular Medicine  University of Oxford  John Radcliffe Hospital, Oxford, UK  Tel: +44 1865 222309  Fax: +44 1865 222 500  Email: <a href="mailto:paresh.vyas@imm.ox.ac.uk">paresh.vyas@imm.ox.ac.uk</a></p> <p>D. Reinhardt, AML-BFM Study group  Hannover Medical School  AML-BFM trials  Pediatric Hematology/Oncology  Medical School Hannover  Carl-Neuberg-Straße 1  D-30625 Hannover, Germany  Tel: +49 (0) 511 532 6720 / -9123  Fax +49 (0) 511 532 9029  Email: <a href="mailto:reinhardt.dirk@mh-hannover.de">reinhardt.dirk@mh-hannover.de</a></p>	<p>M. Zimmermann, Hannover  AML-BFM trials  Pediatric Hematology/Oncology  Medical School Hannover  Carl-Neuberg-Straße 1  D-30625 Hannover, Germany  Tel: +49 (0) 511 532 6720 / -9123  Fax +49 (0) 511 532 9029  Email: <a href="mailto:zimmermann.martin@mh-hannover.de">zimmermann.martin@mh-hannover.de</a></p>
<b>MORPHOLOGY REVIEW PANEL</b> H. Löffler Th. Haferlach D. Reinhardt	
<b>PHARMACY ADVISOR:</b> Dr. Claudia Langebrake, University Hospital Hamburg Eppendorf 20000 Hamburg	<b>MRD Laboratories:</b> GATA1s: Hannover; Oxford; Rotterdam, Paris WT1: Aarhus; Frankfurt Immunophenotyping: Hannover

<b>Cytogenetics</b> <b>Germany/ Europe:</b> J. Harbott, Giessen B. Schlegelberger, Hannover <b>Netherlands</b> Rotterdam <b>UK:</b> Oxford <b>France:</b> Paris <b>NOPHO:</b> Erik Forestier	
--	--



## 4 List of Abbreviations

A	Cytarabine, cytosine arabinoside, ARA-C	HD	high-dose
AE	adverse reaction	I	idarubicin
AI	cytarabine/idarubicin	IT	intrathecal
AIE	cytarabine/idarubicin/etoposide-phosphate	IV	intravenous
ALL	acute lymphoblastic leukemia	LP	lumbar puncture
AML	acute myeloid leukemia	M	mitoxantrone
BM	bone marrow	MDS	myelodysplastic syndrome
BME	Bone marrow aspirate	ML	myeloid leukemia
CA	competent authorities	MMR	
CNS	Central nervous system	MRD	minimal residual disease
CR	complete remission	NR	non-responder
CRP	C-reactive protein	OS	overall survival
CSF	cerebral spinal fluid	p	probability, significance
CT	computer tomography	pEFS	probability of event-free survival
DFS	Disease free survival	PO	oral
ML DS	myeloid leukemia of Down Syndrome	PR	partial remission
E	etoposide-phosphate	SAE	Serious adverse event
EC	ethics committee	SC	subcutaneous
ECHO	ECHO-cardiography	SE	standard error
EFS	event-free survival	SUSAR	Suspected unexpected adverse reaction
FAB M0	acute myeloid leukemia with minimal differentiation	TMD	Transient Myeloproliferative Disorder
FAB M1	acute myeloid leukemia without maturation	TRM	Treatment related mortality
FAB M2	acute myeloid leukemia with maturation	WBC	White Blood Count
FAB M3	acute promyelocytic leukemia (APL)	µl	microlitre
FAB M4	acute myelomonoblastic leukemia		
FAB M5	acute monoblastic leukemia		
FAB M6	acute erythroblastic leukemia		
FAB M7	acute megakaryoblastic leukemia		
GCP	Good clinical practice		
ha	Intermediate-dose cytarabine		
HA	high-dose cytarabine		

## 5 Synopsis

The study ML DS 2006 aims to standardize the treatment of children with DS ML in the participating countries. The aim is to achieve an 85% overall survival in this patient population.

### Primary Objectives:

- Standardization of treatment for all children with Down Syndrome and ML
- Optimization of the quality of supportive therapy
- Achievement of an overall survival of 85% in all participating institutions
- Establishment of an international network of coordinated research in ML DS

### Secondary Objectives:

- Reduction of toxicity without impairment of outcome
- Identification of prognostic factors concerning the risk of relapse, toxicity and poor outcome
- Establishment of a basis for further sequential trials with modified treatment

### Trial Design:

Multi-center, open-label, non-randomized trial with direct individual benefit

End-points:	Response rate (CR), event-free survival (EFS), disease-free survival (DFS), overall survival (OS), Treatment related mortality (TRM)
Projected accrual:	Estimated 25 patients/year
Projected total accrual:	150 patients
Expected duration of the trial:	6 years

### Selection of Patients:

#### Inclusion Criteria

- Age: > 6 months to 4 years of age with/without GATA1 mutation OR >4 years of age to 18 years of age with GATA1 mutation
- Patients, in the above age group, must have Down syndrome (DS) and Myeloid Leukemia (ML)
- Written informed consent

#### Exclusion Criteria

- Children without Down Syndrome
- Children with Down Syndrome and Transient Myeloproliferative Disorder (TMD)
- Children with Down Syndrome and Acute Lymphoblastic Leukemia (ALL)
- Accompanying diseases which do not allow therapy according to the protocol
- Pre-treatment >14 days with intensive induction therapy

Children with DS and AML lacking GATA1 mutation but older than 4 years will be registered as observation patient. Treatment has to be adapted individually by the clinician for these non-trial patients.

#### **Trial Treatment:**

Trial treatment consists of four blocks of chemotherapy (see. Fig.1):

Course 1	AIE (cytarabine/idarubicin/etoposide-phosphate)
Course 2	AI (cytarabine/idarubicin)
Course 3	hAM (intermediate-dose cytarabine (1g)/mitoxantrone)
Course 4	HA (high-dose cytarabine)

#### **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 (principle investigator) will evaluate toxicity reports on a regular basis and may stop the trial 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.

#### **Trial Conduct:**

The trial will be carried out according to the Declaration of Helsinki, the principles of Good Clinical Practice and the Directive 2001/20/EC of the European Parliament and of the Council of 4 April 2001.

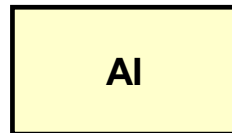
# Down Syndrome ML 2006

## Course 1



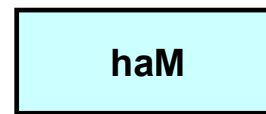
	day
cytarabine 100 mg/m <sup>2</sup> /d	1,2
cytarabine 100 mg/m <sup>2</sup> /12h	3-8
idarubicin 8 mg/m <sup>2</sup> /d	3,5,7
etoposide 150 mg/m <sup>2</sup> /d	6,7,8
cytarabine i.th.	1

## Course 2



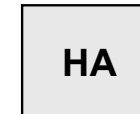
	day
cytarabine 500 mg/m <sup>2</sup> /d	1-4
idarubicin 5 mg/m <sup>2</sup> /d	3,5
cytarabine i.th.	1

## Course 3



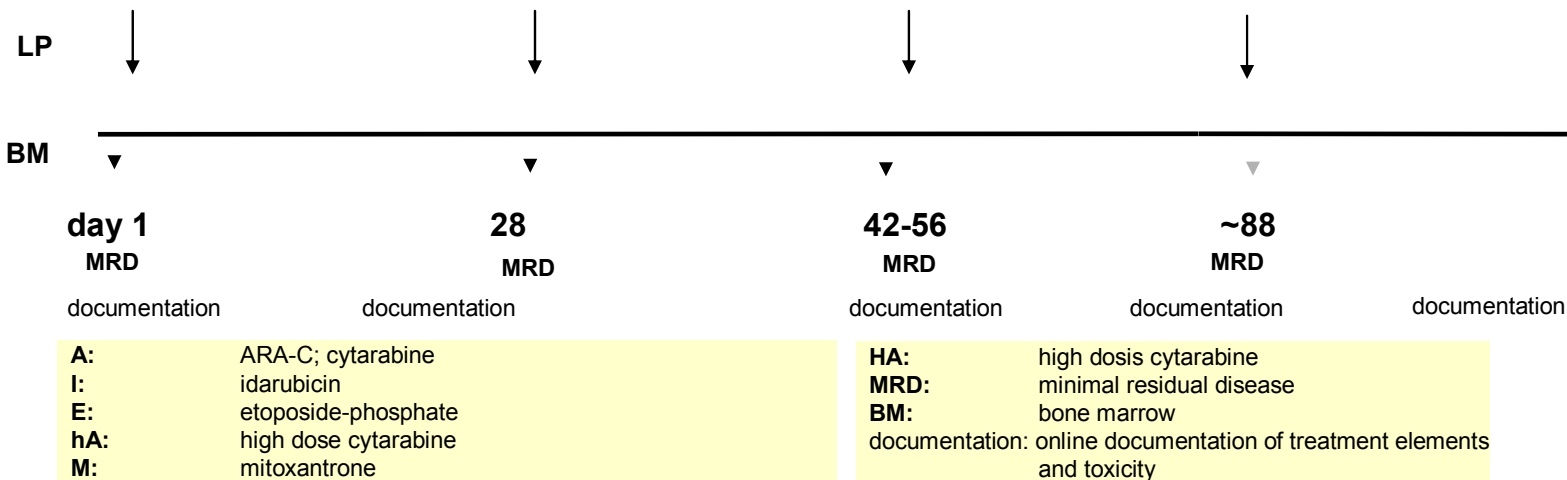
	day
HD-cytarabine 1 g/m <sup>2</sup> /12h	1-3
mitoxantrone 7 mg/m <sup>2</sup> /d	3, 4
cytarabine i.th.	1

## Course 4



	day
HD-cytarabine 3 g/m <sup>2</sup> /12h	1-3
cytarabine i.th.	1

In children with a bodyweight  $\leq 12$  kg, the dosages are calculated according to body weight



## 6 Introduction

Children with DS have a 40 fold increased risk of acute lymphoblastic Leukemia (ALL) and a 150 fold increased risk of myeloid leukemia (ML) before 5 years of age<sup>1</sup>. The peak incidence of ML in children with DS occurs under 4 years of age<sup>2,3</sup>, and in these patients a marked increase of the megakaryoblastic subtype (FAB M7) is seen<sup>4</sup>. Frequently, DS patients show a myelodysplastic phase preceding the development of AML<sup>4-6</sup> This should not be considered as separate disease but as the beginning of ML.

Ten percent of newborns with DS suffer a transient myeloproliferative disorder (TMD) which normally disappears spontaneously<sup>7</sup>. The median age at diagnosis is within the first week of life, and it is rarely diagnosed beyond 4 months of life.

In both, ML DS and TMD, mutations in exon2 of the hematopoietic transcriptions factor GATA1 have been detected. The resulting GATA1 s (short) lacks the activation domain.

TMD and ML DS show unique morphological features and a typical immunophenotype.

### Background

In the 1970s and 1980s, outcome in children with DS and ML was thought to be poor. First reports of high rates of event-free survival (EFS) for patients with DS and ML having intensive AML treatment were submitted by the Pediatric Oncology Group<sup>8</sup>. However, in children with DS, therapy was often suspended, sometimes due to diagnostic difficulties or due to the expectation of a lower tolerance of chemotherapy. During the 1980s, many patients did not receive any or only received symptomatic treatment and subsequently died. Only patients treated according to AML protocols showed an outcome comparable to patients without DS<sup>9</sup>. In patients with DS and Acute Lymphoblastic Leukemia (ALL), an approximately five-fold increased mortality during induction therapy, due to infections, has been reported<sup>2</sup>. In ML, children with DS suffered more toxicity when treated similarly to non-DS patients. This may be due to a higher constitutional susceptibility to cytotoxic drugs, especially cytarabine and anthracyclines.

### Results from the various AML study groups:

#### Medical Research Council (MRC): MRC AML 10 and AML 12 trials (1988-2002)

Children with DS and AML were treated according to the standard protocols, with no recommendation for dose reduction. The combined CR rate for both trials was 90%, with no resistant disease, however there were 10% induction deaths. Deaths in remission were 14% and relapse rate 2%. Overall and event free survival at 4 years were 76%.

There was a significant reduction in induction deaths and deaths in remission between trials, so that in AML 12 (1995 – 2002) overall and event free survival improved to 85%, from 56% in AML 10 (1988- 1995).

### **Dutch Childhood Oncology Group (DCOG)**

14 children with Down Syndrome (DS) and AML were treated according to the DCOG AML 82, 87 and 94 protocols. Half of them were younger than 2 years of age at diagnosis. The FAB-type distribution showed: FAB M0 – 7 patients, FAB M6 – 2 patients and FAB M7 – 5 patients. The clinical outcome improved over time, but numbers were too small for statistical analysis. In AML-87, the 5-yr pEFS 50% (SE 20%) compared to 71% (SE 17%) for AML-92/94 study. Overall the outcome for children with DS and AML was better than that for children without DS and AML [5-year pEFS 57% (SE 13%) vs. 38% (SE 3%)], but the difference did not reach statistical significance ( $p=0.27$ ) probably due to small numbers. From 1997 onwards children with AML were included in the MRC AML12 protocol.

### **German AML-BFM Study Group**

52 patients with DS and ML were entered into the AML-BFM 93 trial and 67 patients with DS and ML were entered into the AML-BFM 98 trial. In both trials, patients with DS and ML were treated according to the standard protocols, with reduced anthracyclines doses, with neither a second induction of high-dose cytarabine/mitoxantrone nor cranial irradiation. The modified protocol provided for longer intervals of recovery between courses of therapy. The cumulative dose of anthracyclines was 220-240mg/m<sup>2</sup> (non-DS patients 320-450mg/m<sup>2</sup>) and the cumulative dose of cytarabine were 23-29g/m<sup>2</sup> (non-DS patients 23-47g/m<sup>2</sup>).

The median age of DS patients was age 1.8; only 5 children were older than 4 years. 16 children had a history of transient myeloproliferative disorder (TMD). The majority of the patients had the typical morphologic picture of DS, i.e. AML FAB M6/M7 or M0. Three patients presented with FAB M1/2 and 2 patients with M4/5. In AML-BFM 93 study, 7 patients did not receive AML specific chemotherapy and treatment modifications in the other patients were more common, whereas in the study AML-BFM 98 study, only 1 child remained untreated. Results in the AML-BFM 98 study improved significantly when compared to AML-BFM 93. 3-year survival rates for patients treated on AML-BFM 98 were 90% (SE 4%) compared to 67% (SE 7%),  $p=0.001$ , in the AML-BFM 93 study. However, for children over 4 years of age (4 patients in the AML-BFM 93 study, 1 in the AML-BFM 98 study) prognosis was inferior (1 patient alive). The cumulative incidence of relapse was significantly lower in DS patients (7% for study 93, and 3% in study 98) compared to non-DS patients (28% in study 98, and 7% in study 98). Therapy related toxicity was lower or in the same range as in non-DS patients<sup>10</sup>.

### **NOPHO**

Fifty-six children with DS were treated on the Nordic Society for Pediatric Hematology and Oncology (NOPHO) AML88 and 93 protocols. In the dose intensive NOPHO-AML88 protocol, 8 out of 15 patients (53%) experienced an event. In the less dose-intensive NOPHO-AML93 protocol, 7 out of 41 patients (17%) had an event. The EFS increased from 47% in NOPHO-AML88 to 85% for children treated on NOPHO-AML93 (Hazard Ratio=4.7,  $p=0.005$ ). Therapy was reduced in 29 patients (52%) with an average of 75% and 67% of the scheduled dose of

anthracycline and cytarabine, respectively. Treatment-related death occurred in 7 patients, whom all received full treatment. Relapse and resistant disease occurred at a similar rate in those receiving full and reduced treatment<sup>11,12</sup>.

### **In Summary**

There is a wide variation in treatment intensity in the various international protocols. The British MRC group treated ML DS to the regular AML schedules with cumulative dosages of anthracyclines of 300mg/m<sup>2</sup> and cytarabine of 30g/m<sup>2</sup>. The NOPHO group administered 300mg/m<sup>2</sup> of anthracyclines and 49.6g/m<sup>2</sup> of cytarabine. The POG 8498 protocols included 405 mg/m<sup>2</sup> of anthracyclines and 38.5 g/m<sup>2</sup> of cytarabine. The CCG 2861 showed that in contrast to other children with AML, the standardized schedule with 350mg/m<sup>2</sup> of anthracyclines and 31.5g/m<sup>2</sup> of cytarabine revealed better results. The AML-BFM 93 and AML-BFM 98 studies recommended dose reduction in ML DS, which resulted in cumulative doses of anthracyclines of 220-240 mg/m<sup>2</sup> and 23-29g/m<sup>2</sup> depending on the treatment arm. This dose reduction did not adversely affect survival rates. Considering these results we conclude that the dogma in AML saying that “more is better”, is not true for DS-AML. Therefore, treatment intensity should be reduced.

## 7 Rational for treatment elements in ML DS 2006

Children with DS and ML have a relatively favourable prognosis if treated with an adequate risk-adapted therapy. This has been shown in several international studies<sup>9,12-15</sup>. These favourable results relate to children with DS, who develop AML FAB type M0/M6/M7 before their 4<sup>th</sup> birthday. Children who are older than 4 years seem to have a prognosis comparable to that of other ML patients and therefore may require individually adapted therapy.

### 7.1 Dose Reduction of Anthracyclines

Both cytarabine and anthracycline have been shown to be very effective in ML DS treatment, *in vivo* as well as *in vitro*<sup>16</sup>. Therefore, treatment elements of this protocol are mainly based on both of these drugs or their analogs. The experiences of the NOPHO and the AML-BFM trials favor a dose reduced regimen for patients with DS and ML compared to children with ML however without Down Syndrome. In the BFM approach, anthracyclines have been reduced to 2/3 of the original dosage. As the German, Austrian and Swiss BFM group includes most of the patients, and as they have achieved good results in the previous study AML-BFM 98, it has been chosen as the backbone of ML DS 2006.

### 7.2 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, DS ML 2006 recommends that children younger than 12 months of age, or weighing less than 12 kg, should be treated according to body weight rather than body surface area. Most pediatricians are more familiar with dose calculation according to body weight than body surface area in infants. In conclusion, the committee decided to calculate the dose reduction in younger children (body weight less than 12 kg) according to body weight.

### 7.3 CNS Therapy

As with the AML-BFM 93 and AML-BFM 98 studies, children with Down Syndrome will not receive CNS radiotherapy. Four doses of intrathecal therapy are included in the DS ML 2006 protocol.

### 7.4 Maintenance Therapy

Another specific treatment element of the BFM protocols is maintenance therapy, which is usually administered for 1 year. Considering the results and the low relapse rates of other study groups (MRC, NOPHO, POG), which treated without maintenance, a maintenance course has not been included in ML DS 2006. This was done in order to reduce toxicity and increase quality of life. The value of maintenance, especially in children with Down Syndrome,



is not finally defined; therefore the study committee has agreed not to reduce further treatment elements.

## **7.5 Continuation of Therapy / Dose Intensity**

The AML-BFM 98 achieved overall 5 year survival rate 89%<sup>17</sup>. The frequency of treatment related mortality in children with Down syndrome was 4.5%<sup>18</sup>, which is within the range of other children who have been treated according to the AML-BFM protocols. This was achieved by a close compliance with strict guidelines for treatment and supportive care and an intensive cooperation between the treating physicians/hospitals and the trial center. In contrast to non-DS children with AML, continuation of therapy was recommended only if the child was in a good general condition without infection, mucositis or fever, even if leukemic blasts had been seen in the bone marrow after induction. In contrast to non-DS children with AML, therefore, the DS ML 2006 protocol requires that each course should be started only if the child is in a good general condition without clinical signs of an infection, mucositis or fever.

## **8 Children with Down Syndrome and myeloid leukemia older than 4 years: the role of GATA1 mutations**

- **GATA1 mutation positive**

In children who are older than 4 years of age accurate diagnosis of GATA1 mutation is highly recommended. If a GATA1 mutation of the malignant clone could be proven, treatment according to this DS ML treatment protocol is recommended, as this probably reflects leukemia with the characteristic DS ML phenotype.

- **GATA1 mutation negative**

Children who are older than 4 years with myeloid leukemia lacking GATA1 mutation seem to have a prognosis comparable to other AML patients, and it is questionable whether they should be treated with reduced intensity therapy. These children will be registered in the protocol, but treatment should be discussed with the national coordinator. In most cases treatment according to the national AML trial for non-DS children will be applied. Leukemic blasts of children older than 4 years will be screened for the ML DS associated GATA1 mutation.

## 9 Study Objectives

The study ML DS 2006 aims at standardizing and optimizing the treatment of children with ML DS in the participating countries. The aim is to maintain at least an 85% overall survival in children with DS and ML, currently achieved in the national trials in several experienced institutions. Prognostic factors for relapse, toxicity and outcome shall be investigated.

Besides the cytotoxic therapy, ML DS 2006 seeks to optimize the quality of supportive therapy by implementing extensive recommendations and measures of quality assurance. This requires complete documentation of each therapy phase. The AML-BFM 98 trial demonstrated that with close compliance to strict guidelines for treatment and supportive care, treatment related mortality in children with Down syndrome was 4.5%<sup>19</sup>, which is similar to other children who have been treated according to the AML-BFM protocols. This was achieved by requesting strict compliance with supportive care guidelines, and an intensive cooperation between the treating physicians/hospital and the trial center/principal investigator. In contrast to non-DS children with AML, continuation of therapy was recommended only if the child was in a good general condition without infection, mucositis or fever, even if leukemic blasts were still present in the bone marrow after induction

A secondary aim is to set a basis for further trials to reduce treatment intensity (and toxicity) without impairing outcome.

### 9.1 Primary study objectives

1. Standardization of treatment for all children with Down Syndrome and ML
2. Achievement of an overall survival of 85% in all participating institutions
3. Optimization of the quality of supportive therapy
4. Establishment of an international network of coordinated research in ML DS

### 9.2 Secondary study objectives

1. Documentation and reduction of toxicity without impairment of outcome
2. Identification of prognostic factors concerning the risk of relapse, toxicity and poor outcome
3. Establishment of an international network for further sequential trials with modified treatment for children with ML DS.

## 10 Study Population

This study is an international multicenter trial with standardized treatment for children with ML DS. The participating countries are Germany, Austria, Switzerland, the Czech Republic (BFM), Great Britain, Ireland (MRC), the Netherlands (DCOG), Scandinavia (NOPHO) and France (SFCE).

### 10.1 Number of Subjects

This is a multi-centre, pan European study with an estimated annual recruitment of 25 children with ML DS. This results in an estimated accrual of 150 patients over 6 years.

### 10.2 Inclusion & Exclusion Criteria

#### Inclusion Criteria

- Patient at one of the participating hospitals
- Date of diagnosis: Diagnosed between 01.10.2006 and 30.09.2012
- Age:
  - > 6 months to 4 years of age
  - $\geq 4$  years of age to 18 years of age with GATA1 mutation
  - $\geq 4$  years without GATA1 mutated DS ML will be registered, but should be  
Offered treatment in consultation with the lead investigator
- Diagnosis of ML DS: Patients, in the above age group, must have Down Syndrome (DS) and Myeloid Leukemia's (ML) with evidence of megakaryoblasts, confirmed by morphology and immunophenotyping in the bone marrow or peripheral blood if bone marrow not possible
- Written informed consent must be obtained

**Children with DS and ML older than 4 years but lacking GATA1 mutation will be registered as observation patients and should not be treated according to this study, but according to regular AML treatment in consultation with the collaborative group lead investigator.**

#### Exclusion Criteria

- Children without Down Syndrome
- Children with Down Syndrome and Transient Myeloproliferative Disorder (TMD)
- Children with Down Syndrome and Acute Lymphoblastic Leukemia (ALL)
- Accompanying diseases which do not allow therapy according to the protocol
- Pre-treatment >14 days with intensive induction therapy
- Refusal of therapy or of important parts of therapy
- Pregnancy (testing only required if clinically indicated)/lactation

Patients who fulfill 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 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.

### **10.3 Transient myeloproliferative disorder (TMD)**

Neonates with Down syndrome often manifest a transient myeloproliferative disorder (TMD). According to a retrospective analysis of studies AML-BFM 87 –98, the median diagnostic age for TMD was 10 days. It is characteristic for TMD patients to show more blasts in the peripheral blood than in the bone marrow. If the child is asymptomatic it is not necessary to start therapy, as the majority of these patients show spontaneous remission. In the case of symptomatic disease, treatment with low-dose cytarabine (0.5 – 2 mg/kg for 4 to 7 days) can be considered. This also applies to cholestatic liver disease which may be indicative of impending liver fibrosis, which is a leading cause of death in children with TMD. As there is an increased risk (20% to 30%) for children with Down syndrome and TMD to develop ML between 1 and 4 years of age, regular blood counts should be undertaken during this period to guarantee the early diagnosis of ML. On condition that informed consent of the parents is obtained, the AML-BFM Trial Centre aims to register children with TMD.

## 11 Patient Registration

For online registration, the user must first apply for authorization to get access to the secured website at the coordinating center (MH-Hannover). A designated representative of a participating country or a collaborative trial group can apply for access at any time.

The website address is: <http://aml.mh-hannover.de/> ==> <sup>DS</sup><sub>ML</sub> ML DS 2006

A patient will be registered as protocol patient if the essential patient characteristics (date of diagnosis, date of birth, sex, confirmation of diagnosis) are provided, and if all inclusion criteria are fulfilled. If all inclusion criteria are fulfilled, but the child will not be treated according to the protocol, he/she will be registered for observation. This includes older children with DS and myeloid leukemia, for whom no results of GATA1 testing are available as yet.

## 12 Treatment Schedule

### 12.1 Assessment prior to starting Treatment

The following investigations are mandatory at the time of diagnosis:

- a) BONE MARROW ASPIRATE, with material being sent for:
  1. Morphology, including cytochemistry (periodic acid schiff, myeloperoxidase, esterase)
  2. Immunophenotyping to detect the following antigens: CD34, CD117, CD7, CD13, CD33, CD15, CD36, CD56, CD41, CD42b, CD61
  3. Cytogenetics
  4. Molecular genetics: GATA1-mutation should be analyzed centrally in the accredited laboratories. (see appendix)
  5. Base line bone marrow sample for MRD (see appendix)

**N.B. if any of these tests have been omitted on the first diagnostic samples, a second sample must be taken before treatment starts, if clinically possible. Analysis of peripheral blood blasts, and trephine biopsy are required if an adequate marrow sample cannot be obtained by aspiration.**

- b) Lumbar Puncture for CSF cytology
- c) Echocardiogram for LV function and to outrule congenital heart disease
- d) Abdominal ultrasound and chest x ray.
- e) FBC and peripheral blood smear for morphology
- f) Viral serologies for EBV, CMV, HSV, HHV6, Parvovirus B19, HIV 1 and 2, HAV, HBV, HCV, VZV.
- g) Weight
- h) Height

### 12.2 Assessment during Treatment

A bone marrow examination (MRD and Morphology) and lumbar puncture must be performed prior to the commencement of each course of chemotherapy.

## 12.3 CNS-prophylaxis

All children should receive CNS prophylaxis intrathecally at the start of each treatment block (4 doses in total). Monotherapy with cytarabine is recommended. Dosages should be adapted to age, as indicated in the table below:

Age	Dosage of intrathecally administered cytarabine for CNS-prophylaxis
< 1 year	20 mg
1-< 2 years of age	26 mg
2-≤ 3 years of age	34 mg
> 3 years of age	40 mg

### 12.3.1 Primary CNS involvement

Patients with primary CNS-involvement must receive weekly intrathecal cytarabine injections until the CSF is cleared of blasts, followed by 1 additional injection. A minimum of three injections should be given. After this initial treatment further intrathecal cytarabine injections are given according to the prophylactic schedule.

## 12.4 Cytoreductive pre-phase

Hyperleukocytosis is a rare event in children with ML DS, the frequency in the BFM group is 4% (WBC more than 100,000/ $\mu$ l) and 11% (WBC > 50,000/ $\mu$ l). Severe coagulopathy is not a major problem. Therefore, cytoreduction prior to definitive therapy in hyperleukocytosis (WBC > 50,000/ $\mu$ l) can be considered. Slow and careful cytoreduction can be achieved with pre-phase therapy consisting of 6-thioguanine (40 mg/m<sup>2</sup>/d PO) and cytarabine (40 mg/m<sup>2</sup>/day SC or IV). Additionally, hydroxyurea (20mg/kg/d) can be used. If there is no noticeable reduction in peripheral blood blasts by day 3, induction therapy should be started immediately. If a continuing risk of hemorrhage exists, it may be started in a modified form, i.e. with 50% of the idarubicin dose (4 mg/m<sup>2</sup>/day) in the AIE induction. The pre-phase should not last more than 7 days.

Other measures to prevent complications due to hyperleukocytosis include hyperhydration (2-3 litres/m<sup>2</sup>/day, please consider whether there are cardiac contra-indications against hyperhydration) and allopurinol administration. In case of hyperuricemia, rasburicase should be administered. Exchange transfusion or leukapheresis may be considered in children with severe clinical symptoms caused by hyperleukocytosis complicated by leucostasis, such as pulmonary or CNS symptoms. Do not perform a diagnostic lumbar puncture in case of hyperleukocytosis, given the risk of introduction of leukemia in the spinal fluid. Lumbar puncture should be delayed until sufficient blast reduction has been achieved, preferably when a WBC <10x10<sup>9</sup>/l has been reached.



## 12.5 Dosages for infants and children $\leq 12$ kg of bodyweight

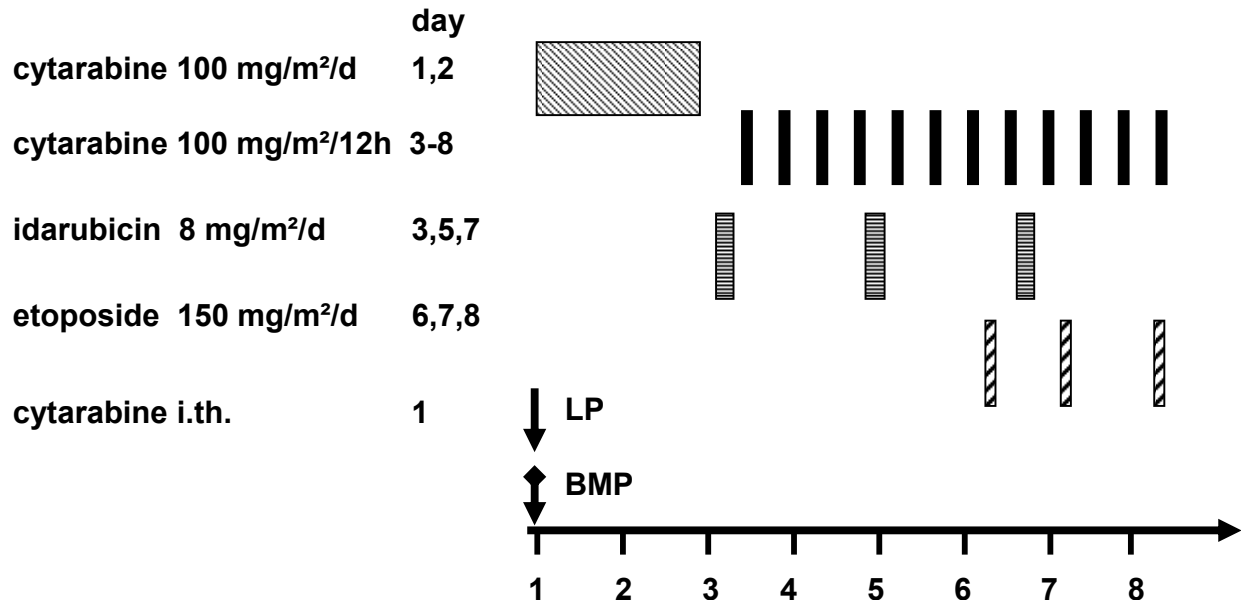
In children with a bodyweight  $\leq 12$  kg, the dosages are usually calculated according to body weight, and not to body surface area. The dosages given per  $m^2$  are to be divided by 30 to obtain the dose per kg., as indicated in the table below.

body weight	cytarabine				etoposide	idarubicin		mitoxantrone
	100 mg/ $m^2$	500 mg/ $m^2$	1000 mg/ $m^2$	3000 mg/ $m^2$	150 mg/ $m^2$	8 mg/ $m^2$	5 mg/ $m^2$	7 mg/ $m^2$
4.0 kg	13.3	67	133	400	20	1.1	0.7	0.9
4.5 kg	15.0	75	150	450	23	1.2	0.8	1.1
5.0 kg	16.7	83	167	500	25	1.3	0.8	1.2
5.5 kg	18.3	92	183	550	28	1.5	0.9	1.3
6.0 kg	20.0	100	200	600	30	1.6	1.0	1.4
6.5 kg	21.7	108	217	650	33	1.7	1.1	1.5
7.0 kg	23.3	117	233	700	35	1.9	1.2	1.6
7.5 kg	25.0	125	250	750	38	2.0	1.3	1.8
8.0 kg	26.7	133	267	800	40	2.1	1.3	1.9
8.5 kg	28.3	142	283	850	43	2.3	1.4	2.0
9.0 kg	30.0	150	300	900	45	2.4	1.5	2.1
9.5 kg	31.7	158	317	950	48	2.5	1.6	2.2
10.0 kg	33.3	167	333	1000	50	2.7	1.7	2.3
10.5 kg	35.0	175	350	1050	53	2.8	1.8	2.5
11.0 kg	36.7	183	367	1100	55	2.9	1.8	2.6
11.5 kg	38.3	192	383	1150	58	3.1	1.9	2.7
12.0 kg	40.0	200	400	1200	60	3.2	2.0	2.8

**Table 1 : Dose reduction in children with a body of less than or equal to 12 kg.**

## 12.6 Induction course 1 - AIE -

Commence Induction chemotherapy at diagnosis or immediately following cytoreductive pre-phase (WBC < 50,000/ $\mu$ l). In case of hyperleucocytosis the initial lumbar puncture should be delayed until sufficient blast reduction has been achieved (WBC < 10,000/ $\mu$ l).



Please note that for children with a body weight  $\leq 12$  kg or age <1 year, dosages if intravenous chemotherapy must be calculated per kg body weight and not according to body surface area.

**Cytarabine** 100 mg/m²/day by continuous IV infusion for 48 hours from morning of day 1 to the morning of day 3.

**Cytarabine** 100 mg/m² every 12 hrs by 30 minute IV infusion from day 3 to day 8 inclusive (12 doses total)

**Etoposide-phosphate or etoposide** 150 mg/m²/day by one hour iV infusion once every 24 hours on days 6, 7 and 8 (3 doses total)

Administer prior to the 8<sup>th</sup>, 10<sup>th</sup>, 12<sup>th</sup> cytarabine infusions.

**Idarubicin** 8 mg/m²/day by 4 hour IV infusion on days 3, 5 and 7, administer immediately prior to cytarabine (3 doses total),

**Cytarabine IT** Administer in age-related doses on Day 1 or at the time of the diagnostic LP (In the case of hyperleukocytosis and peripheral blasts, LP to be deferred until a satisfactory reduction in blasts)

<1 year of age give 20mg. 1-<2 yrs give 26mg. 2-≤ 3 yrs give 34mg. >3 yrs give 40mg.

**Primary CNS involvement**

Patients with primary CNS-involvement must receive weekly intrathecal cytarabine injections until the CSF is cleared of blasts, plus 1 additional injection. A minimum of three injections should be given. After this initial treatment further intrathecal cytarabine injections are given according to the prophylactic schedule. In case of CNS-involvement, triple intrathecal therapy with cytarabine, hydrocortisone and methotrexate can be considered.

## 12.7 Course 2 - AI -

AI therapy commences at least 4 weeks after the start of course 1 (induction).

### To commence this block of treatment the patient must

- be in good general condition and free of active infection, fever, and mucositis (even if >5% persistent blasts in marrow)
- have a neutrophile count  $\geq 1,000/\mu\text{l}$  and platelets  $\geq 80,000/\mu\text{l}$

### Investigations for Consolidation (AI)

- bone marrow exam (MRD and morphology) and lumbar puncture to be done on day 1

Please note that for children with a body weight  $\leq 12$  kg or age <1 year, dosages if intravenous chemotherapy must be calculated per kg body weight and not according to body surface area.

#### AI

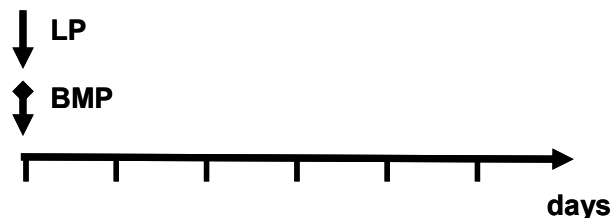
**cytarabine 500 mg/m<sup>2</sup>/day** day 1-4  
96 hrs cont. infusion



**idarubicin 5 mg/m<sup>2</sup>/day** day 3,5  
1 hr.-infusion



**cytarabine i.th.** day 1  
Age-related i.th. Cytarabine  
< 1 year 20 mg; 1-2 years 26 mg; 2-3 years 34 mg; >3 years 40 mg



**Cytarabine** 500 mg/m<sup>2</sup>/day by continuous 24 hour IV infusion on days 1 to 4 inclusive (96 hours total)

**Idarubicin** 5 mg/m<sup>2</sup> by 1hour IV infusion on day 3 and day 5 (2 doses) should be given without interrupting the continuous cytarabine infusion.

**Cytarabine IT** Administer in age-related doses on Day 1 as follows:  
<1 year of age give 20mg. 1-<2 yrs give 26mg. 2-≤ 3 yrs give 34mg.  
>3 yrs give 40mg.

## 12.8 Course 3 – haM -

haM therapy commences at least 4 weeks after start of course 2 (AI)

### To commence this block of treatment the patient must

- be in good general condition and free of active infection, fever, and mucositis
- have a neutrophil count  $\geq 1,000/\mu\text{l}$  and platelets  $\geq 80,000/\mu\text{l}$

### Investigations for Consolidation (hAM)

- Bone marrow exam (MRD and morphology) and lumbar puncture to be done on day 1
- ECHO

Please note that for children with a body weight  $\leq 12$  kg or age  $<1$  year, dosages if intravenous chemotherapy must be calculated per kg body weight and not according to body surface area.

#### hAM

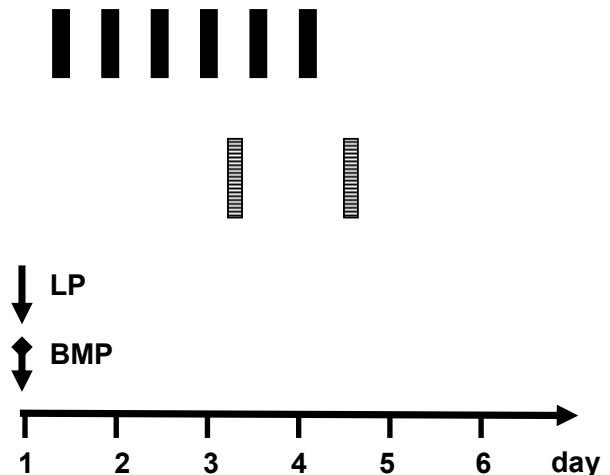
**HD-cytarabine 1 g/m<sup>2</sup> day 1-3**  
every 12 hours: 3 hr-infusion  
totally 6 times

**mitoxantrone 7 mg/m<sup>2</sup>/day day 3, 4**  
30min-infusion

**cytarabine i.th. day 1**

age-related i.th. cytarabine

< 1 year 20 mg; 1-2 years 26 mg; 2-3 years 34 mg; >3 years 40 mg



**Cytarabine** 1 g/m<sup>2</sup> by 3 hour IV infusion every 12 hours on days 1 to 3 inclusive (6 doses in total)

**Mitoxantrone** 7 mg/m<sup>2</sup>/day by 30 minute IV infusion once a day on days 3 and 4 (2 doses total)

**Cytarabine IT** Administer in age-related doses on Day 1 as follows:  
<1 year of age give 20mg. 1-<2 yrs give 26mg. 2-≤ 3 yrs give 34mg.  
>3 yrs give 40mg.

Apply artificial tears 2 drops/eye every 4-6 hours beginning 6 hrs before, and continuing until 12 hrs after the last dose of cytarabine. Alternatively the administration of topical steroids during therapy and for 24 hours after completion could be considered.

## 12.9 Course 4 – HA -

Intensification (HA) therapy commences at least 3 weeks, normally 4 weeks, after course 3

### To commence this block of treatment the patient must

- be in good general condition and free of active infection, fever, and mucositis
- have a neutrophile count  $\geq 1,000/\mu\text{l}$  and platelets  $\geq 80,000/\mu\text{l}$

### Investigations for Intensification (HA)

- bone marrow exam (MRD and morphology) and lumbar puncture to be done on day 1

Please note that for children with a body weight  $\leq 12$  kg or age  $<1$  year, dosages if intravenous chemotherapy must be calculated per kg body weight and not according to body surface area.

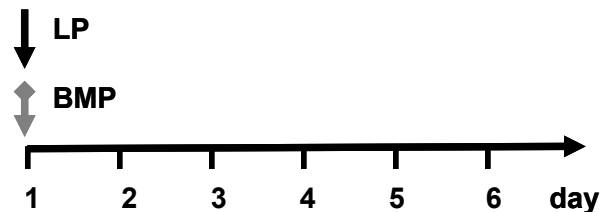
#### HA

**HD-cytarabine 3 g/m<sup>2</sup>**      **day 1-3**  
every 12 hours: 3 hr-infusion  
totally 6 times



#### cytarabine i.th. day 1

age-related i.th. cytarabine  
< 1 year 20 mg; 1-2 years 26 mg; 2-3 years 34 mg; >3 years 40 mg



**HD-Cytarabine**      3 g/m<sup>2</sup> by 3 hour IV infusion every 12 hours on days 1 to 3 inclusive (6 doses total)

**Cytarabine IT**      Administer in age-related doses on Day 1 as follows:

<1 year of age give 20mg. 1-<2 yrs give 26mg. 2-≤ 3 yrs give 34mg.  
>3 yrs give 40mg.

Apply artificial tears 2 drops/eye every 4-6 hours beginning 6 hrs before, and continuing until 12 hrs after the last dose of cytarabine. Alternatively the administration of topical steroids during therapy and for 24 hours after completion could be considered

## 12.10 Duration of participation and follow-up

Documentation for enrolled patients should be provided at diagnosis, during the intensive therapy phases, and 2 months after finishing treatment. After the completion of therapy, patients will be seen at regular intervals for clinical assessment and blood tests as clinically

indicated. An annual follow-up of each patient for the surveillance of late toxicity is part of the study. All cases of relapse or death should be documented at any time.

### **12.11 Relapse therapy**

Children with ML DS, who suffer from relapse, should be treated according to an individualized schedule, which takes the increased risk of toxicity and potential resistant disease into account. Stem cell transplantation should not be a standard practice, given the high risk of procedure related morbidity and mortality in DS children.

### **12.12 Early termination of the study, withdrawal of subjects**

A patient may be withdrawn from the study at any time for any of the following reasons;

1. Withdrawal of informed consent
2. The patient experiences an adverse event which, in the opinion of the investigator, does allow continuation of the trial medications.
3. If the investigator, for any reason, considers that it is not in the patient's best interest to continue.

Early termination of the study will occur in any of the following circumstances;

1. 85% survival rate is not achieved
2. If unacceptable toxicity is reported
3. If a significantly better treatment for children with ML DS becomes available.

## 13 Evaluation of Efficacy

### 13.1 Definitions of efficacy parameters

**Complete Remission (CR)** - The bone marrow is regenerating normal hemopoietic cells and contains <5% blasts cells by morphology, plus peripheral blood neutrophils  $\geq 1,000/\mu\text{l}$  and platelets  $\geq 80,000/\mu\text{l}$ .

**Partial Remission (PR)** - The bone marrow contains <5% blasts cell by morphology, but no hematological recovery (as needed for CR) is seen.

**Non-response (NR)** - Persistence of leukemic blasts (>5%) in bone marrow

**Relapse** - reoccurrence of leukemic blasts after CR was previously obtained (duration of previous CR of at least 4 weeks)

Criteria for the definition of therapy efficacy are the rate of complete remission, as well as the rates of event-free survival, disease-free survival and overall survival.

### 13.2 Assessing treatment response

Day 1 course 1	BME for morphology and MRD Lumbar Puncture
Day 1 of course 2 (AI)	BME for morphology and MRD Lumbar Puncture
Day 1 of course 3 (hAM)	BME for morphology and MRD Lumbar Puncture
Day 1 of course 4 (HA)	BME for morphology and MRD Lumbar Puncture



## 14 Safety evaluation

### 14.1 Definitions

#### 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.

#### 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.
- 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
- 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

#### 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).

### 14.2 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 pdf) 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.

### **14.3 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 the 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

### **14.4 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

## **14.5 SAE Reporting Procedures and Time Limits**

The centre 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 centre to be reviewed by the international principal coordinator. This initial SAE form can be proceeded with a follow-up form detailing any additional information. This follow-up SAE form should be faxed to the Data Centre within 8 days.

## **14.6 SUSAR Reporting Procedures and Time Limits**

The coordinating Principal investigator or designee of each country ensures the competent authorities (CA), main ethics 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 time 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 centre 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 centre.

## **14.7 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 centre. 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 ethics committee (EC).

## **15 Statistics**

### **15.1 Principles and design**

This concerns a non-randomized multicenter registry with treatment advice according to best available treatment.

### **15.2 Target variables and statistical methods**

The target variables of this registry are to document the rate of complete remission and the probabilities of event-free, disease-free and overall survival. The aim is to establish a standard treatment of ML DS in all European countries, and to achieve a 5-year overall survival of at least 85%. The estimated standard error should be less than 5%.

### **15.3 Interim and final evaluations, immediate discontinuation of the whole trial**

Interim analysis of response and safety will be performed after inclusion of 50 and 100 patients. The data monitoring committee will review the response rate, frequency of relapse and severe adverse events. The protocol and registry will be closed if the number of relapses is more than 12 after the first 50 patients and more than 24 after 100 patients. The number of deaths in remission must be less than 10 after inclusion of the first 50 patients, and less than 20 after 100 patients.

### **15.4 Recruitment of patients**

In total 150 children with ML DS will be recruited. The estimated duration of the trial is expected to be 6 years.

## 16 Trial Monitoring & Auditing

Each national leukemia/ collaborative group/ organization is responsible for sufficient monitoring and auditing in accordance with the national requirements and laws.

## 17 Publication Policy

The final report will be published approximately 3 years after enrollment of the last patient.

## 18 Financing

There is no external funding for this trial.

## 19 Data Handling & Record Keeping

Data entry should be performed via a secured web-based data base.

<http://aml.mh-hannover.de> ==> ML DS 2006.

The collaborative groups will define who is responsible for data-entry, depending on local circumstances. After registration, the national and international coordinator will be informed. In principle there are two options:

a) Data collection by the national group and yearly submission of a verified, anonymous dataset for central analysis and statistic reports.

b) direct submission of the data to the international database. Central monitoring of these data will be performed by the European center in Hannover.

## 20 Data monitoring and safety board

Prof. Dr. Robert Arceci

☎ 0251 / 83 52671

e-mail: [arcecro@jhmi.edu](mailto:arcecro@jhmi.edu)

AOR Dr. Achim Heinecke

Institut für medizinische Informatik und Biomathematik

Universitätsklinikum Münster

Von-Esmarch-Str. 62

48129 Münster

☎ 0251 / 83 - 5 52 64

e-mail: [heineck@uni-muenster.de](mailto:heineck@uni-muenster.de)

Prof. Alan Gamis

Children's Mercy Hospital and Clinics,

Kansas City, Missouri, USA.

[agamis@cmh.edu](mailto:agamis@cmh.edu)

## 21 Bibliography

### Reference List

- (1) Hasle H. Pattern of malignant disorders in individuals with Down's syndrome. *Lancet Oncol.* 2001;2:429-436.
- (2) Robison LL, Nesbit MEJ, Sather HN et al. Down syndrome and acute leukemia in children: a 10-year retrospective survey from Childrens Cancer Study Group. *J Pediatr.* 1984;105:235-242.
- (3) Kojima S, Matsuyama T, Sato T et al. Down's syndrome and acute leukemia in children: an analysis of phenotype by use of monoclonal antibodies and electron microscopic platelet peroxidase reaction. *Blood.* 1990;76:2348-2353.
- (4) Zipursky A, Thorner P, De Harven E, Christensen H, Doyle J. Myelodysplasia and acute megakaryoblastic leukemia in Down's syndrome. *Leuk Res.* 1994;18:163-171.
- (5) Zipursky A, Peeters M, Poon A. Megakaryoblastic leukemia and Down's syndrome: a review. *Pediatr Hematol Oncol.* 1987;4:211-230.
- (6) Hasle H, Niemeyer CM, Chessells JM et al. A pediatric approach to the WHO classification of myelodysplastic and myeloproliferative diseases. *Leukemia.* 2003;17:277-282.
- (7) Creutzig U, Ritter J, Vormoor J et al. Transiente Myeloproliferation und akute myeloische Leukämie bei Säuglingen mit Morbus Down. *Klin Pädiatr.* 1990;202:253-257.
- (8) Ravindranath Y, Abella E, Krischer JP et al. Acute myeloid leukemia (AML) in Down's syndrome is highly responsive to chemotherapy: experience on Pediatric Oncology Group AML Study 8498. *Blood.* 1992;80:2210-2214.
- (9) Creutzig U, Ritter J, Vormoor J et al. Myelodysplasia and acute myelogenous leukemia in Down's syndrome. A report of 40 children of the AML-BFM Study Group. *Leukemia.* 1996;10:1677-1686.
- (10) Creutzig U, Reinhardt D, Diekamp S et al. AML patients with Down syndrome have a high cure rate with AML-BFM therapy with reduced dose intensity. *Leukemia.* 2005;19:1355-1360.
- (11) Lie SO, Abrahamsson J, Clausen N et al. Treatment stratification based on initial in vivo response in acute myeloid leukaemia in children without Down's syndrome: results of NOPHO-AML trials. *Br J Haematol.* 2003;122:217-225.
- (12) Zeller B, Gustafsson G, Forestier E et al. Acute leukaemia in children with Down syndrome: a population-based Nordic study. *Br J Haematol.* 2005;128:797-804.
- (13) Lange BJ, Kobrinsky N, Barnard DR et al. Distinctive demography, biology, and outcome of acute myeloid leukemia and myelodysplastic syndrome in children with Down syndrome: Children's Cancer Group Studies 2861 and 2891. *Blood.* 1998;91:608-615.

- (14) Ravindranath Y, Taub JW. Down syndrome and acute myeloid leukemia. Lessons learned from experience with high-dose Ara-C containing regimens. *Adv Exp Med Biol.* 1999;457:409-414.
- (15) Taub JW, Huang X, Matherly LH et al. Expression of chromosome 21-localized genes in acute myeloid leukemia: differences between Down syndrome and non-Down syndrome blast cells and relationship to in vitro sensitivity to cytosine arabinoside and daunorubicin. *Blood.* 1999;94:1393-1400.
- (16) Zwaan CM, Kaspers GJ, Pieters R et al. Different drug sensitivity profiles of acute myeloid and lymphoblastic leukemia and normal peripheral blood mononuclear cells in children with and without Down syndrome. *Blood.* 2002;99:245-251.
- (17) Creutzig U, Reinhardt D, Zimmermann M, Dworzak M, and Sary J. Excellent Results In Patients With Downs Syndrome by Standard AML-BFM Therapy with Reduced Drug Intensity [abstract]. *Blood.* 2003;102.
- (18) Creutzig U, Reinhardt D, Lehnbecher T, and Zimmermann M. Patterns of Failures Related to Intensive Chemotherapy in Childhood AML: Analysis of the AML-BFM Clinical Trials [abstract]. *Blood.* 2002;100:258b.
- (19) Creutzig U, Reinhardt D, Lehnbecher T, and Zimmermann M. Patterns of Failures Related to Intensive Chemotherapy in Childhood AML: Analysis of the AML-BFM Clinical Trials [abstract]. *Blood.* 2002;100:258b.
- (20) Gobel U, Kornhuber B, Schellong G, Winkler K. [Recommendations for the structure and equipment of pediatric oncologic centers]. *Klin Padiatr.* 1991;203:195-205.
- (21) Richards MNCD, NHS Executive. Manual of Cancer Services Standards. National Health Service; 2002.
- (22) Hughes WT, Armstrong D, Bodey GP et al. 2002 guidelines for the use of antimicrobial agents in neutropenic patients with cancer. *Clin Infect Dis.* 2002;34:730-751.
- (23) Hughes WT, Armstrong D, Bodey GP et al. 2002 guidelines for the use of antimicrobial agents in neutropenic patients with cancer. *Clin Infect Dis.* 2002;34:730-751.
- (24) Lehnbecher T. [Intravenous immunoglobulins in the prevention of infection in children with hematologic-oncologic diseases]. *Klin Padiatr.* 2001;213 Suppl 1:A103-A105.
- (25) Lehnbecher T. [Hematopoietic growth factors in the prevention of infections complications in children with hematologic-oncologic diseases]. *Klin Padiatr.* 2001;213 Suppl 1:A88-A102.
- (26) Graubner UB. [Antiviral prophylaxis]. *Klin Pädiatr.* 2001;213 Suppl 1:A69-A76.
- (27) Freifeld AG, Walsh T, Marshall D et al. Monotherapy for fever and neutropenia in cancer patients: a randomized comparison of ceftazidime versus imipenem. *J Clin Oncol.* 1995;13:165-176.

- (28) Groll AH, Walsh TJ. Antifungal chemotherapy: advances and perspectives. Swiss Med Wkly. 2002;132:303-311.
- (29) Groll AH, Ritter J, Müller FM. [Prevention of fungal infections in children and adolescents with cancer]. Klin Pädiatr. 2001;213 Suppl 1:A50-A68.
- (30) Kern WV, Beyer J, Bohme A et al. [Prophylaxis of infection in neutropenic patients. Guidelines of the Working Party on Infections in Hematology and Oncology]. Dtsch Med Wochenschr. 2000;125:1582-1588.
- (31) Heidl M, Scholz H, Dörffel W, Hermann J. Antiviral therapy of varicella-zoster virus infection in immunocompromised children--a prospective randomized study of aciclovir versus brivudin. Infection. 1991;19:401-405.

<b>ICH Topic E 11</b> <b>Clinical Investigation of Medicinal Products in the Paediatric Population</b>
---

ICH Step 4

<b>NOTE FOR GUIDANCE ON CLINICAL INVESTIGATION OF MEDICINAL PRODUCTS IN THE PAEDIATRIC POPULATION (CPMP/ICH/2711/99)</b>
--



## APPENDIX 1

### I. Chemotherapeutic Drugs

NB: Information here is for general use only. Please check most recent manufacturer's information sheets (summary of product characteristics) relevant to the product you are using.

#### a. Cytarabine

Alternative Names:	Ara-C, Cytosar, cytosine arabinoside
Formulation:	Ready diluted vials, containing 100mg/ml. Also available as a 20mg/ml (100mg/5ml) which is used for low dose boluses.
Storage:	At room temperature.
Reconstitution:	Can further dilute with 0.9% saline for infusions
Stability:	According to local policy e.g. 7 days in fridge
Administration:	By direct IV injection (slow bolus) 100mg/m <sup>2</sup> 12 hourly
Toxicity:	Myelosuppression, nausea, vomiting, diarrhoea, gastro-intestinal inflammation and ulceration, abnormal liver function, fever, myalgia (difficulty walking or writing) and arthralgia (flu-like syndrome), sepsis, abdominal pain, urticaria and skin ulcers, abnormal renal function (painful urination or red urine), neuritis and CNS toxicity, headaches, pneumonia, shortness of breath, conjunctivitis, thinned or brittle hair, headache, loss of appetite or weight, fatigue, cough, sore throat, unusual bruising or bleeding, yellowing of the skin or eyes, blurred vision, black tarry stools
Precautions:	Co-administration with steroids relieves "flu" symptoms. Prednisone eye drops prevent/relieve ocular irritation at high doses > 1g/m <sup>2</sup> /day.

#### b. High-dose cytarabine (HD-cytarabine, >1g/m<sup>2</sup>)

See above, plus

**Effect on the nervous system.** At high doses, cytarabine can cause some damage to the nerves inside and outside the brain. This may cause a variety of symptoms, including fits (seizures) and mood changes.

#### c. Etoposide-phosphate

Alternative Names:	etopophos, Etoposide (VP-16)
Formulation:	20mg/ml solution
Reconstitution:	Ready mixed, but needs further dilution with 0.9% saline. Ideal final concentration should be 0.2-0.4 mg/ml
Storage:	At room temperature for 48 hours at this concentration.

Toxicity:	Myelosuppression, emesis, diarrhea, mucositis, anorexia, alopecia, hypertension following rapid intravenous infusion. Transient liver function abnormalities. Anaphylactic-like reaction with fever, chills, bronchospasm, dyspnea and tachycardia. Peripheral neuropathy, nausea and vomiting, loss of appetite, constipation, abdominal pain, changes in taste, fatigue, mouth blistering, unusual bruising or bleeding, dizziness, lightheadedness, or feeling of faintness pain at the injection site , persistent diarrhea or any change in normal bowel habits for more than 2 days, fever, chills, sore throat, shortness of breath, breathing, discomfort, rash, itching. This medicine often causes a temporary loss of hair. After treatment with etoposide has ended, normal hair growth should return.
Precautions:	Always check before and during infusion that the drug has not precipitated

#### **d. Idarubicin**

Alternative Names:	
Formulation:	5mg and 10mg vials as orange-red powder for reconstitution
Reconstitution:	Reconstitute powder with WFI then add 0.9% saline for infusion
Storage:	At 2-8°C in refrigerator
Stability:	Depends on local policy e.g. 7 days once reconstitution
Administration:	IV infusion (as per protocol)
Toxicity:	Black, tarry stools; blood in urine or stools; cough or hoarseness; fever or chills; lower back or side pain; painful or difficult urination; pinpoint red spots on skin; unusual bleeding or bruising , Sores in mouth and on lips, Diarrhea or stomach cramps; headache ; nausea and vomiting, Less common Joint pain , Darkening or redness of skin (after x-ray treatment); numbness or tingling of fingers, toes, or face, Fast or irregular heartbeat; shortness of breath; swelling of feet and lower legs, Fast or irregular heartbeat; pain at place of injection; shortness of breath; swelling of feet and lower legs, Rare Skin rash or hives, Stomach pain (severe) .This medicine often causes a temporary and total loss of hair. After treatment with idarubicin has ended, normal hair growth should return. Dose reduction may be required in hepatic or renal insufficiency. Cardiotoxicity – NB cumulative doses including other anthracyclines
Precautions:	Idarubicin causes the urine to turn reddish in color, which may stain clothes. This is not blood. It is perfectly normal and lasts for only a day or two after each dose is given

#### **e. Mitoxantrone**

Formulation:	10, 12.5 and 15ml vials at 2mg/ml concentration available.
--------------	--

Reconstitution:	Ready diluted – should be diluted further with at least 50ml of 0.9% saline or 5% dextrose
Storage:	At room temperature
Stability:	Depends on local policy and actual product used – varies between different manufacturers
Administration:	IV infusion (as per protocol)
Toxicity:	Myelosuppression, nausea, vomiting, cardiac toxicity, alopecia and mucositis.
Precautions:	Discolouration of urine and rarely, nails and skin (blue)

#### **f. 6-Thioguanine**

Alternative Names:	
Formulation:	40mg scored tablets and 10mg capsules on special order only
Storage:	At room temperature
Administration:	40mg/m <sup>2</sup> /day orally. Doses to be taken once a day one hour after food in the evening
Toxicity:	Bone marrow suppression, stomatitis, severe diarrhoea, hepatic toxicity, loss of vibration sense and unsteady gait
Precautions:	Thioguanine may be made up in a liquid form for those patients who cannot swallow tablets. The preparation once made up has a limited shelf life.

## APPENDIX 2

### II. Dose Modifications for Toxicity

#### a. Cytarabine

##### 'Ara-C Syndrome'

For fever, do not withhold cytarabine if fever is likely from cytarabine. Obtain blood culture if central line present. For rash or conjunctivitis, withhold for grade 3-4 toxicity until resolved.

##### Liver Dysfunction

If increase in hepatic transaminases (SGPT/ALT or SGOT/AST) to greater than 200U/L, obtain total bilirubin. Monitor SGPT/ALT or SGOT/ALT and total bilirubin, before each course of Ara-C. Continue full dose therapy unless either of the following occur:

1. Bilirubin >40
2. SGPT/ALT or SGOT/AST >100

on two determinations at least one week apart. If either of these occur, hold therapy with cytarabine and monitor as above weekly. Restart full dose therapy when transaminase is less than 200U/L if bilirubin is normal. Notify if the elevations persist for greater than two weeks

#### b. Etoposide-phosphate

##### Kidney Dysfunction

Grade 3-4 – Reduce dose to 75%

#### c. Etoposide

#### d. Idarubicin

Each dose of idarubicin of 10mg/m<sup>2</sup> should be tabulated as the isotoxic equivalent of 50mg/m<sup>2</sup> of daunorubicin or doxorubicin towards the lifetime maximum of 550mg/m<sup>2</sup> in patients with no prior cardiac irradiation. An echocardiogram should precede anthracycline therapy. Cardiac re-evaluation is recommended at a cumulative exposure of 270mg/m<sup>2</sup> and for each additional 50mg/m<sup>2</sup>.

If the maximum cumulative dose is achieved or the shortening fraction of ECHO decreases to < 25% or the ejection fraction decreases to < 55%, inform Trial Coordinator.

##### Hyperbilirubinemia

If total bilirubin >120 omit dose;  
if >90 but ≤ 120 give 25% of dose;  
if >50 but ≤ 90 give 50% of dose and  
if ≤ 50 give full dose.

##### Mucositis

Maintain strict oral hygiene

### **e. Mitoxantrone**

Each dose of mitoxantrone of  $10\text{mg}/\text{m}^2$  should be tabulated as the isotoxic equivalent of  $50\text{mg}/\text{m}^2$  of daunorubicin or adriamycin towards the lifetime maximum of  $550\text{mg}/\text{m}^2$  in patients with no prior cardiac irradiation.

An echocardiogram should precede anthracycline therapy. Prior anthracycline exposure, the initial baseline echocardiogram should be reviewed.

Cardiac re-evaluation is recommended at a cumulative exposure of  $270\text{mg}/\text{m}^2$  and each  $50\text{mg}/\text{m}^2$  following.

If the maximum cumulative dose is achieved or the shortening fraction of ECHO decreases to  $< 25\%$  or the ejection fraction decreases to  $< 55\%$ , inform Trial Coordinator.

#### Hyperbilirubinemia

- If total bilirubin  $>120$  omit dose;
- if  $>90$  but  $\leq 120$  give 25% of dose;
- if  $>50$  but  $\leq 90$  give 50% of dose and
- if  $\leq 50$  give full dose.

## **APPENDIX 3**

### **f. Drug Interactions**

## APPENDIX 4

### g. Toxicity Grading

According to the guideline of the National Cancer Institute



**Common Terminology Criteria for Adverse Events v3.0 (CTCAE)**  
Publish Date: August 9, 2006

**Link:**

[http://ctep.cancer.gov/reporting/ctc\\_v30.html](http://ctep.cancer.gov/reporting/ctc_v30.html)

## Appendix 5

### III. Supportive Care Guidelines

#### a. Introduction

Children with Down syndrome are at high risk for severe toxicity from intensive cytotoxic drugs. This is matched, however, by a high likelihood of cure. Supportive care is therefore of major importance in minimizing the effects of toxicity and increasing the likelihood of long term survival.

#### Therefore:

- Children with ML DS should only be treated in specialized pediatric hematology/oncology centers.
- Hospitals with little experience in treating AML should transfer the children to specialized centers.
- The treatment causes long-lasting periods of myelosuppression, and is associated with severe infections in the majority of children. Infections with streptococcus viridans and gram negative septicemia are common, and these infections have contributed to a treatment related mortality rate of approximately 5%.
- In children with Down syndrome, AML treatment should be continued only if the child is in a good general condition. Otherwise, infections or other complications such as mucositis should be resolved before the next course is commenced.
- 

#### b. Specialized centers

In view of the intensity of the AML treatment, a hospital can only be considered as experienced if it treats more than 15 children with intensive chemotherapy per annum.<sup>20,21</sup>

At least two experienced paediatric haematologists/oncologists are required to ensure optimal treatment and supportive care during the whole treatment phase. At any time, an experienced paediatric haematology/oncology nurse should be available. Laboratory facilities (and services) as well as radiological diagnostics (X-ray, computed tomography, ultrasound) must be available 24 hours a day including weekends.

#### c. Infection Guidelines

#### Tests to be performed at diagnosis and during the course of the disease

Serology: at diagnosis, routine diagnostics include the determination of specific antibodies against: EBV, CMV, HSV, HHV6, Parvo-B19, HIV1/2, HBV, HCV, HAV and VZV. In addition, determination of post-vaccination titers for: MMR, pertussis, poliomyelitis 1/2/3, diphtheria, tetanus and Hemophilus influenza type B, may be useful. During treatment, only serologic parameters which are clinically indicated should be determined.

Microbiological surveillance including regular cultures of urine, stools or nose and mouth swabs did not show to decrease toxicity, and can therefore are deemed unnecessary.

Radiology: Routine X-rays or ultrasound is not necessary in patients without clinical symptoms.



Infection parameters (CRP, procalcitonin, interleukin 6 or interleukin 8) should only be done in case of clinical symptoms, and not on a routine basis.

### Prophylactic antibiotic therapy

Prophylaxis with trimethoprim-sulfamethoxazole once daily on 3 days per week against *Pneumocystis carinii* pneumonia is useful and should be applied from the 1st course until three months after discontinuation of chemotherapy.

### Penicillin prophylaxis after high-dose cytarabine

According to our data and experience, penicillin prophylaxis following treatment with high-dose cytarabine can be useful if the local resistance profile is known. In hospitals with a high percentage of penicillin resistant species, oral cephalosporin therapy can be used.

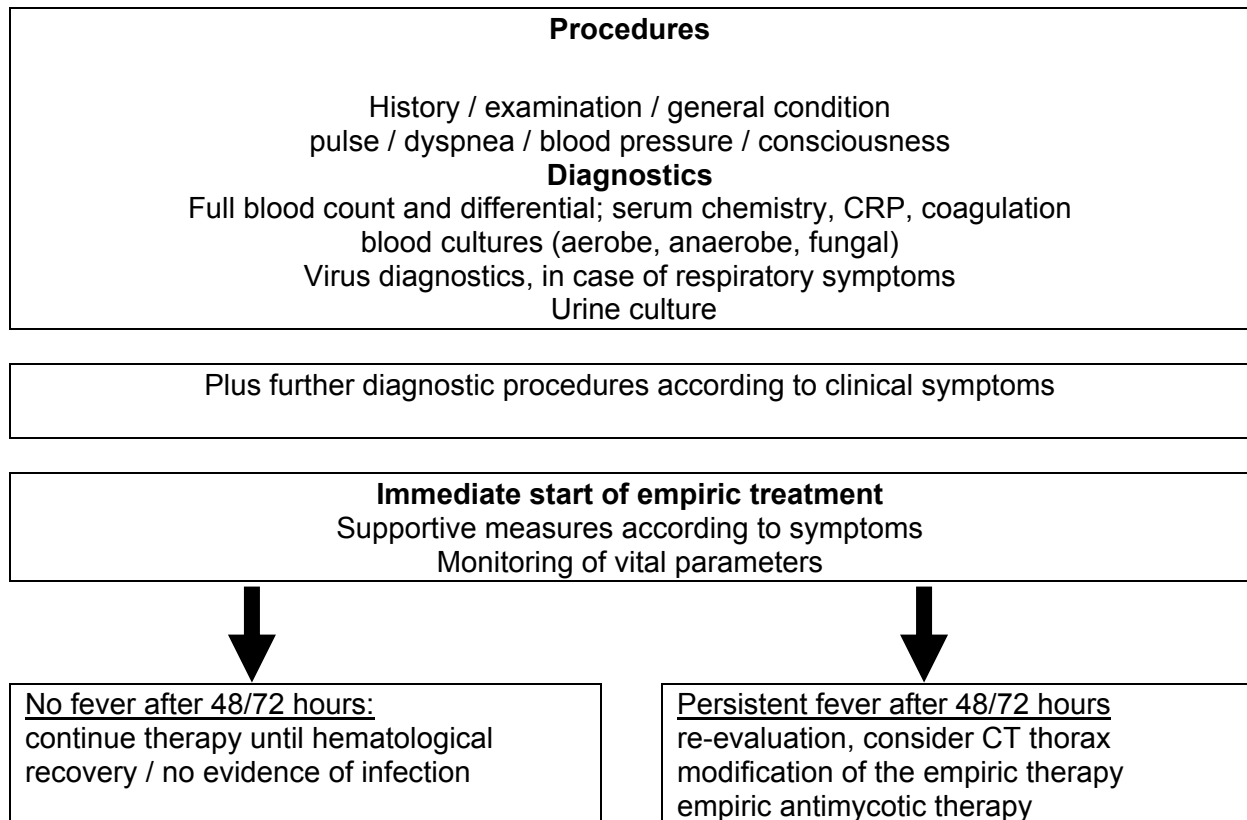
### Antimycotic prophylaxis

In view of the high morbidity and mortality due to invasive fungal infections, systemic oral prophylaxis with fluconazole (8 mg/kg/day; maximum 400 mg/day) should be used. In centres with a high incidence of aspergillosis, itraconazole or voriconazole is recommended. Antimycotic prophylaxis should be commenced after the end of the first course of therapy. It should not be given on days when chemotherapy is given, and it should be continued until 3 months after the discontinuation of chemotherapy.

Drug	Dosage		Comment
	oral	IV.	
Fluconazole	8 mg/kg/day (max. 400 mg/d)	4-8mg	<ul style="list-style-type: none"> <li>• approved for children</li> </ul>
Itraconazole	Loading dose 2x5 mg/kg, continue with 2x 2.5 mg/kg (level control at day 7)		<ul style="list-style-type: none"> <li>• in centers with a high incidence of aspergillosis</li> <li>• not approved for children <sup>1</sup></li> <li>• Solution is better absorbable.</li> </ul>
Voriconazole	day 1: 2x 6 mg/kg/day, continue with : 2x 4 mg/kg/day	day 1: 2x 6 mg/kg/day, continue with 2x 4 mg/kg/d	<ul style="list-style-type: none"> <li>• children &gt; 13 years day 1: 2x400 mg/d PO, continue with: 2x200 mg/day PO</li> <li>• approved for children older than 2 years</li> </ul>

<sup>1</sup> Several trials in children have supported the usefulness of the drug.

## Empiric therapy for febrile neutropaenia



## Empiric antibiotics

The following combinations are recommended as initial empiric therapy for febrile neutropenia

1. an aminoglycoside (gentamycin, tobramycin, amikacin) and a carboxy or ureido-penicillin (mezlocillin, piperacillin), or a 3<sup>rd</sup> generation cephalosporin <sup>22</sup>.

or

2. Monotherapy with Imipenem, Meropenem or Ceftazidime might be an alternative.

A glycopeptide should be used in fever and neutropenia following high-dose cytarabine or in hospitals with a high prevalence of betalactam resistant alpha-hemolytic streptococcus, and in children with severe infections of central catheters <sup>23</sup>.

Empiric therapy should be modified according to the results of microbiology, resistance testing, and clinical response. In children with symptoms of a septicemia or septic shock, a broad combination of imipenem or meropenem plus aminoglycoside and vancomycin or teicoplanin should be considered <sup>24-27</sup>.

**Suggested modifications of the empiric regimen**

<b><u>Results / symptoms</u></b>	<b><u>Modification</u></b>
<b>Blood culture positive:</b> Gram-positive species Gram-negative species Fungus	add a glycopeptide add an aminoglycoside add antifungal therapy
<b>Severe mucositis / gingivitis</b>	add metronidazole or clindamycin (for anaerobes);  add acyclovir (for herpes simplex)
<b>Esophageal symptoms</b>	add fluconazole (for candida); if refractory, add amphotericin B; consider acyclovir for herpes
<b>Abdominal, perirectal or perianal pain</b>	add metronidazol, clindamycin, or a glycopeptide
<b>Focal pulmonary infiltration</b>	diagnostics: broncho alveolar lavage (BAL) add antifungal therapy; if necessary consider mycoplasma, chlamydia, legionella and add macrolides / chinolones
<b>Interstitial pneumonitis</b>	consider CMV, PCP, adenovirus, others; consider empiric start of therapeutic dose of TMP/SMZ.
<b>sepsis, septic shock</b>	Add carbapenem/ aminoglycoside/ glycopeptide

### Empiric antifungal therapy

Antifungal therapy should be considered in children with radiological symptoms of invasive fungal infection or in children with persistent fever and neutropaenia which is refractory to empiric antibiotic therapy. The following table summarizes some antifungal agents that may be used.

Drug	Dosage		Comment
	oral	IV	
Amphotericin B		0.6 mg/kg/day	<ul style="list-style-type: none"> <li>approved for children</li> </ul>
Fluconazole	8 mg/kg/day (max. 400 mg/day)	8 mg/kg/day (max. 400 mg/day)	
Itraconazole	loading dose 2x5 mg/kg afterwards 2x 2.5 mg/kg (level control day 7)		<ul style="list-style-type: none"> <li>High incidence of aspergillosis</li> <li>not approved for children <sup>1</sup></li> </ul>
Voriconazole	day 1: 2x 6 mg/kg/d > day 2: 2x 4 mg/kg/d	day1: 2x 6 mg/kg/d > day 2: 2x 4 mg/kg/day	<ul style="list-style-type: none"> <li>children &gt; 13 years day 1: 2x400 mg/d &gt; day 2: 2x200 mg/day</li> <li>approved for children &gt; 2 years; CNS effective</li> </ul>
Liposomal amphotericin		3-5 mg/kg	<ul style="list-style-type: none"> <li>high dose in case of CNS infection</li> <li>6-10 mg/kg/day</li> </ul>
Caspofungin		Day 1, 70 mg/day afterwards 50 mg/day	<ul style="list-style-type: none"> <li>1 – 1.5 mg/kg/d max. daily dose: 50 mg<sup>2</sup></li> <li>not tested for CNS infection</li> </ul>

<sup>1</sup> Several trials in children have supported the usefulness of the drug.

<sup>2</sup> Ongoing phase I study: 1 – 1.5 mg/kg/day, maximum dose: 50 mg/day

## Treatment of proven invasive fungal infections

For invasive candida infection, use amphotericin B or, if not previously treated with azoles, fluconazole. Alternatives: liposomal amphotericin B, voriconazole, or caspofungin (not approved for children to date).

For invasive aspergillosis, use voriconazole, amphotericin B, liposomal amphotericin B, IV itraconazole or IV caspofungin.

Infection	Drug	Dosage	Comment
		IV	
candidiasis	amphotericin B	0.7 to 1.0 mg/kg	
	fluconazole	12 mg/kg max. 16 mg/kg	<ul style="list-style-type: none"> <li>max. 800 mg/day</li> <li>If not pretreated with azoles</li> </ul>
	voriconazole	day 1: 2x 6 mg/kg/day > day 2: 2x 4 mg/kg/day	<ul style="list-style-type: none"> <li>children &gt; 13 years. day 1: 2x400 mg/d &gt; day 2: 2x200 mg/day</li> <li>approved for children &gt; 2 years</li> </ul>
	liposomal amphotericin B	3-5 mg/kg	
	caspofungin	not known	<ul style="list-style-type: none"> <li>1 – 1.5 mg/kg/day max. daily dose: 50 mg<sup>2</sup></li> </ul>
aspergillosis	amphotericin B	1.0 to 1.5 mg/kg	
	voriconazole	day 1: 2x 6 mg/kg/day > day 2: 2x 4 mg/kg/day	<ul style="list-style-type: none"> <li>children &gt; 13 years. day 1: 2x400 mg/d &gt; day 2: 2x200 mg/day</li> <li>approved for children &gt; 2 years</li> </ul>
	Liposomal amphotericin B	5 mg/kg	
	itraconazole	loading dose 2x5 mg/kg 2x 2,5 mg/kg	<ul style="list-style-type: none"> <li>not approved <sup>1</sup></li> </ul>
	caspofungin	day 1:70 mg/day, afterwards 50mg/day	<ul style="list-style-type: none"> <li>1 – 1.5 mg/kg/day, max. daily dose: 50 mg<sup>2</sup></li> </ul>

<sup>1</sup> Several trials in children have supported the usefulness of the drug.

<sup>2</sup> Ongoing phase I study: 1 – 1.5 mg/kg/day, maximum dose. 50 mg/day

Groll AH, Walsh TJ. Antifungal Chemotherapy: Recent advances and current perspectives. Swiss Medical Weekly 2002; 132: 303-311 <sup>28</sup>

Groll AH, Ritter J, Mueller FMC. Prevention of fungal infections in children and adolescents with cancer. German Society for Pediatric Oncology and Hematology/ German Pediatric Infectious Disease Society. Klin Paediatr 2001; 213S1: A50-A68 <sup>29</sup>

Kern WV et al. [Prophylaxis of infection in neutropenic patients. Guidelines of the Working Party on Infections in Hematology and Oncology] Dtsch Med Wochenschr. 2000 22;125(51-52):1582-8<sup>30</sup>

## **Further supportive care**

### **Immunoglobulins**

The routine use of prophylactic administration of immunoglobulins is not recommended.

### **Cytokines (G-CSF)**

There is no evidence that the routine use of G-CSF increases survival or reduces morbidity. There might be a limited shortening of neutropenia duration, but this does not translate into increased survival. Colony stimulating factors may be used in case of severe infections on an individual basis.

### **Antiviral prophylaxis**

Antiviral prophylaxis is not recommended. However, in children with a history of a severe HSV infection during treatment, a secondary prophylaxis with acyclovir may be considered. During chemotherapy, patients exposed to other people suffering from chickenpox or varicella zoster may be treated with varizella-zoster hyper-immunoglobulin (1 ml/kg within the first 24 to 72 hours [maximum: 96 hours] after the contact). Oral brivudin might be an alternative.<sup>31</sup>

## APPENDIX 6

### IV. Biological Tests and Sampling

The concerted action of this international group should optimize the network for basic and translational research. Patients' samples and material should be provided to qualified groups of scientists. The different groups should exchange and combine their data to achieve synergistic progress in leukemogenesis, epidemiology and treatment. All available leukemic cell samples (usually left-over cells after performing the required diagnostic procedures) should be registered in a central database, to which all members of the scientific committee will have access (<http://aml.mh-hannover.de/> ==> **material bank**). Gathering, preparation and storing of material should be organized centrally. The coordinator is responsible to the study committee and has to report annually on ongoing projects and research results. Storage of these cells can only take place when informed consent of the legal guardians is available. These cells can be provided to promising research projects. Interested groups should submit a proposal of their planned project, which will be reviewed by an external expert group. The projects will be coordinated by scientific committee (D. Reinhardt, P. Vyas, CM Zwaan). The scientific committee will decide about the amount, priority and kind of material which can be provided according to the recommendation of the external review panel.

#### a. Storage and documentation

Left-over cells from diagnostic procedures which is not necessary for diagnosis or patient care should be stored by the national reference laboratories, according to the standard operating procedures. Storage of these cells can only take place when informed consent of the legal guardians is available. The nature and amount of this left-over material should be registered in the central web-based data base: The national coordinators and the scientific groups have access to the database to keep overview about available samples. Scientists / research groups who provide an interesting research proposal which is supported by the external scientific advisors will be supplied with this material to perform biological studies. The scientific committee is responsible for the optimal use of material and the prevention of overlapping projects.



## **Appendix 7**

### **V. Add-on studies**

CM. Zwaan and MM. van den Heuvel-Eibrink: **Clinical relevance of genetic alterations in Myeloid Leukemia of Down syndrome (DS ML)**

Henrik Hasle: **Minimal residual disease measured by WT1 expression in patients with Down syndrome and myeloid leukemia treated on the international Down Syndrome Myeloid Leukemia 2006 study**

combined with:

Reinhardt/ Reinhardt: **Minimal residual disease measured by the quantification of the patient specific GATA1s (RT-PCR) and by immunophenotyping**

Langebrake/ Reinhardt: **Influence of GATA1s on leukemiogenesis in ML DS**

Paresh Vyas: **Defining the hemopoietic defect in patients with Down syndrome and myeloid leukaemia treated on the international Down syndrome Myeloid Leukemia 2006 study**  
(tissue culture system)

## **APPENDIX 8**

### **VI. Ethical Considerations (inc. Information sheets and Consent Forms)**

### **VII. Ethical and National Regulatory Approval**

This trial must have the approval of the local ethics committee and national regulatory body before patients can be enrolled in any participating country.

### **VIII. Concordance with the Declaration of Helsinki and Good Clinical Practice (GCP)**

The trial will be conducted in accordance with the Declaration of Helsinki (1964), amended Republic of South Africa (1996). The European ICH guidelines for “Good Clinical Practice” will be adopted. The patient is informed of the objectives and of the contents of the trial in order to give a conscious and reliable consent to participation in this study. The study protocol is submitted to the competent regional local committees in accordance with the current national statutory and/or regional laws and regulations.

### **IX. Consent forms and patient information**

Prior to obtaining the patient’s and/or guardian’s written consent to participate in the trial, a full explanation, in language and terms they are able to understand, must be given of the treatment options, including the conventional and generally accepted methods of treatment and the manner of treatment allocation. If the patient is a minor (under 16 years of age) and does not have the capacity to understand the trial, fully informed consent must be received from his/her guardian. However, if a minor has the capacity to understand the treatment and its outcome, assent should be obtained from the patient, along with consent from his/her guardian.

In all cases, participants must be made aware of their rights to decline to participate or to withdraw from the study at any time. This right to refuse to participate in the trial without giving reasons must be respected. In addition, the patient has the right to withdraw his/her data from the study without giving reasons and without prejudicing his/her further treatment.

All patients and/or their guardians must give written consent to inclusion in the trial, data processing, sending of diagnostic material to reference institutions. This property must be handled in accordance with national data protection legislation.

## **X. Confidentiality of Patient Information**

In order to preserve the confidentiality with respect to patient taking part in this study, a unique code will be assigned to each patient. Only the patients' initials, date of birth, gender and the unique number will be used to identify the patient.

## **Down Syndrome Myeloid Leukaemia 2006 for the treatment of Myeloid in children with Down Syndrome (ML DS 2006)**

### **XI. Information Sheets**

**Dear Parent / Guardian,**

We would like to invite your child to take part in a research study. Before you decide whether you would like your child to take part it is important for you to understand why the research is being done and what it will involve. Please take time to read the following information carefully and discuss it with others if you wish. Ask us if there is anything that is not clear or if you would like more information. Take time to decide whether or not you wish your child to take part.

- 1. What is the purpose of the study?**
- 2. Why has my child been chosen?**
- 3. Does my child have to take part?**
- 4. What will happen if my child does take part?**
- 5. Will the study restrict my child's lifestyle?**
- 6. What is the drug being tested?**
- 7. What are the alternatives for treatment?**
- 8. What are the main side effects?**
- 9. Are there any risks? (What are the possible disadvantages and risks of taking part?)**
- 10. Are there any benefits?**
- 11. What if new information becomes available?**
- 12. What happens when the study stops?**
- 13. What if something goes wrong?**
- 14. Will taking part in this study be kept confidential?**
- 15. What will happen to the results of the research study?**
- 16. Who is organising this research?**
- 17. What if I have any concerns?**

Thank you for reading this information

(Form to be on Institution headed paper)

Addressogram:

**PARENT/CHILD CONSENT FORM**  
(Version No., Date)

**Title of Project: Down Syndrome Myeloid Leukaemia 2006 for the treatment of Myeloid Leukaemia in children with Down Syndrome (ML DS 2006)**

Name of Local Researcher:

*Please initial boxes*

1. I confirm that I have read and understood the information sheet(s), version no., dated...and have had the opportunity to ask questions and had satisfactory answers to them. ☐
2. I understand that my child's participation is voluntary and that I am free to withdraw at any time, without giving any reason, without his/her medical care or legal rights being affected. ☐
3. I understand that information from my child's medical notes may be looked at and information passed on to responsible individuals from cancer research bodies, where it is relevant to research, to the national registration bodies and to regulatory authorities where it is relevant to my child's participation in research. Relevant information will be collected and held securely and kept confidential. ☐
4. I agree for my child's additional tissue to be collected, stored and used for ethically-approved research. ☐
5. I agree for my child to take part in the above study ☐

\_\_\_\_\_  
Name of patient

\_\_\_\_\_  
Name of parent/guardian

\_\_\_\_\_  
Date

\_\_\_\_\_  
Signature

\_\_\_\_\_  
Researcher

\_\_\_\_\_  
Date

\_\_\_\_\_  
Signature

## **APPENDIX 9**

**XII. Trial Forms (treatment should be documented via remote-data entry ==>  
<http://aml.mh-hannover.de> => ML-DS 2006**

### **Registration form**

Documentation of chemotherapy

Documentation of toxicity / late toxicity