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

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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.

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2 Table of Contents

1 Confidentiality Statement / Disclaimer....3

2 Table of Contents....4

3 Key contact names & addresses....6

4 List of Abbreviations....9

5 Synopsis....10

6 Introduction....13

7 Rational for treatment elements in ML DS 2006....16

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

8 Children with Down Syndrome and myeloid leukemia older than 4 years: the role of GATA1 mutations....18

9 Study Objectives....19

9.1 Primary study objectives....19

9.2 Secondary study objectives....19

10 Study Population....20

10.1 Number of Subjects....20

10.2 Inclusion & Exclusion Criteria....20

10.3 Transient myeloproliferative disorder (TMD)....21

11 Patient Registration....22

12 Treatment Schedule....23

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

13 Evaluation of Efficacy.....32
13.1 Definitions of efficacy parameters.....32
13.2 Assessing treatment response.....32

14 Safety evaluation.....33
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

15 Statistics.....36
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

16 Trial Monitoring & Auditing.....37

17 Publication Policy.....37

18 Financing.....37

19 Data Handling & Record Keeping.....37

20 Data monitoring and safety board.....37

21 Bibliography.....38

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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

AIE

	day
cytarabine 100 mg/m²/d	1,2
cytarabine 100 mg/m²/12h	3-8
idarubicin 8 mg/m²/d	3,5,7
etoposide 150 mg/m²/d	6,7,8
cytarabine i.th.	1

Course 2

AI

	day
cytarabine 500 mg/m²/d	1-4
idarubicin 5 mg/m²/d	3,5
cytarabine i.th.	1

Course 3

haM

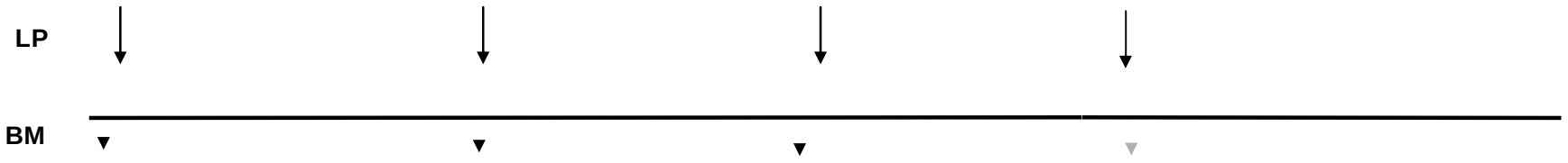
	day
HD-cytarabine 1 g/m²/12h	1-3
mitoxantrone 7 mg/m²/d	3, 4
cytarabine i.th.	1

Course 4

HA

	day
HD-cytarabine 3 g/m²/12h	1-3
cytarabine i.th.	1

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



A:	ARA-C; cytarabine
I:	idarubicin
E:	etoposide-phosphate
hA:	high dose cytarabine
M:	mitoxantrone

HA:	high dosis cytarabine
MRD:	minimal residual disease
BM:	bone marrow
documentation:	online documentation of treatment elements and toxicity

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 [1]. The peak incidence of ML in children with DS occurs under 4 years of age[2,3], and in these patients a marked increase of the megakaryoblastic subtype (FAB M7) is seen [4]. Frequently, DS patients show a myelodysplastic phase preceding the development of AML [4-6]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[7]. 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[8]. 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[9].
In patients with DS and Acute Lymphoblastic Leukemia (ALL), an approximately five-fold increased mortality during induction therapy, due to infections, has been reported[2]. 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/m² (non-DS patients 320-450mg/m²) and the cumulative dose of cytarabine were 23-29g/m² (non-DS patients 23-47g/m²).

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[10].

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[11,12].

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/m² and cytarabine of 30g/m². The NOPHO group administered 300mg/m² of anthracyclines and 49.6g/m² of cytarabine. The POG 8498 protocols included 405 mg/m² of anthracyclines and 38.5 g/m² of cytarabine. The CCG 2861 showed that in contrast to other children with AML, the standardized schedule with 350mg/m² of anthracyclines and 31.5g/m² 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/m² and 23-29g/m² 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[9,12-15]. These favourable results relate to children with DS, who develop AML FAB type M0/M6/M7 before their 4th 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* [16]. 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%[17]. The frequency of treatment related mortality in children with Down syndrome was 4.5%[18], 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 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%[19], 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
  - ≥ 4 years of age to 18 years of age with GATA1 mutation
  - ≥ 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/ ==> DS ML 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/µl) and 11% (WBC > 50,000/µl). Severe coagulopathy is not a major problem. Therefore, cytoredution prior to definitive therapy in hyperleukocytosis (WBC > 50,000/µl) can be considered. Slow and careful cytoredution can be achieved with pre-phase therapy consisting of 6-thioguanine (40 mg/m²/d PO) and cytarabine (40 mg/m²/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^2/day) in the AIE induction. The pre-phase should not last more than 7 days.

Other measures to prevent complications due to hyperleucocytosis include hyperhydration (2-3 litres/m^2/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 hyperleucocytosis 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 <$10x10^9$/l has been reached.

12.5 Dosages for infants and children ≤ 12 kg of bodyweight

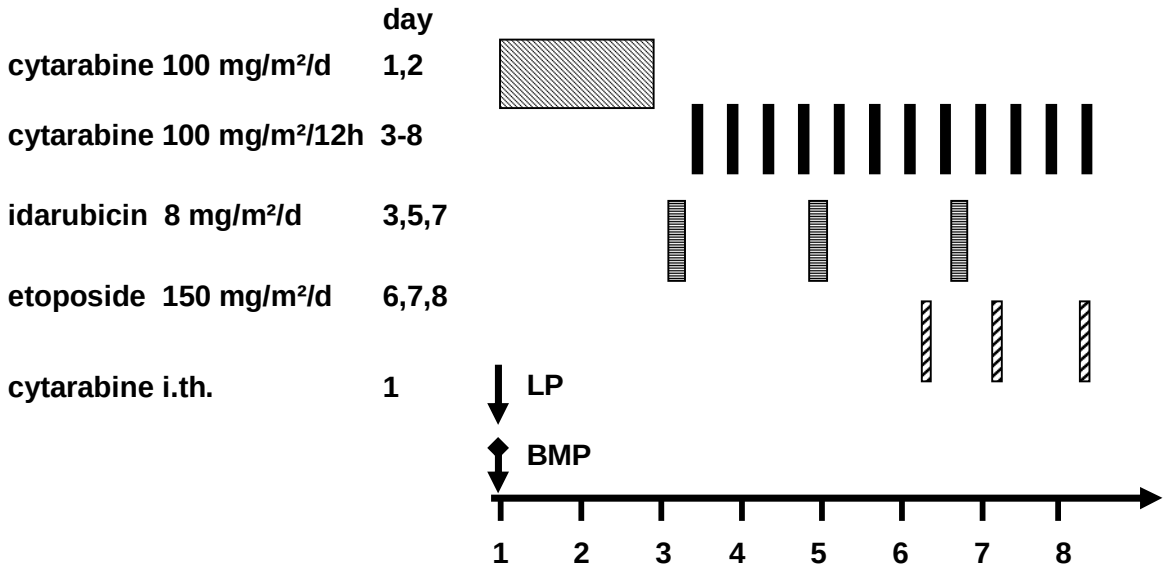
In children with a bodyweight ≤12 kg, the dosages are usually calculated according to body weight, and not to body surface area. The dosages given per m² are to be divided by 30 to obtain the dose per kg., as indicated in the table below.

		cytarabine			etoposide	idarubicin		mitoxantrone
body weight	**100 mg/m²**	**500 mg/m²**	**1000 mg/m²**	**3000 mg/m²**	**150 mg/m²**	**8 mg/m²**	**5 mg/m²**	**7 mg/m²**
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/µl). In case of hyperleucocytosis the initial lumbar puncture should be delayed until sufficient blast reduction has been achieved (WBC < 10,000/µl)).



Please note that for children with a body weight ≤ 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 8th, 10th, 12th 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 ≥1,000/µl and platelets ≥ 80,000/µl

Investigations for Consolidation (AI)

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

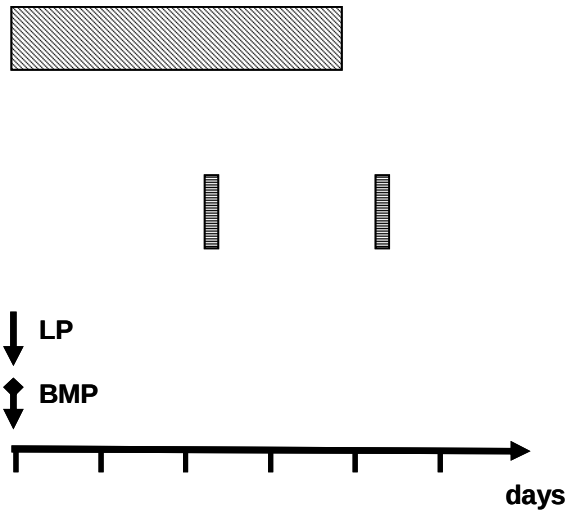
Please note that for children with a body weight ≤ 12 kg or age <1 year, dosages if intravenous chemotherapy must be calculated per kg body weight and not according to body surface area.

AI

cytarabine 500 mg/m²/day day 1-4
96 hrs cont. infusion

idarubicin 5 mg/m²/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/m²/day by continuous 24 hour IV infusion on days 1 to 4 inclusive (96 hours total)

Idarubicin 5 mg/m² 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 neutrophile count ≥1,000/µl and platelets ≥ 80,000/µl

Investigations for Consolidation (hAM)

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

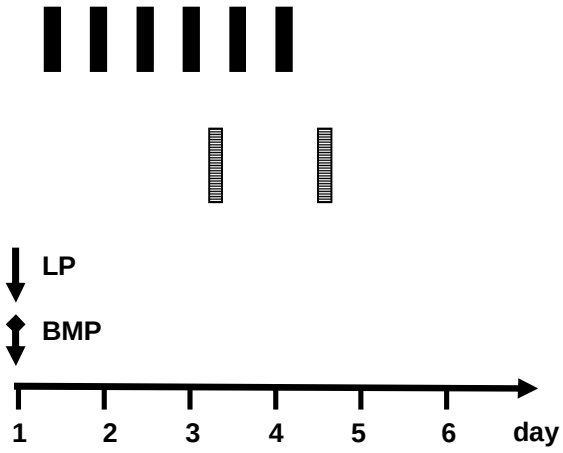
Please note that for children with a body weight ≤ 12 kg or age <1 year, dosages if intravenous chemotherapy must be calculated per kg body weight and not according to body surface area.

hAM

HD-cytarabine 1 g/m² **day 1-3**
every 12 hours: 3 hr-infusion
totally 6 times

mitoxantrone 7 mg/m²/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/m² by 3 hour IV infusion every 12 hours on days 1 to 3 inclusive (6 doses in total)
Mitoxantrone	7 mg/m²/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 ≥1,000/µl and platelets ≥ 80,000/µl

Investigations for Intensification (HA)

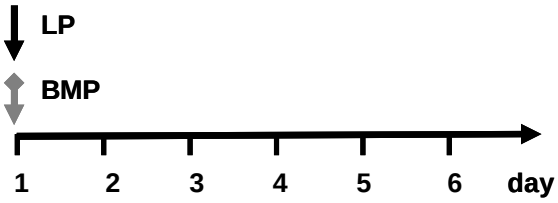
- bone marrow exam (MRD and morphology) and lumbar puncture to be done on day 1

Please note that for children with a body weight ≤ 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/m² **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/m² 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 form 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 ≥1,000/µl and platelets ≥ 80,000/µl.

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:

- o 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.
- o 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.
- o 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.
- o 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.
- o Congenital anomaly/birth defect
- o 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 pfd) and instruction how to use NCI CTCAE v3.0 can be

found on http://ctep.info.nih.gov/reporting/ctc.html. This will not be incorporated into the protocol.

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

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

Grade 1 mild AE
Grade 2 Moderate AE
Grade 3 Severe AE
Grade 4 Life Threatening or disabling AE
Grade 5 Death related to AE

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

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 lime limits set by the EU-directive 2001/20/EC as implemented into National Laws
All relevant information about SUSARs that are fatal or life-threatening must be reported as soon as possible to the CA, Main EC and to all investigators involved in the clinical trial and in any case no later than seven days after knowledge by the data 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

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ICH Topic E 11
Clinical Investigation of Medicinal Products in the Paediatric Population

ICH Step 4

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

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^2 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^2/day.

b. High-dose cytarabine (HD-cytarabine, >1g/m2))

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^{o}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^2/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 Dysfuction

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 Dysfuction

Grade 3-4 – Reduce dose to 75%

c. Etoposide

d. Idarubicin

Each dose of idarubicin of 10mg/m^2 should be tabulated as the isotoxic equivalent of 50mg/m^2 of daunorubicin or doxorubicin towards the lifetime maximum of 550mg/m^2 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^2 and for each additional 50mg/m^2.

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 10mg/m^2 should be tabulated as the isotoxic equivalent of 50mg/m^2 of daunorubicin or adriamycin towards the lifetime maximum of 550mg/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 270mg/m^2 and each 50mg/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 ≤ 120 give 25% of dose;
if >50 but ≤ 90 give 50% of dose and
if ≤ 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

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. [20,21]. 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 commended 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	• approved for children
Itraconazole	Loading dose 2x5 mg/kg, continue with 2x 2.5 mg/kg (level control at day 7)		• in centers with a high incidence of aspergillosis • not approved for children [1] • Solution is better absorbable.
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	• children > 13 years day 1: 2x400 mg/d PO, continue with: 2x200 mg/day PO • approved for children older than 2 years

[1] Several trials in children have supported the usefulness of the drug.

Empiric therapy for febrile neutropaenia

Procedures

History / examination / general condition
pulse / dyspnea / blood pressure / consciousness
Diagnostics
Full blood count and differential; serum chemistry, CRP, coagulation
blood cultures (aerobe, anaerobe, fungal)
Virus diagnostics, in case of respiratory symptoms
Urine culture

Plus further diagnostic procedures according to clinical symptoms

Immediate start of empiric treatment
Supportive measures according to symptoms
Monitoring of vital parameters

No fever after 48/72 hours:
continue therapy until hematological recovery / no evidence of infection

Persistent fever after 48/72 hours
re-evaluation, consider CT thorax
modification of the empiric therapy
empiric antimycotic therapy

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 a3rd generation cephalosporin [22].
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 [23].
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 [24-27].

Suggested modifications of the empiric regimen

Results / symptoms	**Modification**
Blood culture positive: Gram-positive species Gram-negative species Fungus	 add a glycopeptide add an aminoglycoside add antifungal therapy
Severe mucositis / gingivitis	add metronidazole or clindamycin (for anaerobes); add acyclovir (for herpes simplex)
Esophageal symptoms	add fluconazole (for candida); if refractory, add amphotericin B; consider acyclovir for herpes
Abdominal, perirectal or perianal pain	add metronidazol, clindamycin, or a glycopeptide
Focal pulmonary infiltration	diagnostics: broncho alveolar lavage (BAL) add antifungal therapy; if necessary consider mycoplasma, chlamydia, legionella and add macrolides / chinolones
Interstitial pneumonitis	consider CMV, PCP, adenovirus, others; consider empiric start of therapeutic dose of TMP/SMZ.
sepsis, septic shock	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	• approved for children
Fluconazole	8 mg/kg/day (max. 400 mg/day)	8 mg/kg/day (max. 400 mg/day)	
Itraconazole	loading dose 2x5 mg/kg afterwards2x 2.5 mg/kg (level control day 7)		• High incidence of aspergillosis • not approved for children [1]
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	• children > 13 years day 1: 2x400 mg/d > day 2: 2x200 mg/day • approved for children > 2 years; CNS effective
Liposomal amphotericin		3-5 mg/kg	• high dose in case of CNS infection • 6-10 mg/kg/day
Caspofungin		Day 1, 70 mg/day afterwards 50 mg/day	• 1 – 1.5 mg/kg/d max. daily dose: 50 mg[2] • not tested for CNS infection

[1] Several trials in children have supported the usefulness of the drug.

[2] 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, fluconazol. Alternatives: liposomal amphotericin B, voriconazole, or caspofungin (not approved for children to date).

For invasive aspergillosis, use voriconazole, amphotericin B, liposomal amphotericin B, IV itraconazol 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	• max. 800 mg/day • If not pretreated with azoles
	voriconazole	day 1: 2x 6 mg/kg/day > day 2: 2x 4 mg/kg/day	• children > 13 years. day 1: 2x400 mg/d > day 2: 2x200 mg/day • approved for children > 2 years
	liposomal amphotericin B	3-5 mg/kg	
	caspofungin	not known	• 1 – 1.5 mg/kg/day max. daily dose: 50 mg[2]
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	• children > 13 years. day 1: 2x400 mg/d > day 2: 2x200 mg/day • approved for children > 2 years
	Liposomal amphotericin B	5 mg/kg	
	itraconazole	loading dose 2x5 mg/kg 2x 2,5 mg/kg	• not approved [1]
	caspofungin	day 1:70 mg/day, afterwards 50mg/day	• 1 – 1.5 mg/kg/day, max. daily dose: 50 mg[2]

[1] Several trials in children have supported the usefulness of the drug.

[2] 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 [28]

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 [29]

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 [30]

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-SCF 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.[31]

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.

Apendix 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 form 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

Stichting Kinderoncologie Nederland
SKION
Dutch Childhood Oncology Group
DCOG

Leyweg 299, 2545 CJ The Hague
PO box 43515, 2504 AM The Hague
Tel. +31 (0)70 367 45 45
Fax +31 (0)70 367 08 68
E-mail: info@skion.nl

PROTOCOL ML DS 2006

For the treatment of Myeloid Leukemia in children with Down Syndrome

International Cooperative Pediatric AML Study Group

Nederlandse Bijlagen

SKION
Versie 1.0 (oktober 2007)
Implementatiedatum: 20-05-2007

STICHTING KINDERONCOLOGIE NEDERLAND

ML DS 2006
For the treatment of Myeloid Leukemia in children with Down Syndrome

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INDEX

1. Nederlandse Bijlagen

1.1 Myeloid Leukemia in Down Syndrome, begeleidende SKION- richtlijnen…...9

1.2 Informatie voor ouders en patiënt……...18

1.3 Add-on studie Ch.M. Zwaan en M.M. van den Heuvel-Eibrink 'Clinical relvance of genetic alterations in Myeloid Leukemia of Down Syndrome (ML DS)'..……..25

1.4 Add-on studie H. Hasle 'Minimal residual disease measured by WT1 expression in patients with myeloid leukaemia of Down syndrome treated on the international Down Syndrome Myeloid Leukemia 2006 study…….…...35

1.5 Add-on studie C. Langebrake en D. Reinhardt 'Influence of GATA1s on Leukemiogenesis in DS-AML……..……....42

1.6 Add-on studie D. Reinhardt, K. Reinhardt en C. Langebrake 'Minimal residual disease measured by the quantification of the patient specific GATA1s (RT-PCR) and by immunophenotyping'…….....46

1.7 Add-on studie P. Vyas 'Defining the haemopoietic defect in patients with Down syndrome and myeloid leukaemia treated on the international Down Syndrome Myeloid Leukemia 2006 study'……50

1.8 Verklaring van geen bezwaar……57

1. Nederlandse Bijlagen

1.1 Myeloid leukemia in Down Syndrome, begeleidende SKION-richtlijnen

INHOUDSOPGAVE

1.0	GATA-1 mutatie diagnostiek voor SKION centra	blz. 10
2.0	Kinderen ouder dan 4 jaar	blz. 10
3.0	Centraal zenuwstelsel uitbreiding	blz. 10
4.0	Aanvullende supportive care richtlijnen	blz. 10
5.0	Late effecten	blz. 11
6.0	'Early termination of the study'	blz. 12
7.0	Add-on studies	blz. 12
8.0	Registratie en CRFs	blz. 12
9.0	Ethische aspecten en toetsing	blz. 13
10.0	Logistiek sample verzending naar SKION	blz. 13
11.0	Differentiatiestadia en immunologische markers bij AML	blz. 17

NEDERLANDSE RICHTLIJNEN

1.GATA1 MUTATIE DIAGNOSTIEK.
Voor de Nederlandse patiënten zal gecentraliseerd GATA1 mutatie diagnostiek gedaan worden in Rotterdam op het laboratorium kinderoncologie (Dr. M.L. den Boer). Hiervoor zal via de SKION materiaal doorgestuurd worden naar Rotterdam, en de uitslag zal via de SKION naar de behandelend arts worden gerapporteerd.

2. KINDEREN OUDER DAN 4 JAAR.
Indien een patiënt ouder dan 4 jaar is, en er is geen GATA1 mutatie aantoonbaar, is er waarschijnlijk sprake van een sporadische AML bij een kind met Down syndroom. Er mag dan niet uitgegaan worden van een 'gevoelig drug resistentie profiel', en dus dient de patiënt in principe (voor zover de bijwerkingen dat toelaten) behandeld te worden volgens het vigerende protocol voor AML bij patiënten zonder Down syndroom. Overigens moeten deze patiënten NIET getransplanteerd worden in CR1 – omdat de procedure gerelateerde mortaliteit te hoog is. Als er wel een GATA1 mutatie aantoonbaar is heeft de patiënt waarschijnlijk een typische ML DS, en kan hij via dit protocol behandeld gaan worden. De verwachting is echter dat dit niet of slechts bij hoge uitzondering voorkomt.

3. CENTRAAL ZENUWSTELSEL UITBREIDING

In het protocol mist een definitie van centraal zenuwstelsel (CZS) uitbreiding.

CZS uitbreiding is zeldzaam bij DS ML, en minimale hoeveelheden blasten (CNS2) zijn voor zover bekend niet van prognostisch belang.

CZS uitbreiding wordt daarom gedefinieerd volgens de klassieke definities:
- pleiocytose $\geq$ 5 leukocyten per mm^3 met morfologisch aantoonbare leukemische blasten in een cytospin, en zonder bloedcontaminatie (<15 erytrocyten/mm^3)
- klinische symptomen zoals bijvoorbeeld hersenzenuwuitval, door leukemische infiltratie, die aangetoond moet worden op een MRI scan.

4. AANVULLENDE SUPPORTIVE CARE RICHTLIJNEN (bij appendix V van het protocol)

4.1. Algemeen: de meest belangrijke regel bij kinderen met Down syndroom is dat de patiënten voor het starten van een vervolgkuur niet alleen moeten voldoen aan de criteria voor hematologisch herstel, maar dat de patiënt zelf ook volledig klinisch moet zijn hersteld en in goede conditie moet zijn. Bij twijfel is uitstel aan te bevelen boven het streven naar dosisintensiteit en doorbehandelen.

4.2. Echocardiografie: geadviseerd wordt om bij initiële diagnose en voor de 3e kuur een hartecho te maken ter beoordeling van de hartfunctie. Op dat moment is cumulatief 34 mg/m2 idarubicine gegeven (~170 mg/m2 daunorubicine equivalent). Voor kinderen zonder DS wordt een echo pas aanbevolen na 60 mg/m2 idarubicine, maar het is niet goed bekend of kinderen met Down syndroom gevoeliger zijn voor anthracyclines (zie ook bij 'late effecten' hieronder). Bij een shortening fraction <28%, of een daling van >10%, dient dosisreductie en/of staken van anthracyclines overwogen te worden.

4.3. Preventie van chemotherapie geïnduceerde misselijkheid- en braken met een 5HT3-antagonist wordt geadviseerd, eventueel ondersteund met dexamethason, conform institutionele richtlijnen.

4.4. Bij hoge-dosis cytarabine behandeling is hyperhydratie aanbevolen (2.5-3.0 liter/m2/dag), alsmede corticosteroïden oogdruppels 4dd tijdens de kuur. Er is onvoldoende bewijs voor pyridoxine profylaxe. Zie ook de protocol richtlijnen voor penicilline profylaxe.

4.5. Etoposide kan allergische reacties veroorzaken, onderbreek dan het infuus, en hervat, indien klinisch niet gecontraindiceerd, op lagere snelheid, eventueel na anti-histaminica of hydrocortison toediening.

4.6. Voor het toedienen van intrathecale medicatie is adequate sedatie en analgesie een vereiste.Voor verspreiding van het cytostaticum dient men minimal 4 uur plat te liggen na toediening. De toediening vereist het aanleveren van de cytostatica aan een 3-kranenblok toedieningssysteem – om verwisseling met IV te geven cytostatica te voorkomen.

4.7 Kinderen met DS hebben een verhoogd risico op bijwerkingen, onder andere mucositis. De behandeling hiervan is ondersteunend, met adequate pijnbestrijding (eventueel morfine IV), alsmede sondevoeding of eventueel totaal parenterale voeding.

4.8 Tumor-lysis syndroom: ter voorkoming dient voor start van de anti-leukemische therapie gestart te worden met hyperhydratie en goede controle van de diurese. Ter voorkoming van uraat nefropathie wordt gestart met allopurinol (200-500 mg/m^2/dag 2 dd oraal) in combinatie met Natriumbicarbonaat (streef urine pH 6.5 – 7) of met rasburicase bij een leucocyten aantal > 50-100 x 10^9/ l (of een urinezuur > 0.45 mmol/l). Bij rasburicase hoeft de urine niet gealkaliniseerd te worden (geen bicarbonaat dus). Naast bovengenoemde moet men rekening houden met het ontstaan van een hyperkaliaemie, hypocalciaemie of een hyperfosfataemie. In die gevallen dienen specifieke maatregelen genomen te worden.

4.9. Extravasatie: in verband met ernstige lokale necrose bij extravasatie van anthracyclines wordt een centraal veneuze catheter aanbevolen. Bij extravasatie: koeling met ijskompressen, lokale applicatie van 99% DMSO, raadpleeg (plastisch) chirurg. Bij extravasatie van etopside of cytarabine zijn geen speciale maatregelen nodig.

5. LATE EFFECTEN.

In het protocol mist een paragraaf over late effecten. Recentelijk (O'Brien, ASH 2006, abstract 559) is er een abstract gepubliceerd over cardiotoxiciteit bij kinderen met Down syndroom en myeloide leukemie. Van de 57 patiënten bleken er 19 bekend te zijn met preëxistente hartafwijkingen, maar met een goede pompfunctie voorafgaand aan de leukemie behandeling. Die bestond uit 135 mg/m2 DNR en 80 mg/m2 Mitoxantrone. Maar liefst 21% (n=12, waarvan 8 met voorafgaande structurele afwijkingen; waarvan er 4 een functioneel niet relevant ASD of VSD hadden) van de patiënten had gedocumenteerd hartfalen met diuretica gebruik of inotropica en verminderde shortenings fractie bij echocardiografisch onderzoek. Vier patiënten overleden aan hartfalen. De cardiomyopathie ontwikkelde zich over het algemeen snel na de behandeling van deze kinderen.

In dit protocol worden cumulatief de volgende cumulatieve doseringen gegeven:

- cytarabine 27.8 gram/m^2
- anthracyclines: idarubicine 34 mg/m^2 en mitoxantrone 14 mg/m^2 (omgerekend met een factor 5 zou dat op 240 mg/m2 daunorubicine equivalent neerkomen, alhoewel dat geen evidence-based guideline is)
- VP-16: 450 mg/m^2.

Gezien de veel lagere dosering anthracyclines verwachten wij geen uitgebreide cardiotoxiciteit. Dit is ook geen groot probleem in de ervaring van de AML-BFM studiegroep (mondelinge informatie, Dirk Reinhardt).

De belangrijkste potentiële late effecten en de aandachtspunten voor follow-up zijn de volgende (bron: Long term follow-up guidelines van de COG)
a. VP16: secundaire AML, alhoewel de cumulatieve dosering laag is en het risico dus wellicht beperkt.
b. Anthracyclines: cardiomyopathie. Hiervoor wordt jaarlijks een hartecho geadviseerd in de eerste 5 jaar na diagnose. Dat is frequenter dan wat in de COG guidelines bij deze dosering en leeftijd wordt aangehouden (nl. 2 jaar), maar lijkt gezien bovenstaand abstract en eventuele bijkomende structurele afwijkingen geïndiceerd. Indien na 5 jaar geen cardiomyopathie is vastgesteld kan de frequentie worden teruggebracht naar eens per 2 jaar.
c. Cytarabine geeft met name een verhoogd risico op neurocognitieve defecten en leukoencephalopathie. De neurocognitieve defecten zijn in deze doelgroep niet eenvoudig te evalueren, en dit behoeft geen standaard follow-up onderzoek. Leukoencephalopathie kan zich manifesteren met symptomen als spasticiteit, epilepsie, ataxie, dysarthrie, dysfagie en/of hemiparese. Behoudens jaarlijks klinisch neurologisch onderzoek moet er op indicatie neurologische consultatie en MRI-onderzoek van het cerebrum plaats vinden.
d. Het risico op trombose is bij kinderen met Down syndroom waarschijnlijk verhoogd (Journeycake et al., ASH 2006, abstract 1489), evenals bij kinderen met kanker. Dat betekent dat gelet moet worden op klinische tekenen van (doorgemaakte) trombose en/of veneuze insufficiëntie.
e. Daarnaast moeten controles worden uitgevoerd specifiek gericht op Down syndroom. Veel kinderen met Down syndroom zijn hiervoor onder controle bij gespecialiseerde Down syndroom teams, dan wel een algemeen kinderarts. Deze controles dienen gecontinueerd te worden, aangezien dit niet specifiek tot het expertise terrein van de kinderoncoloog behoort.

6. EARLY TERMINATION OF THE STUDY (paragraaf 12.12.).
Hier staat en schrijffout, en de zin moet als volgt worden gelezen: “The patient experiences an adverse event which, in the opinion of the investigator, does NOT allow continuation of the trial medications”.

7. WETENSCHAPPELIJKE ADD-ON STUDIES.
Materiaal voor de door de SKION onderzoekscommissie goedgekeurde add-on studies (dd. 10-04-07) op restmateriaal van bloed/beenmerg zal via de gebruikelijke wijze door de SKION verzorgd worden. Het betreft de volgende studies, die als bijlage aan het protocol zijn toegevoegd:

a. Zwaan CM, Van den Heuvel MM, et al. Clinical relevance of genetic alterations in ML DS,
b. Hasle H, et al. MRD measured by WT1 expression in patients with ML DS,
c. Reinhardt D, et al. MRD measured by the quantification of the patient-specific GATA1s and by immunophenotyping,
d. Langebrake C, et al. Influence of GATA1-s on leukemogenesis in ML-DS,
e. Vyas P, et al. Defining the hematopoietic defect in patients with ML-DS.

Een deel van de MRD-bepalingen zal verricht worden op het ‘Laboratorium Leukemie en Lymfoom diagnostiek’, Erasmus MC, Rotterdam, in nauwe samenwerking met Frankfurt en Aarhus.
Overigens zullen de MRD-bepalingen niet gebruikt worden om de behandeling aan te passen; MRD-uitslagen worden dus in principe niet aan de behandelend arts of patiënt verstrekt.

8. REGISTRATIE EN CRFS.
De voor de Nederlandse patiënten benodigde registratie en de CRFs zullen door de SKION in de internationale database worden ingevoerd. U moet de patiënt dus aanmelden via de SKION op de gebruikelijke wijze.

9. ETHISCHE ASPECTEN.

Het gaat bij dit protocol om een behandelrichtlijn ('best available treatment') zonder gerandomiseerde vraagstelling. Wel is er sprake van centrale gegevens verzameling en van wetenschappelijk onderzoek op restmateriaal, waarvoor toestemming gevraagd moet worden aan de ouders/verzorgers. In dat kader wordt ook een zorgvuldigheids-toetsing uitgevoerd door de toetsingscommissie van het Erasmus MC. Er is geen aparte kinderinformatie beschikbaar, omdat er geen kinderen van 12 jaar of ouder in het protocol geincludeerd zullen worden.

10. LOGISTIEK SAMPLES VERZENDEN NAAR SKION

10.1. Cytologische diagnostiek (uitstrijkpreparaten)

Benodigd materiaal

- Voorafgaande aan de behandeling worden 6 ongekleurde beenmerguitstrijkjes en 3 ongekleurde bloeduitstrijkjes gestuurd naar het laboratorium van de SKION. De uitstrijkjes moeten bij voorkeur zijn afgenomen vóór eventuele transfusie van bloed of bloedprodukten.
- Tijdens en na behandeling volstaan 3 ongekleurde beenmergpreparaten en 1 ongekleurd bloedpreparaat. Bij (verdenking) recidief dient dezelfde procedure te worden gevolgd als bij diagnose.

Richtlijnen voor het vervaardigen van bloed- en beenmerguitstrijken
Ter realisatie van de gewenste uniformiteit van bloed- en beenmergpreparaten gaarne aandacht voor de volgende richtlijnen voor bloed- en beenmerguitstrijken:

- het opbrengen van slechts een kleine druppel op het objectglas.
- het uitstrijken met een glaasje dat smaller is dan het objectglas onder een hoek van 45°, langzaam uitstrijken.
- zodanig uitstrijken dat het einde van de film ongeveer halverwege het objectglas komt te liggen.

Pathologische cellen zijn vaak erg kwetsbaar en vallen spoedig uiteen bij snelle verplaatsing.
De hoeveelheid plasma dient gering te zijn. Indien het plasma meer dan enkele seconden nodig heeft om op te drogen, gaan de cellen door osmotische invloed schrompelen. Men gebruike een geslepen dekglaasje van een telkamer of een geslepen objectglas.

Doel
Op de uitstrijkpreparaten wordt standaard een May-Grünwald-Giemsa kleuring gedaan voor het tellen van het percentage blasten. Voor het classificeren van de leukemie worden tevens een Sudan-Black B, een Peroxidase en gecombineerde naftol AS-D chloroacetaat esterase en α-naftyl-acetaat-esterase verricht. Beoordeling en typering geschiedt volgens de WHO-classificatie (WHO classification of Tumours: Pathology and Genetics of Tumours of Haematopoietic and Lymphoid Tissues. Edited by ES Jaffe, NL Harris, H Stein, JW Vardiman. IARC press, Lyon 2001).

Uitslag
De uitslag wordt telefonisch en schriftelijk doorgegeven aan de behandelend kinderarts.

10.2. Immunophenotypering (Hemoblok – SKION)

Benodigd materiaal

- heparine beenmerg: 5 ml
- heparine bloed: 20 ml

Werkwijze
Hiervoor zijn in het zgn. hemoblok heparinebuizen aanwezig; na afname goed mengen om stolling te voorkomen. Zonodig een tweede beenmergpunctie op een andere plaats uitvoeren, om teveel bloedbijmenging te voorkomen. Het materiaal dient te worden bewaard en getransporteerd op kamertemperatuur.

Doel

Immunofenotypering geschiedt op het laboratorium van de SKION in meervoudige labeling en volgens de richtlijnen van de SKML (www.skml.nl) in een gefaseerde aanpak. De volgende markers worden in elk geval gebruikt:

-	Niet specifiek	CD45, CD34, CD117, HLA-DR
-	B-cel markers	CD19, CD10, CD20
-	T-cel markers	CD2, CD3, CD7
-	Myelo-monocytaire marker	CD13, CD33, CD14, CD15, MPO (CD61, CD235a)

Uitslag
De uitslag wordt schriftelijk aan de behandelend arts meegedeeld.

10.3. Liquordiagnostiek (SKION-liquorblok)

Benodigd materiaal
- Bij diagnose 2,5 ml liquor
- Indien tijdens de behandeling door de behandelend kinderarts een celaantal van ≥15/3 (of ≥5/mm^3) in de liquor gevonden wordt, dient eveneens liquor naar het laboratorium van de SKION te worden gezonden.

Werkwijze
Bij de lumbaalpunctie wordt als 2^e of 3^e afnamemateriaal, het buisje uit het "liquorblok" van de SKION gevuld. Dit wordt aangevuld tot aan de aangegeven streep met liquor.
Eventueel is het mogelijk ongekleurde cytospinpreparaten naar het laboratorium te sturen, mits deze van goede kwaliteit zijn.

Doel
Bepaald worden het aantal cellen in de liquor en de cytomorfologie (cytospinpreparaten MGG gekleurd).

10.4. Cytogenetisch onderzoek

Het chromosomenonderzoek geschiedt in 9 cytogenetische laboratoria in Nederland. Deze zijn hiervoor een gezamenlijk te volgen procedure overeengekomen voor het verkrijgen van materiaal. Voor het doen verrichten van cytogenetisch onderzoek neme men telefonisch contact op met één der onderstaande personen en instituten:

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De uitslagen worden rechtstreeks aan de behandelend kinderarts toegestuurd.
De SKION ontvangt eveneens de uitslag van het desbetreffende cytogenetisch laboratorium.

10.5. Checklist SKION bij diagnose en follow-up

Patiënt telefonisch aanmelden bij de SKION (070-3674545). Hierbij worden naam, geboortedatum en indien van toepassing de (voorlopige) diagnose gemeld, alsmede gegevens over het afgenomen materiaal.

Afnamen bij diagnose en follow-up:

Tijdstip	Preparaten (ongekleurd)		Hemoblok		Liquorblok
	Bm	Bloed	Bm (heparine buis)	Bloed (heparine buis)	Buis met medium (diagnostiek)
Diagnose en (verdenking) recidief	6	3	5 ml	10-20 ml	2,5 ml tot aan de streep op de buis
Overige tijdstippen	3	3	2 ml	10 ml	

10.6. Verzenden per Fiege (BLS koerier) naar het SKION laboratorium

Instructies voor verzenden materiaal naar de SKION (zie ook www.skion.nl):

Hemoblok: Bloed en/of beenmerg kunnen in een, door de SKION verstrekt hemoblok worden verstuurd.
Liquorblok: Voor het verzenden van liquor is een liquorblok beschikbaar. Deze zijn in de regel in het laboratorium van het ziekenhuis van de kinderarts aanwezig (evt. aanvragen bij het laboratorium van de SKION). In de blokken zit een formulier met instructies waarop tevens enkele gegevens van de patiënt moeten worden ingevuld.
Controle preparaten: Deze kunnen in de daartoe ontworpen goedgekeurde verpakking per gewone post worden verstuurd.

Telefonisch wordt de verzending vóóraf gemeld aan de SKION:

Op werkdagen van 09.00-17.00 uur: telefoon 070 - 367 45 45 (buiten deze uren kan een boodschap worden ingesproken op het antwoordapparaat).

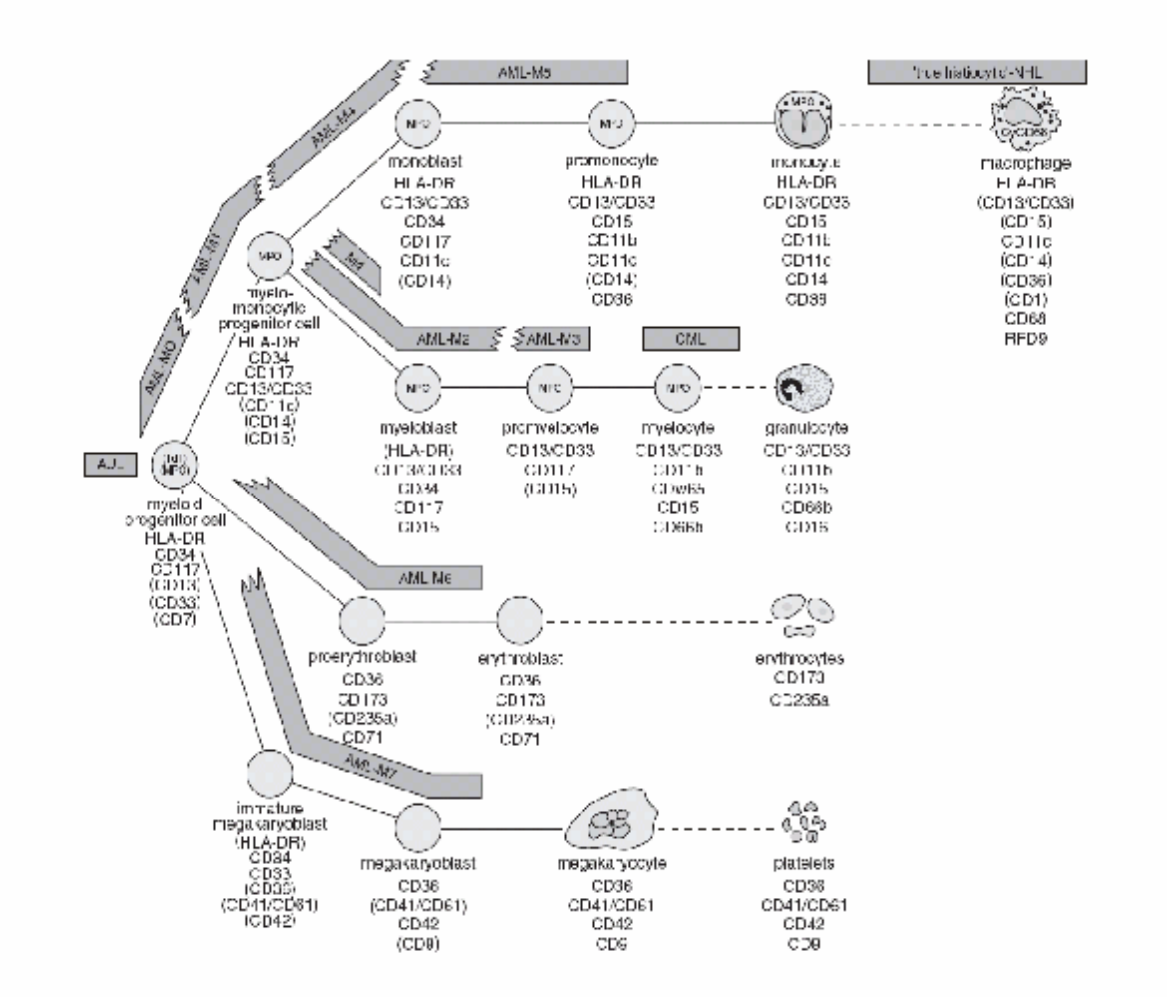
Op zaterdag van 08.30-17.30 uur via telefoon 06 - 5120 12 97 de dienstdoende analist(e) waarschuwen. Indien de analist(e) niet reageert via dit telefoonnummer (b.v. omdat ze bezig is), dan graag uw boodschap op de voice-mail inspreken, opdat u kan worden teruggebeld.

Verzending hemo-, liquorblokken en diagnosepreparaten via Fiege (BLS-Koerier):
Aanmelding voor vervoer dient te geschieden per fax 075 - 650 16 98 of per e-mail (jdewijn@fiege.nl) bij voorkeur vóór 16.00 uur en met een speciaal daarvoor ontworpen opdrachtformulier.

Eventueel tot 21.00 uur bellen naar nummer: 075 - 650 16 19; bgg 06 - 432 71 741, of 06 – 432 71 751.
Formulieren en verpakkingsmateriaal zijn indien nodig aan te vragen bij het SKION laboratorium.

U dient ervoor te zorgen, dat het pakket in SKION verpakking, lekdicht en met absorberend materiaal verpakt, klaar ligt op de met de koeriersdienst afgesproken plaats.

11.0 DIFFERENTIATIESTADIA EN IMMUNOLOGISCHE MARKERS VAN AML



1.2 Informatie over de behandeling en wetenschappelijk onderzoek bij kinderen met Down syndroom en myeloïde leukemie volgens het SKION-ML-DS 2006 protocol

Officiële titel: ”SKION-Myeloid Leukemia Down Syndrome 2006 (ML-DS 2006) for the treatment of Myeloid Leukemia in children with Down syndrome”

Geachte ouder(s) – verzorger(s),

Inleiding
Bij uw kind is onlangs een vorm van bloedkanker vastgesteld, namelijk acute myeloïde leukemie (AML). Daarnaast is al langer bekend dat uw kind het syndroom van Down heeft. Kinderen met Down syndroom hebben een grotere kans op het ontwikkelen van leukemie dan kinderen zonder Down syndroom. De oorzaak hiervan is nog niet opgehelderd.

Bij kinderen met het syndroom van Down komt een speciale vorm van acute myeloïde leukemie voor, waarbij de leukemie cellen gevoeliger zijn voor de anti-kanker medicijnen (chemotherapie, ook wel cytostatica genoemd) dan bij kinderen zonder Down syndroom. Goede gevoeligheid voor chemotherapie betekent goede overlevingskansen. De keerzijde van de grote gevoeligheid van leukemiecellen bij kinderen met Down syndroom, is echter dat er ook een grotere kans bestaat op bijwerkingen. Kinderen met Down syndroom die behandeld worden met chemotherapie zijn namelijk ook gevoeliger voor het optreden van (ernstige) infecties of slijmvlies beschadiging dan kinderen zonder Down syndroom.

Om die reden is er een apart Europees behandelprotocol gemaakt voor kinderen met AML en Down syndroom. Een protocol is een handboek voor de behandelend kinderoncoloog met richtlijnen voor behandeling en onderzoek. Het protocol voor kinderen met AML en Down syndroom schrijft minder chemotherapie voor dan gewoonlijk gegeven wordt aan kinderen met AML die niet het syndroom van Down hebben. Het doel van de behandeling is de juiste balans te vinden tussen anti-leukemische werking en bijwerkingen: kinderen moeten voldoende medicijnen krijgen om te genezen, maar ook niet zoveel zodat er te veel ernstige bijwerkingen ontstaan.

In Duitsland is er al ervaring opgedaan met deze behandeling bij kinderen met AML en het syndroom van Down. Daarbij werd ongeveer 90% van de kinderen beter. In dit Duitse protocol kregen de kinderen na een intensieve behandelfase een zogenaamde onderhoudsbehandeling met milde chemotherapie. In het huidige protocol wordt deze onderhoudsbehandeling niet meer gegeven, omdat die zeer waarschijnlijk niet nodig is om beter te worden. De Stichting Kinderoncologie Nederland (SKION), het samenwerkingsverband van de Nederlandse kinderartsen die zorgen voor kinderen met kanker, heeft besloten mee te doen met dit behandelprotocol en kinderen met AML en Down syndroom voortaan zo te behandelen.

Om de behandelingsmogelijkheden voor kinderen met kanker nog verder te verbeteren, wordt wetenschappelijk onderzoek gedaan. De behandelend arts van uw kind heeft u geïnformeerd over bovengenoemd behandelingsprotocol. Met dit protocol hopen wij ook enkele belangrijke wetenschappelijke vragen te beantwoorden over de behandeling van kinderen met Down syndroom. Voor toestemming of weigering daarvoor is goede voorlichting van onze kant nodig en een zorgvuldige afweging van uw kant nodig. Vandaar dat u deze schriftelijke informatie ontvangt. U kunt die rustig (her)lezen en in eigen kring bespreken. Ook daarna kunt u nog altijd vragen stellen aan de artsen die aan het eind van deze informatie genoemd staan.

Wat is acute myeloïde leukemie?
Leukemie is kanker van bloedcellen. Bloedcellen (en dus ook leukemiecellen) worden in het beenmerg gemaakt, wat in alle botten van het lichaam zit. Normaal gesproken ontstaan de bloedcellen eerst als onrijpe cellen en rijpen ze vervolgens uit in het beenmerg. Daarna worden ze losgelaten in het bloed. Via het bloed kunnen de cellen vervolgens door het hele lichaam vervoerd worden om hun functies uit te voeren.

Er bestaan verschillende soorten normale bloedcellen:

- Rode bloedcellen (erythrocyten): deze cellen nemen vanuit de longen zuurstof op en transporteren dit naar alle organen in het hele lichaam. De zuurstof wordt gebonden aan een bepaald eiwit, het zogenaamde hemoglobine (afgekort als Hb). Een tekort aan rode bloedcellen of een laag Hb wordt bloedarmoede of anemie genoemd. De bijbehorende klachten zijn bleek zien en moeheid.
- Witte bloedcellen (leukocyten): deze cellen verzorgen de afweer van ons lichaam tegen infecties door bijvoorbeeld bacteriën en virussen. Er bestaan verschillende soorten normale witte bloedcellen:
  - Lymfocyten: deze witte cellen komen in het bloed, maar ook in de lymfklieren en milt voor. Van de lymfocyten bestaan weer T-lymfocyten en B-lymfocyten, die belangrijk zijn voor het maken van antistoffen tegen ziekteverwekkers. De meest voorkomende vorm van leukemie bij kinderen ontstaat in deze cellen en heet daarom dan ook lymfatische leukemie.
  - Granulocyten: deze witte bloedcellen spelen ook een belangrijke rol bij de afweer, met name voor het opeten van bacteriën.
  - Monocyten: ook deze grote witte bloedcellen spelen een belangrijke rol bij de afweer, met name voor het opeten van bacteriën en virussen.

  Een tekort aan witte bloedcellen leidt tot een verminderde afweer; er ontstaan dan vaak infecties met daarbij koorts.
- Bloedplaatjes (trombocyten): deze zijn belangrijk voor de bloedstolling. Als een kind te weinig trombocyten heeft, leidt dit dus tot bloedingen. Dit kan zich uiten in bloedneuzen en spontane (grote) blauwe plekken op plaatsen waar deze normaal gesproken zelden ontstaan. Soms zien we ook zeer kleine zogeheten puntbloedingen in de huid of het slijmvlies van de mond.

Acute myeloïde leukemie bij kinderen met Down syndroom ontstaat meestal uit voorlopers van de bloedplaatjes. De zieke (kwaadaardige) bloedcellen heten blasten. Als ze uit voorlopers van de bloedplaatjes ontstaan heten het megakaryoblasten. Deze blasten kunnen niet goed uitrijpen en blijven dus jong, onrijp. Eerst stapelen ze zich op in het beenmerg, zodat daar minder ruimte overblijft om gezonde bloedcellen te maken. Later verspreiden de blasten zich ook verder in het lichaam, bijvoorbeeld in het bloed, lever, milt en lymfeklieren en soms ook in het hersenvocht. De blasten hebben geen functie, maar ze staan gezonde bloedcellen in de weg.

Diagnose
Bij de verdenking op leukemie zal eerst bloedonderzoek gedaan worden door de bloedcellen onder de microscoop te bekijken. Hierbij wordt onder meer het aantal bloedcellen van de verschillende soorten bepaald. Daarna wordt een beenmergprik (beenmergpunctie) verricht om vast te stellen of er leukemie is en zo ja, van welk type leukemie er sprake is. Om de uitbreiding van de ziekte te beoordelen wordt ook een ruggenprik (lumbaalpunctie) verricht om te onderzoeken of er leukemiecellen aanwezig zijn in het hersenvocht (liquor). Tevens wordt er een longfoto gemaakt en wordt ook een echo van de buik gemaakt ter beoordeling van orgaanvergroting in de buik.

Tijdens de behandeling vinden bloedafnames en infuustherapie plaats. Daarom wordt bij de meeste kinderen een speciaal infuussysteem ingebracht. Dit systeem wordt ook wel een lange infuuslijn of een centraal veneuze katheter genoemd. Er zijn twee systemen: een Port-A-Cath (PAC), die onder de huid wordt geplaatst en door de huid heen moet worden aangeprikt, of een broviac-catheter, die door de huid naar buiten komt. De kinderoncoloog bespreekt met u of dit nodig is en welk systeem voor uw kind het beste is. Beide systemen zijn om de toediening van medicijnen en het afnemen van bloed te vergemakkelijken.

Behandeling
Kinderen met AML en het syndroom van Down worden behandeld volgens het SKION-ML-DS 2006 protocol. De SKION heeft een centraal bureau waar gegevens over de ziekte en de behandeling van kinderen met kanker worden verzameld en geregistreerd. Via de SKION worden deze gegevens gerapporteerd aan de International Cooperative Paediatric AML Study Group, de Europese organisatie waar gegevens over de ziekte en de behandeling van kinderen met AML en het syndroom van Down worden verzameld en geregistreerd. Meer informatie over registratie van gegevens kunt u vinden in het aan u uitgereikte informatieformulier "informatie over SKION registratie van de behandeling van kinderen met een kinderoncologische aandoening".

Het SKION-ML-DS 2006 protocol is een handboek voor de behandelend kinderoncoloog met richtlijnen voor behandeling en onderzoek. De huidige behandeling voor AML bij kinderen met het syndroom van Down is gebaseerd op de resultaten van protocollen die eerder met name in Duitsland werden toegepast bij kinderen met AML. Het SKION-ML-DS 2006 protocol biedt naar de huidige inzichten de beste kans op genezing en bestaat uit vier achtereenvolgende kuren chemotherapie met combinaties van geneesmiddelen, die de groei en de vermeerdering van kwaadaardige cellen stoppen. De kuren worden meestal aangeduid met de beginletters van de toe te dienen geneesmiddelen:
Kuur 1: AIE (cytarabine, idarubicine en etoposide)
Kuur 2: AI (cytarabine en idarubicine)
Kuur 3: hAM (cytarabine en mitoxantrone)
Kuur 4: HA (cytarabine).

Bij de chemotherapiekuren wordt ook een aantal keren chemotherapie toegediend via een lumbaalpunctie (ruggenprik). Het aantal injecties hangt af van de uitslag van het onderzoek van de liquor bij diagnose.

Na het onderzoek bij diagnose, wordt tijdens de behandeling voor iedere kuur een beenmergpunctie verricht om te beoordelen of de behandeling het gewenste resultaat heeft. De artsen zullen in overleg met u zorgen voor een zo goed mogelijke pijnbestrijding voor uw kind tijdens de beenmergpuncties en lumbaalpuncties. Indien mogelijk gebeurt dit onder narcose.

Bijwerkingen
Chemotherapie kan helaas gepaard gaan met ernstige bijwerkingen. Daarom wordt de gezondheidstoestand van uw kind nauwlettend in de gaten gehouden. Behalve de leukemiecellen, kunnen ook normale bloedcellen worden aangetast. Als gevolg hiervan zal er geregeld sprake zijn van een sterk verminderd aantal normale bloedcellen. Daardoor bestaat een verhoogde kans op infecties en/of bloedingen. Andere bijwerkingen zijn verminderde eetlust, misselijkheid, braken, obstipatie of diarree, kaalheid, mondslijmvlies beschadiging en koorts. De toediening van sommige cytostatica kan gepaard gaan met een overgevoeligheidsreactie, hart- en leverfunctiestoornissen, branderige ogen, of (zeer zelden) een tijdelijk verminderd bewustzijn. Mogelijke gevolgen van de behandeling op lange termijn zijn een verminderde vruchtbaarheid en vertraging van de groei. Er is een geringe kans op het optreden van een tweede gezwel of hartafwijkingen op latere leeftijd. Deze hartafwijkingen zijn echter anders van aard dan de aangeboren hartafwijkingen die veelvuldig bij kinderen met het syndroom van Down voorkomen.

De mogelijke bijwerkingen kunnen per kind verschillen. Voor meer informatie over de verschillende soorten medicijnen verwijzen we u naar de dagboekagenda van de vereniging ouders, kinderen en kanker (VOKK) die u bij het begin van de behandeling krijgt.

Wetenschappelijk onderzoek
Dankzij onderzoek in het verleden is het percentage genezen kinderen met AML in de loop van enkele decennia gestegen van minder dan 10% tot ruim 80%. Het is van groot belang onderzoek te blijven doen om een verdere verbetering van deze behandelingsresultaten te bereiken.

Evaluatie van de behandeling
Met betrekking tot de behandeling volgens dit protocol wordt gekeken naar de volgende punten:
1. Effectiviteit
   Gedurende de behandeling zal de effectiviteit (werkzaamheid) van het behandelschema worden bepaald door middel van de uitslagen van de leukemiecellen in bloed en beenmerg.
2. Verdraagzaamheid
   De mogelijke bijwerkingen worden geregistreerd aan de hand van bloeduitslagen. Daarnaast controleert de behandelend arts uw kind regelmatig door middel van lichamelijk onderzoek. Ook uw eigen bevindingen tijdens het gebruik van de medicatie worden geregistreerd.
3. Verzamelen van gegevens
   Alle gegevens over het verloop van de behandeling bij uw kind worden verzameld en gecodeerd opgeslagen in een database, mits u daar toestemming voor geeft. Deze database bevat alle gegevens van alle kinderoncologische centra in Europa die meewerken aan dit onderzoek- en behandelprotocol. De resultaten zullen uiteindelijk wetenschappelijk beoordeeld worden.

Aanvullende onderzoeksprojecten
Daarnaast wordt, indien u daar toestemming voor geeft, restmateriaal verzameld. Met restmateriaal wordt bloed, beenmerg en liquor bedoeld, wat overblijft nadat dit bij u kind was afgenomen in het kader van de behandeling. Uw kind wordt er dus niet extra voor geprikt en er wordt geen extra materiaal afgenomen. Reden voor de opslag van restmateriaal is dat voor de verbetering van de behandeling van een zeldzame ziekte als AML bij kinderen met Down syndroom altijd onderzoek nodig zal zijn. Vooral het laboratoriumonderzoek naar de biologische eigenschappen van de leukemiecellen is hierbij van groot belang. Het doel van dergelijk aanvullend onderzoek is het ontwikkelen van nieuwe behandelingen voor kinderen met leukemie.

Voor- en nadelen
Zoals gezegd vinden er geen extra afnames plaats. Er zijn daarom dan ook geen risico's verbonden aan deelname aan dit protocol.

Uw kind heeft geen direct voordeel van deelname aan onderzoek aan de aanvullende onderzoeksprojecten met restmateriaal van bloed, beenmerg en liquor. Wel vergroot het de mogelijkheid om de kwaliteit van behandeling van kinderen met AML en het syndroom van Down in de toekomst te verbeteren.

Vrijwillige deelname en toestemming
Voordat we met de behandeling van uw kind beginnen vragen we u een toestemmingsformulier te ondertekenen waarin staat dat u weet wat de behandeling inhoudt. Tevens vragen wij uw toestemming voor het verzamelen van de gegevens van uw kind over het verloop van de behandeling en voor het bewaren van restmateriaal.

Het behandelteam van de afdeling kinderoncologie begeleidt u en uw kind gedurende het onderzoek zo goed mogelijk. Dit team is multidisciplinair samengesteld en bestaat onder meer uit gespecialiseerde artsen,verpleegkundigen en een psycholoog. Het behandelteam ziet nauwlettend toe op de belasting die de behandeling voor kinderen en ouders met zich meebrengt. We werken daarbij volgens de landelijke afspraken zoals die door de Nederlandse Vereniging voor Kindergeneeskunde (NVK) zijn vastgelegd ter bescherming van minderjarige onderzoeksdeelnemers. Voor meer informatie hierover verwijzen wij u naar de volgende website: www.ccmo.nl (wet- en regelgeving/gedragscode verzet: minderjarigen).

Als u besluit niet aan de gegevensverzameling en het bewaren van restmateriaal mee te doen, dan is dat niet van invloed op de behandeling die uw kind krijgt. De behandeling gaat dan wel volgens het SKION-ML-DS 2006 protocol verder, want dat is de standaard behandeling van dit moment voor kinderen met AML en het syndroom van Down. U krijgt voldoende tijd om hierover na te denken en u kunt te allen tijde om extra informatie vragen of op eenmaal genomen beslissingen terugkomen.

Verantwoording en vertrouwelijkheid
Tot uw kind herleidbare onderzoeksgegevens kunnen slechts met uw toestemming door daartoe bevoegde personen worden ingezien. Deze personen zijn medewerkers van het onderzoeksteam, medewerkers van de Inspectie voor de Gezondheidszorg of bevoegde inspecteurs van een buitenlandse overheid, en leden van de Medisch Ethische Toetsings Commissie (METC).

Inzage kan nodig zijn om de betrouwbaarheid en kwaliteit van het onderzoek na te gaan. Onderzoeksgegevens zullen worden gehanteerd met inachtneming van de Wet Bescherming Persoonsgegevens en het privacyreglement van het Erasmus MC.

Persoonsgegevens die tijdens deze studie worden verzameld, zullen worden vervangen door een code, bestaande uit een studienummer en de initialen van uw kind. De sleutel van deze code zal alleen toegankelijk zijn voor de onderzoeker, de behandelend arts of de researchverpleegkundige/ datamanager. Alleen deze gecodeerde gegevens zullen gebruikt worden voor studiedocumentatie, in rapporten of publicaties over dit protocol. De vertrouwelijkheid van de gegevens blijft hierbij gewaarborgd. De gecodeerde gegevens worden opgeslagen in een computerbestand en verwerkt bij de SKION. De gegevens worden, indien u daar toestemming voor geeft, gedurende 15 jaar bewaard.

Lichaamsmaterialen die tijdens deze studie worden verzameld, worden gecodeerd opgeslagen. Na afloop van het protocol worden de opgeslagen lichaamsmaterialen vernietigd of, als u daarvoor toestemming geeft, gedurende maximaal 15 jaar na afloop van de studie bewaard. Het opgeslagen lichaamsmateriaal kan dan eventueel in een later stadium worden gebruikt voor onderzoek met als doelstelling de toekomstige behandeling van kinderen met leukemie verder te verbeteren.

De resultaten van dit protocol worden gerapporteerd in medisch-wetenschappelijke literatuur en/of op medische congressen.

In het kader van zorgvuldigheidstoetsing is het SKION-ML-DS 2006 protocol voorgelegd aan de METC van het Erasmus MC. De voor dit protocol geldende internationale richtlijnen zullen nauwkeurig in acht worden genomen.

Contactpersonen
Bij vragen of opmerkingen kunt u contact opnemen met dr. C.M. Zwaan, kinderarts-oncoloog, tel. 010-4636691, of met de researchverpleegkundigen van de afdeling kinderoncologie, Inekee van der Vaart of Eline Visser, tel. 010-4636402. Als u twijfelt over deelname van uw kind aan deze studie dan kunt u een onafhankelijke arts raadplegen die zelf niet bij het onderzoek is betrokken maar wel deskundig is op dit gebied: Dr. J.B. van Goudoever, kinderarts, tel. 010 4636077. Ook indien u voor of tijdens het onderzoek vragen heeft die u liever niet aan de onderzoekers stelt dan kunt u contact opnemen met de onafhankelijke arts.

Neemt u de tijd om deze informatie door te spreken en aarzel niet de behandelend arts van uw kind te raadplegen als u vragen heeft.

Wanneer u besluit deel te nemen ontvangt u een kopie van dit document, nadat u en de behandelend arts van uw kind beiden voor deelname getekend hebben.

Met vriendelijke groet,

Dr. C.M. Zwaan,
Kinderarts-oncoloog

Toestemmingsformulier ouders/voogd

behorende bij de patiënteninformatie over de behandeling en wetenschappelijk onderzoek bij kinderen met acute myeloïde leukemie volgens het SKION- ML-DS 2006 protocol

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Titel van het onderzoek: "SKION-Myeloid Leukemia Down Syndrome 2006 (ML-DS 2006) for the treatment of Myeloid Leukemia in children with Down syndrome"

Mij is gevraagd toestemming te verlenen voor deelname aan bovengenoemd protocol ten behoeve van:

Naam kind: Geboortedatum: __ / __ / __

Ik bevestig, dat ik het informatieformulier voor mijn kind heb gelezen. Ik begrijp de informatie. Ik heb de gelegenheid gehad om aanvullende vragen te stellen. Deze vragen zijn naar tevredenheid beantwoord. Ik heb voldoende tijd gehad om over deelname van mijn kind na te denken. Ik weet dat deelname geheel vrijwillig is en dat ik mijn toestemming op ieder moment kan intrekken zonder dat ik daarvoor een reden hoef te geven.

Ik geef toestemming voor deelname van mijn kind aan behandeling volgens het SKION-ML-DS 2006 protocol onder de omstandigheden zoals die mij zijn uitgelegd.

Ik geef wel/geen* toestemming om de gegevens van mijn kind gedurende 15 jaar na afloop van het protocol te bewaren.

Ik geef wel/geen* toestemming voor het langdurig (maximaal 15 jaar) bewaren van restweefsel, waar mogelijk in de toekomst verder onderzoek naar leukemie mee gedaan wordt.

Ik geef toestemming om de huisarts van mijn kind op de hoogte te brengen van zijn/haar deelname aan dit protocol.

Ik geef toestemming voor het gecodeerd verzamelen en verwerken van de gegevens over het verloop van de behandeling, zoals in deze informatiebrief beschreven is. De gegevens zullen worden opgeslagen in een database. De resultaten zullen voor wetenschappelijke doeleinden worden gebruikt waarbij de vertrouwelijkheid gewaarborgd wordt.

Ik geef toestemming, dat daartoe bevoegde medewerkers van het onderzoeksteam, bevoegde personen van de SKION, medewerkers van de Inspectie voor de Gezondheidszorg, bevoegde inspecteurs van een buitenlandse overheid of leden van de medisch-ethische toetsingscommissie inzage kunnen krijgen in de medische gegevens en onderzoeksgegevens van mijn kind.

Naam ouder/voogd **:
Handtekening: Datum : __ / __ / __

Naam ouder/voogd **:
Handtekening: Datum : __ / __ / __

Naam onderzoeker/behandelend arts:
Handtekening: Datum : __ / __ / __

* Doorhalen wat niet van toepassing is.

1.3 Title study: Clinical relevance of genetic alterations in Myeloid Leukemia of Down syndrome (ML DS)

Name applicant: Ch.M. Zwaan and M.M. van den Heuvel-Eibrink

In collaboration with: Dept. of Pediatric Oncology: ML den Boer, Prof.dr R. Pieters
In collaboration with the AML-BFM SG investigators (headed by D. Reinhardt).

To be performed as an add-on study of the "International cooperative childhood AML study group" European DS AML 2006 study

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INTRODUCTION

Children with Down syndrome (DS) have an increased risk of developing both acute myeloid leukemia (AML) and acute lymphoblastic leukemia (ALL).[1,2] Of interest, prognosis of children with AML and DS is much better (80-90% survival) than AML in children without DS (50-70% survival).[3,4] However, in DS ALL prognosis is worse than for children with sporadic ALL.[5] This may be related to the unique type of myeloid leukemia that children with DS develop, which is referred to as "Myeloid Leukemia of Down syndrome (ML DS)".[2,3,6-9]

Before the 1980s, children with ML DS were often treated with palliative care only. Ravindranath and Lie were the first to report stable remissions in ML DS patients who were treated with chemotherapy.[10,11] However, when full dose chemotherapy was applied, many children developed severe side-effects, and the incidence of treatment related mortality was increased compared to sporadic AML.[3,12] Of interest, chemotherapy dose-reductions appeared to be safe in terms of anti-leukemic efficacy, and also diminished treatment related mortality, leading to the current excellent outcome of approximately 80% survival for ML DS patients.[3,4] This may be due to the sensitivity of the ML DS blasts for chemotherapeutic drugs when compared with sporadic AML cells, as demonstrated by us and others using in vitro total cell-kill assays.[13-16] The dose-reductions in the clinic, however, were introduced empirically and, so far, no prognostic factors have been identified which allow stratification of therapy for ML DS patients. Moreover, it is unknown which subgroup of patients may still be cured with further therapy reduction, nor what characterizes the subgroup of approximately 10-20% of ML DS patients with poor treatment outcome due to refractory disease or relapse, who might benefit from more regular AML chemotherapy. Therefore, one of the aims of this project is to study ML DS samples for secondary genetic abnormalities that may potentially be used for identification of risk-groups in ML DS. In addition, information about abnormally expressed genes may identify drugable targets – which could, in the long run, lead to the introduction of targeted therapy in ML DS.

Some children with DS develop ML following a transient leukemia in the neonatal period, which is usually a self-limiting disease, and which is referred to as 'transient myeloproliferative disorder' (TMD) or 'transient leukemia (TL)'.[17,18] It occurs in approximately 5-10% of neonates with DS, although the true frequncy is unknown as only selected populations have been studied. It is not understood why this leukemia is transient – nor is it understood why only 20% of these children subsequently develop ML DS at later age and others do not. It is anticipated that this is due to secondary genetic abnormalities, but the identity of these genetic abnormalities is currently

unknown.[18] Therefore, we want to study which genetic alterations may play a role in the progression from TL to ML DS.

BACKGROUND OF RESEARCH-OBJECTIVES

Genetic background of AML

AML can be classified according to morphological or (cyto-)genetic criteria. Recently, genetic abnormalities in AML have been further subdivided in type I and type II abnormalities.[19-21] The type 1 abnormalities consist of mutations in tyrosine kinases, phosphatases or oncogenes such as RAS, which promote cellular proliferation and survival. Type 2 abnormalities result in differentiation/maturation defects, and typically consist of either mutations in transcription factors [such as C/EPBα, GATA1 or PU.1], or chromosomal translocations [such as t(8;21) or inv(16)], resulting in loss of function of such transcription factors. Type 1 and 2 abnormalities by themselves are not sufficient to induce leukemia, and it is hypothesized that overt AML only results from cooperative genetic alterations in both classes.[19,22-24] The collaboration between type 1 and type 2 abnormalities is not a random process, but certain type 1 abnormalities cluster with certain type 2 abnormalities. For instance, Flt3/ITD is mainly found in AML with normal karyotype and in APL, whereas KIT-mutations occur predominantly in core-binding factor leukemias [CBF-AML, i.e. (t8;21) and inv(16)].[21,25,26]

Type 1 abnormalities in ML DS

In previous studies, focusing at receptor-tyrosine kinase (RTK) abnormalities (e.g. FLT3 and KIT), RAS oncogenes (K and N-RAS) and phosphatase (PTPN-11) mutations, Goemans et al. found mutations in only 2 out of 14 tested (14%) ML DS samples, versus 44% in sporadic AML.[21,27] The mutations in ML DS samples consisted of one N-RAS and one K-RAS mutation. No FLT3, KIT or PTPN11 mutations were found. Other studies on RTK-abnormalities in DS AML are lacking.[28] However, in sporadic AML M7, mutations in JAK-2 have been described in 18% of samples, and recently also mutations in JAK3 have been described.[29,30] In the majority of the ML DS cases the proliferation enhancing type 1 mutations are still unknown, although they may play a role in progression from TL to ML DS. In addition, they may have important prognostic impact or may represent drugable targets, similar to FLT3 or KIT mutations. Therefore, we aim at identifying such abnormalities in these DS patients.

Type 2 abnormalities in ML DS

It was recently discovered that GATA1, a gene that encodes an essential hematopoietic transcription factor, is mutated in the leukemic of patients with ML DS, but not in other leukemias.[31] This concerns various deletions, insertions and point mutations in exon 2, which result in a premature stop codon. GATA1 mutations result in the synthesis of a shorter GATA1 protein (GATA1s), which is characterized by a dysfunctional transactivation domain. Further studies have shown that GATA1 mutations can also be found in DS TL-samples, and already arise in utero in the fetal hematopoietic cells from which TL originates.[31-34] Therefore these mutations are not involved in the progression of TL to ML DS, nor does it provide a possibility to stratify patients.

In a BFM series of 137 ML DS patients, 94 samples were successfully karyotyped. In 25 patients no additional abnormalities (apart from constitutional trisomy 21) were found, whereas in 69 patients various additional abnormalities were detected: 14 patients showed trisomy 8; 10 patients showed an extra non-constitutional copy of chromosome 21; 4 patients showed del 1q and 2 patients showed 7q-/-7 (Figure 1; Reinhardt et al, personal communication).

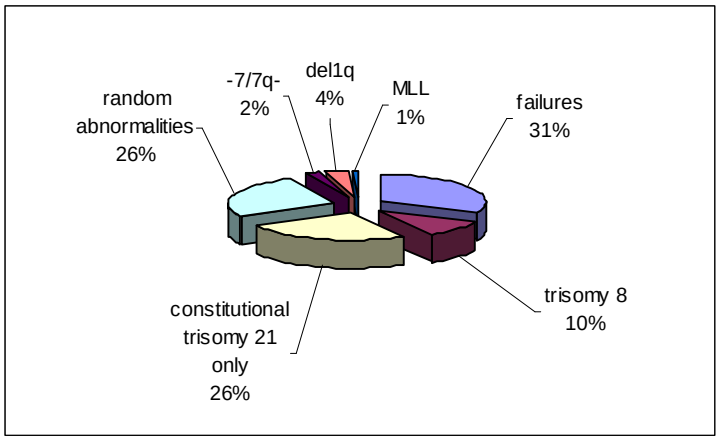

Figure 1. Type 2 genetic abnormalities in 137 ML DS samples (all have contstitutional trisomy 21). In 31% no karyotype was available.

Hence, secondary abnormalities appear to be heterogeneous, and so far have not been linked to prognosis in ML DS. In this study we aim at identifying new genetic abnormalities (deletions and amplifications) by array-CGH in both TL and ML DS samples, to analyze whether they are associated with disease progression and/or prognosis.

Drug resistance studies

Using in-vitro total cell-kill assays, we and others have demonstrated hypersensitivity of the ML DS blasts for chemotherapeutic drugs regularly used in the treatment of ML DS.[13-16] However, a large heterogeneity can be observed for individual sensitivity to these drugs. For instance for cytarabine, approximately 70% of the ML DS samples are hypersensitive (defined as an LC50 value below the 3rd percentile for sporadic AML patients), whereas the other 30% is in the same range as sporadic AML.[15] For daunorubicin however, only 16% display hypersensitivity, whereas 84% of LC50 values is in the same range as sporadic AML. Studies from Taub et al. may explain some of the observed variations in drug sensitivity. They have shown that chromosome 21 localized genes involved in the metabolism of cytarabine and daunorubicin are differentially expressed between ML DS and sporadic AML cases.[35] Of interest, transcript levels in ML DS cases varied from 0 to 12-fold higher levels, hence different from the expected 1.5-fold as predicted by gene copy number. Taken together, these data suggest that ML DS itself is a heterogeneous disease, and that further therapy reduction may be warranted for some, but nor for all patients.

MicroRNAs

It was recently recognized that naturally occurring non-coding small RNAs – so called microRNAs – are involved in controlling gene expression at the posttranscriptional level.[36] Approximately 50% of the genome is transcribed into RNA, but only 2% is being translated into proteins, which might be regulated by these microRNAs (miRNA). MiRNAs are 22 nucleotide RNAs that bind to the target mRNA and prevent its translation. miRNAs inhibit target gene expression at the protein level, and loss of miRNA expression results in increased expression of proto-oncogene proteins.[37] Hence, these miRNAs may function as tumor suppressor or oncogenes.

Knowledge on miRNAs and their relevance for hematological malignancies is still very limited, but some of the miRNAs have been associated with hematopoiesis. For example, CLL is characterized by 13q14 deletions in >50% of cases, and this region was recently discovered to encode for 2 different miRNAs (miR-15 and 16).[36,38] A recently published study showed differential expression of miRNAs across cancer subtypes, and paralleled the developmental origin of tissues.[37] Differential miRNA expression was detected between BCR-ABL, TEL-AML1, T-ALL and MLL-rearranged ALL samples.[37]

Intriguingly, four of the recently discovered miRNAs, miR-99a, miR-125b, miR-155 and let-7c, are encoded on chromosome 21.[39] MiR-125b and miR-155 are overexpressed in megakaryoblastic leukemias. Interestingly, among the miR-125b and miR-155 target genes, there are several hematopoietic transcription factors and regulators such as PU.1, FLI1, LIF, c-myc, IL13, IL6, which have been shown to be involved in the development of different leukemias.[39] In ALL, we recently cloned new miRNAs and identified 87 known and 43 new human mature miRNAs.[40] Quantification of miRNA expression (RT-PCR) revealed that miRNAs were expressed at higher levels in MLL and B-ALL cells when compared to normal CD34+ bone marrow cells.[40] In this project we want to identify whether miRNAs are involved in DS ML, and whether they play a role in the transformation from TL to ML DS.

AIMS OF THE STUDY

The main aims of this study are:
1. To identify new genetic markers (mutations, deletions, amplifications) that allow stratification of patients with ML DS in different risk groups, and that provide a biological rationale in which patients further therapy reduction may be warranted
2. To detect genetic abnormalities related to the progression from TL to ML DS
3. To identify microRNAs that may be involved in ML DS pathogenesis or in the progression from TL to ML DS.
4. To identify drugable targets that may lead to targeted therapy in ML DS patients, which may allow further reduction of intensive chemotherapy and reduce side effects.

MATERIALS AND METHODS AND PRELIMINARY RESULTS IN OTHER SUBSETS OF LEUKEMIA

To answer the questions raised above we will apply the following techniques:

1. Array-CGH, to detect deletions and/or amplifications that are currently unknown. Subsequent FISH studies will be performed to study which genes may be affected.
2. We will seek for mutations in tyrosine kinases (identification of kinases on interest will be derived from the literature and our earlier gene expression profiling study[41]), using dHPLC technology, followed by sequencing, and also determine mRNA expression levels by Taqman technology. In case new tyrosine kinase abnormalities are identified, we will study their sensitivity to known tyrosine kinase-inhibitors (Western blot, cell-kill assays).
3. MicroRNA identification by cloning and expression analysis using stem-loop based real-time Taqman technology for the currently well-characterized and novel miRNAs.

These techniques will be applied in both ML DS and TL samples, addressing the following issues:

(a) To identify subgroups of ML DS patients with different prognosis, which may lead to a better classification of patients with ML DS in risk-groups,
(b) To detect genetic abnormalities that may explain the progression from TL to ML DS,
(c) To detect genetic abnormalities that may be involved in response to chemotherapeutics.

BACKGROUND TECHNIQUES

Comparative genomic hybridization (CGH)

The recent applicability of the array-CGH technique with a much higher resolution than conventional CGH (10-20 Mb), may further lead to the identification of prognostically important chromosomal regions. This technique is currently being used in the PhD-project of Peter van Vlierberghe “Genetic determinants for treatment outcome in pediatric Acute Lymphoblastic Leukemia”, and in 2 AML projects. Various abnormalities were found, that were in agreement with

previous karyotypic data, thereby confirming the specificity of this technique. Interestingly, many clonal and subclonal chromosomal amplification and deletion areas were found that have never been linked to human T-ALL. A particular abnormality that was found in 12 out of 36 T-ALL patients was a subclonal chromosomal duplication involving the 9q34 region.[42] It was demonstrated that this 9q34 duplication was independent from the presence of activating mutations in the NOTCH1 gene, as present in about 50% of T-ALL, and also independent from the recently described episomal amplified NUP214-ABL1 fusion gene in human T-ALL. In a recent paper, we report on a cryptic deletion in chromosome 11, which leads to LMO2 activation in T-ALL.[43] In brief, labeled DNA samples are hybridized in a one to one ratio onto a DNA-chip that contains about 100.000 oligo-nucleotide probes (Agilent oligo-array-CGH). For each chromosomal abnormality that will be identified by array-CGH, a specific FISH procedure will be developed for validation and further pinpointing of the exact chromosomal regions involved, as described in more detail before.[43]

Tyrosine kinase activation

Tyrosine kinases of interest will be studied by direct sequencing or dHPLC technology. These methods are fully operational at our laboratory, as we characterize most AML samples for molecular abnormalities, such as mutations in KIT, RAS, FLT3, NPM1, etc.[44]

Micro-RNAs

RNA will be extracted using Trizol reagents (Gibco, NL), and RNA integrity will be checked before proceeding with miRNA expression analysis. The mature miRNA fraction (~22mer) will be isolated by gel electrophoresis using ^{32}P-labeled size markers. After sequential ligation of 3' 20mer and 5' 18mer adaptor sequences and purification of ligated miRNAs using mobility shift gel electrophoresis, the resulting small RNA (~60mer) is subjected to reverse transcriptase-PCR and, after digestion with Ban-I, small dsDNA fragments are concatemerized using T4 DNA ligase. Gel purified concatemers (200-700mer) are isolated and ligated into a bacterial cloning vector which is used to transform competent E.coli bacteria. The concatemers-containing vector will be purified out of positive E.coli clones, which then can be used for sequence analysis of inserted miRNAs. The sequence will be used to localize encoded miRNAs in the genome using bio-informatic analyses (sequence comparison of our cloned miRNAs to the total human genome). Next, the sequence of each miRNA is used to identify the target gene using complementary, thermodynamic and evolutionary conservation rules.[Lewis, Cell 2003; He & Hannon, Nat Rev Genetics, 2004]. Applying this procedure, we found 94 novel and 101 known miRNAs for which the frequency of expression differed between pilot patients with different genetic subtype (Figure 2).[40] It is the aim of this project to identify miRNAs relevant to TL and ML DS.

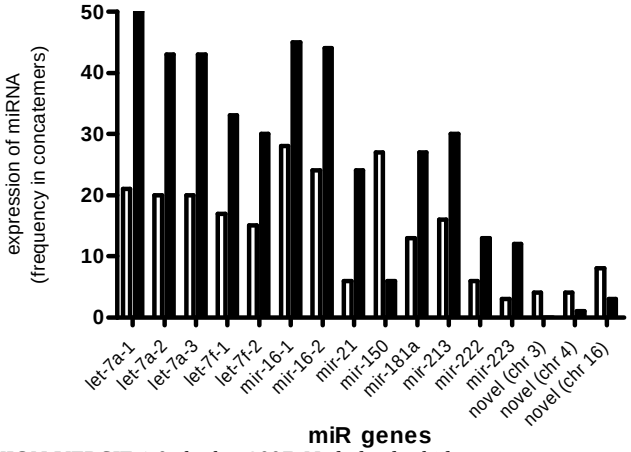

Figure 2.

Selection of miRNAs that are differentially expressed between 2 patients with ALL. White bars represent a patient with an MLL-rearrangement, black bars represent a patient with a t(1;9) translocation. Expression of 3 newly identified miRNAs are included at the right part of the X-axis.

Once identified, the expression of miRNAs and targeted genes will be analyzed in a larger number of patients representing both TL and ML DS. Preferably, we would like to use a miRNA array to detect expression differences between these subgroups. However, at present, the miRNA array technology is not quantitative due to non-linear amplification efficiency and, hence, we prefer to use real-time quantitative (RTQ) PCR. Since mature miRNAs are too small (~22mer) for a traditional RTQ-PCR, a special stem-loop based primer will be used (Applied Biosystems, USA). MiRNA and targeted gene mRNA expression will be quantified by RTQ-PCR. Differences in protein expression levels of targeted genes will be determined by Western blot. The miRNAs, targeted genes and pathways will be identified by applying biostatistical tools similar to the gene expression profiling studies we previously performed to classify leukemias and find drug resistance associated genes.[45,46]

RELEVANCE OF STUDY FOR ML DS PATIENTS

Although many children with ML DS are cured with current modified treatment regimens, this is still achieved at the expense of considerable acute and long-term morbidity. It is currently not known whether for some children with ML DS therapy could be further reduced without compromising anti-leukemic efficacy. The availability of genetic or other markers may be very useful in this respect. Moreover, some of these markers may be drugable and allow molecularly targeted therapy, for instance drugs directed against activated tyrosine kinases. This might in the long run improve prognosis and reduce chemotherapy-related morbidity. This study may also provide further insight in the underlying biology of the progression from TL to true leukemia in children with DS, which so far is not very well understood.

NUMBER OF REQUESTED SAMPLES AND DATA

Requested samples

1) TL peripheral blood samples will be obtained from a population-based study in the Netherlands (organized by the Dutch Childhood Oncology Group) which aims to screen 811 children with DS (in approximately 3 years) for the occurrence of TL, which is estimated to be 5-10%. In addition, samples will be provided by our collaboration with the AML-BFM-SG (principal investigator D. Reinhardt). Samples are shipped to the central laboratory in Hannover, and will also be made available for this study. We aim at studying approximately 30 patients with TL, including at least 10 paired TL and ML DS samples. This will allow us to study differences between TL samples that either undergo spontaneous disappearance versus those that progress to ML DS at a later stage.

2) ML DS samples from bone marrow and or peripheral blood will be obtained from the collaborative groups participating in this European ML DS study, which will include 150 patients. Children with ML DS often have myelofibrosis, complicating the harvest of sufficient amounts of marrow. In addition, the blast percentages may be relatively low. However, in a previous study using a total cell-kill assay, we were able to test 59% of ML DS samples successfully (this test requires samples to have at least 80% blast purity at the start of cell-culture).[15] Therefore we expect to be able to include at least 30 samples of ML DS with sufficient cells to perform the above mentioned studies. Additional samples may come from the cell-bank form the DCOG and AML-BFM SG.

3) Sporadic, FAB-type matched, AML samples (controls) will be obtained from the DCOG and the AML-BFM SG cell banks. These samples may be needed to prove whether abnormalities are specific for ML DS, or also occur in sporadic AML FAB-type matched samples.

4) Normal BM-cells as well as remission samples are available through a collaboration with H. Hasle from the NOPHO, as well as the minimal residual disease studies performed in both the TL and the ML DS studies mentioned before.

Number of cells needed

For array CGH 50µg of DNA is needed, which can usually be obtained from $10x10^6$ viable leukemic cells. This will also allow us to extract sufficient RNA for mRNA and miRNA expression and mutation analysis. For the tyrosine kinase activation assay we need only small amounts of DNA for sequencing of dHPLC analysis. For FISH analysis of genetic deleted/amplified regions we will run prepare cytospin slides. For Western blotting approximately 10x10.6 cells are needed. Therefore these studies can be done with 20x10.6 viable leukemic cells in total, corresponding with approximately 30-40x10.6 cryopreserved cells. In case further sensitivity testing to RTK-inhibitors is warranted (in selected samples with new tyrosine kinase mutations only), we would need $5x10^6$ additional cells per tested inhibitor. In such cases we will ask for additional cells from the cell-bank.

Clinical and cell-biological data

We would like to obtain clinical and cell-biological data for all included patient samples, consisting of: date of birth, date of diagnosis, WBC, CNS-status, sex, FAB-type, cytogenetic and molecular genetic data, date of CR, date and type of event, date of last follow-up.

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1.4 Minimal residual disease measured by WT1 expression in patients with myeloid leukemia of Down syndrome treated on the international Down Syndrome Myeloid Leukemia 2006 study

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Introduction
Children with Down syndrome (DS) have a significantly increased risk of developing myeloid leukemia described as myelodysplastic syndrome (MDS) and acute myeloid leukemia (AML). Due to the unique features of MDS and AML in DS children, including the presence of GATA1 mutation in the vast majority [1], the unifying term myeloid leukemia of DS (ML-DS) has been used for this disorder [2]. Recent years have seen a number of studies with AML therapy producing event free survival (EFS) of more than 75% [3-5] in ML-DS compared with EFS around 50% in AML children without DS [6-8]. The favorable outcome in ML-DS has been explained by the increased sensitivity to cytarabine and daunorubicin [9-11] as well as a higher susceptibility of DS-cells to apoptosis [4;12].

Very intensive therapy is associated with a significantly higher mortality in ML-DS [13] and chemotherapy doses are often reduced in DS patients. The optimal balance between dose intensity and the risk of treatment-related toxicity has not yet been defined.

One of the primary objectives of the international Down Syndrome Myeloid Leukemia 2006 study is to reduce therapy in DS but still achieve an overall survival of 85% in all participating institutions. A few patients have relapsed on previous protocols, we don’t know if the present protocol will increase the risk of relapse. Apart from age [3] no data are available about risk factors for relapse.
The Wilms tumor gene (WT1) is a tumor suppressor gene over-expressed in several types of solid tumors and in leukemias. The high expression of WT1 in patients with AML is useful for minimal residual disease monitoring [14;15]. We have recently shown that WT1 gene expression was increased in five newborns with DS and transient myeloproliferative disease and persistently elevated values may predict subsequent recurrence of leukemia [16].
GATA1 mutation was recently reported as a marker of minimal residual disease in ML-DS [17;18]. Sensitive GATA1 analyses using RQ-PCR need to be patient specific and thereby relatively laborious. The WT1 gene expression analyses are not patient specific and may be an attractive alternative to GATA1 for monitoring minimal residual disease.

Aims of the study

Study WT1 expression in PB and BM of ML-DS patients at diagnosis and follow-up
Identify patients who are under treated on the reduced intensity ML-DS 2006 protocol.
Identify patients who may be candidates for even further dose reductions.

Patients and Methods

Patients

All patients treated according to the Down Syndrome Myeloid Leukemia 2006 study are eligible for the present study on WT1 expression. Patients are eligible regardless of GATA1 mutation status
Samples from peripheral blood (PB) and bone marrow (BM) should be taken at diagnosis, day 28 (before AM), before haM, and before HA as indicated in the therapy flow (appendix 1). An additional PB sample should be sent 3 months from last course (HA).
If the BM material at diagnosis is too sparse to reserve material for the study PB may be sent alone. In such patients follow-up studies will be based upon PB only.
Patients without significantly increased WT1 expression at diagnosis do not need to have samples taken at the three follow-up time points.

Methods

DNA purification, RNA purification, and cDNA synthesis

Mononuclear cells (MNCs) are isolated from PB and BM by Lymphoprep (Axis-Shields PoC AS, Norway) density centrifugation, resuspended in lysis buffer (MagNA Pure LC mRNA Isolation Kit I, Roche Diagnostics GmbH, Mannheim, Germany) and frozen at -80°C until DNA or mRNA purification on a MagNA Pure LC robot (Roche Diagnostics GmbH, Mannheim). Approximately 200 ng mRNA in 19 µl MagNA Pure RNA elution buffer is reverse transcribed in a final volume of 40.6 µl [15].

WT1 quantification by real-time quantitative RT-PCR

Design and validation of WT1 primers and TaqMan probe, as well as assay conditions have been described in detail earlier [15]. Shortly, for all samples, the level of WT1 and the internal control genes β-2-microglobulin (β2M) and Abelson (ABL) are analyzed.
A Ct value of 40 is defined as detection limit, and Ct values above this limit are set to 40 for sensitivity calculations. WT1 expression is determined as ABL-normalized WT1 levels using the following equation: $z = 2^{\Delta CtNORM} \times 1000$, where $\Delta Ct_{NORM} = Ct_{ABL} - Ct_{WT1}$, and z is the number of WT1 copies/1000 ABL copies.
The assay sensitivity, which reveals the maximal decline in WT1 level compared to diagnostic level that may be measured within the individual sample, is calculated from the equation: $y = 10^{(40 - AveCtrlAct - \Delta Ct,Diag)/-3.3}$, where $AveCtrl_{Act} = (Ct_{\beta} + Ct_{ABL})/2$ in the actual sample, $\Delta Ct_{Diag} = Ct_{WT1} - (Ct_{\beta} + Ct_{ABL})/2$ in the diagnostic sample, and –3.3 is the slope of the WT1 standard curve, y^{-1} equals the fold reduction possible.

Statistics

It is planned to open the study in 2006 and the participating countries are estimated to contribute a total of 25 patients per year leading to a total of 150 children with DS-ML after 6 years.
The expected relapse rate is very low. It is hypothesized that the majority of those who relapse will be MRD positive.
Example 1. In a cohort of 88 patients 5 of 8 (63%) MRD positive patients experience a relapse and 2 of 80 (3%) MRD negative patients experience a relapse. The difference in positive predictive value will be 60% determined with a 95% confidence interval of 26-94%.

Example 2. In a cohort of 88 patients 4 of 8 (50%) MRD positive patients experience a relapse and 4 of 80 (3%) MRD negative patients experience a relapse. The difference in positive predictive value will be 45% determined with a 95% confidence interval of 10-80%.

Feasibility and logistics

The methods to perform the study are well established in Aarhus [15] and Frankfurt [19]. The study will be an add-on study to the Down Syndrome Myeloid Leukemia 2006 study.

Clinics participating in the BFM-AML study will send material to Hanover where cells will be frozen and forwarded to the laboratory in Frankfurt where RNA will be isolated.

Patient samples of PB and BM in EDTA from participating non-BFM clinics should be sent with overnight courier to Aarhus (appendix 2). It is recommended to avoid sending samples on a Friday.

At each sample date at least 1,000,000 cells from blood and from BM (i.e. 2 ml PB and 2 ml BM) are sampled and send to one of the laboratories mentioned above. From blood and BM samples at least 1 μg of RNA are isolated for the WT1 studies.

RNA isolation and WT1 quantification at 0 and 48 hrs after BM puncture have been performed comparing stabilized (PaxGene) to unstabilized RNA. Preliminary data indicate that stabilized RNA does not provide any advantage compared to stabilized RNA concerning WT1 quantification (Peter Bader personal communication).

MRD studies are for research only. Recommendations of changes in therapy should not rely on MRD results. It is recommended that the MRD results are not given to the clinicians.

Ethical considerations

Participation in the present study implies an extra 2 ml of PB and 2 ml of BM at four time points when samples are taken for other reasons. The extra amount of PB and BM sampled is not considered to pose any risk for the children. The study results are for research only and treatment changes should not be based on these results. Participation in the study has no direct consequences for the patients (neither negative nor positive). The results of the study may help therapy guidance for future patients.

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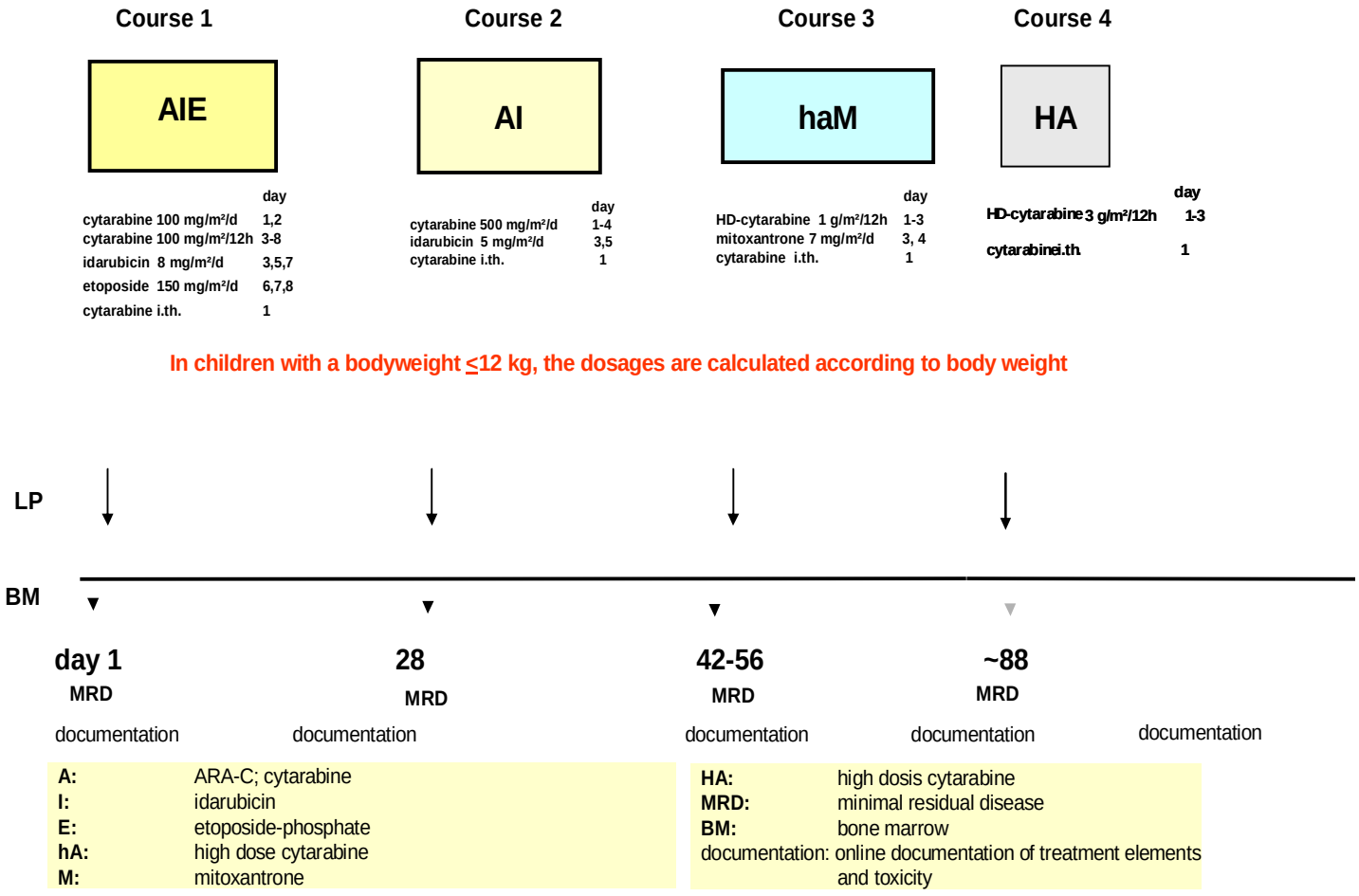
Appendix 1. Flow chart of ML-DS 2006 and time points for MRD samples.

Samples from peripheral blood (PB) and bone marrow (BM) should be taken at diagnosis, day 28 (before AM), before haM, and before HA. One additional PB sample is taken 3 months from last course (HA).
If the BM material at diagnosis is too sparse to reserve material for the study PB may be sent alone. In such patients follow-up studies will be based upon PB only



Appendix 2. Invoice for MRD WT1 studies - non-BFM patients - ML-DS 2006 study

To:
Immunohematologic Laboratory
att: WT1 study
Aarhus University Hospital
Tage-Hansens Gade 2,
DK-8000 Aarhus C
Denmark

Patient Name: _____

Date of birth: _____

Date of ML-DS diagnosis: _____

Mailed material
Date of sampling: _____
□Diagnostic sample □Day 28 sample □Start of HAM □Start of HA

Please send 2 ml of peripheral blood (PB) and 2 ml of bone marrow (BM) in EDTA tubes.
Send the samples by express courier (arrival within 24 hours) at room temperature.
Please avoid sending samples on a Friday.

Please announce the lab before mailing the samples by faxing this invoice to: +45 8949 7598

Date _____ Signature _____
Address of the sending institution (stamp):
Contact person:

Contact persons Aarhus:
Mette Østergaard: mette.oestergaard@ki.au.dk phone: +45 8949 7577
Henrik Hasle: hasle@dadlnet.dk phone +45 8949 6716

1.5 TITLE OF THE STUDY: INFLUENCE OF GATA1S ON LEUKEMIOGENESIS IN DS-AML

NAME APPLICANT(S): DR. CLAUDIA LANGEBRAKE, PD DR. DIRK REINHARDT

INSTITUTE(S): MEDIZINISCHE HOCHSCHULE HANNOVER, PÄDIATRISCHE HÄMATOLOGIE/ONKOLOGIE

1. INTRODUCTION

Children with Down Syndrome (DS) - one of the most frequent chromosomal abnormalities and characterized by constitutional trisomy 21 - have a 20-40 fold increased risk for acute childhood leukemia, mostly of the acute megakaryoblastic leukemia (AMKL) subtype.[1-3] The morphology of the megakaryoblasts by microscopy and flow cytometry is very similar to the blasts of patients without DS, and is characterized by the expression of megakaryocytic/erythroid antigens.[4,5] The peak of incidence is observed between the 1st and 4th year of life. The fact that the outcome of DS patients with AMKL after treatment with chemotherapy is significantly better when compared to patients without this genetic syndrome points to a separate well defined biological entity.
Furthermore about 10% of newborns with DS present with transient myeloproliferative disease (TMD), a preleukemia with spontaneous regression in most cases, but progression to AMKL in about 30% of the patients within 3 years.
Recently, mutations in exon 2 of the transcription factor GATA1, that is involved in megakaryocytic and erythroid development, have been described in both diseases.[6-8] These mutations result in the introduction of a stop codon in exon2, that leads to a shorter GATA1 protein lacking the N-terminal transactivation domain. Up to now, it is unknown, whether these mutations in combination with a trisomy 21 are sufficient, to induce leukemic transformation.
In normal megakaryopoiesis, starting from the hematopoietic stem cell (CD34+/CD38-/CD41-) mature megakaryocytes (CD34-/CD38+/CD41+/CD42b+) develop via the stadium of CFU-MK (colony forming unit megakaryocyte) and promegakaryoblasts under the influence of different transcription factors. Especially RUNX1, PU.1, GATA1, GATA2, NF-E2 and Fli-1 seem to play important rolls in megakaryopoiesis. The activity of a transcription factor is not only dependent on its expression level, but is also influenced by protein-protein interactions. Cross antagonism and activation are therefore decisive effects for lineage differentiation and for leukemiogenesis. A very good characterized phenomenon is the cross antagonism between GATA1 and PU.1.[9]
Human cord blood (CB) is a rich source of hematopoietic progenitor cells. By sorting CD34+/CD41- megakaryocyte progenitors (MK prog) one can differentiate these cells into the megakaryocytic lineage using thrombopoietin (TPO)[10].

2. AIM(S) OF THE STUDY

Within this project it will be analyzed, whether the GATA1 mutation – in addition to the trisomy 21 - is responsible for the malignant transformation, or if further predisposing factors (PU.1, RUNX1) are required.

3. RELEVANCE OF STUDY FOR PEDIATRIC AML PATIENTS

Within this project it will be analyzed, whether the GATA1 mutation – in addition to the trisomy 21 - is responsible for the malignant transformation, or if further predisposing factors (e.g. PU.1 downregulation) are required. The results will give further insight into the predisposing factors for leukemiogenesis and are a prerequisiste for potential new treatment options.

4. PRELIMINARY RESULTS

We could show, that in the blast cells of DS-AMKL, GATA1s gene expression is significantly increased and PU.1 expression is significantly decreased as compared to non-DS AML-M7 or hematologically normal BM cells from children with DS.[11,12]

Furthermore, in regenerating bone marrow of children with DS-AMKL in remission, there is a high amount of cells coexpressing CD33 and CD56 (NCAM), an adhesion molecule, that is normally expressed by natural killer cells. These CD33/56+ cells look morphologically like monocytes and granulocytes and are no malignant cells. We could show that the expression of RUNX1 is highly elevated in these cells. In no other entity, it was possible to detect these CD33/56+ granulocytes/monocytes.[13] Recently, Gattenlöhner et al. could show a correlation between RUNX1 and NCAM expression in cardiomyocytes.[14]

Using a mouse model with an in-frame deletion of the codons encoding amino acids 3-63 in exon 2 of GATA1 ($GATA1^{\Delta N}$), Li et al. could show, that GATA1s leads to hyperproliferation of megakaryocyte progenitor cells derived from fetal liver cell, but not from adult bone marrow.[15]

5. MATERIALS AND METHODS

We will retrovirally transduce cord blood and fetal liver cells from individuals with trisomy 21 with GATA1s. During in-vitro megakaryopoiesis, we will characterize the different maturation and differentiation stages by morphology, immunology (multiparametric immunophenotyping, western blot) and molecular genetics (gene expression analysis using microarray technique and quantitative RT-PCR). For selective inhibition of PU.1, we will use specific PU.1 siRNA (kindly provided by M. Scheer, Medizinische Hochschule Hannover) at different stages of differentiation. The investigation of the impact on transcriptional regulation of hematopoiesis and leukemiogenesis will take a center stage. For comparison, we will also analyze hematopoiesis in children with DS and leukemiogenesis in DS-AMKL.

6. ETHICAL ASPECTS

This project has been approved by the ethic committee (University of Muenster); informed parental and patient consent will be obtained.

7. NUMBER OF REQUESTED SAMPLES AND DATA (INCLUDING STATISTICAL ASPECTS)

- cord blood from 5 newborns with Down Syndrome
- cord blood from 5 newborns without Down Syndrome
- fetal liver cells from 5 fetuses with Down Synrome
- hematopoietic stem cells from 5 children with TMD
- hematopoietic stem cells from 5 children with DS-AMKL

8. IS THE PROPOSED RESEARCH PART OF A PHD-PROJECT ?

no

9. HOW WILL THE PROJECT BE FUNDED ?

For this project, a research grant has been submitted to the José Carreras Leukämiestiftung

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Li Z, Godinho FJ, Klusmann JH* et al. Developmental stage-selective effect of somatically mutated leukemogenic transcription factor GATA1. Nat Genet. 2005;37:613-619

* JH Klusmann is medical student, supervised by D. Reinhardt, who worked at Dana Farber Cancer Institute (Prof. St. Orkin) from 6/2004 to 6/2005. He will continue his studies at the MHH.

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1.6 **TITLE OF THE STUDY: Minimal residual disease measured by the quantification of the patient specific GATA1s (RT-PCR) and by immunophenotyping**

NAME APPLICANT(S): DIRK REINHARDT / KATARINA REINHARDT / C. LANGEBRAKE:

INSTITUTE(S) MEDICAL SCHOOL HANNOVER
PEDIATRIC HEMATOLOGY AND ONCOLOGY

1. INTRODUCTION

Children with Down syndrome (DS) have a significantly increased risk of developing myeloid leukemia. Recent years have seen a number of studies with AML therapy producing event free survival (EFS) of more than 75% [1-3] in ML-DS compared with EFS around 50% in AML children without DS [4-6]. The favorable outcome in ML-DS has been explained by the increased sensitivity to cytarabine and daunorubicin [78] as well as a higher susceptibility of DS-cells to apoptosis [2,9].

Very intensive therapy is associated with a significantly higher mortality in ML-DS [10] and chemotherapy doses are often reduced in DS patients. The optimal balance between dose intensity and the risk of treatment-related toxicity has not yet been defined.

One of the primary objectives of the international Down Syndrome Myeloid Leukemia 2007 study is to reduce therapy in DS but still achieve an overall survival of 85% in all participating institutions. A few patients have relapsed on previous protocols, we don't know if the present protocol will increase the risk of relapse. Apart from age [1] no data are available about risk factors for relapse.

2. AIM(S) OF THE STUDY

Feasibility of MRD-monitoring of the GATA1s positive blasts in DS-ML by quantitative RT-PCR and be immunophenotyping-
Comparison to MRD diagnostics by immunophenotyping and by using WT1 (H.Hasle)
Prognostic value of MRD diagnostics

3. RELEVANCE OF STUDY FOR PEDIATRIC AML PATIENTS

Identification patients with a low MRD burden and a favourable outcome, which might justify further reduction of therapy.
Identification of children with a high MRD burden and a poor outcome, who might need intensified treatment

4. PRELIMINARY RESULTS

Minimal residual disease (MRD) diagnostics, i.e. monitoring of the aberrant GATA1s clone, could be performed by immunophenotyping or molecular methods[11-15]. The specific immunophenotype in Down syndrome allows a high sensitivity and specificity at a level of 1 to $5*10^{-3}$. We demonstrated this as well by stepwise dilution experiments as in patient samples of both DS-ML or TMD children and children with leukemia but without Down syndrome (Fig. 1).

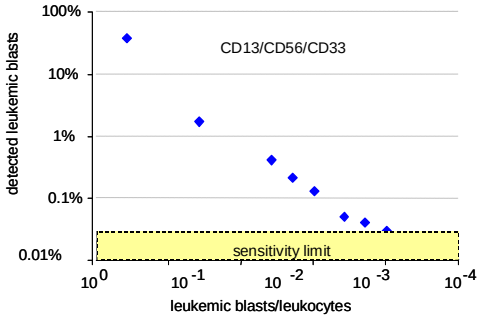


Figure 2: Dilution of myeloid blasts with high specificity

However, as a specific mutation is available, the monitoring of the GATA1s mutation (Fig.2) has an even better sensitivity ($10^{-3}/^{-4}$)[14].
Retrospective analysis of stored samples of children with TMD (n=4), morphological remission and no development of DS-ML revealed no evidence of a preleukemic clone at week 12, neither by immunophenotyping nor by quantitative RT-PCR of the GATA1s clone.

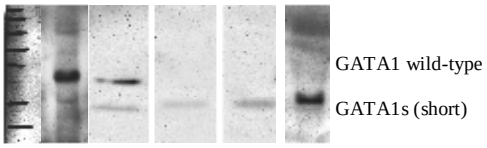


Figure 2: Detection of GATA1 transcription factor

5. METHODS

MRD GATA1s

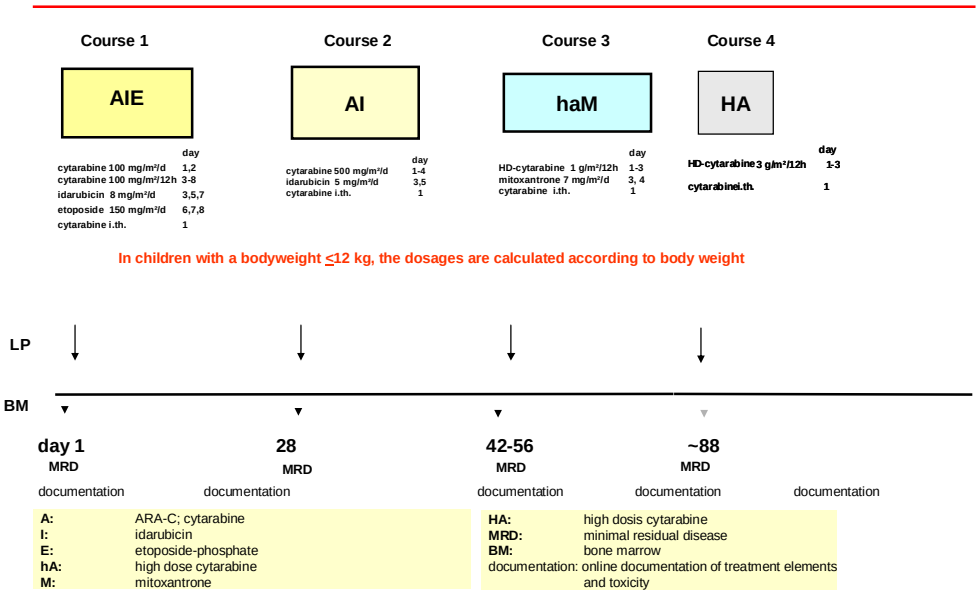
The GATA1s mutation will be detected according to the established SOP (P. Vyas): Patient specific primers will be design and quatitative RT-PCR (Taqman) will be performed.

Immunophenotyping

The specific immunophenotype of leukemic blasts from children with Down syndrome[16] allows a high sensitive MRD-diagnostics (figure 2). MRD diagnostics will be accessed by a 4-color immunophenotyping. Detailed methods and standardisation have been published recently[15].

6. ETHICAL ASPECTS

Parental informed consents for diagnostics, treatment and data storage have to be obtained. Participation in this study will require blood sampling at defined time points (similar to the MRD-proposal of H. Hasle: MRD Day 1; 28; 42 to 56; 88; see schedule). There is no additional harm to the children.
For molecular and immunological GATA1s monitoring, 2 ml of EDTA anti-coagulated blood will be necessary.



7. NUMBER OF REQUESTED SAMPLES AND DATA (INCLUDING STATISTICAL ASPECTS)

It is planned to open the study in 2007 and the participating countries are estimated to contribute a total of 25 patients per year leading to a total of 150 children with DS-ML after 6 years.

9. HOW WILL THE PROJECT BE FUNDED ?

Schlag-Stiftung

10. SELECTED REFERENCES FROM THE INVESTIGATORS INVOLVED IN THE PROJECT

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1.7 Defining the haemopoietic defect in patients with Down syndrome and myeloid leukaemia treated on the international Down Syndrome Myeloid Leukemia 2006 study

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Introduction

Mutations in transcription factors in leukaemia Normal blood stem/progenitor cells have four principal cell fate options; they can remain quiescent, self-renew, proliferate and/or differentiate along a lineage pathway and finally undergo apoptosis. In leukaemia, a number of genetic or epigenetic changes sequentially corrupt these options to progressively impair blood cell differentiation. One class of molecules commonly implicated in leukaemogenesis is a subset of critical haematopoietic transcription factors that regulate normal cell fate choice [1]. Aberrant activity of an individual transcription factor often affects cell fate of a restricted blood cell type suggesting cell context is important for oncogenesis. Mutations associated with transcription factors are often early events in the leukaemic process and in childhood leukaemia can occur in fetal life [2-6]. In some cases these early events result in clonal expansion of blood cells or a 'preleukaemic state', providing the cellular substrate for additional mutations to transform preleukaemia to leukaemia. Understanding how transcription factors corrupt cell fate choice in leukaemia is also very likely to be informative of their normal function in haematopoiesis. Taken together, studying the role of a particular transcription factor in leukaemia, may provide general principles about how transcription factors are oncogenic and separately control normal haematopoiesis.
To date, there is incomplete understanding of how transcription factors function in these roles. In this application, we study the role of one crucial transcription factor, GATA1, in the megakaryocytic/erythroid preleukaemia, TMD (transient myeloproliferative disorder), and leukaemia, AML, (acute myeloid leukaemia) associated with neonates and children with Down syndrome (DS). The study has clearly defined aim:-

1. To identify in which haematopoietic stem/progenitor compartment(s) GATA1 mutations occur and determine the effect on cell fate in each compartment we will study diagnostic AML bone marrow samples. This will be the first study of the impact of cell context on the effects of aberrant transcription factor activity on cell fate in primary paediatric acute leukaemia cells.

Background

i) GATA1 function in normal megakaryocyte and red cell differentiation GATA1 is a double zinc finger DNA-binding transcription factor encoded on the X-chromosome that is principally expressed in haematopoietic cells where it promotes specification and terminal maturation of myeloid progenitor cells to red cells and megakaryocytes [7-13]. In mice, germ line ablation of GATA1 function results in perturbed megakaryocyte and red cell differentiation. There are increased numbers of arrested megakaryoblasts in bone marrow (10-fold increased) and spleen (100-fold increased) with abnormal platelet maturation [12,13]. We have recently shown that this is due

to increased proliferation of GATA1 megakaryocyte progenitors rather than decreased apoptosis [14]. Red cell precursors are also blocked in differentiation but, in contrast to megakaryocytes, GATA1-null erythroid cells apoptose [15]. In humans, germ line missense GATA1 mutations have established its central role in regulating megakaryoblast numbers and ensuring normal megakaryocyte, platelet and red cell differentiation [16-20]. The differentiation block is explicable, in large part, because expression of most megakaryocyte- and red cell-specific genes requires GATA1 [13,21,22]. Previously, we showed that expression of inositol polyphosphate 4-phosphatase type 1 is markedly reduced, most likely indirectly, in primary GATA1-deficient megakaryocytes and restoration of phosphatase expression in these cells led to inhibition of megakaryocyte growth [23]. This phosphatase regulates mitogenic P-I-3 kinase activity suggesting deregulated P-I-3 kinase activity maybe important for abnormal megakaryocyte growth. However, it is unclear if this is the only mitogenic pathway interfacing with megakaryocyte GATA1 and what sequence of events leads to increased proliferation after loss of GATA1 function. In red cells expression of anti-apoptotic BCL-x_L is reduced in GATA1-null red cell progenitors [24]. Though, once again, a more complete understanding of the relationship between GATA1 and apoptosis has yet to be established.

ii) Leukaemia in DS Children with DS are uniquely predisposed to two clonal disorders of the megakaryocyte/red cell lineages; TMD and AML (reviewed in [25-30]). TMD presents in either the fetal or neonatal period suggesting it is a disorder of fetal hematopoiesis [29]. TMD has a variable clinical presentation, ranging from infants who are clinically well with circulating blasts detected only as incidental finding to rare fetuses and neonates that die from fulminant hepatic failure secondary to fibrosis. As blood counts and smears are not routinely performed on all DS newborns (see below), neonates with the more subtle presentation of TMD are likely to escape notice. Given this, though 10% of DS neonates are said to develop TMD [30] and a further 10% of DS foetuses die in utero, possibly due to TMD [31], the true incidence of TMD is unknown.
TMD is an extremely important model to understand the natural history of leukaemia because in ~30% of cases, AML develops either by overt progression or after an apparent remission. In either case AML usually presents before the child is 4 years old [25]. DS children have a ~150-fold increased risk of developing AML compared to the general paediatric population [25,Zipursky, 1992 #1743,32,33]. Several features suggest DS AML and TMD are linked conditions with a distinct pathogenetic basis [27,28]. First, TMD and AML blast cells usually have a very defined immunophenotype with expression of both megakaryocytic and erythroid markers suggesting that TMD and AML are derived from a transformed bipotent erythroid-megakaryocytic cell. Second, in cases where AML is preceded by TMD, the AML and TMD megakaryoblasts are morphologically, immunophenotypically and ultrastructurally similar[34-37]. Third, and possibly most importantly, John Crispino's group and then other groups (including our group) have shown that acquired somatic mutations are present in one copy of GATA1 specifically in TMD and AML cases but not other DS and non-DS leukaemias [38-44]. In cases of TMD that progressed to AML, the same GATA1 mutations were found in both TMD and AML, suggesting a molecular link between the two disorders. All of this suggests that TMD and AML are a unique and highly informative model of preleukaemia and leukaemia where some key pathogenetic events are known.

iii) GATA1 mutations in DS TMD/AML All reported GATA1 mutations occur in the 5' end of the gene, mainly in the first coding exon, exon 2. Most mutations either introduce a stop codon before codon 84 or disrupt splicing of exon 2 to the rest of GATA1 mRNA and thus remove the first translational start codon. In either case, the predicted GATA1 protein, GATA1s or GATA1short, would be translated from an alternative translational start codon, codon 84, and GATA1 protein would be truncated at the N-terminus. GATA1 mutations are disease specific as they are not detectable in remission samples. As GATA1 is on the X-chromosome, in both males and females (due to X-inactivation), the mutant clone expresses only the mutant allele. Finally, given the megakaryocyte/erythroid phenotype of AML, it is likely that mutation of GATA1 would help determine the lineage phenotype of the leukaemia [45,46]. This hypothesis is strengthened by our studies on a rare DS patient that presented with TMD, 2 years later developed AML and at 5 years

of age was diagnosed with ALL (acute lymphoblastic leukaemia). A GATA1 mutation was detected in the AML diagnostic sample but not ALL sample [47]. Thus, at least three events are required for AML. First, trisomy 21 has to be present in fetal life (see next paragraph) and need only be present in fetal blood cells. We and another group have studied rare TMD and AML patients with the hallmark who did not have germline trisomy 21 but had acquired trisomy 21 in the TMD/AML clone [42,48] and had GATA1 mutations. Second, fetal trisomy 21 haematopoietic cells then acquire GATA1 mutations, which are likely to confer a selective advantage. The combination of trisomy 21 and a GATA1 mutation is required for TMD. Third, additional genetic or epigenetic events are required for AML to develop, as not all cases of TMD progress to AML.

Proposed study

Experimental plan

The biological effect of aberrant transcription factor activity is modulated by cell context (see Introduction). To date the effect of deregulated transcription factor activity on cell fate in different haematopoietic stem/progenitor compartments has been not studied in any primary human leukaemia cells. We propose to study this question in DS AML. We will purify the hierarchy of myeloid progenitors during megakaryocyte differentiation from stem cells to the most committed (common megakaryocyte progenitor, CMkP), from bone marrow of both DS children with AML and as controls DS children without AML. Using these purified progenitors we will:

i) Determine when GATA1 mutation occurs in DS AML samples

ii) Compare frequencies of progenitors in DS children with and without AML (expansion of a particular progenitor compartment will implicate where a block in differentiation may occur)

iii) Document the proliferation and differentiation potential of each progenitor compartment and

iv) Determine self renewal within each compartment

v) Study apoptosis in each compartment.

These studies will provide the first detailed insight of how cell fate is altered in different compartments, for any human leukaemia. Unique to our study, a close collaborator and co-applicant Professor Irene Roberts (Imperial College and Hammersmith Hospital, London), is conducting a similar study on the cell context effect of mutant GATA1 in DS fetal haematopoiesis ±TMD. Comparison of data from post natal DS (±AML) and fetal DS (±TMD) samples will be informative of i) consequences of GATA1 mutation in highly defined cell compartments in TMD and AML ii) between fetal and post-natal DS samples. Thus the effect of i) preleukaemia versus leukaemia ii) development (fetal versus post-natal) can be compared. Moreover, by contrasting haemopoiesis in non-leukaemic DS fetuses and children it may provide insight into why fetal DS progenitors but not post-natal DS progenitors are targeted by the GATA1s oncoprotein and more generally into widely acknowledged but poorly understood differences between fetal and adult haemopoiesis [49].

Methods and samples to be used:

Samples

i) We will study both freshly isolated and stored AML bone marrow samples obtained from throughout Europe treated on the international Down Syndrome Myeloid leukaemia 2006 study. As patients with GATA1 mutation DS AML respond well to chemotherapy, the main aim of this protocol is reduce the amount of chemotherapy given to minimise treatment toxicity. To ensure that only good risk patients (i.e. DS patients with GATA1 mutation AML) are entered into this protocol, all DS AML cases will have GATA1 mutation analysis. Bone marrow samples will be collected on all cases. Additionally, we have access to frozen samples from our collaborators.

ii) Freshly isolated sternal control bone marrow samples from DS children undergoing cardiac surgery (collaborators Mr Pillai, Dr Archer, Consultant Surgeon and Cardiologist, Oxford)

iii) adult non-DS bone marrow samples from patients undergoing hip replacements will be used to set up assays (collaborator Professor Andrew Carr, Oxford).

We have ethical approval from the UK, Irish and Belgian local and national ethics committees and informed parental and patient consent will be obtained. This study has been approved by the UKCCSG (UK Childhood Cancer Study Group).

Methods

We will use published FACS sorting protocols to purify $CD34^{+}CD38^{-}$ (stem cell enriched), human common myeloid progenitor (CMP) and megakaryocyte erythroid progenitor (MEP) [50] and will adapt protocols for isolation of mouse common megakaryocyte progenitor (CMkP) that we have implemented [14] to isolate human CMkP [51]. We will use the high-speed Cytomation Mo-Flo sorters in our laboratory for these experiments. In pilot experiments, we will use aliquots of marrow from non-DS adults. Functionality of isolated stem/progenitor will be validated by LTC-IC (long term colony initiating cell culture assays) and colony assays, which we have established in our laboratory [52]. Once FACS-sorting protocols are implemented, we will work with DS material to isolate $CD34^{+}CD38^{-}$ cells, CMP, MEP and CMkP from normal and AML DS marrow samples. For each progenitor compartment, we will:

i) Establish GATA1 mutation status using our published PCR protocols [44]. This will tell us when in haemopoietic differentiation GATA1 mutations are first detected in DS AML.

ii) Compare the frequencies and absolute numbers of normal DS and DS AML stem and progenitor cells at different stages of differentiation. This will demonstrate if there is expansion of a particular progenitor compartment and suggest where perturbation in differentiation/proliferation may occur.

iii) Allow isolated stem/progenitor cells to recover for 12 hours in cytokine-supplemented media and then assay the proportion of proliferating cells (BrdU incorporation and FACS analysis) [53].

iv) Place stem cells in LTC-IC assays and progenitor cells in liquid culture and colony assays to document the extent of differentiation (MGG staining and expression of lineage markers by FACS and Real-Time PCR) and assess replating potential [52].

One issue to consider is whether sufficient cells will be available. We only need between 500 cells (for colony assays) to 5000 cells (for BrdU analysis, MGG staining, GATA1 mutation and lineage marker PCR analysis). The frequency of CD34+CD38- cells in marrow is 0.05-0.1%, CMP in adult marrow and cord blood is 0.28% and 0.4% respectively [50] and MEP frequencies are 0.13% and 0.05% respectively [50]. In a child's marrow sample ($5x10^{6}$-10^{7} cells), we will have between 2000 and 40000 stem and progenitor cells. Therefore, for some experiments we will have to pool samples.

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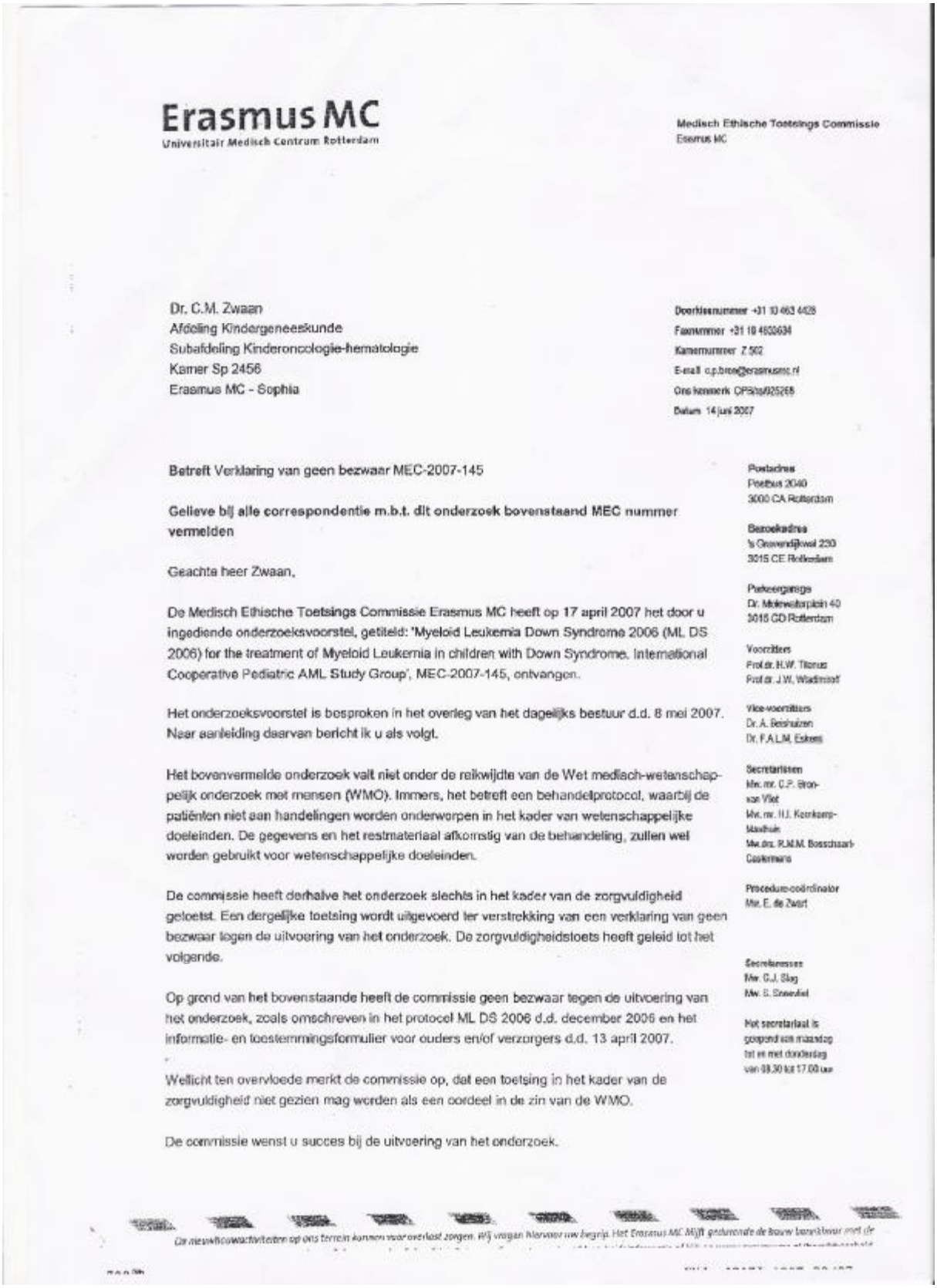
1.8 Verklaring van geen bezwaar



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Datum 14 juni 2007

Betreft Verklaring van geen bezwaar MEC-2007-145

Gelieve bij alle correspondentie m.b.t. dit onderzoek bovenstaand MEC nummer vermelden

Geachte heer Zwaan,

De Medisch Ethische Toetsings Commissie Erasmus MC heeft op 17 april 2007 het door u ingediende onderzoeksvoorstel, getiteld: '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', MEC-2007-145, ontvangen.

Het onderzoeksvoorstel is besproken in het overleg van het dagelijks bestuur d.d. 8 mei 2007. Naar aanleiding daarvan bericht ik u als volgt.

Het bovenvermelde onderzoek valt niet onder de reikwijdte van de Wet medisch-wetenschappelijk onderzoek met mensen (WMO). Immers, het betreft een behandelprotocol, waarbij de patiënten niet aan handelingen worden onderworpen in het kader van wetenschappelijke doeleinden. De gegevens en het restmateriaal afkomstig van de behandeling, zullen wel worden gebruikt voor wetenschappelijke doeleinden.

De commissie heeft derhalve het onderzoek slechts in het kader van de zorgvuldigheid getoetst. Een dergelijke toetsing wordt uitgevoerd ter verstrekking van een verklaring van geen bezwaar tegen de uitvoering van het onderzoek. De zorgvuldigheidstoets heeft geleid tot het volgende.

Op grond van het bovenstaande heeft de commissie geen bezwaar tegen de uitvoering van het onderzoek, zoals omschreven in het protocol ML DS 2006 d.d. december 2006 en het informatie- en toestemmingsformulier voor ouders en/of verzorgers d.d. 13 april 2007.

Wellicht ten overvloede merkt de commissie op, dat een toetsing in het kader van de zorgvuldigheid niet gezien mag worden als een oordeel in de zin van de WMO.

De commissie wenst u succes bij de uitvoering van het onderzoek.

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Mw. E. de Zwart

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Mw. C.J. Slag
Mw. S. Snaterse

Het secretariaat is geopend van maandag tot en met donderdag van 09.30 tot 17.00 uur

De nieuwbouwactiviteiten op ons terrein kunnen voor overlast zorgen. Wij vragen hiervoor uw begrip. Het Erasmus MC blijft gestimuleerde de bouw bereikbaar met de

Erasmus MC

Met vriendelijke groet,
namens de Medisch Ethische Toetsings Commissie Erasmus MC,

Mw.mr. C.P. Bron-van Vliet
Secretaris

Cc. Mw. E.G. Visser, Researchverpleegkundige Kinderoncologie
Prof.dr. A.J. van der Heijden
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