DB AML-01

Dutch-Belgian pediatric AML protocol for children with newly diagnosed acute myeloid leukaemia.

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Sponsor: SKION
Protocolcommissie: AML 2007
Coördinerend PI: Dr. E.S.J.M. de Bont

Dutch-Belgian pediatric AML protocol for children with newly diagnosed acute myeloid leukaemia,

based on the NOPHO-AML 2004 study

DB AML-01

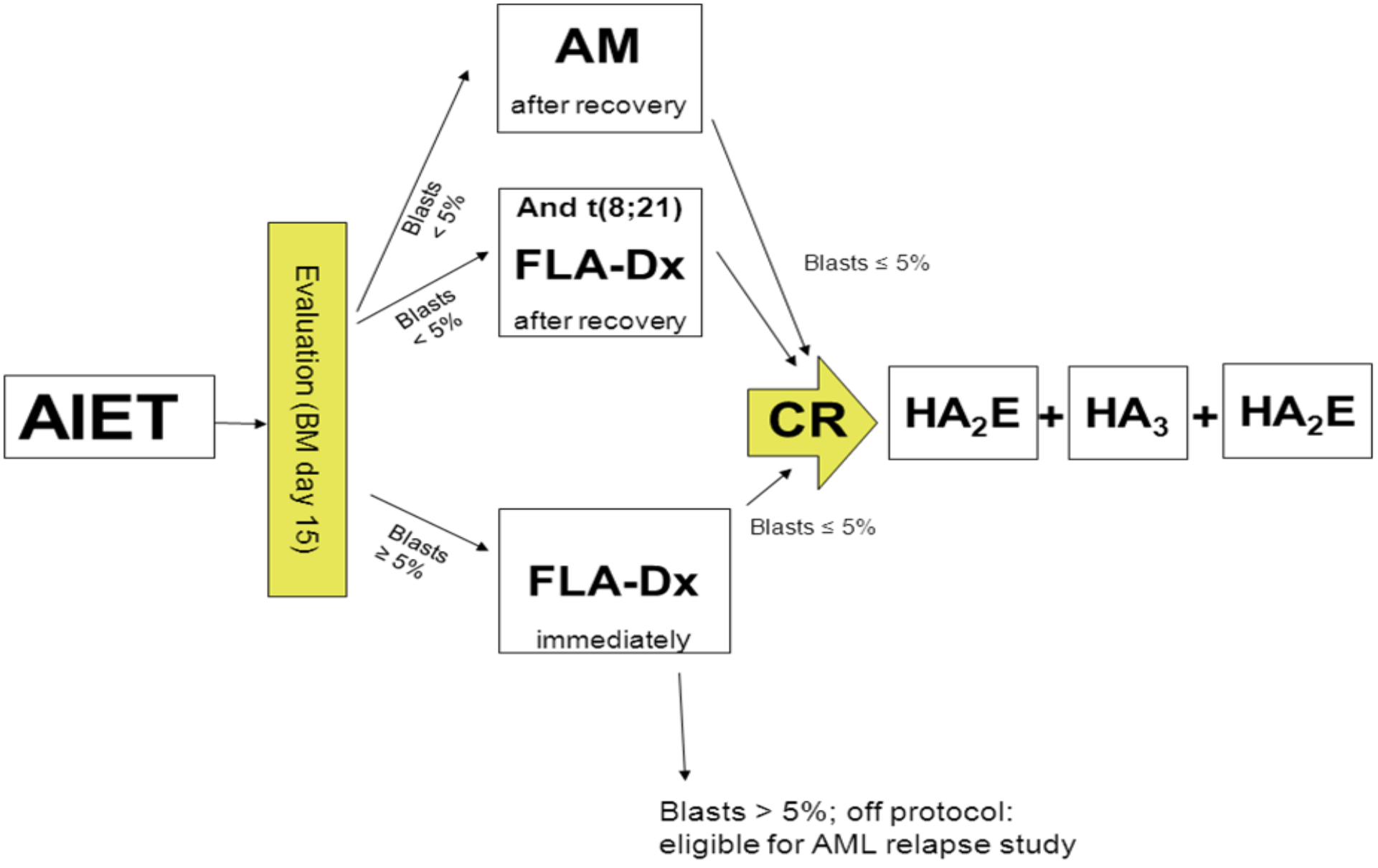
EudraCT Number: 2009-014462-26
Sponsor: DCOG, the Netherlands

Dutch Childhood Oncology Group (DCOG)
Belgian Society of Paediatric Haematology-Oncology (BSPHO)

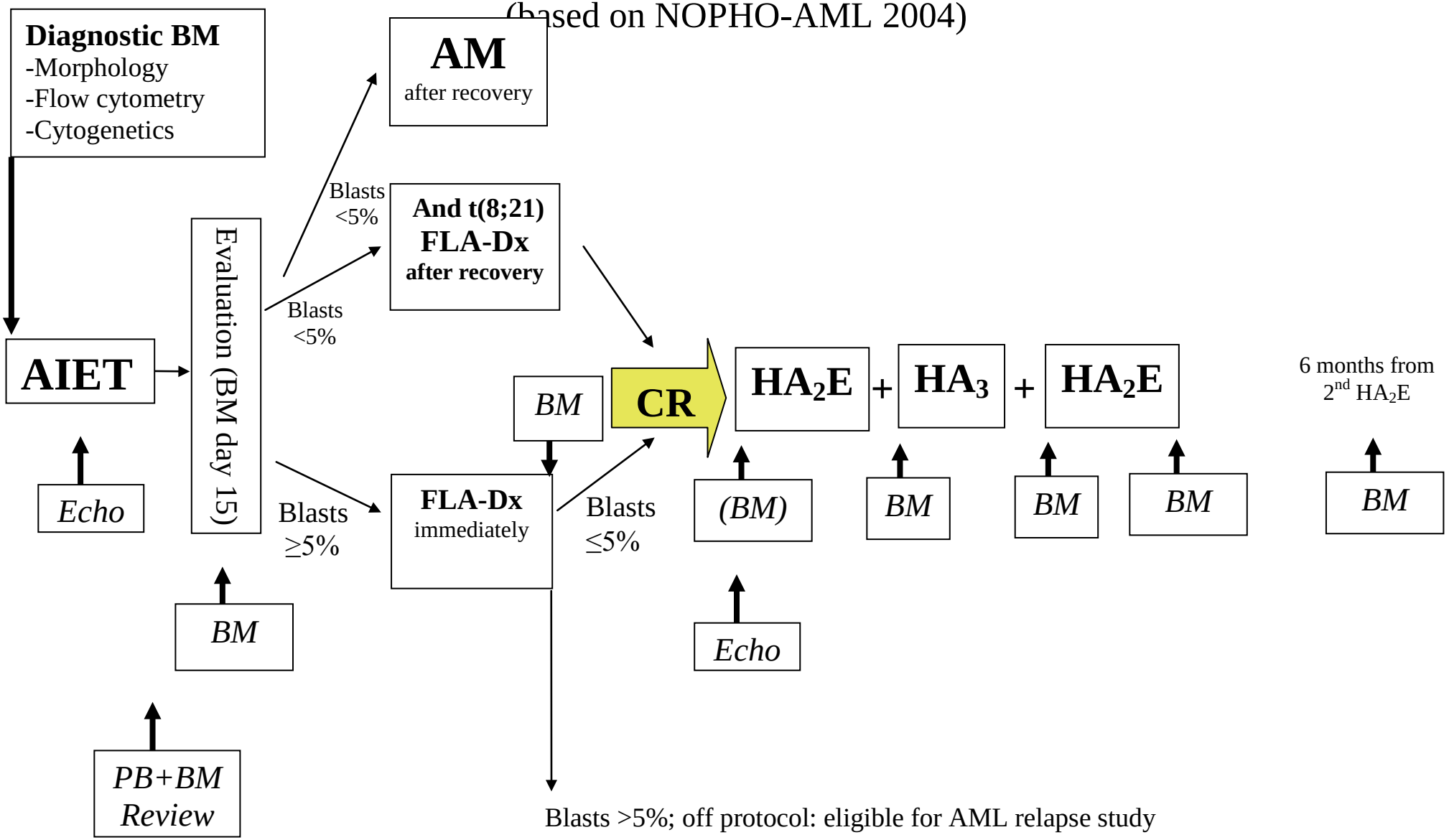
In collaboration with
The Nordic Society for Paediatric Haematology and Oncology

Version 3, April 2011
Final, amendment FLA-Dx as second induction course for intermediate and poor responders and t(8;21) included as well as the more precise procedure for Patient safety and event reporting (chapter 12)

Dutch-Belgian pediatric AML protocol
Flowchart of Standard treatment
(based on NOPHO-AML 2004)



Dutch-Belgian pediatric AML protocol
Examinations overview
(based on NOPHO-AML 2004)



INTRODUCTION

This protocol concerns the management of children with newly diagnosed acute myeloid leukemia (AML). The protocolcommittee AML 2007 started with formulating criteria for the upcoming Dutch trial. These main points were: preferably include patients into a collaborative group trial, no cranial irradiation, no maintenance treatment, moderate dose of antracyclins, stem cell transplantion only in a trial otherwise not in first remission and establish a collaboration with future possibilities. Various collaborative european groups (BFM, MRC and NOPHO) have good and nearly identical outcome rates.
These criteria made us choose for a collaboration with the NOPHO AML trial. The latest Nordic AML 1993 protocol (NOPHO) has proved to have a very good overall survival rate and to be among the best in Europe (1). This protocol is characterized as being centred around high dose cytarabine-arabinoside, high dose etoposide and relatively high doses of anthracyclins. The Nordic group successor trial, the NOPHO AML 2004 protocol, has been used as the basis for this Dutch–Belgian Pediatric AML treatment protocol. Some changes have been made on account of the most recent literature up to 2009 (see summary below). When still successful within the international field of AML trials, the future AML protocol in this newly established collaboration (NOPHO, DCOG, BSPHO) will prolongate the five courses of chemotherapy as given in DB AML-01 as the backbone. The NOPHO AML 2004 protocol ends with a randomisation for Mylotarg postconsolidation.

Table Outcome data of the most recently completed and matured studies from major groups, concerning the well-defined core-group of de novo AML patients below 15 years of age (see Table 1) *modified from Kaspers and Creutzig, Leukemia 2005 (2)*

Study, years of enrolment and reference	***Patient number***	***Early death rate (%)***	***CR rate (%)***	***5-year pEFS (%, with s.e.)***	***5-year pOS (%, with s.e.)***	***% death rate in CR***
AIEOP92 (1992–2001)[10]	160	6	89	54 (4)	60 (4)	7
AML-BFM93 (1993–1998)[3]	427	7	83	51 (3)	58 (2)	4
DCOG-ANLL 92/94 (1992–1998)[7]	78	10	82	42 (6)	42 (6)	16
EORTC-CLG 58921 (1993–2000)[5]	166	2	84	48 (4)	62 (4)	6
LAME91 (1991–1998)[9]	247	4	91	48 (4)	62 (4)	6
NOPHO-AML93 (1993–2001)[8]	223	2	92	50 (3)	66 (3)	2
PPLLSG98 (1998–2002)[4]	104	8	80	47 (5)	50 (5)	10
St. Jude-AML91 (1991–1997)[13]	62	3	79	44 (15)	57 (11)	?
UK-MRC AML10 (1988–1995)[6]	303	4	93	49	58	10

SUMMARY

Acute myeloid leukemia (AML) is a sporadic disease in children. In the Netherlands and Belgium approximately 30-35 children will be diagnosed with AML every year (ages 0–18).
Complete remission (CR) can be achieved in 85–90% of children. However, the five year overall survival rate (OS) is 50–60% due to a high relapse frequency, especially during the first and second years after diagnosis. The results of the latest NOPHO protocol 1993 were for five year OS 65% and for five year event free survival (EFS) 48%, combined with a CR rate of 92% (1). These results are among the best in Europe (2). The NOPHO complete remission rates are comparable to those of the BFM and MRC pediatric study groups. A special characteristic of the NOPHO strategy is the timing of the second course. Timing is intensive: for instance, at day 15 after the first course when the bone marrow reveals 5% blasts or more the second course starts immediately. The NOPHO demonstrated the feasibility of this approach in their last two trials. In view of this CR rate, a major therapeutic issue is to prevent relapses. There is evidence that increasing the total dose of ARA-C reduces the relapse rate, whereas the complete remission rate is not increased further (3;4). The NOPHO backbone is centred around high total doses of ARA-C during consolidation. This may be related to its success.
In general, over the past twenty years there has been an important improvement in therapeutic outcome due to the intensification of treatment based upon high doses of cytarabine-arabinoside and anthracyclins during induction and consolidation.
Early studies established the cardiotoxic threshold dose of 550 mg/m^2 in adults. In children even lower doses of anthracyclins risks exhibiting subclinical cardiovascular dysfunction and clinically significant cardiomyopathy (5). Relatively limited data are available from studies including the cardiovascular status of survivors more than ten to fifteen years after completion of therapy (6-8). Currently available studies show progressive cardiovascular dysfunction over time for anthracyclin doses of over 300 mg/m^2. Altogether, the results available to date have made us aware of possible cardiac damage in upcoming long-term survivors after AML treatment. The success of the NOPHO backbone is thought to lie in the very high cumulative dose of ARA-C. The NOPHO-AML 2004 trial still uses anthracyclin doses of 450 mg/m^2. On the basis of the findings current up to 2009 we limited the cumulative dose of anthracyclins to 330 mg/m^2 in this study protocol.

In international studies two collaborative groups (BFM and MRC) have shown identical good results when the number of courses is reduced to four or five. The original NOPHO-AML 2004 protocol is designed with six intensive courses. To limit the cumulative anthracyclin dose while preserving high cumulative doses of ARA-C, we decided to skip the most toxic course, which frequently resulted in a delay in treatment, and proposed a study protocol with five intensive chemotherapeutic courses.
The role of allogeneic SCT is controversial. It has been accepted practice for several years to offer allogeneic transplantation to all AML patients with an HLA-identical sibling donor. Updates from the larger international collaborative study groups have shown no significant benefit for sibling-SCT in standard risk or high risk groups. While outcomes have improved with more effective chemotherapy, a more restricted attitude towards allogeneic SCT in AML patients has been adopted in several study groups and also by us. Allogeneic SCT in the first CR is not recommended in this study protocol when patients achieve complete remission as described.

In summary, we propose a non-randomized single-arm study for newly diagnosed pediatric AML, which we consider to represent best available treatment. The study is based on the NOPHO 2004 backbone with various modifications, including: omission of 1 course of chemotherapy (5 instead of 6 courses), reduction of the total cumulative anthracycline dose, allo-SCT is not recommended in first CR. Since it is not known whether these modifications will have impact on the relapse rate (which approximates 40% in the current NOPHO study) strict stopping guidelines are provided to ensure that an increased relapse rate will timely be noticed, which will lead to the termination of this protocol. Given the number of patients available for this study a direct comparison with the NOPHO 2004 outcome data is not possible as this comparison will be underpowered.
The results of the interim analysis for the NOPHO AML 1993 and 2004 demonstrated good overall survival for the 'good responders' (blasts < 5% at day 15). Though, subgroup analysis demonstrated an inferior outcome for patients with a translocation (8;21), EFS 35%. The patients with a blast count at day 15 ≥5% also had a poor outcome, EFS 35%.
Based on these results, the NOPHO 2004 protocol as well as the DB AML-01 protocol will be amended. For the patients with t(8;21) and for the patients with a blast count at day 15 ≥ 5% the second course will be FLA-Dx.

Dutch-Belgian pediatric AML protocol
based on the NOPHO-AML 2004 study
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LIST OF ABBREVIATIONS

AIEOP	Associazione Italiana Ematologia ed Oncologia Pediatrica
ALL	Acute lymphoblastic leukemia
AML	Acute myeloid leukemia
AML-BFM	AML Berlin-Frankfurt-Münster group
ANC	Absolute neutrophil count
APL	Acute promyelocytic leukemia
BM	Bone marrow
BSPHO	Belgian Society of Paediatric Haematology-Oncology
CCG	Children's Cancer Group (USA)
CCR	Continued complete remission
CNS	Central nervous system
COG	Children's Oncology Group (USA)
CR	Complete remission
CSF	Cerebrospinal fluid
DCOG	Dutch Childhood Oncology Group
DFS	Disease-free survival
DS	Down syndrome
EFS	Event-free survival
FAB	French American British
FISH	Fluorescent in situ hybridization
GO	Gemtuzumab ozogamicin
IBFM-SG	International Berlin-Frankfurt-Münster Study Group
ICC (APL)	International Consortium on Childhood (APL)
JMML	Juvenile myelomonocytic leukemia
MDS	Myelodysplastic syndrome
MRC	Medical research council (UK)
MRD	Minimal residual disease
MSD	HLA-matched sibling donor
MUD	HLA-matched unrelated donor
NOPHO	Nordic Society for Paediatric Haematology and Oncology
NR	No response
OS	Overall survival
POG	Pediatric Oncology Group (USA)
PR	Partial response
SCT	Stem cell transplantation
VOD	Veno-occlusive disease of the liver
WBC	White blood cell count
WHO	World Health Organization

TABLE OF CONTENTS

1 OBJECTIVES

This study is an international single arm study consisting of 5 intensive chemotherapy courses, modified from the NOPHO AML 2004 protocol (consisting of 6 chemotherapy courses). This study will answer the question whether treatment with these 5 intensive courses demonstrate a relapse rate of 40% or less. This study will be monitored by stopping rules. The inclusion time is restricted to a maximum of 4 years and/or 120 evaluable patients. The ongoing NOPHO 2004 AML protocol consists of a mylotarg postconsolidation randomisation. This will be set up separately and is expected to be fully operational in the second half of 2010.

1.1 Aims

Primary Objectives:
To conduct an international pediatric study for AML based on a modified NOPHO-AML 2004 protocol which we consider best available treatment with optimal outcome and less toxicity
To investigate whether reduction of the number of intensive courses to five and a reduction of the total antracyclins dosage is feasible with a safe cumulative 3-years relapse rate of 40%. .
To decrease toxicity in patients without an increased relapse rate.

Secondary Objectives:
To decrease long term effects of treatment such as cardiac toxicity.

Endpoints:
The main endpoints will be:
Complete remission (CR) achievement and reasons for failure
Duration of remission, rates of relapse and deaths in first CR
Overall survival
Toxicity, both hematological and non-hematological, including cardiac toxicity

1.2 Biological research aims

Various biological research studies will be performed on the material left after adequate diagnostic procedures. In the appendix the various biological research studies are described.

2 BACKGROUND AND RATIONALE

2.1 Experiences from previous NOPHO-AML studies

The outcome of intensive AML treatment in children remains disappointing, with survival being limited to 60% of all patients and toxicity being considerable. It was not until the late 1970s that some progress in AML therapy was made with the introduction of intensive therapy (9). A pilot study, started in Oslo in 1981, used a modified induction therapy from the ongoing MRC trial, combined with doxorubicin, cytarabine, and 6-thioguanine. For the first time in children, the consolidation therapy was based on high-dose cytarabine, 2000 mg/m^2 twice daily for three days repeated four times. Maintenance consisted of monthly courses of cytarabine and 6-thioguanine for one year. The results were promising, with the first eight patients remaining in remission 5-29 months after diagnosis (10).

A common Nordic protocol for AML based on the Oslo pilot was opened 1 July 1984 (NOPHO-AML84). The results of NOPHO-AML84 formed the basis for the next protocol (NOPHO-AML88) (11). Compared to other international series, the frequency of resistant disease (15/105) was too high in NOPHO-AML84 and many patients experienced relapse (45/82). The NOPHO-AML88 induction therapy was intensified through the addition of mitoxantrone. The interval between the first and second courses was intended to be as brief as possible. The consolidation was intensified by adding mitoxantrone and etoposide to alternate courses of high-dose cytarabine.

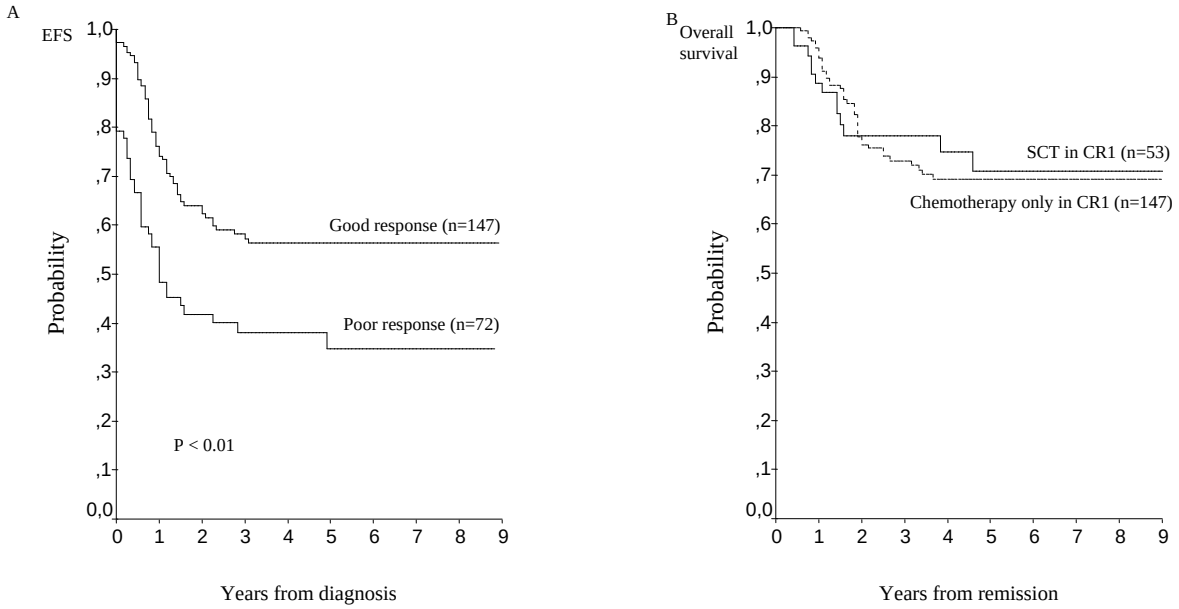
NOPHO-AML88 showed a non-significant trend of increasing EFS (42% vs. 32%) compared with NOPHO-AML84 (11). The toxicity of the NOPHO-AML88 was not acceptable, with 14/118 dying in aplasia, and 10 of 58 dying in CCR. It was obvious, however, that the antileukemic effect was significant.

The NOPHO-AML93 study used the same therapeutic blocks as NOPHO-AML88, but the approach was altered (12). After the first course the patients were observed until BM showed persistent disease or CR. Those who achieved CR (67%) after the first course were given a second identical course. Patients with persistent disease received mitoxantrone and cytarabine as the second course. The consolidation therapy remained the same as that in NOPHO-AML88.

Up to December 2000, 219 children were enrolled on NOPHO-AML93. Of the 219 children, the 7-year EFS increased from 41 to 49%. Toxic death during induction was reduced to 3% and 91% achieved remission. The OS increased from 47% in NOPHO-AML88 to 64% in NOPHO-AML93 (12). With these results the CR rates are comparable to the BFM and MRC study group results for pediatric patients. It is obvious that the first induction course has a moderate ARA-C dose but is combined with three other drugs. In the consolidation courses the total dose of ARA-C is very high. Earlier reports discussed the total dose of ARA-C in relation to CR and relapse rates. High doses of ARA-C increased the complete remission rate in some studies, others could not demonstrate identical results (3;4;13;14). However, later studies with randomized ARA-C doses demonstrated that the relapse rate was significantly reduced, with a high total ARA-C dose (3;4). A high total ARA-C dose is preserved at the core of this protocol.

The main prognostic factor in NOPHO-AML93 was the in vivo response to the first course of therapy. For those achieving remission after one course (67%), the EFS was 56% compared to 35% in those not in remission after the first induction (Figure Survival, figure a). (Described in more detail by *Lie et al., Br J Haematol 2003 (12) and Lie et al., Leukemia 2005 (1).*)

Figure Survival:



A. EFS in NOPHO-AML93 according to response to the first course of chemotherapy. Good response: BM blasts <5% (n=147), poor response (n=72).
B. EFS was superior in those receiving SCT in the first complete remission (CR1) but the OS did not differ between SCT and chemotherapy only in CR1.

Informative cytogenetics was obtained in 91% of the NOPHO-AML93 patients (12). Patients with t(9;11)(p22;q23) had significantly better EFS (86%) than other cytogenetic groups, and t(8;21) and inv(16) had intermediate prognoses. A very poor prognosis was found for patients with 11q23 aberrations other than t(9;11), and in a small group of patients with a high-hyperdiploid karyotype. Results are summarized in subsequent table.

Table: Cytogenetic findings in NOPHO-AML93 with EFS and overall survival (OS) at seven years from diagnosis.

	N	**%**	**Events**	**EFS (%)**	**OS (%)**
diploid	55	27	30	42	54
t(8;21) (q22;q22)	18	9	8	56	77
11q23 abnormality t(9;21) (p22;q23)	16	8	2	86	94
non-t(9;11)	16	8	10	36	44
inv(16)/ t(16;16)	10	5	4	60	77
t(15;17) (q22;q12)	8	4	4	47	63
> 50 chromosomes	6	3	4	33	50
other abnormalities	78	38	39	48	63

The Nordic studies have documented that children with DS represent a large subgroup in AML. In their population-based material they found that children with DS constitute almost 15% of the AML cases (11). NOPHO was among the first to show that children with DS have a very special form of AML with a remarkably good prognosis (15). Several other cooperative groups have now confirmed this. For this reason, myeloid leukemia in children with DS is now considered a separate entity (16) and is treated internationally with a special protocol (DCOG Myeloid leukemia for children with Down's syndrome). Myeloid leukemia with a specific GATA1 mutation in Down's syndrome is an exclusion criterion for this protocol (see also Chapter 4 Patient Eligibility Criteria).

The major findings of the NOPHO studies for the international debate on the therapy of childhood AML can be summarized as follows:

- The induction regimen in NOPHO-AML88 was too toxic when the second induction was administered shortly after the first induction. Postponing the second induction course to hematological recovery (NOPHO-AML93) resulted in reduced toxicity and improved outcome.
- The response to the initial course of therapy is the most important prognostic factor.
- The use of high-dose cytarabine as consolidation therapy is now well established and is part of most protocols. In NOPHO-AML88 the consolidation therapy was strengthened by adding mitoxantrone and etoposide. As long as recovery was complete after the previous course, the toxicity was manageable (this is underscored by the results from the MRC trials). The individual courses from NOPHO AML 2004 have manageable toxicity profiles.

2.2 Summary of the interim analysis of first years of NOPHO-AML 2004:

Early death in the ongoing NOPHO-AML 2004 study is low: 3% in 143 consecutive patients. Death in CR is 3% and EFS (3yrs) and OS (3yrs) respectively 59% and 71%.
The toxicity is toxic as expected. Four children died due to bleeding shortly after diagnosis. Five patients died due to therapy-related complications. All patients except one had fever and required antibiotics following AIET. The infection was considered as life-threatening in 10% of the patients but there were no deaths due to neutropenia following AIET (personal communication of Principal Investigator H.Hasle, NOPHO). In general, these preliminary results are favourable comparing to the previous NOPHO trials. These results are in line with the results of the other international collaborating group trials such as MRC and BFM.

2.3 Selected experiences from other AML study groups

Table Outcome data of the most recently completed and matured studies from major groups, concerning the well-defined core-group of de novo AML patients below 15 years of age (see Table 1) *modified from Kaspers and Creutzig, Leukemia 2005 (2)*

Study, years of enrolment and reference	***Patient number***	***Early death rate (%)***	***CR rate (%)***	***5-year pEFS (%, with s.e.)***	***5-year pOS (%, with s.e.)***	***% death rate in CR***
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Idarubicin

A collaborative overview comparing idarubicin with daunorubicin or other anthracyclins showed better remission rates and better OS in those treated with idarubicin (17). Idarubicin 12 mg/m^2/day for three days has manageable toxicity and substantial anti-leukemic activity in pediatric patients with AML (18). The AML-BFM 93 trial compared at random with daunorubicin 60 mg/m^2/day and idarubicin 12 mg/m^2/day for three days each, combined with cytarabine and etoposide during induction. A significantly better blast cell reduction in the BM on day 15 was observed in patients treated with idarubicin (17% had blasts ≥5% compared to 31% of patients treated with daunorubicin). The effect was most pronounced in high risk patients. The EFS and DFS at five years were similar for patients treated with daunorubicin or idarubicin (49% +/- 4% vs 55% +/- 4% and 57% +/- 4% vs. 64% +/- 4%). However, in patients with more than 5% blasts on day 15, there was a trend towards better outcomes after treatment with idarubicin. Along with the early effect observed in high risk patients, these results indicated a better efficacy for idarubicin than for daunorubicin during induction, with a similar rate of toxicity including cardiotoxicity (19). Idarubicin doses up to 150 mg have been tolerated in adults without significant cardiotoxicity (20). However, in a smaller group of patients the Australian and New Zealand AML groups found no survival benefit for idarubicin but more toxicity, especially in those receiving idarubicin 12 mg/m^2/day for three days compared with those receiving 10 mg, and a lower EFS in those receiving the highest dose of idarubicin (21).

Concerns about the total dosage of anthracyclins

Early studies established the cardiotoxic threshold dose of 550 mg/m^2 in adults. In children even lower doses of anthracyclins risk causing subclinical cardiovascular dysfunction and clinically significant cardiomyopathy (5). Relatively limited data are available from studies including the cardiovascular status of survivors more than ten to fifteen years after completion of therapy (6-8). Currently available studies show progressive cardiovascular dysfunction over time for treatment with anthracyclin doses of over 300 mg/m^2. Recently, the BFM reported a cumulative incidence of late cardiomyopathy of 5% in AML patients given anthracycline doses between 300–450 mg/m^2, with a median follow-up of 5.3 years (22). For AML patients the follow-up time is too short to permit the investigation of the very late effects on cardiac and vascular function. However, the results obtained have made us aware of possible cardiac damage in upcoming long-term survivors.

The EORTC study no. 58921 using a dose of 380 mg/m^2 had an overall survival of 62% (23). The MRC AML15 trial demonstrated no inferior EFS and OS in their latest randomization: high dose ARA-C courses (total dose of anthracyclins lowered to 240 mg/m^2) versus standard MACE-MIDAC (total dose of anthracyclins of 550 mg/m^2) *(personal communication Brenda Gibson)*.

In the subsequent table all anthracyclins dosages are calculated for the larger collaborative pediatric treatment groups. Cumulative doses were calculated as equivalence doses of doxorubicin using a 1:5 ratio of idarubicin and mitoxantrone. This ratio was preferred by the AML Collaborative Group and represents good equivalent doses with respect to toxicity (17;19).

Table: Cumulative dosages of anthracyclins, ARA-C and VP16 in ongoing international pediatric AML treatments regimens. Abbr: HR= high risk defined by each protocol, SR=standard risk defined by each protocol.

	Risk group	**Anthracyclins (mg/m^2)**	**ARA-C (gr/m^2)**	**VP-16 (gr/m^2)**
AML 15	HR	240–300	3.6–16	0–1
	SR	300–550	10.6–40.6	1–1.5
	rand	240–490	23–52	0–0.5
AML-BFM 2004 interim	SR	350	26.8	0.950
	HR	450	44.8	0.950
AML-BFM 2004	SR	350–410	29.32	?
	HR	450–510	47.32	?
St Jude	HR	300	3.6–19.6	0.800
	SR	450–550	12.0–46.0	0.8–1.3
COG	HR	300	13.6	1.75
	SR	600	45.6	1.75
NOPHO	HR	450	7.3	0.4
	SR	450	49.3	1.2
Japan	HR	250	39.4	1.25
	IR	375	77.4	1.75
	LR	225	77.4	1.75
ELAM 02	IR/SR	DNR160 Amsa 300 Mitox 60	44.4	0.4

Therefore, we conclude that at present, lowering the total dose of anthracyclins from 480 mg/m^2 in the original NOPHO 2004 protocol to 330mg/m^2 is reasonable in combination with the high cumulative ARA-C dose. This reduction is achieved by omitting the most toxic course of the original NOPHO-AML 2004 protocol.

Risk group definitions

Several groups have identified children whose prognosis is so good on chemotherapy alone that SCT may be deferred until after a first relapse. The MRC identified t(8;21), inv(16), t(15;17), and those with a favourable response to initial chemotherapy as good risk patients (24). The AML-BFM group defined patients with FAB M1 or M2 with Auer rods, M3, and M4Eo with BM blasts ≤5% on day 15 as standard risk (25). The therapeutic outcome of AML characterized by t(15;17), termed acute promyelocytic leukemia (APL), is improved by the introduction of ATRA. This survival advantage remains although anthracyclins were lowered. Accordingly, APL is excluded from this trial and is internationally treated under a separate international study with limited anthracyclin dosages and high ATRA dosing (DCOG and ICC APL study 01).

The most important prognostic factor in NOPHO-AML93 was the response to the first course of therapy. Good responders had significantly better EFS than poor responders (56% vs 35%). The MRC10 study showed a strong prognostic impact for status after first induction, with a survival of 51% in patients in CR, 42% in PR (5–15% blasts), and 20% in patients with refractory disease (24). The difference was mainly due to increased relapse rates in those with more than 15% blasts after the first course. The BFM study

showed a survival of 61% in those in CR on day 15 vs 40% in those not in CR (25). The CCG study showed a survival of 40% in those with less than 15% blasts on day 14 vs 27% in those with more than 15% blasts (26). The NOPHO approach of delivering a second course directly after day 15 when bone marrow blasts exceed 5% will be continued.

A recent international pediatric collaborative study of 11q23-rearranged AML (n=756) showed that patients with t(1;11)(q21;q23) had a 5-yr EFS of 92%, whereas those with t(6;11)(q27;q23) had the worst outcome (5-yr EFS of 11%). In addition, the subgroups t(10;11)(p12;q23), t(10;11)(p11.2;q23) and t(4;11)(q21;q23) showed a 5-yr EFS of 31%, 17% and 29% respectively (27). Subanalyses showed that these poor prognostic subgroups did not seem to benefit from allogenic stem cell transplantation. Previously, NOPHO-AML93 showed a favourable outcome in those with t(9;11)(p22;q23) in contrast to a poor outcome in those with other 11q23 aberrations. St Jude's Children's Research Hospital and the Chicago group confirmed improved survival for t(9;11) (28-30), especially when receiving intensive post-remission chemotherapy with high-dose cytarabine (31). In vitro sensitivity studies have shown increased cytotoxicity of etoposide and cytarabine in patients with t(9;11) (32). In the collaborative retrospective study by Balgobind et al., patients with t(9;11)(p22;q23) had a 5y-pEFS of 50% and a 5-yr OS of 63% (n=328). Patients with t(9;11)(p22;q23) and FAB-M5 had a 5-yr EFS of 56% (n=254), whereas those with a t(9;11)(p22;q23) and another FAB subtype had a 5-yr EFS of 23% (n=59, p 0.001). Further analysis of the subgroups t(9;11)(p22;q23) and FAB-M5 showed that patients with a WBC of less than 50x109/L had a greater 5-yr EFS (65%; n=166) than those with a WBC greater than $50x10^9$/L (46%; n=87, p=0.04). Stem cell transplantation showed no difference in outcome in the 11q23-rearranged AML patient group. However, the numbers in the t(9;11)(p22;q23) subgroup with a FAB subtype other than FAB-M5 were too low to evaluate the effect of SCT (27).

2.4 Stem cell transplantation

Most multi-centre studies in pediatric patients have shown no advantage for autologous SCT over chemotherapy alone (33-35). The role of allogeneic SCT is more controversial. It is methodologically difficult to evaluate the impact of SCT (36). Most studies show a lower relapse rate after allogeneic SCT. However, this is counterbalanced by a higher treatment-related mortality in this group. To date, no traditional randomized controlled study has been performed to test the efficacy of allogeneic SCT in AML.

For several years it has been accepted practice to offer allogeneic transplantation to all patients on NOPHO-AML studies with an HLA-identical sibling donor. The POG, CCG and AIEOP AML trials have demonstrated that matched sibling transplant in the first CR was superior to chemotherapy (33-35). However, no significant benefit for sibling SCT in a standard risk group was obtained using AML-BFM 93 and MRC AML 10, whereas significant benefit for SCT was obtained in the high risk group (37;38). While the results have improved with more effective chemotherapy, a more restricted attitude towards allogeneic SCT in standard risk patients has been adopted in several study groups. Allogeneic SCT in the first CR is recommended for all intermediate and high risk AML children with HLA-identical sibling donors by the current COG and AIEOP studies but only for the poor risk patients in the latest BFM-SG and MRC studies (39;40).
There are no large studies on unrelated donor SCT in AML children. Acceptable toxicity has been shown in AML and ALL children with MUD-SCT (41-46). In the current BFM 98 protocol, SCT with a related or unrelated donor is recommended for PR/NR patients with $\geq$10% blasts after the second induction course. In the MRC AML 15 study, poor risk patients may be considered for allogeneic transplant from unrelated donors. However, recent updates of the SCT results in BFM and MRC in the pediatric AML treated patients showed that overall survival showed no significant advantage over chemotherapy treatment alone in standard and poor risk AML pediatric patients (47;48).

Recent studies of larger cohorts with specific chromosomal translocations or monosomias show extremely poor overall survival in some subgroups. As mentioned above in paragraph 2.3, the 11q23 rearranged AML patients experience varying outcomes. Patients with t(1;11)(q21;q23) have favourable outcomes whereas patients with t(6;11)(q27;q23), t(10;11)(p12;q23), t(10;11)(p11.2;q23) and t(4;11)(q21;q23) have

poor outcomes. However, the benefit of allo-SCT has not been proven in these subgroups. Therefore, allo-SCT is only advised in cases with poor biological response.
Several studies have investigated the outcome of AML characterized by monosomy 7 of deletion 7q- (49;50). The 5-yr OS was shown to be 39%. The outcome was less inferior in the group with del 7q- versus monosomy 7 (51% vs. 30% respectively). Even del 7q- along with favourable cytogenetic abnormalities demonstrated a DFS of 75%. The unfavourable outcomes for AML patients with monosomy 7 did not change with SCT in the first CR1. However, patients with monosomy 7 and inv(3), or -5/del(5q) or an extra chromosome 21 had a 5-yr survival rate of 5% (50). Although the patient numbers are small, this patient group may benefit from SCT after chemotherapeutic treatment with survival rates of 31% after SCT. We recommend special consideration of AML patients with monosomy 7 who do not reach CR after two induction courses. On the basis of results published in the literature, we strongly advise transplants for these patients (50).
The t(9;22)(q34;q11) represents 2% of all therapy-related MDS and t-AML. It is found to be associated with previous therapy with topoisomerase II inhibitors. t(9;22) is very rare in de novo AML.
Flt3-ITD positivity has been found in around 12–15% of AML cases. FLT3-ITD is strongly related to a poor outcome. Recent studies (51-54) demonstrate an overall survival at five years varying between 32% and 42%, whereas the FLT3-ITD negative AML cases show an OS of 58%–84% in the latest Shimada study (53). The poor outcome has been reflected in a high number of induction failures. Only one study of adult AML patients compared chemotherapy alone with allo-SCT and showed no difference in outcome. Allo-SCT is not recommended for this subgroup of patients.
Inappropriate expression of EVI-1 is found in 7.8% of AML cases and is often observed due to chromosome 3q26 lesions, resulting in the development or progression of high risk AML. EVI-1 may also be highly expressed in subgroups of AML lacking 3q26 rearrangement. Moreover, not all 3q26 rearrangements necessarily result in high EVI1 expression (55). In 50% of the cases with high EVI-1 expression, other cytogenetic lesions are frequently observed in association: -7/7q- deletions and translocations involving 11q23. In this group the poor outcome is also reflected in an increased number of induction failures. No results are available regarding whether allo-SCT produces a more favourable outcome than chemotherapy alone. Allo-SCT is not recommended on the basis of cytogenetic abnormalities alone.

In the light of these recent results we do not offer SCT in first CR in this trial. When patients do not achieve first CR following this protocol after one or two courses of chemohterapy, patients will be off protocol and eligible for relapse AML protocol. However, we ask for special attention in cases of AML patients with monosomy 7 and not in CR after two induction courses. Recently, it was shown that these particular patients achieved an overall survival of 31% when a stem cell transplantation was initiated (50).

2.5 Late effects of treatment

Cardiotoxicity is the main late effect of treatment of childhood AML with chemotherapy alone (56-58). Reduced left ventricular fraction shortening (<28%) was found in 17–35% of patients who received median anthracycline doses of 200–385 mg/m^2 4–16 years earlier(56-58). Other reported late effects after AML treatment without SCT have been sensorineural hearing loss (7–30%) (56;58;59), mild cognitive deficits requiring extra tutoring or special education classes (5–30%) (58;59) and abnormal renal function (4%) (56;57). Growth, endocrine function and fertility have universally been normal in this patient group (56-59).

2.6 Summary of interim analysis 2010 of NOPHO 1993 and 2004

The results of the intermediate responders in NOPHO 1993 and NOPHO 2004 (definition: day 15 blasts 5-15%) are poor with an EFS of 35% (*personal communication).*
A subgroup analysis of inv(16), t(8;21), Flt3 ITD demonstrated a good overall survival. It was obvious that the event free survival of the subgroup t(8;21) was inferior with an EFS of 35%. Also other international studygroups showed less favorable outcomes for t(8;21) characterized AML patients.

Recently, the BFM analyzed their results for t(8;21); in the BFM98 EFS 84% and BFM2004 EFS 60-65% (*personal communication)*. In the BFM2004 the second induction course with high dose AraC was omitted. These results prompted the BFM recently to reintroduce the HAM course as second induction course. In the BFM2004 the t(8;21) patients did better with DaunoXome than with Idarubicin in the first course without the second HAM course. Moreover, no significant differences were found between day 15 blast count and CR rate for the t(8;21) AML patients between the BFM and the NOPHO protocols.
There are strong suggestions that t(8;21) patients need high doses of ARA-C from other study groups as well as in the BFM. MRC group delivers low dose antracyclines and high dose AraC in their courses with slightly better results for the t(8;21) (AML15 and AML17).
It can be concluded that the group of AML patients with intermediate responses and with t(8;21) abnormalities need more therapy in the NOPHO 2004 protocol. And so it is expected that in the DB AML-01 this will be identical.
Therefore, an amendment is made of this protocol. For the patients with a blast count at day 15 ≥5% and for the patients with a t(8;21) the second course will be FLA-Dx.

3 DRUG INFORMATION

CYTARABINE (Ara-C) (Cytosar®)

Source and Pharmacology: Cytarabine is a deoxycytidine analogue. It must be tri-phosphorylated to its active form, Ara-CTP, by deoxycytidine kinase and other nucleotide kinases. Ara-CTP inhibits DNA polymerase. In addition, ara-CTP is incorporated into DNA as a false base, causing inhibition of DNA synthesis. It is S phase specific. Cytarabine penetrates the blood brain barrier. It is converted to its inactive form, uracil arabinoside, by pyrimidine nucleoside deaminase. Approximately 80% of the dose is recovered in the urine, mostly as uracil arabinoside (ara-U).

Formulation and Stability: Cytarabine is available in multi-dose vials containing 100, 500, 1000, and 2000 mg of lyophilized drug. Intact vials can be stored at room temperature. For IV use, either sterile water for injection or bacteriostatic water for injection can be used to reconstitute the lyophilized drug. For intrathecal use, only sterile water for injection should be used for reconstitution. The 100 and 500 mg vials are reconstituted with 2 and 10 ml, respectively, resulting in a final concentration of 50 mg/ml. The 1000 and 2000 mg vials are reconstituted with 20 ml and 40 ml respectively resulting in a final concentration of 50 mg/ml. After reconstitution, the drug is stable for 8 days at room temperature.

Toxicity: Myelosuppression is the dose limiting adverse effect, with leukopenia and thrombocytopenia being predominant. Other common adverse effects include nausea and vomiting (may be severe at high doses), diarrhea, mucositis, anorexia, alopecia, skin rash, and liver dysfunction. A flu-like syndrome characterized by fever and aches in muscle and bone is common. Less common side effects include allergic reactions and cellulitis at the injection site. High doses of cytarabine can cause conjunctivitis, hepatitis, and CNS symptoms including somnolence, peripheral neuropathy, ataxia, and personality changes. CNS symptoms are usually reversible and are more common in the elderly and patients with renal impairment.

Route of Administration: intravenous, subcutaneous, and intrathecal

ETOPOSIDE (VP-16)

Source and Pharmacology: Etoposide is an epipodophyllotoxin derived from *Podophyllum pelatatum*. It is thought to act mainly by inhibiting DNA topoisomerase II, causing double and single strand DNA breaks. Etoposide is phase-specific with activity in the S and G2 phases. It is extensively bound to serum proteins and is metabolized in the liver. Etoposide and its metabolites are excreted mainly in the urine with a smaller amount excreted in the feces. Penetration into the CSF is poor. Dosage adjustments should be considered in patients with liver dysfunction, kidney dysfunction, or hypoalbuminemia.

Formulation and Stability: Available in multi-dose vials containing 100 mg and 500 mg of etoposide as a 20 mg/ml solution in 30% alcohol. The intact vials of etoposide solution should be stored at room temperature. Etoposide solution should be diluted in 5% glucose or 0.9% NaCl prior to administration. Solutions with a final concentration < 0.4 mg/ml are stable at room temperature for 24 hours.

Toxicity: Dose limiting toxicity is myelosuppression. Nausea and vomiting (usually of low to moderate severity), diarrhea, mucositis (particularly with high doses), alopecia, and anorexia are fairly common. Hypotension can occur with rapid infusions. Other side effects reported less commonly include hepatitis, fever and chills, anaphylaxis, and peripheral neuropathy. Secondary leukemia has been reported.

Route of Administration: intravenous and orally

IDARUBICIN (Zavedos®)

Source and Pharmacology: Idarubicin is an anthracycline acting by inhibition of DNA and RNA synthesis. In addition, idarubicin inhibits the action of DNA topoisomerase II. Idarubicin is metabolized in the liver to the active metabolite idarubicinol. The parent drug and metabolites are excreted by urine and hepatobiliary excretion.

Formulation and Stability: Available in vials of 5 and 10 mg of Idarubicin. The intact vials should be stored at room temperature. The drug is diluted in 0.9% NaCl prior to administration. The solution is chemically stable for at least 12 hours.

Toxicity: The major dose-limiting toxicity of idarubicin is leukopenia, thrombocytopenia and anemia. Nausea and vomiting are usually moderate in severity. Other side effects include alopecia, diarrhea, headache, fever, and stomatitis. Congestive heart failure has been reported. Extravasations of idarubicin lead to severe local tissue damage and deep ulcerations.

Route of Administration: intravenous

MITOXANTRONE (Novantrone®)

Source and Pharmacology: Mitoxantrone is an anthracenedione that is structurally similar to the anthracyclins. It is thought to act by intercalating into DNA, causing template disorder, steric obstruction, and inhibition of DNA and RNA synthesis. In addition, mitoxantrone inhibits the action of DNA topoisomerase II. Mitoxantrone is active throughout the cell cycle. Mitoxantrone is about 78% protein bound and crosses the blood brain barrier. Mitoxantrone is metabolized in the liver to inactive metabolites. The parent drug and metabolites are excreted primarily via hepatobiliary excretion with small amounts excreted in the urine. Dosage adjustment is recommended for patients with severe hepatic dysfunction (total bilirubin > 3.4 mg/dl = 58 μmol/L).

Formulation and Stability: Mitoxantrone is available in multi-dose vials containing 5, 10, and 15 ml of Mitoxantrone as a dark blue, aqueous solution at a concentration of 2 mg/ml. The intact vials should be stored at room temperature. Refrigeration may result in precipitation of Mitoxantrone, which will redissolve upon warming to room temperature. The drug should be further diluted to at least 50 ml in 5% glucose or 0.9% NaCl prior to administration. These solutions are chemically stable for at least 7 days when stored at room temperature.

Toxicity: The major dose-limiting toxicity of mitoxantrone is leukopenia and thrombocytopenia. Nausea and vomiting are usually moderate in severity. Other common side effects include alopecia, diarrhea, headache, fever, and stomatitis. Blue to green discoloration of urine and other body fluids occurs. Other side effects reported less commonly include elevated liver function tests, allergic reactions, seizures, jaundice, and renal failure. Congestive heart failure has been reported, but is much less common than with doxorubicin. Heart failure has been reported primarily in patients receiving prior therapy with anthracyclins. Patients with an increased risk of cardiotoxicity include those having received prior therapy with anthracyclins, those with previous mediastinal radiotherapy, and those with pre-existing cardiac disease.

Route of Administration: intravenous

6-THIOGUANINE (TGN) (Lanvis®)

Source and Pharmacology: 6-thioguanine (TGN) is a purine antimetabolite. TGN is incorporated into DNA and RNA and cause inhibition of DNA and RNA synthesis. Thioguanine is S phase specific. Absorption is variable and incomplete (5-37%) and is decreased by the presence of food in the gut. 6-thioguanine undergoes first pass metabolism in the GI mucosa and the liver. It is inactivated to methylated metabolites by TPMT (thiopurine methyl transferase). The TPMT enzyme is deficient in about 1 in 300 persons who cannot tolerate usual doses of TGN. 6-thioguanine is eliminated through the urine mostly as metabolites.

Formulation and Stability: 6-thioguanine is available as a 40 mg tablet. The tablets should be stored at room temperature and protected from light.

Toxicity: The dose-limiting toxicity of 6-thioguanine is myelosuppression. TGN can cause intrahepatic cholestasis and focal centralobular necrosis, which is usually manifested by hyperbilirubinemia and increased liver enzymes. Other toxicities include mild nausea and vomiting, skin rash, hyperuricemia, and mild diarrhea.

Route of Administration: orally

FLUDARABINE (2F-ara-A)

Source and Pharmacology: Fludarabine Ebewe contains fludarabine phosphate, a fluorinated nucleotide analogue of the antiviral agent vidarabine, (9-β-D-arabinofuranosyladenine) that is relatively resistant to deamination by adenosine deaminase. Fludarabine phosphate is rapidly dephosphorylated to fludarabine (2F-ara-A) which is taken up by cells and then phosphorylated intracellularly by deoxycytidine kinase to the active triphosphate, fludarabine triphosphate (2F-ara-ATP). This metabolite has been shown to inhibit ribonucleotide reductase, DNA polymerase α, δ and ε, DNA primase and DNA ligase thereby inhibiting DNA synthesis. Furthermore, partial inhibition of RNA polymerase II and consequent reduction in protein synthesis occurs. Fludarabine elimination is largely by renal excretion.

Formulation and Stability: Fludarabine Ebewe is available in vials containing 50 mg of the active ingredient fludarabine phosphate together with sodium phosphate- dibasic dihydrate, and sodium hydroxide in water for injections to give a solution containing 25 mg/mL of fludarabine phosphate for intravenous administration. Fludarabine Ebewe contains no antimicrobial preservative. To reduce microbiological hazards it is recommended that any dilution should be effected immediately prior to use and infusion commenced as soon as practicable after preparation of infusion solutions. If storage is necessary, store at $2 – 8^0$C (refrigerate, do not freeze) for not more than 8 hours. Any solutions which are discoloured, hazy of contain visible particulate matter should not be used.

Toxicity: Dosage may be decreased or delayed based on evidence of haematological or non-haematological toxicity. Physiscians should consider delaying or discontinuing the medicine if toxicity occurs.

Route of Administration: intravenous

DAUNOXOME (Daunorubicin Citrate Liposome Injection)

Source and Pharmacology: DaunoXome (Daunorubicin citrate liposome injection) is a sterile, pyrogen-free, preservative-free product in a single use vial for intravenous use. Daunorubicin is an anthracycline ntibiotic with antineoplastic activity, originally obtained form *Streptomyces*

peucetius. While in the circulation, the DaunoXome formulation helps to protect the entrapped daunorubicin from chemical and enzymatic degradation, minimizes protein binding, and generally decreases uptake by normal (non-reticuloendothelial system) tissues. The specific mechanism by which DaunoXome is able to deliver daunorubicin to neoplastics is not known. However, it is believed to be a function of increased permeability of the tumor neovasculature to some particles in the size range of DaunoXome. In animal studies, daunorubicin has been shown to accumulate in tumors to a greater extent when administered as DaunoXome than when administered as daunorubicin. Once within the tumor environment, daunorubicin is released over time enabling it to exert its antineoplastic activity.

Formulation and Stability: DaunoXome should be diluted 1:1 with 5% Dextrose Injection (D5W) before administration. Each vial of DaunoXome contains daunorubicin citrate equivalent to 50 mg daunorubicin base, at a concentration of 2 mg/mL. The recommended concentration after dilution is 1 mg daunorubicin/mL of solution. Withdraw the calculated volume of DaunoXome from the vial into a sterile syringe, and transfer it into a sterile infusion bag containing an equivalent amount of D5W. Administer diluted DaunoXome immediately. If not used immediately, diluted DaunoXome should be refrigerated at 2°– 8 °C (36°– 46°F) for a maximum of 6 hours.

Toxicity: The primary toxicity of DaunoXome is myelosuppression, especially of the granulocytic series, which may be severe, and associated with fever and may result in infection. Effects on the platelets and erythroid series are much less marked. Special attention must be given to the potential cardiac toxicity of DaunoXome. Although there is no reliable means of predicting congestive heart failure, cardiomyopathy induced by anthracyclines is usually associated with a decrease of the left ventricular ejection fraction (LVEF). Cardiac function should be evaluated in each patient by means of a history and physical examination before each course of DaunoXome and determination of LVEF should be performed at total cumulative doses of DaunoXome of 320 mg/m2, and every 160 mg/m2 thereafter. Patients who have received prior therapy with anthracyclines (doxorubicin > 300 mg/m2 or equivalent), have pre-existing cardiac disease, or have received previous radiotherapy encompassing the heart may be less "cardiac" tolerant to treatment with DaunoXome. Therefore, monitoring of LVEF at cumulative DaunoXome doses should occur prior to therapy and every 160 mg/m2 of DaunoXome.

Route of Administration: intravenous

4 PATIENT ELIGIBILITY CRITERIA

Inclusion Criteria

AML as defined by the diagnostic criteria (as defines in paragraph 7)
Age,< 18 years at time of study entry
Written informed consent

Exclusion Criteria

Previous chemo- or radiotherapy
AML secondary to previous bone marrow failure syndrome
Down syndrome (DS) with age <5 years, and DS =/>5 yrs with GATA1 mutation
Acute promyelocytic leukemia (APL)
Juvenile myelomonocytic leukemia (JMML)
Myelodysplastic syndrome (MDS)
Fanconi anemia
Positive pregnancy test

All children with myeloid leukemia should be reported to the Dutch or Belgian trial office, even if they are not treated following this treatment protocol. Reporting should be done independently of the therapy received.

5 TREATMENT PLAN

5.1 Risk group definitions

Treatment will be based on cytogenetic and molecular genetic characteristics and response to therapy. Risk groups are defined as follows:

Standard-risk criteria

- t(8;21)(q22;q22)/*RUNX1-CBFA2T1*, inv(16)(p13q22)/t(16;16)(p13;q22)/*CBFB-MYH11* and CR following the 2nd induction course (regardless of the blast count following 1st induction)

OR

- Complete remission after first induction course

OR

- 5-15% blasts after first induction (as determined by the central review laboratory) and CR (< 5% blasts) after second induction (as determined by the central review laboratory)

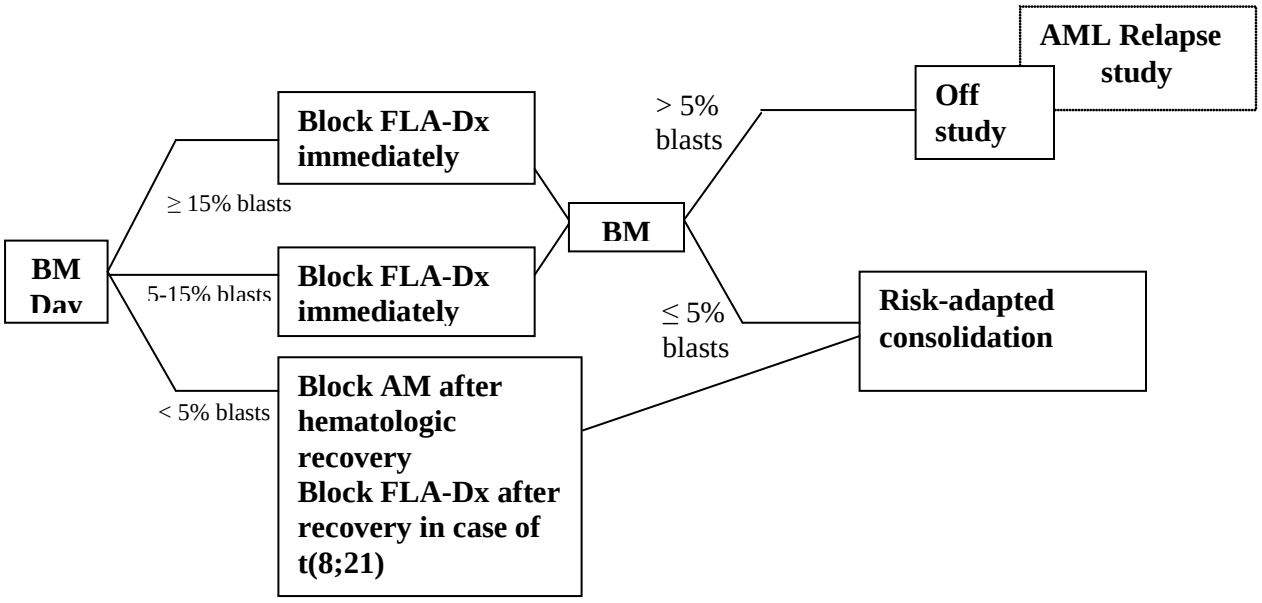
High-risk criteria

- BM blasts > 15% on day 15 after first day of induction therapy but CR after two induction courses and no favorable cytogenetics (as determined by the central review laboratory).

5.2 Therapy overview

A flow chart of the therapy courses and examination points is shown on page 3. After initial evaluation and institution of appropriate supportive measures, patients receive two induction courses starting with course AIET. Response evaluation with BM examination should be performed on day 15 after the beginning of AIET , which will be centrally reviewed. The results of the central laboratory will be used for stratification of the patient. Patients with less than 5% blast cells on morphological examination will receive block AM after hematological recovery (ANC > 1.0 x 10^9/L; platelets > 80 x 10^9/L). Patients with non-evaluable BM due to hypoplasia should have repeat BM examinations performed weekly to search for regrowth of blast cells during the recovery phase.
Based on an interim analysis of the NOPHO-group it was demonstrated that patients with a translocation (8;21) had a poorer survival as other patients with a corebinding factor AML had. Also, the group of patients with ≥5% blasts at day 15 performed worse. Therefore the AM course will be replaced by a FLA-Dx course for the t(8;21) (after recovery) and patients with ≥ 5% blasts at day 15 (immediately).
Children with 5% blast cells or more should, immediately proceed to therapy with block FLA-Dx unless life threatening therapy-related complications necessitate a delay. If 5-15% blasts are found in a hypoplastic BM where it is difficult to differentiate between residual leukemia and regenerating BM it is recommended to postpone therapy and repeat the BM examination on day 21-23.

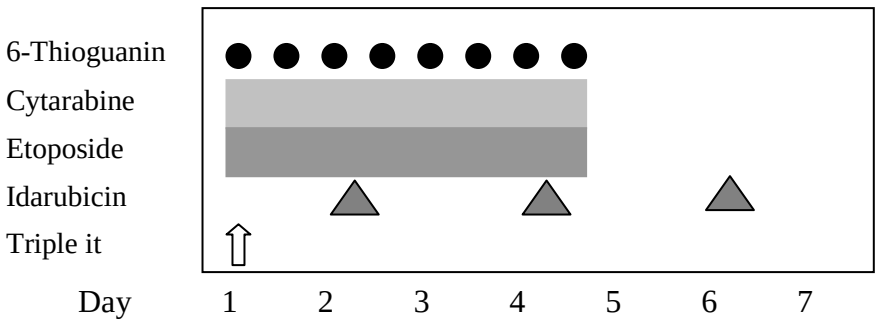
Figure evaluation algorithm after induction AIET and AM/ FLA-DX



Patients still not in remission (> 5% blasts) after block FLA-Dx are eligible for the refractory/relapsed trial AML 2001/01 or a following open Relapse AML study (DCOG and I-BFM-SG). Patients in CR after the second course (AM or FLA-Dx) proceed to the next course.

Definitions for and reporting of serious adverse events, see Chapter 12 Patient Safety and event reporting for details.

5.3 Induction AIET



6-Thioguanin 100 mg/m^2 orally every twelve hours day 1,2,3,4
Cytarabine 200 mg/m^2 per day continuous IV infusion day 1,2,3,4
Etoposide 100 mg/m^2 per day continuous IV infusion day 1,2,3,4

Idarubicin 12 mg/m^2 as a 4-hour IV infusion day 2, 4, and 6
Triple intrathecal injection. Age-adjusted dose; see table triple IT doses in paragraph 5.7.

Note: all drug doses should be reduced for children less than one year or below a bodyweight of 10kg (paragraph 5.10)

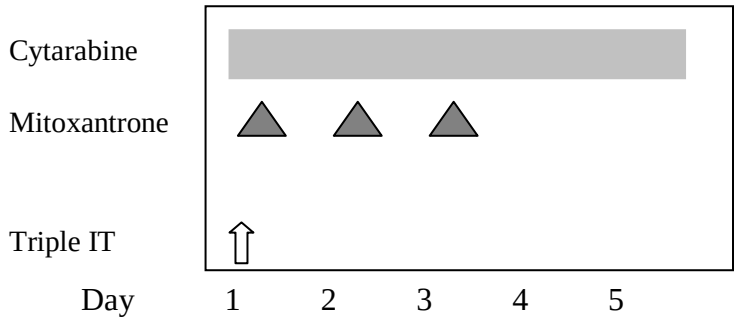
Block AIET should be commenced as soon as the patient is properly hydrated with adequate urinary production. In case of coagulopathy, measures to reduce bleeding tendency should be instituted prior to cytotoxic therapy. In patients with symptoms of sludging, exchange transfusion or leukapheresis should be considered. However, in symptomless patients, also with WBC count > 100 x 10^9/L, we advice to start immediately with the complete induction course AIET. (see also Chapter 8 Supportive Care Guidelines)
Always start or continue measurements to control for the complications of acute tumor lysis (see Chapter 8 Supportive Care Guidelines)
Following induction therapy patients are expected to become severely neutropenic during a prolonged period and the risk of mucositis is high. It is therefore mandatory that all patients receive the highest standard of supportive care (See Chapter 8 Supportive Care Guidelines).

Observations following induction AIET

- Complete blood count, liver tests, and electrolytes should be performed twice weekly until hematological recovery.
- BM aspiration on day 15 after first day of AIET is mandatory. BM examinations from day 14 to day 19 from first day of AIET are accepted as a "day 15 BM". The BM aspirate should be studied by morphology, immunophenotype.
- Smears from PB and BM from diagnosis and day 15 should be sent together for morphological review to the DCOG or laboratory in Ghent.
- Patients with persistent leukemia (unequivocal leukemic blasts by morphology representing ≥ 5%) should, if clinically justifiable, immediately proceed to therapy with the second course FLA-Dx.
- All other patients should begin second course when their ANC is greater than 1.0 x 10^9/L and platelet count is greater than 80 x 10^9/L, AM or FLA-Dx when t(8;21) is present.
- Patients with non-evaluable BM due to hypoplasia should have repeat BM examinations performed weekly to search for regrowth of blast cells during the recovery phase. If 5-15% blasts are found in a hypoplastic BM where it is difficult to differentiate between residual leukemia and regenerating normal BM it is recommended to postpone therapy and repeat the BM examination on day 21-23.
- The second course should generally not be delayed beyond day 36. If there is no evidence of leukemia and the BM is severely hypocellular, induction second course may be delayed at the discretion of the regional coordinator.

5.4.a Second course: Induction AM

Patients in remission after AIET receive AM following hematological recovery, if t(8;21) is not present. .

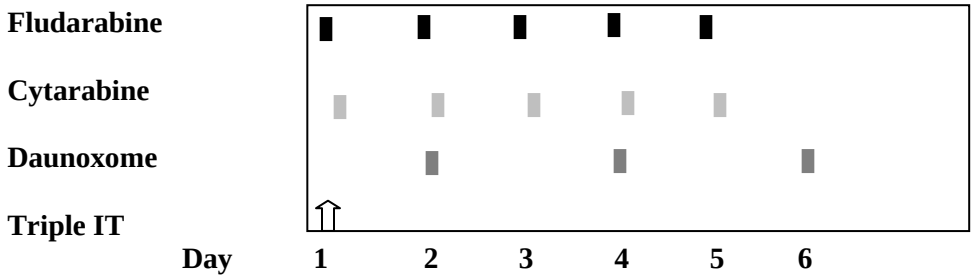


Cytarabine 100 mg/m^2 per day as continuous IV infusion day 12,3,4,5
Mitoxantrone 10 mg/m^2 as a 30-minute IV infusion day 1,2,3
Triple intrathecal injection. Age-adjusted dose; see table triple IT doses in paragraph 5.7 .

Note: all drug doses should be reduced for children less than one year or below a bodyweight of 10kg (paragraph 5.10)

5.4.b Second course: Induction FLA-Dx

Patients with t(8;21) always receive FLA-Dx as a second course. The timing is dependent on the blast count at day 15.
Patients with >5% blasts on day 15 are given FLA-Dx. They preferably start already on day 16 unless life threatening therapy related complications necessitate a delay in administering chemotherapy.



Fludarabine 30 mg/m^2, per day IV infusion (30 min.), day 1, 2, 3, 4, 5
Cytarabine 2000 mg/m^2, per day IV infusion, 4h after fludarabine. day 1, 2, 3, 4 , 5
Daunoxome 60 mg/m^2, IV infusion, 1h immediately after fludarabine, day 2, 4, 6
Triple intrathecal injection. Age-adjusted dose; see table triple IT doses in paragraph 5.7 .

All patients, but especially those not in remission prior to second course are expected to be rendered severely neutropenic for a prolonged period. It is therefore mandatory to assure that each child is given the highest standard of supportive care (See Chapter 8 Supportive Care Guidelines). Children not in remission at the start of FLA-Dx therapy should have a BM evaluation performed between day 28 and 36 irrespective of the blood counts. The bone marrow evaluation will be repeated weekly until hematological recovery is established or until the blast count is equal to or exceeding 5% without full hematological recovery. These patients are off protocol and can be entered into the current protocol for refractory disease.

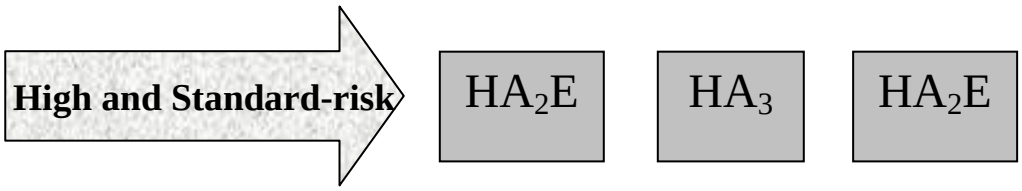
Obsevations following second course

- It is recommended to obtain complete blood count, liver tests, and electrolytes twice weekly until hematological recovery. It is recommended that smears from PB and BM from patients with residual disease following AIET should be sent for morphological review to the DCOG or laboratory in Ghent.
- Patients with persistent leukemia (blasts >5%) will be enrolled on the international protocol for refractory disease (refractory/relapsed AML study 2001/01 or its next protocol).
- A BM examination should be performed in these patients prior to HA_2E. Patients should begin consolidation when ANC is greater than 1.0 x 10^9/L and platelet count is greater than 80 x 10^9/L.
- Echocardiography should be performed before the start of HA_2E.

5.5 Consolidation therapy

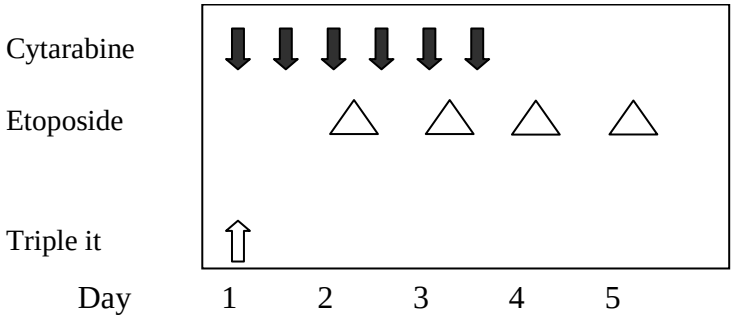
Patients in all risk groups are given a total of three consolidation courses. Each course is started as soon as possible following clinical and hematological recovery (ANC > 1.0 x 10^9/L and platelets > 80 x 10^9/L), which is expected to occur 21-28 days after the start of the previous course.

Figure overview of consolidation therapy



Because relapses still occur we recommend to consider the Myelotarg randomisation after this treatment protocol (see: Protocol post-consolidation randomization for Myelotarg – to be expected in 2010).

Course HA_2E

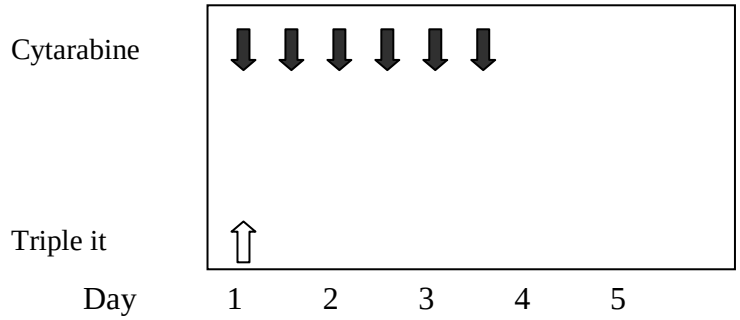


Cytarabine 2 g/m^2 every twelve hours as a 2-hour IV infusion day 1,2,3 (total of six doses)
Etoposide 100 mg/m^2 as a 60-minute IV infusion day 2,3,4,5
Triple intrathecal injection. Age-adjusted dose; see table triple IT doses in paragraph 5.7.

Notes:

- **All drug doses should be reduced for children less than one year or below a bodyweight of 10kg (paragraph 5.10)**
- **All consolidation courses include high-dose cytarabine and patients should be given prophylaxis for chemical conjunctivitis using topical steroids**

Course HA_3

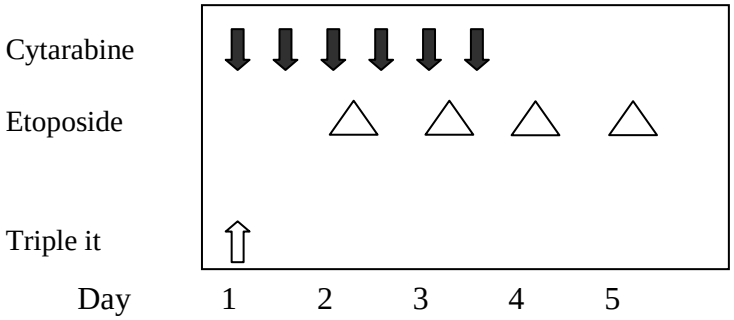


Cytarabine 3 g/m^2 every twelve hours as a 2-hour IV infusion day 1,2,3 (total of six doses)
Triple intrathecal injection. Age-adjusted dose; see table triple IT doses in paragraph 5.7.

Notes:

- **All drug doses should be reduced for children less than one year or below a bodyweight of 10kg (paragraph 5.10)**
- **All consolidation courses include high-dose cytarabine and patients should be given prophylaxis for chemical conjunctivitis using topical steroids**

Course HA$_2$E



Cytarabine 2 g/m^2 every twelve hours as a 2-hour IV infusion day 1,2,3 (total of six doses)
Etoposide 100 mg/m^2 as a 60-minute IV infusion day 2,3,4,5
Triple intrathecal injection. Age-adjusted dose; see table triple IT doses in paragraph 5.7.

Notes:

- **All drug doses should be reduced for children less than one year or below a bodyweight of 10kg (paragraph 5.10)**
- **All consolidation courses include high-dose cytarabine and patients should be given prophylaxis for chemical conjunctivitis using topical steroids**

5.6 Stem cell transplantation

No patients are recommended for SCT in this protocol.

5.7 CNS therapy

Patients without CNS disease (i.e., less than 5 leukocytes per μl of CSF) will receive age-adjusted dose of Triple intrathecal injections (IT) with each course of chemotherapy.

Patients with overt CNS leukemia (≥ 5 leukocytes per µl of CSF and the presence of leukemic blast cells on CSF cytospin) will receive IT triple therapy twice weekly until the CSF is free of blast cells plus two further courses to a minimum of four triple-doses. CNS-irradiation is not advised.
During following courses Triple IT is given according to the treatment schedules.

Table intrathecal triple-doses

Age	MTX	Cytarabine	Prednisolone	Volume
< 1 year	6 mg	15 mg	6 mg	8 ml
1 year	8 mg	20 mg	8 mg	8 ml
2 years	10 mg	25 mg	10 mg	10 ml
3 - < 9 years	12 mg	30 mg	12 mg	12 ml
≥ 9 years	12 mg	30 mg	12 mg	15 ml

5.8 Local therapy

It is not generally recommended to irradiate patients with extramedullary myeloid tumors.
However, patients with extramedullary myeloid tumors may receive low-dose local radiotherapy after consultation with the radiation oncologist when tumors threaten vital organs or neural function. .

5.9 Special treatment groups not eligible for this protocol

Myeloid leukemia in Down syndrome
Myeloid leukemia in Down syndrome has a very good outcome. Myeloid leukemia in children with DS under the age of 5 years is now considered a separate entity and is treated internationally on a special protocol (DCOG Myeloid leukemia for children with Down syndrome) (16). Myeloid leukemia with specific GATA1 mutation in Down syndrome at all ages is an exclusion criterion for this protocol. Only children with Down syndrome of 5 years and older will be treated on this protocol when they do not have a GATA1 mutation.

Acute promyelocytic leukemia
The therapeutic outcome of AML characterized by t(15;17), called acute promyelocytic leukemia (APL) is increased by the introduction of ATRA. This survival advantage remains although anthracyclins were lowered in e.g. AML-BFM studies. So, internationally APL is treated on a separate international study with limited anthracyclin dosages and high ATRA dosing (DCOG and ICC APL study 01) and are excluded from this trial.

MDS and JMML
Both MDS and JMML respond poorly to AML-like chemotherapy. Allogeneic SCT is the therapy of choice. Pre-SCT chemotherapy is of limited benefit. The patients should be referred to and treated according to guidelines from the European Working Group on childhood MDS (EWOG-MDS).
A repeat BM examination after two weeks is recommended in patients with 20-30% blasts at first examination. If the blasts count has increased to 30% or more the case should be considered as AML. Patients with persistent blasts <30% should be referred to the MDS study with exception of patients with t(15;17), inv 16, t(16;16) and t(8;21), which should always be considered AML regardless of blast percentage.

Therapy-related myeloid leukemia
Myeloid leukemia occurring after treatment for a primary malignancy often shares the clinical and biological characteristics of MDS including the poor response to AML therapy. The patients are most appropriately treated with a MDS approach.

Relapsed AML
Patients with relapsed AML should be treated according to the refractory/relapsed trial AML 2001/01 or its successor.

5.10 Treatment modifications

Doses are given adjusted to body surface (BSA) calculated according to the formula:

$$\text{BSA } [m^2] = \sqrt{length(cm)\ x\ weight(kg)/3600} = \sqrt{length(cm)\ x\ weight(kg)}\ /\ 60$$

Dose reduction in infants:
Children under one year of age or below a bodyweight of 10 kg should have doses calculated according to bodyweight with one m^2 equaling 30 kilograms:

6-Thioguanin	100 mg/m^2	corresponding to	3.3 mg/kg
Cytarabine	100 mg/m^2	corresponding to	3.3 mg/kg
	200 mg/m^2	corresponding to	6.7 mg/kg
	1000 mg/m^2	corresponding to	33 mg/kg
	2000 mg/m^2	corresponding to	67 mg/kg
	3000 mg/m^2	corresponding to	100 mg/kg
Etoposide	100 mg/m^2	corresponding to	3.3 mg/kg
Idarubicin	12 mg/m^2	corresponding to	0.4 mg/kg
Mitoxantrone	10 mg/m^2	corresponding to	0.33 mg/kg
Fludarabine	30 mg/m^2	corresponding to	1 mg/kg
Daunoxome	60 mg/m^2	corresponding to	2 mg/kg

Cardiac toxicity
Patients with clinical evidence of congestive heart failure should receive no additional idarubicin or mitoxantrone. In the event of a significant decrease in fractional shortening consideration will be given to discontinuing administration of anthracyclins.
Dexrazoxane (Cardioxane®) may be used for cardioprotection but is not generally recommended.

Cumulated doses of chemotherapy

Treatment according to protocol for a standard-risk patient results in the following cumulated doses:

6-Thioguanin	800 mg/m^2 orally
Cytarabine	43300 mg/m^2 IV
Etoposide	1200 mg/m^2 IV
Idarubicin	36 mg/m^2 IV
Mitoxantrone	30 mg/ m^2 IV
Fludarabine	30 mg/m^2 IV
Daunoxome	60 mg/m^2 IV
Triple intrathecal injections	
methotrexate	6 x 12 mg (3 years and older) intrathecally
ARA-C	6 x 30 mg (3 years and older) intrathecally
Prednison	6 x 12 mg (3 years and older) intrathecally

Total cumulative dosages of Anthracyclins (with AM): 330 mg/m2 (conversion factor 5*)
Total cumulative dosages of Anthracyclins (with FLA-Dx): 180 mg/m2 (conversion factor 5*) + daunoxome 180 mg/m2

*Cumulative doses were calculated as equivalence dose to doxorubicin using a ratio 1:5 for idarubicin and mitoxantrone. This ratio was preferred by the AML Collaborative Group and represents good equivalent doses concerning toxicity (17;19).

6 REQUIRED EVALUATIONS, TESTS, AND OBSERVATIONS

6.1 Initial evaluation

1. History and physical examination.
2. Hemoglobin, WBC with differential count, platelet count.
3. Sodium, potassium, creatinine, urea, uric acid, ALAT or ASAT, alkaline phosphatase, LDH, albumin, bilirubin, calcium, phosphorus, magnesium.
4. Chest x-ray.
5. Echo Abdomen
6. Coagulation screen (prothrombin time, APTT). Consider fibrinogen, plasminogen, AT III, and fibrinogen split products.
7. BM aspirate for diagnosis; tests will include morphologic, immunophenotypic, cytogenetic, and molecular genetic analyses according to the diagnostic guidelines (see Chapter 7 Diagnostic Guidelines).
8. CSF cell count, differential, and protein and immunotyping according to institutional guidelines .
9. Hepatitis B surface antigen, hepatitis A en C, HIV, EBV, CMV, VZV, HSV, parvo, and toxoplasmosis titers.
10. Quantitative immunoglobulin A, G, and M levels.
11. Baseline cardiac evaluation by echocardiography and electrocardiography.
12. Save extra for central review.
13. Send smears of PB and BM for central review to the DCOG for dutch patients or laboratory in Ghent for belgian patients.

6.2 Evaluations after the completion of therapy

- Blood and BM examination should be performed by morphology and MRD 4 weeks after last consolidation.
- A repeat BM examination for morphology and MRD is recommended 6 months from the last consolidation (second HA_2E course).

6.3 Long-term follow-up evaluations

Complete blood count should be obtained as follows:

- First year after the end of therapy: monthly
- Second year: every 2 months
- Third year: every 4 months
- Fourth and fifth years: every 6 months
- Thereafter the Guidelines of the SKION LATER will be followed.
- During the five years of follow-up the consult includes height, weight, pubertal development and questions regarding speech dysfunction, seizure disorder, learning disabilities, problems with social contacts, and need for extra tutoring or special education classes. Patients are referred to further evaluations if indicated. No routine neuropsychological screening is recommended.
- Since cardiac, renal, and audiologic late-effects have shown to be the important late effects of AML treatment a structured follow up is recommended (shown below) and the results reported annually.

Cardiac evaluation

Cardiac evaluation is based on echocardiography and measurement of left ventricular fractional shortening (FS) calculated by the formula: FS = [(left ventricular end diastolic diameter – end systolic diameter)/end diastolic diameter] x 100. The normal range has been reported to be 28%-44% (60).
Patients who show clinical cardiotoxicity during or immediate after therapy or have reduced FS (<28%) one year after therapy have increased risk of late cardiotoxicity and should have closer cardiac follow-up and referred to a pediatric cardiologist.
On the annual report the FS values measured during the year are reported in percentage as well as an indication of whether the patient had clinical symptoms or received therapy for cardiac failure. (See also Appendix 2 Guidelines for Cardiotoxicity Monitoring)

Hearing

Hearing is assessed by pure-tone audiometry and tympanometry performed by trained audiologist.
The values outside of the normal range are indicated as “abnormal” in the report of the respective year.

Renal function

Evaluation of renal function includes measurements of the supine blood pressure, creatinine clearance, serum levels of creatinine, and electrolytes (K, Na, Ca, P, Mg), and capillary or venous blood bicarbonate.

The blood values outside the reference values of the local laboratory and systolic and diastolic blood pressure greater than 95th percentile for age, sex and height percentile are indicated as “abnormal” in the annual patient report.

Table follow-up examinations overview

	At dg	Before HA_2E	1 y	2 y	3 y	4y	5y
Complete blood count	Guidelines in section 6		Every month	Every 2 months	Every 3 months	Every 6 months	
Echocardio-graphy *	X	X	X		X		X
BP, electrolytes, bicarbonate, creatinine, creatinine clearance	X		X				X
Audiometry			X				X

* Regarding Echocardiography:
Additional cardial examinations (Echo Cor) are recommended:
- For any clinical symptom of cardiac problem
- 1 year after the start of puberty and at the age of 18 years (growth spurt can exacerbate cardiac dysfunction) or otherwise following the guidelines defines by SKION LATER
- Before a planned pregnancy and during pregnancy (women previous treated with anthracyclins might go into cardiac failure during pregnancy, during labour, or shortly after labour)

7 DIAGNOSTIC GUIDELINES

Definition of leukemia

The operative definition of leukemia has traditionally been the presence of an identifiable clone of malignant hematopoietic cells in the marrow and/or peripheral blood. When light microscopy was the sole means of detection, the diagnosis required either the presence of increased numbers of primitive blood precursors, i.e. acute leukemia, or a superabundance of differentiated blood cells as in chronic leukemia.
Internationally working groups, such as the FAB Cooperative Group (61) and the National Cancer Institute, established 30% as the minimal level of blast cell infiltration required for the diagnosis of AML. For ALL the definition most frequently employed requires a 25% infiltration of lymphoblasts in the bone marrow.
For AML there has been a continuous debate about the proper classification of cases in which the malignant nature of the immature cells cannot be ascertained by morphology. This has led to the development of a new definition of the hematopoietic malignant neoplasms based on the increasing ability to identify, with near certainty, recurrent genetic abnormalities associated with and often causally related to leukaemia, WHO classification 2001. Recently, a revision has been published: WHO 2008 classification for Acute myeloid leukemia (62). Thus, when such a marker is detected in the setting of an alteration of blood cell production or extramedullary myeloid tumor, the diagnosis of leukemia can be made independently of blast cell counts. In cases in which a marker cannot be demonstrated, diagnosis must depend on the combination of history, morphology, immunophenotype, cytogenetics, and molecular genetics.
The current cytogenetic and molecular approach has changed our concept of AML to a very heterogeneous family of malignant disorders.

Table WHO 2008 classification for Acute myeloid leukaemia

AML with recurrent genetic abnormalities
AML with t(8;21)(q22;q22), RUNX1-RUNX1T1
AML with inv(16)(p13.1q22) or t(16;16)(p13.1;p22); CBFB-MYH11
Acute promyelocytic leukaemia with t(15;17)(q22;q12);PML-RARA
AML with t(9;11)(p22;q23)MLLT3-MLL
AML with t(6:9)(p23;q34); DEK-NUP214
AML with inv(3)(q21q26.2) or t(3.3)(q21;q26.2); RPN1-EVl1
AML (megakaryoblastic) with t(1:22)(p13;q13); RBM15-MKL1
AML with mutated NPM1
AML with mutated CEBPA

AML with myelodysplasia-related changes **Therapy-related myeloid neoplasms**
AML with minimal differentiation
AML without maturation
AML with maturation
Acute myelomonocytic leukaemia
Acute monoblastic and monocytic leukaemia
Acute erythroid leukaemia
Acute megakaryoblastic leukaemia
Acute basophilic leukaemia
Acute panmyelosis with myelofibrosis

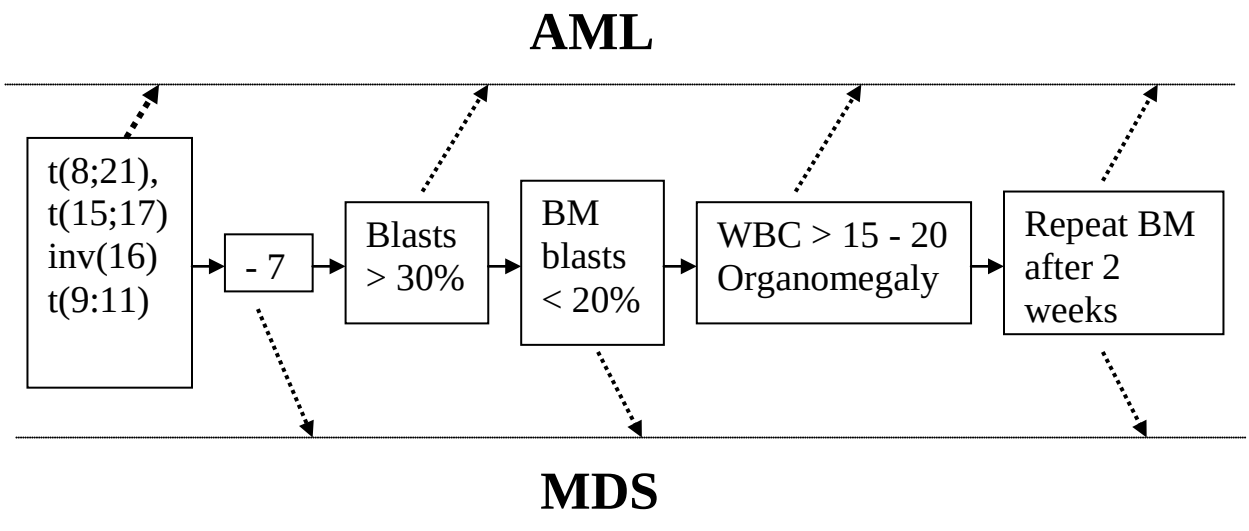
In the borderline group with blasts below 30% and no recurrent AML-specific aberration the differentiating between AML and MDS is done according to the pediatric modification of the WHO classification 2008 (16).
In short:

Table classification of MDS in children (WHO 2008)

Myelodysplastic/myeloproliferative syndromes
Juvenile myelomonocytic leukemia (JMML)
Chronic myelomonocytic leukemia (CMML)
BCR/ABL negative chronic myeloid leukemia (Ph-CML)
Myelodysplastic syndromes (MDS)
Refractory cytopenia (RC): peripheral blood <2% blasts, bone marrow < 5% blasts
Refractory anemia with blast excess (RAEB): peripheral blood 2-19% blasts, bone marrow 5-19% blasts
RAEB in transformation (RAEB-t): peripheral blood or bone marrow 20-29% blasts

A diagnosis of AML requires in most cases that 30% or more of the nucleated cells in the BM are myelo- or monoblasts or 30% of the non-erythroid cells when the BM contains 50% or more erythroblasts.
Promyelocytes should not be counted as blasts.
A repeat BM examination after two weeks is recommended in patients with 20-30% blasts at first examination. If the blasts count has increased to 30% or more the case should be considered as AML. Patients with persistent blasts <30% should be referred to the MDS study.
AML M3 may be diagnosed in patients with less than 30% blasts, when there are abundant abnormal promyelocytes in the BM.
AML M7 is often fibrotic and BM differential cell count may be difficult to assess. Differential count of the biopsy may be helpful.

Algorithm helping to distinguish AML from MDS



Central morphological review of the diagnostic and day 15 BM is recommended. A group of pathologists and pediatric hematologists will receive smears for review (see Appendix 5 for specific laboratory guidelines in the Netherlands or Belgium).

Immunophenotype

Immunophenotyping is a powerful tool for the characterization of the various subpopulations in phenotypically heterogeneous AML (63;64). Triple/quadruple immunologic marker analysis can be especially helpful in discriminating the various immature and more mature subpopulations. Generally CD34, CD117, and TdT are markers for immature AML subpopulations.

The table below summarizes the immunophenotypic characteristics of AML. Virtually all AML are positive for the panmyeloid markers CD13 and CD33. In a minority of cases, the AML cells express only one of these antigens. MPO is the only panmyeloid marker fully specific for myeloid differentiation lineage. However, some AML M0 and AML M5 cases may be negative for MPO. Finally, approximately two-thirds of AML display CD117 positivity. This marker is rarely detected in ALL.

In the table below the three myeloblastic leukemias as well as AML M4 and AML M5 are combined; these AML types cannot be discriminated based on their immunophenotype. Markers that show a high correlation with the FAB classification are expression of the monocytic antigen CD14 in AML M4 and AML M5, glycophorin A expression in AML M6, and expression of the megakaryocytic markers CD41/CD61 and CD42 in AML M7. In AML M4 and AML M5 monocytic differentiation can be confirmed by CD11c and/or CD36. AML M3 is characterized by a homogeneous immunophenotype with negativity for HLA-DR and frequently also CD15, but positivity for CD13/CD33 as well as CD9.

Table immunophenotypic characteristics of AML

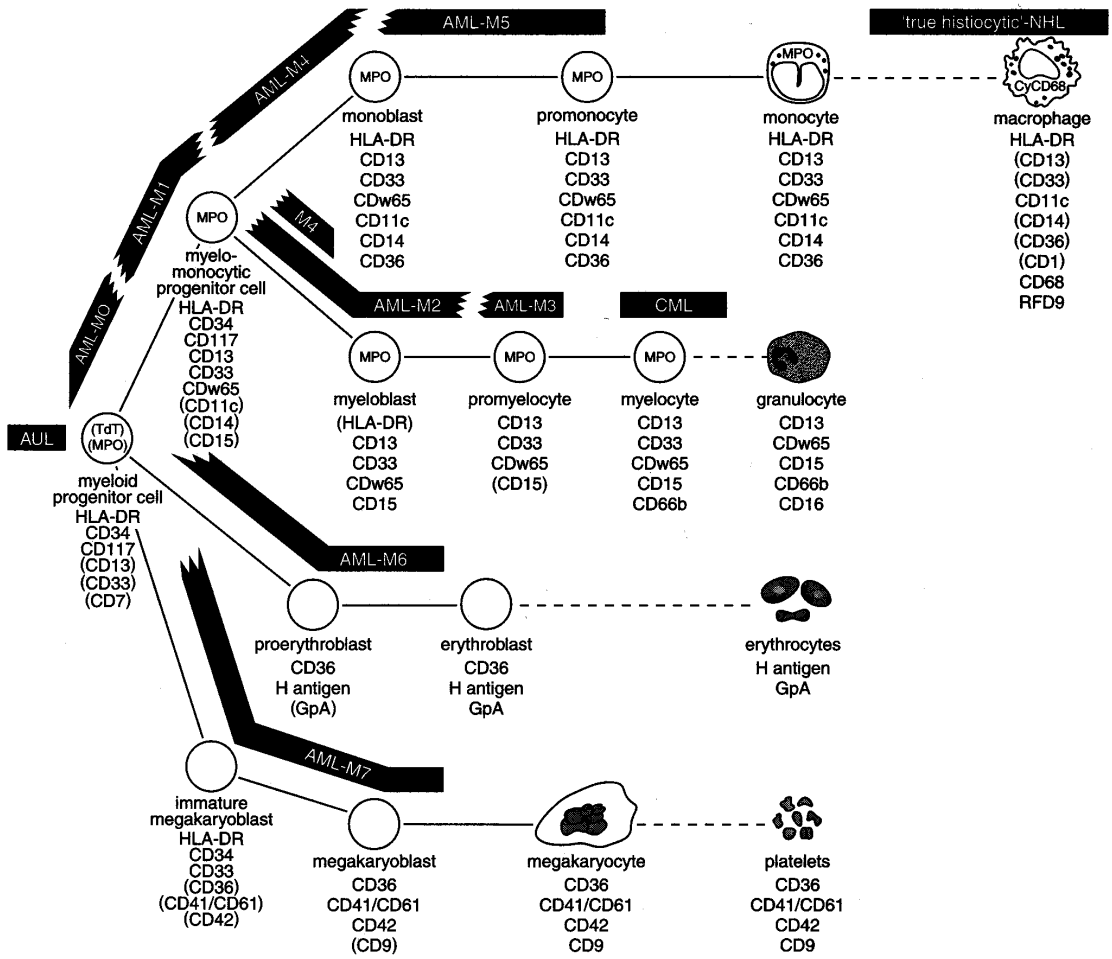
Markers	AML M0/M1/M2	AML M3	AML M4/M5a/M5b	AML M6	AML M7
CD13/CD33	++	++	++	+	++
CD65	±/+/++	+	++	±	±
MPO	-/+/++	++	++	+	-
CD11c	**- or ±**	-	++	-	-
CD14	-	-	+/+/++	-	-
CD15	±/±/++	±	-	-	-
CD36	-	-	+	++	+
H-antigen	-	-	-	++	+
GpA	-	-	-	+	-
CD41/CD61	-	-	-	-	++
CD42	-	-	-	-	+
CD34	++/++/+	±	±/+/±	+	+
CD117	++	+	+	+	+
HLA-DR	++/++/++	-	++	+	++
TdT	+	±	+	+	±

Legend: -, <10% of the leukemias are positive; ±, 10-25% of the leukemias are positive; +, 25%-75% of the leukemias are positive; ++, >75% of the leukemias are positive.

The following represent the minimal requirements to be tested by multicolour immunophenotyping
Non-lineage: HLA-DR, TdT, CD10, CD34, CD45, and CD117
B-lineage: CD19, cytCD22, cytCD79a, kappa, lambda, and cytIgM
T-Lineage: CD7, CD5, CD2, CD3, CD4, CD8, and cytCD3
Myeloid lineage: CD13, CD33, CD14, CD15, CD11c, and MPO
Erythroid lineage: GPA and/or CD71
Megakaryocytic lineage: CD41, CD42b, and CD61
NK-lineage: CD56

The following figure summarizes the expression of relevant immunologic markers for each myeloid differentiation stage as well as the maturation arrest of the various types of AML.

Figure hypothetical scheme of myeloid differentiation.



The expression of relevant immunologic markers is indicated for each differentiation stage; markers in parentheses are not always expressed. The bars represent the various types of leukemias and non-Hodgkin lymphomas (NHL) and indicate where these malignancies can be located according to their maturation arrest. It should be emphasized that most acute leukemia's of the myeloid lineage have a heterogeneous phenotype, i.e. are composed of cells in multiple immature myeloid differentiation stages. To underline this phenotypic heterogeneity, several bars fade into each other. AUL = acute undifferentiated leukemia, CML = chronic myeloid leukemia.

Cytogenetics and molecular genetics

G-banded karyotyping of at least 25 mitoses should be performed (65). Multiplex PCR for recurrent aberrations is recommended. Comparative genomic hybridization (CGG) is recommended as part of a separate NOPHO study.

If the G-banded karyotype or multiplex show no t(8;21), t(15;17), inv(16)/t(16;16), or 11q23 aberrations interphase FISH should be performed to search for these aberrations. If the aberrations are found by multiplex only it is recommended to perform confirmatory FISH studies.

There is no general recommendation of repeated G-banding karyotype examination during the treatment course. If relapse is suspected G-banded karyotype should be performed.

All karyotypes as well as targeted investigations should on a yearly basis be reviewed.

Detection of cytogenetic abnormalities such as t(8;21), inv 16, t(15;17), Flt3 ITD, EVI1, MLL, WT1, cKIT, CEBPa, NPM1, NF, will be organized centrally by the DCOG and laboratory in Ghent.

Extramedullary sites

Cerebrospinal fluid (CSF)

CNS leukemia is defined as at least 5 $x10^6$/L leukocytes in the CSF and leukemic cells detected in the cytospin and/or new neurologic symptoms (e.g. seizures, cranial nerve palsy, and signs of increased intracranial pressure).

Extramedullary myeloid tumors

Biopsy from suspected sites should be performed for morphologic and immuno-histochemical examination and preferentially cytogenetics. The classification of extramedullary myeloid tumors without BM involvement is controversial. In case of AML typical cytogenetics (e.g. t(8;21) or *MLL* rearrangements) it is most appropriate to consider and treat as AML regardless of the BM blast count.

Down syndrome

Myeloid leukemias in patients with DS show unique biological features and they are best described as one disorder termed myeloid leukemia of DS not included as AML (16;66) .

Myeloid leukemia in older DS children (5 years or older) behaves more like AML in patients without DS and has a poorer prognosis when no GATA1 mutation is detected (67). Such patients may present as “true de novo” AML (68) not fulfilling the criteria for myeloid leukemia of DS.

Secondary myeloid leukemia

Myeloid leukemia may occur secondary to a constitutional or acquired bone marrow failure or following chemotherapy. Myeloid neoplasias in patients with predisposing conditions almost always share the biologic characteristics of MDS regardless of the presenting blast count.

Definition of complete remission (CR)

The proportion of normal primitive bone marrow cells was empirically determined to be less than 5%. This remains the most widely used definition of remission (69). In AML CR is defined as ≤5% blasts in a BM by morphology with signs of normal hematopoiesis and with clear signs of regeneration of normal blood cell production in the peripheral blood (platelets > 80 x 10^9/L without transfusions and ANC >1.0 x 10^9/L), and no leukemic cells in the PB or anywhere else.

A low number of blasts (< 5%) may be seen in the PB at day 15-20 during regeneration marrow and especially during G-CSF therapy without indication of residual disease (70;71).

Biobank

To ensure reevaluation of the characteristics of the patients in case of poor response or relapse storage of biological material from diagnosis should be performed in all patients centrally at the DCOG for all patients in the Netherlands and at the Laboratory of the Ghent University for all Belgian patients. When patients are willing to facilitate biological research studies the material left overs might be used for research following the standard operating procedures at the DCOG.

8 SUPPORTIVE CARE GUIDELINES

The treatment to be given is aggressive and immunosuppressive. Therefore, meticulous care is required in the management of patients entering the study. The following represent guidelines for supportive therapy.
Pediatric oncology emergencies arise as a result of space-occupying lesions, metabolic disturbances, and as a consequence of cytopenia. They can be the presenting features of a new malignancy, or they may arise during the treatment.
The following categories of oncology emergencies are the most frequent/ the most important:

1) Disseminated intravascular coagulation (DIC) and bleeding
2) Metabolic complications and tumor lysis
3) Hyperleukocytosis

DIC and bleeding
Risk parameters for early death due to bleeding or leukostasis in childhood AML are:

FAB-type	In combination with
M4 / M5	WBC > 100 x 10^9/L, and in pt with kidney involvement also at lower WBC
M1, M2	WBC > 150 x 10^9/L
M3	All patients

Patients at risk of life threatening bleeding/thrombosis:

1. Analyze coagulation status, including fibrinogen, AT III, and fibrinogen split products.
2. An exchange transfusion is preferable to leukapheresis as treatment for severe leukostasis, because the exchange transfusion can substitute coagulation factors and reduce uric acid and acidosis.
3. Attempt to correct the deficiency of coagulation factors through fresh frozen plasma (FFP) rather than supplementing with single factors since the coagulopathy is multifactorial. Give a daily dose of FFP 30 – 50 ml/kg/24 hr in three doses.
4. During exchange transfusion, the platelet count should be maintained at 60 x 10^9/L.

Non-life-threatening bleedings:

1. Wound bleeding
2. Mucous membrane bleeding: tranexamic acid or “fibrin-kleber”
3. Heparinization is normally not indicated
4. Avoid i.m. injections, acetyl salicylic acid medication, and dental surgery
5. Nose bleeding. Compression (15 min.)

Metabolic complications and tumor lysis
Acute tumor lysis syndrome is a condition caused by the rapid release of intracellular metabolites in quantities exceeding the excretory capacity of the kidneys. These metabolites are uric acid, potassium, and phosphate. Common associated complications are renal failure and hypocalcemia.

Tumor lysis is a result of a) the normal turnover in the blast cell population, already present before therapy, b) massive lysis of leukemic cells after the initiation of chemotherapy.

When to start chemotherapy in patients with tumor lysis?
Chemotherapy must not be initiated until the patient has been metabolically stabilized with hydration, alkalinization, and allopurinol, and until there is adequate urinary output. This will usually take a minimum of 12-24 hours following the guidelines defined in the “Werkboek Supportive Care”.
On the other hand, while waiting, the leukemic burden is constantly increasing; so do not wait too long.
The basic problem lies in the excretion of the big metabolic burden (potassium, phosphate, uric acid) through kidneys functioning poorly just because of the precipitation of these metabolic waste products. Rasburicase is recommended in patients with initial WBC of > 100 x 10^9/L or urine acid > 0.45 mmol/L.

In patients with hyperleukocytosis (WBC of > 100 x 10^9/L) and clinical disturbances exchange transfusion and gentle cytoreduction should be considered (see below).

Hyperleukocytosis

Hyperleukocytosis is defined as a total peripheral WBC above 100 x 10^9/L. Hyperleukocytosis increases the blood viscosity and is associated with the aggregation of leukemic cells in the microcirculation. Stasis of leukemic blasts occurs in the pulmonary vessels, which may block oxygen diffusion and cause respiratory distress. In the CNS, leucostasis may result in CNS hemorrhage or thrombosis. Complications from hyperleukocytosis are much more common in AML than in ALL. Particularly the AML subtypes M3/M4/M5 frequently present with coagulopathy/hyperleukocytosis increasing the risk for both bleeding and microcirculatory thrombosis.

All patients should be carefully evaluated for signs of hypoxia and acidosis: pulmonary insufficiency (dyspnea, tachypnea, and cyanosis), CNS symptoms (level of consciousness, slurred speech, ataxia, nystagmus), and eye symptoms (examine the ocular fundi for papilledema). Chest X-ray is recommended and laboratory tests for evaluation of kidney function and coagulation parameters should be made.

Management of hyperleukocytosis:

1. Avoid nonessential transfusions. Do not raise the hemoglobin concentration above 5-5.5 mmol/L because of the high "cytocrit".
2. Consider an exchange transfusion (particularly in children <20 kg) to decrease the "cytocrit" and blood viscosity when there are clinical symptoms of leukostasis. Part of the blood exchanged should be substituted by albumin or fresh frozen plasma instead of cellular components. Target volume to be exchanged is about two times the blood volume, i.e. 150 ml/kg.
3. Consider leukapheresis when there are clinical symptoms of leukostasis (in children >20 kg).
4. Consider to start the first course of chemotherapy as soon as possible.

The indications for exchange transfusion/leukapheresis depend on the WBC, the clinical condition of the patient, availability of exchange transfusion or leukapheresis, and the potential risks involved. A WBC above 300 x 10^9/L is a very strong indication for leukapheresis, but preferably start with the first course of chemotherapy as soon as possible.

Cardiac toxicity

The following cumulated doses of anthracyclins are given:

Idarubicin	36 mg/m^2 IV
Mitoxantrone	30 mg/m^2 IV

If a conversion factor of 5 is used the corresponding daunorubicin dose is 330 mg/m^2.

Due to unresolved questions about interference with the antileukemic effect the use of Dexrazoxane (Cardioxane®) is not recommended.

Patients with clinical evidence of congestive heart failure should receive no additional idarubicin or mitoxantrone.

Echocardiography is recommended at presentation, before the second mitoxantrone-containing course of chemotherapy, and at one, three, five, and ten years of follow-up or more often as specified in paragraph 6.4.

9 STATISTICAL CONSIDERATIONS

This study is an international single arm study consisting of 5 intensive chemotherapy courses, modified from the NOPHO AML 2004 protocol (giving a sixth course to the 5 identical courses). This study will answer the question whether treatment with these 5 intensive courses demonstrate a relapse rate of 40% or less. This study will be monitored by stopping rules. The inclusion time is restricted to a maximum of 4 years and/or 120 evaluable patients.
The outcome for all patients will be evaluated by using the following endpoints (events): induction failure due to refractory disease or early death, death in continuous complete remission, or relapse.
The Trial Offices at the DCOG with the Ghent University will continuously monitor the rate of treatment related morbidity. The protocol may be stopped in case of too high frequency of excessive toxicity. The protocol can also be stopped in case of too high relapse rates.

9.1 Safety monitoring stopping guideline

The cumulative 3-years relapse rate of the NOPHO-AML93 was 42% (1). Of the NOPHO-AML 2004 it is 36%, but the number of patients in NOPHO-AML 2004 as yet is still small. The hazards (= no. of relapses / total follow-up time) under the NOPHO-AML93 protocol in the first four years were 0.186, 0.143, 0.051 and 0.023. So the yearly hazards decrease during follow up, and the ratios between them are 3.65, 2.80, 1 and 0.45, with year three as the reference. We assume that a safe cumulative 3-years relapse rate of the new protocol is 40%. Assuming also that the ratios between the hazards in the first three years for the new protocol are the same as in the NOPHO-AML93, a 3-years relapse rate of 40% corresponds with yearly hazards of 0.250, 0.192, 0.069 and 0.031. The monitoring boundary provides a guideline for stopping the protocol prematurely if the yearly relapse hazards are too high in comparison to the above mentioned safe hazards. The design is such that after every second relapse a weighted cumulative follow-up time is calculated. Follow-up time in the first year is multiplied by 3.65, in the second year by 2.80, in the third year by 1 and in the fourth year by 0.45. If it falls below the lower limit indicated in the table below, the alarm bell rings and the protocol is declared as unsafe. The stopping guideline is designed such that the probability that this happens under the safe 3-years relapse rate of 40% is equal to 10%.
We expect the duration of the new protocol to be 4 years with an intake of 120 patients in total. Under the above safe yearly hazard rates, it is very unlikely that more than 54 relapses will be observed during this time. Therefore we have chosen 54 relapses as the horizon of the stopping guideline.
The stopping rule is inspired by the Sequential Probability Ratio Test (SPRT) comparing a safe 3-years relapse rate 0.40 with an unsafe rate of 0.45 per year. The standard SPRT does not allow stopping before observing 12 relapses. Therefore we modified the SPRT by allowing stopping at 12 relapses or lower if the one-sided P-value for testing H_0: 3-years relapse rate = 0.40 against H_1: 3-years relapse rate > 0.40 is smaller than 0.001. This modification is inspired by the Peto-Haybittle stopping rule.
The stopping guideline is given in the next table. For instance, if after 16 observed relapses the weighted number of follow-up years is less than 57.458, the guideline advises to stop the protocol declaring it having a 3-years relapse rate larger than 40%. The estimated 3-years relapse rate (extrapolated, because there are no patients yet with 3-years follow-up) is than at least 87%.

Number of relapses	*Total weighted follow-up years Lower limit*	***Corresponding estimated /extrapolated 3-years relapse rate***
2	0.029	100
4	1.324	100
6	5.555	100
8	12.494	99
10	21.556	97
12	32.277	94
14	44.325	90
16	57.458	87
18	73.427	84

20	100.364	77
22	127.300	72
24	154.237	69
26	181.173	66
28	208.109	63
30	235.046	61
32	261.982	60
34	288.918	58
36	315.855	57
38	342.791	56
40	369.728	55
42	396.664	55
44	423.600	54
46	450.537	53
48	477.473	53
50	504.409	52
52	531.346	52
54	558.282	51

Further properties of the design are described in the next table. For instance, if the true 3-years relapse rate is 35%, then the probability that the protocol runs to its natural end is 97%, and the probability that it is claimed to have an unsafe relapse rate larger than 40% is only 3%. If for instance the true 3-years relapse rate is 55%, then the power is 80% that it will be declared unsafe, after observing on average 38 relapses.

Relapse rate 3-years	*Probability declaring unsafe*	*Average no. relapses at time of stopping*
0.35	3%	53.4
0.40 (safe)	10%	52.2
0.45	27%	49.4
0.50	54%	44.4
0.55	79%	38.1

At the end of the study it can be interesting to compare the relapse rate with the relapse rates of the NOPHO 2004 AML protocol. We already checked that when the results of this protocol demonstrate a relapse rate of 40% the power to state that this protocol is non-inferior to the NOPHO 2004 AML protocol is 23%, whereas the power increases to 52% when the relapse rate of this protocol is 36%.

9.2 Data Safety Monitoring Board

A Data Safety Monitoring Board (DSMB) is established to perform ongoing safety surveillance and to perform interim analyses on the safety data and efficacy of treatment. The independent DSMB committee is formed by two clinicians and one statistician who give recommendations about continuation of the trial as planned.
The purpose of this paragraph is to describe the roles and responsibilities of the independent DSMB for the DB AML-01 trial. The aim of the DSMB is to protect and serve trial patients and to assist and advise Principal Investigators so as to protect the safety and monitor the overall conduct of the trial. The conduct of the trial will be assessed by the DMSB in relation to the safety monitoring stopping guidelines (paragraph 9.1). The DMSB will meet at least yearly, or more frequent when necessary according to the safety guidelines. The DMSB will be informed about the conduct of the trial, and interim analysis and suspected (unexpected or expected) serious adverse events with annual reports before their meeting or more frequent when necessary. The DMSB will report each year after their meeting to the Steering committee their conclusions and advices.

10 OPERATIONAL ASPECTS AND DATA MANAGEMENT

Each participating group will refer to the contact person of the group and to the usual network of the clinical centres, data centre and experts for the application of this protocol, the monitoring of the data collection and data quality. The coordinating Investigator, the regional coordinators and the Trial Office DCOG will act as a Coordination Unit for the monitoring and exchange of information and for pooling of the data.

More specifications will follow by the DCOG Trial Office, such as that each group will use the data collection forms designed for this protocol. Each group is required to register each new patient with AML and to report each adverse event, as described in Chapter 12, immediately to the DCOG Trial Office or Chent Trial Office.

Case Report Forms (CRFs) about diagnosis, different treatment phases, toxicity and follow-up should be filled in. Each participating center in the Netherlands submits CRFs to the DCOG Trial Office. In Belgium CRFs are sent to the Ghent Trial Office. After visual checks, the CRFs are sent to the DCOG from there on. At the DCOG Trial Office, the CRFs are entered in a secure database.
The data is checked visually for consistency and completeness when entered in the database. Thereafter, validation checks will be performed.
The following rules for completing paper CRFs have to be observed:

- CRFs are to be filled in with a blue/black ballpoint pen in a clear handwriting.
- Mistakes are to be cancelled by a simple horizontal line and correction is to be written above or next to it.
- The correction has to be signed and dated.
- Data fields which cannot be completed due to missing information have to be marked and commented.
- Every first page of the CRF starts with the patient unique registration number and date of birth.
- Every last page of the CRF ends with the date of completion and signature of the local data manager.
- All requested data fields should be answered completely; even if there is no major change from a previous examination.
- At all times the local investigator is responsible for the accuracy and authenticity of all clinical and laboratory data entered.

Time points for submitting CRFs
CRF about diagnosis: 1 month after initial diagnosis
CRFs about induction treatment (course AIET and AM) and toxicity: 1 month after start AM course.
CRF about consolidation treatment (course HA_2E, HA_3 and HA_2E) and toxicity: 1 month after start second HA_2E course.
CRFs about the follow-up, yearly after treatment CRFs.
CRFs concerning a SAE, immediately after occurrence of SAE. See Chapter 12 Patient safety and event reporting.

DCOG Trial Office, POBox 43515, 2504 AM The Hague

The archiving of all study relevant documents at the local centers and at the DCOG and Ghent trial office will be handled according to national law. Each patient receives an unique patient number (UPN). All study relevant data will be stored electronically and handled confidentially.
The investigators and all members of a trial centre or other persons involved in the trial are obliged to keep study data and information confidential and to grant access only to individuals who are involved in the study.
Annual reports are made for the Study Committee and for the Data Safety Monitoring Board.

11 PATIENT INFORMATION, INFORMED CONSENT, INSURANCE

Before signing the informed consent form the patient and/or his/her parents/legal representatives must be informed about the disease, the treatment according to the clinical trial including estimated duration, possible side and late effects of the treatment, and the assessment required for the treatment and about alternative treatment options. The patients and/or their parents/legal representatives must have sufficient time to decide about trial participation and must have the opportunity to ask all questions they may have concerning the trial treatment before signing the consent form.
Informed consent should be obtained according to national and institutional regulations. The informed consent should ask for permission to send data about the clinical characteristics and outcome to the DCOG and/or Ghent Trial Office and for storing biological material for (future) leukemia-specific studies. The signature of the legal representative is required for children and adolescents below 18 years. Consent can be withdrawn at any time.

Insurance
Each country will organize their own insurance for patients following the regulations in each country. For the participating institutes in the Netherlands regulations and procedures will be followed as mentioned below.
Ingevolge art. 7 van de Wet medisch wetenschappelijk onderzoek met mensen (Stbl. 1998, 161) is voor de deelnemende proefpersonen een verzekering afgesloten die de door het onderzoek veroorzaakte schade door dood of letsel van de deelnemende proefpersonen dekt. Deze verzekering voldoet aan de bepalingen van het Besluit verplichte verzekering bij medisch-wetenschappelijk onderzoek met mensen (Stbl. 2003, 266). Aan het onderzoek deelnemende proefpersonen zullen schriftelijk worden ingelicht over deze verzekering. Elke aan het onderzoek participerende instelling draagt zorg voor de verzekering van de in de eigen instelling te includeren proefpersonen.

12 PATIENT SAFETY AND EVENT REPORTING

Since a more precise procedure was made for this paragraph in October 2010 and the protocol is amended now, this patient safety and event reporting procedure is incorporated in this chapter.

Safety and tolerability of study treatment will be reported for all treated subjects. Each AML patient must be carefully monitored for toxic reactions (adverse events) during the course of the protocol. These reactions must be registered on the toxicity forms. Safety assessments will include physical examinations, vital signs (systolic/diastolic blood pressure, pulse rate, and body temperature), clinical laboratory tests (hematology, serum chemistry), and reported or observed adverse events.

Toxicity Reporting

For every treatment course there is a CRF which has to be filled out together with a toxicity form. In the toxicity forms known side effects of the applied drugs are listed and ranked according to severity from 1, mild severity to 5, death (adapted from NCI Common Toxicity Criteria).

Adverse events

Adverse events (AEs) are defined as any untoward medical occurrence in a patient and which does not necessarily have a causal relationship with the treatment. An AE can therefore be any unfavorable and unintended sign, symptom, or disease temporally associated with the use of a medicinal product, whether or not considered related. Pre-existing conditions which worsen during a study are to be reported as AEs. AEs will be assessed continuously and graded according to NCI Common Terminology Criteria for Adverse Events (CTCAE) version 3.0 (a copy can be downloaded from the CTEP web site (http://ctep.cancer.gov/reporting/ctc.html). Frequency and severity, and outcomes of AEs will be determined.

AEs should be followed to resolution or stabilization, and reported as SAEs as they become serious.

In addition, an event that meets the criteria of serious adverse event should also be reported on a SAE form within 48 hours at the DCOG Trial Office and at the Trial Office in Ghent and at the National Coordinating Investigators for the Netherlands and Belgium.

Serious Adverse Event

The definition of a Serious Adverse Event (SAE) is an adverse event occurring at any dose and resulting in one of the following outcomes: (a) death, (b) is life threatening, (c) an unexpected admission to hospital or unexpected prolongation of existing hospitalization, (d) a persistent or significant disability or incapacity to conduct normal life's functions, (e) a congenital anomaly or birth defect in the offspring. Every SAE can be classified as expected or unexpected. Expected SAEs are toxic reactions described in the drug information.

In case of chemotherapy administration for leukemias, all complications as a result of severe bone marrow failure are expected adverse events, even if they result in death. An unexpected SAE is all toxicity that does not meet the description for expected SAE or the nature/ severity of the event is not consistent with the applicable drug information.

A SAE report should be completed for any event where doubt exists regarding its status of seriousness.

The causality of the relationship with the treatment to the SAEs will be assessed as either:

1. Certain: There is a reasonable causal relationship between the combined drug treatment and the AE. The event responds to withdrawal of study treatment (dechallenge), and recurs with rechallenge when clinically feasible.
2. Probable: There is a reasonable causal relationship between the treatment and the AE. The event responds to dechallenge. Rechallenge is not required.
3. Possible: There is reasonable causal relationship between the treatment and the AE. Dechallenge information is lacking or unclear.
4. Not likely: There is a temporal relationship to treatment administration, but there is not a reasonable causal relationship between the treatment and the AE.
5. Not related: There is not a temporal relationship to treatment administration (too early, or late, or study drug not taken), or there is a reasonable causal relationship between another drug, concurrent disease, or circumstance and the AE.

Protocol specific exceptions of SAE Reporting
The following does not require reporting on the SAE form: (a) hospitalization due to signs and symptoms of disease progression, (b) death due to disease progression, (c) expected hospitalization for procedures such as blood transfusion or unexpected hospitalization for treatment of grade 1-3 toxicities, (d) pre-existing toxicities before entering the study which meet the criteria of a SAE.

Reporting Serious Adverse Events
All AEs and SAE's will be recorded in the patient's file and in the CRF.
All serious adverse events (SAE) and pregnancies occurring in Belgium and the Netherlands during this clinical trial must be reported by the local Principal Investigator within 2 working days after becoming aware of the SAE according to the process as described below :
SAE's occurring within a period of 30 days following the last intake of trial medication will also be handled as such if spontaneously reported to the investigator.

Collection of complete information concerning SAEs is extremely important. If only limited information is initially available, follow-up reports are required. Thus, follow-up information which becomes available as the SAE evolves, as well as supporting documentation (e.g., hospital discharge summaries and autopsy reports), should be collected subsequently. For ongoing SAEs a follow-up report should be sent at least once-monthly. The National Coordinating Investigator is responsible for submitting these follow-up reports for all SAEs, until the SAE has resolved or until the patient's condition stabilizes (in the case of persistent impairment), or the patient dies. A SAE Follow up form can be used.

The local Principal Investigator (in Belgium and the Netherlands) should report all events simultaneously and as soon as possible, at least within 2 working days after becoming aware of the SAE to :

- The National Coordinating Investigators of the Netherlands and Belgium
- Trial Bureau of the Ghent University Hospital
- DCOG Trial Office

SAE reporting by FAX:
The first report of a serious adverse event should be made by facsimile (FAX).

Contact details of the DCOG Trial Office The Hague:
e-mail: trialbureau@skion.nl
tel.: +31 70 367 45 45
fax: +31 70 359 90 63

Contact details of the National Coordinating Investigator for the Netherlands:
e-mail: e.de.bont@bkk.umcg.nl
tel.: +31 50 361 42 13
fax: +31 50 361 42 35

Contact details of the Trial Bureau of the Ghent University Hospital:
e-mail: Trialbureau@uzgent.be
tel.: +32 9 332 05 00
fax: +32 9 332 05 20

Contact details of the National Coordinating Investigator for Belgium:
e-mail: Barbara.demoerloose@ugent.be
Tel.: +32 9 332 64 17
Fax: +32 9 332 34 48

Causality:
The causality of the relationship with the treatment to the SAEs will be assessed by the National Coordinating Investigator of the country where the SAE occurred, in order to determine whether the reported SAE should be handled as SAE or as SUSAR. The expectedness of the SAE should be assessed with regard to the valid Investigator's Brochure (IB) or SmPC (Summary of Product Characteristics).

→ In case the National Coordinating Investigator determines the SAE is NOT a SUSAR:

For SAE's occurring in Belgium :
It is the responsibility of the National Coordinating Investigator of Belgium to report the SAE's simultaneously and within 2 working days after receiving the report to:

- the Central EC in Belgium
- the Trial Bureau of the Ghent University Hospital and the DCOG Trial Office
- all the local Principal Investigators of Belgium

Where required by local regulations, the local Principal investigator is responsible for reporting the SAE to his/her own local Ethics Committee.

For SAE's occurring in the Netherlands :
It is the responsibility of the National Coordinating Investigator of the Netherlands to report the SAE's simultaneously and within 2 working days after receiving the report to:

- all local Principal Investigators in the Netherlands,
- the Central EC in the Netherlands and the Competent Authority (CA) as defined in the Dutch national legislation
- the DCOG Trial Office and the Trial Bureau of the Ghent University Hospital

→ In case the National Coordinating Investigator determines the SAE is a SUSAR:

In addition to the SAE reporting procedure described above, following reporting requirements will be fulfilled in case the National Coordinating Investigator decides the SAE is a SUSAR (Suspected Unexpected Serious Adverse Reaction):

For a SUSAR occurring in Belgium:
In case the National Coordinating Investigator of Belgium decides the SAE is a SUSAR :
- The Trial Bureau of the Ghent University Hospital will report the SUSAR simultaneously and within the timelines as defined in the national legislation of Belgium to:
    - the Central EC and the CA in Belgium.

- Simultaneously, the National Coordinating Investigator of Belgium reports the SUSAR to:
    - all local Principal Investigators in Belgium
    - to the National Coordinating Investigator of the Netherlands and DCOG Trial Office

- It is the responsibility of the DCOG Trial Office to report the SUSAR simultaneously and within the timelines as defined in the national legislation of the Netherlands to:
    - all local Principal Investigators in the Netherlands
    - the METC and the CA as defined in the Dutch national legislation.

In case of a life-threatening SUSAR the entire reporting process must be completed within 7 calendar days.
In case of a non life-threatening SUSAR the reporting process must completed within 15 calendar days.

For a SUSAR occurring in the Netherlands:
In case the National Coordinating Investigator of the Netherlands decides the SAE is a SUSAR :
- The DCOG Trial Office will report the SUSAR simultaneously and within the timelines as defined in the national legislation of the Netherlands to :

- the METC and the CA in the Netherlands.
- All local Principal Investigators in the Netherlands
- The National Coordinating Investigator of Belgium and the Trial Bureau of the Ghent University Hospital

- It is the responsibility of the Trial Bureau of the Ghent University Hospital and the National Coordinating Investigator of Belgium to report the SUSAR simultaneously and within the timelines as defined in the national legislation of Belgium to:

- all local Principal Investigators in Belgium
- the Central EC in Belgium.

In case of a life-threatening SUSAR the entire reporting process must be completed within 7 calendar days.
In case of a non life-threatening SUSAR the reporting process must completed within 15 calendar days.

Upon receiving a SUSAR notification, all investigators need to inform their study team. The SUSAR report will be retained in the Investigator's Brochure.
Where required by local regulations, the local principal investigator submits the SUSAR report to his local Ethics Committee.
The DCOG Trial Office will report a SUSAR report, coming from Belgium, to the METC.
The Trial Bureau UZ Gent will report a SUSAR report, coming from the Netherlands, to their central Ethics Committee.

Annual Safety Reporting
The DCOG will be responsible for the Annual Safety Reporting as the Sponsor of the DB AML-01

protocol. The DCOG Trial Office will provide the National Coordinating Investigators for Belgium and the

Netherlands an annual report containing an overview of all SAE's and SSARs (Suspected Serious Adverse

Reaction) and a summary regarding the safety of the trial subjects. This report will also be send to the Data

Safety Monitoring Board.

The DCOG Trial Office will send this report to the Central EC in the Netherlands within the timelines as

defined in the Dutch national legislation.

The Trial Bureau of the Ghent University Hospital will send this report to the Central EC and the CA in

Belgium within the timelines as defined in the Belgian national legislation.

The National Coordinating Investigators for Belgium and the Netherlands will pass this annual report to all local Principal Investigators in Belgium and the Netherlands

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Bijlagen behorende bij
Dutch- Belgian Pediatric AML protocol

voor kinderen met nieuw gediagnosticeerd
acute myeloïde leukemie

gebaseerd op de NOPHO-AML 2004 studie

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Inhoud Nederlandse Bijlagen **Pagina**

CRF’s van de verschillende behandel fasen volgen nog

Bijlage 1 Nederlandse samenvatting:

Introductie en rationale:
Acute myeloid leukemie (AML) is een sporadische ziekte bij kinderen. In Nederland en België zullen ongeveer 30-35 kinderen met AML worden gediagnosticeerd elk jaar (leeftijden 0-18). Complete remissie (CR), het verdwijnen van de leukemiecellen kan in 85-90% van kinderen worden bereikt. Nochtans, is het totale overlevingstarief van vijf jaar (OS) 50-60% toe te schrijven aan een hoge recidief frequentie, vooral tijdens het eerste en tweede jaar na diagnose. De resultaten van het recentste NOPHO (samenwerkingsverband van scandinavische landen) protocol 1993 waren voor overal survival (OS) bij vijf jaar 65% en voor event vrije overleving (gebeurtenis vrije overleving) van vijf jaar (EFS) 48%, gecombineerd met een CR percentage van 92% (1). Deze resultaten behoren tot de beste in Europa (2). De NOPHO uitkomsten zijn vergelijkbaar met die van de andere pediatrische studiegroepen zoals de BFM (samenwerkingsverband van duitsland, oostenrijk) en MRC (engeland). Gezien het hoge CR percentage, moet er aandacht zijn om recidieven te voorkomen. Het blijkt dat het verhogen van de cumulatieve dosis ARA-C het recidief risico verlaagt, terwijl het percentage CR niet verder wordt verhoogd (3; 4). De backbone van de NOPHO is gecentreerd rond hoge cumulatieve dosis ARA-C tijdens consolidatie. Dit kan het succes van de NOPHO protocollen mede verklaren.

In het algemeen is er in de loop van de afgelopen twintig jaar een belangrijke verbetering van therapeutisch resultaat toe te schrijven aan de intensivering van behandeling gebaseerd op hoge cumulatieve dosis van cytarabine-arabinoside en anthracyclins tijdens inductie en consolidatie. Eerdere studies vestigden de cardiotoxische drempeldosis op 550 mg/m2 in volwassenen. In kinderen zijn er aanwijzingen dat lagere dosissen anthracyclins ook cardiovasculaire dysfunctie zonder duidelijke symptomen en klinisch significante cardiomyopathie kunnen veroorzaken (5). Echter vrij beperkte gegevens zijn beschikbaar van studies betreffende cardiovasculaire status van overlevenden meer dan tien tot vijftien jaar na voltooiing van therapie (6-8). De nu verkrijgbare studies tonen progressieve cardiovasculaire dysfunctie in tijd voor anthracyclindosissen meer dan 300 mg/m2. Alles bij elkaar hebben de beschikbare resultaten tot nu toe ons bewust gemaakt van mogelijke hartschade in aanstaande overlevenden op lange termijn na behandeling. De huidige Nopho-AML 2004 studie gebruikt nog anthracyclin dosissen van 450 mg/m2. Op basis van de bevindingen tot 2009 willen wij de cumulatieve dosis anthracyclins tot 330 mg/m2 in dit nieuwe studieprotocol beperken. In internationale studies hebben twee samenwerkingsgroepen (BFM en MRC) identieke goede resultaten getoond wanneer het aantal kuren tot vier of vijf wordt verminderd. Het originele protocol van Nopho-AML 2004 geeft nog zes intensieve kuren. Om de cumulatieve anthracycline dosis te beperken met behoud van hoge cumulatieve dosissen ARA-C, beslisten wij kuur met de meeste complicaties over te slaan. Deze laatstgenoemde kuur resulteerde vaak in een vertraging in behandeling. Dit studieprotocol stelt vijf intensieve chemotherapeutische kuren voor.
De rol van allogeneic stamceltransplantatie (SCT) is controversieel. Lange tijd was het de gewoonte om allogene SCT aan te bieden aan patiënten met AML en een HLA-Identieke sibling beenmerg donor. De updates van de grotere internationale samenwerkende studiegroepen hebben geen significant voordeel voor sibling-SCT in standaardrisico of hoog risico patienten groepen getoond. Omdat de uitkomsten met efficiëntere chemotherapie zijn verbeterd, is er meer terughoudendheid ten opzichte van allogeneic SCT in patiënten met AML in verscheidene studiegroepen en ook door ons. Allogeneic SCT in eerste CR wordt niet geadviseerd in dit studieprotocol. Wij maximaliseren onze inspanningen om de studie betreffende randomisatie van post-consolidatie Mylotarg later in 2010 te kunnen openen. Aangezien we niet weten of deze wijzigingen effect zullen hebben op het aantal recidieven (dat circa 40% in de NOPHO studie benadert) zijn strikte richtlijnen afgesproken om de veiligheid van patienten te waarborgen.

Doelstelling:
Het doel is te onderzoeken of deze voorgestelde behandeling een recidief percentage van 40% of minder heeft en minder toxiciteit. Alle patiënten in Nederland en België komen voor deze studie in aanmerking. De resultaten zullen bestudeerd worden in samenspraak met de NOPHO groep.

Inclusie:
Patiënten met een nieuw gediagnosticeerde AML, in de leeftijd van 0 tot18 jaar.

Exclusie:
Patiënten met het syndroom van Down en een nieuw gediagnosticeerde AML gekenmerkt door een GATA1 afwijking en patiënten met andere myeloproliferatieve beelden die niet een AML zijn.

Risicogroepen:
In deze trial worden patiënten ingedeeld in standaard risico groep als de leukemische cellen in het beenmerg snel verdwijnen en in hoog risico als dit langer duurt. (definitie hoogrisico: nog 15% blasten op dag 15 in het beenmerg).

Behandeling:
De behandeling bestaat uit 5 intensieve kuren chemotherapie die elkaar gemiddeld iedere 3 a 4 weken zullen opvolgen.

Aanvullend Onderzoek:
Het aanvullend onderzoek is wetenschappelijk onderzoek om de toekomst voor het kind met een AML te verbeteren op het gebied van optimaliseren van therapie voor de individuele patiënt door de verdwijning van de leukemiecellen optimaal te willen meten, het vergroten van de kennis van de afwijkende gedragingen van de leukemiecel om van daaruit aanvullenden behandelingen te kunnen ontwikkelen, maar ook op het gebied van kwaliteit van leven zal onderzoek worden voorgesteld aan de desbetreffende patiënten.

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Bijlage 2 Guidelines for Cardiotoxicity Monitoring

Please use M-mode echo measurements according to the American Heart Association guidelines. Whenever possible the patient should be afebrile with a Hb of >9.0 g/dl (>5.5 mmol/l). An ECG should be monitored simultaneously with the M-mode echo. The blood pressure should be recorded either during the last part of the echocardiograph or immediately afterwards.

What to Measure:

Baseline echocardiogram	- apical 4 chamber view - apical 5 chamber view (with aorta) - short axis left ventricle (papillary muscle level) - short axis left ventricle (aortic valve/pulmonary artery level)
Baseline colour Doppler	- apical 4 chamber (mitral, tricuspid, aortic flows)
Pulsed Doppler	- aortic flow (measurement of Left Ventricular Ejection Time (LVET)) - mitral valve (tips of mitral valve leaflets)
M-mode	- left ventricle – parasternal axis

When to Measure:

If at all possible a baseline echocardiograph should be performed before the start of therapy, or within one week of the initiation of therapy. AML is a hematological emergency, treatment should not be postponed for any reason and AIET should be started as soon as the diagnosis is suspected. Ideally repeat Echo 1 week prior to each consolidation course and 3 months after the completion of therapy. Echocardiography should be repeated 12 months from diagnosis and at 3 yearly intervals thereafter. If the Fractional Shortening (FS) is <28% repeat annually.

Bijlage 3 Advies van Taak- en disciplinegroepen

3.1 Taakgroep Supportive Care

Protocol ondersteunende maatregelen bij kinderoncologische behandeling

Inleiding
De behandeling van maligniteiten vergt een aantal maatregelen in de ondersteunende behandeling. Deze worden ingegeven door de betreffende medicatie, de toedieningsweg, toedieningsperiode en de dosering. Bij radiotherapeutische behandeling zijn het bestralingveld, volume, de dosis en de fractionering bepalend voor de noodzakelijke ondersteunende therapie. De basale preventieve ondersteunende maatregelen treft u navolgend aan.
Een deel van de maatregelen is niet gerelateerd aan een specifiek onderdeel van de behandeling maar geldt als ondersteunend in algemene zin. Deze zijn het laatste onderdeel van deze paragraaf.
Uiteraard bestaat een breed spectrum aan bijwerkingen en complicaties van elk betreffend medicament. Deze zijn onder andere terug te vinden in het Farmacotherapeutisch Kompas en kinderoncologische handboeken. Overigens wordt verwezen naar het werkboek “Ondersteunende behandeling in de kinderoncologie”, onder redactie van W.A. Kamps, M.C. Naafs-Wilstra, A.Y.N. Schouten-van Meeteren en W.J.E. Tissing, eindredactie C.M.F. Kneepkens.

Cytostaticum

Potentiële bijwerking **Symptomen / Therapie**

Anthracyclines: Daunorubicine / doxorubicine / Idarubicine / mitoxantrone

Cardiotoxiciteit

Echografie hartcontractiliteit voor aanvang anthracyclines.
Echocardiografie volgens schema:

Naam	Max. totaal cumulatieve dosis	Dosis waarboven standaard echocardiografie
Idarubicine	125 mg/m^2	60 mg/m^2
Mitoxantrone	160 mg/m^2	60 mg/m^2

Voor de cumulatieve doseringen van anthracycline- combinaties zijn nog geen specifieke afspraken over standaard echocardiografie

Bij shortening fraction < 28% of > 10% reductie: overweeg dosisaanpassing / staken van anthracycline toediening

Emesis — Anti-emeticum 5HT$_3$-antagonist

Extravasatie — In verband met ernstige lokale necrose bij extravasatie wordt een centraal veneuze catheter aanbevolen. Koeling met ijskompressen, lokale applikatie van 99% DMSO, raadpleeg (plastisch) chirurg

Potentiële bijwerking	**Symptomen / Therapie**
Cytosine arabinoside, lage dosis < 1000 mg / m^2 / kuur	Geen aanvullende maatregelen
Cytosine arabinoside, hoge dosis > 1000 mg / m^2 / kuur	
Hydratie	2,5 l/m^2/dag
Emesis	Anti-emeticum $5HT_3$-antagonist
Keratitis/ conjunctivitis	Oogdruppels corticosteroïden 4 dd tijdens kuur
Infectie Streptococ vir	Profylaxe pheneticilline 50 mg / kg in 3 dd tot na herstel uit neutropenie. In geval van peni-resistente streptococ in keelkweek claritromycine overwegen.
CZS & mucosa	In tegenstelling tot voorgaande protocollen wordt geen pyridoxine meer geadviseerd aangezien er geen evidence is dat dit profylactisch werkt.
Extravasatie	Geen maatregelen nodig
Etoposide	
Matig oplosbaar	Concentratie maximaal 0,4 mg/ml
Allergeen Cave hypotensie of allergische reactie	Controle pols, bloeddruk voor en tijdens infusie In geval van allergische reactie: –> onderbreek infusie –> hervat bij herstel op lagere snelheid Eventueel vooraf antihistaminicum, hydrocortison
Extravasatie	Geen maatregelen nodig
Intrathecale medicatie	
Pijn / belasting	Adequate sedatie en analgesie
Emesis	Anti-emeticum $5HT_3$-antagonist
Liquorverdeling	Voor verspreiding cytostaticum in liquor 4 uur platliggen na toediening
Risico toediening foutieve medicatie onjuiste medicatie	 gebruik 3-weg kranenblok-toedieningssysteem geen andere medicatie in de behandelruimte voor de lumbaal punctie
6-Thioguanine	
Individuele gevoeligheid	Controleer TPMT deficientie igv sterke aplasie
Toediening	Avonddosis op 1 uur nuchtere maag Niet innemen met melkproducten
Leucopenie	Met name voor onderhoudstherapie 6-mercaptopurine, voorkom te hoge / lage leucocytenwaarden: streefwaarden volgens protocol
Veno- occlusive disease (VOD)	Verhoogde kans op ontstaan van een VOD. Trias pijnlijk vergrote lever, vochtretentie en icterus.

Potentiële bijwerking	**Symptomen / Therapie**
Algemene maatregelen	
Emesis	Indien 5 HT_3-antagonist ontoereikend is, overweeg dexamethason 10 mg/m^2 in 3 dd en toevoeging van lorazepam.
Pneumocysitis infectie	Cotrimoxazol profylaxe 3 dagen/week, 3/15 mg/kg 1dd gift op 3 aaneengesloten dagen.
Transfusies	bestraalde bloedtransfusieprodukten bij lymfopenie < 500.10^6/l, tot 6 maanden na totaal lichaamsbestraling.
Infertiliteit	Semenpreservatie in geval van beenmergtransplantatie, bij voorkeur voor aanvang van de therapie
Teratogeniciteit	De meeste chemotherapeutica zijn (potentieel) teratogeen. Bij oudere kinderen is het daarom soms zinvol hiervoor te waarschuwen en anticonceptive maatregelen te nemen.
Infectie profylaxe	Overweeg SDD en schimmel / gist profylaxe (itraconazol suspensie) gezien de zeer langdurige neutropenie. Het is aan te bevelen de patiënt bij diagnose te screenen voor infectie foci (KNO gebied, tanden ed) en zo nodig te behandelen
Neutropenie en koorts	Start breed spectrum antibiotica indien de temperatuur een aantal uur achtereen > 38,5 °C is.
Beenmergtransplantatie	Na de beenmergtransplantatie zijn aanvullende supportive care maatregelen nodig. Deze verschillen per soort transplantaat e.d. en zullen dus per patiënt verschillen.
Voeding	Gezien de aard van de behandeling is het te verwachten dat een aanzienlijk deel van de patiënten voedingsproblemen krijgt. Vroegtijdige interventie d.m.v. sondevoeding / parenterale voeding is aangewezen, waarbij enterale voeding de voorkeur heeft.
Mucositis	Verhoogde kans bij hoge dosis ARA-C. Therapie: ondersteunend, adequate pijnstilling.

Potentiele problemen tijdens de inductie therapie

Potentiële bijwerking	**Symptomen / Therapie**
Hyperleucocytose	Hyperleucocytose is een risicofactor bij diagnose ivm verhoogde bloedingsneiging danwel leucostasis. Totdat de leucocyten onder 50 x 10^9/ l zijn gedaald, dient men terughoudend te zijn met erythrocyten transfusies (in ieder geval niet als Hb>5). In zeldzame gevallen kan een wisseltransfusie overwogen worden bij een zeer hoog leucocyten aantal
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 > 100 x 10^9/ l (of een urinezuur > 0.45 mmol/l). Naast bovengenoemde moet men beducht zijn op het ontstaan van een hyperkaliaemie, hypocalciaemie of een hyperfosfataemie. In die gevallen dienen specifieke maatregelen genomen te worden.

.

3.2 Taakgroep Late Effecten

Richtlijnen betreffende de zorg voor 5-jaars overlevenden van Kinderkanker (Richtlijn SKION LATER) zijn vanaf eind 2009 beschikbaar via www.SKION.nl. Deze richtlijnen zijn met financiële ondersteuning van Zonmw ontwikkeld in een samenwerkingsverband van de 7 kinderoncologische centra in Nederland. Op basis van de oncologische behandeling en de hiermee samenhangende potentiële late schadelijke gevolgen wordt aangegeven welke zorg deze kinderen en volwassenen minimaal nodig hebben. Voor de zorg tussen het einde van de behandeling en 5 jaar na diagnose is nog geen richtlijn beschikbaar. In afwachting van de ontwikkeling van een richtlijn voor deze periode kan voorlopig de SKION LATER richtlijn dienen als uitgangspunt voor de zorg.

Behandeling bij kinderen met acute myeloïde leukemie volgens Dutch-Belgian pediatric AML protocol voor pediatrische AML.

Informatie voor ouders / verzorgers

Inleiding

Bij uw kind is onlangs een vorm van bloedkanker, acute myeloïde leukemie (AML), vastgesteld. Om de behandelingsmogelijkheden voor kinderen met kanker te blijven verbeteren en verder te ontwikkelen, wordt wetenschappelijk onderzoek gedaan. De behandelend arts van uw kind heeft u en uw kind geïnformeerd over bovengenoemde behandeling. Hij/zij heeft u al het één en ander uitgelegd. Voor toestemming of weigering is goede voorlichting van onze kant nodig en een zorgvuldige afweging van uw kant. 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 waarna ze losgelaten worden 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. Leukos betekent wit. Omdat bloedkanker voor het eerst ontdekt werd in de witte bloedcellen (en omdat bloedkanker het meest voorkomt in de witte bloedcellen) wordt bloedkanker ook leukemie genoemd. 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 derhalve ook lymfatische leukemie.
- Granulocyten: deze witte bloedcellen spelen ook een belangrijke rol bij de afweer, m.n. voor het opeten van m.n. bacteriën.
- Monocyten: deze grote witte bloedcellen spelen ook een belangrijke rol bij de afweer, m.n. voor het opeten van bacteriën en virussen.
- Te weinig witte bloedcellen leiden tot een verminderde afweer tegen infecties en dus vaak tot het krijgen van infecties met koorts.
- Bloedplaatjes (trombocyten): deze zijn belangrijk voor de bloedstolling. Te weinig trombocyten leiden dus tot bloedingen. Dit uit zich in bijvoorbeeld bloedneuzen of spontane of ongewoon grote blauwe plekken op plaatsen waar deze normaal gesproken zelden ontstaan of zeer kleine zogeheten puntbloedingen in de huid of het slijmvlies van de mond.

Bij **acute myeloïde leukemie** is een bepaald soort witte bloedcel ziekte. De zieke (kwaadaardige) bloedcellen heten **blasten**. 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. De blasten hebben geen functie, maar ze staan gezonde bloedcellen in de weg.

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

Tijdens de behandeling vinden bloedafnames en infuustherapie plaats. De therapie kan ervoor zorgen dat uw kind minder makkelijk te prikken wordt. 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 moet worden aangeprikt, of een broviac-katheter, 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. Dit zijn systemen om de toediening van medicijnen en het afnemen van bloed te vergemakkelijken.

Behandeling
De kinderarts heeft u een behandeling met medicijnen voorgesteld volgens het behandelprotocol Dutch-Belgian pediactric AML protocol, dat is vastgesteld door het bestuur van de Stichting Kinderoncologie Nederland (SKION). In de SKION werken alle Nederlandse kinderartsen die kinderen met kwaadaardige ziekten behandelen samen. Bij de behandeling van AML op de kinder- en adolescenten leeftijd werken de Nederlandse kinderartsen samen met hun collega's uit België en de collega's van de NOPHO. De NOPHO is een groep samenwerkende artsen uit het noorden van Europa te weten Denemarken, Finland, Zweden, IJsland en Groenland. Hun resultaten behoren al langere tijd binnen Europa tot een van de beste.

De **huidige behandeling** voor AML bij kinderen in Nederland is gebaseerd op de eerder verkregen resultaten van de AML protocollen in de NOPHO. De laatste studie NOPHO-AML-2004 van de NOPHO groep bestaat uit 6 intensieve chemotherapie kuren. Een van die 6 kuren gaf veel bijwerkingen op korte en lange termijn. Door die bijwerkingen moest het geven van de volgende kuur vaak uitgesteld worden. Van één van de middelen, de zogenaamde anthracyclines, was de dosering hoog gemiddeld te noemen. Van dit type middel weten we ondertussen dat dit ernstige schade aan de hartspier kan geven bij het ouder worden. De laatste kennis anno 2009 maakt dat we het aantal kuren hebben teruggebracht tot 5 intensieve kuren. De kuur met de meeste bijwerkingen wordt niet gegeven. De totale dosering anthracyclines is op deze manier teruggebracht tot laag gemiddeld. De kuren worden aangeduid met de beginletters van de toe te dienen geneesmiddelen: AIET, AM, HA_2E, HA_3, en HA_2E.
Het geven van een stamcel transplantatie wordt uitgesteld tot het moment dat de ziekte niet onder controle komt na 2 inductie kuren chemotherapie (ofwel: geen complete remissie wordt bereikt) of dat er een recidief ontstaat na een aanvankelijke goede reactie op de behandeling.

Leukemiecellen bevinden zich vooral in het beenmerg en in het bloed, maar de ziekte kan zich ook uitbreiden naar de vliezen rond de hersenen en het ruggenmerg; soms worden er leukemiecellen in het hersenvocht (de liquor) aangetroffen. Bij de chemotherapiekuren wordt daarom ook een aantal keren cytostatica 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 nog acht keer een beenmergpunctie verricht om beenmerg voor onderzoek af te nemen. Dat is nodig om te beoordelen of de behandeling het gewenste resultaat heeft. De artsen zullen in overleg met u zorgen voor een zo goed mogelijke pijnbestrijding tijdens de beenmergpuncties en lumbaalpuncties.

Bijwerkingen
De chemotherapie bestaat uit vijf opeenvolgende kuren met combinaties van geneesmiddelen, die de groei en de vermeerdering van kwaadaardige cellen stoppen. Het is een intensieve behandeling, die helaas vaak gepaard gaat met ernstige bijwerkingen. 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.
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.

Het vastleggen van behandeluitkomsten waarvoor uw goedkeuring wordt gevraagd
Dankzij onderzoek in het verleden is het percentage genezen kinderen met AML in de loop van enkele decennia gestegen van 0% tot ruim 60%. Het is van groot belang onderzoek te blijven doen om een verdere verbetering van deze behandelingsresultaten te bereiken. De gegevens van uw kind worden geregistreerd door de SKION om zo de kwaliteit van de behandeling te kunnen controleren. Deze registratie van het verloop van de ziekte gaat door na afloop van het onderzoek.
Met betrekking tot de behandeling volgens dit protocol wordt gekeken naar de volgende punten die uit het medisch dossier zullen worden gehaald.

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. Bijwerkingen:
   De mogelijke bijwerkingen worden geregistreerd aan de hand van bloeduitslagen en de behandelend arts zal uw kind regelmatig op mogelijke bijwerkingen controleren. Dit gebeurt aan de hand van lichamelijk onderzoek en de eigen bevindingen van u of uw kind tijdens het gebruik van de medicatie.
3. Verzamelen van gegevens:
   Alle gegevens over het verloop van de behandeling bij uw kind worden verzameld en gecodeerd opgeslagen in een database. Deze database bevat alle gegevens van alle kinderoncologische centra in Nederland t.a.v. dit behandelprotocol. De resultaten zullen uiteindelijk wetenschappelijk beoordeeld worden.

Het wetenschappelijk onderzoek waarvoor uw deelname wordt gevraagd

4. Opslag van bloed, beenmerg en liquor
   Overgebleven bloed, beenmerg en liquor dat gedurende deze periode is afgenomen wordt langdurig (tientallen jaren) op het laboratorium bewaard. Reden hiervoor is dat voor de verbetering van de behandeling van een zeldzame ziekte als AML 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.
5. Aanvullend wetenschappelijk onderzoek naar de kwaliteit van leven, slaap en vermoeidheid
   Onderzoek naar het effect van de behandeling op de kwaliteit van leven, slaap en vermoeidheid. Door middel van het afnemen van vragenlijsten bij kinderen en hun ouders willen wij hier meer inzicht in krijgen, zodat er in de toekomst gerichter hulp of begeleiding geboden kan worden. De vragenlijsten worden door ouders en kinderen vanaf 8 jaar tussen de tijd vanaf diagnose tot 1 jaar na het einde van de behandeling viermaal ingevuld. Het invullen van de vragenlijsten zal per keer ongeveer 30 minuten duren voor ouders en 45 minuten voor kinderen.

Toestemming
Wat betekent mee doen voor U?

Uw kind heeft AML en gaan we behandelen volgens de behandelinzichten anno 2010. Voordat we met de behandeling beginnen vragen wij uw goedkeuring om de gegevens over het verloop van de behandeling te mogen gebruiken voor het vastleggen van de behandeluitkomsten.

Voor het onderzoek verzamelen wij algemene patiënt- en behandelgegevens. Alle gegevens van uw kind zullen zorgvuldig en vertrouwelijk worden behandeld. Inzage in de oorspronkelijke medische dossiers is slechts voorbehouden aan daartoe geautoriseerde en gekwalificeerde medewerkers van het behandelende ziekenhuis. Deze gecodeerde en niet tot de persoon herleidbare formulieren zullen worden opgestuurd naar het trialbureau van de SKION. De onderzoeksgegevens kunnen worden gecontroleerd door de SKION en toezichthoudende instanties als bijvoorbeeld de Inspectie voor de Gezondheidszorg. De onderzoeksgegevens kunnen dan worden vergeleken met gegevens uit het medische dossier van uw kind. Degene die deze controles uitvoeren hebben allen een geheimhoudingsplicht. De naam van uw kind zal nooit openbaar worden gemaakt.
Onderzoeksgegevens zullen worden gehanteerd met inachtneming van de Wet Bescherming Persoonsgegevens (WPB) en het privacyreglement van SKION. Zoals boven beschreven zullen alle medische gegevens en lichaamsmaterialen die worden verzameld van een uniek codenummer worden voorzien. Persoonsgegevens (zoals naam en adres) worden niet gebruikt in documentatie, rapporten of publicaties over het onderzoek. De uiteindelijke resultaten van onderzoek worden gerapporteerd in medisch-wetenschappelijke literatuur en/of op medische congressen.

Voor het wetenschappelijk onderzoek vragen wij uw deelname. Van de patiënten die aan dit onderzoek deelnemen zal overgebleven bloed, beenmerg en liquor worden bewaard voor onderzoek waarmee nieuwe behandelmogelijkheden kunnen worden ontwikkeld voor kinderen met Acute Myeloïde Leukemie. Tevens wordt uw deelname gevraagd aan het onderzoek naar Kwaliteit van Leven, slaap en Vermoeidheid.
Meedoen aan het wetenschappelijke onderzoek betekent dus **geen** extra bloed, beenmerg of liquor afnames voor uw kind. Het restmateriaal wordt na de nodige analyses voor de diagnose en behandeling opgeslagen in het laboratorium van de SKION volgens vaste regelgeving. De gegevens die in het kader van wetenschappelijk onderzoek uit restmateriaal worden verkregen zullen vertrouwelijk en anoniem worden behandeld en voorzien van een uniek codenummer. Persoonsgegevens (zoals naam en adres) worden ook niet gebruikt in documentatie, rapporten of publicaties over het onderzoek. De uiteindelijke resultaten van onderzoek worden gerapporteerd in medisch-wetenschappelijke literatuur en/of op medische congressen. De gegevens worden dus anoniem verwerkt.
De behandeling van uw kind is niet anders als u wel of niet meedoet aan het wetenschappelijk onderzoek. Het al dan niet meedoen heeft geen invloed op de zorg en aandacht die uw kind in het kader van zijn behandeling krijgt. Als u niet meedoet wordt uw kind te allen tijde behandeld met de grootst mogelijk zorg en toewijding waarmee we ieder kind behandelen.
Medisch Ethische Toetsing Commissie heeft dit beoordeeld; de zorgvuldigheid is hiermee gewaarborgd. De voor dit onderzoek geldende internationale richtlijnen zullen nauwkeurig in acht worden genomen.

Ook zal uw huisarts van de behandeling op de hoogte worden gebracht. Wanneer dat voor u een probleem is kunt u dat ter sprake brengen met uw behandelend kinderarts-oncoloog/hematoloog.

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. Wanneer uw kind meedoet, kunt u op ieder moment stoppen, zonder opgaaf van redenen.
Na ondertekening zal u een kopie van het ondertekende toestemmingsformulier worden meegegeven.

Slotopmerking
Hoewel de patiënten worden onderzocht en behandeld volgens een van te voren opgesteld plan, kunnen er zich in individuele gevallen omstandigheden voordoen, die een afwijking hiervan gewenst maken. In die gevallen zal het belang van de patiënt altijd worden gesteld boven het belang van het volgen van de behandelrichtlijn. De behandelende arts heeft te allen tijde de plicht aan de patiënt de beste medische zorg te verlenen.

Contactpersonen
Bij vragen of opmerkingen kunt u contact opnemen met uw behandelend kinderarts-oncoloog, bereikbaar via het secretariaat kinderoncologie/hematologie, tel. 050 - 3614213. Buiten normale werktijden is altijd een dienstdoende specialist bereikbaar.

Als u informatie of advies over het onderzoek wilt, kunt u een onafhankelijk arts raadplegen die niet zelf betrokken is bij dit onderzoek maar wel deskundig op dit gebied is: Prof. Dr. H. Kluin-Nelemans, afdeling Hematologie, tel: 050 - 3612354. Ook indien u voor of tijdens het onderzoek vragen heeft die u liever niet aan de onderzoekers stelt dan kunt u contact opnemen met deze onafhankelijke arts.

Neemt u de tijd om deze informatie door te spreken en aarzel niet uw behandelend arts te raadplegen als u vragen heeft. Wanneer u besluit deel te nemen ontvangt u een kopie van dit document, nadat u en uw behandelend arts beiden voor uw deelname getekend hebben.

Instemmingsverklaring betreffende behandeling volgens Dutch-Belgian pediatric AML protocol voor pediatrische AML.

Door dit toestemmingsformulier te tekenen verklaar ik het volgende:

- Ik ben goed geïnformeerd, zowel mondeling als schriftelijk. Ik heb de gelegenheid gehad vragen te stellen en heb de informatie goed begrepen.
- Ik heb begrepen dat deelname aan het onderzoek geheel vrijwillig is en dat ik te allen tijde verdere deelname kan weigeren, zonder dat dit gevolgen heeft voor de verdere behandeling van mijn kind of de relatie met de behandelend arts.
- Ik begrijp dat bevoegde personen, tijdens of na het onderzoek inzage kunnen hebben in het medisch dossier van mijn kind.
- Ik ben ervan op de hoogte dat mijn huisarts wordt geïnformeerd over de behandeling.

1. Ik geef **wel / geen *** toestemming voor behandeling van mijn kind volgens dit protocol.

2. Ik geef **wel / geen *** toestemming dat gegevens over het verloop van de behandeling van mijn kind worden opgeslagen en verwerkt ten behoeve van wetenschappelijk onderzoek, onder de voorwaarden zoals beschreven in de patiënteninformatiebrief.

3. Ik geef **wel / geen *** toestemming dat het diagnostisch materiaal dat bij SKION is opgeslagen kan worden gebruikt ten behoeve van wetenschappelijk onderzoek, onder de voorwaarden zoals beschreven in de patiënteninformatiebrief.

4. Ik geef **wel / geen *** toestemming voor deelname aan het aanvullend onderzoek met vragenlijsten over de kwaliteit van leven, slaap en vermoeidheid van mijn kind tijdens en na de behandeling.

5. Ik geef **wel / geen *** toestemming om ten behoeve van wetenschappelijk onderzoek de databestanden van SKION te combineren met gegevens die elders bekend zijn, bijvoorbeeld bij het Centraal Bureau voor de Statistiek, onder de voorwaarden zoals beschreven in de patiënteninformatiebrief.

(*) *s.v.p. doorhalen wat niet van toepassing is.*

Naam patiënt: _____

Naam ouder/voogd: _____

Handtekening en datum: _____

Naam ouder/voogd: _____

Handtekening en datum: _____

Naam behandelend arts: _____

Handtekening en datum: _____

Behandeling bij kinderen met acute myeloïde leukemie volgens Dutch-Belgian pediatric AML protocol voor pediatrische AML

Informatie voor kinderen vanaf 12 jaar

Inleiding

Jouw arts heeft je verteld dat je acute myeloïde leukemie (AML) hebt. Dat is een soort bloedkanker dat vooral bij volwassenen voorkomt, maar helaas soms ook bij kinderen. Wat je arts met je heeft besproken over je ziekte, staat nog eens in deze brief. Door deze brief te lezen begrijp je misschien beter wat er met je aan de hand is. Je kunt dan ook beter beslissen of je mee wil doen aan het wetenschappelijke onderzoek, dat later nuttig kan zijn voor andere kinderen met dezelfde ziekte als jij. Voor jouw behandeling maakt het geen verschil of je wel of niet meedoet met dit wetenschappelijk onderzoek. Jij krijgt altijd de best mogelijke behandeling.

Wat is acute myeloide leukemie?

Bij leukemie (bloedkanker) is het beenmerg ziek. Het beenmerg zit binnen in je botten en maakt bloedcellen, de bouwstenen van het bloed. De bloedcellen groeien op in het beenmerg en als ze klaar zijn voor hun taak (rijp zijn) gaan zij uit het beenmerg naar het bloed en met het bloed naar alle delen van je lichaam. Als je beenmerg niet goed werkt, wordt dus ook je bloed ziek. Omdat je dan vaak snel ziek wordt noemen we de leukemie **acute** leukemie.

Als je met een microscoop (een soort vergrootglas) naar het bloed kijkt, zie je drie soorten cellen: rode bloedcellen, witte bloedcellen en bloedplaatjes.

- **Rode bloedcellen (erythrocyten, ery's)** maken dat je bloed rood is. De rode kleurstof (hemoglobine, Hb) in je rode bloedcellen brengt zuurstof uit je longen naar alle delen van je lichaam. Als je leukemie hebt, zijn er te weinig rode bloedcellen en krijg je bloedarmoede (anemie). Je bent dan bleek en moe.

- **Witte bloedcellen (leukocyten, leuko's)** zorgen er voor dat je geen ontsteking of infectie krijgt, of anders er zo snel mogelijk weer van geneest. Er zijn verschillende soorten witte bloedcellen, die allemaal op hun eigen manier helpen bij de bestrijding van infecties.

- **Bloedplaatjes (trombocyten, trombo's)** zorgen er voor dat bij een wondje het bloeden stopt en er een korstje op komt. Bij leukemie zijn er te weinig bloedplaatjes en daardoor duurt een bloeding langer. Soms krijg je een bloedneus of een grote blauwe plek.

Bij **acute myeloïde leukemie** is een bepaald soort witte bloedcel ziek. De zieke (kwaadaardige) bloedcellen heten **blasten**. 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 je lichaam, in je bloed, lever, milt, lymfeklieren, enz. De blasten kunnen zelf niks, maar ze staan gezonde bloedcellen in de weg.

Als je leukemie hebt, voel je je niet prettig. Je bent moe en ziet bleek, je hebt vaak koorts en ontstekingen, je krijgt vlug blauwe plekken, je botten doen zeer en het lopen doet pijn. Je lever, milt of lymfeklieren kunnen opgezet zijn.

Diagnose
Om zeker te weten of je leukemie hebt, is je bloed en je beenmerg bekeken. Je behandelend arts heeft het beenmerg met een naald uit het binnenste van je bot bij je heupen gehaald (beenmergpunctie). Door je bloed en beenmerg in het laboratorium te onderzoeken is nu duidelijk geworden dat je acute myeloïde leukemie hebt. Door een lumbaalpunctie (LP, ruggenprik) is liquor (ruggenvocht/hersenvocht) afgenomen om te kijken of de blasten daar ook in zitten. Dit is belangrijk om je zo goed mogelijk te kunnen behandelen.

Tijdens de behandeling wordt je regelmatig geprikt voor bloedafname of een infuus. De therapie kan ervoor zorgen dat je moeilijker te prikken wordt. 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 moet worden aangeprikt, of een broviac-katheter, die door de huid naar buiten komt. De kinderoncoloog bespreekt met je of dit nodig is en welk systeem voor jou het beste is. Deze systemen zijn bedoeld om de toediening van medicijnen en het afnemen van bloed makkelijker te maken.

Behandeling
Kinderen met **acute myeloïde** leukemie worden overal in Nederland behandeld volgens één behandelplan,. Zo'n behandelplan is een boek waarin staat hoe een bepaalde ziekte behandeld moet worden. Artsen in Nederland die kinderen zoals jij behandelen, werken samen in de Stichting Kinderoncologie Nederland (SKION) in Den Haag. Ze hebben met elkaar afgesproken hoe leukemie het beste behandeld kan worden.

Leukemie wordt behandeld door medicijnen te geven tegen de onrijpe cellen (blasten). Deze medicijnen heten cytostatica. De behandeling bestaat uit vier kuren met cytostatica, die meestal via een infuus in het bloed worden toegediend. Zo'n behandeling met cytostatica heet chemotherapie. Elke kuur met cytostatica wordt in het ziekenhuis gegeven en ook na een kuur moet je soms nog een poos in het ziekenhuis blijven. Om te kunnen genezen moeten uiteindelijk alle leukemiecellen worden opgeruimd.

Een paar weken na de eerste kuur, als je genoeg bent opgeknapt, zal je arts weer een beenmergpunctie en een lumbaalpunctie doen om te kijken of al veel van de blasten door de cytostatica zijn opgeruimd (of een **remissie** is bereikt). Ook later wordt af en toe een beenmergpunctie en een lumbaalpunctie gedaan om te kijken hoe het met je gaat.

Bijwerkingen
De behandeling met cytostatica werkt niet alleen tegen de blasten. Ook gezonde cellen worden aangetast. Daardoor kun je je ziek voelen van de behandeling: misselijk, geen zin in eten. Soms moet je overgeven. Verder moet je er op rekenen dat je haar zal uitvallen. Ook kan je snel infecties krijgen en dan heb je medicijnen (antibiotica) nodig om die te bestrijden. Als je te weinig rode bloedlichaampje hebt (bloedarmoede) of als je vaak bloedingen hebt, krijg je wel eens een bloedtransfusie. De meeste vervelende bijwerkingen van de chemotherapie gaan weer weg als de behandeling helemaal klaar is, maar sommige gevolgen van de ziekte en de behandeling kunnen ook langer blijven bestaan. Je arts kan je daar nog wel meer over vertellen.

Evaluatie van de behandeling
Met betrekking tot de behandeling volgens dit protocol wordt gekeken naar de volgende punten:

1. **Werking**
   Als de leukemie in 'remissie' is zijn er nog maar zo weinig blasten in het beenmerg over, dat je ze met een microscoop niet meer kunt zien. De artsen zijn nu bezig om een nieuwe manier te vinden om in het laboratorium deze heel kleine aantallen blasten toch te vinden. Misschien kunnen ze dan in de toekomst beter voorspellen bij welke kinderen de zieke cellen altijd wegblijven en bij welke kinderen ze misschien terugkomen. Als jij het goed vindt, wordt bij elke beenmergpunctie een beetje extra beenmerg en ook wat bloed afgenomen. Je hoeft daar niet extra voor geprikt te worden.

2. **Verdraagzaamheid**
   De artsen willen ook graag meer weten over de verschillen tussen de leukemiecellen bij de ene patiënt en de andere. Met heel nieuwe technieken in het laboratorium kan dat. Maar we weten nog niet hoe we die informatie kunnen gebruiken voor de behandeling. Daarom willen we, als jij het goed vindt, graag wat beenmerg, bloed en liquor dat bij jou is afgenomen, gebruiken voor wetenschappelijk onderzoek in het laboratorium. Je hoeft daar niet extra voor geprikt te worden.

3. **Verzamelen van gegevens**
   We verzamelen alle gegevens over het verloop van de behandeling en slaan deze met een code (zonder je naam) op in een database. In deze database zitten alle gegevens van alle patiënten in de kinderoncologische centra in Nederland. De resultaten zullen uiteindelijk wetenschappelijk besproken worden.

4. **Opslag van bloed, beenmerg en hersenvocht**
   Bloed, beenmerg en liquor (hersenvocht) dat voor andere bepalingen al is afgenomen, willen we langdurig (tientallen jaren) op het laboratorium bewaren. Er zal namelijk altijd laboratorium onderzoek nodig blijven. AML bij kinderen is een zeldzame ziekte. Het doel van deze onderzoeken is het ontwikkelen van nieuwe behandelingen voor kinderen met leukemie.

Aanvullend onderzoek naar de kwaliteit van leven, slaap en vermoeidheid
Onderzoek naar het effect van de behandeling op hoe jij je voelt, hoe je slaapt en op vermoeidheid. Door meer te weten te komen over hoe het met je gaat, kunnen we later andere kinderen en hun ouders beter helpen. Voor dit onderzoek vragen we jou en je ouders om vier keer vragenlijsten in te vullen. Dat gebeurt tussen het moment van diagnose tot 1 jaar na het einde van de behandeling. Het invullen van de vragenlijsten duurt ongeveer 45 minuten per keer.

Toestemming
Omdat onderzoek naar de behandeling van kinderen met AML nodig is, vragen wij jou en je ouders toestemming voor de punten die hierboven geschreven zijn.

Als jij en je ouders nu of later besluiten om hier geen toestemming voor te geven, dan heeft dit geen invloed op de zorg en aandacht die je krijgt.

Als je ouder bent dan 12 jaar moeten jij en je ouders/verzorgers samen beslissen of je aan deze onderzoeken mee wilt doen. Zowel jij als je ouders/verzorgers moeten een handtekening zetten onder het toestemmingsformulier. Wanneer je niet dezelfde mening hebt als je ouders/verzorgers kun je dit bespreken met je behandelend arts.

We zullen je huisarts van deze behandeling op de hoogte brengen. Ook daar moet je toestemming voor geven.

Verantwoording / vertrouwelijkheid
Deelname aan het onderzoek horend bij deze behandelrichtlijn is vrijwillig. Voor het onderzoek verzamelen we algemene gegevens van jou (bijv. bloeduitslagen, röntgenonderzoek). Er worden geen extra onderzoeken gedaan.

Alle verzamelde gegevens worden zorgvuldig en vertrouwelijk behandeld. Onderzoeksgegevens van jou kunnen alleen door medewerkers van de SKION, het datamanagement centrum, worden ingezien, of door medewerkers van de Inspectie voor de Gezondheidszorg . Bij gebruik van onderzoeksgegevens houden wij ons aan de Wet Bescherming persoonsgegevens. Alle medische gegevens die tijdens deze studie worden verzameld zullen van een codenummer worden voorzien. Jouw persoonlijke gegevens (zoals je naam of adres) zullen niet gebruikt worden op studiedocumentatie, in rapporten of publicaties over dit onderzoek. De uiteindelijke resultaten van het onderzoek worden gerapporteerd in medisch-wetenschappelijke literatuur en/of op medische congressen.

Contactpersonen
Bij vragen of opmerkingen kan je contact opnemen met je behandelend arts-oncoloog, bereikbaar via het secretariaat kinderoncologie/hematologie, tel. 050 - 3614213.

Als je twijfelt over deelname kan je een onafhankelijk arts raadplegen die niet zelf betrokken is bij dit onderzoek maar wel deskundig op dit gebied is: Prof. dr. H. Kluin-Nelemans, tel. 050 - 3612354. Ook kan je voor of tijdens het onderzoek aan de onafhankelijke arts vragen stellen die je liever niet aan de onderzoekers wilt stellen.

Neem gerust de tijd om deze informatie door te spreken en aarzel niet je behandelend arts vragen te stellen. Wanneer je besluit deel te nemen krijg je een kopie van dit document, nadat jij en je behandelend arts beiden getekend hebben.

Instemmingsverklaring betreffende behandeling volgens Dutch-Belgian pediatric AML protocol voor pediatrische AML

Toestemmingsformulier voor patiënten van 12 jaar en ouder

Door dit toestemmingsformulier te tekenen verklaar ik het volgende:

- Ik ben goed geïnformeerd, zowel mondeling als schriftelijk. Ik heb de gelegenheid gehad vragen te stellen en heb de informatie goed begrepen.
- Ik heb begrepen dat ik door de onderzoeker op tijd op de hoogte wordt gesteld als er nieuwe informatie beschikbaar komt die van belang is.
- Ik heb begrepen dat deelname aan het onderzoek geheel vrijwillig is en dat ik te allen tijde verdere deelname kan weigeren, zonder dat dit gevolgen heeft voor mijn verdere behandeling of de relatie met mijn behandelend arts.
- Ik begrijp dat bevoegde personen, tijdens of na het onderzoek inzage kunnen hebben in mijn medisch dossier.
- Ik ben ervan op de hoogte dat mijn huisarts wordt geïnformeerd over de behandeling.

1. Ik geef **wel / geen *** toestemming voor behandeling volgens dit protocol.

2. Ik geef **wel / geen *** toestemming dat gegevens over het verloop van mijn behandeling worden opgeslagen en verwerkt ten behoeve van wetenschappelijk onderzoek, onder de voorwaarden zoals beschreven in de patiënteninformatiebrief.

3. Ik geef **wel / geen *** toestemming dat het diagnostisch materiaal dat bij SKION is opgeslagen kan worden gebruikt ten behoeve van wetenschappelijk onderzoek, onder de voorwaarden zoals beschreven in de patiënteninformatiebrief.

4. Ik geef **wel / geen *** toestemming voor deelname aan het aanvullend onderzoek met vragenlijsten over de kwaliteit van leven, slaap en vermoeidheid tijdens en na de behandeling.

5. Ik geef **wel / geen *** toestemming om ten behoeve van wetenschappelijk onderzoek de databestanden van SKION te combineren met gegevens die elders bekend zijn, bijvoorbeeld bij het Centraal Bureau voor de Statistiek, onder de voorwaarden zoals beschreven in de patiënteninformatiebrief.

(*): Svp doorhalen wat niet van toepassing is.

Naam patiënt: _____ Geboortedatum_____

Handtekening en datum: _____

Naam ouder/voogd: _____

Handtekening en datum: _____

Naam behandelend arts: _____

Handtekening en datum: _____

Bijlage 5 Diagnostiek SKION-Laboratorium

1) Cytologische diagnostiek (uitstrijkpreparaten)

Benodigd materiaal
Dit betreft uitstrijkpreparaten van bloed en beenmerg. In voorkomende gevallen kan ascites- en/of pleuravocht worden ingestuurd in de (heparine-)buizen van het SKION-haemoblok. Voorafgaande aan de behandeling worden 6 ongekleurde beenmerguitstrijkjes en 3 ongekleurde bloeduitstrijkjes zo snel mogelijk naar het laboratorium van de SKION gestuurd. De uitstrijkjes moeten zijn afgenomen vóór eventuele transfusie van bloed of bloedproducten .

Werkwijze/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 van de preparaten.
- 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.

Doel
Op de uitstrijkpreparaten wordt standaard een May-Grünwald-Giemsa kleuring gedaan voor het tellen van het percentage blasten. Voor het classificeren van de eventueel pathologische cellen worden tevens een Sudan-Black B en een Peroxidase kleuring gedaan. 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).

2) Immunologisch onderzoek ("Haemoblok")

Benodigd materiaal
Voor immunologisch onderzoek, op cellen in suspensie, dient te worden afgenomen:
Heparine beenmerg: 10-20 ml (2 heparinebuizen)
heparine bloed: 5-10 ml (1 heparinebuis)

Werkwijze
Hiervoor zijn in het zgn. **haemoblok** heparinebuizen aanwezig; na afname goed mengen om stolling te voorkomen. Deze heparinebuizen kunnen ook worden gebruikt voor verzending van pleura- en/of ascitesvocht. Wordt er voor meer doeleinden beenmerg afgenomen, dan zonodig een tweede beenmergpunctie op een andere plaats uitvoeren, om teveel bloedbijmenging te voorkomen. Bewaren en transporteren kan bij kamertemperatuur.

Doel
Immunofenotypering in suspensie, (bloed, beenmerg, ascites- en/of pleuravocht) geschiedt op het laboratorium van de SKION in meervoudige labeling en volgens de richtlijnen van de SKML (www.cyotmetrie.nl) in een gefaseerde aanpak. De volgende markers worden in elk geval gebruikt voor de screening:

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

3) Liquordiagnostiek

Benodigd materiaal
• Bij diagnose 2,5 ml liquor.
• Indien tijdens de behandeling blasten in de liquor gevonden worden, dient eveneens liquor naar het laboratorium van de SKION te worden gezonden.

Werkwijze
Bij de lumbaalpunctie wordt als 2de of 3de 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). Macroscopisch zichtbare bloedbijmenging maakt het stellen van de diagnose CZS uitbreiding/leukemie onmogelijk. Erythrocyten (> 30/3) in de cytospinpreparaten (microscopische bloedbijmenging), maken het resultaat onbetrouwbaar. In geval van dubieuze bevindingen bij het liquoronderzoek of bloedbijmenging wordt na 1 of enkele dagen opnieuw een liquormonster afgenomen en opgestuurd naar het laboratorium van de SKION.

4) Cytogenetisch onderzoek

Cytogenetisch onderzoek, waaronder ook moleculaire diagnostiek voor chromosomenonderzoek geschiedt in 8 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 neemt men telefonisch contact op met één van de betrokken personen en instituten. De uitslagen worden rechtstreeks aan de behandelend kinderarts toegestuurd. De SKION ontvangt eveneens de uitslag van het desbetreffende cytogenetisch laboratorium. Twee-jaarlijks worden deze uitslagen gereviewd door een panel van cytogenetici.

UITSLAGEN

De uitslagen worden bij diagnose telefonisch en schriftelijk aan de behandelend kinderarts doorgegeven. Voor follow-up samples worden uitslagen per fax en schriftelijk doorgegeven.

CHECKLIST VOOR INSTUREN MATERIAAL

- **Patiënt telefonisch aanmelden bij het laboratorium van de SKION.** Hierbij worden naam, geslacht, geboortedatum en (voorlopige) diagnose gemeld, alsmede gegevens over het afgenomen materiaal.
- **Verzending haemo-, liquorblokken en diagnosepreparaten via koerier. Aanmelding voor vervoer dient te geschieden per fax of per email.**
  => zie instructies voor verzenden materiaal SKIONwebsite: www.skion.nl/praktische informatie

AFNAME BIJ DIAGNOSE:

- **Haemoblok** met 10-20 ml beenmerg en 5-10 ml bloed t.b.v. immunofenotypering.
- **Liquorblok** met 2 ml liquor (tot de streep op de buis).
- **Preparaten,** ongekleurd 6 beenmerg en 3 bloedpreparaten.
- **Bloed en beenmerg** naar het **cytogenetisch laboratorium** in eigen centrum na telefonisch overleg.

AFNAME TIJDENS BEHANDELING:

- **Bloed en beenmerg preparaten (**minimaal 3 beenmerg- en 3 bloedpreparaten).
- **Haemoblok** met 10-20 ml beenmerg en 5-10ml bloed.
- **Indien van toepassing: Liquorblok** met 2 ml liquor.

TIJDSTIP	AFNAME	DOEL	Add-on studies
Diagnose	Zie “bij diagnose”	Diagnose	1-2-3-4
Dag15	Zie “tijdens behandeling”	Remissiestatus	1-3 (MRD)-4
Dag 28-36	Zie “tijdens behandeling”	Remissiestatus	1-3 (MRD)-4
Voor start van iedere consolidatie kuur	Zie “tijdens behandeling”	Remissiestatus	1-3 (MRD)-4
4 wkn na 2^{e} HA$_2$E kuur	Zie “tijdens behandeling”	Remissiestatus	1-3 (MRD)-4
6 mnd na einde 2^{e} HA$_2$E kuur	Zie “tijdens behandeling”	Remissiestatus	1-3 (MRD)-4

SAE form

DCOG patient ID ☐☐☐☐☐

INITIAL REPORTING

Please send the SAE form within 48 hours of acknowledgement of Serious Adverse Event by fax to 070 – 3598718. All data should be filled out in English.

Trial
Protocol name:_____ Name investigator: _____ Hospital name.: _____

Patient
Initials (first/surname): ☐☐ ☐☐ Date of birth: _ _ / _ _ / _ _ _ _ (dd/mm/yyyy)

Date of diagnosis: _ _ / _ _ / _ _ _ _ (dd/mm/yyyy) Risk group: _____ Gender: ☐ M ☐ F

Date SAE
Start date SAE:_ _ / _ _ / _ _ _ _ (dd/mm/yyyy) Date investigator informed about SAE:_ _ / _ _ / _ _ _ _ (dd/mm/yyyy)

Stop date SAE*:_ _ / _ _ / _ _ _ _ (dd/mm/yyyy) Ongoing: ☐ CTCAE grade: _____
*When stop date is known, please also fill out follow-up section

Study drug	**Cumulative dose**	**Route** (e.g. PO,IV)	**Start date**	**Stop date**	**Relationship** 1 none 2 doubtful 3 possible 4 probable 5 very likely	**Action taken** 1 none 2 dose reduced 3 delayed** 4 interrupted*** 5 discontinued

Description SAE
(include diagnosis)

SAE category
- ☐ Death
- ☐ Life-threatening
- ☐ Hospitalization or prolongation of existing hospitalization
- ☐ Persistent or significant disability/incapacity
- ☐ Congenital anomaly/birth defect (offspring of patient)

Outcome
- ☐ Not yet recovered
- ☐ Recovered, without sequelae
- ☐ Recovered with sequelae
- ☐ Died

Treatment Course
(describe during which protocol phase the SAE occurred and indicate the week of the protocol phase, if possible)

Expectedness of event
Event was: ☐expected ☐unexpected

delayed=planned treatment is not started due to event, * interrupted =treatment has started but is stopped prematurely due to event.

SAE form

DCOG patient ID ☐☐☐☐☐

INITIAL REPORTING continued

Relevant medical history
(Complete this section)

Investigator	**Signature investigator** _____
Name: _____	**Date** _ _ / _ _ / _ _ _ _ (dd/mm/yyyy)

For DCOG only

Date SAE received _ _ / _ _ / _ _ _ _ (dd/mm/yyyy) Initials DM _____

Date PC chairman notified _ _ / _ _ / _ _ _ _ (dd/mm/yyyy) Initials DM _____

Minimal required information available:
(reporting time starts when these 4 items are available)

- ☐ Information on study product (can be blinded)
- ☐ Description of event (SAE diagnosis, symptoms or signs)
- ☐ Identification of patient (initials, patient number, date of birth)
- ☐ Name of (co-)investigator who has reported the SAE

In case of missing data, date additional data received: _ _ / _ _ / _ _ _ _ (dd/mm/yyyy)

To be filled after receiving comments of PC Chairman:

Type of SAE: ☐SAE ☐SSAR ☐SUSAR *Take appropriate actions see SAE procedure.*

For PC Chairman only

Does the event comply with the definition of SAE according to this protocol? ☐ Yes ☐ No

Necessary to break randomisation code? ☐Yes ☐No ☐NA.
If yes, who are unblinded_____

Do you agree to SAE category as given by investigator ☐Yes ☐No
If no, give SAE category_____

Is the event related to study treatment?

☐Not related or unlikely

☐Possibly, probably or definitely

Or Blinded study:

☐Unknown (code not broken; code breaking required to assess relatedness and/or expectedness)

In case event is related to a specific study drug(s), please give drug(s)_____

_____

Expectedness of event
Event was: ☐expected ☐unexpected

Signature PC Chairman _____ Date _ _ / _ _ / _ _ _ _ (dd/mm/yyyy)

SAE form

DCOG patient ID ☐☐☐☐☐☐

Follow-up

Please send the SAE follow-up form as soon as possible after completion of Serious Adverse Event by fax to 070 – 3598718. All data should be filled out in English.

Trial
Protocol name:_____ Name investigator: _____ Hospital name.: _____

Patient
Date of birth: _ _ / _ _ / _ _ _ _ (dd/mm/yyyy)

Date SAE
Start date SAE:_ _ / _ _ / _ _ _ _ (dd/mm/yyyy)

Stop date SAE:_ _ / _ _ / _ _ _ _ (dd/mm/yyyy)

SAE final diagnosis (give term and description)
SAE final diagnosis date _ _ / _ _ / _ _ _ (dd/mm/yyyy)

Date of death: _ _ / _ _ / _ _ _ _ (dd/mm/yyyy)

Cause of Death:

☐ Malignant disease

☐ Toxicity

☐ Other, _____

Autopsy: ☐ No ☐ Yes
If yes, include autopsy report.

Outcome

☐ Recovered, without sequelae

☐ Recovered with sequelae

☐ Died (fill out next section)

Action taken:

☐ Other action taken concerned to study drugs (only fill out if different from initial report)

Drug_____ Action_____

Drug_____ Action_____

☐ Hospitalisation: Date hosp.: _ _ / _ _ / _ _ _ _ (dd/mm/yyyy) Date discharge: _ _ / _ _ / _ _ _ _ (dd/mm/yyyy)

☐ Non drug therapy given_____

☐ Concomitant medication given (if yes, describe below)

Relevant Concomitant Medication (Complete this section or attach CRF page 'Concomitant Medication')

Name medication	**Route of administration**	**Total daily dosage** (incl. unit)	**Frequency** (e.g. once daily)	**Start date** (day-month-year)	**Stop date** (day-month-year) or 'continued'	**Indication**

SAE form

DCOG patient ID ☐☐☐☐☐

Follow-up continued

Relevant laboratory and diagnostic tests
(Attach reports/results of laboratory and diagnostic tests e.g. laboratory results, ECGs etc.)

Randomisation code broken?

☐ No ☐ Yes, date _ _ / _ _ / _ _ _ _ (dd/mm/yyyy) ☐ N.A.

For DCOG only

Date follow-up SAE received _ _ / _ _ / _ _ _ _ (dd/mm/yyyy) Initials DM _____

Date PC chairman notified _ _ / _ _ / _ _ _ _ (dd/mm/yyyy) Initials DM _____

Completed actions by SKION Trial Office (only required in case of SUSAR):

Date reported to CA _ _ / _ _ / _ _ _ _ (dd/mm/yyyy) by_____

Date reported to EC _ _ / _ _ / _ _ _ _ (dd/mm/yyyy) by_____

Date reported to investigators _ _ / _ _ / _ _ _ _ (dd/mm/yyyy) by_____

Additional actions required ☐Yes,_____ ☐No

Actions performed _ _ / _ _ / _ _ _ _ (dd/mm/yyyy) by_____

Bijlage 7 Add- on biological studies

Biological studies in short

Stem cell frequency and oligoclonality of mutations in childhood AML and their functional consequences for the development of relapse.

The general aim of this study is to increase our knowledge on the frequency of AML stem cells and on the presence of molecular abnormalities in MRD cells and more specifically in AML stem cells. The mutational status will be investigated in relation to its presence in different subclones at initial diagnosis, and thereby the existence of oliglclonality of AML in children and adolescents.
We want to accomplish this by answering the following questions:

1. What is the burden of AML stem cells at initial diagnosis and at the setting of MRD, how does this correlate with well-known clinical and cell biological features such as FAB type and cytogenetics (including molecular abnormalities), and what is the prognostic significance of both factors.
2. What is the correlation between levels of MRD as determined by more conventional techniques, and the leukemic stem cell load, at different time-points.
3. What are the differences in type I and type II abnormalities from initial diagnosis to MRD (and ultimately to relapse), and when comparing the AML stem cells and the more progenitor type AML cells
4. Is there oligoclonality of AML, and how do subclones develop from initial diagnosis to MRD (and ultimately to relapse).

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1. Van Rhenen et al. High stem cell frequency in acute myeloid leukemia at diagnosis predicts high minimal residual disease and poor survival. Clin. Cancer Res., 2005;11:6520-7.
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Integrating proteomics and kinomics in pediatric acute myeloid leukemia (AML): detailed cellular insights to improve outcome.

The goal of this add-on study is to produce an exceptionally detailed map of protein expression, phosphorylation and enzymatic activation in AML that integrates proteomics and the net functional measurement of kinomics. In this add on study we will focus on the stem cell enriched AML cells (CD34+/CD38-).The hypothesis is that this will reveal a phenotypic proteomic based classification system with the ability to discern different means of arriving at the same functional state. This in turn will provide new insights into leukemia biology that can be formally tested in greater detail. These observations will lead to the development of clinical kits based on key measurements that can then be prospectively tested for the ability to classify cases, provide prognostic information and to aid in the rational selection of therapeutic agents.

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Prognostic relevance of detection Minimal Residual Disease for children with acute myeloid leukaemia.

Several studies have shown that detection of minimal residual disease (MRD) in AML is an independent prognostic factor. Recently, retrospective flowcytometric MRD studies showed that the relative risk of relapse in MRD-positive pediatric AML patients is higher in comparison with the risk of relapses in MRD-negative patients. However, more work is required to further optimize the flowcytometric MRD analysis and additional studies are required in order to determine the most optimal time point(s) and cut-off level for MRD analysis in childhood AML. The recent introduction of 8-color flowcytometers has shown to further improve the applicability and sensitivity of MRD detection in childhood acute leukemia. Within the EuroFlow network, 8-color immunostaining protocols have been designed for all hematological malignancies, including AML

This clinical research project has the following aims:

1. To investigate whether flowcytometric MRD detection in childhood AML can be improved in order to detect an aberrant immunophenotype in over 95% of patients and to reach sensitivities which are consistently at least 0.01%, preferably 0.001%.
2. To evaluate the clinical value of MRD in childhood AML by monitoring of MRD during and after treatment at predefined sampling points.
3. To obtain insight in the characteristics of the leukemic cell subsets, particularly leukemic stem cells, at diagnosis and to monitor these subpopulations by flowcytometry during treatment.

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Prognostic significance of early AML blast clearance and of routine bone marrow and peripheral blood monitoring by simple morphology during and after chemotherapy in pediatric AML.

It is clinically relevant to predict relapse early in the treatment, potentially allowing adaptation of treatment in such patients. Well-known prognostic factors in pediatric AML are early treatment response, cytogenetics and minimal residual disease. The latter is cumbersome, and not available in all countries. Moreover, sofar it has not been shown to be as successful as in ALL, and is not being used yet for risk-group adapted therapy. Early treatment response has usually been studied by bone marrow

(BM) examination on days 15 and day 30. To the best of our knowledge, studies on the prognostic significance of the dynamics of clearance of AML blasts from the peripheral blood (PB) have not been performed in pediatric AML.
While searching for prognostic factors, it has not convincingly been shown that early detection of AML relapse is clinically relevant, except for APL. However, BM and PB sampling is routinely being done in many pediatric AML protocols. In fact, without evidence that such routine monitoring (including conventional examination by morphology) is able to detect a relapse in patients in whom otherwise a relapse was not suspected.
This study is not aimed at MRD detection, although correlating morphology and MRD data seems of great interest.

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Kwaliteit van leven en slaap tijdens en na behandeling voor acute myeloide leukemie op de kinderleeftijd.
De relevantie is gelegen in het vroegtijdig opsporen van problemen op het terrein van kwaliteit van leven, ook van problemen die niet blijken te herstellen na de behandeling. Het onderzoek lijkt erg voor de hand liggende vragen op te gaan lossen, maar het onderzoek bij kinderen met ALL heeft toch al geleerd dat er problemen kunnen zijn met veel impact op de kwaliteit van leven, die in de spreekkamer vaak niet worden gemeld. Een goed voorbeeld is slaapproblematiek. De vragenlijsten zoals hier voorgesteld kunnen dergelijke problemen inzichtelijk maken, evenals de incidentie ervan. In de toekomst zou een gevolg van dit onderzoek kunnen zijn dat bij alle kinderen met AML tijdens en na de behandeling dit soort vragenlijsten periodiek worden afgenomen, en dat interventies gericht op het verbeteren van kwaliteit van leven worden ontwikkeld en geïmplementeerd in de reguliere zorg.

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7.1 Stem cell frequency and oligoclonality of mutations in childhood AML and their functional consequences for the development of relapse

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Introduction

We hypothesize that to further improve the outcome of children with AML, we need to increase our knowledge of AML stem cells and of the presence of molecular abnormalities in minimal residual disease and leukemic stem cells. This will be the focus of our research proposal.
The outcome of pediatric AML has improved significantly over the past decades, but seems to have reached a plateau at about 60% survival with contemporary intensive chemotherapy. The leading cause of treatment failure is a relapse. Relapse is thought to arise from minimal residual leukemic cells and leukemic stem cells.
The amount of AML stem cells at initial diagnosis and in minimal residual disease (MRD) might provide a novel prognostic factor, which might be useful in risk-group adapted treatment. Indeed, a high AML stem cell burden at initial diagnosis was recently shown to correlate with high levels of MRD and to be a significant adverse prognostic factor in adult AML (van Rhenen, 2005).
The implementation of novel targeted agents, such as tyrosine kinase inhibitors, may further improve outcome by reducing relapses, without undue toxicity. Thus, it is important to study the presence of these treatment targets in the populations of AML cells, which are most relevant to target minimal residual disease cells and especially the AML stem cells. We (Cloos, 2006) and others (Tiesmeier, 2004) have shown that the presence of treatment targets such as a *FLT3/ITD* or *KIT* mutation is unstable from initial diagnosis to relapse. It is therefore not sufficient to determine the presence of these mutations in the bulk of the leukemic cells at initial diagnosis.
Minimal residual disease and AML stem cells can be detected by extensive immunophenotyping and sorted with modern flowcytometers (Rhenen, 2007). Using flowcytometry, our group has the possibility to reliably identify both AML and normal stem cells within one and the same sample by including CLL-1 and other aberrant markers/marker combinations, together with scatter aberrancies.
The cause of the instability of these mutations between initial diagnosis and relapse is unknown. One hypothesis is that AML is an oligoclonal malignant disease. This implies that treatment targets may be present in part of the AML cells, but not in all of them. This can be studied using the same sophisticated flowcytometrical techniques mentioned above. Such oligoclonality may explain the instability of molecular abnormalities, since certain subclones may disappear from diagnosis to relapse, while other small subclones, containing molecular abnormalities that were not detected at initial diagnosis, may actually become the relapsing clone, leading to the presence of the molecular abnormality in the relapsing AML cells. An alternative explanation for the emergence of molecular abnormalities from diagnosis to relapse is genetic instability of the AML (stem) cells, leading to either spontaneous or drug-induced additional genetic abnormalities over time. It seems important for our understanding of AML to study these phenomena.

Aims of the study

The general aim of this study is to increase our knowledge on the frequency of AML stem cells and on the presence of molecular abnormalities in MRD cells and more specifically in AML stem cells. The mutational status will be investigated in relation to its presence in different subclones at initial diagnosis, and thereby the existence of oliglclonality of AML in children and adolescents.
We want to accomplish this by answering the following questions:

1. What is the burden of AML stem cells at initial diagnosis and at the setting of MRD, how does this correlate with well-known clinical and cell biological features such as FAB type and cytogenetics (including molecular abnormalities), and what is the prognostic significance of both factors.
2. What is the correlation between levels of MRD as determined by more conventional techniques, and the leukemic stem cell load, at different time-points.
3. What are the differences in type I and type II abnormalities from initial diagnosis to MRD (and ultimately to relapse), and when comparing the AML stem cells and the more progenitor type AML cells
4. Is there oligoclonality of AML, and how do subclones develop from initial diagnosis to MRD (and ultimately to relapse).

Relevance of the study for AML patients

The relevance of this research is threefold.
First, we may identify a novel prognostic factor by determining the AML stem cell burden at initial diagnosis and at the situation of MRD.
Second, we will increase our knowledge of the biology of AML in general, especially concerning stem cells and oligoclonality, which might become useful in future treatment and disease monitoring.
Third, this study will provide important information on how and when to apply targeted therapy; especially tyrosine kinase inhibitors in future targeted therapy protocols.

Preliminary results

Stem cell load detection in initial and MRD samples

The prognostic impact of stem cell frequency in CD34-positive AML was investigated by analyzing whether the CD34+/CD38– compartment at diagnosis correlates with MRD frequency after chemotherapy and clinical outcome in 92 adult AML patients (van Rhenen, 2005l). A high percentage of CD34+/CD38– stem cells at diagnosis significantly correlated with a high MRD frequency, especially after the third course of chemotherapy. Also, it directly correlated with poor survival (figure 1).

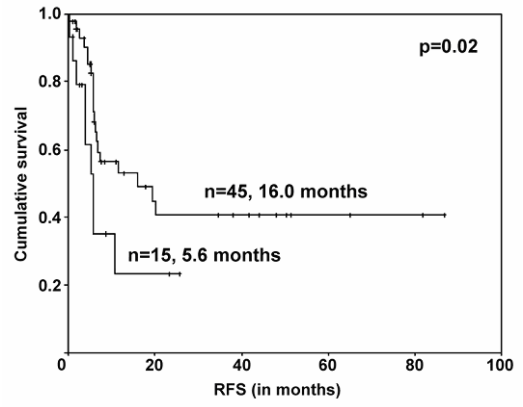

Figure 1: Kaplan-Meier plot of relapse-free survival (n=60). The cutoff used is 3.5%. The patients with a high stem cell frequency at diagnosis (>3.5%) had a median relapse-free survival of 5.6 months (n = 15). This is in strong contrast to the patients with a low stem cell frequency who had a median relapse-free survival of 16.0 months (n = 45). This difference was significant using log-rank statistics (P = 0.02).

In contrast, the percentage of CD34+ AML cells in total percentage showed no such correlations. In addition, *in vivo* data showed that engraftment of AML blasts in non-obese diabetic/severe combined immunodeficient (NOD/SCID) mice directly correlated with stem cell frequency of the graft.

Both *in vivo* data, as well as the correlation studies, show that AML stem cell frequency at diagnosis offers a new prognostic factor. From our data, it is tempting to hypothesize that a large CD34+/CD38– population at diagnosis reflects a higher percentage of chemotherapy-resistant cells that will lead to the outgrowth of MRD, thereby affecting clinical outcome.
Previously, the group of Schuurhuis at VUMC (van Rhenen) found that C-type lectin-like molecule-1 (CLL-1) has high expression on the whole blast compartment in the majority of AML cases. Moreover, CLL-1 expression is also present on the CD34+/CD38- stem- cell compartment in AML (77/89 patients). The CD34+/CLL-1+ population, containing the CD34+/CD38-CLL-1+ cells, does engraft in NOD/SCID mice with outgrowth to CLL-1+ blasts. CLL-1 expression was not different between diagnosis and relapse (n = 9). In remission, both CLL-1- normal and CLL-1+ malignant CD34+/CD38- cells were present. A high CLL-1+ fraction was associated with the development of an earlier relapse. CLL-1 expression is completely absent both on CD34+/CD38- cells in normal (n = 11) and in regenerating bone marrow controls (n = 6).
In addition to the LAP and CLL-1, the stem cell fraction can be even further characterized using scatter properties (figure 2).

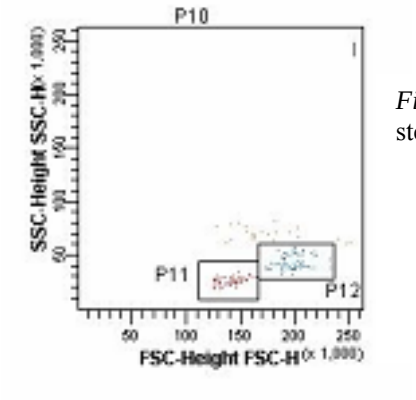

Figure 2: Additional separation of normal (p11) versus malignant (p12) stem cells based on scatter properties.

These results indicate that malignant stem cell frequency may be an important additional prognostic factor and malignant stem cell fractions can be more specifically detected by combining LAP, CLL-1 expression and scatter properties.

Instability of type I mutations in AML
We (Cloos et al.) determined FLT3/ITD and D835 point mutations in paired initial and relapse samples from 80 pediatric and adult AML patients. One D835 point mutation was found in an initial pediatric AML sample. FLT3/ITDs were present in 21 initial and 22 relapse samples (26.3 and 27.5%, respectively). Interestingly, FLT3/ITD positivity was related to a significantly shorter time to relapse, most pronounced when the ITD-positive status was found at relapse (P<0.001). However, FLT3/ITD status changed between diagnosis and relapse in 14 cases. In four patients, the FLT3/ITD became undetectable at relapse, in five patients FLT3/ITDs were only detected at relapse, and in five patients the length or number of FLT3/ITDs changed (table 1).
Since it is not known whether the instability of mutations only occurs in FLT3 we are currently extending this study by determining all relevant type I/II mutations. For this study we selected ± 70 paired pediatric AML samples since for some mutations frequencies are low and differ from adult patients which were formerly included in the FLT3 study. Preliminary analysis suggests that in about 40% of cases changes in mutations occur and that these are associated with the time to relapse.
Our hypothesis is that for some patients the gained mutation at relapse was already present in the initial sample but in a very small subclone. In order to detect the mutations in these very small cell populations, we have set up a method to sort the cells in a 96-wells plate with 25 cells per well. We are able to perform very sensitive mutation analysis on these sorted subpopulations. For example, iIn a sample of leukophoresis material that was supposed not to harbor any tumor cells we have detected the FLT3/ITD in some of the cell fractions.

Table 1 Relation between changes in FLT3/ ITD status and time to relapse

FLT3/ITD status			Number of patients (n = 80)	Median # months to relapse (p25 – p75)	ANOVA (*P*-value)
	Initial	*Relapse*			
I FLT3/ITD status in initial sample					
	Wild type	Any status	59	12.6 (9 – 21)	*P*<0.03
	FLT3/ITD+	Any status	21	7.8 (5 – 9)	
II FLT3/ITD status in relapse sample					
	Any status	Wild type	58	13.5 (9 – 22)	*P*<0.001
	Any status	FLT3/ITD+	22	6.6 (5 – 8)	
III FLT3/ITD changes in initial and relapse sample					
	Wild type	Wild type	54	13.4 (9 – 22)	*P*<0.03
	FLT3/ITD+	FLT3/ITD+	12	7.4 (5 – 8)	
	Wild type	FLT3/ITD+	5	6.3 (5 – 19)	
	FLT3/ITD+	Wild type	4	15.4 (5 – 46)	
	FLT3/ITD+	Different ITD	5	4.7 (4 – 10)	

In an AML sample of which we had frozen material available we performed our new cell sorting and PCR method. We could show that the mutation that was gained at relapse was indeed present in the initial sample (figure 3). However, the mutation was found in the CD38 dim population and not in the CD38- as we would have expected. More samples will be analyzed in the near future to determine if this oligoclonality can be found in all patients. For some patients the concept of genomic instability may still be an explanation for the change in mutations.

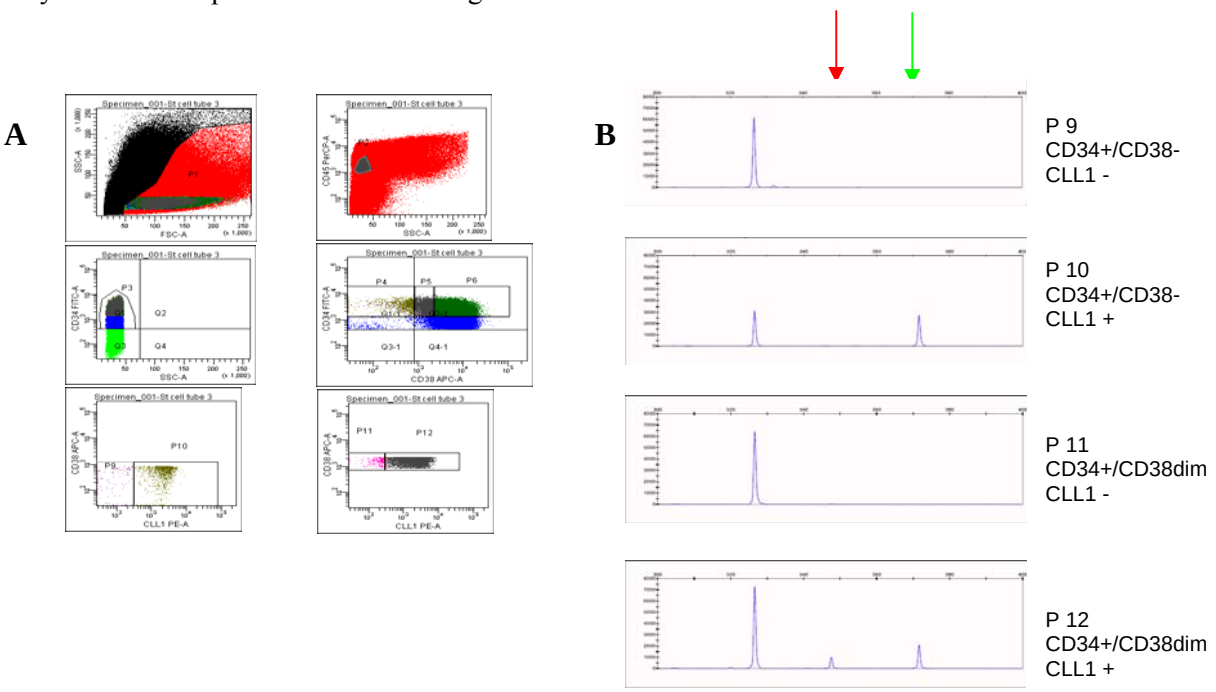

Figure 3: A) Flowcytometric gating strategy of the initial sample.for subpopulation selection. First the viable cells are selected by FCS/SCC (P1), then the blasts as CD45 dim (P2), CD34+ (P3), CD34+CD38- (P4=immature (stem)cells) and CD34+CD38dim (P5=progenitor cells). As additional marker to distinguish malignant stem cells we use CLL1; CLL1- subpopulation contains the normal stem cells while the CLL+ cells are the malignant stem cells. We have compared these populations both in the CD34+CD38- population (P9 and P10 respectively) and in the CD34+CD38dim population (P11 en P12).
B) FLT3/ITD analysis. The red arrow represents the ITD length that was only present in the relapse sample while the green arrow is the ITD length that was found in the bulk of the initial sample. In P9 and P11 we don't expect an ITD since these contain the normal cells. In P10, only the ITD is seen that has been found in the initial cell while in P11 we see both the initial and the relapse ITD.

CLL1- population represent normal cells and do not show ITD as expected. The relapse ITD was found in the more mature CD38dim fraction of this initial sample. Probably these cells do have (acquired) tumor-initiating characteristics. It has to be emphasized that the subpopulation in which the mutation is found is determined by the selection of markers used. They have been selected on malignant (stem)cell characteristics in this study while other studies using different markers might reveal another defined subpopulation.
These results suggest that at least in some patients the subpopulation of cells, harbouring the mutation of the relapse, was already present in the initial sample. This warrants the detection of mutations during treatment and at MRD settings. These data are important for knowledge about relapse development and decision making on targeted intervention for instance using FLT3 inhibitors.

Materials and methods
We will determine stem cell load in initial samples and the subsequent MRD and follow-up samples. We will use flowcytometry methods that were successfully used in our laboratory in adult AML samples. In order to use as few cells as possible we will apply the leukemia associated phenotype (LAP) as determined in The Hague at SKION/DCOG (de Haas et al) in collaboration with the Dept. of Immunology in Rotterdam (van der Velden & Van Dongen et al.). We will determine the stem cell load by FACS analysis using besides lineage markers, CLL-1 and scatter properties to distinguish the MRD/AML cells from the normal stem cells in the regenerating bone marrow. Including these markers is interesting since it may improve the number of patients for whom we can determine the malignant stem cells and it will also enable to quantitate the more specific malignant stem cell load. In addition to the quantification of these stem cells we will sort them and molecularly characterize them for the presence of the known type I and II mutations. We will also determine these mutations in several other leukemic subpopulations, such as the non-stem progenitor cells. Mutation analysis currently included are: FLT3, c-KIT, RAS, NPM1, CEBPα and WT1.

Requested samples and data
Overview of requested samples:

Sample	Fresh/Frozen	Number of cells	Application
Initial	Frozen	1 µg DNA	Mutation profiling of the bulk of cells
	Frozen (DMSO) vial	50.10^6	Subpopulation characterization on mutation profile and determination of stem cell load including CLL-1
MRD	Residual of fresh material*	Depending on cell number and % MRD of sample preferably 8.10^6 cells	Subpopulation characterization on mutation profile and determination of stem cell load including CLL-1

*after $5\text{-}10^6$ cells for immunophenotyping and 5.10^6 cells for molecular diagnostics at DCOG or Rotterdam

To determine the presence of a subclone in the initial sample that was found at relapse we can use frozen material. This will induce a cell loss of ± 50% after thawing but it will not influence the results. Of course, fresh material is preferred in all instances but may be difficult for shipment logistics. For sorting MRD samples, frozen material is not an option due to the limited number of cells

Logistics of material flow to Amsterdam:
Initial sample:

- DNA for mutation analysis
- Results of LAP as determined by immunophenotyping at SKION and Rotterdam will be communicated with Amsterdam

MRD samples at the different time points that bone marrow samples are drawn during the treatment:

- Residual fresh material will be sent to Amsterdam after the SKION and Rotterdam have taken
    - 5-10.106 cells for immunophenotyping
    - 5.106 cells for molecular diagnostics

Cryopreserved cells from initial diagnosis:

- Vials containing 50.106 cells will be send in bulk to Amsterdam at a later stage to analyze subfractions and determine stem cell load. An additional option is to send vials of 70.106 cells so we will sort 20.106 cells on CD34+/CD38- and freeze the pellets to be send to Groningen (see add-on study by de Bont et al.)

In order to determine the association between the development of relapse and mutation profile (including stem cell load) we need to perform our analysis on the whole cohort of patients included in the clinical protocol. This includes the patients with no known type I mutation and those who do not change in their mutational status during MRD of which we expect they will have a lower probability of relapse.

Stem cell frequency
To be able to show a statistically significant difference between the prognosis of patients with a low- versus a high -stem cell load we consider the data as has been published by van Rhenen et al. (2005) from the Hematology Laboratory of our institute. This implies that taking into account the same variation in stem cell frequencies we would need approximately 60 patients of whom we can determine the stem cell fraction (estimated to be 80% of pediatric patients). So a total of 80 patients would be required. Successful identification of the stem cells will differ among patients and depends on the stem cell load and available aberrant markers.

Oligoclonality
To know the standard mutational status of the initial sample we will determine the most relevant type I/II mutations. After analysis these data will be sent to the SKION. Our preliminary studies in pediatric and adult AML have shown that we need 50.10^6 cells to sort different leukemic subpopulations and determine all type I/II mutations. We have set up very sensitive assays with high resolution melting techniques to measure the mutations in at least 1:10,000 cells. Since we sort the cells in portions of about 25 cells per well any well containing a cell with mutation would certainly be detected with these procedures. Pilot seeding experiments have shown that these results can be achieved. We have established collaboration with the Immunology laboratory in Rotterdam (van Dongen/van der Velden) and SKION (de Haas) to use the LAP they define as a starting point for our analyses. In this way we are able to limit the amount of cells needed to a minimum. In addition, we will agree to share the sorted cell populations of the initial samples with other research groups when feasible. We could for instance arrange that when we have $\geq 70.\ 10^6$ cells we will sort 20.10^6 cells on CD34+/CD38- and freeze the cells to be shipped to Groningen.
Despite the collaborations between research groups to reduce the amount of cells needed, it would be very helpful if a second bone marrow aspirate (which can be done without an extra puncture through the skin) will be performed to obtain as many leukemia cells as possible, without diluting the bone marrow with blood in case of one extended aspiration.

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Curriculum vitae applicant

Dr. Jacqueline Cloos (1962) obtained her PhD in 1996 at the VU university medical center at the Department of Otolaryngology, Head and Neck Surgery. During her PhD training period she worked at the MD Anderson Cancer Center, Houston, USA to learn chromosomal instability assays in order to set up these techniques in Amsterdam. As a post-doc in the pediatric oncology laboratory of Prof Dr G.J.L. Kaspers, she continued working on the concept of inherited sensitivity to drug-induced chromosomal damage with main focus on DNA repair. In 2003 she was appointed as laboratory research coordinator for Pediatric Oncology and obtained a "KWF Kankerbestrijding stimuleringssubsidie" grant for pediatric oncology. Her research is focused on *in vitro* models for determining sensitivity for chemotherapeutic drugs, radiation and innovative targeted therapy. These models are valuable for predicting the response of the patient to treatment and for finding more novel treatment targets including DNA repair pathways. The research includes three tumor types: brain tumors, retinoblastoma and leukemia. Recently, the Pediatric Oncology/Hematology laboratory has been integrated within the Hematology Laboratories for mutual optimization of research and increased access to state-of-the-art equipment. These laboratories reside in the new building of the Cancer Center Amsterdam where all cancer research of our institute is united and where more than 250 investigators study cancer. Due to the strong collaboration with the Hematology research group of Dr. GJS. Schuurhuis, there is significant expertise on FACS immunophenotyping, MRD measurements and stem cell detection including subsequent sorting of the subpopulations.

• Invited speaker at the 96th annual meeting of the American Association for Cancer Research (AACR) 2005, Anaheim, USA.
• Selected speaker at the 46th annual meeting of the American Society of Hematology (ASH) 2004, San Diego, USA.
• (Co)author of 47 peer reviewed papers

7.2 Integrating proteomics and kinomics in pediatric acute myeloid leukemia (AML): detailed cellular insights to improve outcome

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Introduction

Despite similar clinical features at presentation, there are many different types of acute myelogenous leukemia (AML). Proteins form complex signalling pathways that control how normal hematopoietic cells respond to signals from the body to regulate proliferation, apoptosis and differentiation into functional blood cells. The amount or activity of these proteins is often abnormal in AML cells, and this can affect the response to therapy. The group of Steve Kornblau utilized a new technique called Reverse Phase Protein Array (RPPA) to measure the level of expression and phosphorylation of 176 cell signalling, apoptosis and cell cycle regulating proteins using less material than was previously required to study one protein. Seven signatures were differentiated based on the overall pattern of expression of all these proteins. These signatures showed a relation with outcome (1,2). Reversible phosphorylation of proteins on serine, threonine or tyrosine residues is a major signalling mechanism in the cell. Kinases are responsible for the reversible phosphorylation of all proteins. The measurement of kinase activity is called kinomics. The groups of Maikel Peppelenbosch, who developed a kinomic array, and of Eveline de Bont, pediatric oncology, have used the kinome array to measure the activity of these kinases in leukemic cells on 1024 different sites on the array (3,4). A common leukemic signalling pathway was elucidated and validated in functional assays using specific inhibitors. These two platforms complement each other as many of the peptides used in the kinomic array are measured with phosphospecific antibodies used in the RPPA, while several others are the downstream targets of these phosphoproteins. In this add on study we propose to perform functional kinomic profiling and proteomic profiling using RPPA on the same samples. This will provide a functional measurement to complement the measurement of expression and phosphorylation levels. Since these proteins are major signalling molecules of pathways or networks, we will perform highly sophisticated statistical network based analysis that combines data from these two arrays. This will create a map of expression, phosphorylation and activity within leukemic cells with a detail and precision that is unprecedented. Previously generated RPPA data are present for adult AML, but we propose to study pediatric AML samples. This will enable us to evaluate the commonalities and distinctions between pediatric/adult cases. These maps will give insight into the different biology present in AML and help to explain why we see differences in response to therapy despite similarity of other clinical features like cytogenetics. The final aim of this add on study is to investigate some of these insights in greater detail to confirm the proteomic and kinomic based observations. There are many emerging "targeted" therapies being developed that aim at many of these same proteins, but we lack the means to intelligently match the correct targeted therapy to the correct patient. We believe that this map will enable us to identify which pathways are crucial to the survival and resistance of individual patients' leukemic cells and that this will facilitate the correct matching of the right targeted therapy to the right patient, thereby improving outcome.

2. Doel/vraagstelling van het onderzoek

The goal of this add-on study is to produce an exceptionally detailed map of protein expression, phosphorylation and enzymatic activation in AML that integrates proteomics and the net functional measurement of kinomics. In this study we will focus on the stem cell enriched AML cells (CD34+/CD38-).The hypothesis is that this will reveal a phenotypic proteomic based classification system with the ability to discern different means of arriving at the same functional state. This in turn will provide new insights into leukemia biology that can be formally tested in greater detail. These observations will lead to the development of clinical kits based on key measurements that can then be prospectively tested for the ability to classify cases, provide prognostic information and to aid in the rational selection of therapeutic agents.

Objectives:

1) Characterize the deregulated signaling pathways in pediatric AML by means of proteomics and kinomics and integrate the results and compare the bulk of AML cells with the CD34+/CD38- fraction.
2) Compare the obtained results in pediatric AML with already generated data of over 250 adult AML samples from the lab of Steven Kornblau (MD Anderson Cancer Center, USA), in both the bulk of AML cells and the CD34+/CD38- fraction.
3) Identify clusters of pediatric AML patients with overlapping signaling pathways and correlate with patient characteristics such as age, cytogenetics, risk groups, remission status and outcome.
4) Identify potential therapeutic targets also related to available small molecule inhibitors and validate these specific inhibitors with targeted therapy with cell survival assays.

The project will start with kinomic profiling on the pediatric AML samples (bulk leukemic cells and CD34+ enriched leukemic cell populations) and the same samples will be utilized for RPPA analysis. This will provide us with an enormous amount of data, which one might suggest will be a problem to analyze. However, through our collaboration we already have a wide experience with RPPA datasets and kinomic datasets. In addition the group of Dana Pe'er will take care of the integrative statistical analysis. The group of Pe'er has wide experience in the use of Bayesian networks for the reconstruction of molecular networks (7) and seems to be the perfect group to analyze these large datasets.

With this detailed map we will be able to compare various populations with each other and correlate the maps to patient details. In the meantime, with these results we will be able to identify clusters of activity and make provisional signal transduction schemes as shown in the prior results. Then we will continue by discerning possible kinase inhibitors upon the given results. With functional assays we will investigate their potential for clinical studies in pediatric AML patients.

Figure of the overall study design:

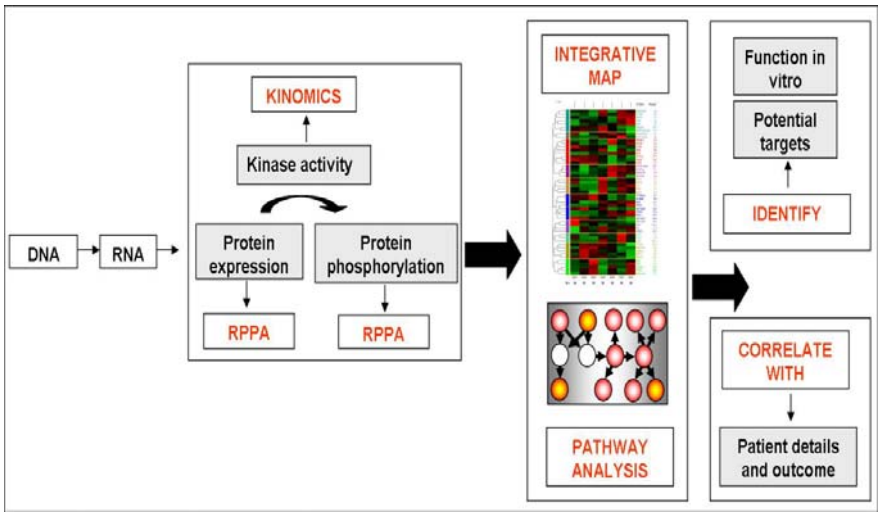


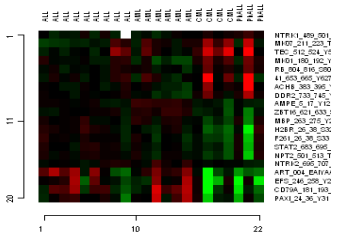
***Figure legend*:** *This figure shows the overall study design of the integration of the kinomics and RPPA, generated in this add on study. We might encounter the criticism that this is a fishing expedition. However, our previous work has been shown that these individual platforms involved have been proven successful in predicting pathways in oncological diseases. We would counter that the bait is appropriate*.

Relevance of the study for AML patients

The identification of active signal transduction pathways can expand our insight in the leukemogenesis of pediatric AML. Our techniques enable us to functionally test AML samples in vitro for differences in active signal transduction pathways. Upon our study, AML samples may be organized into different clusters based on their active signal transduction profile. This might help to predict in vivo responses of subgroups of patients to potential therapeutic targets related to available small molecule inhibitors. The addition of kinase inhibitors to standard chemotherapeutic treatment could improve outcome further.

Preliminary results

Figure 1. Supervised clustering of normalized peptide phosphorylation signals of primary pediatric leukemia



We already have generated kinome profiles in three cohorts of patient samples: pediatric brain tumor tissues, pediatric leukemia samples (Fig. 1) and more specifically pediatric AML samples carrying a MLL translocation using a tyrosine kinome array. In the first study concerning pediatric brain tumor tissue, we demonstrated that peptides with phosphorylation consensus sequences corresponding to Src-family kinases showed remarkably high levels of phosphorylation in pediatric brain tumors. Src activity was confirmed and Src-family kinase inhibitors PP1 and Dasatinib induced substantial tumor cell death in pediatric brain tumor cell lines, but not in control cell lines(3). In the second study we found that AML, ALL and CML samples contain substantial kinase activity; 120 peptides are detected above threshold level in one or more of the samples. Comparison of the kinase activity patterns of the three leukemias identified a common phosphorylation profile containing 44 peptides. Further analysis revealed 25 peptides differentially phosphorylated between the different leukemia subgroups. None of the peptides were exclusively phosphorylated in only one of the subgroups.

Figure 2. Provisional signal transduction scheme of common leukemia signaling pathway. Red kinases, corresponding peptides are phosphorylated in all leukemia subtypes, yellow centred molecules have been used in cell survival assays

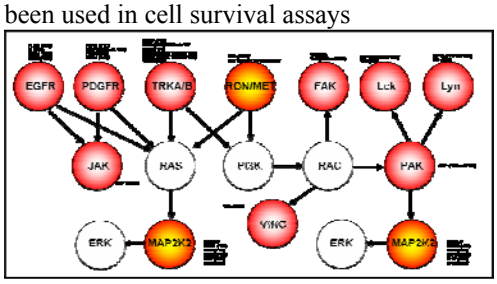
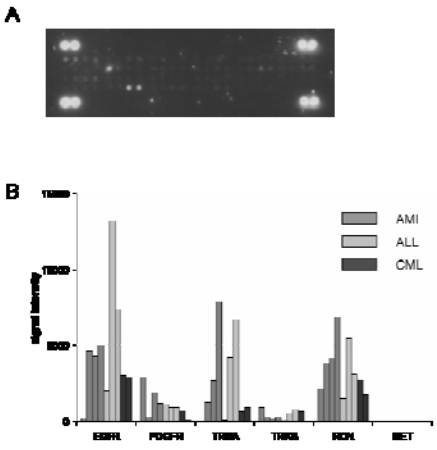
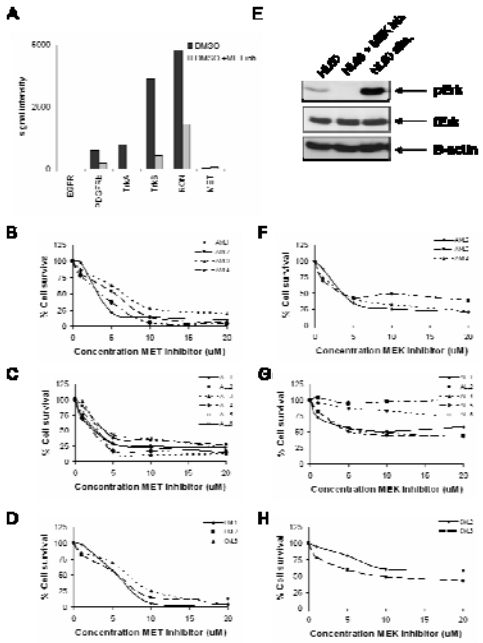


Figure 3. Phosphorylation signal intensity of nine leukemia samples from the six receptor tyrosine kinases represented in the provisional scheme.



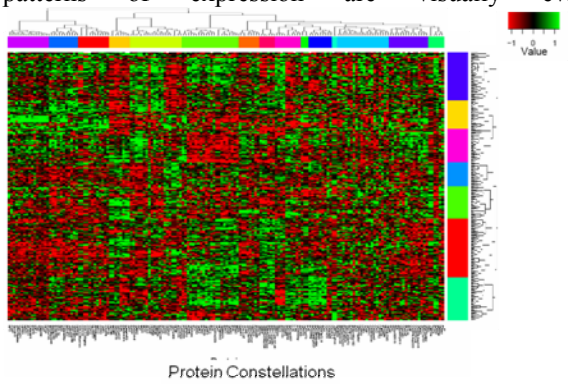
A provisional signal transduction pathway (Fig. 2) was constructed to show the common leukemia signal transduction pathway (4). The 14 leukemia samples measured seem to be characterized by activation of the MEK pathway induced by the neurotrophic tyrosine kinase receptors (TRKA/B) and the hepatocyte growth factor receptor (MET). Phospho-Receptor Tyrosine Kinase Proteome profiler array analyses confirmed phosphorylation of key tyrosine kinase receptors EGFR, PDGFR, NTRK (TRKA/B), and RON included in the provisional scheme (Fig. 3).

Figure 4. Decreased leukemia cell survival in response to MET and MAP2K2 (MEK) inhibition



No phosphorylation of MET could be detected. Inhibitors against two key signalling molecules from the constructed provisional pathway, MET/RON and MAP2K2 (MEK) inhibited phosphorylation of RON and ERK (downstream target of MEK) (Fig. 4A and E) and induced substantial tumor cell death in primary AML, ALL and CML patient samples (Fig. 4 B-D and F-H) AML samples are highly sensitive to the MEK inhibitor, whereas ALL samples are most sensitive to the MET inhibitor. MET inhibitors SGX523 and AMG 208 are currently being tested in phase I trials to treat solid tumors. MEK inhibitor AZD6244 is currently being tested in phase I and phase II trials in solid tumors and recurrent or refractory AML. In the third study the activity of AML cases with MLL rearrangements was measured against the complete kinome array (tyrosine, serine and threonine peptides). We constructed a provisional signal transduction scheme of the common phosphorylated peptides of 15 poor risk AML patients carrying a MLL translocation. We found that in MLL patients both the MAPK pathway and the PI3K signaling pathway were active, promoting the survival of AML blasts. In addition, we found an active TGFβ/LKB1 signalling pathway, which results in a decreased chemotherapeutic sensitivity. These results correlate with the poor risk phenotype of MLL patients. Overall, these kinomic data indicate the feasibility of identifying new targets with kinome profiling for the treatment of AML patients.

Figure 5. Heat map showing protein expression of 176 proteins in 256 AML cases, clustered by protein constellation (x-axis) and ProExpSig (y-axis). Repetitive patterns of expression are visually evident.



By using RPPA the group of Kornblau demonstrated that there were recurrent patterns of protein expression in adult AML samples (Fig 5). Moreover, these signatures have significant correlation with outcome (1,2). Beyond the classification and pattern a more focused analysis of these signatures reveals insight into AML cell biology that provide therapeutically targetable information. For instance, AML can be divided into cases characterized by activation of the STAT proteins, but not the PI3K/AKT or MEK/ERK STPs and a second group characterized by the opposite pattern. The reason for this pathway exclusivity requires investigation. The efficacy of blocking a quiescent pathway is uncertain. We also examined how protein expression differs between bulk leukemia cells and CD34+ or CD34+/CD38- stem cell enriched populations, isolated by sequential magnetic antibody selection using anti-CD34 and then anti-CD38 beads (MACS, Miltenyi Biotec, Auburn CA). Although stem cell enriched populations comprise a tiny fraction of marrow cells we purified a median of 2x105 CD34+/CD38- from AML patient marrows. We made a RPPA with bulk, CD34+ and CD34+/CD38- from the same sample on them. We observed highly significant and consistent differences in expression for 70 of these proteins between the various populations (all below the Bonferroni corrected P <0.0001). Comparison of CD34+ to bulk leukemic cells also detects most of these differences. Relapse is thought to arise from leukemic stem cells that survive therapy and repopulate the marrow suggesting that it may be relevant to study both leukemic stem cell enriched sorted populations as well as bulk

leukemic cells. Both the kinomic array and RPPA are capable of providing profiling data on very small numbers of cells. This makes these methodologies uniquely suited for performing profiling on stem cell enriched populations. While the kinomic array and RPPA on the bulk population provided insights, we hypothesize that therapy directed at the characteristics of the results of the stem cell enriched populations may have more profound efficacy in eliminating them.

Materials and methods

Prior results RPPA: We are able to use the results of over 700 adult AML samples already analyzed for RPPA (*1*) and 250 identical samples analyzed for kinomics in the near future. A first pilot experiment of shipping material across the ocean demonstrated reproducible results. Both methods, kinomics as well as proteomics, use a very low amount of material as mentioned above. These two platforms complement each other as many of the peptides used in the kinomics array are measured with phosphospecific antibodies used in the RPPA, while many others are the downstream targets of these phosphoproteins.

Kinomics with Pepscan methodology: Arrays were constructed by chemically synthesizing soluble pseudo-peptides, which were covalently coupled to glass substrates. If the design of our peptide array is appropriate, addition of a purified kinase in the presence of ATP should result in the phosphorylation of the appropriate consensus peptide sequences without concomitant phosphorylation of other peptides. The protocol of the kinome array is described in detail at this website: www.pepscanpresto.com/index.php?id=15. Peptide sequences are derived from the human protein reference database: http://www.hprd.org, and in vitro phosphorylation of these peptides is described. Additional verification can come from the fact that for all peptides a corresponding phosphospecific antibody is available. The peptide arrays will be used and analysed as described earlier(*3,9*). Statistical analysis will be ongoing with the group of RC Jansen, Groningen.

As shown in the prior result section clusters of activity will be identified and provisional signal transduction schemes will be made. Potential targets will be analysed in more detail and functional assays for specific inhibitors will be performed.

Proteomics with RPPA methodology. To enable us to perform proteomic profiling we optimized the techniques necessary to perform RPPA on samples from patients with hematological malignancies. The methodology and validation of the technique are fully described in publications from the Kornblau laboratory.(*1,2*) The stained slides are analyzed using Microvigene® software to produce quantified data. Supercurve algorithms are used to generate a single value from the 5 serial dilutions. Loading control and topographical normalization (Neeley, *In Press,* Bioinformatics) procedures account for protein concentration and background staining variations. Analysis using unbiased clustering, perturbation bootstrap clustering and principle component analysis is then performed as fully described.(*1*)

Integrating statistics. The statistical analysis as used before *(1)* of each dataset will include univariate analysis of each protein/peptide ascertaining expression level relative to the median group expression and correlation with clinical, laboratory and cytogenetic features as well as with outcome. Next we will perform unbiased clustering to look for constellations of proteins/peptides that have correlated expression followed by principle component analysis to look for recurrent signatures. Supervised clustering will also be performed to look for expression patterns associated with cytogenetics, progression to AML, response and relapse to different therapies, outcome and other clinical events. Comparison of the pediatric and adult cases will provide insight on the changes in expression associated with age. We anticipate that some patterns will be age specific and others age independent and that these will associate with age related differences in response. The key underlying principle is that influences and interactions between biological entities generate statistical dependencies in the observed data (e.g. if protein A activates protein B, then we expect to see high levels of protein B whenever levels of A are high). This is the foundation upon which we develop the algorithms for the data analysis and modeling in this proposal. A key feature of our probabilistic modeling language is its ability to integrate heterogeneous types of biological data, *e.g.* protein expression, kinome profiles in this proposal. An essential point in the start up of the analysis is the knowledge that the peptide and the phosphospecific antibody are based upon identical proteins. We will learn a Bayesian network that integrates both the protein expression and kinomics in a step-wise manner as follows: We begin with the RPPA data alone and learn network structure as described in[8]. This structure will be the initial

starting point of the second phase, in which we add a random variable for each peptide in the kinome array. Let X_p be a peptide corresponding to phospho-protein X and let Reg(X) be X's regulators in the Bayesian network, then we set Reg(X) to be X_p's regulators as well. We use Bayesian network structure learning, enforcing shared regulators between X and X_p during the learning. Given two independent and complimentary measurements for X (total expression and kinomic potential) we gain a much more robust evaluation of the factors influencing X, the combination being more than a sum of each piece of evidence alone. This will provide insights into altered networks at the single patient level.

Ex vivo drug target assay.For quantification of leukemia cell viability after drug inhibition, cell survival assays will be performed on primary leukemia samples. A WST-1 colorimetric viability assay protocol will be used (Roche). Cells will be seeded at a density of 4x10E4 cells per well in medium supplemented with 1% FBS. The cells will be subjected in quadruple to different concentrations of inhibitor and incubated at 37 °C for 48 hours. Absorbance will be measured at 450 nm in a microplate reader (Benchmark; Bio-Rad, Veenendaal, the Netherlands). The data are presented as the cell survival percentage relative to untreated cells. The LC50 value (drug concentration needed to kill 50% of the leukemic cells) will be used to compare differences between patients and/or various drugs combinations. LC50 value equation: ([% leukemic cell survival>50%]-50)/([% leukemic cell survival>50%]-[% leukemic cell survival<50%])×(drug concentration when leukemic cell survival<50%-drug concentration when leukemic cell survival>50%)+(drug concentration when leukemic cell survival>50%).

Requisted samples and data

We would need fresh material to be able to sort CD34+/CD38- cells, as described above, for analysis of signal transduction pathways on the stem cell fraction.

120 patient samples are needed to be able to perform an unsupervised clustering and subsequent principle component analysis. To calculate the number of patients needed we have used the sample size calculation tool provided on the bioinformatics website of MD Anderson. Our arrays contain 1024 peptides and in previous experiments we assume the standard deviation to be about 0,7. We would like a power of 0,8, and the number of false positives should not exceed 1% (acceptable number of false positives is therefore 10). We want to be able to detect a 1.7-fold difference between groups. This results in a sample size of 20 patients with a per-peptide significance level of $p = 0.0097$. The bonferoni corrected p-value based upon 6 subgroups will entail $p= 0.05$.

This results in a sample size of 20 patient samples times six subgroups is in total 120 patient samples.

For kinome profiling and RPPA of CD34+/CD38- cells 0,4 x 10E6 viable leukemic CD34+/CD38- cells will be needed per patient sample (previous experiments have yielded 0,4 x 10E6 CD34+/CD38- cells out of 20 x 10E6 cells). We are of course willing to collaborate with other groups (e.g. VU Medical center) for logistical purposes and to minimize the amount of material lost by sorting the cells for CD34+/CD38-.

For kinome profiling and RPPA on the bulk of cells and for additional conformational techniques 10 x 10E6 viable leukemic cells will be needed.

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Curriculum vitae applicant

known

7.3 Prognostic relevance of detection Minimal Residual Disease for children with acute myeloid leukemia

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Prof. Dr. J.J.M. van Dongen (Immunology, ErasmusMC)

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Introduction

Clinical significance of MRD in childhood AML

The prognosis of childhood acute myeloid leukemia (AML) has improved considerably over the past decades. The improvements are not only due to intensified chemotherapy, but also to improvements in supportive care, such as antifungal prophylaxis and treatment, as well intensive care and blood product support. Despite these improvements, 30-40% of patients suffer from a relapse, indicating that the current cytotoxic therapy regimens are not always able to kill all malignant cells. Furthermore, the improvement in prognosis is achieved using very intensive chemotherapy, which may result in significant acute toxicity (e.g. mucositis and infections) and late toxicity (e.g. anthracycline-induced cardiomyopathy). To limit unwanted side-effects, it is important to recognize those patients that need intensive therapy for survival and those patients who can be cured with the current treatment protocols, or who may even profit from treatment reduction.

Several studies have shown that detection of minimal residual disease (MRD) in AML is an independent prognostic factor. [1-9] Most MRD studies in AML have focused on adults, and relatively little is known about MRD in childhood AML. Sievers *et al*. showed that, in a retrospective flowcytometric MRD study, the relative risk of relapse in MRD-positive pediatric AML patients was 2.8 times the risk of relapses in MRD-negative patients. [3] MRD was monitored using a standard 3-color immunophenotyping protocol and occult leukemia was defined as more than or equal to 0.5% blasts with aberrant surface antigen expression. Also in a more recent study from the same group, using a comparable flowcytometric approach and the same cut-off level of 0.5%, it could be shown that patients with occult leukemia after induction therapy were almost 5 times more likely to relapse than those lacking detectable MRD. [4] A flowcytometric study by Coustan-Smith *et al*. showed that pediatric AML patients with MRD levels >0.1% after induction therapy had a 2-year survival estimate of 33%, whereas this was 72% for MRD-negative patients. [1] In this 4-color flowcytometric study, an leukemia-associated immunophenotype (LAIP) was detected in 85% of patients and, using patient-tailored labelings, sensitivities of 0.1%-0.01% were obtained. In the recent study of the MRD-AML-BFM Study Group [2], a standard four-color flowcytometric analysis was used with cut-off values dependent on the time-point and specificity of the LAIP. MRD positivity before second induction was associated with a more than 2-fold risk of relapse, but MRD was no longer significant in multivariate analysis. [2]

In conclusion, currently available data show that MRD analysis in childhood AML can be of prognostic relevance. However, more work is required to further optimize the flowcytometric MRD analysis and additional studies are required in order to determine the most optimal time point(s) and cut-off level for MRD analysis in childhood AML.

Recent developments in flowcytometric MRD analysis

Flowcytometric immunophenotyping so far has mainly been limited due to its applicability and sensitivity. However, the introduction of 3-color immunophenotyping in the early nineties and the introduction of 4-color immunophenotyping in the late nineties have significantly improved its applicability and sensitivity. When using 3-color immunophenotyping, an aberrant immunophenotype could only be found in about 60% of childhood ALL cases,[10] but the introduction of 4-color

immunophenotyping increased this frequency to over 90%.[11] Consequently, it can be expected that the recent introduction of 8-color flowcytometers will further improve the applicability and sensitivity of MRD detection in childhood acute leukemia. Within the EuroFlow network, [12] 8-color immunostaining protocols have been designed for all hematological malignancies, including AML. These 8-color immunostaining protocol have been optimized for use with the new Infinicyt software, which allows true multiparameter analysis of flowcytometric data.[13-14]
Flowcytometric immunophenotyping not only has the advantage of being fast en cheap, it also has the advantage of analysis at the single cell level. This is particularly important because childhood acute leukemia cells (especially AML) may be heterogeneous [15] and different subpopulations may respond to therapy differently.[16-18] This heterogeneity may be due to the fact that an acute leukemia arises from a small population of 'cancer stem cells' that gives rise to phenotypically diverse cancer cells, with less proliferative potential. Several studies have shown that such 'cancer stem cells' can be found in AML [19-22] and that these leukemic stem cells possess extensive proliferative capacity and the potential for self-renewal. The possibility that only a small minority of AML cells (<0.01%-10%) have the ability to act as stem cells in vivo and maintain the malignant population has important therapeutic significance, as these cells may be the only relevant target cells for treatment protocols.[23] Furthermore, characterization of the leukemic stem cell in AML is fundamental in order to gain insight into the composition of the leukemic clone and into the cellular and molecular mechanisms that underlie leukemogenesis. Immunophenotyping, culture of sorted cells, and leukemic repopulation in NOD/SCID mice, revealed that the immunophenotype of the stem cell probably is CD34+/CD38-/lin-/HLA-DR-/CD71-/Hoechst 33342 efflux+/CD90+/CD123-.[19,20,22-25] Also CD117 and CD133 may be expressed on normal stem cells.[26] Interestingly, leukemic stem cells may show a slightly different immunophenotype, being Hoechst 33342-efflux-/CD90-/CD123+;[22,24-26] also CD132 may only be present on leukemic stem cells.[27] Identification of leukemic stem cells at diagnosis and flowcytometric monitoring of these cells during treatment should give more insight in true treatment efficacy, because it might well be that leukemic stem cells and the other (more mature) leukemic cells differ in treatment sensitivity.[28-29]

Aims of the study
The here proposed clinical research project has the following aims:
1. To investigate whether flowcytometric MRD detection in childhood AML can be improved in order to detect an aberrant immunophenotype in over 95% of patients and to reach sensitivities which are consistently at least 0.01%, preferably 0.001%. Such improvement should particularly be possible by the introduction of 8-color flowcytometric immunophenotyping in combination with the new Infinicyt software. To support the flowcytometric MRD data, MRD data obtained via RT-PCR analysis of fusion gene transcripts derived from chromosome aberrations and/or *FLT3*-ITD will be used.
2. To evaluate the clinical value of MRD in childhood AML by monitoring of MRD during treatment at predefined sampling points.
    - To determine the prognostic value of the dynamics of tumor-load reduction during induction therapy and thereafter and to analyze whether MRD information can be used for risk group stratification (recognition of low-risk, intermediate-risk, and high-risk groups).
    - To determine whether a reappearance of MRD or increase in MRD after stop of therapy is related to relapse.
    - To determine the prognostic sigmicicance of MRD prior to and after stem cell transplantation.
    - To determine the correlation between MRD results and known prognostic factors like FAB classification and cytogenetic data.
2. To obtain insight in the characteristics of the leukemic cell subsets, particularly leukemic stem cells, at diagnosis and to monitor these subpopulations by flowcytometry during treatment.

Relevance of the study for AML patients
In tegenstelling tot acute lymfatische leukemie, is er bij AML een veel grotere recifiefkans. Zelfs in de "good risk" groep bestaat nog een aanzienlijke kans op recidief. Daarnaast is vroege detectie van een recidief klinisch relevant omdat met een kleinere tumormassa de kans op remissie-herinductie

mogelijk groter is en omdat meer tijd beschikbaar is voor het uitwerken van alternatieve behandelingsopties.
Gezien onze eerdere resultaten van flowcytometrische bepaling van MRD bij kinderen met ANLL, behandeld met de voorafgaande protocollen (SNWLK ANLL-97, MRC AML 12 en 15), is het zinvol om deze resultaten uit te breiden. Inmiddels is de techniek veel gevoeliger geworden (uitbreiding van 4-kleuren naar 8-kleuren flow) en dit zal bijdragen tot een optimalisatie van techniek en uitkomsten.
Dit biedt de mogelijkheid vroeger in de behandeling in te grijpen in de therapie, daarnaast zal het mogelijk zijn een patient met een verdenking recidief sneller op te sporen en dus adequater te behandelen.

Preliminary results

A. Data from the MRC AML12/DCOG ANLL97 study

During the last years we evaluated MRD in 98 pediatric AML patients treated within the AML12/ANLL97 protocol. Bone marrow samples were obtained at diagnosis, before the second course (TP2; n=61), before the third (MACE) course (TP3; n=31), before the second randomisation (TP4; n=27), and at the end of treatment (TP5; n=31).

Identification of LAIP at diagnosis

Flowcytometric immunophenotyping at diagnosis confirmed the presence of an acute myeloid leukemia in all cases. In 85% of patients, subpopulations (≥20% of leukemic cells) could be observed for at least one antigen. In order to limit the risk of false negative MRD results, preferably two or more LAIPs were therefore monitored per patient. This was possible in 68% of cases; in 32% of patients only one LAIP could be defined, and no LAIP could be defined in 6% of patients (this concerned one FAB-M1, one FAB-M2, two FAB-M4, and two FAB-M5). In 61% of LAIPs a sensitivity of at least 0.01% was achieved, in all cases the sensitivity was at least 0.1%. In conclusion, flowcytometric MRD analysis reaching a sensitivity of minimally 0.1% (10^{-3}) is possible in the vast majority of pediatric AML patients.

MRD levels at various time-points

Out of the 96 patients alive after the first course, 11 patients not being in complete hematological remission were excluded for MRD analysis. As shown in **Figure 1A**, MRD levels slowly decreased at later time points. Comparison between patients who ultimately relapsed and those who remained in remission showed that MRD levels before the second course (TP2) and before the third course (TP3) were significantly higher in relapsing patients (**Figure 1B**), whereas no difference was observed before second randomization (TP4) and at the end of therapy (TP5).

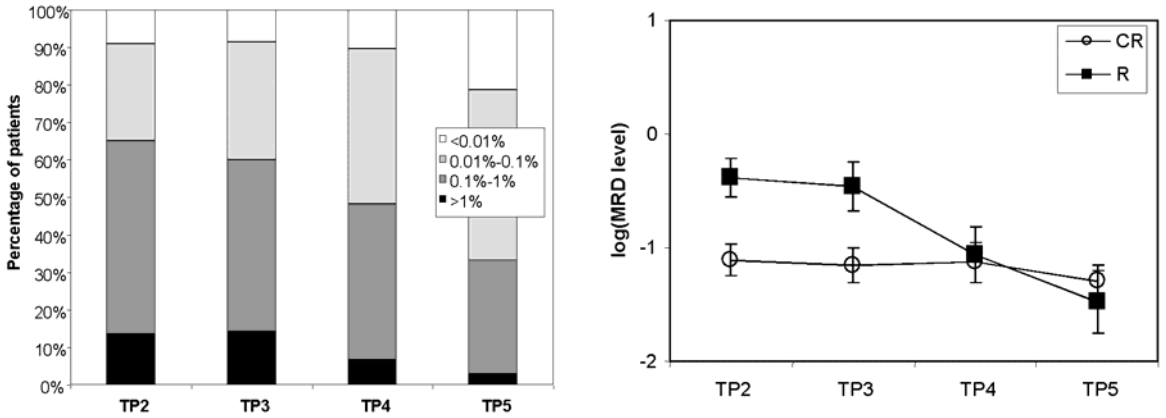

Figure 1. MRD levels during follow-up. A. MRD levels before the second course (TP2), before the thirds course (TP3), before the second randomization (TP4), and at the end of treatment (TP5). **B.** Mean (±SE) MRD levels during and after therapy for patients remaining in remission (open symbols) and patients who relapsed (closed symbols). *: $p<0.05$ between both groups (Mann Whitney test).

Prognostic significance of MRD in the MRC-AML12/DCOG ANLL97 protocol

For all time points evaluated, patients were classified in three groups according to their MRD level: MRD-Low risk if the MRD level was within the first tertile; MRD-Medium risk if the MRD level was within the second tertile; and MRD-High risk if the MRD level was within the third tertile. As shown in **Figure 2A**, MRD levels before the second course (TP2) were significantly related to the risk of relapse. MRD-Low risk patients (n=20) had a 5-year relapse free survival of 81%±9%, MRD-Medium risk patients (n=21) had a 5-y RFS of 65%±11%, and MRD-High risk patients (n=20) had a 5-y RFS of only 15%±8% (p[log rank]<0.0001). MRD-based risk group classification at TP2 was also significantly related to overall survival (p[log rank]<0.0001) (**Figure 2B**). At TP3 MRD levels were significantly related to the risk of relapse: the 5-y RFS for the MRD-Low risk (n=10), Medium risk (n=11), and High risk (n=10) was 67%±16%, 90%±9%, and 27%±13%, respectively (p[log rank]<0.01). MRD-based risk group classification at TP3 was also significantly related to overall survival: MRD-Low risk, Medium risk, and High risk had on overall survival of 56%±17%, 91%±9%, and 20%±13%, respectively (p[log rank]<0.05)

MRD at later time points was not significantly associated with relapse-free or overall survival, also not if other cut-off points were used (data not shown). Combining MRD information at two subsequent time points did not provide additional prognostic information (data not shown).

Since the minimum sensitivity of the flowcytometric MRD analysis was 0.1%, we also grouped patients for the various time-points in MRD negative (MRD level <0.1%) and MRD-positive (MRD level≥0.1%). At TP2, MRD-negative patients (n=22; 35%) had a significant higher RFS than MRD positive patients (n=40; 65%): 81%±9% versus 38%±8% (p[log rank]<0.01)(**Figure 2C**). Also overall survival was significantly better in the MRD-negative patients (85%±8% versus 50%±8%; p[log rank]<0.01) (**Figure 2D**). At other time-points, this classification had no prognostics significance.

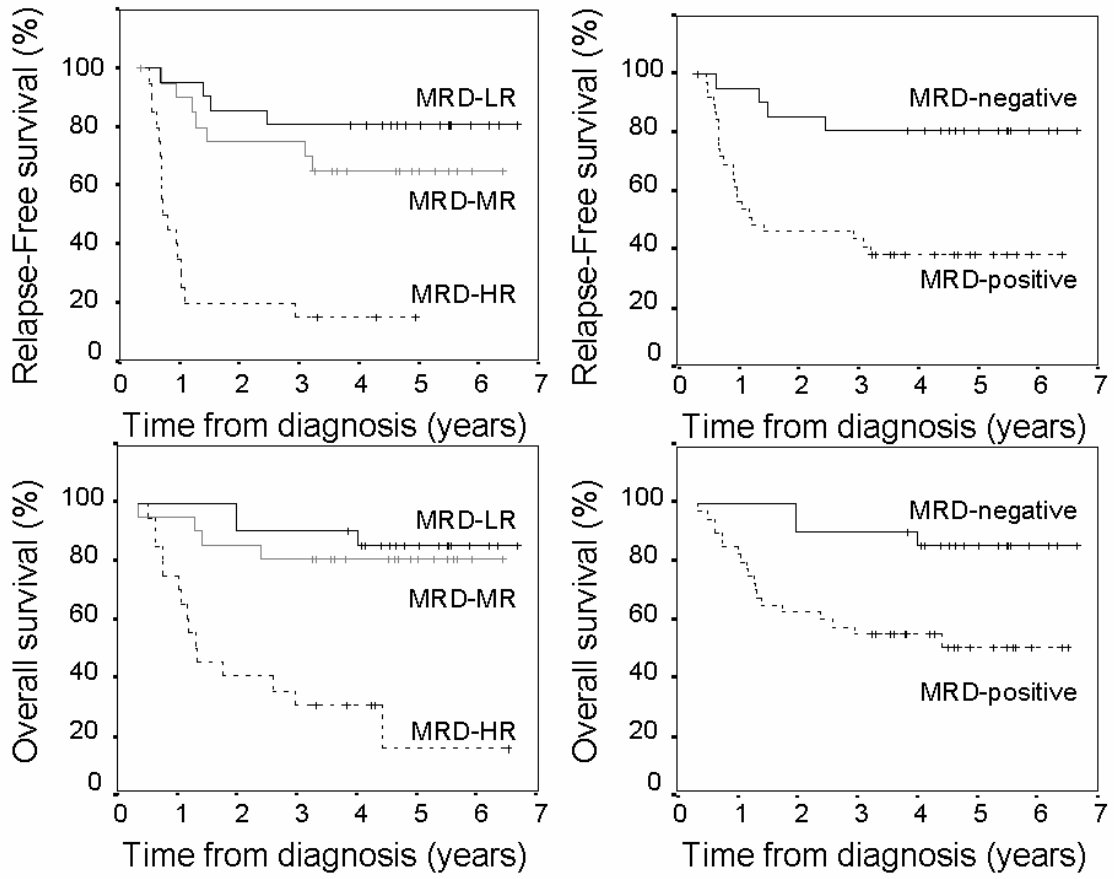

Figure 2. Probability of Relapse-free and overall survival according to MRD. Based on MRD levels before the second course (TP2), patients were classified as MRD-high risk, MRD-medium risk, or MRD-low risk (according to MRD tertiles, see text for details) or classified as MRD-negative (MRD<0.1%) or MRD positive(MRD≥0.1%). All patients included were in morphological remission at TP2. A and B: Relapse-free survival for the various MRD-based risk groups; C and D: Overall survival for the various MRD-based risk groups.

MRD is an independent prognostic factor
To evaluate whether MRD (positive *vs*. negative) was an independent prognostic factor, multivariate analysis including age, the presence or absence of fusion gene transcripts, the presence of absence of *FLT3*-ITD and MRD before the second course was performed. This analysis showed that MRD at TP2 was an independent prognostic parameters for relapse-free survival. MRD at TP2 also maintained it prognostic significance for overall survival in multivariate analysis.

Stability of LAIP between diagnosis and relapse
To investigate whether immunophenotypic shifts occurred between diagnosis and relapse, we compared the immunophenotypes of 27 out of 41 relapsed patients from who paired diagnosis and relapse samples were available. Immunophenotypic shifts at relapse were frequently observed: in 21 out of 27 paired diagnosis and relapse samples (78%), an immunophenotypic shift was observed. We observed that CD34, CD117, and HLA-DR were more frequently gained at relapse, whereas CD14, CD11b, and CD15 were more frequently lost at relapse, suggesting that the immunophenotype at relapse was more immature.
Of importance, in 23 out of the 27 (85%) relapsed AML patients in our study, detection of MRD would have been possible using the patient-tailored labeling(s). In four patients, a part of the leukemic cells at relapse would have been missed using the LAIP labelings. However, all these four relapsed patients were classified as MRD-High risk at TP2, indicating that the immunophenotypic shift at relapse did not hamper reliable MRD detection at this early time point. At TP3, two patients were classified as MRD-High risk and one patient as MRD-Low risk (no data available for fourth patient).

B. Molecular MRD analysis
Our laboratory has ample experience in the detection of fusion gene transcripts associated with chromosomal translocations, including AML1-ETO, PML-RARA, and CBFB-MYH11, and coordinated several international networks in this files (BIOMED-1 Concerted Action.[30], 'Europe Against Cancer' study.[31,32]). In addition, the presence of FLT3-ITD is analyzed by RT-PCR analysis and expression of WT1 is determined according to international standardization protocols (European LeukemiaNet;).[33,34]
In the 98 pediatric AML patients analyzed within the AML12/ANLL97 protocol, AML1-ETO and CBFB-MYH11 fusion gene transcripts were detected in 15% and 5% of patients, respectively. FLT3-ITD were detected in 19% of patients, including one patient with AML1-ETO. FLT3-ITD were however lost at relapse in four out of seven patients (57%) in our study; this instability of FLT3-ITD between diagnosis and relapse may hamper its use as MRD target.

C. New flowcytometric approaches
Within the EuroFlow network (EU-FP6 LSHC-CT-2006-018708), coordinated by the Department of Immunology, Erasmus MC, and with participation by the DCOG, 8-color immunophenotyping protocols have been designed and standardized. Using new Infinicyt software tools, markers most informative in separating AML cells from normal myeloid cells can automatically be obtained, thereby allowing a more objective and reliable MRD analysis. In addition, the new Infinicyt software will facilitate the recognition of low numbers of residual AML cells between normal (regenerating) myeloid cells.13-14

Materials and methods
Voor de MRD detectie zal vooral gebruik worden gemaakt van flowcytometrische MRD detectie en van RQ-PCR gemediëerde MRD detectie van fusiegentranscripten of andere genetische afwijkingen, zoals *FLT3*-ITD en *WT-1*.
Voor de flowcytometrische MRD analyse zullen patiënten bij diagnose worden getypeerd middels de EuroFlow 8-kleuren panels. Op basis van het immunofenotype zullen middels de Infinicyt software markers worden gedefinieerd die het bese de AML cellen onderscheiden van normale myeloide cellen. Op basis van deze gegevens zullen patient-specifieke 8-kleuren labelingen worden ontwikkeld en ingezet bij diagnose en later tijdens follow-up. De Infinicyt software zal gebruikt worden om residuele

AML cellen te onderscheiden van aanwezige normale myeloide cellen. Daarnaast zullen AML stamcellen zowel bij diagnose als tijdens follow-up worden geanalyseerd middels een 8-kleuren labeling.

Hoewel het percentage ANLL patiënten met een geschikt RT-PCR target beperkt is (25 à 50%), kan deze MRD techniek wel degelijk relevant zijn voor de studie als aanvulling op en ondersteuning van de flowcytometrische MRD detectie. Toevoegen van deze MRD techniek is makkelijk haalbaar, omdat binnen de BIOMED-1 Concerted Action gestandaardiseerde primer sets zijn ontwikkeld voor de meest voorkomende chromosoomafwijkingen bij ANLL. Daarnaast zijn binnen het 'Europe Against Cancer Project' primer/probe sets ontworpen voor de gevoelige en kwantitatieve detectie van fusiegentranscripten gedurende follow-up. De aanwezigheid van *FLT3*-ITD en overexpressie van *WT-1* kunnen worden gedetecteerd met recent opgezette RQ-PCR technieken.

Requested samples and data

Bij diagnose wordt het aan te leveren bloed- en beenmergmonster onderzocht op het voorkomen van een aberrant immunofenotype. Naar aanleiding hiervan zullen de beide laboratoria gezamenlijk "patiënt-specifieke" achtvoudige labelingen samenstellen voor flowcytometrische MRD detectie in follow-up monsters. Daarnaast zal bij diagnose worden vastgesteld of er fusiegentranscripten, *FLT3*-ITD, of *WT-1* overexpressie aanwezig zijn.
Het MRD onderzoek zal plaatsvinden bij alle beenmergmonsters, die in het kader van de behandeling worden afgenomen: na 15 dagen inductietherapie, na ieder behandelingsblok, bij einde behandeling en vervolgens 3-maandelijks in het eerste jaar. Daarnaast zullen ook eventuele recidiefmonsters worden geanalyseerd om vast te stellen of, en zo ja in welke mate, het immunofenotype is veranderd. Deze tijdstipppen vallen samen met de reguliere beenmergpuncties om de remissie-status vast te stellen.
Om zo zorgvuldig mogelijk met materiaal om te springen, is deze add-on studie volledig afgestemd met de add-on studie van Cloos et al., waarbij uitwisseling van gegevens wederzijds zal gebeuren, alsmede zn uitwisseling van restmateriaal. Voor uitgebreide beschrijving van deze logistiek, verwijzen wij u naar de add-on studie van Cloos et al.

In Nederland worden ongeveer 25-30 kinderen met ANLL per jaar gediagnosticeerd. Daarnaast zullen ook de Belgische patiënten deelnemen aan deze studie, hun aantal wordt geschat op 10-15 patienten per jaar. Dit betekent reëel een inclusie van ongeveer 30 patienten (uitval door te weinig materiaal, geen informed consent). Totaal zal in een periode van vier jaar daarom maximaal 120 kinderen in de MRD studie worden opgenomen. Alhoewel deze aantallen vrij beperkt zijn om de klinische betekenis van MRD in kinderen met AML zeer betrouwbaar vast te stellen, zullen ze in combinatie met de MRD data verkregen in AML12/ANLL97 en AML15 mogelijk voldoende basis vormen voor MRD-gebaseerde stratificatie in toekomstige Nederlandse AML behandelingsprotocollen.

Coordination

Het flowcytometrisch MRD onderzoek van de Nederlandse kinderen zou moeten worden uitgevoerd door het SKION laboratorium in nauwe samenwerking met afdeling Immunologie van het Erasmus MC. De RT-PCR studies van de Nederlandse kinderen met AML zullen worden uitgevoerd door de afdeling Immunologie van het Erasmus MC.

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7.4 Prognostic significance of early AML blast clearance and of routine bone marrow and peripheral blood monitoring by simple morphology during and after chemotherapy in pediatric AML

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Introduction

The cure rate of pediatric AML has improved significantly, but relapses still occur in 30-40% of patients in high-income countries, and in an even higher percentage of patients in low-income countries. It is clinically relevant to predict relapse early in the treatment, potentially allowing adaptation of treatment in such patients. Well-known prognostic factors in pediatric AML are early treatment response, cytogenetics and minimal residual disease. The latter is cumbersome, and not available in all countries. Moreover, sofar it has not been shown to be as successful as in ALL, and is not being used yet for risk-group adapted therapy. Early treatment response has usually been studied by bone marrow (BM) examination on days 15 and "30". To the best of our knowledge, studies on the prognostic significance of the dynamics of clearance of AML blasts from the peripheral blood (PB) have not been performed in pediatric AML.
While searching for prognostic factors, it has not convincingly been shown that early detection of AML relapse is clinically relevant, except for APL (AML FAB type M3). However, BM and PB sampling is routinely being done in many pediatric AML protocols. In fact, without evidence that such routine monitoring (including conventional examination by morphology) is able to detect a relapse in patients in whom otherwise a relapse was not suspected.
This study is not aimed at MRD detection, although correlating morphology and MRD data seems of great interest.

Aims of the study

1. To prospectively study the prognostic significance of early clearance of the PB from AML blasts, in association with the prognostic significance of day 8, 15 and 33 BM morphology results. The hypothesis is that early clearance of blasts from the PB will be of prognostic significance.
2. To prospectively determine the usefulness of routine BM and PB examination by morphology during and after treatment, done according to routine protocol guidelines, in detecting AML relapse, that was not suspected otherwise (based on signs or symptoms already suggesting a relapse). The hypothesis is that such routine monitoring is not useful in non-M3 AML patients.

Relevance of the study

This study may provide a novel prognostic factor that will be available very early in therapy, and that potentially will enable adapting the treatment accordingly. In view of its simplicity, these factors (early examination of BM and PB) will also be available to low-income countries, which can not afford expensive techniques to detect MRD.
Moreover, the study may learn whether it is useful for relapse detection to routinely sample BM and PB in children that have no complaints or signs at physical examination suggesting a relapse. Such

procedures should only be done if such usefulness has been demonstrated, and currently that evidence is lacking. This study aims to provide evidence pro or against routine monitoring..

Materials and Methods

1. Pediatric AML patients being treated according to protocol DB-AML-01.
2. BM on days 8, 15, 33 and other time-points as described in the protocol already; examined by simple morphology. After achieving hematologic remission, this concerns a BM before each consolidation course, 4 weeks after the 2nd HA2E course, and 6 months after the end of the 2nd HA2E course.
3. PB: daily during hospital admission for the first course of therapy, and twice weekly afterwards, until clearance of the PB from AML blasts as determined by simple morphology. Routine PB sampling will be done with each BM examination, and in the first year after completion of therapy every month, in the second year every 2 months, in the third year every 4 months, and in the fourth and fifth years every 6 months.

Statistics

Descriptive, prospective study, in all children with pediatric AML that will be treated according to protocol DB-AML-01. Power-analysis does not seem possible, but this study will enable such calculations in a next study that should prospectively confirm the results. Although the number of patients will be limited (on average, 35 patients per year), each patient provides many data because of sequential sampling.

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7.5 Kwaliteit van leven en slaap tijdens en na behandeling voor acute myeloide leukemie op de kinderleeftijd

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Introduction

Acute Myeloide Leukemie (AML) is een zeldzame aandoening op de kinderleeftijd, met circa 20-25 nieuwe gevallen per jaar in Nederland. Een groot deel van de kinderen geraakt in complete remissie (85-90%), overall survival is echter 50 tot 60% in verband met een hoog aantal recidieven. Ondanks de toenemende overleving is er in de literatuur maar weinig aandacht voor de late effecten van AML op de kinderleeftijd. Weinig studies richten zich specifiek op deze groep overlevers, al zijn er wel aanwijzigingen voor late effecten zowel op medisch als op psychosociaal gebied. (1)
Binnen de (kinder)oncologie is toenemende aandacht voor het effect van behandeling op kwaliteit van leven en psychosociaal functioneren, slaap en vermoeidheid, tijdens en na behandeling. Onderzoek richt zich met name op die aandoeningen die een heel goede prognose hebben, zoals acute lymfatische leukemie (ALL). Tijdens behandeling voor aandoeningen met een minder goede prognose, zoals AML, staan deze factoren minder op de voorgrond en wordt hier in de internationale literatuur niet of nauwelijks over gerapporteerd.
Behandeling voor AML is relatief kortdurend, maar intensief. Verwacht wordt dat kinderen een duidelijk verminderde kwaliteit van leven hebben tijdens behandeling, zowel ten opzichte van de norm als ten opzichte van bepaalde andere maligniteiten op de kinderleeftijd, bijvoorbeeld ALL. Kwaliteit van leven zal vermoedelijk na het einde van de behandeling verbeteren, mits er geen recidief optreedt.
Er is weinig literatuur bekend over vermoeidheid en slaap bij kinderen met leukemie. Over vermoeidheid en slaap bij kinderen onder behandeling voor ALL is wel, maar weinig en wisselend, bericht. (2-6) Aangezien vermoeidheid een belangrijke bijwerking is van oncologische behandelingen bij volwassenen, zou het kunnen dat vermoeidheid wellicht een onderschat probleem bij (jonge) kinderen is. Er zijn in ieder geval aanwijzingen dat vermoeidheid anders omschreven of beleefd wordt door kinderen en zorgverleners.(7)
De incidentie van vermoeidheid en slaapproblemen zal naar verwachting bij AML patiënten hoger zijn ten opzichte van de incidentie in de normpopulatie. Aangezien meer in het algemeen aanhoudende problemen op het gebied van vermoeidheid en slaap worden gevonden bij overlevers van kanker op de kinderleeftijd (8), is de verwachting dat dit ook geldt voor kinderen na behandeling voor AML.
Mede gezien de toenemende overleving is meer kennis over het beloop van de kwaliteit van leven, slaap en vermoeidheid tijdens behandeling essentieel om gerichter hulp te kunnen bieden, tijdens en na de behandeling.

Aims of the study

Vraagstellingen:

1. Hoe ontwikkelt zich de kwaliteit van leven van kinderen met AML tijdens en na behandeling?
   a. Verbetert en/of normaliseert de kwaliteit van leven na het einde van de behandeling?
   b. Is er verschil met kwaliteit van leven zoals gevonden bij kinderen met andere maligniteiten (bijvoorbeeld ALL)?

2. Hoe is de slaap van kinderen met AML tijdens en na behandeling?
    a. Verschilt de incidentie of het karakter van slaapproblemen van de norm?
    b. Verbeteren of normaliseren aanwezige slaapproblemen na het einde van de behandeling?
    c. Is de incidentie of het karakter van slaapproblemen verschillend ten opzichte van kinderen die worden behandeld voor andere maligniteiten?

3. Is er sprake van (toegenomen) vermoeidheid bij kinderen met AML tijdens en na behandeling?
    a. Is de incidentie van vermoeidheid verschillend van de norm?
    b. Is de incidentie van vermoeidheid verschillend ten opzichte van kinderen behandeld voor andere maligniteiten?
    c. Verbetert of normaliseert vermoeidheid na het einde van de behandeling?

Materials and methods

Alle ouders van nieuw gediagnosticeerde patiënten met AML in de leeftijd van 2-18 jaar die in de deelnemende Nederlandse kinderoncologische centra worden behandeld met het AML protocol zullen worden gevraagd om deel te nemen. Om de ontwikkeling van de uitkomstmaten (kwaliteit van leven, slaap en vermoeidheid) longitudinaal te vervolgen, zullen vragenlijsten worden uitgedeeld of toegestuurd: bij diagnose (T=0), halverwege (T=1) en aan het einde (T=2) van de behandeling, 6 maanden na het stoppen van de behandeling (T=3) en 12 maanden na het stoppen van de behandeling (T=4).

Binnen de kindergeneeskunde is men voor het verkrijgen van gegevens over het kind vaak op de ouders/verzorgers aangewezen. Over de betrouwbaarheid van het gebruik van proxy-respondenten (bijvoorbeeld ouders in plaats van het kind) bestaat geen consensus. (9, 10) In het algemeen wordt geadviseerd ook het kind bij het beoordelen van zijn of haar kwaliteit van leven te betrekken, indien de leeftijd en fysieke toestand van het kind het toelaten. Zo mogelijk zullen vragenlijsten door zowel ouders als kind (indien 8 jaar of ouder) ingevuld worden.

In het afgelopen decennium zijn een aantal betrouwbare en valide instrumenten ontwikkeld waarmee kwaliteit van leven van kinderen door ouders en door kinderen zelf kan worden beoordeeld. Deze instrumenten zijn meerdimensioneel en bieden uitkomsten op verschillende aspecten van kwaliteit van leven: medisch, functioneel en psychosociaal.

1. Kwaliteit van leven zal worden bepaald met behulp van 2 soorten meetinstrumenten: een generiek meetinstrument en een ziekte-specifiek meetinstrument. De duur van het invullen van deze twee instrumenten wordt geschat op ca. 15 min voor ouders. De vragenlijst voor kinderen is langer, invulduur wordt geschat op 30 minuten. Om redenen van internationale vergelijkbaarheid van de Nederlandse gegevens bestaat voor de meetinstrumenten voor kwaliteit van leven een voorkeur voor het gebruik van Angelsaksische instrumenten.
    a. Child Health Questionnaire (CHQ) voor ouders van kinderen vanaf 5 jaar en de CHQ versie voor kinderen vanaf 8 jaar. Dit zijn van de SF-36 afgeleid generieke (i.e. niet ziekte-specifiek) instrumenten die, mede vanwege de goede ontwikkeling en validering, toepassing vinden in uiteenlopende taalgebieden.(11) De CHQ is in het Nederlands vertaald en gevalideerd door één van de aanvragers van deze ALL-10 add-on studie in nauwe samenwerking met de oorspronkelijke (Amerikaanse) auteurs. (12-14)
    b. Voor ouders van kinderen onder de 5 jaar zal gebruik gemaakt worden van de Infant Toddler Quality of Life Questionnaire (ITQOL). Voor deze leeftijdscategorie zijn slechts weinig QoL vragenlijsten beschikbaar. Deze vragenlijst is conceptueel gelijk aan de CHQ en geschikt voor ouders van kinderen van 2 maanden tot 5 jaar. Een Nederlandse vertaling met adequate betrouwbaarheid en validiteit en leeftijdspecifieke normgegevens is beschikbaar. (15)
    c. Als ziekte-specifiek instrument zal de Pediatric Cancer Quality of Life Inventory (PedsQL) worden gebruikt. (16) Dit instrument is één van de weinige kanker-specifieke kwaliteit van leven meetinstrumenten voor kinderen en een aantal jaar geleden in overleg

met de auteur door onze groep vertaald naar het Nederlands. Dit instrument is ook gebruikt in een aantal USA kinderoncologie studies en tijdens het Nederlandse ALL10 protocol. Hiermee zullen de Nederlandse resultaten kunnen worden vergeleken. De PedsQL kan door alle ouders van kinderen ≥2 jaar en door kinderen vanaf 5 jaar worden ingevuld.

2. Slaapproblemen zullen worden geïnventariseerd door middel van:
    a. Children's Sleep Habits Questionnaire (CSHQ), abbreviated version, voor ouders van kinderen van 2 tot 12 jaar en de Sleep Self Report (SSR) voor kinderen van 8 tot 12 jaar. Door het ontbreken van een Nederlandse vragenlijst gericht op slaapproblemen bij kinderen werd deze van origine Amerikaanse vragenlijst met toestemming van de oorspronkelijke auteur recent in het Nederlands vertaald. De vragenlijst heeft een adequate betrouwbaarheid en validiteit.(17) [Waumans, van Litsenburg, submitted] De slaapgewoonten van meerdere populaties kinderen werden hiermee in kaart gebracht.(18-20) Nederlandse normgegevens voor de CSHQ werden recent door aanvragers verkregen uit een gezonde populatie kinderen. [van Litsenburg, submitted]
    b. CSHQ adolescent versie voor ouders van kinderen vanaf 12 jaar en voor kinderen vanaf 12 jaar. De vragenlijst is gebaseerd op de CSHQ en kan op itemniveau vergeleken worden. Deze versie werd recent met toestemming van de originele auteur naar het Nederlands vertaald. Nederlandse normgegevens worden momenteel verzameld. Deze (originele) versie van de CSHQ is (nog) niet gevalideerd, maar een beter alternatief is nog niet voor handen.
3. Vermoeidheid
   Voor het objectiveren van vermoeidheid zal gebruik gemaakt worden van de PedsQL Multidimensional Fatigue Scale (MFS), een vragenlijst met adequate validiteit en betrouwbaarheid. Deze vragenlijst werd al in meerdere pediatrisch oncologische studies gebruikt. (2, 21) De MFS kan door alle ouders van kinderen ≥2 jaar en door kinderen vanaf 5 jaar worden ingevuld.

De bovenstaande instrumenten onder punt 1 en 3 bestaan uit leeftijdsspecifieke versies. De versies worden op dezelfde manier verwerkt en leiden tot een vergelijkbare totaalscore en/of subschaalscores. Dat betekent dat voor de twee kwaliteit van leven instrumenten (CHQ/ITQOL en PedsQL cancer version) en de vermoeidheidsvragenlijst (PedsQL MFS) één totaal en/of subschaal score voor ouders en een voor kinderen voor de gehele leeftijdsrange wordt verkregen.
Voor de vragenlijst genoemd onder 2, de CSHQ adolescenten versie en de SRR, geldt dit (nog) niet, maar bestaat de mogelijkheid om een item-tot-item "best fit" model te construeren, zoals al eerder voor de originele versie is gedaan door de oorspronkelijke auteur. Nederlandse normdata voor deze vragenlijst zijn/worden momenteel verzameld en dus bestaat de mogelijkheid om hierdoor een uniformere score weer te geven voor de gehele leeftijdsrange.

Tabel 1 geeft een overzicht van vragenlijsten en afnamemomenten.

Tabel 1. Overzicht te gebruiken vragenlijsten bij het onderzoek naar Kwaliteit van leven en slaap tijdens en na behandeling voor acute myeloide leukemie op de kinderleeftijd.

	T0 Na diagnose		T1 Halverwege behandeling		T2 Einde behandeling		T3 6 mnd na einde behandeling		T4 12 mnd na einde behandeling	
	Ouder	Kind (≥8 jr)	Ouder	Kind (≥8 jr)	Ouder	Kind (≥8 jr)	Ouder	Kind (≥8 jr)	Ouder	Kind (≥8 jr)
CHQ/ITQOL	+		+		+		+		+	
CHQ		+		+		+		+		+
PedsQL cancer	+	+	+	+	+	+				
CSHQ	+		+		+		+		+	
SSR/CQSH adolescenten versie		+		+		+		+		+
PedsQL MFS	+	+	+	+	+	+	+	+	+	+

Exclusiecriteria zijn:
- Belangrijke preëxistente co-morbiditeit (zoals b.v. Syndroom van Down).
- Het niet goed beheersen van de Nederlandse taal in woord en geschrift.
- Leeftijd ≤ 2 jaar.

Relevance of the study for AML patients
De relevantie is gelegen in het vroegtijdig opsporen van problemen op het terrein van kwaliteit van leven, ook van problemen die niet blijken te herstellen na de behandeling. Het onderzoek lijkt erg voor de hand liggende vragen op te gaan lossen, maar het onderzoek bij kinderen met ALL heeft toch al geleerd dat er problemen kunnen zijn met veel impact op de kwaliteit van leven, die in de spreekkamer vaak niet worden gemeld. Een goed voorbeeld is slaapproblematiek. De vragenlijsten zoals hier voorgesteld kunnen dergelijke problemen inzichtelijk maken, evenals de incidentie ervan. In de toekomst zou een gevolg van dit onderzoek kunnen zijn dat bij alle kinderen met AML tijdens en na de behandeling dit soort vragenlijsten periodiek worden afgenomen, en dat interventies gericht op het verbeteren van kwaliteit van leven worden ontwikkeld en geïmplementeerd in de reguliere zorg.

Requested samples and data
Participatie:
Op basis van deelname aan de SKION studie "ALL-10 behandeling bij kinderen met acute lymfatische leukemie: een studie naar aanpassing en kwaliteit van leven en de determinanten hiervan", deels uitgevoerd door de aanvragers van deze studie, wordt een hoge participatiegraad verwacht. 97% van alle ouders die werden uitgenodigd aan deze ALL-10 add-on studie deel te nemen, deed daadwerkelijk mee. Van alle kinderen gediagnosticeerd met ALL en behandeld volgens het ALL-10 protocol in de deelnemende centra, voldeed 12% aan de exclusiecriteria (5,4% taalproblematiek, 3,8% Down syndroom of andere belangrijke co-morbiditeit, 2,7% leeftijdscriterium). Gezien het vergelijkbare karakter van deze studie, is de verwachting dat de participatiegraad ook voor deze studie hoog zal zijn.

Analyseplan:
- Verschillen in scores op de verschillende vragenlijsten tussen kinderen met AML, kinderen met andere maligniteiten (zoals kinderen behandeld volgens het ALL-9 of ALL-10 protocol) en de gezonde controlegroep zullen worden geanalyseerd door middel van t-toetsen. De klinische relevantie zal worden bepaald door middel van effect sizes: volgens Cohen's regels voor de berekening van effect size wordt een effect size > 0.2 en < 0.5 beschouwd als een klein effect, > 0.5 en < 0.8 een matig effect en > 0.8 een groot effect. (22, 23)
- Veranderingen van de eerder genoemde uitkomstmaten over tijd, zullen geanalyseerd worden door middel van repeated measure ANOVA en generalized estimating equations, waarbij effecten van andere factoren (zoals leeftijd, complicaties, socio-economische factoren) kunnen worden meegenomen.
- Samenhang tussen de verschillende uitkomstmaten kwaliteit van leven, slaap, en vermoeidheid zullen geanalyseerd worden door middel van Pearson's productmoment-correlatie en multipele regressie analyses.

Powerberekening:
Door het ontbreken van gegevens over de uitkomstmaten bij kinderen met AML, kan geen powerberekening obv AML behandeling worden verricht. Met gegevens verkregen uit de add-on studie studie "ALL-10 behandeling bij kinderen met acute lymfatische leukemie: een studie naar aanpassing en kwaliteit van leven en de determinanten hiervan" kan een aanname worden gedaan met betrekking tot benodigde sample size. Op basis van verandering in kwaliteit van leven tussen meting na diagnose en halverwege de behandeling, is voor het aantonen van een klein effect van 0.3 en een beta van 0.2 (power 0.8), afhankelijk van het gemeten aspect van kwaliteit van leven, een sample size van 20 (fysieke schaal) tot 50 (psycho-sociale schaal) patiënten nodig. Voorgesteld wordt circa 50 kinderen te includeren. Indien alle kinderoncologische centra in Nederland deelnemen aan deze add-on studie en deelname en exclusie gelijk zijn aan de ALL-10 add-on studie, kunnen er in 3 jaar voldoende patiënten geincludeerd worden.

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