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

NOPHO

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)

AIET → Evaluation (BM day 15)

- AM after recovery
  - And t(8;21)
  - FLA-Dx after recovery
  - FLA-Dx immediately

CR → HA₂E + HA₃ + HA₂E

- Blasts ≤ 5%

- Blasts > 5%; off protocol: eligible for AML relapse study
Dutch-Belgian pediatric AML protocol
Examinations overview
(based on NOPHO-AML 2004)

**Diagnostic BM**
- Morphology
- Flow cytometry
- Cytogenetics

**AIET**

**Evaluation (BM day 15)**

- **Echo**

- **BM**

**AM**
- After recovery

- **Blasts ≤5%**

- **FLA-Dx**
- And t(8;21)
- FLA-Dx after recovery

**Blasts ≥5%; off protocol: eligible for AML relapse study**

**BM**

**CR**

- **HA₂E**
- **HA₃**
- **HA₂E**

**Echo**

**(BM)**

**BM**

**6 months from 2nd HA₂E**

**BM**

**PB+BM Review**
INTRODUCTION

This protocol concerns the management of children with newly diagnosed acute myeloid leukemia (AML). The protocol committee 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 transplantation 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)

<table>
<thead>
<tr>
<th>Study, years of enrolment and reference</th>
<th>Patient number</th>
<th>Early death rate (%)</th>
<th>CR rate (%)</th>
<th>5-year pEFS (% with s.e.)</th>
<th>5-year pOS (% with s.e.)</th>
<th>% death rate in CR</th>
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<td>AIEOP92 (1992–2001)10</td>
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<td>DCOG-ANLL 92/94 (1992–1998)7</td>
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<td>82</td>
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<td>42 (6)</td>
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<tr>
<td>EORTC-CLG 58921 (1993–2000)3</td>
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<td>2</td>
<td>84</td>
<td>48 (4)</td>
<td>62 (4)</td>
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<td>NOPHO-AML93 (1993–2001)8</td>
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<td>92</td>
<td>50 (3)</td>
<td>66 (3)</td>
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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/m² 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/m². 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/m². On the basis of the findings current up to 2009 we limited the cumulative dose of anthracyclins to 330 mg/m² 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**

<table>
<thead>
<tr>
<th>AIEOP</th>
<th>Associazione Italiana Ematologia ed Oncologia Pediatrica</th>
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<tbody>
<tr>
<td>ALL</td>
<td>Acute lymphoblastic leukemia</td>
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<tr>
<td>AML</td>
<td>Acute myeloid leukemia</td>
</tr>
<tr>
<td>AML-BFM</td>
<td>AML Berlin-Frankfurt-Münster group</td>
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<tr>
<td>ANC</td>
<td>Absolute neutrophil count</td>
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<tr>
<td>APL</td>
<td>Acute promyelocytic leukemia</td>
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<td>BM</td>
<td>Bone marrow</td>
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<td>BSPHO</td>
<td>Belgian Society of Paediatric Haematology-Oncology</td>
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<tr>
<td>CCG</td>
<td>Children's Cancer Group (USA)</td>
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<tr>
<td>CCR</td>
<td>Continued complete remission</td>
</tr>
<tr>
<td>CNS</td>
<td>Central nervous system</td>
</tr>
<tr>
<td>COG</td>
<td>Children’s Oncology Group (USA)</td>
</tr>
<tr>
<td>CR</td>
<td>Complete remission</td>
</tr>
<tr>
<td>CSF</td>
<td>Cerebrospinal fluid</td>
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<tr>
<td>DCOG</td>
<td>Dutch Childhood Oncology Group</td>
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<tr>
<td>DFS</td>
<td>Disease-free survival</td>
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<td>DS</td>
<td>Down syndrome</td>
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<tr>
<td>EFS</td>
<td>Event-free survival</td>
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<tr>
<td>FAB</td>
<td>French American British</td>
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<tr>
<td>FISH</td>
<td>Fluorescent in situ hybridization</td>
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<tr>
<td>GO</td>
<td>Gemtuzumab ozogamicin</td>
</tr>
<tr>
<td>IBFM-SG</td>
<td>International Berlin-Frankfurt-Münster Study Group</td>
</tr>
<tr>
<td>ICC (APL)</td>
<td>International Consortium on Childhood (APL)</td>
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<tr>
<td>JMML</td>
<td>Juvenile myelomonocytic leukemia</td>
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<td>MDS</td>
<td>Myelodysplastic syndrome</td>
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<tr>
<td>MRC</td>
<td>Medical research council (UK)</td>
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<tr>
<td>MRD</td>
<td>Minimal residual disease</td>
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<tr>
<td>MSD</td>
<td>HLA-matched sibling donor</td>
</tr>
<tr>
<td>MUD</td>
<td>HLA-matched unrelated donor</td>
</tr>
<tr>
<td>NOPHO</td>
<td>Nordic Society for Paediatric Haematology and Oncology</td>
</tr>
<tr>
<td>NR</td>
<td>No response</td>
</tr>
<tr>
<td>OS</td>
<td>Overall survival</td>
</tr>
<tr>
<td>POG</td>
<td>Pediatric Oncology Group (USA)</td>
</tr>
<tr>
<td>PR</td>
<td>Partial response</td>
</tr>
<tr>
<td>SCT</td>
<td>Stem cell transplantation</td>
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<tr>
<td>VOD</td>
<td>Veno-occlusive disease of the liver</td>
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<tr>
<td>WBC</td>
<td>White blood cell count</td>
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<td>WHO</td>
<td>World Health Organization</td>
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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/m² 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>
<thead>
<tr>
<th>Cytogenetic Finding</th>
<th>N</th>
<th>%</th>
<th>Events</th>
<th>EFS (%)</th>
<th>OS (%)</th>
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<td>diploid</td>
<td>55</td>
<td>27</td>
<td>30</td>
<td>42</td>
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<tr>
<td>t(8;21) (q22;q22)</td>
<td>18</td>
<td>9</td>
<td>8</td>
<td>56</td>
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<td>11q23 abnormality</td>
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<td>t(9;21) (p22;q23)</td>
<td>16</td>
<td>8</td>
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<td>non-t(9;11)</td>
<td>16</td>
<td>8</td>
<td>10</td>
<td>36</td>
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<td>inv(16)/ t(16;16)</td>
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<td>5</td>
<td>4</td>
<td>60</td>
<td>77</td>
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<td>t(15;17) (q22;q12)</td>
<td>8</td>
<td>4</td>
<td>4</td>
<td>47</td>
<td>63</td>
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<tr>
<td>&gt; 50 chromosomes</td>
<td>6</td>
<td>3</td>
<td>4</td>
<td>33</td>
<td>50</td>
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<tr>
<td>other abnormalities</td>
<td>78</td>
<td>38</td>
<td>39</td>
<td>48</td>
<td>63</td>
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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>
<thead>
<tr>
<th>Study, years of enrolment and reference</th>
<th>Patient number</th>
<th>Early death rate (%)</th>
<th>CR rate (%)</th>
<th>5-year pEFS (% with s.e.)</th>
<th>5-year pOS (% with s.e.)</th>
<th>% death rate in CR</th>
</tr>
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<tbody>
<tr>
<td>AIEOP92 (1992–2001)10</td>
<td>160</td>
<td>6</td>
<td>89</td>
<td>54 (4)</td>
<td>60 (4)</td>
<td>7</td>
</tr>
<tr>
<td>AML-BFM93 (1993–1998)3</td>
<td>427</td>
<td>7</td>
<td>83</td>
<td>51 (3)</td>
<td>58 (2)</td>
<td>4</td>
</tr>
<tr>
<td>DCOG-ANLL 92/94 (1992–1998)7</td>
<td>78</td>
<td>10</td>
<td>82</td>
<td>42 (6)</td>
<td>42 (6)</td>
<td>16</td>
</tr>
<tr>
<td>EORTC-CLG 58921 (1993–2000)5</td>
<td>166</td>
<td>2</td>
<td>84</td>
<td>48 (4)</td>
<td>62 (4)</td>
<td>6</td>
</tr>
</tbody>
</table>
### 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/m²/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/m²/day and idarubicin 12 mg/m²/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/m²/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/m² 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/m². Recently, the BFM reported a cumulative incidence of late cardiomyopathy of 5% in AML patients given anthracyline doses between 300–450 mg/m², 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/m² 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/m²) versus standard MACE-MIDAC (total dose of anthracyclins of 550 mg/m²) (personal communication Brenda Gibson).

In the subsequent table all anthracyclins dosages are calculated for the larger collaborative pediatric treatment groups. Cumulative dosages 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.

<table>
<thead>
<tr>
<th>Risk group</th>
<th>Anthracyclins (mg/m²)</th>
<th>ARA-C (gr/m²)</th>
<th>VP-16 (gr/m²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>AML 15</td>
<td>HR 240–300 3.6–16</td>
<td>0–1</td>
<td></td>
</tr>
<tr>
<td></td>
<td>SR 300–550 10.6–40.6</td>
<td>1–1.5</td>
<td></td>
</tr>
<tr>
<td></td>
<td>rand 240–490 23–52</td>
<td>0–0.5</td>
<td></td>
</tr>
<tr>
<td>AML-BFM 2004 interim</td>
<td>SR 350 26.8</td>
<td>0.950</td>
<td></td>
</tr>
<tr>
<td></td>
<td>HR 450 44.8</td>
<td>0.950</td>
<td></td>
</tr>
<tr>
<td>AML-BFM 2004</td>
<td>SR 350–410 29.32</td>
<td>?</td>
<td></td>
</tr>
<tr>
<td></td>
<td>HR 450–510 47.32</td>
<td>?</td>
<td></td>
</tr>
<tr>
<td>St Jude</td>
<td>HR 300 3.6–19.6</td>
<td>0.800</td>
<td></td>
</tr>
<tr>
<td></td>
<td>SR 450–550 12.0–46.0</td>
<td>0.8–1.3</td>
<td></td>
</tr>
<tr>
<td>COG</td>
<td>HR 300 13.6</td>
<td>1.75</td>
<td></td>
</tr>
<tr>
<td></td>
<td>SR 600 45.6</td>
<td>1.75</td>
<td></td>
</tr>
<tr>
<td>NOPHO</td>
<td>HR 450 7.3</td>
<td>0.4</td>
<td></td>
</tr>
<tr>
<td></td>
<td>SR 450 49.3</td>
<td>1.2</td>
<td></td>
</tr>
<tr>
<td>Japan</td>
<td>HR 250 39.4</td>
<td>1.25</td>
<td></td>
</tr>
<tr>
<td></td>
<td>IR 375 77.4</td>
<td>1.75</td>
<td></td>
</tr>
<tr>
<td></td>
<td>LR 225 77.4</td>
<td>1.75</td>
<td></td>
</tr>
<tr>
<td>ELAM 02</td>
<td>IR/SR DNR160 Amsa 300</td>
<td>44.4</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Mitox 60</td>
<td>0.4</td>
<td></td>
</tr>
</tbody>
</table>

Therefore, we conclude that at present, lowering the total dose of anthracyclins from 480 mg/m² in the original NOPHO 2004 protocol to 330mg/m² 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 allogeneic 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 50x10^9/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 ≥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 chemotherapy, 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/m² 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 peltatum*. 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°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. Physicians 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 antibiotics with antineoplastic activity, originally obtained from *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)
- Complete remission after first induction course
- 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.
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/m² orally every twelve hours day 1,2,3,4
Cytarabine 200 mg/m² per day continuous IV infusion day 1,2,3,4
Etoposide 100 mg/m² per day continuous IV infusion day 1,2,3,4
Idarubicin 12 mg/m² 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 10⁹/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 10⁹/L and platelet count is greater than 80 x 10⁹/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/m² per day as continuous IV infusion day 12,3,4,5
Mitoxantrone 10 mg/m² 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.

<table>
<thead>
<tr>
<th>Fludarabine</th>
<th>Cytarabine</th>
<th>Daunoxome</th>
<th>Triple IT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day</td>
<td>1</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>Fludarabine</td>
<td>30 mg/m²</td>
<td>per day</td>
<td>IV infusion (30 min.), day 1, 2, 3, 4, 5</td>
</tr>
<tr>
<td>Cytarabine</td>
<td>2000 mg/m²</td>
<td>per day</td>
<td>IV infusion, 4h after fludarabine. day 1, 2, 3, 4, 5</td>
</tr>
<tr>
<td>Daunoxome</td>
<td>60 mg/m²</td>
<td>IV infusion, 1h immediately after fludarabine, day 2, 4, 6</td>
<td></td>
</tr>
</tbody>
</table>

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.

Observations 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 HA₂E. Patients should begin consolidation when ANC is greater than 1.0 x 10⁹/L and platelet count is greater than 80 x 10⁹/L.
- Echocardiography should be performed before the start of HA₂E.
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 10⁹/L and platelets > 80 x 10⁹/L), which is expected to occur 21-28 days after the start of the previous course.

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 HA₂E

Cytarabine 2 g/m² every twelve hours as a 2-hour IV infusion day 1,2,3 (total of six doses)
Etoposide 100 mg/m² 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 HA₃
Cytarabine 3 g/m² 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 HA₂E

Cytarabine 2 g/m² every twelve hours as a 2-hour IV infusion day 1,2,3 (total of six doses)
Etoposide 100 mg/m² 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>
<thead>
<tr>
<th>Table intrathecal triple-doses</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age</strong></td>
</tr>
<tr>
<td>&lt; 1 year</td>
</tr>
<tr>
<td>1 year</td>
</tr>
<tr>
<td>2 years</td>
</tr>
<tr>
<td>3 - &lt; 9 years</td>
</tr>
<tr>
<td>≥ 9 years</td>
</tr>
</tbody>
</table>

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:

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

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:

<table>
<thead>
<tr>
<th>Drug</th>
<th>Dose per m$^2$</th>
<th>Corresponding to</th>
</tr>
</thead>
<tbody>
<tr>
<td>6-Thioguanin</td>
<td>100 mg/m$^2$</td>
<td>3.3 mg/kg</td>
</tr>
<tr>
<td>Cytarabine</td>
<td>100 mg/m$^2$</td>
<td>3.3 mg/kg</td>
</tr>
<tr>
<td></td>
<td>200 mg/m$^2$</td>
<td>6.7 mg/kg</td>
</tr>
<tr>
<td></td>
<td>1000 mg/m$^2$</td>
<td>33 mg/kg</td>
</tr>
<tr>
<td></td>
<td>2000 mg/m$^2$</td>
<td>67 mg/kg</td>
</tr>
<tr>
<td></td>
<td>3000 mg/m$^2$</td>
<td>100 mg/kg</td>
</tr>
<tr>
<td>Etoposide</td>
<td>100 mg/m$^2$</td>
<td>3.3 mg/kg</td>
</tr>
<tr>
<td>Idarubicin</td>
<td>12 mg/m$^2$</td>
<td>0.4 mg/kg</td>
</tr>
<tr>
<td>Mitoxantrone</td>
<td>10 mg/m$^2$</td>
<td>0.33 mg/kg</td>
</tr>
<tr>
<td>Fludarabine</td>
<td>30 mg/m$^2$</td>
<td>1 mg/kg</td>
</tr>
<tr>
<td>Daunoxome</td>
<td>60 mg/m$^2$</td>
<td>2 mg/kg</td>
</tr>
</tbody>
</table>

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/m² orally
- Cytarabine  43300 mg/m² IV
- Etoposide  1200 mg/m² IV
- Idarubicin  36 mg/m² IV
- Mitoxantrone  30 mg/m² IV
- Fludarabine  30 mg/m² IV
- Daunoxome  60 mg/m² 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.
5. Echo Abdomen
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.
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 HA2E 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: $FS = \frac{[(\text{left ventricular end diastolic diameter} - \text{end systolic diameter})/\text{end diastolic diameter}]}{100}$. 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>
<thead>
<tr>
<th>Table follow-up examinations overview</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
</tr>
<tr>
<td>At dg</td>
</tr>
<tr>
<td>Complete blood count</td>
</tr>
<tr>
<td>Echocardiography *</td>
</tr>
<tr>
<td>BP, electrolytes, bicarbonate, creatinine, creatinine clearance</td>
</tr>
<tr>
<td>Audiometry</td>
</tr>
</tbody>
</table>

* 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>
<thead>
<tr>
<th>Table WHO 2008 classification for Acute myeloid leukaemia</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>AML with recurrent genetic abnormalities</strong></td>
</tr>
<tr>
<td>- AML with t(8;21)(q22;q22), RUNX1-RUNX1T1</td>
</tr>
<tr>
<td>- AML with inv(16)(p13.1;q22) or t(16;16)(p13.1;q22); CBFB-MYH11</td>
</tr>
<tr>
<td>- Acute promyelocytic leukaemia with t(15;17)(q22;q12);PML-RARA</td>
</tr>
<tr>
<td>- AML with t(9;11)(p22;q23)MLLT3-MLL</td>
</tr>
<tr>
<td>- AML with t(6;9)(p23;q34); DEK-NUP214</td>
</tr>
<tr>
<td>- AML with inv(3)(q21q26.2) or t(3;3)(q21;q26.2); RPN1-EVI1</td>
</tr>
<tr>
<td>- AML (megakaryoblastic) with t(1:22)(p13;q13); RBM15-MKL1</td>
</tr>
<tr>
<td>- AML with mutated NPM1</td>
</tr>
<tr>
<td>- AML with mutated CEBPA</td>
</tr>
</tbody>
</table>

| **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)

<table>
<thead>
<tr>
<th>Myelodysplastic/myeloproliferative syndromes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Juvenile myelomonocytic leukemia (JMML)</td>
</tr>
<tr>
<td>Chronic myelomonocytic leukemia (CMML)</td>
</tr>
<tr>
<td>BCR/ABL negative chronic myeloid leukemia (Ph-CML)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Myelodysplastic syndromes (MDS)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Refractory cytopenia (RC): peripheral blood &lt; 2% blasts, bone marrow &lt; 5% blasts</td>
</tr>
<tr>
<td>Refractory anemia with blast excess (RAEB): peripheral blood 2-19% blasts, bone marrow 5-19% blasts</td>
</tr>
<tr>
<td>RAEB in transformation (RAEB-t): peripheral blood or bone marrow 20-29% blasts</td>
</tr>
</tbody>
</table>

A diagnosis of AML requires in most cases that 30% or more of the nucleated cells in the BM are myeloid 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

![Algorithm](image)

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

<table>
<thead>
<tr>
<th>Markers</th>
<th>AML M0/M1/M2</th>
<th>AML M3</th>
<th>AML M4/M5a/M5b</th>
<th>AML M6</th>
<th>AML M7</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD13/CD33</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>+</td>
<td>++</td>
</tr>
<tr>
<td>CD65</td>
<td>±/+/+++</td>
<td>+</td>
<td>++</td>
<td>±</td>
<td>±</td>
</tr>
<tr>
<td>MPO</td>
<td>-/+/+</td>
<td>++</td>
<td>++</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>CD11c</td>
<td>- or ±</td>
<td>-</td>
<td>++</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>CD14</td>
<td>-</td>
<td>-</td>
<td>+/-+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>CD15</td>
<td>±/±/+</td>
<td>±</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>CD36</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>++</td>
<td>+</td>
</tr>
<tr>
<td>H-antigen</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>++</td>
<td>+</td>
</tr>
<tr>
<td>GPA</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>CD41/CD61</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>++</td>
</tr>
<tr>
<td>CD42</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>CD34</td>
<td>++/+/+</td>
<td>±</td>
<td>±/±/±</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>CD117</td>
<td>++</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>HLA-DR</td>
<td>++/+/+</td>
<td>-</td>
<td>++</td>
<td>+</td>
<td>++</td>
</tr>
<tr>
<td>TdT</td>
<td>+</td>
<td>±</td>
<td>+</td>
<td>+</td>
<td>±</td>
</tr>
</tbody>
</table>

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 (CGH) 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 $5 \times 10^6$/L leukocytes in the CSF and leukemic cells detected in the cytopsin 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 $\leq 5\%$ 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 $> 80 \times 10^9$/L without transfusions and ANC $>1.0 \times 10^9$/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:

<table>
<thead>
<tr>
<th>FAB-type</th>
<th>In combination with</th>
</tr>
</thead>
<tbody>
<tr>
<td>M4 / M5</td>
<td>WBC &gt; 100 x 10^9/L, and in pt with kidney involvement also at lower WBC</td>
</tr>
<tr>
<td>M1, M2</td>
<td>WBC &gt; 150 x 10^9/L</td>
</tr>
<tr>
<td>M3</td>
<td>All patients</td>
</tr>
</tbody>
</table>

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 \times 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 \times 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, leukostasis 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 \times 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:

<table>
<thead>
<tr>
<th>Drug</th>
<th>Dose</th>
<th>Route</th>
</tr>
</thead>
<tbody>
<tr>
<td>Idarubicin</td>
<td>36 mg/m^2</td>
<td>IV</td>
</tr>
<tr>
<td>Mitoxantrone</td>
<td>30 mg/m^2</td>
<td>IV</td>
</tr>
</tbody>
</table>

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 then at least 87%.

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

<table>
<thead>
<tr>
<th>Relapse rate 3-years</th>
<th>Probability declaring unsafe</th>
<th>Average no. relapses at time of stopping</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.35</td>
<td>3%</td>
<td>53.4</td>
</tr>
<tr>
<td>0.40 (safe)</td>
<td>10%</td>
<td>52.2</td>
</tr>
<tr>
<td>0.45</td>
<td>27%</td>
<td>49.4</td>
</tr>
<tr>
<td>0.50</td>
<td>54%</td>
<td>44.4</td>
</tr>
<tr>
<td>0.55</td>
<td>79%</td>
<td>38.1</td>
</tr>
</tbody>
</table>

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.

<table>
<thead>
<tr>
<th>Time points for submitting CRFs</th>
</tr>
</thead>
<tbody>
<tr>
<td>CRF about diagnosis: 1 month after initial diagnosis</td>
</tr>
<tr>
<td>CRFs about induction treatment (course AIET and AM) and toxicity: 1 month after start AM course.</td>
</tr>
<tr>
<td>CRF about consolidation treatment (course HA₂E, HA₃ and HA₂E) and toxicity: 1 month after start second HA₂E course.</td>
</tr>
<tr>
<td>CRFs about the follow-up, yearly after treatment CRFs.</td>
</tr>
<tr>
<td>CRFs concerning a SAE, immediately after occurrence of SAE. See Chapter 12 Patient safety and event reporting.</td>
</tr>
</tbody>
</table>

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.
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 adverse Event 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.demoeerloose@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
REFERENCES


