NOPHO-DBH AML 2012 Protocol

Research study for treatment of children and adolescents with acute myeloid leukaemia
0-18 years

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Administration

The study is a cooperative protocol including NOPHO, BSPHO, DCOG, Estonia and Hong Kong.

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Minimal residual disease – flow cytometry

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<tr>
<td>AML</td>
<td>Acute myeloid leukaemia</td>
</tr>
<tr>
<td>ANC</td>
<td>Absolute neutrophil count</td>
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<td>APL</td>
<td>Acute promyelocytic leukaemia</td>
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<td>ARDS</td>
<td>Adult respiratory distress syndrome</td>
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<tr>
<td>BFM</td>
<td>Berlin Frankfurt Münster study group</td>
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<tr>
<td>BM</td>
<td>Bone marrow</td>
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<tr>
<td>BSA</td>
<td>Body surface area</td>
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<tr>
<td>BSPHO</td>
<td>Belgian Society of Paediatric Hematology Oncology</td>
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<tr>
<td>BW</td>
<td>Body weight</td>
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<tr>
<td>CNS</td>
<td>Central nervous system</td>
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<tr>
<td>CR</td>
<td>Complete remission</td>
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<td>CRF</td>
<td>Case report form</td>
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<td>CSF</td>
<td>Cerebrospinal fluid</td>
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<td>DCOG</td>
<td>Dutch Childhood Oncology Group</td>
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<td>DS</td>
<td>Down syndrome</td>
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<td>ECHO</td>
<td>Echocardiography</td>
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<td>EFS</td>
<td>Event-free survival</td>
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<td>EWOG-MDS</td>
<td>European working group of MDS in childhood</td>
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<td>FISH</td>
<td>Fluorescence in situ hybridisation</td>
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<td>FLT3-ITD</td>
<td>fms-like tyrosine kinase receptor-3 internal tandem duplication</td>
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<tr>
<td>G-CSF</td>
<td>Granulocyte colony stimulating factor</td>
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<tr>
<td>GO</td>
<td>Gemtuzumab ozogamicin</td>
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<td>GCP</td>
<td>Good Clinical practice</td>
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<tr>
<td>HEPA</td>
<td>High-efficiency particulate air</td>
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<td>IT</td>
<td>Intrathecal</td>
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<tr>
<td>JMML</td>
<td>Juvenile myelomonocytic leukaemia</td>
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<tr>
<td>LAIP</td>
<td>Leukaemia aberrant immunophenotype</td>
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<tr>
<td>LC</td>
<td>Leukaemic cell(s)</td>
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<td>MDS</td>
<td>Myelodysplastic syndrome</td>
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<tr>
<td>MLL</td>
<td>Mixed leukemia lineage gene</td>
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<td>MPAL</td>
<td>Mixed phenotype acute leukaemia</td>
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<td>MRC</td>
<td>Medical Research Council</td>
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<tr>
<td>MRD</td>
<td>Minimal residual disease</td>
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<td>NOPHO</td>
<td>Nordic society for paediatric haematology and oncology</td>
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<td>NPM1</td>
<td>Nucleophosmin gene</td>
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<td>OS</td>
<td>Overall survival</td>
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<td>PCR</td>
<td>Polymerase chain reaction</td>
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<td>qPCR</td>
<td>Quantitative polymerase chain reaction</td>
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<tr>
<td>RD</td>
<td>Resistant disease</td>
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<td>SAE</td>
<td>Severe adverse event</td>
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<td>SCT</td>
<td>Stem cell transplantation</td>
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<td>SUSAR</td>
<td>Suspected unexpected severe adverse events</td>
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<td>TLS</td>
<td>Tumour lysis syndrome</td>
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2. Important note
The NOPHO-DBH AML2012 protocol has the overall aim of increasing the cure rate of children with AML. It is a collaborative research protocol but also provides a standard treatment, which is considered as best available treatment. Although the protocol contains recommendations for standard therapy, it is not intended for use in unregistered patients. Treatment with this protocol is not recommended unless the AML protocol group has been consulted and the group will not take any responsibility, legal or otherwise for the use of the protocol in unregistered patients. The protocol includes two randomised studies, which will be conducted according to Good Clinical Practice (GCP) guidelines after approval from the national legal authorities. The study aims to improve therapy but also to increase the quality of diagnostic and response evaluation as well as data registration particularly regarding toxicity. This will increase the demands on the treating centres and physicians. To ensure that the intended high standards will be reached all participating centres must adhere to the following principles:

- All centres will sign a contract stating their commitment to perform the study and identifying the laboratories that will carry out diagnostic evaluation and minimal residual disease MRD monitoring for the centre (appendix 1).
- All patients should be registered in the NOPHO-AML registry within five days of commencing therapy.
- All deaths and Suspected Unexpected Severe Adverse Reactions (SUSARs) must be reported to the AML registry within 48 hours.
- Patients for whom mandatory data are lacking cannot enter the randomised studies.
- In all patients with an informative leukaemia aberrant immunophenotype (LAIP), MRD must be monitored with flow cytometry, standardised according to appendix 4.
- Significant treatment modifications, including dose reductions and delays should not be based only on personal views but on protocol recommendations and discussions with the study and/or national coordinator.

The protocol has been prepared with great care but amendments may be necessary. These will be circulated to known participants in the trial, but institutions entering patients are in case of doubt advised to consult with the NOPHO website www.nopho.org, where the most current version of the protocol and all relevant appendices and other documents are available for NOPHO members and associated partners. Despite our best efforts, the possibility of errors within this document cannot be excluded. We remind investigators that the responsibility for any therapy given lies with the attending physician and that any adverse consequences arising from application of this treatment should be regulated within the framework of regulations and insurance.
policies in the individual countries and ICH-GCP guidelines. The content of the protocol is confidential and may not be distributed outside NOPHO treatment centres without the approval of the NOPHO-DBH AML 2012 Study Committee. For centres that for any reason do not participate in the randomised trials, treatment should be given according to the standard arms. This also applies to individual patients not taking part in the randomisations for other reasons (e.g., patient refusal). For each of the randomised studies a standard arm is depicted in the protocol.
3. Protocol outline

*BM evaluation with MRD is done day 22. Patients with ≥ 5% leukaemic cells proceed immediately to course 2. Patients with < 5% receive course 2 after recovery but weekly controls of BM with MRD should be performed.**

**BM evaluation in patients who started course 2 early (i.e. had ≥ 5% LC after course 1) is performed on day 22 after course 2 whereas patients with < 5% LC after course 1 are evaluated immediately before start of consolidation (HAM).

Echocardiography should be performed prior to course 1, 2 and 3.
4. Checklist AML 2012

**Diagnosis**
- BM morphology
- BM immunophenotype and MRD flow marker
- BM FISH/PCR genetic markers and qPCR marker
- BM Cytogenetics
- CSF cell count
- CSF cytology
- If CNS symptoms MRI
- HLA typing of patient
- Echocardiography

Register patient in AML database
- Check incl/excl criteria
- Obtain informed consent
- Randomise DNX study

**Day 22 evaluation**
- BM MRD Flow and morphology
- BM MRD PCR or storage
- Echocardiography

Yes: BM flow ≥ 5% LC?
- Randomise ADxE/FLADx

- Start Course 2 immediately

No: Repeat BM + MRD weekly
- until increase of LC ≥ 5%
- or peripheral regeneration

- BM evaluation + MRD d22

**Evaluation Before consolidation**
- BM MRD Flow and morphology
- BM MRD PCR or storage
- Echocardiography

**Risk Grouping**
- Resistant disease ≥ 5% after course 2
- High-risk if either
  - MRD≥15% at any time after course 1
  - MRD ≥0.1% before consolidation
  - FLT3-ITD without NPM1
- All other patients standard-risk
5. Summary of protocol

The outcome of paediatric acute myeloid leukaemia is still unsatisfactory with an overall survival around 70% and a relapse rate of 30-40% after primary treatment. The primary aim of the NOPHO-DBH AML 2012 study is to improve EFS and OS in children with AML. The protocol is a collaborative research study including the BSPHO, DCOG, Hong Kong and NOPHO paediatric AML groups.

The patient group includes children and adolescents up to 19 years of age with de novo AML excluding patients with MDS-AML, myeloid leukaemia of Down syndrome and acute promyelocytic leukaemia.

To improve outcome, an intensified induction regimen will be given and a response guided risk-group stratification using flow cytometric minimal residual disease measurements to evaluate therapy response will be used. Patients with a poor response to the two induction courses will be assigned to the high-risk group and receive consolidation therapy including stem cell transplantation whereas those with a good response will be given three chemotherapy courses as consolidation therapy. An exception are patients with good response and inv(16), who will not be given HAM and thus receive two consolidation courses only. The only other cytogenetic feature that will affect risk stratification is the presence of an FLT3-ITD mutation which, when not associated with concomitant nucleophosmin (NPM1) mutation, will stratify patients to the high-risk group.

Effective induction therapy is crucial for outcome in AML and MRD levels following induction are highly predictive of outcome. AML 2012 includes two randomised studies that both address the efficacy of induction therapy.

The first study is based on the induction course from the Japanese AML99 trial and compares the efficacy of mitoxantrone and DaunoXome in the first treatment course. The primary outcome measure is the MRD level on day 22 from start of the course.

The second study compares ADxE (low-dose cytarabine, DaunoXome and etoposide) with FLADx (fludarabine, high-dose cytarabine and DaunoXome) as the second induction course. The primary endpoint is the MRD level at day 22 from start of the course.

Secondary outcome measures in both studies include EFS, OS, remission rate and toxicity. AML 2012 is scheduled to run between five and six years and expected to recruit at least 300 patients. The study incorporates strict guidelines for cytogenetic characterisation of the disease and a standardised approach to flow cytometric MRD determination with central review of data files. Toxicity will be carefully monitored and the study will be conducted according to GCP guidelines.
6. Objectives of NOPHO-DBH AML 2012

The AML 2012 study is a treatment and research protocol with the overall aim of improving prognosis for children and adolescents with AML. This is to be achieved by better risk stratification based on MRD quantification and more intensive induction compared to previous NOPHO protocols.

The protocol contains two randomised studies. The first compares the efficacy of mitoxantrone vs. liposomal daunorubicin in the first induction course (DNX study) and the second compares the efficacy of two courses, ADxE vs. FLADx, as the second induction course (FLADx study). Minimal residual disease measurement by flow cytometry will be employed for evaluation of early response to therapy and provide the main means for evaluation of the randomised studies and for treatment stratification.

The protocol also includes studies addressing genetic and epigenetic aberrations in AML and the prognostic impact of minimal residual disease as determined by flow cytometry and/or PCR for specific genetic aberrations.

The specific aims of the randomised studies are

1) To investigate if either DaunoXome or Mitoxantrone, when given in course 1, is more effective in reducing the MRD level to < 0.1% as measured on day 22.
2) To investigate if either of the courses ADxE or FLADx is more effective in reducing the MRD level to < 0.1% after the second induction course.

The protocol will also compare the efficacy and toxicity of the treatment between the randomised arms and with previous NOPHO-AML protocols with the aims of

1) Improving both EFS and OS as compared to NOPHO-AML 93 and 2004.
2) Improving EFS and OS for patients with intermediate response (5-14.9%) blasts after course 1 and patients with t(8;21).
3) Achieving improved anti-leukaemic effect with no increase or a decrease in early toxic deaths and deaths in CR.
4) Comparing outcome in subgroups of patients as defined by characteristics of both patients and disease such as age, FAB type and cytogenetics (e.g. t(8;21, inv(16) and MLL rearrangements).

The protocol will also explore and when applicable compare the randomised treatment arms for

1) The incidence of severe infections and severe organ toxicity.
2) The feasibility of obtaining an informative leukemic immunophenotype in at least 80% of patients.
3) The correlation between MRD measurement by PCR and flow cytometry and the prognostic impact of MRD with either method after course 1 and 2 respectively.
7. Background

7.1. Early NOPHO AML protocols (NOPHO-AML 84, 88 and 93)
Cure rates in paediatric AML were very low until the introduction of intensive chemotherapy in the late 1970s(1). In 1981, a pilot study in Oslo, introducing consolidation therapy with high-dose (HD) cytarabine in children, showed promising results and was used as the base for the first Nordic collaborative paediatric AML protocol(2). NOPHO-AML84 used an induction with three courses of doxorubicin, low-dose cytarabine and 6-thioguanine and consolidation with four courses of high-dose cytarabine and resulted in an EFS of 29%(3). Since the number of patients with resistant disease (15%) and relapse (46%) was high, the next protocol, NOPHO-AML88 was intensified. Etoposide, continuous infusion of cytarabine and mitoxantrone were added to induction and HD cytarabine was combined with etoposide or mitoxantrone in consolidation. Intensive timing was used for the first two induction courses. NOPHO-AML88 had a strong anti-leukaemic effect and the rate of resistant disease was reduced to 4% and relapse rate to 36%. However, although EFS increased to 41%, the treatment was very toxic and the frequency of early death and death in CR was unacceptably high at 9.3% each(3).

NOPHO-AML93 used a response-guided approach to retain the anti-leukemic effect while reducing toxicity. Thus, only patients who did not respond with <5% blast cells after the first course with doxorubicin, low-dose cytarabine, etoposide and 6-thioguanine (ATEDox), received the second course early after 14 days. This course was low-dose cytarabine and mitoxantrone (AM). In contrast, good responders repeated the first course but only after peripheral regeneration. NOPHO-AML93 was highly successful with an EFS of 51% and an OS of 65% at five years, results which at the time were world-leading(3).

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**Figure 2. Event-free survival in the four consecutive NOPHO AML studies.**
7.2. NOPHO-AML 2004

AML 2004 used idarubicin instead of doxorubicin in the first induction course, since other studies suggested that idarubicin could be more effective than other anthracyclines\(^4\),\(^5\). The response-guided approach from the 1993 protocol was retained but all patients were given AM as the second induction course. Patients were allocated to the high-risk group if they had >15% blasts after the first course or did not achieve CR after the second course. Until 2009, patients with 11q23 abnormalities other than t(9;11) were also classified as HR. However, the majority of these patients are under 2 years and compliance to the SCT recommendation was poor. An interim analysis showed, that the outcome of these patients was good even without SCT and an amendment was made removing these patients from the HR group. In contrast to NOPHO-AML93, in which only high-risk patients with matched siblings were eligible for stem cell transplant in CR1, all high-risk patients were recommended SCT if they had a matched related or unrelated donor. AML 2004 had a very low rate of resistant disease (RD) and overall 95% of patients entered CR. The toxic death rate was acceptable with 2.6% early deaths and 2.6% dead in CR\(^6\). However, although overall survival was fairly satisfying at 70% the EFS did not improve compared to NOPHO-AML 93 (figure 2). The large difference between EFS and OS reflects a better outcome of relapsed patients, which most likely depends on improvements in stem cell transplant procedures and supportive care (figure 3).

7.2.1. Therapy response in AML 2004

The low rate of RD indicated that the induction regimen in AML2004 could be more effective than in AML93 and could translate into a better outcome. Therefore, the EFS of 47% was disappointing. An analysis in 2010 (figure 4), including 152 patients, showed that those with an intermediate response to the first course (5-15% blasts) had a very high relapse rate whereas patients with a poor response (>15% blasts), who were stratified to the HR group, did well with an EFS of

![Graph](image)

Figure 3. Overall survival at 5 years in the four consecutive NOPHO AML studies.

![Graph](image)

Figure 4. Event-free survival in patients with good, intermediate or poor response to course 1 (AIET).
80% [6]. The CR rate after the first course AIET was 70% and the AM course resulted in CR in 92%. Thus, although the total CR rate was high, the quality of remission was low following AM, particularly in the intermediate responders, leading to a high relapse rate. On the other hand, the good results in the poor risk group indicate that SCT, as opposed to conventional consolidation therapy, can compensate for the lower quality of remission. A repeat evaluation, now including 261 patients, also showed that patients who reach CR after the second course have much worse EFS than those who enter CR after the first course. As shown in figure 5, the overall survival of patients who enter remission after two courses is only slightly lower but of the 60 patients in this group, 33 had relapse (of which 18 had SCT) and 16 received SCT in CR1.

Our conclusion is that AM is not sufficiently intensive for induction therapy of paediatric AML and today, when comparing with current intensive induction protocols, AM stands out as a weak course. An amendment was made in 2011 replacing the AM course with FLADx (fludarabine, HD cytarabine, DaunoXome) in patients with an intermediate response to AIET. Still, the relapse rate even for good responders is high and it is likely that these patients also would benefit from a more intensive 2nd course.

7.2.2. Minimal residual disease in AML 2004

The AML 2004 protocol aimed at evaluating the prognostic impact of early treatment response by measuring minimal residual disease (MRD) by flow cytometry. MRD flow is much more difficult in AML than in ALL and, in combination with suboptimal standardisation of the techniques used in different laboratories, reliable results were only available in a subset of 97 patients. However, the results from a central review of all MRD data files strengthen the conclusions from the evaluation of response on bone marrow (BM) morphology. Even with the limitations given above, 78% of the patients had an informative leukemic aberrant immunophenotype (LAIP) on day 15 after AIET and 75% on the last examination following AM (figure 6). Patients with MRD < 0.1% had an EFS of 64% compared to only
24% in those with $\geq 0.1\%$ on day 15 after AIET (figure 7). An MRD $\geq 0.1\%$, measured immediately before consolidation was associated with an EFS of 16% and all but one of twelve SR patients relapsed (figure 9). The effect of MRD levels on survival were also striking. As shown in figure 8, OS was

![Figure 7. EFS in 41 patients with MRD <0.1% and 35 with ≥0.1% leukemic cells at day 15 after AIET.](image)

73% in those with MRD < 0.1% on day 15 compared to 45% in those with MRD ≥ 0.1%. As expected, high MRD levels had an even more pronounced effect on survival when measured before consolidation (BC) and the OS for patients with MRD ≥ 0.1% was extremely poor (figure 9). The NOPHO-AML 2004 clearly shows that determination of early therapy response with MRD quantification by flow is feasible in the majority of patients and identifies patients with a very poor prognosis.

![Figure 8. OS in 41 patients with MRD <0.1% and 35 with ≥0.1% leukemic cells at day 15 after AIET.](image)

![Figure 9. OS in 56 patients with MRD <0.1% and 18 with ≥0.1% leukemic cells before consolidation.](image)

![Figure 10. EFS in 56 patients with MRD <0.1% and 18 with ≥0.1% leukemic cells before consolidation.](image)
7.2.3. Stem cell transplantation in AML 2004

In AML 2004 all patients in the HR group were recommended SCT in CR1. Also patients with resistant disease, the majority of whom entered CR after FLAG as the third induction course should receive SCT. In total, 44 patients had SCT in CR1 including 11/14 with resistant disease and 33/55 patients in the high-risk group. The majority of the patients in the HR group who did not receive a transplant were either patients with 11q23 as sole HR criteria or patients with a relapse prior to SCT. Ten had a sibling donor and 34 an unrelated donor. The survival in the transplanted patients was 80% showing that SCT is effective in patients with a poor initial treatment response (figure 11).

Importantly, patients who entered CR first after two induction courses and received SCT, had an OS of 86% (N=16) compared to 59% (N=41) in patients treated with chemotherapy only. Patients entering CR after two induction courses and not receiving SCT, had an EFS of only 16% and the majority of patients surviving in this group do so after relapse therapy. Furthermore, seven of eight patients who obtained CR after three courses survive after SCT in CR1, again showing that SCT can overcome the negative impact of a poor initial treatment response. The excellent outcome after SCT most likely reflects improvements in transplant procedures and supportive care and shows that a matched unrelated donor is as suitable as a sibling donor in high-risk patients.

7.2.4. Cytogenetics in AML 2004

Almost all patients in the protocol had successful karyotyping and FISH or PCR results for t(8;21), inv(16) and 11q23 aberrations. Analysis of FLT3 mutations were only mandatory from 2011 when patients with FLT3-ITD mutation and normal NPM1 were stratified to the HR group. The frequency and survival in some genetic subgroups are shown in table 1. While the EFS in 11q23 aberrations other than t(9;11) seems to have improved, the EFS for both t(8;21) and t(9;11) is lower. The discussion in the next session on t(8;21) regarding possible reasons for the decreased outcome largely applies to patients with t(9;11) as well. Patients with inv(16) had an EFS of 74%.
Table 1. Event-free survival in patients with inv(16), t(9;11), other 11q23 aberrations and normal karyotype in NOPHO-AML93 and 2004.

<table>
<thead>
<tr>
<th></th>
<th>NOPHO-AML93</th>
<th>NOPHO-AML 2004</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N (%)</td>
<td>EFS (%)</td>
</tr>
<tr>
<td>t(8;21) yes</td>
<td>24 (8.1)</td>
<td>67</td>
</tr>
<tr>
<td>t(8;21) no</td>
<td>272</td>
<td>50</td>
</tr>
<tr>
<td>t(9;11) yes</td>
<td>25 (8.4)</td>
<td>80</td>
</tr>
<tr>
<td>t(9;11) no</td>
<td>271</td>
<td>48</td>
</tr>
<tr>
<td>inv(16) yes</td>
<td>18 (6.1)</td>
<td>72</td>
</tr>
<tr>
<td>inv(16) no</td>
<td>278</td>
<td>50</td>
</tr>
<tr>
<td>Other 11q23 yes</td>
<td>29 (9.8)</td>
<td>31</td>
</tr>
<tr>
<td>Other 11q23 no</td>
<td>267</td>
<td>53</td>
</tr>
<tr>
<td>Normal karyotype yes</td>
<td>74 (25)</td>
<td>43</td>
</tr>
<tr>
<td>Normal karyotype no</td>
<td>222</td>
<td>54</td>
</tr>
</tbody>
</table>

7.2.4.1. AML with t(8;21)

The outcome in AML 2004 for patients with t(8;21) was significantly inferior to the AML93 protocol and the EFS was only 34.7% and, although many relapsed patients could be salvaged, the OS was only 60.6% compared to 71.2% in patients without t(8;21). 89% (40/45) achieved remission after AIET. The reason for the decrease in EFS compared to AML93 can be that the majority of these patients received the weak AM as the 2nd induction course. It is also possible that idarubicin has a lower effect than doxorubicin in AML with t(8;21). In 2010, an amendment was made that replaced AM with FLADx in patients with t(8;21). The time is too short to allow evaluation of this change.

7.2.5. Gemtuzumab ozogamicin randomisation

NOPHO-AML 2004 included a post-consolidation randomisation between treatment with gemtuzumab ozogamicin (GO) or no further treatment. 120 patients were randomised and although toxicity was well tolerable GO could not prevent relapses and the outcome was similar in treated and non-treated patients (figure 12)(7)

Figure 12. EFS and OS in patients randomised to receive gemtuzumab ozogamicin or no further therapy as post-consolidation in standard-risk patients.
7.3. Relevant experiences from other study groups

7.3.1. Recent large treatment studies
Since the publication of the issue on paediatric AML in Leukaemia 2005, where most of the cooperative paediatric AML groups published their results, four new treatment studies from major paediatric AML study groups have been published(8). The CCG-2961 study showed an EFS of 42% and OS of 52% and also employed a first induction course very similar to AIET. Though the study was large including 901 patients the results are difficult to interpret due to a high proportion of patients withdrawing, lack of cytogenetic data in a substantial proportion of patients and a time-dependent effect on outcome(9). The Japanese AML02 study used cytarabine, mitoxantrone and etoposide in induction and risk groups stratification based on initial treatment response, age, WBC and cytogenetics(10). The study enrolled 240 children and, although the frequency of t(8;21) was 32%, the results are impressive with an EFS of 61.6% and OS of 75.6%. In 2010, the AML02 study from St Jude was published. Induction consisted of two courses of ADE (cytarabine, daunorubicin and etoposide) with a randomisation between HD cytarabine and conventional cytarabine dosing in the first course(11). The study used MRD flow to evaluate treatment response after the induction courses and guided the timing of the 2nd course after MRD results. Patients with high MRD after course 1 had GO added to the 2nd course. The study recruited 232 patients and EFS and OS at three years were 63.3 and 71.1% respectively. Finally, the BFM study group reported their AML-2004 study at the ASH meeting 2010(12). This study randomised between idarubicin and DaunoXome in the first ADE-like course. The risk stratification was based on morphology, genetics and therapy response on day 15 after the first course. The study enrolled 566 patients and EFS and OS were 54% and 72%. There was a trend to better EFS and OS in the DaunoXome arm with lower treatment related mortality. At present, the latter three studies show the best overall results in paediatric AML.

7.3.2. Induction therapy
It is generally accepted that cytarabine combined with an anthracycline are the most essential components of AML induction therapy. In paediatric protocols, although based on much less evidence, etoposide is often incorporated as a third drug. Accordingly, AML99, AML02, BFM-AML2004 and NOPHO-AML2004 all use cytarabine and etoposide in course 1 but the anthracycline differs. The results from NOPHO-AML 2004 indicate that, although a high CR rate is obtained, the induction therapy is insufficient, leading to a low quality of remission.

7.3.2.1. The length of the first induction course
When comparing with induction regimens in studies with good outcome it is evident that the courses in AML-2004 are shorter (AIET 6 days, AM 5 days). The first course in AML99 is twelve days, in AML02 ten days and in BFM2004 eight days. The assumption for the advantage of longer courses is that damaged leukaemic cells will be exposed to repeated insults finally causing them to die.
Table 2. Comparison of the first induction course in selected studies.

<table>
<thead>
<tr>
<th>Course 1</th>
<th>NOPHO-AML 2004</th>
<th>AML99</th>
<th>AML02</th>
<th>BFM-AML2004</th>
</tr>
</thead>
<tbody>
<tr>
<td>Length (days)</td>
<td>6</td>
<td>12</td>
<td>10</td>
<td>8</td>
</tr>
<tr>
<td>Cytarabine (mg/m²)</td>
<td>800</td>
<td>1400</td>
<td>2000</td>
<td>1400</td>
</tr>
<tr>
<td>administration</td>
<td>96 h ci</td>
<td>7 x 12 h inf</td>
<td>20 x 0.5h</td>
<td>48 h ci + 12 x 0.5h</td>
</tr>
<tr>
<td>Etoposide (mg/m²)</td>
<td>400</td>
<td>750</td>
<td>500</td>
<td>450</td>
</tr>
<tr>
<td>Anthracycline</td>
<td>Idarubicin</td>
<td>Mitoxantrone</td>
<td>Daunorubicin</td>
<td>Idarubicin / DaunoXome</td>
</tr>
<tr>
<td>Dose (mg/m²)</td>
<td>3 x 12</td>
<td>5 x 5</td>
<td>3 x 60</td>
<td>3 x 12 / 3 x 60</td>
</tr>
<tr>
<td>CR rate</td>
<td>70</td>
<td>86</td>
<td>80</td>
<td>83*</td>
</tr>
</tbody>
</table>

* Reported for idarubicin arm in BFM-AML98.(13)

As shown in table 2, all the other studies have a higher CR rate than AIET and the common denominator is the length of the course.

7.3.2.2. Anthracyclines

At present, there is no consensus on which anthracycline to use in neither paediatric nor adult AML. The main issues are whether any of the drugs are more effective and if any of the drugs have a lower risk of the cardiac toxicity which is associated with all of the drugs.

In adults, the 7+3 regimen with cytarabine and daunorubicin, at a dose of 45-60 mg/m², was initially the standard induction. Randomised studies in the late 80s comparing idarubicin (12mg/m² x III) with daunorubicin (45-50 mg/m²) showed a higher CR rate with idarubicin(14, 15). However, the doses compared may not have been equivalent and more recent studies show that higher doses of daunorubicin (90 mg/m² x III) are more effective(16) and a large Japanese study showed that daunorubicin at 50 mg/m² x V was as effective as idarubicin 12 mg/m² x III(17). Although numerous studies on cardiac toxicity have been performed in adults, patient numbers, design and follow-up have often been suboptimal precluding definite conclusions particularly on long-term effects. However, a recent meta-analysis of 55 randomised controlled trials could show lower cardiac toxicity for continuous infusion vs bolus, mitoxantrone vs anthracyclines, liposomal doxorubicin vs doxorubicin and epirubicin vs doxorubicin(18).

In children, the AML-BFM93 study compared idarubicin (12 mg/m² x III) vs daunorubicin (60 mg/m² x III) and found no significant effect on EFS but since idarubicin gave an increased early blast clearance and had a trend to better outcome it was used as the standard drug in AML-BFM98(4). The MRC AML 12 study compared mitoxantrone (12 mg/m² x III) with daunorubicin (50 mg/m² x III) in an ADE setting and found ten year estimates of significantly higher DFS₁₀ (63% vs 55%), a lower relapse rate (32% vs 39%) but not significantly increased EFS₁₀ (57% vs 51%) or OS₁₀ (65% vs 61%) for mitoxantrone and CR rates were 67% and 63% after the first course(19). There was no difference in treatment related mortality (TRM 10%) or non-haematological toxicity but haematological toxicity was higher for mitoxantrone. The conclusion was that mitoxantrone might be more effective, particularly in younger children, but the increased toxicity prompted MRC to continue with daunorubicin in the next study (AML MRC 15). The EORTC compared idarubicin (10 mg/m² x III) and mitoxantrone (10 mg/m² x III) and found no difference in outcome (EFS 42% vs 48.4% ; OS 59.8% vs 57.5%), acute or cardiac toxicity(20). The AML-BFM 2004 study randomised idarubicin
(12 mg/m$^2$ x III) vs liposomal daunorubicin (DaunoXome 80 mg/m$^2$ x III)(12). There was a trend for better outcome (EFS 60% vs 54%, p=0.17 ; OS 78% vs 70%, p=0.15) for DaunoXome and also lower TRM and less grade III/IV cardiotoxicity. Long-term follow-up of cardiac toxicity is not published for any of these studies.

**7.3.2.3. Liposomal daunorubicin (DaunoXome)**
Animal studies and pharmacokinetic studies in humans have shown that liposomal daunorubicin, in comparison to free drug, gives higher plasma levels and increased area under the curve and a lower conversion to the toxic metabolite daunorubicinol(21, 22). Uptake in non-reticuloendothelial tissue is decreased with a reduction in toxicity. Also, liposomal doxorubicin has been found less cardiotoxic than free drug(18). Animal studies have shown reduced cardiac toxicity(23) and in patients with Kaposi's sarcoma endomyocardial biopsies show less damage after liposomal daunorubicin than free drug(24). Liposomal daunorubicin has been administered in very high doses to adults with AML in both single courses (150 mg/m$^2$ x III)(25) and with cumulative doses in excess of 750 mg/m$^2$ showing low cardiotoxicity(22). Thus, although not proven in randomised trials and lacking long-term follow-up, liposomal daunorubicin has the potential to be significantly less cardiotoxic than conventional anthracyclines.

In children with AML, liposomal daunorubicin has been investigated in two phase III studies. The I-BFM-SG Relapsed AML 2001 study could show that addition of DaunoXome (60 mg/m2 x III) to FLAG (fludarabine, cytarabine, G-CSF) gave a better early treatment response than FLAG alone(26). In patients with de novo AML, the BFM-AML 2004 study compared idarubicin to DaunoXome administered together with low-dose cytarabine and etoposide(12). As mentioned above this study showed a tendency to reduced toxicity and better outcome with DaunoXome.

**7.3.2.4. Implications for NOPHO-DBH AML 2012**
The results from other studies clearly indicate that a higher CR rate can be obtained than seen after AIET in NOPHO-AML 2004. Since the first course in AML99 has the highest published CR rate with very good EFS and OS in the protocol, we have decided to use this as the standard arm in AML 2012. Furthermore, the favourable tumour biological and pharmacokinetic profile of DaunoXome together with the very promising results from the European relapse study and BFM-AML 2004 makes it compelling to test DaunoXome against mitoxantrone using the AML99 induction backbone. It is obvious that AM is a too weak second course. Similarly to the AML02 protocol and its successor AML05 and MRC AML trials, NOPHO will continue to use a second induction course. Both these groups give an eight day ADE variant and we will use an eight day ADE course as the standard treatment but, aiming for better efficacy and lower cardiac toxicity, replace daunorubicin with DaunoXome. The combination of fludarabine and high-dose cytarabine with or without added anthracycline has repeatedly been shown effective in children with refractory or relapsed AML(26, 27). In adults, the MRC AML 15 study randomised FLAG-IDA and ADE in induction and showed that the FLAG regimen was associated with a lower relapse rate but also increased toxic deaths(28). Despite this therapeutic potential, no study in children has tested this drug combination in upfront therapy. We will therefore randomise between ADxE or FLADx as the 2nd course. Using the development of sensitive techniques for MRD quantification with flow cytometry we will have sufficient power to evaluate both these randomisations regarding efficacy.
Since the concept of intensively timed induction has proven successful in obtaining high CR rates we will continue to give the 2nd course early to patients responding poorly (9, 29).

7.3.3. Minimal residual disease (MRD)

Many studies have demonstrated the prognostic significance of early marrow response by bone marrow morphology (6, 30). A number of studies have used flow cytometry for MRD quantification of early response to therapy and, in line with our findings in the AML 2004 protocol, all have demonstrated that rapid early clearance is associated with a better outcome (11, 31-34). PCR-MRD is more sensitive than flow MRD but the applicability is lower since recurrent mutational targets, using conventional technology, are present in only 40-50% of patients (35). Therefore, few studies have investigated the significance of early response by PCR-MRD (36, 37). Although the identification of a leukaemic aberrant immunophenotype (LAIP) is more difficult in AML than ALL, the development in flow technology now allows for the detection of MRD at a sensitivity of at least 0.1% in over 90% of patients (34, 38). One study in 150 patients in the BFM-AML 98 study showed that the 2-year overall survival was 30% in MRD positive (≥0.1%) compared to 72% in MRD negative patients after induction (31). Similarly, in 94 children treated with the MRC AML12 protocol, MRD levels after the first course were highly predictive of outcome. The 3-year relapse-free survival was 85% in MRD-negative (<0.1%) compared to only 14% in those with MRD ≥ 0.5% (34). Finally AML02, the most recent and largest study to date, could identify an informative LAIP in 94.9% of 215 patients and the 3-year cumulative incidence of relapse or induction failure was 16.9% in MRD-negative and 38.6% in MRD positive patients measured after the first induction (11).

Thus, it is clear that early response evaluation by MRD flow is feasible and carries significant prognostic information. Since data from the NOPHO-AML 2004 show that a poor initial treatment response can be salvaged by stem cell transplantation we will use MRD flow for treatment stratification in the current protocol.

7.3.4. Consolidation therapy

Although there is general consensus that consolidation therapy should include high-dose cytarabine there is considerable debate as to the number of consolidation courses and the role of stem cell transplant in first remission.

7.3.4.1. Allogeneic stem cell transplantation

Studies comparing consolidation with SCT or conventional chemotherapy in CR1 have mainly employed biological randomisation such that patients with a matched sibling donor have been given SCT and those without have received chemotherapy. Most studies have shown that, although the relapse rate is lower and disease-free survival is higher following SCT, no significant effect of SCT on overall survival has been observed (39). This has been interpreted such that SCT has a higher anti-leukemic effect which reduces the relapse rate but that this is balanced by the increased TRM together with the increased salvage rate in patients who receive chemotherapy only. The same pattern has been observed when studying patients with high- and low-risk AML (40, 41). However,
most studies have been low-powered and the risk groups, which have mainly been based on cytogenetics, have been heterogeneously defined. Importantly, no large or randomised study has investigated the effect of SCT in patients with a suboptimal response to induction therapy. Since several studies now convincingly show that a high MRD level after induction therapy is associated with a very low EFS and OS, and NOPHO data demonstrate that the effect of a poor initial response can be overcome by SCT the AML2012 protocol will use SCT in patients with a poor response to induction therapy. The issue of the optimal treatment of patients with FLT3-ITD is still controversial(42). However, previous NOPHO studies confirm the poor prognosis in patients with FLT3-ITD, but also indicate, although patient numbers are small, that SCT may improve prognosis in this group. Thus, patients with FLT3-ITD without concomitant nucleophosmin mutation, will be given SCT in CR1. There are several other cytogenetic aberrations (e.g. -7, t(6;9), t(7;12), t(10;11), del(12p)) that have been associated with very poor outcome in which SCT may be beneficial. However, the outcome in these rare aberrations has mainly been studied over a long time span and in the context of collaborative inter-group studies with diverse treatments. Thus, for most of these aberrations it is uncertain if modern, highly intensive chemotherapy may improve prognosis and there is no data regarding the effect of SCT. Therefore, in AML2012, these patients will be treated with SCT only if they have a poor response to induction therapy. In AML2012 it is expected that between 7-10% of patients will be eligible for SCT due to poor treatment response and in addition not more than 5% due to FLT3-ITD mutation.

7.3.4.2. Consolidation with conventional chemotherapy

Most paediatric AML study groups today use a total of five courses and the consolidation courses are usually based on high-dose cytarabine. The MRC AML12 trial showed equal outcome after randomising between four or five courses(19). However, the study was not powered for the paediatric cohort alone and the course tested was CLASP (high-dose cytarabine and asparaginase) which is not used in either NOPHO, DCOG or BFM protocols. Our conclusion is that it seems safe to reduce the number of courses from six to five. All major treatment protocols, including NOPHO-AML 2004, now consistently show a high EFS for patients with inv(16)/t(16;16). Since both MRC12 and AML-BFM 2004 show good results in these patients using only four courses (43, 44) the AML 2012 protocol will omit the HAM course in patients with inv(16) and a good response to induction therapy. Thus, these patients will receive a total of four courses.

8. Study population

All centres participating in the randomised studies must be committed to follow the instructions in the protocol and to offer participation in the randomised trials for their patients. Patients not included in the randomised studies should be treated according to the respective control arms (MEC as course 1 and ADxE as course 2). Centres may be excluded from study participation, after discussion in the NOPHO-AML 2012 group, in
case of repetitive protocol violations including poor compliance to randomisation and
data base registration.

8.1. Inclusion Criteria
Patients are eligible for the study if they fulfil all three criteria below

1) AML as defined by the diagnostic criteria in section 16
2) Age < 19 years at time of diagnosis
3) Written informed consent

8.2. Exclusion Criteria
Patients are excluded if any of the criteria below are present

1) Previous chemotherapy or radiotherapy. This includes patient with secondary
AML after previous cancer therapy. They can be treated according to the protocol
but will not be included in the study population. Secondary AML has a poorer
response to chemotherapy but may benefit from SCT if the procedure can be
tolerated.
2) AML secondary to previous bone marrow failure syndrome.
3) Down syndrome (DS). Patients with myeloid leukaemia of Down syndrome are
recommended to be treated according to the international ML-DS protocol.
Patients with AML and DS older than 5 years who often lack GATA1 mutation and
do not have typical myeloid leukaemia of DS may be treated according to the
protocol but will not be included in the study population.
4) Acute promyelocytic leukaemia (APL). These patients are recommended
treatment according to the international APL Study.
5) Myelodysplastic syndrome (MDS). These patients are recommended treatment
according to EWOG-MDS.
6) Juvenile Myelomonocytic Leukaemia (JMML). These patients are recommended
treatment according to EWOG-MDS.
7) Known intolerance to any of the chemotherapeutic drugs in the protocol.
8) Fanconi anaemia.
9) Major organ failure precluding administration of planned chemotherapy.
10) Positive pregnancy test.
11) Lactating female or female of childbearing potential not using adequate
contraception.

All patients with myeloid leukaemia or MDS, including those fulfilling any exclusion
criteria, should be reported to the NOPHO-AML database. Reporting should be done
regardless of therapy given in order to obtain a population-based database.

8.3. Withdrawal of consent
Patients participating in the randomised studies can at any time choose to leave the
studies without being required to specify a reason. These patients are recommended to
be treated according to the control arm. If consent within either of the two studies is withdrawn at a time when they have started the experimental therapy the course in question should in general be concluded. All patients will be subject to continued registration in the database to allow for intention-to-treat analysis.
9. Risk Grouping

Risk stratification in AML 2012 is based on treatment response and the presence of a FLT3-ITD mutation without concurrent NPM1 mutation and the presence of inv(16) [(including t(16;16)]. Treatment response is primarily evaluated by flow cytometry and every effort should be made at time of diagnosis to identify a leukaemia associated immunophenotype (LAIP) allowing for MRD determination at a sensitivity of at least 0.1% (see guidelines in section 16.4.2). This will not be feasible in approximately 5-20% of patients. In these patients treatment response will be evaluated by bone marrow morphology but in patients with fusion genes (t(8;21), inv(16) and some of the MLL rearrangements) qPCR may be used to assist morphological evaluation. However, caution must be applied since the correlation between qPCR results and fraction of remaining leukemic cells at this time point is largely unknown.

The presence of FLT3-ITD mutations and NPM1 mutations should be assessed by PCR as described in section 16.1.3.

9.1. High-risk group.

Patients are allocated to the high-risk group (HR) if they achieve CR after two induction courses and either of the following criteria are fulfilled

1. Poor response after course 1 defined by the presence of ≥15% leukemic cells at BM evaluation on day 22, or at any subsequent evaluation prior to course 2. In all patients with an informative LAIP the flow cytometric MRD results should be used for evaluation, else traditional morphological analysis will be employed.

2. Intermediate response after the first two induction courses. In patients with an informative flow LAIP this is defined as the presence of ≥0.1%-4.9% leukemic cells before start of the first consolidation. In patients without an informative LAIP this criterion cannot be evaluated.

3. The presence of a FLT3-ITD mutation without concurrent NPM1 mutation.

Note that patients who after the second induction have ≥ 5% leukemic cells on MRD flow or, in patients with non-informative flow, ≥ 5% blast cells by morphology, will be classified as having resistant disease and thus not classified to the high-risk group. They will be off protocol therapy and eligible for salvage therapy such as according to protocol Relapsed AML 2010/01 (see section 12.13).

Based on historical data it is expected that approximately 15% of patients will be stratified to the high-risk group in NOPHO-AML 2012.

9.2. Standard-risk group

All patients that do not fulfil any of the high-risk criteria and have complete remission after two induction courses will be assigned to the standard-risk group (SR). Note that standard risk patients with inv(16) [(including t(16;16)] will only receive two consolidation courses by omission of the first consolidation course HAM.
10. Patient and data registration

In order to participate in the randomised studies the patient must be registered and mandatory data entered in the NOPHO-AML database within set time limits.

10.1. Mandatory data

The main purpose of registration of mandatory data is to ensure that the entire protocol and the randomised studies can be conducted according to good clinical practice guidelines and to guarantee appropriate risk stratification.

10.1.1. Mandatory data before randomisation in DNX study

Mandatory data prior to first randomisation should be entered within four days of diagnosis so that randomisation can be done at the latest on the fifth day of the first course. The following data must be provided

1. Patient identification data (name, date of birth, patient identification number, sex)
2. Centre identification data
3. Inclusion criteria
   a) Diagnosis of AML and date of diagnosis
   b) Written informed consent obtained for DNX study (yes/no)
   c) if no consent – reason for non-inclusion
4. Exclusion criteria
   a) Previous chemo- or radiotherapy (yes/no)
   b) AML secondary to previous bone marrow failure syndrome (yes/no)
   c) Downs syndrome (yes/no)
   d) APL (yes/no)
   e) MDS (yes/no)
   f) JMML (yes/no)
   g) Known intolerance to components of chemotherapy (yes/no)
   h) Fanconi anaemia (yes/no)
   i) positive pregnancy test (yes/no)
   j) lactating female or female of childbearing potential not using adequate contraception (yes/no)
5. ECHO (ejection fraction and fractional shortening)

10.1.2. Additional mandatory data at diagnosis

Results of the additional mandatory investigations at diagnosis should be entered in the database within the first three weeks from start of therapy. These comprise

1. Immunophenotype and LAIP
2. Karyotype
3. CNS status (note that it is recommended to perform evaluation of CSF on day 6 of the first course)
   a) clinical findings of CNS involvement (e.g. cranial nerve palsy) (yes/no, description)
b) CSF cell count (RBC, WBC) before first intrathecal chemotherapy
c) CSF cytology before first intrathecal chemotherapy
4. Presence of other extramedullary involvement (yes/no)
   Site, method of evaluation.
5. Hepatomegaly (yes/no)
6. Splenomegaly (yes/no)
7. Peripheral blood values (hemoglobin, white blood cell count, platelet count, peripheral blast cell count)
8. Other associated anomalies (yes/no, specify if yes)

10.1.3. Mandatory data at day 22 after course 1 (before FLADx randomisation)
These data evaluate the outcome of course 1 and provide baseline data for the randomisation in the FLADx study and risk grouping.

1. Result of flow MRD quantification in BM
   a) The centre registers result (<0.1 / 0.1 - 4.9 / 5-14.9 / ≥15% / no LAIP)
   b) The laboratory registers if an informative LAIP was detected (yes, sensitivity ≤ 0.1%/yes, sensitivity ≤ 5%/no)
      if yes -> result is given
2. Bone marrow cellularity (aplastic/hypoplastic/normal)
3. Bone marrow blast count (<5% / 5-14.9% / ≥ 15% and absolute value)
   Genetic abnormalities at diagnosis (PCR or FISH)
   t(8;21) yes/no
   t(15;17) yes/no
   Inv(16) yes/no
   FLT3 mutation FLT3-ITD/point mutation/wild type
   NPM1 mutation yes/no
4. Written consent for randomisation in the FLADx study (yes/no)
   if no consent – reason for non-inclusion
5. Cardiac evaluation after course 1
   Clinical signs of cardiac dysfunction (arrhythmia, cardiac failure)
   ECHO prior to course 2 (ejection fraction and fractional shortening)

10.1.4. Mandatory data before consolidation (at start of HAM\textsuperscript{*})
These data evaluate the outcome of the whole induction including the randomised studies and provide information necessary for risk grouping.

1. Result of flow MRD quantification in BM
   a) The centre registers result (<0.1 / 0.1 - 4.9 / ≥5% / no LAIP)
   b) The laboratory registers if an informative LAIP was detected (yes, sensitivity ≤ 0.1%/yes, sensitivity ≤ 5%/no)
      if yes -> result
2. Bone marrow cellularity (aplastic/hypoplastic/normal)
3. Bone marrow blast count (<5% / ≥ 5% and absolute value)

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\textsuperscript{*} For standard risk patients with inv(16)/t(16;16) - HA\textsubscript{3}E
4. If CNS positive at diagnosis
   a) time-point of negative cytology
   b) evaluation of tumour mass in CNS if applicable
5. If other extramedullary involvement at diagnosis
   radiological evaluation of tumour
6. Cardiac evaluation after course 2
   Clinical signs of cardiac dysfunction (arrhythmia, cardiac failure)
   ECHO prior to HAM (ejection fraction and fractional shortening)
11. **Required evaluations, procedures and documentation**

11.1. **Initial evaluation**

1. History and physical examination.
2. Haemoglobin, WBC with differential count, platelet count.
3. Sodium, potassium, creatinine, urea, uric acid, ALAT, LDH, bilirubin, calcium, phosphorus, magnesium.
5. Coagulation screen (prothrombin time, APTT, fibrinogen and fibrinogen split products). If abnormal, perform additional investigations including plasminogen and AT III.
6. BM aspirate and biopsy for diagnosis. Morphological, immunophenotypic, cytogenetic, and molecular genetic analyses will be assessed according to the diagnostic guidelines (see section 16).
7. CSF cell count, differential, and protein, cytological examination of cytospin preparation. Note that it is recommended to perform evaluation of CSF on day 6 of the first course.
8. HLA typing of patient is strongly recommended.
9. Hepatitis B surface antigen, HIV, EBV, CMV, VZV, and HSV titres.
10. Quantitative immunoglobulin levels.
11. Baseline cardiac evaluation by echocardiography.
12. Serum, PB and BM cells should be sent to the central bio bank (appendix 8).
13. Randomisation in the DNX study. After giving information and obtaining consent the mandatory data should be entered in the NOPHO database and the patient randomised. If consent is not obtained the reason should be registered.

The results of the above investigations should be documented in the online registry when appropriate.

11.2. **Evaluation and procedures following induction course 1 (MEC or DxEC)**

11.2.1. **Toxicity.**

It is anticipated that almost all children will require care at the paediatric unit for the majority of time between course 1 and 2. The toxicity after MEC or DxEC should be registered online according to the SAE registration form (appendix 2).

11.2.2. **Bone marrow evaluation**

On day 22 from start of course 1 a bone marrow aspirate should be taken with evaluation of morphology (cellularity and blast count) and flow MRD quantification. If present, qPCR for t(8;21) or inv(16) must be performed. It is strongly recommended to investigate qPCR for other fusion genes when possible. It is also recommended to perform a biopsy with touch preparations. If flow MRD quantification shows ≥ 5% leukemic cells the patients will proceed to receive the second course. Children without a sufficiently sensitive LAIP and with a blast count on morphology of ≥5% will also proceed immediately to the second course.
Patients with an MRD with <5% leukemic cells (or <5% blasts on morphology in those with no sensitive LAIP) will not start course 2 until they have regeneration of peripheral blood counts (rising counts with ANC ≥ 0.5 10⁹/L and platelets ≥ 80 10⁹/L). These patients should repeat bone marrow aspiration with MRD quantification once weekly in order to detect regrowth of the leukemic clone. When regeneration occurs a final bone marrow examination with MRD flow must be done if the previous bone marrow investigation was performed more than five days prior to the planned start of the second course. If regrowth of LC to ≥ 5% occurs course 2 should be started immediately.

11.2.3 Evaluation of extramedullary disease
Patients with CNS involvement or CNS2 (cytospin positive for leukemic cells with < 5 white blood cells/mm³) at diagnosis should have a lumbar puncture on day 22 with CSF cell count and cytology. Those with other extramedullary involvement will primarily be investigated by clinical examination at this point unless it for clinical reasons is important to map the extramedullary tumour (e.g. intraspinal tumour with risk of medullary or nerve compression).

11.2.4 Randomisation in the FLADx study
The children and guardians should be informed of the FLADx study and after obtaining consent the mandatory data should be entered in the NOPHO database and the patient randomised. If consent is not obtained the reason should be registered.

11.2.5 Cardiac evaluation
A repeat echo should be performed prior to start of course 2. Clinical signs of heart dysfunction should be documented in the NOPHO database together with a measure of cardiac contractility (fractional shortening and ejection fraction).

11.3 Evaluation and procedures following induction course two (ADxE or FLADx)

11.3.1 Toxicity
The toxicity after ADxE or FLADx should be registered online according to the SAE registration form (appendix 2).

11.3.2 Bone marrow evaluation

11.3.2.1 Patients with ≥ 5% leukemic cells after course 1
A BM aspirate is done on day 22 from start of course 2 and evaluated for morphology (cellularity and blast count), flow MRD quantification and, when applicable, MRD quantification of fusion transcripts with qPCR. It is also recommended to perform a biopsy with touch preparations.

If flow MRD quantification shows ≥ 5% leukemic cells after the 2nd course the disease is classified as resistant disease and salvage therapy should be given. Children without a sufficiently sensitive LAIP and with a morphology blast count of ≥5% will also be classified as having resistant disease and proceed immediately to salvage.
In patients who have a very aplastic marrow and thus have a low absolute number of leukaemic cells, it can be wise, after discussing with the study and/or national coordinator, to withhold salvage therapy and repeat a BM within a week. At this time-point the children are very heavily treated and occasionally the fraction of LC will decrease on peripheral regeneration. If the second BM shows <5% LC the patient should have repeated BM examinations until peripheral regeneration has occurred (rising counts with ANC ≥ 0.5 × 10^9/L and platelets ≥ 80 × 10^9/L) and consolidation can be started. For risk stratification the results of the last BM, which should be performed within five days before start of consolidation, will be used. When regeneration occurs a final bone marrow examination with MRD flow must be done if the previous BM MRD investigation was performed more than five days prior to the planned start of HAM.

If any of the repeat examinations show ≥ 5% LC the patient is classified as having resistant disease and proceeds to salvage therapy. The results of the last BM examination should be available before starting therapy.

11.3.2.2. Patients with < 5% leukemic cells after course 1
The children will start consolidation therapy when peripheral regeneration of blood counts has occurred (rising counts with ANC ≥ 0.5 and platelets ≥ 80) and the clinical condition is good. A BM aspirate is done immediately prior to start of course 2 and evaluated for morphology (cellularity and blast count), flow MRD quantification and when applicable MRD quantification of fusion transcripts with qPCR. It is also recommended to perform a biopsy with touch preparations. If there is no clinical suspicion of relapse, HAM can be started prior to obtaining results from the BM examination. Patients with inv(16) [including patients with t(16;16)(p13;q22)] will receive HA\textsubscript{3}E instead of HAM.

Note: Since it, in patients with a good response to course 1, is allowed to start consolidation before the MRD results after course 2 are known, it is possible but unlikely that a patient with inv(16)/t(16;16) will have an MRD ≥ 0.1%. These rare patients should be assigned to the HR arm and receive treatment with SCT. While waiting for SCT they continue chemotherapy as depicted for inv(16)/t(16;16).

11.3.3. Evaluation of extramedullary disease
Patients with CNS involvement at diagnosis should have a lumbar puncture with cell count and cytology prior to consolidation. This will, for patients with ≥5% LC after course one be done on day 22 after course 2 whereas all others will do it at the time of BM immediately preceding consolidation. Those with other extramedullary involvement will be evaluated by radiology as appropriate at the same time point. In case of residual disease contact with the study and/or national coordinator should be taken.

11.3.4. Cardiac evaluation
An ECHO should be performed prior to start of consolidation. Clinical signs of heart dysfunction should be documented in the NOPHO database together with a measure of cardiac contractility (fractional shortening and ejection fraction).
11.3.5. Risk Stratification

When the final results of the response evaluation after course 2, together with the evaluation after course 1 and cytogenetic investigations of FLT3-ITD, NPM1 and inv(16) are available, risk grouping can be done (see section 9). Since the stratification relies on MRD flow investigation, which is technically demanding, it is mandatory for the laboratory to send the data files from the MRD flow investigations at diagnosis, from day 22 after course 1 and from the last BM performed prior to consolidation or start of salvage therapy for central review. This should be done within days after analysis and the results of the review will be returned within two weeks and entered in the database by the reviewer (appendix 4 describes the procedure for review).

11.4. Evaluation and procedures during consolidation

11.4.1. Toxicity.

The toxicity after each course should be registered online as depicted in 14.2.3. Prior to each consolidation course peripheral regeneration with rising counts and ANC ≥ 0.5 \(10^9\)/L and platelets ≥ 80 \(10^9\)/L is required. Serum levels of creatinine, liver enzymes and bilirubin should be checked before each course.

11.4.2. Bone marrow evaluation

11.4.2.1. High-risk patients

BM aspirates, with evaluation of morphology (cellularity and blast count), flow MRD quantification and when applicable MRD quantification of fusion transcripts with qPCR, should be performed prior to each consolidation course and within two weeks prior to SCT. This is also recommended in patients with resistant disease.

11.4.2.2. Standard-risk patients

Unless clinically indicated, e.g. exceptionally long aplasia or symptoms suggestive of relapse, BM evaluation with analysis of morphology (cellularity and blast count), flow MRD quantification and when applicable MRD quantification of fusion transcripts with qPCR, is optional before HA3E but mandatory prior to the last consolidation course (FLA).

11.4.3. Evaluation of CNS disease

Patients with CNS involvement at diagnosis should have a lumbar puncture with analysis of cell count and blasts on cytospin at the time of the intrathecal injection of each consolidation course.

11.5. Evaluation and procedures after completion of therapy

The aim of follow-up is to early detect disease recurrence and late effects of therapy. It is also essential to obtain data for evaluation of the entire protocol and the randomised studies.

For patients with isolated extramedullary disease, other than in CNS, adequate radiological investigations should be done before the last course (FLA).
11.5.1. Early disease detection

Only patients who have a positive MRD flow prior to FLA should perform a routine bone marrow evaluation two months after completion of therapy.

In all patients with a fusion gene marker, it is recommended to quantify the transcript in PB by qPCR every second month until 18 months from diagnosis. In case a more than one-log rise in transcript levels occurs, a BM examination with MRD flow and MRD qPCR should be performed. The patient should be discussed with the study coordinator and assessed for eligibility for pre-emptive therapy. A donor search for SCT should be initiated in these patients. Children treated with SCT in first remission should also be followed with bimonthly qPCR.

Except for the patients with MRD flow positivity prior to FLA (see above), patients lacking PCR markers will only be subject to BM examination if relapse is suspected on clinical or laboratory grounds.

11.5.2. Late effects of therapy

Patients who have received SCT should be subject to follow-up according to specific guidelines from the transplant centre. However, ECHO at one, five and ten years after diagnosis is mandatory.

Following recovery after the last consolidation, including laboratory testing for parameters of haematological, liver and kidney function, all patients not receiving SCT, should be monitored clinically at an initial interval of 6-8 weeks. Even after regeneration of haematological parameters, the patients will be immunocompromised for an extended period and protective antibodies from previous immunisations are often decreased or absent. Patients are recommended to either be evaluated and/or re-immunized for diphteria, tetanus, polio and hemophilus influenzae at 3-6 months after completion of therapy and tested for measles and rubella antibodies after one year. Further antibody measurements or immunisations may be done at the discretion of individual centres/countries.

Previous experience from patients treated for AML with only chemotherapy is that long-term side effects are few and health quality good(45). Nonetheless, severe long-term effects may develop in some patients. At appropriate intervals the patients should be assessed for growth and pubertal development.

Given the high nominal anthracycline load it is important to question the patient on physical capacity. Cardiac function should be assessed by echo at one, five and ten years after diagnosis.

11.5.3. Evaluation of protocol and randomised studies

Cardiac function, as assessed by ECHO, should be performed and documented in the online database at one, five and ten years following diagnosis. Relapses, second malignancies and serious late-effects should be registered on occurrence. For the first five years follow-up should be updated at least semi-annually in the database.
12. Treatment plan

12.1. Therapy overview
AML requires very intensive therapy with a high risk of severe toxicity and treatment is therefore sometimes subject to individual modification. This protocol offers guidelines and general recommendations but the treating physician may, mainly depending on the clinical parameters of the individual patient, make modifications. All major decisions e.g. regarding disease evaluation and delay or modification of chemotherapy must be taken at a paediatric oncology unit. In case problems arise, that are not addressed in the protocol, contact should be taken with the national and/or study coordinator. Deaths and SUSARs should be reported to the study centre through the online leukaemia registry within 48 hours.

12.1.1. Initiation of therapy
Figure 1 shows a flow chart over therapy in AML 2012. After initial evaluation, as depicted in section 11.1 and institution of supportive therapy, randomisation can be performed in eligible patients. Course one (MEC or DxEC) can be started already prior to completed randomisation since treatment is identical for the first five days. Figure 13 shows how therapy response during induction affects repeat BM evaluations, timing of courses and risk stratification.

*Note that patients with inv(16) with good response should receive HA₃E instead of HAM.*
### 12.1.2. Evaluation after course 1 (MEC or DxEC)

On day 22 from start of course 1, the first evaluation of bone marrow response is done. Bone marrow morphology, MRD by flow cytometric analysis and, in patients with a fusion gene allowing quantification, qPCR should be performed. In patients with an informative LAIP, flow cytometry shall be used to determine treatment response. In patients with non-informative flow results bone marrow morphology, supported by qPCR when applicable, will be used.

Patients with ≥ 5% leukaemic cells by MRD flow or, in patients where flow is non-informative, with ≥ 5% blasts on bone marrow morphology should immediately be randomised and start treatment with course 2 (ADxE or FLADx).

Patients with < 5% leukaemic cells by MRD flow or, in patients where flow is non-informative, with < 5% blasts on bone marrow morphology should be randomised but not receive treatment with course 2 until peripheral regeneration of blood counts has occurred (rising counts with ANC ≥ 0.5 \(10^9\)/L and platelets ≥ 80 \(10^9\)/L) and the clinical condition is good. However, weekly examinations of bone marrow should be performed in these patients in order to detect regrowth of the leukaemic clone during aplasia. If an increase in leukaemic cells to ≥ 5% occurs, course 2 should be started immediately. In patients with repeated BM examinations between course 1 and 2 it is mandatory to register the BM on day 22 and the last BM prior to course 2 in the database. When regeneration occurs a final bone marrow examination with MRD flow must be done if the previous bone marrow investigation was performed more than five days prior to the planned start of the second course.

Poor response after course 1 is defined as ≥ 15% leukaemic cells or, in patients with non-informative LAIP, ≥15% blast cells at any evaluation time prior to course 2. Intermediate response after course 1 is defined as 5–14.9% leukaemic cells or, in patients with non-informative LAIP, 5–14.9% blast cells at any evaluation time prior to course 2. Good response is defined as < 5% leukaemic cells or, in patients with non-informative LAIP, < 5% blast cells at the final evaluation prior to course 2.

### 12.1.3. Evaluation after course 2 (ADxE or FLADx)

Depending on response to the first treatment course, evaluation will be done in two different ways.

#### 12.1.3.1. Patients with ≥ 5% leukaemic cells after course one

In patients with poor or intermediate response to course 1, a bone marrow evaluation should be performed on day 22 after course 2. If they have ≥ 5% leukaemic cells in the bone marrow they are classified to have resistant disease and should proceed to salvage therapy as outlined in section 12.13. If the day 22 bone marrow is very aplastic, it may at this time sometimes be prudent to withhold therapy even if leukaemic cell counts are ≥ 5%. At this point the children are heavily treated and have in general experienced a large reduction in tumour cell burden and sometimes, in those with very aplastic marrows, the fraction of leukaemic cells may be reduced upon regeneration. These patients should be discussed with the national or...
study coordinator and, in case of withholding therapy, a repeat marrow should be performed after one week. If this shows < 5% leukaemic cells they can proceed according to protocol guidelines.

Patients with < 5% leukaemic cells on day 22 will start consolidation therapy when peripheral regeneration of blood counts has occurred (rising counts with ANC ≥ 0.5 $10^9$/L and platelets ≥ 80 $10^9$/L) and the clinical condition is good. However, weekly examinations of bone marrow should be performed in these patients in order to detect regrowth of the leukaemic clone during aplasia. When regeneration occurs a final bone marrow examination with MRD flow must be done if the previous bone marrow investigation was performed more than five days prior to the planned start of HAM. If an increase in leukaemic cells to ≥ 5% occurs, the patient will be classified as having resistant disease and proceed to salvage therapy. It is recommended that these patients be discussed with the study and/or national coordinator.

12.1.3.2. Patients with < 5% leukaemic cells after course one
In patients with good response to course 1 the bone marrow will, unless clinically indicated, only be evaluated following regeneration of peripheral blood counts immediately prior to starting the first consolidation course. Patients with inv(16) will not receive HAM but HA₃E as their first consolidation course. The first consolidation course may be started as soon as the BM examination has been performed.

12.1.4. Consolidation therapy
Immediately prior to starting the first consolidation course the final bone marrow with MRD determination required for risk grouping is performed. If the patient at this point has ≥ 5% leukaemic cells the patient has resistant disease and salvage therapy should be given with the aim of proceeding to stem cell transplant (see section 12.12). Since MRD by flow is technically demanding and is crucial for risk grouping, the flow data files obtained at diagnosis, at day 22 after course 1 and before consolidation will be reviewed by the protocol MRD group within two weeks after start of consolidation (see section 16.4.2).

Standard-risk patients will receive three consolidation courses (HAM, HA₃E, FLA) except for patients with inv(16) who are given two courses (HA₃E, FLA). High-risk patients will receive HAM and then proceed to SCT as soon as possible if an adequate donor is identified. If a donor is not identified, consolidation should proceed according to the standard risk arm (see section 12.12). Also patients with inv(16)/t(16;16) with high-risk should proceed to SCT.
12.1.5. Overview of MRD flow sampling during treatment

Figure 14. Time points of MRD measurement in AML 2012. The exact number of MRD is dependent on the time required for regeneration in individual patients with good response after the respective courses.
* For standard risk patients with inv(16) the last MRD is performed before the 4th course.
12.2. Induction course 1 MEC

This is the standard treatment for course 1 and should be given to patients who are not participating in the DNX study. Patients randomised to mitoxantrone in course 1 should also receive this course.

Treatment schedule for course 1 – MEC standard arm in the DNX study.
Etoposide 150 mg/m² as a 2 hour IV infusion day 1-5 (5 mg/kg for age < 1y or BW < 10kg).
Mitoxantrone 5 mg/m² as a 1 hour IV infusion day 6-10 (0.17 mg/kg for age < 1y or BW < 10kg).
Cytarabine 200 mg/m² as a 12 hour IV infusion day 6-12 (6.7 mg/kg for age < 1y or BW < 10kg).
For children with initial CNS involvement Mtx it is replaced with triple it.

Course MEC should be commenced as soon as the patient is properly hydrated with adequate urinary output. In case of coagulopathy, measures to reduce bleeding tendency should be instituted prior to starting chemotherapy. In general, a cytoreductive pre-phase is not recommended even with very high WBC counts. Adequate hydration (3 L/m²) and close follow-up of tumour lysis and coagulation abnormalities should be maintained during the course. Following this course, patients are expected to be severely neutropenic for an extended time and the risk of mucositis is high. It is mandatory that all patients receive the highest standard of supportive care.
12.3. Induction course 1 DxEC

Patients randomised to receive DaunoXome in course 1 should receive the course DxEC. This is the experimental arm in the DNX study and should not be given to any patient who is not randomised to this arm.

* If Mtx it given at diagnosis omit it therapy on day 6 unless in case of CNS involvement.

Treatment schedule for course 1 – DxEC experimental arm in the DNX study.

- Etoposide 150 mg/m² as a 2 hour IV infusion day 1-5 (5 mg/kg for age < 1y or BW < 10kg).
- DaunoXome 60 mg/m² as a 1 hour IV infusion day 6,8 and 10 (2 mg/kg for age < 1y or BW < 10kg).
- Cytarabine 200 mg/m² as a 12 hour IV infusion day 6-12 (6.7 mg/kg for age < 1y or BW < 10kg).

For children with initial CNS involvement Mtx it is replaced with triple it.

Course DxEC should be commenced as soon as the patient is properly hydrated with adequate urinary output. In case of coagulopathy, measures to reduce bleeding tendency should be instituted prior to starting chemotherapy. In general, a cytoreductive pre-phase is not recommended even with very high WBC counts. Adequate hydration (3 L/m²) and close follow-up of tumour lysis and coagulation abnormalities should be maintained during the course. Following this course, patients are expected to be severely neutropenic for an extended time and the risk of mucositis is high. It is mandatory that all patients receive the highest standard of supportive care. In case of hepatic dysfunction the dose of DaunoXome should be adjusted according to section 12.14.4.
12.4. Induction course 2 ADxE
This is the standard therapy for course 2. Patients randomised in the FLADx study to receive ADxE and patients not participating in the study will receive ADxE. Patients who have ≥ 5% leukaemic cells on the day 22 evaluation after course 1 and are randomised to this treatment, should receive ADxE immediately whereas patients with < 5% leukaemic cells will receive therapy after recovery. The course can then be given when the patient is in clinical condition to tolerate therapy and peripheral blood counts are rising with ANC ≥ 0.5 x 10⁹/L and platelets ≥ 80 x 10⁹/L. An ECHO should be performed prior to starting ADxE. Adequate hydration (2-3 L/m²) should be maintained throughout the course.

![Treatment schedule for course 2 - ADxE standard arm in the FLADx study.](image)

Cytarabine 100 mg/m² as a continuous IV infusion day 1-2 (3.3 mg/kg for age < 1y or BW < 10kg).
Cytarabine 100 mg/m² as a 30 min IV infusion every 12 hours day 3-8 (12 doses) (3.3 mg/kg for age < 1y or BW < 10kg).
Etoposide 150 mg/m² as a 2 hour IV infusion day 6-8 (5 mg/kg for age < 1y or BW < 10kg).
DaunoXome 60 mg/m² as a 1 hour IV infusion day 2,4 and 6 (2 mg/kg for age < 1y or BW < 10kg).

For children with initial CNS involvement Mtx it is replaced with triple it.

All patients, but especially those that commence ADxE early due to poor or intermediate response to course 1, are expected to have severe neutropenia for a prolonged period. It is mandatory to assure that all children receive the highest quality of supportive care. Patients with poor or intermediate response to course 1 should following ADxE have a bone marrow evaluation performed on day 22. In case of hepatic dysfunction the dose of DaunoXome should be adjusted according to section 12.14.4.
12.5. Induction course 2 FLADx
This is the experimental arm for course 2. Only patients randomised in the FLADx study to receive FLADx should receive this course. Patients who have ≥ 5% leukaemic cells on the day 22 evaluation after course 1 and are randomised to this treatment should receive FLADx immediately whereas patients with < 5% leukaemic cells will receive therapy after recovery. The course can then be given when the patient is in clinical condition to tolerate therapy and peripheral blood counts are rising with ANC ≥ 0.5 x 10⁹/L and platelets ≥ 80 x 10⁹/L. An ECHO should be performed prior to start of FLADx. Steroid eye drops should be given daily to prevent cytarabine induced conjunctivitis. Adequate hydration (2-3 L/m²) should be maintained throughout the course.

Treatment schedule for course 2 – FLADx experimental arm in the FLADx study.
Fludarabine 30 mg/m² as a 30 min IV infusion day 1-5 (1 mg/kg for age < 1y or BW < 10kg).
Cytarabine 2000 mg/m² as a 3 hour IV infusion starting 4 hours after fludarabine day 1-5 (67 mg/kg for age < 1y or BW < 10kg).
DaunoXome 60 mg/m² as a 1 hour IV infusion immediately after fludarabine day 2,4 and 6 (2 mg/kg for age < 1y or BW < 10kg).
For children with initial CNS involvement Mtx it is replaced with triple it.

All patients, but especially those that commence FLADx early due to poor or intermediate response to course 1, are expected to have severe neutropenia for a prolonged period. It is mandatory to assure that all children receive the highest quality of supportive care. Patients with poor or intermediate response to course 1 should following FLADx have a bone marrow evaluation performed on day 22. In case of hepatic dysfunction the dose of DaunoXome should be adjusted according to section 12.14.4.
12.6. Consolidation

Only patients that respond to induction therapy with < 5% leukaemic cells will proceed to consolidation therapy. A bone marrow examination should be performed immediately prior to HAM. In patients with good response to course 1 this BM can be performed at the same time as HAM is commenced. Patients with inv(16) [including patients with t(16;16)] and < 5% LC after the first course 2 will receive HA3E as the first consolidation course whereas all other patients will receive HAM. In patients with ≥ 5% LC after course 1, the last bone marrow results after course 2 should be available before starting HAM. With the exception of standard risk patients with inv(16), who receive two consolidation blocks starting with HA3E, all other standard and high-risk patients will receive HAM after which high-risk patients should proceed to stem cell transplant as soon as possible (see section 12.12). If a patient with inv(16) who responded with < 5% LC after the first course and has started consolidation with HA3E, proves to have MRD ≥ 0.1% after course 2 the patient should be assigned to the high risk group including therapy with SCT.

**Figure 15. Overview of consolidation therapy in AML 2012. Standard risk patients with inv(16)/t(16;16) receive two consolidation blocks, all other SR patients three blocks.**

Before each consolidation course the children should be in good clinical condition and have recovery of peripheral blood cell counts. Haematological criteria for start of each course are rising counts with ANC ≥ 0.5 x 10^9/L and platelet count ≥ 80 x 10^9/L. ECHO should be performed before HAM. Serum levels of liver enzymes, creatinine and bilirubin should be checked prior to assess toxicity. All consolidation courses are toxic and children are expected to have prolonged neutropenia after each course. It is expected that the recovery time will be between 3-4 weeks in most cases. No course should be started earlier than three weeks from start of the previous course.
12.7. Consolidation course 1 HAM

Treatment schedule for course 3 – HAM.
Cytarabine 1000 mg/m² as a 2 hour IV infusion every 12 hours day 1-3 (six doses) (33 mg/kg for age < 1y or BW < 10kg).
Mitoxantrone 10 mg/m² as a one hour IV infusion day 3-5 (0.33 mg/kg for age < 1y or BW < 10kg).
For children with initial CNS involvement Mtx it is replaced with triple it.

Prior to HAM an ECHO should be done. Adequate hydration (2-3 L/m²) should be maintained throughout the course and steroid eye drops should be given daily to prevent cytarabine induced conjunctivitis.

12.7.1. Laboratory requirements before start of the course
Rising peripheral blood counts with ANC ≥ 0.5 x 10⁹/L and platelets ≥ 80 x 10⁹/L. Check S-bilirubin, S-ALAT, S-potassium, S-sodium and S-creatinine.
12.8. Consolidation course 2 HA₃E

Treatment schedule for course 4 – HA₃E.
Cytarabine 3000 mg/m² as a 2 hour IV infusion every 12 hours day 1-3 (six doses) (100 mg/kg for age < 1y or BW < 10kg).
Etoposide 100 mg/m² as a 1 hour IV infusion day 1-5 (3.3 mg/kg for age < 1y or BW < 10kg).
For children with initial CNS involvement Mtx it is replaced with triple it.

Adequate hydration (2-3 L/m²) should be maintained throughout the course and steroid eye drops should be given daily to prevent cytarabine induced conjunctivitis.

12.8.1. Laboratory requirements before start of the course
Rising peripheral blood counts with ANC ≥ 0.5 x 10⁹/L and platelets ≥ 80 x 10⁹/L. Check S-bilirubin, S-ALAT, S-potassium, S-sodium and S-creatinine.
12.9. Consolidation course 3 FLA

Treatment schedule for course 5 – FLA.
Fludarabine 30 mg/m² as a 30 min IV infusion day 1-5 (1 mg/kg for age < 1y or BW < 10kg).
Cytarabine 2000 mg/m² as a 3 hour IV infusion starting 4 hours after fludarabine day 1-5 (67 mg/kg for age < 1y or BW < 10kg).
For children with initial CNS involvement Mtx it is replaced with triple it.

Adequate hydration (2-3 L/m²) should be maintained throughout the course and steroid eye drops should be given daily to prevent cytarabine induced conjunctivitis.

12.9.1. Laboratory requirements before start of the course
Rising peripheral blood counts with ANC ≥ 0.5 x 10⁹/L and platelets ≥ 80 x 10⁹/L. Check S-bilirubin, S-ALAT, S-potassium, S-sodium and S-creatinine.

12.10. CNS Therapy
CNS disease is defined according to the diagnostic guidelines (section 16.2).

Patients without CNS disease and where no malignant cells are found in CSF receive one mtx intrathecal (it) injection on day 6 of course one and subsequently one injection at the start of each course. In patients, who in association with diagnostic procedures received mtx it, the injection day 6 should be omitted. No further evaluation of CSF-cytology needs to be done in these patients.

Patients without CNS disease but with presence of low levels of malignant cells in CSF (< 5 white blood cells/mm³) will have the same intrathecal treatment as patients without detectable blasts but should be evaluated with a lumbar puncture on day 22 after course one. If this is negative they will be treated and followed as patients without CNS disease.

Patients with CNS disease should do an MRI of the brain at diagnosis. They will receive age-adjusted intrathecal triple injections twice weekly until the CSF is free from blasts followed by two additional injections. A minimum of four injections should be given. Thereafter one
triple it is given at the start of each course. They will be evaluated with CSF cytology at day 22 after course one and before consolidation. If these are negative evaluation will be done at the start of the fifth course. Cranial irradiation is not indicated. If cytology is not negative following the third injection the patient should be discussed with the study and/or national coordinator.

Patients with CNS disease with focal symptoms without malignant blasts in CSF will receive triple it twice weekly for two weeks (i.e. a total of four injections) and then receive one triple it injection with each course.

If a mass is present on MRI this should be evaluated after two induction courses. A persisting mass should be discussed with the study and/or national coordinator.

**Table 3.** Age-adjusted doses for intrathecal methotrexate therapy in patients without CNS disease.

<table>
<thead>
<tr>
<th>Age</th>
<th>Methotrexate</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;1y</td>
<td>6 mg</td>
</tr>
<tr>
<td>1-&lt;2y</td>
<td>8 mg</td>
</tr>
<tr>
<td>2-&lt;3y</td>
<td>10 mg</td>
</tr>
<tr>
<td>≥3y</td>
<td>12 mg</td>
</tr>
</tbody>
</table>

**Table 4.** Age-adjusted doses for intrathecal triple therapy in patients with CNS disease.

<table>
<thead>
<tr>
<th>Age</th>
<th>Methotrexate</th>
<th>Cytarabine</th>
<th>Prednisolone</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;1y</td>
<td>6 mg</td>
<td>16 mg</td>
<td>4 mg</td>
</tr>
<tr>
<td>1-&lt;2y</td>
<td>8 mg</td>
<td>20 mg</td>
<td>6 mg</td>
</tr>
<tr>
<td>2-&lt;3y</td>
<td>10 mg</td>
<td>26 mg</td>
<td>8 mg</td>
</tr>
<tr>
<td>≥3y</td>
<td>12 mg</td>
<td>30 mg</td>
<td>10 mg</td>
</tr>
</tbody>
</table>

**12.11. Therapy of isolated extramedullary disease (non-CNS)**

Patients with isolated extramedullary disease and AML specific gene aberrations [t(8;21), inv(16) and MLL rearrangements where lymphoid lineage can be ruled out] can be enrolled and will be evaluated in the whole protocol and also be included in the randomised studies although they cannot be evaluated by the primary endpoint MRD flow. They should, prior to each course, be monitored by qPCR of the fusion gene transcript in PB.

Patients without AML specific gene aberrations but with clear evidence of myeloid malignant cells may be registered in the database and treated according to the standard treatment but they will not be included in the evaluation of the protocol. There is no evidence that radiation improves outcome but radiation may be indicated as emergency treatment in case the tumour presents with organ- or life-threatening symptoms (e.g. spinal compression). However, in most situations the tumours respond rapidly to chemotherapy.
12.11.1. Evaluation of therapy response

After course one (MEC) it is often sufficient to evaluate the extramedullary tumour by clinical investigation unless signs of progression are evident or the tumour presents a threat to organ function. Following course two (ADxE) appropriate radiological investigations should be performed and tumour volume measured. If the tumour has disappeared at this point, repeat radiological evaluation will only be performed prior to the last course (FLA) else radiology will be repeated prior to each course until the tumour has disappeared. Patients in whom the tumour is still present after induction should be discussed with the study and/or national coordinator. All patients with a quantifiable fusion gene should be monitored by qPCR on peripheral blood for 18 months at two-monthly intervals after completion of therapy.

12.12. Stem Cell Transplantation

Although induction therapy in AML 2012 is intensified some patients will have resistant disease and the number is expected to be around 5%. These patients will be off protocol. Based on AML2004 an additional 10-12% will have LC ≥ 0.1% after the second induction and around 5% will have an FLT3-ITD without a concurrent NPM1 mutation. Considering the overlap between suboptimal response and FLT3 mutation the total number of patients in the high risk group is expected to be around 15%. Patients in the high-risk group should, whenever possible, receive an allogeneic SCT with an HLA matched related or unrelated donor. In all high-risk patients a donor search should be commenced as soon as possible. Allele compatibility for at least the HLA-A/B/C/DRB1/DQB1 loci (which forms the basis for the traditional 10/10 match) should be investigated by high resolution techniques and donors with a 9/10 or 10/10 identity are considered matched. If a suitable donor is not identified, SCT using adequately matched umbilical cord cells may be performed. Although SCT techniques are developing rapidly, more experimental SCT approaches are not presently recommended for high-risk patients, but should be considered for patients proceeding to salvage therapy following the first two induction courses.

For high-risk patients the SCT should be done as soon as possible after course 3 (HAM), but may be given after the fourth or fifth course if the donor is not available until then. However, the indication for transplant should be re-evaluated in patients who have completed all three consolidation courses. Although disease status at time of SCT has been shown to influence outcome, there is at present not enough evidence to give recommendations for timing of transplant in relation to the level of disease(46). However, disease status (morphology, flow and when applicable PCR results) immediately prior to SCT should be documented in the database. The source of stem cells may be bone marrow, peripheral blood or cord blood. The recommended conditioning regimen is busulfan/cyclophosphamide/melphalan in patients who tolerate full conditioning. Reduced intensity regimens may be considered in selected patients with severe organ toxicity.

Autologous transplants should not be performed.
12.13. **Salvage therapy**

It is expected that 90-95% of patients will have an adequate response (i.e. < 5% LC) and proceed to standard or high-risk consolidation after induction therapy. In patients with resistant disease in the AML 2004 protocol, FLA was shown to be effective in achieving remission. Therefore, patients with resistant disease (≥5% LC after induction, see figure 2) who not have received FLADx as their second induction course are eligible for the I-BFM-SG Relapsed AML 2010/01 study. In these patients FLA based therapy has been proven effective. For patients randomised to FLADx, evidence-based treatment recommendations are more difficult to give, but CloEC (clofarabine, etoposide, cyclophosphamide), CLARA-X (clofarabine, cytarabine, DaunoXome) or MACE (amsacrine, cytarabine, etoposide ± Gemtuzumab) may be alternatives in this situation. These patients should be discussed with the study and/or national coordinator. If patients respond, treatment should aim at SCT, conventional or experimental. Even patients who respond poorly and continue to have significant residual disease may in this situation benefit from SCT(46).

12.14. **Treatment modifications**

AML therapy is very intensive and sometimes severe and/or unpredictable organ dysfunction occurs either due to the disease or to direct or indirect consequences of chemotherapy. Examples are kidney failure caused by TLS, severe pulmonary insufficiency due to infection, ARDS, neurotoxicity or fulminant liver toxicity. Patients with severe organ toxicity requiring extensive treatment modification should be discussed with the study and/or national coordinator.

For children above one year and body weight (BW) above 10 kg all doses are calculated according to body surface area (BSA). BSA in m² is calculated by Mosteller’s formula(47).

\[
BSA(m^2) = \sqrt{\frac{BW(kg) \times \text{Length(cm)}}{3600}}
\]
12.14.1. Dose reduction in children < 1 year or < 10 kg
Children below 1y or < 10 kg should receive doses according to body weight. The per kg doses of each drug are calculated by setting $1 \text{ m}^2 = 30 \text{ kg}$ obtaining the doses given in table 5.

**Table 5.** Children below 1 year or < 10 kg should receive doses according to body weight (column with bold numbers).

<table>
<thead>
<tr>
<th>Drug</th>
<th>Course</th>
<th>Dose mg/m² (≥ 1y and ≥10 kg)</th>
<th>Dose mg/kg &lt; 1y OR &lt; 10 kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cytarabine</td>
<td>ADxE</td>
<td>100</td>
<td>3.3</td>
</tr>
<tr>
<td>Cytarabine</td>
<td>MEC/DxEC</td>
<td>200</td>
<td>6.7</td>
</tr>
<tr>
<td>Cytarabine</td>
<td>HAM</td>
<td>1000</td>
<td>33</td>
</tr>
<tr>
<td>Cytarabine</td>
<td>FLA/FLADx</td>
<td>2000</td>
<td>67</td>
</tr>
<tr>
<td>Cytarabine</td>
<td>HA₃E</td>
<td>3000</td>
<td>100</td>
</tr>
<tr>
<td>DaunoXome</td>
<td>ADxE/FLADx</td>
<td>60</td>
<td>2</td>
</tr>
<tr>
<td>Etoposide</td>
<td>HA₃E</td>
<td>100</td>
<td>3.3</td>
</tr>
<tr>
<td>Etoposide</td>
<td>MEC/DxEC/ADxE</td>
<td>150</td>
<td>5</td>
</tr>
<tr>
<td>Fludarabine</td>
<td>FLADx/FLA</td>
<td>30</td>
<td>1</td>
</tr>
<tr>
<td>Mitoxantrone</td>
<td>MEC</td>
<td>5</td>
<td>0.17</td>
</tr>
<tr>
<td>Mitoxantrone</td>
<td>HAM</td>
<td>10</td>
<td>0.33</td>
</tr>
</tbody>
</table>

12.14.2. Obesity
Some studies indicate that obesity may affect drug disposition and a US study shows that obesity reduces outcome in paediatric AML(48). However, no firm data regarding the pros and cons of adjusting doses for obesity, including capping, exist. Therefore the protocol does not recommend any dose modification in these patients.

12.14.3. Cardiac toxicity
Children with congestive heart failure that is likely to be caused by anthracyclines should receive no further liposomal daunorubicin or mitoxantrone. In children with significant decrease in either fractional shortening or ejection fraction it should be considered to discontinue anthracyclines.

12.14.4. DaunoXome
Due to limited experience in patients with hepatic dysfunction a reduction in doses is recommended if patients before the start of each course have
- S-Bilirubin 25-50 µmol/L give 75% of dose
- S-Bilirubin 50-100 µmol/L give 50% of dose
- S-Bilirubin >100 µmol/L discuss with study coordinator

In patients with severe renal impairment a reduction of the dose may be appropriate and should be discussed with the study and/or national coordinator.
13. Randomised studies

Two randomised studies both investigating the efficacy of induction treatment are integrated in the AML 2012 protocol. Patients are eligible for the studies if they fulfil inclusion and lack exclusion criteria as listed in sections 8.1 and 8.2 and if all mandatory data are available (section 10.1).

13.1. Comparison of efficacy of DaunoXome and Mitoxantrone in course 1 (DNX study)

13.1.1. Short rationale
Despite intensive therapy, at least 1/3 of all children with AML will experience a relapse. Therapy response is the most important predictor of relapse and recent studies show that early response to induction therapy can be evaluated by MRD quantification using flow cytometry to identify cells with a LAIP. Furthermore, the MRD levels both after the first and second induction courses have a large prognostic impact.(11, 31).
In almost all protocols, induction therapy in paediatric AML is based on cytarabine and an anthracycline, commonly with the addition of etoposide. Few paediatric studies have compared the efficacy of the different anthracyclines in AML induction and it is still unresolved how the cardiotoxicity of different anthracyclines is related to the anti-leukemic effect. Liposomal daunorubicin (L-DNR) has been used in adults at high doses with low cardiotoxicity, but follow-up has in general been short in these studies(22, 49). It has also been used in the paediatric AML2001/01 relapse study with a low incidence of cardiac toxicity despite heavy pre-treatment(50). Recently, the BFM published a randomised study comparing L-DNR with idarubicin and could show a trend towards better EFS and OS with no increase in toxicity(12).

The Japanese AML99 protocol used a twelve day long induction course with cytarabine, etoposide and mitoxantrone and could show a very high CR rate of 86% after the first course and also very good EFS of 62% and OS of 76% at 5 years.(10).

Since L-DNR has been shown to have a strong anti-leukemic effect in paediatric AML and may be less cardiotoxic than other anthracyclines it is essential to further investigate the drug in paediatric AML(51). We therefore want to investigate drug efficacy and compare cardiac toxicity in the context of a very effective induction course namely the MEC course from the AML99 study. Patients will be randomised to receive mitoxantrone, which is the standard arm, or liposomal daunorubicin.

13.1.2. Primary objective
To investigate if liposomal daunorubicin is more effective than mitoxantrone in reducing leukaemic cell numbers when used in the first induction course.

13.1.3. Secondary objective
- To investigate if liposomal daunorubicin improves CR rates, EFS and OS.
- To investigate and compare severe toxicity and mortality in the treatment arms.
- To compare cardiac toxicity in the treatment arms.
13.1.4. Primary endpoint
The fraction of patients who achieve an MRD level below 0.1%, as quantified by flow cytometry, after the first induction course.

13.1.5. Secondary endpoints
- Event-free survival and overall survival at five years.
- The median MRD after course 1 and course 2.
- The rate of CR after one and two induction courses.
- Cardiac function after one and five years.
- Frequency of severe adverse events as defined in 14.2.3, early death and death in CR.

13.1.6. Patient numbers and power calculation
The study is expected to enrol 300-350 patients during a 5-6 year period depending on the final number of countries participating. We expect that the number of early deaths will be less than 5% and that over 90% will accept randomisation. Assuming that a LAIP can be found in 85% this will give at least 255 patients evaluable for the primary endpoint. Based on previous data the fraction of MRD negative patients after course 1 is between 30-60% and with 100 patients in each arm we will, at a two-sided alpha of 5% obtain a power of more than 80% to detect an increase in MRD negativity with 20%.

![Power calculation graph]

Figure 16. Power calculation based on an effect size of 50% MRD negativity in one arm and 30% in the other after course one. For a two-sided alpha of 0.05 a power of 80% is reached at 92 patients in each group.

For the secondary endpoints EFS and OS, comparison of cardiac and other toxicity the entire cohort will be evaluable.
13.1.7. Study procedure
At diagnosis, after checking eligibility criteria and providing mandatory data to the AML leukaemia registry, randomisation can be performed online. In case of inability to perform randomisation online a fax can be sent to the registry to +46 (0)8 51773184. The patient will then receive the allocated treatment. Response to therapy and toxicity will be registered on case report forms online. All deaths and SUSARs must be reported within 48 hours to the registry.

13.2. Comparison of the efficacy of ADxE and FLADx as the second induction course (FLADx study).

13.2.1. Short rational
ADE (low-dose cytarabine, daunorubicin and etoposide) has been and still is used as induction therapy in many adult and paediatric AML protocols. The BFM has, in an ADE course setting, in consecutive studies tested first idarubicin versus daunorubicin in the BFM AML98 study and could show an increased blast clearance with idarubicin(4). This was followed by the BFM AML2004 study which compared idarubicin and liposomal daunorubicin (L-DNR) and showed a trend to higher EFS and OS in the DaunoXome arm without higher toxicity(12). Thus it seems as if the effect of the traditional ADE can be increased by replacing daunorubicin with liposomal daunorubicin.

FLAG (fludarabine + high-dose cytarabine + G-CSF) regimens with and without addition of anthracyclines have been frequently used for treatment of relapsed paediatric AML(27, 52). Recently FLAG + L-DNR has been shown to be more effective than FLAG in relapsed paediatric AML(50). The use of G-CSF in the FLAG regimen has recently been questioned and will be abandoned in the next European relapse protocol, since patients with isoform IV of the G-CSF receptor may have a high relapse rate when treated with G-CSF(53).

Despite the proven benefits in relapsed AML, different FLA regimens have hardly been investigated in paediatric de novo AML. Since several relapse studies report a high efficacy in combination with a favourable toxicity profile it is warranted to test this drug combination in upfront therapy(27, 50). This study will therefore randomise patients to receive either ADxE or FLADx. ADxE is the standard arm.

13.2.2. Primary objective
To investigate if FLADx is more effective than ADxE in reducing leukaemic cell numbers when used in the second induction course.

13.2.3. Secondary objective
- To investigate if FLADx improves CR rates, EFS and OS.
- To investigate and compare severe toxicity and mortality in the treatment arms.
- To compare cardiac toxicity in the treatment arms.

13.2.4. Primary endpoint
The fraction of patients who achieve an MRD level below 0.1%, as quantified by flow cytometry, after the second induction course.
13.2.5. Secondary endpoints
- Event-free survival and overall survival at five years.
- The median MRD after course 2.
- The rate of CR after two induction courses.
- Cardiac function after one and five years.
- Frequency of severe adverse events as defined in 14.2.3, early death and deaths in CR.

13.2.6. Patient numbers and power calculation
The study is expected to enrol 300-350 patients during a 5-6 year period. Assuming that a LAIP can be found in 85% this will give at least 255 patients. We expect that the number of early deaths will be less than 5% and that over 90% will accept randomisation. Assuming that 40% will be MRD positive after the first induction course this will give approximately 100 patients evaluable for the primary endpoint. Given the importance of the study questions and the small number of randomised treatment studies undertaken in this rare disease we consider it justifiable to accept a two-sided alpha of 0.1. We will then obtain a power of 80% in detecting a reduction by 2/3 in MRD levels at a sample size of 46 patients in each group. The 2nd randomisation will be stratified on the treatment in course one (MEC / DxEC / no randomisation) and on the day 22 response to the first course (LC < 0.1% / LC ≥ 0.1% / no LAIP).

Figure 17. Power calculation based on an effect size of 30% remaining MRD negativity in one arm and 10% in the other arm after course two. For a two-sided alpha of 0.1 a power of 80% is reached at 46 patients in each group.

For the secondary endpoints EFS and OS and for evaluation of toxicity the entire cohort will be evaluable.
13.2.7. Study procedure
As soon as the results of the evaluation on day 22 after the first course are available and after checking eligibility criteria and confirming that cardiac function is normal, randomisation can be performed online. In case of inability to perform randomisation online a fax can be sent to the registry to +46 (0)8 51773184. The patient will then receive the allocated treatment. Response to therapy and toxicity will be registered on case report forms online. All deaths and SUSARs must be reported within 48 hours to the registry.
14. Quality control and toxicity registration

The AML 2012 protocol will be conducted according to the declaration of Helsinki and ICH Guidelines for Good Clinical Practice (GCP). GCP is a scientific and ethical standard for the design, conduction and reporting of clinical trials involving human subjects. Compliance with these standards will assure that the rights, safety and wellbeing of the subjects are protected and that the trial data are credible.

The NOPHO-DBH AML 2012 protocol contains the standard therapy for paediatric AML in the participating countries and is thus regarded as the best available therapy. Therefore, as soon as all the logistics are implemented (including laboratory requirements for diagnostic evaluation, response evaluation and data registration) children can be treated according to the standard arm of the protocol. Following approval of all relevant national authorities the two randomised studies will commence.

The trial will be conducted in accordance with the protocol, legal requirements, and GCP quality assurance and control procedures. To obtain the necessary documentation and monitoring, each National Principal Investigator will establish an agreement with national independent GCP units to perform GCP monitoring. The national coordinators are responsible for that each participating trial centre has the facilities to conduct the study according to protocol guidelines and meet any emergency that may arise during the study. Data retention including clinical trial records and other essential documents will follow national laws.


The overall monitoring of the randomised trials will be performed at four levels

- GCP monitoring
- Supervision by the protocol committee
- Supervision by the data safety monitoring committee (DSMC)
- Monitoring of essential laboratory investigations

To facilitate monitoring and to comply with EU-Directive 2001/20/EC and ICH Guidelines for Good Clinical Practice a standardised toxicity registration will be established including reporting of severe adverse events (SAEs) and suspected unexpected severe adverse reactions (SUSARs). Also, a central review of MRD flow results will be implemented (appendix 4).

14.1.1. GCP monitoring

The GCP monitoring provides a systematic and independent examination of trial-related activities and documents to determine, whether the evaluated trial-related activities were conducted, and the data were recorded, analysed, and accurately reported according to the protocol, Good Clinical Practice (GCP), and the applicable regulatory requirements. To achieve these goals the monitoring should evaluate essential protocol issues. Therefore the following will be monitored in the AML 2012 protocol:

1. Documentation of informed consent for the AML 2012 study with separate specification of each of the randomised trials. These will be filed by the local investigator at each centre and/or in the individual patients charts.
2. Documentation and validity of the inclusion criteria of each patient in the different treatment arms. This information will be held at the NOPHO Leukaemia Registry in Stockholm.

3. Documentation that the treatment was given according to the allocated treatment arm. This will be included in the online Case Report Forms (appendicies 6 and 7).

4. Documentation of data regarding study treatment endpoints in particular date and type of events, MRD flow results, and toxicities as specified in the protocol.

5. Documentation and reporting of SAE as defined in the protocol.

14.1.2. Supervision by protocol committee
The protocol committee will meet semi-annually and discuss progress of the trial including accrual, events and toxicity. The protocol chair is responsible to initiate additional meetings in case of unexpected problems with the protocol. Local investigators are encouraged to contact the study and/or national coordinators for guidance. An electronic help desk, accessible for the treating physicians, will be established on the NOPHO web.

14.1.3. Data safety monitoring committee (DSMC)
The DSMC will annually receive a full report of the outcome data of the treatment arms and a toxicity report. The DSMC will together with the study chair monitor the outcome of the different treatment arms. While the studies are running, only the data manager, the chair of the protocol and the members of the DSMC will have access to outcome data of the individual treatment arms. None of the DSMC members are involved in the treatment of study patients. When obtaining written informed consent for the studies, an authorisation from the trial subject will be acquired to allow third party (monitor, auditor or inspector from the authorities) to access information on trial person’s health data.

14.1.4. Monitoring of essential laboratory investigations
MRD flow is the cornerstone of evaluation of response to therapy and the primary endpoint of the randomised studies. Since MRD flow examination is technically demanding and, despite standardisation, subject to individual interpretation, the data files obtained from analysis at diagnosis, day 22 after course 1 and the last bone marrow sample prior to start of consolidation or salvage therapy will be rapidly reviewed by the MRD flow committee. The results will be returned within two weeks to assist the treating physician in risk stratification.

Immunophenotyping at diagnosis will be reviewed once yearly by the NOPHO immunophenotyping group and karyotype data will be reviewed by the NOPHO cytogenetic group.

14.2. Toxicity registration
AML in children requires very intensive therapy and a high level of toxicity is expected. The main purpose of the registration is to early detect unacceptably high and/or unexpected toxicity from the therapy in order to ensure the safety of the trial subjects. In compliance with EU-Directive 2001/20/EC and ICH Guidelines for Good Clinical Practice (GCP), deaths and SUSARs must be documented and reported to the sponsor’s delegate immediately.

Further aims of the registration is to compare the toxicity in the different treatment arms in the randomised studies and with previous treatment protocols.
All registration will be performed online but there will be an option to report deaths and SUSARs by fax (+46 (0)8 51773184) in case of local problems of accessing the registry immediately.

The toxicity reporting will be divided into three main categories. Reporting of

- SUSARs and deaths
- SAEs following each treatment course
- Long-term toxicity

14.2.1. Suspected unexpected severe adverse events (SUSARs) and deaths

A SUSAR is defined as a serious adverse reaction which is not consistent with the product information and either

- Results in death.
- Is life threatening or requires inpatient hospitalisation or prolongation of existing hospitalisation.
- Results in persistent or significant disability/incapacity.
- Causes congenital malformation.

Thus, severe SAE that are well-known side effects of the anti-leukemic therapy are not to be registered as SUSARs.

All SUSARs or deaths, unrelated to death from progressive leukaemia, must be reported on the online SUSAR/death form or by faxing the form in appendix 3 to the NOPHO leukaemia registry that automatically will forward the information to the study and national coordinators. In case of SUSAR, the study chair will inform the national medicine agency in Sweden and the ethics committee in Gothenburg, Sweden, and the national investigators will contact their national regulatory agencies if required by national laws. The study chair will in each case of toxic death, if necessary, institute an investigation as to probable cause and make a report to the protocol committee. It is recommended that patients are subjected to autopsy if the cause of death is not certain.

14.2.1.1. Stopping rules

The study chair and data manager will analyse the cumulative death rate in relation to the rate of resistant disease, event-free survival and overall survival at every toxic death. To provide an additional safety measure to detect poor protocol performance one stopping rule for detection of excessive toxicity and one rule for detection of a high relapse rate have been devised.

14.2.1.1.1. Stopping rule for induction deaths and deaths in complete remission

NOPHO-AML 2004 had an induction death rate of 2.6% and also 2.6% deaths in CR. The AML99 study had 5.1% toxic deaths\(^{10}\). These rates are low compared to most other paediatric AML studies which have reported rates around 8-10\%\(^{11, 40}\). The number of deaths in AML 2012 should therefore not reach significantly beyond 5\% and we have therefore calculated a Wald sequential plan based on a \(p_0\) of 5\% and a \(p_1\) of 8\%. We use an \(\alpha\) of 5\% and \(\beta\) of 1\% giving a power of 99\% to detect a toxic death rate exceeding 8\%. Table 6 shows the number of toxic deaths “allowed” in relation to patients treated and if this limit is exceeded then the Study Committee will put the study on hold, perform a rapid analysis and decide upon interim treatment, while the DSMC reviews the data and decide on continuation.
Table 6. The limits for the number of toxic deaths that are acceptable in relation to the number of patients treated.

<table>
<thead>
<tr>
<th>N</th>
<th>Maximum number accepted deaths</th>
<th>N</th>
<th>Maximum number accepted deaths</th>
</tr>
</thead>
<tbody>
<tr>
<td>-17</td>
<td>6</td>
<td>157-172</td>
<td>16</td>
</tr>
<tr>
<td>18-32</td>
<td>7</td>
<td>173-187</td>
<td>17</td>
</tr>
<tr>
<td>33-48</td>
<td>8</td>
<td>188-203</td>
<td>18</td>
</tr>
<tr>
<td>49-64</td>
<td>9</td>
<td>204-219</td>
<td>19</td>
</tr>
<tr>
<td>65-78</td>
<td>10</td>
<td>220-234</td>
<td>20</td>
</tr>
<tr>
<td>79-93</td>
<td>11</td>
<td>235-250</td>
<td>21</td>
</tr>
<tr>
<td>94-109</td>
<td>12</td>
<td>251-266</td>
<td>22</td>
</tr>
<tr>
<td>110-125</td>
<td>13</td>
<td>267-281</td>
<td>23</td>
</tr>
<tr>
<td>126-140</td>
<td>14</td>
<td>282-297</td>
<td>24</td>
</tr>
<tr>
<td>141-156</td>
<td>15</td>
<td>298-312</td>
<td>25</td>
</tr>
</tbody>
</table>

14.2.1.1.2. Stopping rule for relapse

The cumulative 3-years relapse rate of the NOPHO-AML93 was 42% and the hazards (= no. of relapses / total follow-up time) in the NOPHO-AML93 protocol during the first four years were 0.186, 0.143, 0.051 and 0.023. 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. Hazard rates in NOPHO AML-2004 were very similar to NOPHO AML93.

Assuming that a safe cumulative 3-year relapse rate in AML 2012 is 40% and 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 table 7, the Study Committee will put the study on hold, perform a rapid analysis and decide upon interim treatment, while the DSMC reviews the data and decide on continuation. 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%.

The stopping rule is based on 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 the SPRT was modified 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 table 7. 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 7.** Stopping rule for relapses. At defined numbers of relapses the table gives the lower acceptable limit for the weighted follow-up time in years.

<table>
<thead>
<tr>
<th>Number of relapses</th>
<th>Total weighted follow-up years</th>
<th>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>
<td></td>
</tr>
<tr>
<td>4</td>
<td>1.324</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>5.555</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>12.494</td>
<td>99</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>21.556</td>
<td>97</td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>32.277</td>
<td>94</td>
<td></td>
</tr>
<tr>
<td>14</td>
<td>44.325</td>
<td>90</td>
<td></td>
</tr>
<tr>
<td>16</td>
<td>57.458</td>
<td>87</td>
<td></td>
</tr>
<tr>
<td>18</td>
<td>73.427</td>
<td>84</td>
<td></td>
</tr>
<tr>
<td>20</td>
<td>100.364</td>
<td>77</td>
<td></td>
</tr>
<tr>
<td>22</td>
<td>127.300</td>
<td>72</td>
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</tr>
<tr>
<td>24</td>
<td>154.237</td>
<td>69</td>
<td></td>
</tr>
<tr>
<td>26</td>
<td>181.173</td>
<td>66</td>
<td></td>
</tr>
<tr>
<td>28</td>
<td>208.109</td>
<td>63</td>
<td></td>
</tr>
<tr>
<td>30</td>
<td>235.046</td>
<td>61</td>
<td></td>
</tr>
<tr>
<td>32</td>
<td>261.982</td>
<td>60</td>
<td></td>
</tr>
<tr>
<td>34</td>
<td>288.918</td>
<td>58</td>
<td></td>
</tr>
<tr>
<td>36</td>
<td>315.855</td>
<td>57</td>
<td></td>
</tr>
<tr>
<td>38</td>
<td>342.791</td>
<td>56</td>
<td></td>
</tr>
<tr>
<td>40</td>
<td>369.728</td>
<td>55</td>
<td></td>
</tr>
<tr>
<td>42</td>
<td>396.664</td>
<td>55</td>
<td></td>
</tr>
<tr>
<td>44</td>
<td>423.600</td>
<td>54</td>
<td></td>
</tr>
<tr>
<td>46</td>
<td>450.537</td>
<td>53</td>
<td></td>
</tr>
<tr>
<td>48</td>
<td>477.473</td>
<td>53</td>
<td></td>
</tr>
<tr>
<td>50</td>
<td>504.409</td>
<td>52</td>
<td></td>
</tr>
<tr>
<td>52</td>
<td>531.346</td>
<td>52</td>
<td></td>
</tr>
<tr>
<td>54</td>
<td>558.282</td>
<td>51</td>
<td></td>
</tr>
</tbody>
</table>

14.2.2. **Severe adverse events (SAEs)**

Toxicity reporting will be performed on NOPHO toxicity online forms developed from the criteria of the US National Cancer Institute Terminology Criteria for Adverse Events Common Toxicity Criteria (CTC) [http://ctep.cancer.gov/reporting/ctc.html](http://ctep.cancer.gov/reporting/ctc.html).

An SAE is defined as any untoward medical occurrence that at any dose either

- Results in death.
- Is life threatening or requires inpatient hospitalisation or prolongation of existing hospitalisation.
Results in persistent or significant disability/incapacity.
Causes congenital malformation

To obtain high cure rates, AML therapy needs to be directed towards the limit of toxicity. All patients are expected to suffer from certain SAEs (e.g. febrile neutropenia) and will be informed of this before starting therapy. The frequency of SAEs represents the overall treatment intensity and must be balanced against the anti-leukemic efficacy when evaluating the performance and benefits of the protocol. The protocol committee and the DSMC will carefully monitor that the frequency of SAEs is acceptable in relation to the outcome of therapy and that any of the randomised arms do not have an excess toxicity.

14.2.3. Reporting of severe adverse events after induction and consolidation courses

The SAEs occurring during AML therapy are well known. After each course the following SAEs will be reported according to appendix 2:

- Need of intensive care (transfer to an ICU) with or without need of assisted ventilation. Days at ICU and days on ventilatory support will be recorded.
- Infections requiring iv antibiotics with documentation of proven sepsis, septic shock and invasive fungal infection.
- Congestive heart failure.
- Cardiac arrhythmia.
- Hypoxia (CTC grade 3 or 4).
- Anaphylaxis (CTC grade 3 or 4).
- Abdominal pain.
- Abdominal symptoms leading to laparotomy
- Typhlitis (CTC grade 3 or 4).
- Multi-organ failure (CTC grade 3 or 4).
- Increased bilirubin levels bilirubin >5x upper normal limit
- Severe thrombosis causing organ dysfunction and/or requiring systemic anticoagulation (CTC grade 3 or 4)
- Renal dysfunction with an increase in creatinine > 3 UNL.
- Catastrophic bleeding with documentation of organ involved.
- Disseminated intravascular coagulation.
- Central neurotoxicity.

In addition, the number of days with ANC < 0.5 x 10^9/L and the days until last platelet transfusion for each course will be reported. Some adverse events that are common in AML and expected to occur in the majority of patients, e.g. anaemia, mucositis and nausea, will not be reported.

14.2.4. Reporting of long-term toxicity

Patients who receive SCT are recommended to be followed according to the guidelines at the treatment centre. The AML database will include registration of the extent of acute and chronic graft versus host disease and results of ECHO at one, five and ten years from diagnosis.
For patients not treated with SCT the registration will include data on persistent renal or hepatic dysfunction as well as registration of symptoms of cardiac failure and results of ECHO at one, five and ten years from diagnosis.
15. Definitions

15.1. Event definition

15.1.1. Relapse
For patients who have entered remission the following relapse definitions apply:

- Bone marrow relapse is defined as the presence of ≥ 5% leukaemic cells in the bone marrow. In patients with an informative LAIP, quantification of blasts will be done by flow cytometry.
- Molecular relapse is defined as a more than tenfold increase (> one-log) in transcript level in patients who have obtained complete remission as defined by MRD flow or morphology. The finding must be confirmed in an independent sample.
- CNS relapse is defined either as the presence of leukaemic cells, detected on cytospin preparations of CSF with a cell count of ≥ 5 white blood cells/µL or when a tumour mass is detected in CNS with identical or very similar characteristics on biopsy (morphology, immunophenotype and cytogenetic analysis) as the original malignant cells.
- Extramedullary relapse is diagnosed when a tumour mass is detected with identical or very similar characteristics on biopsy (morphology, immunophenotype and cytogenetic analysis) as the original malignant cells.

15.1.2. Resistant disease
Resistant disease (RD) is, in patients with an informative LAIP, defined as ≥ 5% leukaemic cells in the bone marrow after the second induction course. In patients with non-informative LAIP it is defined as ≥ 5% blast cells on bone marrow morphology at the same time point. In patients with extramedullary disease, resistant disease is defined as unequivocal demonstration of residual leukemic cells at evaluation after two induction courses.

15.1.3. Early death
Early death is defined as any death occurring in patients before achieving remission unless they have been classified as having resistant disease.

15.1.4. Death in CR
This is defined as death from any cause occurring when the patient is in CR.

15.1.5. SMN
This defined as any malignancy during or following therapy that is not a relapse of the original myeloid leukaemia.

15.2. Therapy response definitions

15.2.1. Poor response to induction 1
In patients with an informative LAIP, this is defined as ≥15% leukaemic cells after the first induction course. In patients with non-informative flow results, this is defined as ≥ 15% blast cells on morphology. These patients will start their second course as soon as the poor
response is reported from the laboratory and be assigned to the high risk group provided they achieve CR after the second course (if not they will by definition have resistant disease).

15.2.2. Intermediate response to induction 1
In patients with an informative LAIP, this is defined as 5 – 14.9% leukaemic cells after the first induction course. In patients with non-informative flow results, this is defined as 5 – 14.9% blast cells on morphology. These patients will start their second course as soon as the intermediate response is reported from the laboratory.

15.2.3. Good response to induction 1
In patients with an informative LAIP, this is defined as < 5% leukaemic cells after the first induction course. In patients with non-informative flow results, this is defined as <5% blast cells on morphology. These patients will start their second course after haematological recovery.

15.2.4. Poor response to induction 2
In patients with an informative LAIP, this is defined as ≥5% leukaemic cells after the second induction course. In patients with non-informative flow results, this is defined as ≥ 5% blast cells on morphology. These patients will be classified as having resistant disease and proceed to salvage therapy.

15.2.5. Intermediate response to induction 2
This definition only applies to patients with an informative LAIP. It is defined as 0.1-4.9% leukaemic cells after the second induction course. These patients will start consolidation after haematological recovery and be assigned to the high-risk group.

15.2.6. Good response to induction 2
This definition only applies to patients with an informative LAIP. It is defined as <0.1% leukaemic cells after the second induction course. These patients will start consolidation after haematological recovery and be assigned to the standard risk group providing they did not have a poor response to course 1 and do not have an FLT3-ITD mutation without NPM1 mutation.

15.2.7. Complete remission (CR)
This is defined as < 5% leukemic cells or in patient with non-informative LAIP < 5% blast cells with clear evidence of haematological regeneration in the bone marrow and peripheral levels of ANC ≥ 0.5 x 10⁹/L and platelets ≥ 80 x 10⁹/L and no signs of leukaemia elsewhere.
16. Diagnostic guidelines

The diagnosis of AML is based on the revised WHO classification from 2008 which incorporates morphology, cytochemistry, immunophenotype, genetics and clinical features to define AML disease groups(54). The classification introduced AML subtypes characterized by genetics and classified by their recurrent genetic aberration independent of other criteria.

16.1. The diagnosis of AML

16.1.1. Bone marrow morphology

The BM diagnosis is based on morphological examination of peripheral blood and bone marrow prepared with Wright-Giemsa or similar stains in which blast counts are performed. Myeloblasts, monoblasts, promonocytes and megakaryoblasts are summed to obtain the blast percentage. Promyelocytes should not be included as blasts. Proerythroblasts are not counted except in the rare instance of pure erythroleukaemia. Cytochemistry confirms lineage affiliation and classifies myeloid [myeloperoxidase (MPO) positive] and monoblastic differentiation [nonspecific esterase (NSE) positive] AML blasts. However, cytochemistry results should not be considered independent denominators of final AML diagnosis.

Differentiating between AML and advanced MDS may be difficult in children with a low percentage of blasts but is important for treatment decisions. In adults, a blast threshold of 20% is used to differentiate between the diseases, but in children with blast percentages between 20% and 30% additional features should be considered and repeat BM after e.g. two weeks is necessary to make a diagnosis(55).

16.1.2. Blast lineage - immunophenotype

Multiparameter flow cytometric analysis is used to determine the leukaemic cell differentiation and maturation as well as to assess the leukaemia-associated immunophenotype for MRD evaluation. A standard 8-colour antibody (Ab) panel will be applied both at diagnosis and follow-up. It comprises marker combinations aimed at identifying LAIP such as cross lineage and discordant antigen expression in the blast population and maturing granulocytic and monocytic cells. Immunophenotyping to be used in MRD assessment is difficult and should be centralized in a few dedicated and experienced labs.

Immunophenotyping is a rapid method to distinguish between AML and ALL. According to the WHO 2008 classification, only the markers MPO, lysozyme, CD11c, CD14, CD64, (cy)CD3; CD19, cyCD22, cyCD79, and CD10 are essential to assign lineage affiliations. Mixed phenotype acute leukaemias (MPAL) is defined as otherwise not specified (NOS) including biphenotypic leukaemia with one blast population, and bi-lineage leukaemia with distinctly differentiated blast populations. Additional markers may be needed to accurately distinguish AML and ALL. For example, classification of AML FAB subtypes M0 (negative MPO activity by cytochemistry but positive by immunophenotyping for myeloid markers such as MPO [proenzyme] and/or CD13, CD33, CD117) as well as M7 (positive for platelet markers such as CD41 and/or CD61) relies on immunophenotypic assessment.
16.1.3. Genetic aberrations

16.1.3.1. Cytogenetics

Cytogenetics is an important feature in the diagnosis and therapeutic risk assignment of childhood AML. Conventional cytogenetics can detect abnormalities in 80% of children with AML. The WHO 2008 classification further expanded the number of cytogenetic abnormalities associated with AML classification (54,56).

Directed analysis with FISH or PCR should be done to search for RUNX1-RUNX1T1, CBFB-MYH11, PML-RARA and MLL aberrations.

16.1.3.2. Molecular genetics

Several gene mutations and aberrant gene expression profiles have been recognized in pediatric AML, further unraveling the heterogeneity of the disease. The prognostically important type I/type II mutations are most frequent in those with cytogenetically normal AML (CN-AML).

Mutational screening should include FLT3, NPM1, and CEBPA. A number of additional abnormalities have been described but are not yet routinely screened for or included in treatment stratification; KIT, RAS, TET2, IDH1, PTPN11, and DNMT3A. Gene expression of ERG, MN1, EVI1, BAALC, and WT1 may be of prognostic importance but the final role is not clarified. Several gene expression profiling (GEP) studies have been conducted in recent years in pediatric AML to study their diagnostic potential and to determine whether they can replace current labor-intensive diagnostic procedures. GEP is not yet recommended for routine evaluation in the diagnosis of children with AML.
### Table 8. WHO Classification (2008) of paediatric AML.

<table>
<thead>
<tr>
<th>Main Category</th>
<th>Subgroup</th>
</tr>
</thead>
<tbody>
<tr>
<td>AML with recurrent genetic abnormalities</td>
<td>t(8;21)(q22;q22), RUNX1-RUNX1T1(CBFA/ETO inv(16)(p13q22) or t(16;16)(p13;q22), CBFB-MYH11</td>
</tr>
<tr>
<td></td>
<td>* APL t(15;17)(q22;q11-12), PML-RARA</td>
</tr>
<tr>
<td></td>
<td>t(9;11)(p22;q23), MLLT3-MLL</td>
</tr>
<tr>
<td></td>
<td>with t(6;9)(p23;q34); DEK-NUP214</td>
</tr>
<tr>
<td></td>
<td>inv(3)(q21q26.2) or t(3;3)(q21q26.2), RPN1-EVI1</td>
</tr>
<tr>
<td></td>
<td>t(1;22)(p13;q13), RBM15-MKL1</td>
</tr>
<tr>
<td></td>
<td>mutated NPM1</td>
</tr>
<tr>
<td></td>
<td>mutated CEBPA</td>
</tr>
<tr>
<td>AML with myelodysplasia-related features</td>
<td></td>
</tr>
<tr>
<td>Therapy-related myeloid neoplasms*</td>
<td></td>
</tr>
<tr>
<td>AML, not otherwise specified</td>
<td>AML with minimal differentiation</td>
</tr>
<tr>
<td></td>
<td>AML without maturation</td>
</tr>
<tr>
<td></td>
<td>AML with maturation</td>
</tr>
<tr>
<td></td>
<td>Acute myelomonocytic leukaemia</td>
</tr>
<tr>
<td></td>
<td>Acute monoblastic and monocytic leukaemia</td>
</tr>
<tr>
<td></td>
<td>Acute erythroid leukaemia</td>
</tr>
<tr>
<td></td>
<td>Acute megakaryoblastic leukaemia</td>
</tr>
<tr>
<td></td>
<td>Acute basophilic leukaemia</td>
</tr>
<tr>
<td></td>
<td>Acute panmyelosis with myelofibrosis</td>
</tr>
<tr>
<td>Myeloid sarcoma</td>
<td></td>
</tr>
<tr>
<td>Myeloid proliferations related to Downs syndrome*</td>
<td></td>
</tr>
<tr>
<td>Blastic plasmacytoid dendritic cell neoplasm</td>
<td></td>
</tr>
<tr>
<td></td>
<td>* not eligible for NOPHO-AML 2012</td>
</tr>
</tbody>
</table>

A diagnosis of AML can be established regardless of peripheral or BM blast count if t(8;21) or inv(16) is detected. For all other AML subtypes it is required that the blast count in PB or BM is at least 20%.

### 16.2. CNS disease diagnostics

CNS disease is defined if either of the following are present
- ≥ 5 white blood cells/mm³ and unequivocal evidence of blasts on cytospin examination,
- clinical signs/symptoms not obviously caused by another disease (seizures, cranial nerve palsy and symptoms of increased cranial pressure)
- radiological evidence of leukemic infiltration in the central nervous system.

Patients with CNS disease should be evaluated with an MRI of the CNS. Contrary to in ALL,
there is no evidence that the presence of low number of malignant cells (<5 /mm$^3$) in CSF affects outcome (57).

16.3. Biobank
Biobanking is the most relevant source to identify or confirm new genetic aberrations and prognostic subgroups (i.e. mutations, gene expression) and primary samples are an indispensable source for experimental and translational research. Central biobanking is therefore an integrated part of the protocol (appendix 8).

16.4. Minimal residual disease

16.4.1. Flow cytometry for quantification of cells with LAIP
Since risk stratification in AML 2012 and the evaluation of the randomised studies relies on MRD quantification by flow cytometry (MRD flow) it is essential that all treating centres establish clear logistics for the collection and rapid analysis at appropriate time points. MRD flow in AML is challenging and therefore detailed guidelines are provided in appendix 4 and a central review of the datafiles of samples at the previously outlined MRD time-points will be performed.

16.4.2. MRD flow guidelines
It is mandatory to adhere strictly to standard operating procedures (SOPs) as detailed in appendix 4. These include instructions for the collection of the samples, transportation, standardisation of the analyses and reporting of the MRD results to the attending physician and registry. The results of the analyses at the critical time-points in the protocol should be reported within 48 hours after sample collection (at day 22 after course one and at repeat examinations prior to course two, at day 22 and repeat examinations following course two in those with ≥5% LC after course one). Standardisation of the analytic phase will include specifications of instrument set-up, preparation of the samples, acquisition of the data and data analysis.

As soon as the final analysis after course two is performed the laboratory should send the data files from diagnosis, day 22 after course one and the final analysis after course two for central review according to the instructions in appendix 4. After the review the registration of MRD results in the database will be checked and risk group assignment confirmed.

16.4.3. qPCR for fusion gene transcripts
When applicable, quantification of fusion gene transcripts by PCR gives a higher sensitivity than MRD flow. Despite the fact that up to 40% of children with AML have genetic aberrations suitable for quantification of MRD with PCR (MRD PCR), very few studies have investigated early treatment response and both the kinetics and prognostic significance of qPCR are largely unknown. The design of the AML 2012 study offers a very good background to study both of these factors and it seems likely that early measurement of transcript levels carries strong prognostic information.
In contrast to the unclear prognostic value in early treatment, data are accumulating on the role of qPCR as an early predictor of relapse during and after therapy. Relatively large studies in APL measuring t(15;17) but also studies in t(8;21) and inv(16) indicate that a more than one-log increase in transcript levels is strongly predictive of overt bone marrow relapse(58). Studies are in progress that will aim at treating the children while the disease still is at a molecular level. The ultimate purpose is to thereby increase cure rates and reduce the treatment burden(59).

The AML 2012 protocol includes three research aims in relation to qPCR of fusion gene transcripts.

- To investigate the prognostic significance of MRD PCR in PB and BM on day 22 after course one and before start of consolidation.
- To investigate the correlation between MRD flow in BM and MRD PCR in PB and BM during induction.
- To investigate the sensitivity of bimonthly qPCR measurements in PB during 18 months to detect relapse.

To achieve these aims it will be mandatory to perform qPCR in BM and PB at diagnosis, on day 22 after course one and before consolidation in patients with t(8;21), t(9;11) and inv(16) for which the methodology is well established at several labs within NOPHO. It is strongly recommended, but not mandatory, that other fusion gene transcripts also be monitored. To facilitate qPCR MRD analysis, guidelines are provided in appendix 5. Each country will provide a reference laboratory to which samples may be sent for analysis.
17. Drug information

17.1. Cumulative doses

Table 9a. Cumulative doses (mg/m²) of the drugs in the different treatment arms for standard risk patients without inv(16).

<table>
<thead>
<tr>
<th>Drug (mg/m²)</th>
<th>Treatment arm</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Standard arm</td>
</tr>
<tr>
<td>Cytarabine</td>
<td>36800</td>
</tr>
<tr>
<td>Etoposide</td>
<td>1700</td>
</tr>
<tr>
<td>Fludarabine</td>
<td>150</td>
</tr>
<tr>
<td>Liposomal daunorubicin</td>
<td>180</td>
</tr>
<tr>
<td>Mitoxantrone</td>
<td>55</td>
</tr>
</tbody>
</table>

Table 9b. Cumulative doses (mg/m²) of the drugs in the different treatment arms for standard risk patients with inv(16).

<table>
<thead>
<tr>
<th>Drug (mg/m²)</th>
<th>Treatment arm</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Standard arm</td>
</tr>
<tr>
<td>Cytarabine</td>
<td>30800</td>
</tr>
<tr>
<td>Etoposide</td>
<td>1700</td>
</tr>
<tr>
<td>Fludarabine</td>
<td>150</td>
</tr>
<tr>
<td>Liposomal daunorubicin</td>
<td>180</td>
</tr>
<tr>
<td>Mitoxantrone</td>
<td>25</td>
</tr>
</tbody>
</table>

Table 9c. Cumulative doses (mg/m²) of the drugs in the different treatment arms for high risk patients who receive SCT after the third course (HAM).

<table>
<thead>
<tr>
<th>Drug (mg/m²)</th>
<th>Treatment arm</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Standard arm</td>
</tr>
<tr>
<td>Cytarabine</td>
<td>8800</td>
</tr>
<tr>
<td>Etoposide</td>
<td>1200</td>
</tr>
<tr>
<td>Fludarabine</td>
<td>0</td>
</tr>
<tr>
<td>Liposomal daunorubicin</td>
<td>180</td>
</tr>
<tr>
<td>Mitoxantrone</td>
<td>55</td>
</tr>
</tbody>
</table>

17.2. Cytarabine

Cytarabine is an deoxycytidine analogue to the nucleotide cytosine. Cytarabine is converted into deoxycytosine triphosphate (dCTP) that competes with natural cytosine for DNA-
incorporation. 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**
Cytarabine is available in 20 mg/ml or 100 mg/ml vials. Intact vials can be stored at room temperature. For i.v. use, the solution can be diluted with 0.9% sodium chloride or glucose 50 mg/ml. For intrathecal injections, be sure to use **unpreserved** solutions only.

**Dose**
100 mg/m\(^2\) 30 min iv infusion (course ADxE)
100 mg/m\(^2\) iv 24 hour continuous infusion (course ADxE)
200 mg/m\(^2\) 12h iv infusion (Course MEC/DxEC)
1000 mg/m\(^2\) 2 hour infusion (Course HAM)
2000 mg/m\(^2\) 3 hour infusion (Course FLA/FLADx)
3000 mg/m\(^2\) 2 hour infusion (Course HA\(^3\)E)
Age-adjusted dose for intrathecal administration. See table 4.

**Administration**
Intravenous and intrathecal. For intrathecal administration preservation-free methotrexate, preservation-free cytarabine, and glucocorticosteroids can be given in the same syringe.

**Storage**
Intact vials can be stored 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), diarrhoea, mucositis, anorexia, alopecia, skin rash, and liver dysfunction. Fever, myalgia, rash, muscle and bone pain and increased CRP are not uncommon after the start of cytosine arabinoside treatment (Ara-C syndrome). In very severe cases systemic corticosteroids can be considered, as it has been shown to be beneficial in treating or preventing this syndrome. Cytarabine treatment usually doesn’t need to be discontinued. It is however important to consider infection as a differential diagnosis. 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 in patients with renal impairment.

**Precautions**
Keratitis/conjunctivitis can be caused by high-dose ara-C treatment (≥1000 mg/m\(^2\)/day) and prophylactic treatment with ophthalmic corticosteroids is recommended, starting before the first cytarabine dose and continuing until 24 hours after the last one.
17.3. Etoposide
Etoposide (=VP16) is an epipodophyllotoxin. In the AML 2012 protocol, the drug Etopophos (etoposide phosphate) will be recommended. In contrast to Etoposide for injection, Etopophos does not contain Macrogol 300, citric acid, polysorbate 80, or ethanol (water free). Etoposide phosphate is a water soluble ester prodrug of etoposide, which is a semi-synthetic derivate of podophyllotoxin. The better water solubility of etoposide phosphate minimises the risks for precipitation both when diluted and during intravenous administration. In vivo, Etoposide phosphate is converted to its active substance etoposide by dephosphorylation. Etoposide inhibit DNA synthesis by binding to topoisomerase II, which results in apoptotic cell death. At high concentrations (>10 μg/ml) lysis of cells that are entering the mitosis is seen. At low concentration (0.3-10 μg/ml), cells do not enter the mitotic prophase.

**Formulation**
Etoposide phosphate is available in vials that contain 100 mg etoposide (equivalent), 32.7 mg sodium citrate, and 300 mg dextran 40. For injection the vial must be reconstituted with 5-10 ml sterile water, 5 % dextrose, or 0.9 % sodium chloride.

**Dose**
100 mg/m² 1 hour iv infusion (Course HA3E).
150 mg/m² 2 hour iv infusion (course MEC/EDxE/ADxE)

**Administration**
Intravenously as a 1 or 2 hour infusion.

**Storage**
Stored unopened vials are stable at 2-8 degrees C in the refrigerator. Keep in original package to protect from light. When opened the diluted solution for intravenous use can be kept in room temperature for up to 12 hours.

**Toxicity**
Myelosuppression is the dose limiting adverse effect. In addition, gastrointestinal toxicity, blood pressure changes, allergic reactions, alopecia, abdominal pain, constipation, fever, and blindness (transient and rare). Second myeloid malignancies has been linked to intensive dose schedules.

17.4. Fludarabine
This nucleoside analogue (purine antagonist) is an antimetabolite. It is administered as Fludarabine phosphate, which is rapidly dephosphorylated to 2-fluoro-ara-A, and then phosphorylated intracellularly by deoxycytidine kinase to the active triphosphate, 2-fluoro-ara-ATP. This metabolite appears to act by inhibiting DNA synthesis. Fludarabine is mainly excreted in the urine.

**Formulation**
Each vial contains Fludarabine phosphate 50 mg, Mannitol 50 mg Sodium hydroxide to adjust pH to 7.7.

**Dose**
30 mg/m² intravenous infusion over 30 minutes.

**Administration**
Intravenous infusion over 30 minutes. Add 2 ml of sterile water to the vials, and further diluted in 100 ml 0.9 mg/ml NaCl for i.v. infusion.

**Storage**
Store refrigerated, 2° to 8°C. Fludarabine should be used within 8 hours of reconstitution.

**Toxicity**
Frequent side effects are myelosuppression, fever, chills, malaise, nausea, vomiting. Rarely autoimmune phenomena (hemolytic anemia), pneumonitis, and CNS symptoms such as agitation can occur. It causes profound and long-term lymphocytopenia.

### 17.5. Liposomal daunorubicin (DaunoXome)
DaunoXome is a liposomal preparation of daunorubicin. DaunoXome has a pharmacokinetic profile significantly different from that of conventional daunorubicin with increased peak plasma levels and mean area under the plasma curve. Daunorubicin is released slowly, and then mainly excreted in the bile, less so in the urine. Daunorubicin is metabolized to daunorubicinol, which is again mainly excreted in the urine and in the bile. Daunorubicin is an anthracycline antibiotic with antimitotic and cytotoxic activity with a number of proposed mechanisms of action. Daunorubicin forms complexes with DNA by intercalation between base pairs. It inhibits topoisomerase II activity by stabilizing the DNA-topoisomerase II complex, preventing the religation portion of the ligation-religation reaction that topoisomerase II catalyzes. Single strand and double strand DNA breaks result. Daunorubicin hydrochloride may also inhibit polymerase activity, affect regulation of gene expression, and produce free radical damage to DNA.

**Formulation**
DaunoXome is available in 2 mg/ml vials with a preservative-free liposomal emulsion.

**Dose**
60 mg/m² one hour iv infusion (course DxEC/ADxE/FLADx).

**Administration**
DaunoXome should only be used iv and only diluted with 5% dextrose. Other solvents may cause agglutination. The recommended concentration in the diluted preparation is 0.2 – 1 mg/ml. Heparin or dexamethasone should not be used simultaneously since precipitation may occur.

**Storage**
Intact vials shall be stored refrigerated at 2-8 degrees C. The prepared solution is stable for 24 hours if kept at 2-8°C and protected from light but recommended to give immediately after preparation from a microbial point of view.

**Toxicity**
Myelosuppression is the dose-limiting toxicity. Neutropenic fever, nausea, vomiting, diarrhoea, alopecia and hepatotoxicity are common side effects. An acute infusion associated reaction with back pain, flushing, chest tightness and dyspnoea is common and can occur at the first dose. It usually starts within 10 minutes of start of infusion and generally subsides when the infusion is slowed or halted. The risk of anthracycline induced cardiomyopathy requires that cardiac function is carefully monitored.
17.6. Methotrexate
Methotrexate (MTX) is a folate analogue that inhibits enzyme dihydrofolate reductase, which is important in conversion of folic acid to tetrahydrofolic acid, and several enzymes involved in purine de novo synthesis.

Formulation
Solution 2.5 mg/ml for injection/infusion. The product is a MTX sodium salt, yellow liquid with or without a preservative. Use only preservative-free product for intrathecal injection.

Dose
Age-adjusted dose for intrathecal administration. See table 3.

Storage
At room temperature.

Administration
For intrathecal administration preservation-free methotrexate, preservation-free cytarabine, and glucocorticosteroids can be given in the same syringe.

Toxicity
Systemic toxicity following intrathecal administration is very rare. Chemical arachnoiditis may occur after intrathecal administration.

17.7. Mitoxantrone
Mitoxantrone is an anthracenedione that is structurally similar to the anthracyclines. 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
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 drug should be further diluted to at least 50 ml in 5% glucose or 0.9% NaCl prior to administration.

Dose
5 mg/m² 1 hour iv infusion (course MEC).
10 mg/m² 1 hour iv infusion (course HAM).

Administration
The drug should be further diluted to at least 50 ml in 5% glucose or 0.9% NaCl prior to administration.

Storage
The intact vials should be stored at room temperature. Refrigeration may result in precipitation of Mitoxantrone, which will redissolve upon warming to room temperature.
Diluted preparations 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, diarrhoea, 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 anthracyclines. Patients with an increased risk of cardiotoxicity include those having received prior therapy with anthracyclines, those with previous mediastinal radiotherapy, and those with pre-existing cardiac disease.
18. Supportive Care

Although the AML 2012 protocol provides guidelines and recommendations for several topics of supportive care the responsibility for all therapy lies with the attending physician alone and the authors of this protocol do not take responsibility, legal or otherwise, for any adverse consequences arising from application of this treatment.

18.1. General recommendations.
At initial presentation it is important to bear in mind that most complications are caused by the leukaemic disease and that it is crucial to start chemotherapy as soon as possible. All children with AML have a high risk of initial complications such as severe infections, bleeding, respiratory problems, renal dysfunction and metabolic disturbances. Occasionally general anaesthesia may be life-threatening, in which case diagnostic work-up can be made by performing bone marrow puncture under mild sedation and local anaesthesia or in peripheral blood if blast counts are high.
All children with AML should be carefully evaluated for signs of pulmonary insufficiency with hypoxia and acidosis (dyspnoea, tachypnea and cyanosis), CNS symptoms (level of consciousness, slurred speech, ataxia, nystagmus) and eye symptoms (papilledema). Chest X-ray is recommended and prothrombine time, partial prothrombin time, antithrombin III, fibrinogen and fibrin split products should be evaluated.
For venous access the placement of a double or triple lumen central venous catheter for administration of chemotherapy, nutrients, antibiotics, and blood products is strongly advised.

18.2. Hyperleukocytosis
Around 15-20% of children with AML have WBC > 100 x 10^9/L at presentation(3). These children have a high risk of life-threatening complications and it is essential that chemotherapy is started as soon as possible. In the lungs, stasis of leukaemic cells in the vessels, possibly in combination with release of inflammatory mediators, can cause respiratory distress. In the CNS, leukostasis may cause both bleeding and thrombosis. Compared to children with ALL, associated coagulopathy is more common and the risk of severe bleeding is higher (see below). Hyperleukocytosis is also associated with an increased risk of renal dysfunction and metabolic disturbances.
The mainstay of treatment is to reduce hyperviscosity in the blood by hydration. A volume of 3000-4500 ml/m² (in children below 10 kg 150-200 ml/kg) should be administered (see section 17.3). Since erythrocytes increase viscosity, blood transfusions should be avoided unless the patient has critical anaemia and even in this situation it is often prudent to transfuse smaller volumes e.g. 5 ml/kg. Platelets do not substantially increase blood viscosity and should be liberally administered.
Although it has been used in children with very high WBC, there is no proven benefit of performing either leukapheresis or partial exchange transfusion.
18.3. Tumour lysis syndrome (TLS)
Although less frequent than in ALL, severe TLS may be seen in children with AML and a high tumour burden. TLS presents with hyperuricemia, hyperphosphatemia with secondary hypocalcaemia, hyperkalemia, acidosis and renal failure. The condition is caused by the spontaneous or chemotherapy induced lysis of leukaemic cells and is aggravated by precipitation of calcium phosphate salts, uric acid or xanthin crystals in the kidneys. Renal function may be further compromised by leukaemic cell infiltration in the kidneys and by leukostasis in the small renal vessels.

18.3.1. Treatment.
All patients need careful monitoring of blood pressure, fluid input and diuresis and management of electrolyte disturbances. It is essential to closely follow serum levels of creatinine, uric acid, potassium, calcium and phosphate. Despite the best care some patients proceed to acute renal failure and will require dialysis or haemofiltration (60).

18.3.1.1. Hydration and diuresis.
The early establishment of a high urinary output is the most important factor in preventing and treating TLS. Intravenous fluids should be given at a rate of 3000-4500 ml/m²/24h (in children below 10 kg 200 ml/kg/24h) while checking that urinary output is ≥ 80% of fluids administered. Furosemide may be needed to maintain diuresis. There is no evidence that alkalisation of the urine improves outcome of TLS (61). Although the solubility of uric acid is maximal at pH 7.5 the solubility of xanthin, hypoxanthin and calcium phosphate decreases and since high uric acid levels can be easily managed with rasburicase (or allopurinol in mild cases), hyperphosphatemia is the major threat to kidney function.

18.3.1.2. Hyperuricemia.
In mild cases allopurinol may be used at 100-150 mg/m²/dose every 8th hour. One should bear in mind that allopurinol does not reduce already produced uric acid and that it causes elevated levels of xanthine and hypoxanthine which may cause obstructive uropathy. In more severe cases and in patients with a high risk of TLS, rasburicase (0.05)-0.2 mg/kg i.v. over 30 minutes is recommended and this is repeated daily if uric acid levels remain high (after rasburicase samples for uric acid analysis need to be placed on ice to avoid ex vivo degradation). Rasburicase should be avoided in patients with known G6PDH deficiency.

18.3.1.3. Hyperphosphatemia
Hyperphosphatemia is defined as serum levels ≥ 2.1 mmol/L and can cause nausea, vomiting, diarrhoea, lethargy and seizures. More importantly, it can cause renal precipitation of calcium phosphate. Treatment consists of avoiding administration of phosphate. Oral phosphate binders such as aluminium hydroxide can be given at a dose of 12.5-37.5 mg/kg every 6th hour. Patients often have secondary hypocalcaemia but only patients with symptomatic hypocalcaemia should receive treatment with calcium gluconate 50-100 mg/kg/dose i.v.

18.3.1.4. Hyperkalemia
Hyperkalemia is defined as serum levels ≥ 6.0 mmol/L and can cause neuromuscular and cardiac abnormalities. Asymptomatic patients can be treated with sodium polystyrene sorbitol (1g/kg with sorbitol) orally or per rectum. In symptomatic patients more aggressive
therapy is needed. An emergency bolus of insulin actrapid 0.1 unit/kg together with 25% glucose 2 ml/kg can be given and continue with 5% glucose with 1 unit of insulin per 5 g glucose and infuse at about 0.1 units of insulin/kg/hour. Monitor serum glucose closely and adjust insulin infusion rates accordingly.

Patients with hyperkalemia should have ECG monitoring. For the prevention of life-threatening arrhythmia, administer calcium chloride IV 0.1 mmol/kg per dose. IV administration of calcium is the fastest means of reversing the cardiac effects of hyperkalemia. The onset of action is within minutes, but the duration only about one 1/2 hour. Do not use calcium i.v prophylactically! Do not administer in the same line as sodium bicarbonate. Uncontrollable hyperkalemia is an indication for peritoneal or haemodialysis.

18.4. Coagulation abnormalities and bleeding
Children with AML, especially those with promyelocytic (APL), myelomonocytic or monocytic AML (M4 and M5), have a high incidence of coagulation disturbances, which can cause disseminated intravasal coagulation. The risk of bleeding or microvascular thrombosis increases further in case of hyperleukocytosis. All patients should be screened for coagulopathy (prothrombine time, partial prothrombin time, antithrombin III, fibrinogen and fibrin split products) and if present, fresh frozen plasma 10-15 ml/kg 2-3 times daily should be administered and platelets given to maintain platelet counts over 50 x 10^9/L. Bleeding should be aggressively managed with platelet transfusions. The use of salicylic acid and NSAID preparations is contraindicated.

18.5. Infection
Children with AML have severe immunosuppression already at time of diagnosis and it is not uncommon that they present with life-threatening infections. The chemotherapy is very intensive and the children are expected to have prolonged periods of neutropenia after each treatment course with a high risk of bacterial and fungal infection. The risk of severe infection is particularly high during induction.

18.5.1. Prevention of infection
If available, HEPA air filtration in the nursing room should be used. At diagnosis, potential infectious foci e.g. dental should be identified and if possible treated. Dental extractions may be necessary. Antibodies for hepatitis viruses, CMV and varicella, should be evaluated. In case of surgery with high-risk of bacteraemia prophylactic antibiotics is recommended. The issue of prophylactic antibiotics to prevent sepsis is still controversial. It is likely that the incidence of sepsis can be reduced in these patients by prophylactic treatment. Thus, a retrospective study from the AML02 protocol showed that prophylaxis with either cefepime i.v. or vancomycin i.v. + oral quinolone gave a substantial reduction in sepsis (62). Furthermore, a large Cochran review of studies of prophylactic antibiotics in patients with neutropenia, demonstrated that quinolone or trimethoprim/sulfamethax prophylaxis was associated with a reduction of both all cause and infection related mortality in neutropenic patients (63). However, most studies were performed on hospitalised patients, transplant patients were also included and the number of children was comparatively low making
inferences to paediatric AML patients uncertain. In combination with concerns regarding the risk of emergence of resistant bacterial strains from prolonged quinolone administration, no recommendations for antibacterial prophylaxis is given in AML 2012 but prophylaxis may be employed at the discretion of the individual centres.

Pneumocystis jiroveci prophylaxis is advised with sulfamethoxazole-trimethoprim (e.g. 25/5 mg/kg divided in two daily doses orally two consecutive days a week)[64, 65]. AML therapy has a high risk of fungal infections and although larger randomised studies on efficacy are lacking, prophylactic anti-fungal therapy is recommended. Fluconazole 5 mg/kg/day has been most commonly employed. However, fluconazole lacks effect on moulds and Candida Krusei and institutions may, depending on local incidence of causative fungi, choose an alternative agent such as liposomal amphotericin i.v. twice weekly, voriconazole, itraconazole or posaconazole.[62, 66]

Administration of varicella zoster hyper immune globulin within 72 hours is recommended after probable varicella exposure of a seronegative patient. An alternate practice, which is increasingly common but poorly evidence-based, is to administer a 14 day course of aciclovir following exposure[67]. Vaccination of unprotected siblings is recommended.

The prophylactic use of myeloid growth factors is not recommended since no study has documented a benefit in survival and children with the isoform IV of the G-CSF receptor may have an increased risk of relapse when prophylactically treated with G-CSF[53].

18.5.2. Neutropenic fever
Neutropenic fever is a medical emergency and the children should be urgently and carefully evaluated for signs of shock or pre-shock (hypoxia, reduced capillary blood flow, blood pressure, level of consciousness), pulmonary insufficiency (dyspnoea, tachypnea, hypoxia, acidosis) and focal signs of infection. Empiric treatment should be started rapidly with broad-spectrum antibiotics i.v. according to institutional guidelines but it is essential that the drugs have good activity against Gram negative rods and alpha-haemolytic streptococci. The children should be carefully monitored for signs of deterioration of circulatory, pulmonary or mental function.

The empiric treatment may be adjusted when a specific cause for the infectious symptoms is found, although broad-spectrum antibiotics should be continued if the patient is still neutropenic.

The risk of fungal infection is high and children with persisting fever after 48-72 hours should be carefully re-evaluated and empiric antifungal therapy with activity against both yeast and mould species instituted. Every effort should be made to try to identify the site and cause of infection in these patients and CT scans of the chest and abdomen are often informative.

18.5.2.1. Pulmonary symptoms
Respiratory insufficiency in AML can have numerous causes and always requires rapid and careful work-up. At diagnosis, leukostasis can cause occlusion of pulmonary vessels and induce an ARDS-like reaction which often necessitates care at the intensive care unit. In
these patients early institution of chemotherapy is particularly important and
dexamethasone may be beneficial(68).

AML treatment is associated with a high risk of pulmonary aspergillosis. However,
pulmonary infiltrates can also be caused by bacteria, virus (CMV), legionella, and
*pneumocystis jiroveci.* Therefore, aggressive diagnostic measures including (repeated) high
resolution CT scans, bronchoscopy with biopsy or bronchoalveolar lavage are essential, and
open lung biopsy should be considered. Severe infections may also induce ARDS particularly
after high-dose cytarabine and/or in association with sepsis caused by alpha-haemolytic
streptococci(69, 70).

**18.5.2.2. Abdominal infection - typhlitis**

Nausea, vomiting and diarrhoea are common symptoms related to chemotherapy induced
mucositis. If abdominal pain and/or fever also is present the child may have neutropenic
enterocolitis (typhlitis), which is an inflammation of the bowel wall most often caused by
infection. The causative agents include gram negative or anaerobic bacteria and fungi. The
diagnosis can be confirmed by demonstrating increased bowel wall thickness on CT scan or
ultrasonography. Treatment relies on conservative management with broad-spectrum
antibiotics i.v., antifungal treatment, meticulous administration of fluids and electrolytes and
analgesics. A paediatric surgeon should be involved in assessment. The patients should be
carefully monitored for clinical deterioration since intestinal perforation requiring
emergency surgery may occur. There is no consensus regarding enteral feedings during
typhlitis but most surgeons tend to recommend withholding oral nutrition during the first
24-48 hours(70, 71).

**18.6. Transfusion support**

It is recommended that the platelet count be prophylactically maintained above 10 x 10⁹/L.
Higher thresholds may be indicated, e.g. during lumbar punctures, surgical procedures and
infections. Bleeding due to thrombocytopenia should be treated promptly. In case of
alloimmunisation HLA-matched platelets may be required.
Packed red blood cell transfusions should be used to correct anaemia (Hb < 5 mmol/L = 8.0
g/dl). Higher threshold levels may be indicated in case of pneumonitis or other causes of a
compromised clinical condition.
The blood products should be leukocyte-depleted to reduce the risk of HLA sensitisation and
CMV infection. Some institutions use irradiation (15 Gy) of cellular blood products to avoid
transfusion related graft versus host disease but with adequate methods for leukocyte
depletion the risk of GvHD is very low(72) and there is no consensus of the need of
irradiation except for patients undergoing SCT.

**18.7. Nutrition**

The combination of chemotherapy-induced vomiting, mucositis, infection, and haemorrhage
may result in significant weight loss. Progressive weight loss should be treated aggressively
with supplemental enteral or parenteral nutrition. Enteral feedings are preferred to
parenteral but adjustment of the diet (i.e. no lactose or semi elementary) may be beneficial.
Physical therapy may also improve the general condition, appetite and prevent the loss of
motor skills.
18.8. Mucositis
The incidence of mucositis is high after chemotherapy for AML, particularly after courses including anthracyclines. Meticulous oral hygiene is required; tooth brushing, hydrogen peroxide, saline and bicarbonate rinses and chlorhexidine solution. The liberal use of pain medication for this condition is encouraged. Stomatitis due to herpes virus may be confused with drug-induced mucositis. Therefore, viral cultures should be obtained frequently. Anti-herpetic and anti-fungal therapy should be given as indicated.

18.9. Emesis
All chemotherapeutic drugs used in this protocol are highly emetogenic and all patients should receive anti-emetic therapy according to local institutional guidelines.

19. Ethical considerations
The study will be submitted for approval to the National and/or local Scientific Ethical Committees and the National Medicine Agencies in all participating countries. The patients will be recruited by their attending physician. Patients will participate only after receiving proper oral and written information and after their parents/guardians and (when appropriate) also themselves have given oral and written consent. All sampling from the bone marrow, blood and CSF will be obtained in relation to routine sampling as part of their treatment. Trial subjects and families will be able to contact both the study and national coordinators if they require additional information.

19.1. Possible benefits and risks for patients
Cooperative treatment studies have been performed in paediatric AML since the late 1970s. These studies are the main reason that outcome in thirty years has increased from being an almost incurable disease to having an OS approaching 70%. The AML 2012 protocol is a carefully prepared research protocol, which applies recent advanced techniques for response evaluation with the hope of giving a better risk stratification. It also incorporates some of the most efficacious treatment elements from NOPHO and other collaborative group studies. We therefore have good reason to hope that this will translate into an improved outcome for the protocol as a whole. We have no reason to believe, although this has to be supervised, that the study treatment will be more toxic than previous protocols. This protocol will be carefully monitored and follow GCP guidelines which will provide additional safety for the patients. The study includes two randomised studies investigating important questions regarding treatment efficacy and cardiac toxicity. The study questions are completely open i.e. we have no preformed opinion or belief as to which treatment arms are the best. What we do know is that we offer very effective anti-leukemic therapy in all treatment arms. Thus, this research protocol has the potential of increasing the cure rates without increasing toxicity for all children participating.

19.2. Informed consent
The parents/guardians/legal representatives of the child and the child will receive both written and oral information of the trial including study objectives, study procedure and
possible risks. Only physicians with experience in communicating with children and adolescents and with an in depth knowledge of the study will give information. All information will be given in compliance with guidelines from the national Scientific Ethical Committees. Both oral and written information will be adjusted for age and written in an easily understandable language. Information will be given at a dedicated meeting and they will be given ample time to make their decision. This will most often require that the family after the first information are given time to read the written information and think about the study and that the final consent is obtained at a second meeting. Consent will be obtained from the parents and when appropriate from the child. If a child is capable to understand the study and rejects participation while the parents give consent, the child will not be included in the study. Families should be assured that they at any time can choose to withdraw the consent. Information will also include that those that decide not to participate will be treated according to the control arms of the protocol.

20. Financial issues
All expenses for submission for approval of the trial to the relevant authorities of a country is the responsibility of the Principal Investigator of the country in question. The National Principal Investigators, the research laboratories, the administrative groups can apply for external funding from research foundations and similar non-profit organization to cover expenses linked to the implementation of the protocol, running of the infrastructure (including registries), and for the research activities. Applications for funding from commercial entities (e.g. pharmaceutical companies) can only be done after approval from the NOPHO-DBH AML 2012 study committee.

21. Publication guidelines

The overall results and results of the randomised studies will be published in international peer-reviewed journals. The responsibility for writing these publications rests on the study chair and all members of the NOPHO-DBH AML 2012 study committee will be included as co-authors. Additional co-authors may be included if they have made significant scientific contributions. All authorships should be approved by the NOPHO AML group.

Data from the study can and will be used in other publications, abstracts and presentations after approval of the NOPHO-DBH AML 2012 study committee.

Results of the add-on studies will be written by the principal investigators of the respective studies. Co-authors that have contributed significantly and scientifically should be included.
22. List of Appendices

1. AML Study Contract
2. Toxicity registration
3. SUSAR/Death report form
4. MRD flow guidelines
5. MRD PCR guidelines
6. Case Report Form DNX study
7. Case Report Form FLADx study
8. Instructions for bio bank sampling
9. Guidelines for patient information
23. References

42. Levis M. (2011) Blood 117(26),6987-6990