Important points in this amendment for AML2012

- The randomization in course one (R1) between mitoxantrone (MEC) and DaunoXome (DxEC) will be closed. All patients should receive MEC as first course.

- It is important to continue enrolling patients in the overall AML2012 protocol during the first course as it is essential to continue evaluating the effect of MRD after first induction course and fusion gene PCR on event-free and overall survival.

- The randomization in course 2 (ADxE vs FLADx) will allow replacement of liposomal daunorubicin with daunorubicin if DaunoXome is not available. Therefore R2 can be immediately resumed using liposomal daunorubicin if available otherwise using daunorubicin.

Rationale for amendment – termination of randomization 1 (mitoxantrone vs liposomal daunorubicin)

Due to manufacturing problems for liposomal daunorubicin, both randomizations in AML2012 have been on hold since Nov 2017. At this time, 194 patients had been included in the R1 randomization. Since Nov 2017, all patients have been treated with the standard arm with mitoxantrone (MEC) in the first course and with ADE in the second course. In course ADE, daunorubicin replaces liposomal daunorubicin given at the same dose and timepoints. A yearly report was prepared for the DMC based on results up to Oct 10 2018. At this time point, all patients randomized for course 1 had a minimum follow-up of almost 12 months with a median follow-up time of 30 months in patients without event. For the primary endpoint, the fraction of patients with MRD < 0.1% on day 22 after start of course 1, there was no difference between the treatment arms. However, as shown in figure 1 there was a large and statistically significant difference in event-free survival, estimated at three years, between the treatment arms. Thus EFS$_{3y}$ was 76.6±4.4% for the MEC arm and 57.0±5.6% for the DxEC arm (Log rank P = 0.017).
Figure 1. EFS in 194 patients randomized between MEC and DxEC in R1 and with outcome data. MEC (blue line) had an EFS$_{3y}$ of 76.6±4.4% and DxEC had an EFS$_{3y}$ of 57.0±5.6%. Log rank test is significant with $P=0.017$. The number at risk at three years was 27 for MEC and 23 for DxEC with a median observation time of 30 months in patients without events.

Event analysis showed that the difference in EFS$_{3y}$ between the treatment arms was caused by a higher rate of relapses in the DxEC arm. Figure 2 shows the distribution of events in the treatment arms.

Figure 2. Distribution of events in patients randomized to MEC or DxEC. The difference in total events is statistically significant ($P=0.021$ Chi$^2$).

In total, there were 39 events in the DxEC arm and 22 in the MEC which is a statistically significant difference ($P=0.021$ Chi$^2$). Further analysis of the events showed, as demonstrated in figure 3, that the difference in EFS$_{3y}$ was caused by a higher cumulative incidence of relapses (CIR$_{3y}$) in the DxEC arm. CIR$_{3y}$ was 17.4±4.0% for MEC and 39.1±5.7% for DxEC ($P=0.004$).
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Figure 3. CIR$_{3y}$ in 194 patients randomized between MEC and DxEC in R1 and with outcome data. MEC (blue line) had a CIR$_{3y}$ of 17.4±4.0% and DxEC had an CIR$_{3y}$ of 39.1±5.7%. Log rank is significant with $P=0.004$. Since the sum of the cumulative incidences of cause-specific events and EFS at three years was close to 100% no attempt to correct for competing events was made.

To evaluate the effect of randomization on EFS$_{3y}$ in relation to other risk factors a Cox regression including age, gender, white blood cells at diagnosis, presence of CBF AML and FLT3-ITD without concomitant NPM1 mutation was performed. The regression gave a model with an overall significance $P=0.034$. Results are shown in table 1 and demonstrate that significant effects were found for treatment arm (HR 1.77 [CI 1.04–2.99] for DxEC arm) and for CBF leukemia (HR 0.43 [CI 0.19–0.96]).

Table 1. Cox regression showing effects of factors on EFS at three years.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Sig</th>
<th>Exp(b)</th>
<th>Lower 95% CI</th>
<th>Upper 95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>MEC$/DxEC$</td>
<td>.034</td>
<td>1.77</td>
<td>1.04</td>
<td>2.99</td>
</tr>
<tr>
<td>CBF</td>
<td>.039</td>
<td>0.43</td>
<td>0.19</td>
<td>0.96</td>
</tr>
<tr>
<td>Gender$^1$</td>
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<td>1.46</td>
<td>0.87</td>
<td>2.46</td>
</tr>
<tr>
<td>WBC</td>
<td>.64</td>
<td>1.00</td>
<td>1.00</td>
<td>1.00</td>
</tr>
<tr>
<td>Age</td>
<td>.64</td>
<td>1.01</td>
<td>0.96</td>
<td>1.06</td>
</tr>
<tr>
<td>FLT3</td>
<td>.84</td>
<td>0.92</td>
<td>0.40</td>
<td>2.12</td>
</tr>
</tbody>
</table>

1. MEC and male gender are the respective reference categories

Finally, the effect of treatment arm on survival was investigated. Figure 4 shows that there is a trend to higher survival in the MEC arm (OS$_{3y}$ 85.9±3.9% vs 75.2±5.0% $P=0.105$).
It is likely that the lower relapse rate in MEC causes the trend to higher survival and, assuming a salvage rate of 40% in patients who relapse, the 10% increase in OS corresponds well to the observed >20% difference in relapse rates. However, to obtain significance for a 10% “true” increase in OS it will require randomization of a substantial number of additional patients potentially exposing half of these to an inferior treatment. The data on survival are however less reliable than EFS data since deaths from relapse occur at a later time point.

Conclusions – randomization between MEC and DxEC
EFS\textsubscript{3y} is significantly lower and CIR\textsubscript{3y} significantly higher in patients treated with liposomal daunorubicin indicating that, with the dosing employed, mitoxantrone is superior to liposomal daunorubicin. Since relapses in AML occur early at a median time of 9 months with 44/46 relapses occurring within two years the estimates of EFS are likely to be stable with time. Cox regression demonstrates that the effect of treatment arm remains significant when taking into account potential confounding factors. Furthermore, although not statistically significant, the trend to difference in survival corresponds well to the observed difference in relapses.

The DMC, which also had access to interaction data with the second randomization (not disclosed here for confidentiality) strongly recommended a termination of the randomization between mitoxantrone and liposomal daunorubicin. The protocol steering group thus has decided to close the R1 randomization. However, patients should continue to be enrolled in the overall AML2012 protocol since this will enable us to analyze the relation between MRD response after induction course one, fusion gene transcript levels and EFS and OS. Furthermore, this will ensure that patients fulfil eligibility criteria for randomization in the second course.
Rationale for amendment – continuing randomization between ADxE and FLADx but allowing for substitution of liposomal daunorubicin with daunorubicin

The aim of the randomization of the second course between ADxE and FLADx is to evaluate if a FLA (fludarabine, high-dose cytarabine + anthracycline) based regimen is more effective and/or less toxic than conventional ADE (low-dose cytarabine, etoposide + anthracycline) regimens. Liposomal daunorubicin was chosen since preliminary data indicated that it may be less cardiotoxic than other anthracyclines. A randomized BFM study showed that there was no difference in OS and EFS when comparing liposomal daunorubicin (80 mg/m² x III) with idarubicin (12 mg/m² x III) but found better EFS in patients with RUNX1/RUNX1T1(1). One direct comparison in adults with free daunorubicin has been published but is difficult to interpret since a much lower dose of daunorubicin was used (45 mg/m² x III versus 80 mg/m² x III(2). There has been no other studies comparing liposomal daunorubicin with other anthracyclines since the start of AML2012.

We still consider the main study question for the second randomization, i.e. to investigate if a FLA based regimen is beneficial in terms of effect and toxicity for children with de novo AML, to be important. Given the facts that supply of liposomal daunorubicin is unreliable and that the drug had inferior efficacy in course one in AML2012 we find it reasonable to continue the study using free daunorubicin as an alternative to the liposomal formulation. The active drug is the same and the differences relate to pharmacokinetics and tissue distribution.

The DMC has reviewed the data on efficacy and toxicity for the 194 patients currently randomized between ADxE and FLADx. They support a continuation of the study and also allowing for substitution of liposomal daunorubicin with daunorubicin if the former is not available.

Conclusions – randomization between ADxE and FLADx

Since it is important to define the role of FLA based regimens in induction therapy for children with AML and the issues with supply of liposomal daunorubicin jeopardize the study, conventional daunorubicin may be used instead of liposomal daunorubicin but only when liposomal daunorubicin is unavailable.

The dose and scheduling of daunorubicin is the same as for liposomal daunorubicin. Thus, in the ADxE course daunorubicin will be given as a one hour infusion at a dose of 60 mg/m² on days 2, 4 and 6. In the FLADx course daunorubicin will be given as a one hour infusion at a dose of 60 mg/m² on days 2, 4 and 6. In both courses, for children below one year or 10 kg the daunorubicin dose will be 2 mg/kg at each administration.