PROTOCOL FOR PATIENTS AGED UNDER 60
(Trial Reference ISRCTN55675535)

Through the use of a risk based approach AML17 will evaluate several relevant therapeutic questions in acute myeloid leukaemia (AML) as defined by WHO, and high risk Myelodysplastic Syndrome. The trial is open to all adult patients aged less than 60 years, and also to patients aged 60 years or over for whom intensive therapy is considered appropriate. At least 2800 patients will be recruited. For patients who do not have the Acute Promyelocytic Leukaemia (APL) subtype, an induction randomisation will compare two courses of the standard **DA with the daunorubicin dose being either 60mg/m^2 or 90mg/m^2 in course 1 (two options).** In Patients who are not high risk, consolidation in adults will compare **one course with two courses of High Dose Ara-C.** After course 1 of treatment, patients will be segregated based on their molecular-genetic characteristics, and a validated risk score. Patients who are at high risk of relapse based on the AML Risk Score and patients who have a FLT3 mutation without an NPM1c mutation irrespective of risk score, will be eligible for a myelo-ablative or reduced intensity conditioned allogeneic stem cell transplant if a donor is available. These patients will be randomised between **FLAG-Ida** (standard arm) **vs Daunorubicin/Clofarabine** with the aim of maximising the number of patients receiving an allogeneic transplant. Adult patients who have **Core Binding Factor (CBF) leukaemias** ie favourable risk disease, will be randomised only to the 3 versus 4 comparison, but they will all receive gemtuzumab ozogamicin on day 1 of course 2

Patients who are not high risk, or favourable risk (Core Binding Factor (CBF) leukaemias are defined as intermediate risk and will be randomised to the 3 versus 4 comparison. Patients in this group who are > 40 years of age should be considered for a Reduced Intensity Allograft (RIC) transplant if a fully matched sibling donor is available. Investigators will be informed about eligible patients.

For patients with APL, the Italian **AIDA** anthracycline plus ATRA based chemotherapy approach will be compared with the chemotherapy-free combination of **ATRA plus Arsenic Trioxide. Children with APL are not eligible for AML 17.**

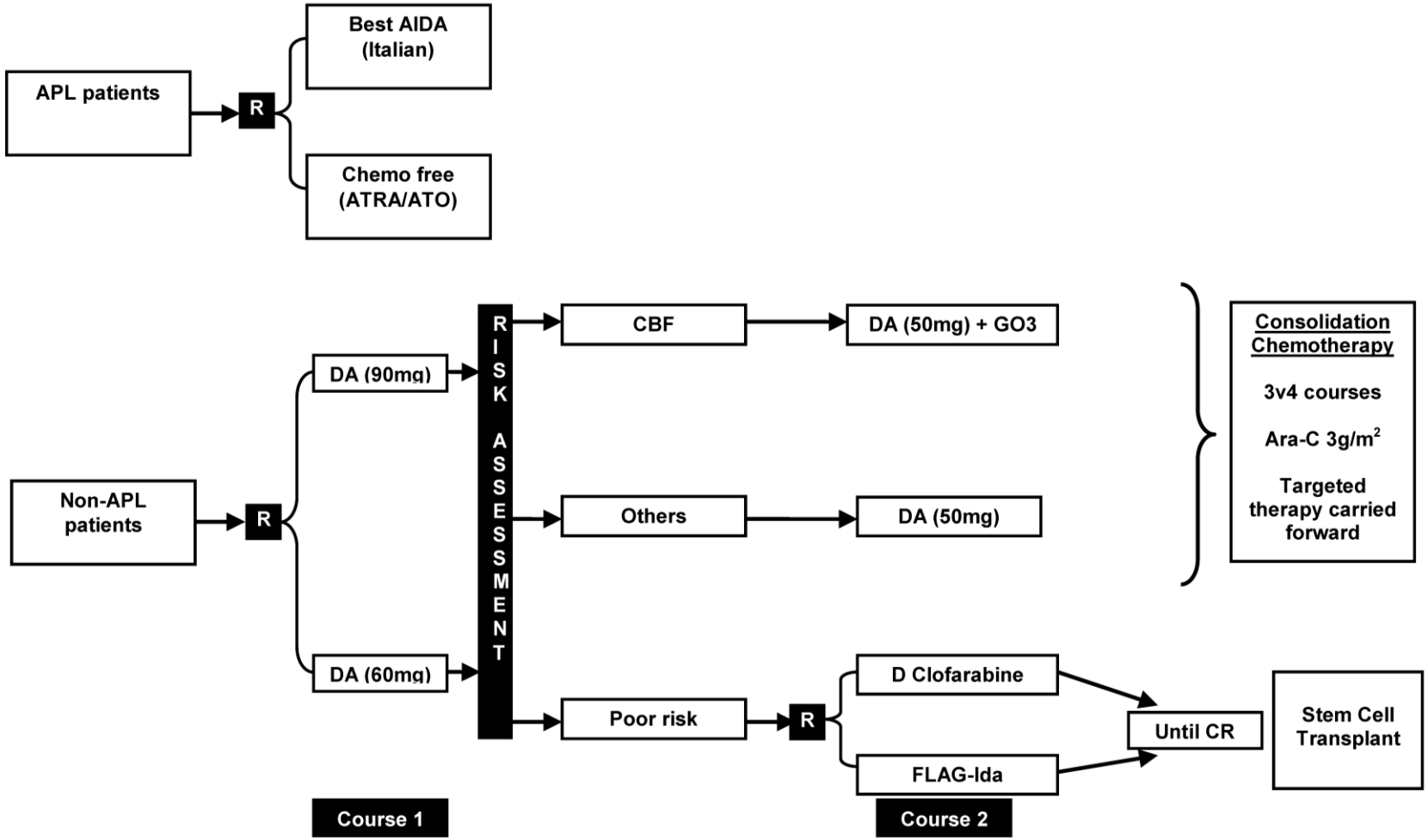
At diagnosis, material will be sent to reference labs for molecular and immunophenotypic characterisation and the identification of markers of minimal residual disease (MRD) detection. The predictive value of these markers will be validated in the early part of the trial, and the clinical impact of this information will be tested in a **monitor versus not monitor** randomisation in a later patient cohort.

There are about 700 cases of AML aged 16-59 years per annum in the British Isles alone. About 650 patients entered AML15 annually, so with a continuation of accrual at this, or a higher level, clear evidence on the relative benefits of the therapeutic options being tested in AML17 will be obtained in just a few years. This information will contribute to the continuing improvement of the treatment available to many future patients with AML.

This protocol is intended to describe a trial conducted by the AML Working Group of the National Cancer Research Institute (NCRI) Haematological Oncology Study Group in Acute Myeloid Leukaemia and high risk Myelodysplastic Syndrome in adults under the sponsorship of Cardiff University. It provides information about procedures for the entry, treatment and follow-up of patients. It is not intended that this protocol should be used as an *aide-memoire* or guide for the treatment of other patients. Every care has been taken in its drafting, but corrections or amendments may be necessary. Before entering patients into the trial, clinicians must ensure that the trial protocol has received clearance from their Local Research Ethics Committee and the participating Institution's Research and Development Office. During the course of this 6-year trial, not all randomisation options will be open at all times and some additional options may be included by protocol amendment.

Clinicians are required to read the whole protocol before commencing treatment

Flow chart for adult patients



Adult patients who have an HLA-matched sibling or volunteer unrelated donor and who are designated to have a high risk score or a FLT3 ITD mutant, NPM1 WT can proceed to allogeneic transplantation (myeloablative for the ITD+/NPM1c-). Recent maturing data suggests that patients who have intermediate risk defined by the risk score who are >40 years will benefit from a Reduced Intensity allograft from a matched sibling donor.

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09.00-17.00 hours, Monday to Friday (except bank holidays)

24 hour internet randomisation and data entry: http://AML17.cardiff.ac.uk

Contents

1. ETHICAL CONSIDERATIONS

The AML17 Trial Protocol has been approved by the Wales Multicentre Research Ethics Committee (NRES). Centres are required to go through a registration process with the Trial Office before recruitment is started. The institution's Research and Development Office must complete the site agreement with Cardiff University.

The right of a patient to refuse to participate in the trial without giving reasons must be respected. After the patient has entered the trial, the clinician is free to give alternative treatment to that specified in the protocol at any stage if he/she feels it to be in the patient's best interest, and the reason for doing so should be recorded. Similarly, the patient must remain free to withdraw at any time from protocol treatment without giving reasons and without prejudicing any further treatment. All patients who come off protocol therapy for whatever reason will still need to remain within the study for the purposes of follow-up and data analysis.

The AML17 trial will be conducted in accordance with the Medical Research Council's Guidelines for Good Clinical Practice in Clinical Trials (a copy of these may be obtained from the MRC or from the Trial Office).

Section A:
TRIAL SUMMARY

2. OBJECTIVES

The AML17 trial has two distinct parts:

i. For patients with acute myeloid leukaemia (AML), (other than acute promyelocytic leukaemia) and High Risk Myelodysplasia, as defined by the WHO Classification (2008) (Appendix A).

ii. For adults with acute promyelocytic leukaemia (APL).

The objectives for each of these components are summarised below.

2.1 Therapeutic questions for adult patients with non-APL AML and High Risk Myelodysplastic Syndrome

For patients with acute myeloid leukaemia (AML) the aims of the AML17 trial are:

- To compare two induction chemotherapy schedules D(90)A versus D(60)A in course 1, in each case followed by D(50)A as course 2 in both arms.

- To compare a total of **three versus four courses** of treatment in total, comparing one versus two courses of **HD-Ara-C** in consolidation.

- In high risk patients to compare novel treatment, **Daunorubicin/Clofarabine** vs standard **FLAG-Ida.**

- In high risk patients, to evaluate, the value of **allogeneic stem cell transplantation** (SCT), whether standard allogeneic (allo-SCT) or non-myeloablative “mini” allogeneic (mini-SCT).

- To assess the clinical value of **minimal residual disease (MRD) monitoring** for patients’ overall survival.

2.2 Therapeutic questions for patients with APL

For adult patients with APL the aims of the AML17 trial are:

- To compare the Idarubicin based, **AIDA** Schedule with the chemo-free combination of **ATRA and Arsenic Trioxide.**

- A full description of the trial intentions for patients with APL are set out in Section 20.

2.3 Endpoints for Patients who have non-APL AML

The main endpoints for each comparison will be:

- Complete remission (CR) achievement and reasons for failure (for induction questions).
- Duration of remission, relapse rates and deaths in first CR.
- Overall survival.
- Toxicity, both haematological and non-haematological
- Quality of life and Health Economics assessments for patients in the disease monitoring randomisation
- Supportive care requirements (and other aspects of health economics).

2.4 Subsidiary objectives

Blood and bone marrow will be required at diagnosis, during remission and at relapse to evaluate the therapeutic relevance of morphological, cytogenetic, molecular-genetic and immunophenotypic assessments, with particular respect to:

- The relevance of the molecular and immunophenotypic detection of minimal residual disease
- The relevance of the presence of a cytogenetic abnormality in the bone marrow of patients in morphological remission.
- To correlate plasma arsenic levels with disease response and treatment-related toxicities including differentiation syndrome in APL patients allocated to receive ATO therapy
- To store excess diagnostic material for future research.

3. TRIAL DESIGN

AML17 is a randomised, controlled, open label Phase III trial for patients with AML and High Risk Myelodysplastic Syndrome (MDS). The design may, at first sight, appear complicated. However, if the trial is broken down into separate sections, each phase is straightforward and should be readily understandable to both clinicians and patients and of similar complexity to other NCRI AML trials:

3.1 Summary of comparisons

AML (other than APL):

A. Induction phase: one randomisation to one of two arms.
B. Consolidation phase: for patients who are not high risk two versus one further treatment courses of high dose Ara-C (two arms)
C. For high risk adult patients standard therapy (FLAG-Ida) vs D/Clofarabine (two arms)

APL:

A. AIDA versus ATRA plus Arsenic Trioxide (two arms)

3.2 AML (other than APL)

There are four randomised comparisons for adults within the trial:

At diagnosis:	i)	D(90)A versus D(60)A (two comparisons)
End of Course 1	ii)	FLAG-Ida versus D/Clofarabine for high risk score cases, and patients with a FLT3+/NPM1c⁻ genotype.
	iii)	The trial management system will inform investigators of which intermediate risk patients should be considered for myeloablative or Reduced Intensity Transplant.
After Course 2	iv)	1 versus 2 additional courses (i.e. 3 versus 4 courses of therapy =in total) for patients who are not poor risk who have entered complete remission. Chemotherapy will be high dose Ara-C
	v)	Patients will be invited to enter a randomisation between minimal residual disease monitoring or no monitoring.

In poor risk patients, defined by the risk score or the presence of an FLT3+/NPM1c⁻ genotype, the role of allogeneic SCT of either Standard or Reduced intensity will be assessed by means of a Mantel Byar analysis of transplant given versus not given. Some, but not all patients, with intermediate risk over 40 years of age may benefit from a reduced intensity allograft if a matched sibling donor is available which will also be assessed by a Mantel Byar analysis. The management system will inform investigators which patients >40 years should be considered.

Full details of the rationale for these comparisons, progress through the trial and treatment can be found in the relevant sections of the protocol, but are summarised below (and in the flow diagrams at the front and back of the protocol):

1. At diagnosis in adults: randomise between D(90)A and D(60)A as induction chemotherapy.

   The Two induction treatment arms will therefore be:

   Arm A One Course of D(90)A followed by a course of D(50)A.
   Arm B One Course of D(60)A followed by a course of D(50)A

2. By the end of the first course of induction chemotherapy (day 20), the FLT3 and NPM1 mutation status will be known in the reference labs, allowing the poor risk FLT3+/NPM1c- patients to be identified as candidates for stem cell transplant. On recovery from course 1 cytogenetics and molecular screening (Core Binding Factor) and Risk Index status of each non-APL patient will be available (the risk score is provided by the online system which must be used). At this point patients who are candidates for a myeloablative transplant (High risk and FLT3+/NPM1c-) and which standard risk patients > 40 years should be considered for a reduced Intensity Allograft from a matched sibling donor identified and indicated to the local team.

   i) Patients who have a high risk score and FLT+/NPM1c- genotype will enter the comparison of Daunorubicin/Clofarabine versus FLAG-Ida (Section 11.3)

   ii) Core Binding Factor Leukaemias will receive mylotarg 3mg/m^2 on day 1 of course 2 and will be randomised after course 2 to one or two more courses of treatment .i.e a total of three or four total courses of chemotherapy.

   iii) Other patients who are not involved in the options (i) and (ii), will be randomised after course 2 to one or two more courses of treatment, i.e a total of three or four total courses of chemotherapy.

   iv) All patients except the High Risk Index and FLT3+/NPM1c- genotype patients will receive the second induction treatment course.

3. Following the first and second course of treatment, patients should have a bone marrow (and paired blood sample) for MRD assessment (see Section 15).

4. On recovery from course two, patients who are not high risk will be randomised to one versus two further treatment courses in total.

The consolidation will be one or two courses of high dose Ara-C

Arm C: High Dose Ara- C
or
Arm D: High Dose Ara-C + High Dose Ara-C

5. Patients who are not in CR following the second course of treatment ie have refractory disease, are also eligible to enter the high risk randomisation.

3.3 Acute Promyelocytic Leukaemia (APL)

There is one randomisation within the trial for **adults only**:

At diagnosis: **(i) AIDA versus ATRA plus Arsenic Trioxide.**

Full details of the rationale for these comparisons, progress through the trial and treatment can be found in the relevant sections of the protocol, but are summarised below (and in the flow diagrams in the front):

1. At diagnosis: Adults only will be randomised between the AIDA (4 courses of Ida based chemotherapy) and the chemotherapy free approach of four courses of ATRA combined with Arsenic Trioxide.

2. Patients who present with a white cell count of $>10x10^9$/l are at a slightly higher risk of relapse and should receive Mylotarg ($3mg/m^2$) to reduce the WBC in addition to the allocated treatment.

3. Patients randomised to the chemotherapy free approach are eligible for monitoring of plasma arsenic levels during course 1 of therapy (see Section 190.5.4)

4. After 55 to 60 days assess remission status (see Section 19).

5. After Course 2, reassess remission status for minimal residual disease monitoring:

   - If in morphological CR, continue with AML17 protocol.
   - If not in morphological or molecular CR, the patient should be treated with Arsenic Trioxide or Mylotarg.
   - Bone marrow should be sent for MRD monitoring.

6. After courses three and four and at subsequent specified intervals, bone marrow should be sent for molecular monitoring (see section 15)

Section B:
RATIONALE FOR TREATMENT INTERVENTIONS

4. JUSTIFICATION OF TRIAL DESIGN AND TREATMENT SCHEDULES

4.1 AML (excluding APL)

Experience from AML15

It is clear that AML15 was a highly successful trial with recruitment at an unprecedented level (60 patients per month), a high overall CR rate of 84%, and survival which is significantly improved compared with the previous MRC AML12 trial and which compares very favourably with any international protocol. Thus, the therapy used in AML15 forms the backbone of the AML17 trial.

The theme for AML 17 is best available chemotherapy with or without molecular intervention, and, for patients who are at high risk of relapse, novel treatment will be assessed in a "pick a winner" design. The choice of induction treatments was informed by the preliminary experience from AML15. Although longer follow up is required there is ample evidence that the FLAG-Ida schedule was significantly more myelosuppressive and required more supportive care with the associated economic implications. Preliminary analysis does not suggest that any potential benefit would outweigh this. It is possible that later benefits may emerge. The addition of Mylotarg to induction course 1, initially at least, has significantly reduced the risk of relapse and improved the disease free survival/m, which translated into a significant overall survival advantage for 70% of the patients[(1)]. The first part of the AML17 trial has completed a comparison of two doses (3mg/m^2 versus 6mg/m^2). In the next phase of AML 17 two dose levels of daunoribicin will be compared in course 1 in combination with standard dose Ara-C.

Recent studies have raised the issue of whether the standard dose of Daunorubicin (45 or 50mg/m^2) is optimal. In a randomised comparison in patients <60 years the ECOG Group compared a 90mg/m^2 X 3 with 45mg/m^2 X 3 in course 1[(2)]. Patients not in complete remission after course 1 received a second DA course with the Daunorubicin dose at 45mg/m^2. The remission rate was superior in the 90mg arm (71% vs 57%) with a higher proportion entering remission with one course. This resulted in an improved median survival (23.7months vs 15.7 months)
(Clin Trials.Gov.NTC00049517)

A second similar trial (90mg vs 45mg) conducted by the HOVON-SAKK in patients over 60 years[(3)], there was a significantly better CR rate overall (64% vs 54%) of whom 52% and 35% achieved CR with one course. However, this only converted into a survival advantage (38% vs 23%) in patients aged 60 to 65 years (ISRCTN77039377). In a third trial from Korea[(4)] (submitted for publication)(NCT00474006), 383 patients were randomised between a 90mg and 45mg dose in patients under 60 years. Here the remission rate was improved (83% vs 72%) and the survival from 35% to 47%. The survival benefit predominated in the intermediate and poor risk cytogenetic groups. Standard of care for Daunorubicin at a 90mg dose has not been universally accepted, because of an unsubstantiated view that it may not be superior to a 60mg dose. This randomisation has not been studied to date. There has been historical concern that there is a limit to the total dose of Daunorubicin which can be given to avoid an increased risk of cardiac toxicity. In none of these three trials was cardiac toxicity reported, however short term assessments were not routinely carried out. By protocol amendment the AML 17 trial will ask this question in course 1 only.

In consolidation, around 1000 patients were randomised in MRC AML15 between MRC consolidation (MACE/MidAc) and high dose Ara-C (HD-AraC),. Longer follow up will be required

of the randomised patients to establish if one or other approach is superior. However amsacrine is now unavailable so high dose Ara-C will be adopted as the consolidation schedule. There is uncertainty as to how many total courses of chemotherapy are optimal. This clearly has significant importance for the patient's experience and the associated resource use. Both the AML12 and AML15 trials compared four versus five courses and have not found a significant benefit of adding a fifth treatment course. For various reasons, in both trials some patients only received 3 treatment courses. We have conducted a careful retrospective comparison of these patient groups, excluding only patients who could not have received the fourth course of treatment, and, using an analysis adjusted for risk factors, we have evidence that the survival in both good and standard risk patients was comparable whether 3 or 4 courses were given. This is an imperfect comparison, but it justifies a prospective evaluation of this question. Therefore the AML 17 trial will randomise patients after course 2 to one or two more courses of treatment (i.e. a total of three versus four courses). Children will not be randomised in consolidation, but will receive two courses of high dose Ara-C

Interventions Based on Molecular Genetic Characteristics and Risk Score

The genetic and molecular heterogeneity of AML is well known[5]. To date consolidation treatment in our group's trials have been guided by the cytogenetic information, such that patients with adverse cytogenetics, or with inadequate responses to induction chemotherapy, were segregated off to receive an allogeneic stem cell transplant or alternative chemotherapy, while good risk patients were advised not to undergo transplantation.

Recently, we have had concerns that the cytogenetic prognostic score is not sufficiently sensitive to the risk profile of individual patients who have entered complete remission (CR). In part this was based on the lack of a demonstrable survival advantage in any of the three risk groups for transplantation. To that end we have devised a new risk score based on modelling outcomes of patients entering AML10 and AML12 (described in appendix G), which divides patients into three groups with 5-year survivals of 63%, 47% and 24%, and which was prospectively validated using data from AML15[6]. The important effect when compared with the cytogenetic risk definition is to move approximately one sixth of the patients who were previously standard risk into the high risk category and to move about one tenth of previously poor risk patients into the standard risk group. The net effect is that 27% of patients in AML10, & 12 are now defined as high risk compared with 17% previously. When we examine the role of transplantation on the new high risk group, Mantel-Byar analysis shows a significant survival advantage, although in the light of possible selection biases this result needs to be interpreted cautiously. , In a recent review of the accumulating data from our database there is emerging evidence that, whereas to date the role of transplantation in patients with the high risk FLT3+/NPM1c- genotype was uncertain, there is now evidence that this subgroup also benefit from a myeloablative stem cell transplant. The AML17 trial, therefore, compares a novel combination (Daunorubicin/ Clofarabine) with FLAG-Ida, in adults, with a view to proceeding to allogeneic transplantation.

Core Binding Factor Leukaemias

This subgroup is characterised by having either the t(8;21) or inv(16)/t(16;16) balanced chromosomal rearrangements which result in the production of a fusion transcript namely the AML1-ETO and CBFβ-MYHII respectively. These provide potentially useful molecular targets for monitoring minimal residual disease (MRD).

Patients with these lesions have tended to be more sensitive to intensive treatment with a 5-year survival of around 65%. Nevertheless, there is still a significant chance of relapse. Approximately 30 to 35% of cases have a c-KIT mutation which is associated with a significantly increased risk of relapse[7], and, therefore, the addition of a tyrosine kinase inhibitor with anti-KIT activity, such as Dasatinib or PKC412, would be a potential new treatment option for the AML17 trial. However the data from AML15 concerning Mylotarg in this subgroup suggests that they appear to benefit particularly from the administration of Mylotarg in course 1. The recent analysis of AML15 indicates that the survival of Core Binding Factor Leukaemia patients who have received Mylotarg in course 1 is 87% at 4 years. This means that a comparative study of Dasatinib/PKC 412 is not statistically viable in AML17. In the June 2011 amendment of AML17 CBF leukaemias will receive mylotarg (3mg/m^2) on day 1 of course 2.

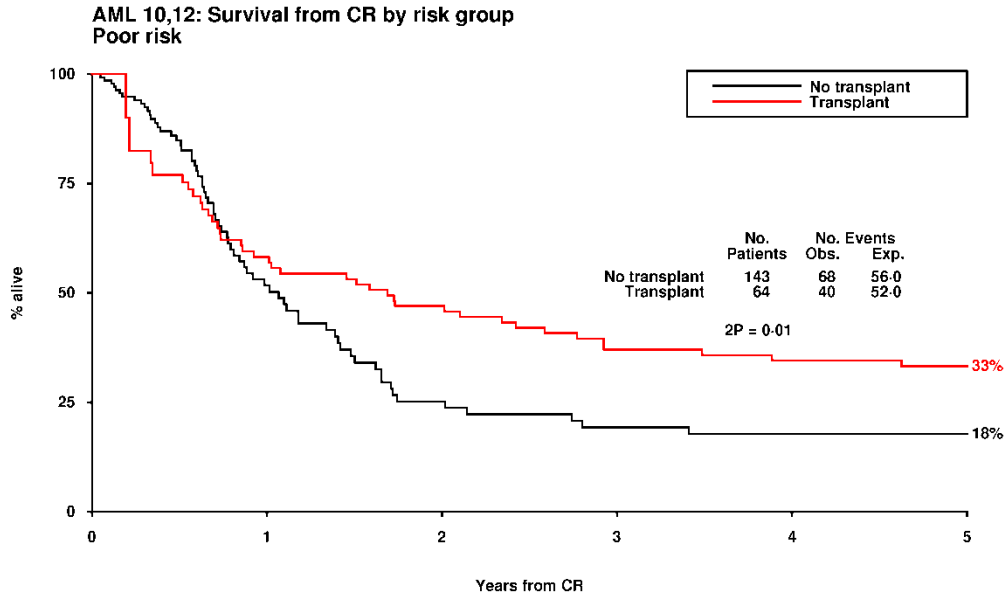
Other Patients
Approximately 80% of all non-APL patients do not have Core Binding Factor Leukaemia. Approximately half of these adult patients will have high risk disease as defined by our new risk score. These patients merit evaluation of novel treatment approaches and/or should be offered stem cell transplantation.

High Risk Score
To date post induction treatment decisions have been substantially based on cytogenetics. Because of concerns that this definition was not sensitive enough at an individual patient level a retrospective analysis was undertaken on patients in the AML10 & 12 trials using a Cox proportional hazards model to provide a number of weighted factors which would be available after treatment course 1 which could provide a risk index for survival from CR. The central concern was whether there were subgroups of patients who were missing out on an effective treatment eg stem cell transplantation. The parameters in the index and the derivation of the score are shown in Appendix D. The cut points for designating patients as good, standard or high risk are to an extent arbitrary, and the index could be refined as new prognostic markers are incorporated e g FLT3 status. FLT3 has been excluded from the score to be used in AML17 but it is now recognised that non-high risk patients with an FLT3+/NPM1c- genotype may also benefit from stem cell transplant..

For the purposes of the AML17 trial patients who have a risk score of greater than 2.667 or the FLT3+/NPM1c- genotype will be designated as high risk with a predicted survival at 5 years of 24% (based on AML10, 12). This will comprise approximately 30% of all patients who enter CR. Retrospective information indicates that this group of patients may have an improved survival following transplant (33% vs 20%), so at the present level of knowledge a stem cell transplant from a sibling or unrelated matched donor may well be indicated. However new treatments need to be found for these patients to improve outcome per se, or to increase the number who can get to transplant. The new generation nucleoside analogue, Clofarabine has proved to be an effective agent as monotherapy, particularly in patients with high risk cytogenetics. As a prelude to the NCRI AML16 trial, we developed the combination of Daunorubicin and Clofarabine. In the pilot study this proved to be both effective and tolerated without additional toxicity. More than 100 patients in AML16 have now received this combination without difficulty. This combination will therefore be compared to the FLAG-Ida schedule which in the AML15 trial appeared to give a trend to superiority over the DA or ADE combination. The aim of this comparison is to increase the number of patients reaching transplant and to reduce the risk of relapse. It is expected that a donor (sibling or unrelated) will be found for most patients.

4.2 Stem Cell Transplantation
There was a modest overall survival advantage of allogeneic SCT in the MRC AML10 Trial, but there was sufficient uncertainty to justify continuing to address the question in standard and high risk patients in the MRC AML12 trial. In the AML12 trial where risk was defined only on cytogenetics and morphological response to course 1, there was no overall survival benefit for transplant in either risk group. Nevertheless the AML15 trial permitted standard risk patients who had a matched sibling donor to go forward to transplantation including a reduced intensity allograft, and for high risk patients a matched unrelated donor was permitted. The comparative results of transplantation in the AML15 trial are not yet available, but both the reduced intensity allograft and transplant from an unrelated donor deliver a similar survival to a matched sibling transplant.

In this large dataset the new risk score was used, in a retrospective analysis, to re-examine the role of transplantation. In patients with an intermediate score there was again no survival benefit from transplantation, however in the newly defined high risk score patients there was a significant survival difference (33% vs 18%, p=0.01). This leads to the conclusion that the risk score can identify a population of patients which benefits from transplantation, and comprises a larger population than defined as high risk by previous criteria. However only 30% of such patients received a transplant



and relapse after transplant is still an important reason for patients failing. The aim of the AML17 trial in this group is to develop novel treatments which are better able to get a patient to transplant, by reducing early relapse, and similarly to reduce the risk of post transplant relapse. The value of transplantation will continue to be assessed by a comparison of patients who were and were not transplanted using the methods described in the statistical plan.

4.3 Acute Promyelocytic Leukaemia (APL)

Acute promyelocytic leukaemia (APL) is a particular subtype of acute myelogenous leukaemia (AML) characterised by consistent clinical, morphologic, and genetic features. These features include the frequent association at diagnosis of a severe hemorrhagic diathesis, a striking sensitivity to anthracyclines, and the response *in vitro* and *in vivo* to differentiation therapy with retinoid derivatives such as all-*trans* retinoic acid (ATRA)[8-10]. At the molecular level, APL blasts are characterised by a specific chromosomal translocation t(15;17) resulting in a hybrid *PML/RARα* gene which is readily identified by reverse-transcriptase polymerase chain reaction (RT-PCR)[11-14]. In addition to its diagnostic relevance, detection of the *PML/RARα* hybrid by sensitive RT-PCR techniques is relevant to assess response to therapy and for the monitoring of minimal residual disease (MRD) during follow-up. In fact, several prospective studies using RT-PCR methods with sensitivity between 10^{-3} and 10^{-4} have shown that the achievement of PCR-negative status is associated with prolonged survival and higher probability of cure, whereas persistence of, or conversion to PCR-positivity in bone marrow after consolidation is invariably associated with subsequent haematologic relapse (reviewed in[15,16]). As a consequence, the achievement of molecular remission is nowadays universally considered as a therapeutic objective in this disease[15]. Furthermore, preliminary evidence from the pre-arsenic era has suggested that early therapy of APL recurrence at time of molecular relapse is advantageous over delaying treatment until haematologic relapse[17,18]. The development of real-time quantitative PCR (RQ-PCR) methods has recently provided an opportunity to better assess at the quantitative level the kinetics of PML/RARa reduction and that of disease relapse in the individual patient. In addition, RQ-PCR permits the identification of poor quality samples which give rise to "false negative" results and facilitates the standardised analysis of samples in the context of multi-centre clinical trials[15,16].

As reported in several large multicentre trials, front-line use of combined ATRA and anthracycline chemotherapy results in long-term remission and potential cure in >80% of newly diagnosed APL patients[17-29]. The Italian multicentre Group GIMEMA reported in 1997 high rates of molecular remission in newly diagnosed and genetically confirmed APL using a simultaneous ATRA plus Idarubicin (AIDA) combination for induction treatment, followed by 3 courses of intensive chemotherapy as consolidation[17]. This protocol, with slight modifications, was subsequently adopted by other groups including the Spanish PETHEMA cooperative group who reported similar antileukaemic efficacy despite omitting Ara-C and other non-intercalating agents from the

original AIDA, with the advantage of sparing toxicity and increasing compliance to treatment[29]. Based on a meta-analysis of the two studies, a stratification score was developed which distinguished patients into high, intermediate and low relapse risk categories according to initial WBC and platelet counts. According to this system, patients with WBC >10x10^9/L had significantly higher relapse risk[28] . Two independent risk-adapted studies were therefore designed by the PETHEMA and GIMEMA in which treatment intensification was planned according to the relapse risk. The results of both studies showed improved outcomes by adding ATRA for consolidation to the original AIDA scheme[29,30]. In particular, the GIMEMA reported significantly improved antileukaemic efficacy and reduction of the relapse rate in the high risk group by administering ATRA in addition to Ara-C[30] In line with these findings, most studies nowadays include risk-adapted approaches in which treatment intensification is based on initial WBC counts[26].

Despite the dramatic progress achieved in front-line therapy of APL with the ATRA/chemotherapy combination, treatment failure still occurs in approximately 15% of patients. Moreover, these regimens are associated with significant toxicity due to severe myelosuppression frequently resulting in life-threatening infections, and with serious, though infrequent complications such as cardiomyopathy and the occurrence of secondary myelodysplastic syndromes and/or acute myeloid leukaemias[31,32].

Several means are available to decrease toxicity in the treatment of newly diagnosed APL, including the availability of less toxic and highly effective agents such as arsenic trioxide (ATO) and the possibility of stringent MRD monitoring offered by RT-PCR.

Following the demonstration of its striking activity in relapsed patients[33-41] arsenic trioxide (ATO) has been licensed in the USA and Europe for the treatment of relapsed and refractory APL. Arsenic derivatives had been used since ancient times in Chinese medicine for the treatment of malignant and inflammatory diseases. The mechanism of action of ATO in APL is complex and not yet known in detail. At a high concentration (0.5 to 2.0 µmol/L) ATO induces apoptosis *in vitro*, through induction of caspases 2 and 3, while at lower concentrations (0.1 to 0.5 µmol/L) it induces partial differentiation of leukaemic promyelocytes through PML/RAR degradation; furthermore, ATO is known to inhibit angiogenesis via down-regulation of vascular endothelial growth factor (VEGF)[41-44]. Concerning its toxicity profile, ATO is usually well tolerated and its use is associated with a series of manageable adverse events including hyperleucocytosis, the APL differentiation syndrome, prolongation of the QT interval, peripheral neuropathy, mild myelosuppression, hyperglycaemia and hypokalaemia[45]. Of these, QT prolongation and, particularly, the so called *APL differentiation syndrome* are the most serious as they can evolve into severe and potentially fatal ventricular arrhythmias (*torsade de points*) or respiratory failure, respectively[46-49]. The APL differentiation syndrome (formerly known as retinoic acid syndrome) results from APL cell activation during the differentiation process. It is characterised by fever, dyspnoea, weight gain, pulmonary infiltrates and pleural or pericardial effusion[49]. Early recognition of this complication and prompt institution of treatment with high-dose steroids is mandatory because it results in resolution of the syndrome in the vast majority of cases.

Severe QT prolongation leading to fatal *torsade de points* has been reported in patients treated with locally formulated arsenic but never with arsenic trioxide used in clinical trials during post-marketing surveillance[45-47]. However, stringent monitoring of serum electrolyte levels (Mg^{2+}, K^+) is recommended during therapy with ATO to minimise the risk of severe arrhythmias, particularly in patients receiving concomitant drugs that induce hypokalemia or hypomagnesemia. Other adverse events mentioned above are usually mild and manageable.

According to original clinical trials reported from China[33,34], ATO was able to induce hematologic CR in >85% patients who relapsed after front-line ATRA. These results were subsequently reproduced in the USA first in a pilot[35], then in an expanded multicentre trial for patients relapsed after ATRA[37]. In the pilot study, hematologic CR was achieved in 91.6% of patients after a median of 33 days of treatment using 10 mg/d as an intravenous infusion[35]. A CR rate of 86% was reported subsequently in the US multicentre study[37]. Significantly, unlike ATRA, ATO as a single agent was able to induce durable molecular remission after two cycles in the majority of patients treated for disease recurrence. Confirmation of the high efficacy of ATO in relapsed APL

was provided successively by several trials conducted worldwide which reported CR rates >70% and 1 to 3-year survival rates in the range of 50-70%[36,38-40].

Arsenic Trioxide in Combination

In addition to trials in which ATO was used a single agent, some studies investigated its efficacy and toxicity profile in combination with other agents including ATRA. Synergism with ATRA and increased anti-leukaemic efficacy in APL was demonstrated in a Chinese randomised study comparing ATO+ATRA vs. either ATO or ATRA used as single agents[65]. No significant additional toxicity was reported in this or in other studies which analysed the effect of ATRA and ATO combination[39,41,424]. Following the experience in relapsed patients and based on the favourable toxicity profile, several investigators have more recently explored the effect of ATO in newly diagnosed APL patients and reported preliminary findings in front-line therapy[41-46]. Results of studies from Shanghai, Houston, India and Iran conducted with ATO as single agent or combined with ATRA for newly diagnosed patients reported CR rates of 86-95%, molecular remission rates after two cycles of 76-100% and survival rates of 86-88%, with significantly better responses being obtained in patients with low and intermediate-risk disease as compared to high-risk patients[41-46]. Although these data need to be strengthened by studies in larger series and with more prolonged observation, they strongly suggest that at least non-high risk APL patients may be cured without chemotherapy[45,47]. However, this possibility has never been tested in a randomised trial which compares this approach with the current standard ATRA plus chemotherapy front-line therapy.

The AML 17 trial will therefore compare the anthracycline approach (AIDA) with the chemotherapy – free ATRA with Arsenic Trioxide combination. The trial is being done in collaboration with the GIMEMA Collaborative Group. The ATO dosing schedule used in AML17, which is easier to administer than the traditional daily dosing schedule, involves a five day loading period (0.3mg/kg/day) followed by twice weekly maintenance (0.25mg/kg). This schedule was initially developed in the treatment of MDS [48] and subsequently studied in relapsed APL in the MRC AML15 Trial where it was found to be effective in inducing molecular remission with no excess toxicity as compared to the standard daily regimen [48,49]. The optimal ATO dosing schedule for APL is not yet firmly established, however, particularly in patients with high body mass index – a population significantly over-represented in APL [50] As an adjunct to the enhanced pharmacovigilance monitoring for APL patients receiving ATO in AML17, plasma arsenic levels measured during induction therapy will be correlated with disease response (morphological and molecular) and treatment-related toxicity (particularly hyperleucocytosis and differentiation syndrome) to better inform future APL arsenic dosing schedules. Children with APL will not enter the AML17 trial.

4.4 Molecular Screening and Minimal Residual Disease Monitoring

At diagnosis all cases will have molecular screening. The particular target lesions concern the definition of favourable genetic abnormalities, i.e. AML1-ETO, CBFβ-MYHII and PML-RARα corresponding to t(8;21)(q22;q22), inv(16)(p13q22)/t(16;16)(p13;q22) and t(15;17)(q22;q12-21) respectively. Previous analyses suggest that approximately 15% of cases with these lesions that were not detected by conventional cytogenetics can be detected molecularly. In several cases this was due to technical failure, but may also be explained by more complex rearrangements. Although the number of cases is small they seem to respond in a similar way to cases defined by cytogenetics, and therefore can be used to define the favourable risk group.

Recent studies have revealed that 20-27% of AML cases are associated with a mutation of the FLT3 gene, which is an independent prognostic factor. All samples will be sent to the two reference labs (at UCL or Cardiff) will be analysed for FLT3 mutations as a quality control for banked nucleic acid and to establish the mutation status to enable patients to enter the inhibitor randomisation. Samples will be routinely screened for other mutations eg NPM1, CEBPalpha and *RAS* which in some studies have been shown to have prognostic value (reviewed [51])and will be necessary in evaluating the planned interventions and may contribute to a revised risk score for future treatment choices.

Minimal Residual Disease Monitoring

The AML17 trial will provide an opportunity to continue to evaluate and validate techniques of minimal residual disease monitoring in AML. Within the AML15 trial much information was collected to define and validate the value of RQ-PCR monitoring in APL where there is strong evidence and opinion that intervention at the point of molecular persistence or recurrence is clinically useful, not least because Arsenic Trioxide or Mylotarg are effective at re-instating molecular negativity. MRD monitoring will be incorporated as an inherent part of treating patients in the arms of the APL comparison.

Less clear-cut information is available for the Core Binding Factor (CBF) leukaemias. Considerable information has been collected in serial monitoring in the AML15 trial and criteria which predict the risk of relapse have been defined. However these criteria have yet to be prospectively validated. In the case of Core Binding Factor leukaemias, it is far from clear whether therapeutic intervention at the time these criteria are met, rather than intervening at the time of relapse, is of benefit. The facility to monitor CBF leukaemias in patients who enter the AML 17 trial will be available on a commercial basis from the reference lab in Manchester for those who wish to have the information. Other molecular lesions e.g. NPM1, may also serve as stable markers of MRD and will, in the early part of the AML 17 trial, be assessed for its prognostic value with respect to utility as a marker for molecular monitoring.

A more universal target, is the leukaemia specific immunophenotype which can be established in over 90% of cases[52]. There are now several reports which suggest that immunophenotypic phenotypes can be characterised in almost all cases of AML and furthermore the persistence of the phenotype can predict relapse[85]. This approach will also be used in AML17 as an extension of the study already initiated in the AML16 trial. In the early part of the AML17 trial this approach will be validated in the four reference labs which have been established for AML16.

Assessment of the Value of Minimal Residual Disease Detection

Although various techniques have the potential to detect residual disease which predicts impending relapse, such monitoring requires considerable organisational and technical resource as well as potential inconvenience and possible anxiety for patients undergoing serial marrow examinations. It is important to establish whether having this clinical information improves the patient's prognosis. Apart from the case of Acute Promyelocytic Leukaemia there is no therapeutic intervention which is of proven value in the treatment of residual disease. An aim of the AML17 trial is to determine the clinical value of knowing the MRD status, when detected by any validated method. The chosen method of doing this, once a validated method has been identified, is to randomise patients to be **monitored or not to be monitored**. Within the AML17 protocol non-APL patients who are monitored, and who are thought by the individual investigator to be at high risk because they have been found to have MRD detected, can enter the high risk component of the trial.

5. RANDOMISATION AVAILABILITY

Investigators are invited to regard this protocol as an evolving investigation into AML treatment. The statistical power calculations differ with each randomisation, so recruitment to some randomisations may be completed before others. This will mean that a randomised component of the trial may close or be changed before completion of the trial as a whole. Similarly, because individual components might require alteration in the light of trial monitoring or other experience this will be a feature of the trial. It is possible that for these or other reasons not all of the randomisations will be available at all times. When such circumstances arise investigators will be informed.

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Section C:

NON – APL AML and HIGH RISK MYELODYSPLASTIC SYNDROME

7. INCLUSION AND EXCLUSION CRITERIA

Instructions relevant to patients who have Acute Promyelocytic Leukaemia are given in Section 19 of the protocol.

7.1 Inclusion Criteria Non APL Leukaemia

Patients are eligible for the AML17 trial if:

- They have one of the forms of acute myeloid leukaemia as defined by the WHO Classification (Appendix A) — this can be any type of *de novo* or secondary AML or high risk Myelodysplastic Syndrome (defined as >10% bone marrow blasts).

- Patients with acute promyelocytic leukaemia (APL) are eligible and should be entered into the randomisations specifically for APL (see Section 19).

- They are considered suitable for intensive chemotherapy.

- They should normally be under the age of 60, but patients over this age are eligible if intensive therapy is considered a suitable option.

- Patients must have liver function tests within twice the upper limit of the normal local range to receive Mylotarg in course 2 for the Core Binding Factor Leukaemia subset.

- Women of child-bearing potential (ie women who are pre-menopausal or not surgically sterile) must use acceptable contraceptive methods (abstinence. Intrauterine device (IUD) and must have a negative pregnancy test within 2 weeks of trial entry. Pregnant or nursing patients are excluded. Sexually active men must also use acceptable contraceptive methods

- They have given written informed consent.

7.2 Exclusion criteria

Patients are not eligible for the AML17 trial if:

- They have previously received cytotoxic chemotherapy for AML. [Hydroxycarbamide, or similar low-dose therapy, to control the white count prior to initiation of intensive therapy is *not* an exclusion.]

- They are in blast transformation of chronic myeloid leukaemia (CML).

- Have a LV ejection fraction of <45% (such patients can be placed on to the D(60)A arm

- They have a concurrent active malignancy.

- They are pregnant or lactating.

- The physician and patient consider that intensive therapy is not an appropriate treatment option. **(Such patients should be considered for current NCRI trial for older or less fit patients).**

8. PROCEDURES FOR ENTRY INTO THE TRIAL AND DATA RECORDING

8.1 Centre Registration

Centres will be sent trial information by way of an invitation to participate in the trial. New regulations on the conduct of clinical trials place obligations on the investigators. In order to be registered as a trial centre, investigators (as an institution) will be asked to confirm: (1) that they have received and have read the MRC guidelines for good clinical practice in clinical trials, (2 that the institution has accepted the responsibilities under the Research Governance Framework, (3) that written consent will be obtained for each patient and a copy retained in the notes, (4) that they agree to report serious unexpected adverse events as set out in Section 21 of this protocol, or in any subsequent guidance, (5) that they agree to participate in random audit carried out by the sponsor or its representative, if requested, (6) that they will report data in a timely fashion using the internet data collection system, (7) that material to be stored for research is obtained using the trial consent documentation.

For administrative reasons, investigators will also be asked to confirm that they will transmit data using the web based data collection system (it is intended to use the electronic data capture system for trial data collection), and to supply details of the location of their immunophenotyping, cytogenetic, genetic, pharmacy, tissue typing and transplant services, and investigator contact e-mail addresses. In addition a limited amount of biochemical data will be collected and, as part of the centre registration process, relevant institutional normal ranges (bilirubin, AST, ALT and LDH) will be recorded.

8.1.1 Patient Recruitment

Patients may be recruited only once a centre is fully registered. Patients should be consented for overall entry into the trial using **Patient Information Sheet 1 and Consent Form 1.** Further consent documents will be used at each randomisation point. **For APL patients see section 19 of the protocol.**

8.2 Randomisation

There are four randomisation points in the trial for which contact must be made with the Wales Cancer Trials Unit (WCTU). Patients fulfilling the criteria for entry into the trial (see Section 7) should be entered into the first randomisation by telephoning the WCTU in Cardiff (tel: 029 2064 5500). Telephone randomisation is available Monday to Friday, 09.00–17.00; internet randomisation is available seven days a week at: **website: http://AML17.cardiff.ac.uk**.

8.2.1 First randomisation

Note: For this randomisation **Patient Information Sheet 2** and **Consent Form 2** should be used. During the course of the trial certain randomisation options may not be available permanently or on a temporary basis. Investigators will be informed in advance so that only relevant information is given to the patient during the consent procedure.

Induction chemotherapy allocation will be given once the required patient details have been supplied. Patients will be allocated to one of the two induction chemotherapy treatment arms.

Arm A: D(90)A in course 1, followed by D(50)A as course 2
or
Arm B: D(60)A in course 1, followed by D(50)A as course

Patients have a 50% chance of receiving each of the treatments.

8.2.2 Information required at first randomisation

- Centre and name of consultant in charge of management
- Patient's name (family name and given name)
- Sex
- Date of birth
- WHO performance status:
  - 0=normal activity
  - 1=restricted activity
  - 2=in bed <50% waking hours
  - 3=in bed >50% waking hours
  - 4=completely disabled.
- Type of disease: *de novo* AML / secondary AML /High Risk MDS
- Whether APL (FAB type M3) or not
- Baseline White Blood Count
- Height
- Weight
- Confirmation that the patient is eligible for the D(90)A arm.
- Confirmation that diagnostic samples of bone marrow and/or blood will be sent to the reference labs for mutation analysis and immunophenotyping.

8.3 Diagnostic material

One objective of the trial is to investigate the therapeutic relevance of new techniques for detecting minimal residual disease and the quality of remission. Diagnostic material is essential for these studies. It is of particular importance to define the cytogenetic abnormalities, and where possible the molecular characteristics, of each patient as this may be relevant to the treatment strategy.

8.3.1 Cytogenetics

Cytogenetics should be carried out locally. The trial office will email the appropriate local lab to indicate that a patient has entered. The lab will be requested to complete the electronic form which will be incorporated into the database and used to inform the patients' risk score. To allow risk stratification, cytogenetic results will be required before randomisation at the end of course 1. Cell pellets should be stored locally.

8.3.2 FLT3/NPM1c- Mutation Status and Molecular Screening

Molecular definition is intended for all patients, initially for characterisation of FLT3 mutation, for identification of cases with cryptic gene rearrangements that reassign patients to the favourable risk group, and for the identification of cases suitable for minimal residual disease monitoring by molecular methods. To enable this to be achieved in the timescale required samples should be sent to either Dr P White in Cardiff or Professor R Gale at University College Hospital using the dispatch methods currently in place. Investigators will be informed of the FLT3/NPM1c- mutation status of patients. Additionally, they will be told of patients in whom molecular screening alters the risk group assignment. All cases of AML will be candidates for MRD assessment using one of a range of molecular or immunophenotypic targets and separate paired marrow and blood samples should be routinely sent following induction to Prof. Grimwade (molecular markers) and to the relevant reference immunophenotyping centre (see Section 15).

FLT3 mutation analysis will be analysed in real time at two reference laboratories (see below). Diagnostic material will also be stored for studies which may include resistance proteins, WT-1 gene expression, DNA microarray and future research studies, for which patient informed consent must be obtained (use **Patient Information Sheet 9 and Consent Form 9).** Molecular screening will be carried out in the reference molecular labs.

It is essential that a sample is sent to a designated laboratory for the identification of patients with a FLT3/NPM1c- mutations. These laboratories will pass samples on to the laboratories designated for MRD monitoring. It is intended that investigators will have the results of FLT3 assays within approximately two weeks of the end of the first course of chemotherapy.

Laboratories for FLT3/NPM1c- Mutation Analysis and Molecular Screening:

Department of Haematology, University College Hospital, London.
(Professor R Gale)

Department of Haematology, University Hospital of Wales, Cardiff
(Ms M Gilkes)

Samples at diagnosis for **molecular analysis** (To be sent to UCL or Cardiff Labs):

4 ml of bone marrow and 30ml of blood in EDTA.

Samples at diagnosis for **cytogenetic analysis (local labs)**:

4 ml of bone marrow in tissue culture medium with preservative-free heparin
30 ml of heparinised blood

Ideally, both marrow and blood should be sent, but if only one is available please send that.

8.3.3 Immunophenotyping

Immunological definition is essential and a diagnostic bone marrow and blood sample should be sent to the designated reference laboratory in order to establish the leukaemia associated aberrant immunophenotype (LAIP) as a target for subsequent MRD monitoring. This involves the use of standardised methodology with an extended range of antibody panels and hence this information cannot be provided by non-designated labs.

Laboratories for Immunophenotypic Characterisation and Monitoring:

Dr Sylvie Freeman
Clinical Immunology
Division of Infection and Immunity
University of Birmingham
P.O. Box 1894
Vincent Drive
Edgbaston
Birmingham, B15 2SZ
Tel: 0121 415 8759
Mob: 07884310528
Fax: 0121 414 3069
Email: s.freeman@bham.ac.uk

Dr Paul White
Department of Haematology
University Hospital of Wales
Heath Park
Cardiff
CF14 4XN
Tel: 029 2074 2370
Fax : 029 2074 5084
Email: whitepc@cardiff.ac.uk

Mr Paul Virgo
Department of Immunology
Southmead Hospital
Westbury on Trym
Bristol
BS10 5NB
Tel: 0117 323 6306
Fax: 0117 323 6062
Email: Paul.Virgo@nbt.nhs.uk

Arrangements will be made to allocate individual sites to one of these labs

8.3.4 Follow-up Material

All patients should be considered eligible for MRD monitoring. At diagnosis **Investigators should send one molecular sample to Cardiff or UCL and the immunophenotyping sample to one of the three reference labs. Sites will be informed of which labs they should associate with.**

The majority of non-APL patients will have a molecular and immunophenotypic marker, potentially allowing more accurate assessment of remission status following Course 1. Results of these analyses may ultimately enhance the risk score and may be used to inform risk stratification later in the trial. Therefore separate **paired marrow and blood samples** should be routinely sent on regeneration following induction to the relevant reference immunophenotyping lab (see above) Prof Grimwade for detection of molecular markers (see section 19.10 for addresses). Clinicians will be informed if their patient is most appropriately monitored by immunophenotyping or a molecular marker and the laboratory to which subsequent MRD samples should be sent. Arrangements for monitoring these patients are set out in Section 15. The labs undertaking initial characterisation and MRD are listed above.

The immunophenotyping labs are **not** providing a diagnostic service under these arrangements.

8.4 Data recording

It is intended to develop data recording for this trial as a web-based system. This is a secure encrypted system accessed by an individual password, and complies with Data Protection Act standards. The system can be accessed on:

http://AML17.cardiff.ac.uk

A user password will be supplied to investigators on receipt of the letters of LREC approval and site specific assessment, and centre registration information (see Section 8.1).

Investigators who do not wish to use the internet system should make arrangements with the trial centre in Cardiff.

Web based data collection forms should be completed as follows:

Notification of Entry (Form A) - return when all the diagnostic data requested are available (but not later than 1 month after entry).

Induction Chemotherapy (Form B) - return when blood counts have recovered after the second induction course, or at prior death (but not later than 2 months after completion of Course 2).

Consolidation Chemotherapy (Form C) - return when blood counts have recovered after the final course of consolidation chemotherapy, or at prior death (but not later than 2 months after the final course).

Transplant (Form D only for patients receiving a transplant) - return when blood counts have recovered post transplant, or at prior death (but not later than 3 months after transplant).

One Year Follow-up (Form E) - return at one year after the end of treatment in 1st CR (i.e. last consolidation chemotherapy or transplant), or at death if the patient dies within 1 year of finishing therapy.

Relapse (Form F) - return at the completion of re-induction (and consolidation) therapy or at death (but not later than 4 months after relapse).

8.5 Health Economics

Basic information on resource usage will be collected in the data forms B to F on all patients. Selected patients will be invited to provide additional information in the form of a patient diary that will be issued to the patient by the investigator. Health economic data collection will be more comprehensive as part of the "monitor vs no monitor" assessment of the clinical value of minimal residual disease at a later stage of the trial.

Once a patient has been randomised, it is very important to have full details of the subsequent course of events, even if allocated therapy has been abandoned. Although clinical decisions remain with the physician (see Section 1, Ethical Considerations), follow-up data must continue to be collected on such patients and trial forms must be filled in, as far as possible, giving details of the therapy actually received and its outcome.

9. INDUCTION CHEMOTHERAPY: Courses 1 and 2

Each induction schedule comprises two courses of allocated chemotherapy. Remission status will be determined after each course. After Course 1, the additional or alternative treatments will be decided as patients are characterised as having Core Binding Factor leukaemia, a high risk score, a FLT3/NPM1c- genotype or none of these. The additional interventions are described in section 11 of the protocol. If a patient is not in complete remission after course 2, they may enter the high risk randomisation (section 11.3).

9.1 D(90)A schedule

Course 1 **D(90)A 3+10**

Cytosine Arabinoside 100 mg/m^2 12-hourly by i.v. push on days 1-10 inclusive (20 doses).

Daunorubicin 90 mg/m^2 daily by slow (1 hour) i.v. infusion on days 1, 3 and 5 (3 doses).

Course 2 **DA 3+8**

Cytosine Arabinoside 100 mg/m^2 12-hourly by i.v. push on days 1-8 inclusive (16 1doses).

Daunorubicin 50 mg/m^2 daily by slow (1 hour) i.v. infusion on days 1, 3 and 5 (3 doses).

9.2 D(60)A schedule

Course 1 **DA 3+10**

Daunorubicin 60 mg/m^2 daily by slow (1 hour) i.v. infusion on days 1, 3 and 5 (3 doses).

Cytosine Arabinoside 100 mg/m^2 12-hourly by i.v. push on days 1-10 inclusive (20 doses).

Course 2 **DA 3+8**

Daunorubicin 50 mg/m^2 daily by slow (1 hour) i.v. infusion on days 1, 3 and 5 (3 doses).

Cytosine Arabinoside 100 mg/m^2 12-hourly by i.v. push on days 1-8 inclusive (16 doses).

NB: Some sites have an established practice if giving Daunorubicin in a longer infusion time. Up to four hours will be permissible..

Seven to 10 days before the commencement of course 2, patients should have a troponin level and an ejection fraction done, to monitor for subclinical cardiac effects. It is intended that Investigators will be offered access to a more detailed companion project monitoring the cardiac function of patients in the Daunorubicin randomisation. This will be co-ordinated by Dr Ann Hunter.

10. ASSESSMENT OF RESPONSE

A bone marrow aspirate to assess remission status should be carried out at 18-21 days after the end of Course 1. If the bone marrow is of adequate cellularity for the assessment of haematopoiesis, the patient's remission status should be ascertained. If the marrow is hypoplastic and assessment of status is not possible, a repeat marrow should be performed after a further 7-10 days and remission status be assessed. The level and date of the maximum level of neutrophil and platelet recovery should be recorded.

In order to achieve a subsidiary aim of the trial (i.e. assessing the relevance of residual cytogenetic or molecular existence of disease in morphological CR) investigators should also request cytogenetic analysis on this sample. In addition a paired marrow and blood sample should be sent to the relevant reference immunophenotyping laboratory and a separate paired sample to Prof Grimwade for molecular assessment of MRD (see Sections 8.3.4, 11.4 and 19.10 for addresses). Favourable risk patients should also be randomised to receive 3mg/m^2 of Mylotarg on day 1 of course 2..

10.1 Definitions of Complete Remission, Partial Remission and Resistant Disease

Complete Remission (CR): The bone marrow is regenerating normal haemopoietic cells and contains <5% blast cells by morphology in an aspirate sample with at least 200 nucleated cells. Additionally there is an absolute neutrophil count of more than 1.0 x 10^9/l and platelet count of at least 100 x 10^9/l

Complete Remission with incomplete recovery (CRi): Fulfilling all criteria for CR except for residual neutropenia (<1000/µL) or thrombocytopenia (<100,000/µL)

Partial Remission (PR): The bone marrow is regenerating normal haemopoietic cells and blast count has reduced by at least half, to a value between 5 and 15% leukaemic cells.

Resistant Disease (RD): The bone marrow shows persistent AML, and patient survives at least 7 days beyond end of course.

Once blood counts have recovered after the second course of induction therapy, the completed "Induction Chemotherapy" form (Section B) should be completed on the web-based data collection system.

11. SUBSEQUENT TREATMENTS

11.1 Subsequent Treatments

After recovery of blood counts and marrow assessment of response additional information will be available. Patients with Core Binding Factor Leukaemias will be identified and sufficient information will be available to calculate the individual patient's risk score. The investigator should ascertain the risk score which is only calculated and provided by the internet data system which will inform investigators if the patient has a high risk score or not , and what treatment options the patient is eligible for. (for high risk see Section 11.3The computer randomisation system will

identify which randomisation patients are eligible to enter (by calculating risk score and identifying patients who are either CBF or have a FLT3+/NPM1c- genotype).

Patients in the high risk group will be randomised in a 2:1 fashion, so that there is a two out of three chance of receiving Daunorubicin/Clofarabine in the high risk option.
Some patients will be considered to be primarily refractory if the marrow blast count has not been reduced by >50% with course 1. These patients can enter the high risk option irrespective of other score parameters.

- For patients eligible for the high risk treatments use **Patient Information Sheet 4 and Consent Form 4**

11.2 CORE BINDING FACTOR LEUKAEMIA

Patients who have Core Binding Factor Leukaemia, t(8;21)/AML1-ETO and inv(16)/t(16;16)/CBFB-MYH11, will continue with the chemotherapy as allocated for course 2. **They will receive mylotarg 3mg/m^2 on day 1 of course 2**. Samples of bone marrow and blood at time of routine assessment for remission may be sent to the molecular laboratory in London who can provide information on the patient's molecular response. Core Binding Factor Leukaemia minimal residual disease monitoring is **not part of the formal assessment of the value of minimal residual disease monitoring** being evaluated in the trial.

Laboratory for Core Binding Factor Leukaemia Molecular Monitoring:

Professor Rosemary Gale
Cancer Institute, Department of Haematology
Paul O'Gorman Building
University College London
72 Huntley Street
London
WC1E 6DD
Tel: 0207 679 6232
Fax: 0207 679 6222
E-mail: rosemary.gale@ucl.ac.uk

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11.3 HIGH RISK SCORE PATIENTS.

After course 1, sufficient information will be available to assign a risk score to individual patients. This is based on age, de novo or secondary disease, cytogenetics, white blood count, sex and response to course 1. This will be allocated by providing the required information to the trial office/internet system. The additional information required, in addition to what was provided in Form A is the cytogenetic result and marrow response after course 1. The cytogenetic result will be automatically entered by the relevant cytogenetic lab, but **the investigator is responsible for entering the marrow response to course 1**.The molecular screening labs in Cardiff and UCH will automatically inform the database of the FLT3/NPM1c- mutation genotype and the system will inform the site if they should be managed as high risk. The internet system will allocate the risk category and indicate what treatment options are available.

N.B. Treatment of patients with Core Binding Factor leukaemias is not influenced by the risk score, and risk score is not validated for such patients.

Adult patients who are defined as high risk will enter the high risk treatment randomisation with the expectation that they should proceed to transplantation. Patients who relapse are eligible for this high risk randomisation with a view of going to transplant. For these patients **Form F** should be used.

The standard arm in this patient group is FLAG-Ida. Patients will be randomised to treatment in a 1:2: manner, so the chance of receiving the standard arm (FLAG-Ida) is 1 in 3, and of receiving the novel combination of Daunorubicin/Clofarabine 2 in 3. For entry into this randomisation **Patient Information Sheet 5 and Consent Form 5** should be used**.** The treatments are:

Up to three courses of FLAG-Ida [standard arm]
Vs
Up to three Courses of Daunorubicin/Clofarabine

FLAG-Ida: Fludarabine 30 mg/m^2 daily by 30-minute i.v infusion on days 2-6 inclusive (5 doses).

Cytosine Arabinoside 2 g/m^2 daily over 4 hours starting 4 hours after Fludarabine on days 2-6 inclusive (5 doses).

G-CSF [Lenograstim 263μg (1 vial)] s.c. daily days 1-7 inclusive (7 doses). (In children the dose will be 5 μg/kg to a maximum of 1 vial given as a 30 minute i.v. infusion.)

Idarubicin 8 mg/m^2 i.v. daily on days 4, 5 and 6 (3 doses). (In children Idarubicin is to be given as a 1 hour infusion.)

Patients should receive up to 3 courses of FLAG-Ida but should proceed to transplant as soon as practical if the option is available.

Daunorubicin/Clofarabine:

Course 1 Daunorubicin 50 mg/m2 daily by i.v. infusion on days 1, 3 and 5 (3 doses).

Clofarabine 20mg/m2 by i.v. infusion over 1 hour daily on days 1 to 5

Course 2 Daunorubicin 50 mg/m^2 daily by i.v. infusion on days 1, 3 and 5 (3 doses).

Clofarabine 20mg/m^2 by i.v. infusion over 1 hour daily on days 1 to 5

Course 3 Daunorubicin 50 mg/m^2 daily by i.v. infusion on days 1, 3 and 5 (3 doses).

Clofarabine 20mg/m^2 by i.v. infusion over 1 hour daily on days 1 to 5.

Patients should receive up to 3 courses of Daunorubicin/Clofarabine but should proceed to transplant as soon as practical if the option is available.

The main side effect of Clofarabine will be myelosuppression, which can be quite variable in duration. It is therefore recommended that if patients whose marrow is cleared of blast cells, but have failed to regenerate neutrophils to $1x10^9$/l by day 32 from the end of treatment (by which time 95% of patients on standard treatment would have regenerated), should have the dose of Clofarabine in course 2 reduced to 15 mg/m^2 daily for 5 days. Patients who enter the Clofarabine randomisation are required to have a serum creatinine within the normal range. Serum creatinine should also be measured on each treatment day and the Clofarabine withheld if the level rises above the upper limit of normal. Patients should be well hydrated during Clofarabine treatment.

In light of new observations blood products for patients receiving Clofarabine should be irradiated

Patients who are at high risk are recommended to receive an **allogeneic transplant** from a matched sibling or volunteer donor either with standard or reduced intensity conditioning**.** However it is recognised that it takes time for the arrangements for transplant to be made, and that there will be a number of patients for who a donor cannot be identified. Therefore patients

should continue with the allocated treatment courses until the transplant can be delivered. It is recommended that patients in whom a reduced intensity allograft is intended should receive a minimum of two high risk treatment courses.

11.6 Progression Through Induction Therapy

FLT3/NPM1c mutation status should be available by the end of the first course of chemotherapy. After recovery from course 1 and assessment of response, the risk score can be provided for individual patients who do not have Core Binding Factor Leukaemia. This will automatically appear on the website when the investigator completes the response information to course 1 (form B1). Those with high risk disease should enter the randomisation detailed in Section 11.3. All except the high risk or refractory patients should receive the second chemotherapy course.

The marrow should be re-assessed at 18-21 days after the end of Course 2 for the assessment of morphological, immunophenotypic and molecular response.

After Course 2, when patients in complete remission have regenerated to 1.0 x 10^9/l neutrophils and 100 x 10^9/l platelets, they are ready for the consolidation randomisation (see Section 12) and commencement of consolidation treatment, i.e. Course 3 (see Section 12)

For patients who are not in complete remission after Course 2 treatment will be deemed to have failed. They may be entered into the high risk arm or withdrawn from the trial and treated at the investigator's discretion. All patients off protocol will still continue to be followed up within AML17.

12. CONSOLIDATION RANDOMISATION

Note: For this randomisation **Patient Information Sheet 7** and **Consent Form 7** should be used.

12.1 Randomisation Options for Adults:

The consolidation randomisation is available to patients who have achieved complete remission within 2 courses and are not candidates for the high risk score randomisation. The randomisation is to **one** (course 3) or **two** (courses 3 and 4) courses of consolidation treatment. The treatment to be used is the MRC consolidation (**high dose Ara-C**)**.** Patients allocated to one course will receive one course of high dose Ara-C and those allocated to two courses will receive high dose Ara-C followed by high dose Ara-C. .

12.2 Timing of Consolidation Randomisation

Statistically, it is preferable for the randomisation to take place as close as possible to the start of consolidation course 1 (Course 3). This will reduce non-compliance, which would have an adverse impact on the power of the trial.

Although randomisation should be carried out as close to Course 3 as possible, it is recommended that the options available are discussed with the patient at an earlier stage, e.g. during induction therapy, in order to ensure that the patient has plenty of time to consider the options and arrive at an informed decision. This should reduce the risk of non-compliance with allocated treatment.

12.3 Information Required at Consolidation Randomisation

Before carrying out the consolidation randomisation please make sure that:

a) The patient is in complete remission

b) The patient's risk group is known.

c) It has been decided whether the patient is willing to be randomised between one or two courses of high dose Ara-C consolidation chemotherapy.

For randomisation: (i) telephone the WCTU (tel: 029 2064 5500) during office hours (09:00 to 17:00 hrs, Monday to Friday); or (ii) use the 24 hour internet randomisation available at: **http://AML17.cardiff.ac.uk**

Treatment allocation will be given once the following patient details have been supplied:

- AML17 trial number (or full name and date of birth).
- Confirmation that the patient has received two courses of induction therapy, and is currently in complete remission.
- The patient is not high risk
- Whether the patient is to be randomised between one and two additional courses (if not the patient should receive 2 more courses).

13. CONSOLIDATION CHEMOTHERAPY: Courses 3 and 4

Consolidation schedule comprises one or two courses of chemotherapy. If allocated to one course, the patient will receive the High Dose Ara-C treatment. Patients who do not wish to be randomised for the consolidation options should be allocated to receive both courses of High Dose Ara-C.

13.1 Consolidation

Course 3 **High Dose Ara-C**

Cytosine Arabinoside 3.0 g/m^2 12-hourly by 4 hour i.v. infusion on days 1, 3 and 5 (6 doses).

Course 4 **High Dose Ara-C**

Cytosine Arabinoside 3.0 g/m^2 12-hourly by 4 hour i.v. infusion on days 1, 3 and 5 (6 doses).

NB. If a patient is randomised to receive one consolidation course they should receive course 3 only

Course 4 should ideally be given once counts have recovered to 1.0x10^9/l neutrophils and 100x10^9/l platelets following Course 3. Delay in count recovery regularly occurs, and problem cases should be discussed with the clinical coordinators.

Once blood counts have recovered after the fourth course of chemotherapy, the "Consolidation" form (Form C) on the web based data collection system should be completed.

14 STEM CELL TRANSPLANTATION

The protocol provides for allogeneic transplantation for all adult patients who have an HLA-matched sibling or volunteer unrelated donor and who are designated to have a **high risk score or a FLT3+/NPM1c- genotype**. **Recent maturing data suggests that some patients who have intermediate risk defined by the risk score who are >40 years will benefit from a Reduced Intensity allograft from a matched sibling donor.** The management system will inform investigators at the time of risk assessment which older standard risk patients should be considered. As soon as a potential donor is identified the transplant centre should be informed. The transplant should be carried out 6-8 weeks after the final course of chemotherapy. The type

of transplant and the transplant protocol will be determined by the transplant centre's usual policy. As a guide based on prior evidence:

1. Patients <35 years should receive a conventional allogeneic transplant with Cyclophosphamide and Total Body Irradiation (8 x 180cGy fractions).
2. Patients 35-40 years can receive a conventional allogeneic transplant or a reduced intensity allograft (RIC) depending on investigator or patient choice.
3. Patients ≥40 years should receive a Reduced Intensity allograft (RIC)..

14.1 Conventional Myelo-Ablative Allogeneic Transplantation

If the patient meets the criteria of the transplant centre, he/she will receive the transplant as soon as is practical. It is expected that they will have received one or two of the allocated treatment courses in the high risk arm. The most widely used myeloablative schedule is Cyclophosphamide and Total Body Irradiation (8 x 180 cGy). The source of stem cells can be bone marrow or peripheral blood. If peripheral blood is used, a dose of at least 4 x 10^6 CD34 cells/kg should be given. Graft versus host prophylaxis will be determined by the transplant centre, but the most widely used is Methotrexate and Cyclosporin. It is required that patients who receive a transplant will provide written consent in line with the transplant centre policy

14.2 Reduced Intensity Allograft Schedules

Patients who will receive a reduced intensity allograft must first receive two courses of the high risk arm and the **mini-allograft as Course 4**. The mini-allograft should only be carried out at centres with experience of this approach and should **not be carried out in centres who do not perform conventional allografts**. The precise protocol to be used in the AML17 trial will be that chosen by the transplant centre, but may be subject to change in light of emerging evidence in the field. . For patients with intermediate risk cytogenetics either the FMC or FBC protocol (see below) is recommended. Emerging evidence suggests that patients with poor risk disease may benefit from a more intensive conditioning regimen and the "FLAMSA-Bu" schedule should be considered (see below). Supervision of this schedule is being undertaken by Prof C Craddock (page 5 for contact details) who should be informed of each patient who is planned for this approach to RIC transplant.

14.2.1 Reduced Intensity Protocols for Patients with Intermediate Risk Disease

a) FBC Protocol:

Fludarabine	30 mg/m^2/day	days –9 to –5 inclusive
Busulphan	4 mg/kg/day	days –3 and –2
Campath 1H	20 mg/day i.v.	days –5 to –1 inclusive

(use of phenytoin and low molecular weight heparin for VOD prophylaxis is optional)

b) Fludara, Melphalan, Campath (UCL) Protocol:

Fludarabine	30 mg/m^2/day	days –7 to –3 inclusive
Melphalan	140 mg/m^2	on day –2
Campath 1H	20 mg/day	days –8 to –4 inclusive

14.2.2 FLAMSA-Bu Schedule for Patients 60 years old with High Risk Disease and under who are fit for transplant:

Eligible patients 60 years or younger with high risk disease and an available matched sibling or 8/8 or 7/8 adult volunteer unrelated donor will undergo transplantation utilising the following regimen:

Day -12 to -9: Intravenous chemotherapy
Day -12 to -9: Fludarabine 30 mg/m²/d
Day -12 to -9: Cytarabine 2 g/m²/d

Day -12 to -9: Amsacrine 100 mg/m²/d
Day -8 to -6: Rest day
Day -5 to –2: Conditioning:
IV Busulphan, total dose 11. 2 mg/kg
IV Fludarabine, total dose 60 mg/m²
Day -5: IV Bu 3.2 mg/kg/day once-daily over 3 hours
Day -4: IV Bu 3.2 mg/kg/day once-daily over 3 hours
Day -3: IV Bu 3.2 mg/kg/day once-daily over 3 hours
Day -2: IV Bu 1.6 mg/kg/d for once-daily over 3 hours
Day -3 to -2: Flu 30 mg/m²/day once daily IV over 1 hour

ATG (Fresenius) on day -3, -2 and -1,
(dose adapted to the donor type. Total dose 10 mg/kg for patients with a sibling donor or Total dose 20 mg/kg for patients with unrelated donors)

Day -1: Initiation of GVH disease prophylaxis with Cyclosporin
Day 0: Initiation of GVH disease prophylaxis with MMF
Day 0: Infusion of sibling or unrelated donor PBSCT or BMT

14.2.3 Patients over 60 years old with High Risk Disease who are fit for transplant:

Eligible patients over 60 years of age with high risk disease with an available matched sibling or 8/8 or 7/8 adult volunteer unrelated donor will undergo transplantation utilising the following regimen:

Day -12 to -9: **Intravenous chemotherapy**
Day -12 to -9: Fludarabine 30 mg/m²/d
Day -12 to -9: Cytarabine 2 g/m²/d
Day -12 to -9: Amsacrine 100 mg/m²/d
Day -8 to -5: Rest day
Day -4 to -2**:** **Conditioning**:
IV Busulphan total doses 8 mg/kg
IV Fludarabine total dose 60 mg/m²
Day -4: IV Bu at 3.2 mg/kg/d in 3 hours
Day -3: IV Bu at 3.2 mg/kg/ in 3 hours
Day -2: IV Bu at 1.6 mg/kg/d in 3 hours
Day -3 to-2: IV Flu 30 mg/m²/d once daily over 1 hour
ATG (Fresenius) on day -3, -2 and -1, (dose adapted to the donor type. **Total dose** 10 mg/kg for patients with a sibling donor **or Total dose** 20 mg/kg for patients with unrelated donors)

Day -1: Initiation of GVH disease prophylaxis with Cyclosporin
Day 0: Initiation of GVH disease prophylaxis with MMF
Day 0: **Infusion of sibling or unrelated donor PBSCT or BMT**

Donor lymphocyte infusions (DLI) to be administered at day +120 post transplant in patients in remission if there is no history of GVHD and immunosuppression has been discontinued. Up to three transfusions will be scheduled using an escalating dose regimen until 100 donor T cell chimerism is achieved. Patients with a related donor will receive an incremental dose schedule of 1 x 106, 5 x 106 and 1 x 107 CD3+ cells/kg administered every 2 months. Patients with an unrelated donor will receive an escalating schedule of 5 x 105, 1 x 106 and 5 x 106 CD3+ cells/kg.

Since patient and donor will require time to be counselled about the transplant option which may be delivered as early as course 3, investigators are encouraged to identify donor availability as soon as possible after diagnosis. Collection of Autologous stem cells is not an inherent part of the AML17 trial but nor is it proscribed. On completion of the transplant the “Transplant” form (Form D) should be completed via the web-based system.

15 ARRANGEMENTS FOR MOLECULAR SCREENING AND MINIMAL RESIDUAL DISEASE MONITORING

15.1 Molecular Screening

All diagnostic material will be collected into the AML cell bank at the UCL (Professor R Gale) or Cardiff (Ms. M Gilkes) Labs, from where it will immediately be analysed for FLT3 status and subsequent molecular screening and also stored for future research. Investigators should note that patients' consent must be given for this donation, and documentation concerning this is included in the main trial consent documentation **(Patient Information Sheet 9 and Consent Form 9).** Molecular screening for the more common mutations is intended on all patients. The reference labs do not require to have a copy of the consent documentation but are working on the assumption that the sending of the sample constitutes consent. **It is the responsibility of the investigator to ensure that when excess sample is sent that consent has been obtained**. If this is not the case the reference labs must be informed to enable the sample to be destroyed.

These labs will undertake the FLT3/NPM1c mutation assessment.

Laboratory Contacts:

Dr Mandy Gilkes
Department of Haematology
Cardiff University School of Medicine
Heath Park
Cardiff
CF14 4XN
Tel: 029 20744522
Fax: 029 2074 4655
gilkes@cardiff.ac.uk

Prof R Gale
Cancer Institute, Department of Haematology
Paul O'Gorman Building
University College London
72 Huntley Street
London
WC1E 6DD
Tel: 0207 679 6232
Fax: 0207 679 6222
Email: rosemary.gale@ucl.ac.uk

15.2 Minimal Residual Disease Monitoring

A major question to be addressed in the AML17 trial is the **clinical value** of disease monitoring using molecular and immunophenotypic approaches. The referred sample to UCL or Cardiff will identify patients who are candidates for molecular monitoring of MRD. In patients consenting to MRD assessment **(using PIS and consent form 8**), paired marrow and blood samples should be sent following each course of chemotherapy. Post-induction samples from all patients should be sent to Professor Grimwade at Guy's Hospital to assess molecular response (in addition a separate paired marrow and blood sample should be sent to the designated immunophenotyping lab, see Sections 8 and 10). After induction, investigators will be informed if a patient has a relevant marker and subsequent samples should be sent to the appropriate lab i.e., to Professor Grimwade in London for other molecular markers, and to the designated immunophenotyping laboratory. Investigators will receive requests for further follow up samples in relevant patients who have given consent to be monitored (PIS and consent form 8).

Investigators should note that PIS 8 has two parts. In Part A patients are being asked to consent to samples being sent to the labs for monitoring tests. In Part B they are being asked to be randomised between being monitored versus not being monitored

Molecular Targets:

Prof David Grimwade
Department of Medical & Molecular Genetics
8th Floor, Tower Wing
Guy's Hospital
London
SE1 9RT
Tel: 0207 188 3699 (lab)
Fax: 0207 188 2585
Email: david.grimwade@genetics.kcl.ac.uk

15.3 Frequency of Molecular Monitoring

On entering AML17, it should be explained to patients that their leukaemia cells are likely to have an appropriate target for minimal residual disease monitoring and will be invited to participate in this aspect of the trial. Investigators will be alerted by the molecular monitoring group (Professor Grimwade/ or Tissue Co-ordinator), should any additional markers be identified and follow-up samples should be sent to the appropriate lab as detailed above (Section 15.2). Since in patients with APL the strategy of treatment reduction is being tested, molecular monitoring is an inherent part of the APL treatment. In the non-APL patients the intention is routinely to monitor blood and bone marrow after each course of chemotherapy, and at regular intervals (3-4 monthly) until 2 years following consolidation, to establish the most appropriate monitoring schedule for any given target. The frequency of monitoring may change during the trial as new information or new markers becomes available. Since it has become clear that persistent MRD or molecular relapse with rising transcript level powerfully predicts relapse, it is important to ensure that the test is completely reliable for that patient. This may result in advice to repeat the test within the interval planned. The issue of sequential testing is incorporated in the **Patient Information Sheet 8 and Consent Sheets 8.** Investigators are reminded to be aware of the consent being requested in PIS 8a and 8B

15.4 Monitoring by Immunophenotyping

Monitoring by immunophenotypic techniques can also predict relapse. A specific phenotype will be defined for each patient by sending a separate sample at diagnosis to the designated reference lab. It is expected that a suitable phenotype will be established for the majority of patients. Investigators will subsequently be asked to send a sample of bone marrow collected at the time of routine disease assessments to the reference labs for follow up monitoring.

Laboratories for Immunophenotypic Characterisation and Monitoring:

Dr Sylvie Freeman
Clinical Immunology
Division of Infection and Immunity
University of Birmingham
P.O. Box 1894
Vincent Drive
Edgbaston
Birmingham, B15 2SZ
Tel: 0121 415 8759
Mob: 07884310528
Fax: 0121 414 3069
Email: s.freeman@bham.ac.uk

Mr Paul Virgo
Department of Immunology
Southmead Hospital
Westbury on Trym
Bristol
BS10 5NB
Tel: 0117 959 6306
Fax: 0117 959 6062
Email: Paul.Virgo@nbt.nhs.uk

Dr Paul White
Department of Haematology
University Hospital of Wales
Heath Park, Cardiff
CF14 4XN
Tel: 029 2074 2370
Fax: 029 2074 5084
Email: whitepc@cardiff.ac.uk

15.5 Assessment of the Clinical Value of Minimal Residual Disease Monitoring

Studies using molecular or immunophenotypic techniques have been shown in a number of retrospective studies to be capable of predicting relapse. During the initial phase of the AML17 trial the techniques established in the reference labs will go through three phases of development. In **phase 1**, techniques to establish the prognostic relationship to relapse will be established for each technique/marker. In **phase 2** this prognostic value will be prospectively validated within a new patient cohort to ensure that it is valid in the context of the AML17 treatment schedules. During these phases the reference labs **will not be feeding back** information to the investigators. This is explicit in the **Patient Consent Form 8.** In **phase 3**, the aim is to establish clinically whether having information that a patient has evidence of residual disease at very low levels is clinically useful. This stage is anticipated to be underway at a later time point in the trial, however work on the first two phases for some of the markers are already well progressed.

In order to meet the objectives of phase 3, patients will be asked to consent to be randomised to be **monitored or not to be monitored**. Patients allocated to the monitored arm will be required to agree to regular blood & marrow tests. All patients who are randomised i.e both the monitored and not monitored groups will be expected to complete a periodic Quality of Life assessment. They will also have information collected about medical interventions.

When initiated, the clinical value of monitoring will be assessed by randomising patients shortly after diagnosis and before the initial response marrow is assessed, to be monitored or not to be monitored. In these circumstances patients will be asked to consent to be randomised to be monitored or not to be monitored. The monitored patients will be required to agree to samples being taken according to the prescribed monitoring schedule, which will be established for each marker in phases 1 and 2. In the monitored cohort the monitoring lab will provide the investigator with each test result. If and when a patient is found to have a significant level of MRD by any informative method (patients may well be being monitored using more than one marker), the investigator will be given this information, and will be asked to confirm that they have received this information. The protocol leaves the question of therapeutic intervention to the discretion of

the investigator and the monitoring labs are forbidden from making any treatment recommendations. If new information emerges during the study that a particular course of action is validated, then the investigator will be informed and advised. They can, for example enter the treatment options provided for high risk score patients.

It is recognised that repeated testing of this nature could cause patients extra anxiety, but it could also provide reassurance. In order to assess this, all patients in this randomisation (monitored or not monitored) will also be asked to participate in a **Quality of Life** assessment at 3, 6 and 12 months after the completion of chemotherapy

It is of the utmost importance that this assessment is carefully explained to patients and consent should be obtained using **Patient Information Sheet 8B and Consent Sheet 8B** when this part of the trial opens**.**

16 MANAGEMENT OF PATIENTS WHO RELAPSE or are REFRACTORY

Patients who are entered into AML17 who are refractory (ie who have had less than a 50% reduction in marrow blasts after course 1 or have not achieved complete remission after the second course of induction chemotherapy) **or subsequently relapse** will be eligible to be randomised to the high risk treatment options i.e. to receive either FLAG-Ida or Daunorubicin/Clofarabine, with a view to progressing to stem cell transplant (section 11.5). For patients with AML recurrence, it is becoming apparent that some "relapses" are genetically distinct from the features detected at original diagnosis and most likely represent therapy-related leukaemias following first-line therapy. This is a recognised cause of treatment failure in ~2% of APL with chemotherapy-based regimens, although its frequency outside APL is unknown. Since this is clinically relevant, **bone marrow or peripheral blood taken to diagnose relapse should also be sent for local cytogenetic analysis**. **In addition samples should be sent to one of the two reference laboratories for evaluation of molecular progression of the disease and to one of the reference immunophenotyping laboratories to assess stability of the immunophenotype**. During the course of the trial newer molecularly targeted treatments are likely to become available and could be provided to patients who have entered the AML17 trial. Investigators will be informed of developments in this area by way of the regular newsletters and should discuss relevant cases with one of the Chief Investigators.

17 SUPPORTIVE CARE

The remission induction and consolidation phases of therapy are intensive and will be associated with a risk of infection and haemorrhage. The care of patients will make stringent demands on supportive care. Some information regarding aspects of supportive care will be collected in the patient record books, since this will be one factor to be taken into account in assessing the schedules.

Participants should have local supportive care protocols. It is considered that policies related to the following aspects should be decided in advance to ensure that treatment-related complications are minimised.

1. Venous access via Hickman-type catheter
2. Control of nausea and vomiting
3. Mouth care
4. Prophylactic gut decontamination (if considered appropriate)
5. Antifungal prophylaxis
6. Response to a significant pyrexia — i.e. two readings of ≥38°C two hours apart, or a single reading ≥39°C
7. Antibiotic treatment of febrile episodes — including antibiotic choice(s) and monitoring, duration of therapy, and the treatment of non-response
8. G-CSF therapy [Lenograstim 263 µg (1 vial) s.c. daily in adults or 5µg/kg i.v. in children] may be given in case of prolonged neutropenia but it is **not** intended that it should be part of routine supportive care

9. Irradiated blood products should be given to patients who receive Clofarabine or Fludarabine or Stem Cell Transplant.

18 CNS TREATMENT FOR ADULTS

The routine administration of treatment to the central nervous system is not recommended for patients with no evidence of CNS disease at diagnosis. Routine CNS investigation at diagnosis for patients without CNS symptoms is not recommended, but this should be considered for APL patients who relapse.

Patients who present with CNS disease may be entered into the trial and be randomised at the same points as patients without obvious CNS involvement. If a patient presents with physical signs suggesting CNS disease, an intrathecal injection of Cytosine Arabinoside (50 mg) should be given when the diagnostic lumbar puncture is performed. If blast cells are identified in the CSF sample, a series of intrathecal injections with Cytosine Arabinoside should be given on 3 days each week until CSF samples are clear. This may need to be modified if the platelet count is very low or coagulation is abnormal. Thereafter, treatment should be repeated at intervals of approximately 2 weeks until consolidation treatment has been completed.

Section D:
ACUTE PROMYELOCYTIC LEUKAEMIA

19 ACUTE PROMYELOCYTIC LEUKAEMIA

19.1 APL

Patients will enter this part of the protocol at diagnosis with de novo or secondary acute promyelocytic leukaemia (APL) recognised morphologically as FAB-M3/M3v. Treatment with ATRA and supportive care for coagulopathy should be started as soon as the diagnosis is suspected, without awaiting results of cytogenetic/FISH/PCR analyses (see BCSH AML guideline (Appendix H). Diagnostic bone marrow (4mls in EDTA) and peripheral blood (30mls in EDTA) from all patients with suspected APL should be sent to Prof David Grimwade at Guy's Hospital (see Section 19.10 for address). Arrangements can be made for rapid confirmation of presence of PML-RARA fusion by PML immunofluorescence testing by contacting Prof David Grimwade, whose laboratory is also responsible for MRD testing. Confirmation of the molecular lesion is important because cases lacking the PML-RARA fusion will be under treated. Patients who enter the APL part of this trial will be monitored for minimal residual disease (MRD) (Section 15) with the aim of identifying patients failing first line therapy who require additional therapy in first CR (see Section 19.9) and will be assessed for Quality of Life and Health Economics impact.

Patients entering this randomisation should use **Patient Information Sheet 3 and Consent Form 3.** Patients who are subsequently allocated to receive chemo-free treatment of ATRA + Arsenic Trioxide are eligible for plasma arsenic level measurement during induction therapy and should go on to use **Patient Information Sheet 3a and Consent Form 3a.**

19.2 Objectives:

The primary objective of the trial is:

- To compare quality of life and toxicity and resource usage of patients receiving the AIDA or the chemo-free treatment of ATRA + Arsenic Trioxide.

The secondary objectives are:

- To compare CR, OS and relapse rates in the two arms
- To compare the kinetics of MRD in the two arms.
- To correlate plasma arsenic levels with disease response and treatment-related toxicities in APL patients allocated to receive arsenic trioxide therapy.

19.3 Entry Criteria:

Inclusion criteria:

- Signed written informed consent
- Clinical diagnosis of APL and subsequently confirmed to have PML-RARA fusion
- Age > 15 years
- WHO performance status 0-2
- Serum total bilirubin $\leq$ 2.0 mg/dL (≤51 umol/L)
- Serum creatinine $\leq$ 3.0 mg/dL ($\leq$ 260 µmol/L)
- Women of child-bearing potential (ie women who are pre-menopausal or not surgically sterile) must use acceptable contraceptive methods (abstinence. Intrauterine device (IUD) and must have a negative pregnancy test within 2 weeks of trial entry. Pregnant or nursing patients are excluded. Sexually active men must also use acceptable contraceptive methods

Exclusion criteria:

- Age < 16
- Active malignancy at time of study entry
- Lack of subsequent diagnostic confirmation of PML-RARA fusion at molecular level
- Significant arrhythmias, ECG abnormalities or neuropathy
- Cardiac contraindications for intensive chemotherapy (L-VEF <50%)
- Uncontrolled, life-threatening infections.
- Severe uncontrolled pulmonary or cardiac disease.
- Pregnant or lactating.

19.4 ARM A: AIDA Treatment

19.4.1 Induction

All-transretinoic acid, 45 mg/m²/day will be administered orally in two equally divided doses and rounded to the nearest 10 mg increment, starting on day 1. ATRA treatment will be continued until haematologic CR and for a maximum of 60 days.

Idarubicin, 12 mg/m² on days 2, 4, 6 and 8 usually by a short (20 minute) intravenous infusion. Idarubicin doses should be brought forward by one day in patients presenting with WBC>10, with first dose given within a few hours of starting ATRA. If marrow appearances are equivocal at around d30, then ATRA is continued. If haematological CR is not achieved by 60 days after the start of induction the patient will go off-study (and would be eligible for "High risk" APL protocols, see Section 20.9).

19.4.2 Consolidation Therapy

After the achievement of haematological CR, patients will receive three successive courses of consolidation chemotherapy and ATRA. Each course will be initiated at haematological recovery from the previous course defined as: ANC >$1.5x10^9$/L and platelets >$100x10^9$/L. In case of toxicity requiring a delay of more than 3 months from the initiation of the previous course, consolidation treatment will be discontinued and management discussed with a trial coordinator.

First consolidation cycle
Idarubicin, 5 mg/m^2/d by short (20 minute) intravenous infusion on days 1, 2, 3, 4.
ATRA, 45 mg/m^2/d, will be administered orally in two equally divided doses and rounded to the nearest 10 mg increment, given from day 1 to day 15.

Second consolidation cycle
Mitoxantrone, 10 mg/m^2/d as 30 minute intravenous infusion on days 1, 2, 3, 4, and 5.
ATRA, 45 mg/m^2/d will be administered orally in two equally divided doses and rounded to the nearest 10 mg increment, given from day 1 to day 15.

Third consolidation cycle
Idarubicin, 12 mg/m^2/d as short (20 minute) intravenous infusion only on day 1.
ATRA, 45 mg/m^2/d will be administered orally in two equally divided doses and rounded to the nearest 10 mg increment, given from day 1 to day 15.

Marrow samples will be collected around day 60 (i.e. following course 1 in patients requiring prolonged ATRA to achieve CR, or following course 2 in those with earlier documentation of CR) and on regeneration following each consolidation course for testing by real-time quantitative PCR (RQ-PCR) by the reference laboratory for assessment of molecular remission. Patients who do not achieve molecular remission by the end of the 3rd consolidation cycle will be considered as molecular resistant and will go off study (eligible for protocols in Section 20.9). Marrow samples collected at earlier time points are used to

measure disease response and provide early indication of patients at risk of failing first line therapy.

19.5 ARM B: ATRA with Arsenic Trioxide

19.5.1 Induction

All-transretinoic acid (ATRA): 45 mg/m²/day will be administered orally in two equally divided doses and rounded to the nearest 10 mg increment, starting on day 1. ATRA treatment will be continued until haematological complete remission (CR, see section 10.1 for definition) or for a maximum of 60 days.

Arsenic Trioxide (**As_2O_3=ATO**): 0.30 mg/kg IV over 2 hours daily for 5 days (days 1-5) in week 1, and thereafter 0.25mg/kg IV over 2 hours twice a week for an additional seven weeks.

If haematologic CR is not achieved by 60 days after start of induction, patient will go off-study.

19.5.2 Consolidation

ATRA: 45 mg/m²/day will be administered orally in two equally divided doses and rounded to the nearest 10 mg increment. Treatment will be administered for 2 weeks on followed by 2 weeks off, for a total of 7 cycles (last cycle administered on weeks 25 - 26).

ATO: 0.30 mg/kg IV over 2 hours daily for 5 days, in week 1. In weeks two to four ATO will be given on 2 days a week in a dose of 0.25mg/kg. This is followed by four weeks with no treatment. This will be repeated for a total of 4 cycles (last cycle administered on weeks 25 - 28).

Marrow samples will be collected at day 60 and after the end of consolidation cycles of ATO, to be tested by RQ-PCR for assessment of molecular remission (see below for definition). Patients who do not achieve molecular remission by the end of the 3rd consolidation cycle will be considered as molecular resistant and will go off study. Marrow and peripheral blood samples collected at earlier time points are used to measure disease response and provide early indication of patients at risk of failing first line therapy.

19.5.3 Patients with High White Counts at Diagnosis

Patients who present with a peripheral white cell count of >10x10^9/l have a higher chance of developing differentiation syndrome if allocated to the ATRA plus Arsenic treatment. These patients should receive Mylotarg 3mg/m^2 on day 1 of treatment and on day 4 if the white count has not fallen below 10x 10^9/l. In addition two doses of Rasburicase can be given on day 1 to prevent tumour lysis. These patients require close clinical and biochemical monitoring for evidence of differentiation syndrome and/or tumour lysis syndrome.

19.5.4 Analysis of Plasma Arsenic Levels during Induction

In a subsidiary study patients consenting to blood sampling for arsenic level assessment (using PIS and consent form 3a) paired peripheral blood samples should be taken at 3 timepoints during ATO induction therapy (see schedule below). At each timepoint blood samples (4ml in EDTA) should be drawn from a peripheral vein (to avoid sampling from any lines used for ATO administration) immediately prior to ATO infusion (trough level) and 1 hour following ATO infusion (peak level).

Sample timepoints

- **Week 1**: taken **between day 3 and day 5** of ATO therapy (one 4ml PB sample pre ATO infusion, one 4ml PB sample 1hr post ATO infusion)
- **Week 2**: taken on **first day of twice-weekly dosing** (one 4ml PB sample pre ATO infusion, one 4ml PB sample 1hr post ATO infusion)
- **Week 3**: taken around **either week 3 ATO dose** according to convenience (one 4ml PB sample pre ATO infusion, one 4ml PB sample 1hr post ATO infusion)

Samples should be clearly labelled with the patient's trial number, initials and date of birth and dated, clearly indicating which is the 'trough' sample, which is the 'peak' sample and the week and day of the ATO induction schedule on which they were taken. A sampling kit with transport pre-labelling will be provided to investigators who have consented to this study. Each pair of samples should be sent by first class post in the packs provided to:

Mr Steve Smith
Trace Metals Laboratory
Department of Biochemistry
University Hospital of Wales
Heath Park
Cardiff CF14 4XW

Where possible, samples should be sent in the first half of the working week. In the event of a delay in sending samples, blood should be kept refrigerated at 4°C. Advice regarding arsenic monitoring will be provided by Dr Steve Knapper (contact details p.6).

19.6 Quality of Life Assessments

All patients in the APL component of the trial will have a Quality of Life assessment at **baseline and 3, 6, 12, and 24 months** from diagnosis. This will take the form of the EORTC QLQC-30 questionnaire plus the Leukaemia Specific Module which has been used in previous MRC AML trials, and the Hospital Anxiety and Depression Scale (HADS). This will be sent to the investigator at the appropriate times, who should arrange for the patient to receive it. The patient will complete the questionnaire and return it (Freepost) to the Trials Office.

19.7 Health Economics Assessment

Information will be collected on all patients as surrogates for resource usage. This will include time to neutrophil and platelet recovery, days in hospital, blood product usage, and days on antibiotics. This will be collected by the data collection system (internet or record books).

In addition selected patients may be asked to complete a patient diary concerning medical events. For patients who are selected the diary will be sent to the investigator.

19.8 Treatment Modification

During induction treatment, ATRA may be temporarily discontinued in the presence of one of the following complications: Differentiation syndrome, pseudotumour cerebri, hepatotoxicity. ATO may be temporarily discontinued in the presence of differentiation syndrome, QT prolongation on ECG or hepatotoxicity; the drug will need to be discontinued permanently in the event of cardiac arrhythmias or severe neurological toxicity. If QTc prolongation is observed ensure that electrolyte level including Mg are corrected

19.8.1 Differentiation (ATRA) Syndrome

This is accurately defined by the presence of: unexplained fever, weight gain, respiratory distress, interstitial pulmonary infiltrates, and pleural or pericardial effusion, with or without hyperleukocytosis. No single sign or symptom itself may be considered diagnostic of the syndrome. However, at the earliest manifestations of suspected Differentiation Syndrome (e.g. unexplained respiratory distress), and prior to development of a fulminant syndrome, the following measures should be immediately undertaken:

- temporary discontinuation of ATRA and ATO treatment.
- prompt initiation of dexamethasone 10 mg i.v. 12-hourly until disappearance of symptoms and signs, and for a minimum of 3 days.
- frusemide when clinically required.

In patients treated with ATRA+ATO for induction, it is anticipated that induction of hyperleucocytosis (WBC >10) associated with induction of differentiation may occur in a proportion of patients. This does not require any change in therapy, beyond careful vigilance for development of differentiation syndrome.

19.8.2 Pseudotumour Cerebri

This is defined as presence of: severe headaches with nausea, vomiting, and visual disorders. In this case, generally developing in patients under 20 years of age, it is often necessary to discontinue ATRA treatment temporarily and to administer opiates.

19.8.3 Hepatotoxicity

This is defined as: an increase in serum bilirubin, AST/ALT, or alkaline phosphatase >5 times the normal upper level. This requires a temporary suspension of the ATRA. If hepatoxicity persists following discontinuation of ATRA, ATO should be temporarily discontinued in patients assigned to ATRA+ATO. The Idarubicin doses should not be changed on the AIDA arm.

As soon as the symptoms and the patient's clinical condition improves, treatment with ATRA will be resumed at 50% of the previous dose during the first 4 days after the disappearance of retinoic acid syndrome, amelioration of pseudotumour cerebri or when serum bilirubin, AST/ALT or alkaline phosphatase are reduced to <4 times the normal upper level. Thereafter, in absence of worsening of the previous toxicity, ATRA should be resumed at full dosage.

In case of reappearance of signs and symptoms of ATRA toxicity, the drug must be discontinued indefinitely during induction therapy.

19.9 Treatment of High Risk APL (relapse, molecular relapse, or persistent MRD positivity)

Initial treatment of APL may fail, in which case patients will either relapse or be at high risk of relapse. In this study adult patients who relapse, or who are deemed to be at high risk of relapse based on molecular data, should be treated with Arsenic Trioxide or Gemtuzumab Ozogamicin (Mylotarg) and stem cell transplant options discussed with a trial co-ordinator. It is anticipated that during the course of the trial molecular criteria will become more precise as a result of the monitoring data (Section 19.10). As this evidence emerges investigators will be informed of patients who are considered high risk and who should be offered further treatment.

Note: At relapse, CNS should be checked for occult disease.

19.10 Molecular Diagnosis and Monitoring

Minimal residual disease monitoring is well established in APL and is an integral part of the treatment of patients in the AML17 trial. This is particularly important since overall the treatment strategy aims at a de-intensification of treatment, with omission of maintenance from the original AIDA schedule and removal of chemotherapy from the ATO+ATRA arm. Patients should be advised, as is stated in the information and consent form that this is the case and it will involve marrow samples (2-3mls first pull into EDTA) being taken after each consolidation course and then at 3-monthly intervals for 36 months, and that for technical or confirmatory reasons extra tests may be recommended.

Samples should be sent to Professor David Grimwade **at the address below.** Medical supervision of molecular diagnostics and MRD monitoring and advice will be undertaken by Professor David Grimwade:

Dr Yvonne Morgan
Molecular Oncology Diagnostics Unit,
Clinical Laboratory Services
4th Floor
Southwark Wing,
Guys Hospital,
Great Maze Pond,
London SE1 9RT
Tel: 0207 188 7188 (Etn 51060)
Mobile: 0780 329 3372
Email: david.grimwade@nhs.net

19.11 Supportive Care for APL Patients

APL treatment has some particular requirements with respect to supportive care which are described in the **BCSH Guideline (www.bcsguidelines.com)** an extract of which is shown in **Appendix H.**

Section E:
STATISTICS & TRIAL GOVERNANCE

20 STATISTICAL CONSIDERATIONS

20.1 Patient numbers

Over the last 40 years, 5-year survival of younger patients in MRC AML trials has gone from 0% in AML4 to about 45% in AML12 and AML15. This dramatic improvement, which has changed AML from an invariably fatal disease into a potentially curable one, has been achieved not by any single major advance but through a series of small, but nonetheless important, increases in survival over a number of trials. However, there is great heterogeneity of outcome between different types of patient, and this is reflected in the design of the AML17 trial.

There are approximately 700 cases of AML diagnosed each year in patients under the age of 60 in the British Isles, of whom about 15% have the APL sub-type. It is hoped that the majority of suitable patients will be entered into the trial. Indeed, recruitment to AML15 has typically run at around 650 patients per annum, so that over the course of the recruitment period it should be possible to randomise at least 300 APL patients and 2700 non-APL patients.

For the APL randomisation, it is anticipated that a similar number of patients will be recruited from both the UK and GIMEMA and East German networks, giving a total of 600 patients for analysis. Outcomes are typically very good for this group of patients. It is anticipated that survival will be similar for these two treatment arms, however with 600 patients there will be over 80% power to establish equivalence based on a 7.5% difference in survival. However survival will remain a secondary objective in this part of the trial. Instead, the study will, as in AML15, use quality of life and resource usage as primary endpoints. With 600 patients it is possible to detect, with 80% power at $p<0.05$, a small-to-moderate difference of 0.25 standard deviations, and there is 90% power to detect a standardised difference of 0.27. Interim data from AML15 indicate that the standard deviation of the EORTC-QLQ30 global score is approximately 20 points, indicating that the trial will be powered to detect a 5 to 6 point difference in quality of life. The use of repeated measures modelling for the quality of life outcomes should increase the power to detect smaller differences.

Following the closure of the original induction randomisation, the remaining recruitment period should see another 3.5 years of recruitment, equating to a further 1700 non-APL patients recruited to the study based on current recruitment rates. Taking a baseline survival of 45% at 5 years, as for the current mylotarg randomisation, there will be more than 90% power to detect a hazard ratio of 0.80 (i.e. a 20% proportional reduction in mortality, equating to an 8% absolute difference in 5-year survival). Such a comparison would require a total of just over 800 deaths to provide sufficient power. As before, any interactions between treatments or between treatment and baseline covariates will be tested here using standard techniques

Just under 30% of patients starting their second course will be poor risk (i.e. approximately 700 patients). In the first instance, patients will be randomised between DClo v FLAG-Ida in a 2:1 randomisation. In the first half of the trial, therefore, one might expect a recruitment of around 300 patients. Five-year survival of this group of patients is currently 30%, so recruiting 315 patients in the first half of the trial will give 80% power to detect a clinically meaningful 15% improvement in survival from 30% to 45%. If the randomisation ran for the entirety of the trial, recruiting approaximately 480 patients, then there would be 90% power to detect a 15% difference in 3-year survival difference from 40% to 55% with 234 deaths. Should other new therapies suitable for this group of patients become available during the course of the trial these can then be introduced by protocol modification. At present only around 40% of poor risk patients entering CR receive a transplant; with 360 patients randomised there will be approximately 80% power to

detect an improvement from 40% to 55% in the numbers being transplanted, and 90% power to detect the same improvement with 480 patients.

Of the patients who are not considered to be “poor risk”, at least 80% should enter CR and therefore be eligible for the 3 vs 4 course randomisation. This equates to around 1600 patients. Even if only two-thirds of such patients are randomised, there will be 1000 patients for the 3v4 course randomisation. This will be powered as a non-inferiority trial with a one-sided significance level of p=0.025. With 90% power there will be sufficient power to detect or rule out inferiority in 5 year survival from CR (the primary endpoint) of 65% versus 55%.

To investigate the effect of MRD monitoring, the project will run in several stages. Initially, the best cut-offs will be identified; because a number of different time-points will be investigated, all analyses will be performed at a 1% significance level. Around 80% of patients enter CR, and it is anticipated that about 50% of these will achieve MRD negativity. Approximately half of all patients will relapse in the first 3 years. With a total of 360 patients entering CR (i.e. 450 patients with suitable markers), there will be 90% power to detect a difference between groups of 20% (40% versus 60% relapsing). Thus, it is planned that the first stage of the process will run for the first 600 patients, to allow for 20% of patients not having suitable markers.

Sequential monitoring has proved feasible in about 50% of patients; and these patients have 5 year survival of approximately 55%. There should be approximately 600 patients eligible to be randomised during the course of the trial in a 2:1 ratio (monitor vs no monitor). This is sufficient with 80% power to detect an increase from 55% survival to 67%, with 198 deaths overall.

20.2 Data analysis

Interim analyses of the main endpoints will be supplied periodically, in strict confidence, to the MRC Leukaemia Data Monitoring and Ethics Committee (DMEC). In the light of these interim analyses, the DMEC will advise the chairman of the Trial Steering Committee and Chief Investigator if, in their view, one or more of the randomised comparisons in the trial have provided proof beyond reasonable doubt* that for all, or for some, types of patient one treatment is clearly indicated or clearly contraindicated.

The main analyses will be based on the intention to treat - i.e. **all** patients believed to be eligible at the time of randomisation will be included in the analysis, irrespective of protocol compliance, early death, etc. Comparisons of randomised treatments will be made using the log-rank test for time to event outcomes; and the Mantel-Haenszel test for dichotomous outcomes. Resource usage data will be compared using Wilcoxon rank-sum tests or t-tests as appropriate. The primary outcome is survival for all randomisations except the APL randomisation (see below), The APL randomisation has quality of life as primary outcome, which will be analysed using Multilevel Models Repeated Measures techniques adjusted for baseline. The randomisations will be stratified by age (0-15, 16-29, 30-39, 40-49, 50-59, 60+), performance status, and type of disease (*de novo*/secondary AML). Consolidation randomisations will also be stratified by initial allocation and by risk group. All stratification variables used at randomisation will be used in analyses: in addition any analyses of treatment effectiveness will be stratified by cytogenetic risk group, and any relevant molecular markers (including, but not limited to FLT3-ITD, FLT-3 TKD and NPM1 status). All stratified analyses will assume that there may be some **quantitative** differences in the size of any treatment effects in these different strata, but that there is unlikely to be any **qualitative** difference (i.e. harm in one group, benefit in another). Interactions will be tested using standard techniques developed by the Early Breast Cancer Trialists Collaborative

* Appropriate criteria of proof beyond reasonable doubt cannot be specified precisely, but a difference of at least three standard deviations in an interim analysis of a major endpoint may be needed to justify halting, or modifying, a randomisation prematurely. If this criterion were to be adopted, it would have the practical advantage that the exact number of interim analyses would be of little importance, and so no precise schedule is proposed.

Group; simultaneous adjustment for more than one stratification variable will be by means of logistic or Cox regression analysis.

21. TRIAL GOVERNANCE AND ADVERSE EVENT REPORTING

Cardiff University is the Trial Sponsor and has delegated certain responsibilities to participating sites. These define the responsibilities of the Principal Investigator on each site. The trial will be conducted in compliance with the MRC Guidelines for Good Clinical Practice in Clinical Trials copies of which are available from the MRC or the Trial Office. In the use of unlicensed drugs the trial is conducted under a CTA issued by the MHRA which requires the investigators to report Serious Adverse Events (SAEs) as described in below. The trial will be monitored by an independent Data Monitoring and Ethics Committee.

21.1 Adverse Event Reporting

Principal Investigators at each participating institution have an obligation to report relevant Serious Adverse Events (SAEs) which occur in this trial to the Haematology Clinical Trial Office in a timely manner. It is recognised that adverse events which may be life-threatening are a normal consequence of acute myeloid leukaemia or its effective treatment, and many clinical changes in the patient's condition are expected. Adverse events as defined should be reported up to 1 month from the conclusion of all protocol defined therapy.

21.1.1 Definitions:

For the purpose of this trial a **Serious Adverse Event** is defined as:

- Development of a non-haematological toxicity of grade 3 as defined in the NCI Common Toxicity Criteria Version 3**, which does not resolve to grade 2 or less within 7 days

- Development of any grade 4 non-haematological toxicity (excluding alopecia) (this includes any life threatening event)

- Development of neutropenia (<1.0 x 109/L) or thrombocytopenia (<50 x 109/L) for longer than 42 days after the end of chemotherapy in the absence of significant disease in the bone marrow (>5% blasts)

- Events which are not related to AML or its treatment which result in hospitalisation or prolongation of hospitalisation.

- Any event which results in persistent or significant disability or incapacity

- Any event which results in a congenital abnormality or birth defect

- Death from any cause including persistent or progressive disease

- Other Medically important event*

The following **do not** require to be reported as **SAEs**:

- Grade 4 haematological toxicity is an expected consequence of effective treatment, and is only required to be reported if it fulfils the criteria as defined above

** A copy of the NCI Common Toxicity Criteria is available from the Trial Office and on the website.

- Patients may present with some pre-existing toxicities which meet the criteria set out above, but it is only the development of these toxicities after entering the trial which should be reported

- Neutropenic fever is an expected severe adverse event which may occur as a result of the disease or the treatment. This or its consequences do not have to be reported unless fulfilling the criteria set out above

* Note: other events that may not result in death are not life threatening, or do not require hospitalisation may be considered as a serious adverse experience when, based upon appropriate medical judgement, the event may jeopardise the patient and may require medical or surgical intervention to prevent one of the outcomes listed above (excluding new cancers or result of overdose).

Serious Adverse Reactions (SARs): SARs are **SAEs** which are considered by the investigator to be possibly/probably/definitely related to the trial treatment.

Suspected Unexpected Serious Adverse Reactions (SUSAR): These are **SARs** which are classified as 'unexpected' i.e. an adverse reaction the nature and severity of which is not consistent with the information about the medicinal product in question, set out in the summary of product characteristics (SpC) for that product. The current SpC can be accessed at www.emc.medicines.org.uk and a copy will be kept in each centre's site file.

Please refer to the Individual Investigator Brochures (IB) or Summary of Product Characteristics (SpC) for a list of expected adverse reactions

21.1.2 Causality
Investigators will be asked to record their opinion as to whether the SAE as defined above was related to the study medication. This will be further reviewed by the Trial Management Group.

21.1.3 Collection of Data
Preliminary discussion of the event may take place with a clinical co-ordinator. SAEs should be recorded on the Adverse Event Form which is available on the trial website, and sent to the Trial Office in Cardiff. SAE Fax Number 029 2074 2289

21.1.4 Time of Report
Any death that is clearly **not** due to, or associated with**,** persistent or progressive disease should be reported to the trial office within 24 hours.

21.1.5 Enhanced Pharmaco-Vigilance
For patients allocated to IMPs (Investigational Medicinal Products) there will be enhanced vigilance. This will involve a telephone enquiry from the Cardiff Trial Office weekly for up to 4 weeks after the administration of the IMP. The pharmaco-vigilance officer or her nominee will seek information of any treatment adverse effects or compliance difficulties.

21.1.6 Reporting to the Regulatory Authorities
The Chief Investigator or his nominee will review and record all SAEs. He will be responsible for reporting the events to the MHRA, COREC, and the Trial Steering Committee in the appropriate timelines. He will also report, where relevant, to the provider of the IMP (Investigational Medicinal Product) and produce periodic reports for all investigators to forward to the LREC.

APPENDIX A: WHO Histological Classification of Acute Myeloid Leukaemias

	ICD Code
Acute myeloid leukaemia with recurrent genetic abnormalities	
Acute myeloid leukaemia with t(8;21)(q22;q22); (AML1(CBFα)/ETO)	9896/3
Acute myeloid leukaemia with abnormal bone marrow eosinophils Inv(16)(p13q22) or t(16;16)(p13;q22); (CBFß/MYHII)	9871/3
Acute Promyelocytic leukaemia (AML with t(15;17)(q22;q12-21),	9866/3
(PML/RARα) and variants.	
Acute myeloid leukaemia with 11q23 (MLL) abnormalities	9897/3
Acute myeloid leukaemia with multilineage dysplasia	9895/3
Acute myeloid leukaemia and myelodysplastic syndromes, therapy-related	9920/3
Acute myeloid leukaemia not otherwise categorised	
Acute myeloid leukaemia minimally differentiated	9872/3
Acute myeloid leukaemia without maturation	9873/3
Acute myeloid leukaemia with maturation	9874/3
Acute myelomonocytic leukaemia	9867/3
Acute monoblastic and monocytic leukaemia	9891/3
Acute erythroid leukaemias	9840/3
Acute megakaryoblastic leukaemia	9910/3
Acute basophilic leukaemia	9870/3
Acute panmyelosis with myelofibrosis	9931/3
Myeloid sarcoma	9930/3
Acute leukaemia of ambiguous lineage	9805/3
Undifferentiated acute leukaemia	9801/3
Bilineal acute leukaemia	9805/3
Biphenotypic acute leukaemia	9805/3

APPENDIX B: Preparation, Administration and Toxicity of Drugs used in AML17

Daunorubicin (Cerubidin™ - May & Baker Ltd)
Daunorubicin is presented as a red powder in glass vials containing 20 mg with mannitol as a stabilising agent. The drug is reconstituted in sodium chloride 0.9% or water for injection. Following reconstitution, further dilution with sodium chloride 0.9% to a concentration of 1mg/ml is recommended. The resultant solution is given by a one hour infusion into a swiftly flowing drip. In children Daunorubicin should be administered as a 1 hour infusion. For hepatic dysfunction with a bilirubin 20-50 µmol/L reduce by 25%; for bilirubin >50 µmol/L reduce by 50%. In patients with renal impairment dose reduction should take place: Serum Creatinine 105-265µmol/L, reduce dose by 25%; Serum Creatinine >265µmol/L reduce dose by 50%.

Side effects include nausea, alopecia, chronic and acute cardiac failure and dysrrhythmias. Subcutaneous extravasation may cause severe tissue necrosis.

Centres may have an established practice of administering Daunorubcin over a longer period (up to 4 hours) than written in the protocol. This is permissible.

Cytosine Arabinoside - Ara-C, Cytarabine (Cytosar™ – Pharmacia & Upjohn)
Cytosar is available as a freeze dried powder containing 100 mg or 500 mg of Cytosine Arabinoside in a rubber capped vial. The diluent provided in the drug pack is water for injection containing 0.9% w/v benzyl-alcohol. Following reconstitution with the manufacturer's diluent the solution contains 20 mg/ml of Cytosine Arabinoside. At this concentration it is suitable for direct intravenous bolus injection into a central or peripheral line.

Cytarabine solution is also available in a non-proprietary form from Pharmacia & Upjohn and Faulding DBL. These are presented as 20mg/ml and 100mg/ml solutions of cytarabine in a variety of vial sizes. It is recommended that before administration by intravenous bolus injection the hypertonic 100mg/ml solution is further diluted in water for injection, sodium chloride, 0.9%, or glucose, 5% solution, to produce a solution of 20mg/ml concentration.
In patients with impaired hepatic function (bilirubin >34µmol/L) the dose should be reduced by 50%. No reductions are necessary for renal impairment.

Side effects at the doses prescribed for remission induction include nausea, diarrhoea, oral ulceration and hepatic dysfunction. A Cytosine syndrome has also been described. It is characterised by fever, myalgia, bone pain, occasional chest pains, maculopapular rash, conjunctivitis and malaise. It usually occurs 6-12 hours following administration, and is more common with higher doses.

Gemtuzumab Ozogamicin — MylotargTM (Pfizer) Research
MYLOTARG (gemtuzumab ozogamicin for Injection) is supplied as an amber glass vial containing 5mg of MYLOTARG lyophilised powder. This vial should be refrigerated (2-8ºC).

Preparation
The drug product is light sensitive and must be protected from direct and indirect sunlight and unshielded fluorescent light during the preparation and administration of the infusion. **All preparation should take place in a biologic safety hood with the fluorescent light off**. Reconstitute the contents of each vial with 5ml Water for Injection. Gently swirl each vial. Each vial should be inspected to ensure dissolution and for particulates. (The final concentration of drug in the vial is 1mg/ml). This solution may be stored refrigerated (2-8° C) and protected from light for up to 8 hours. (Reconstituted vials of drug should not be frozen.)

Before administration, withdraw the desired volume from each vial and inject into a 100ml IV bag of 0.9% Sodium Chloride Injection. Place the 100ml IV bag into an UV protectant bag. The following time intervals for reconstitution, dilution, and administration should be followed for

storage of the reconstituted solution: reconstitution ≤ 2 hours; dilution ≤ 16 hours at room temperature: administration; 2 hour infusion; i.e. **a total of a maximum of 20 hours.**

Administration
DO NOT ADMINISTER AS AN INTRAVENOUS PUSH OR BOLUS

Once the reconstituted Mylotarg™ is diluted in 100ml sodium chloride 0.9% for infusion, the resulting solution should be infused over 2 hours. Prior to infusion inspect visually for particulate matter and discoloration.

A separate IV line equipped with a low protein-binding 1.2-micron terminal filter must be used for administration of the drug (see note). MYLOTARG may be given peripherally or through a central line.

Premedication, consisting of an antihistamine (such as chlorpheniramine), should be given before each infusion to reduce the incidence of a post-infusion symptom complex. Methylprednisolone 50mg may also be used. Vital signs should be monitored during infusion and for four hours following infusion.

Instructions for Use, Handling and for Disposal
Mylotarg should be inspected visually for particulate matter and discoloration, once in the transfer syringe. Additionally, the diluted admixture solution should be inspected visually for particulate matter and discoloration. Protect from light and use an UV protective bag over the IV bag during infusion. Vials are for single use. Aseptic technique must be strictly observed throughout the handling of Mylotarg since no bacteriostatic agent or preservative is present. Institutional procedures for handling and disposal of cytotoxic drugs should be used.

Cautions

Hepatic Insufficiency: Patients with hepatic impairment will not be included in the clinical studies if the abnormality is greater than twice the local normal range..

Renal Insufficiency: Patients with renal impairment will not be included in the clinical studies.

Note: The recommended in-line filter for Mylotarg administration is a 1.2-micron polyether sulfone (PES) filter, e.g. “intrapurlipid” (Braun product number 4099702). If that filter is not available, the following filters may be used: 0.22 micron PES, 0.20 micron cellulose acetate, 0.8 to 1.2 micron cellulose acetate/cellulose nitrate (mixed ester), or 1.2 micron acrylic copolymer.

Adverse Events: The most important serious adverse event may be hepatotoxicity or myelosuppression. These should be reported to the Chief Investigator as described in Section 22. Other events which have been reported in at least 10% of recipients of single agent Mylotarg include fever, nausea, chills, vomiting, headache, dyspnoea, hypotension, and hyperglycaemia. It is not necessary to report these events.

Fludarabine (Fludara™ - Schering-Plough)
Fludara contains 50mg fludarabine phosphate per vial. It should be given by slow intravenous infusion after dilution in 2ml water for injection.

For hepatic dysfunction no dose change is required. For renal impairment a Cr Cl of 30 – 70 ml/min requires a dose reduction of 50%; greater impairment excludes the administration.
The most frequent adverse event is myelosuppression. Patients less commonly suffer nausea, vomiting or alopecia. Fludarabine is a prolonged inhibitor of T-cells and has been associated with the development of transfusional GVHD and pneumocystis pneumonia. Rarely fludarabine has caused CNS side-effects with agitation, confusion and visual disturbance.

Idarubicin (Zavedos™ - Pharmacia)
Idarubicin is available as a sterile pyrogen-free, orange-red freeze-dried powder, in vials containing 5 or 10 mg of idarubicin hydrochloride with 50 or 100 mg of lactose respectively.

For administration the vial contents should be dissolved in water for injection to give a solution of 1mg/ml. The resultant solution should be administered intravenously into the side arm of a freely running intravenous infusion of 0.9% sodium chloride over 5 to 10 minutes. In children Idarubicin should be given as a 1 hour infusion.

In cases with hepatic dysfunction dose reduction is required: bilirubin 21 – 34umol/L reduce the dose by 50%.Greater rises contraindicate the administration. For renal impairment with a serum creatinine 100 – 175umol/L reduce the dose to 50%.Administration at higher creatinine levels is a clinical decision.

Side-effects: The major side effect is myelosuppression. Cardiac toxicity may occur, manifested by cardiac failure, arrhythmias or cardiomyopathies, either during therapy or several weeks later. The cumulative dose associated with cardiotoxicity is not known, but it is believed that a total dose of 60-80 mg/m^2, which is considerably higher than that used in AML15, is not problematic. Idarubicin may cause a red discoloration of the urine for 1-2 days after administration. Reversible alopecia will occur, and some nausea or vomiting and oral mucositis should be expected. Elevation of liver enzymes and bilirubin may occur in a minority of patients. **Idarubicin should not be given to patients with severe renal or liver impairment**

G-CSF- Human Granulocyte Colony-Stimulating Factor: (Granocyte^{TM} -rHuG-CSF, lenograstim - Chugai Pharma UK Limited)

Granocyte^{TM},lenograstim, rHuG-CSF -Chugai Pharma UK Ltd- available in 2 presentations and the stated G-CSF in the AML17 protocol:-

Presentations:
Granocyte 34, 33.6MIU Lenograstim in 263ug vials supplied in packs of 5 with 5 x 1ml water for injection in pre-filled syringes
Granocyte 13, 13.4MIU Lenograstim in 105ug vials supplied in packs of 5 with 5 x 1ml water for injection in pre-filled syringes.

Dose:
Dose for autologous transplantation or for chemotherapy-induced neutropenia – 150ug/m^2 (or as per local protocol)
Dose for allogeneic transplantation 10ug/kg (or as per local protocol)
Or as per AML17 protocol:

In autologous PBPC mobilisation	1 vial/day sc
In allogeneic PBPC mobilisation	10 µg/kg/day for 4-6 days
Post BMT	1 vial/day sc
Chemotherapy induced neutropenia:	1 vial/day sc days 1-7
In FLAG regimen:	1 vial/day sc days 1-7

Collection of Autologous and Allogeneic Stem Cells:

Autologous stem cell collection:
Mobilisation should be attempted using G-CSF, Lenograstim,150µg/m^2/day

Allogeneic stem cell collection:
Mobilisation should be attempted using G-CSF, Lenograstim,10µg/kg/day

Bone pain and injection site reaction have been associated with Granocyte treatment in some patients.

Granocyte is available at contract prices from AAH Hospital Service in the UK

Cyclophosphamide (Endoxana^{TM} – ASTA Medica)

Endoxana is available as a powder in vials containing 100 mg, 200 mg, 500 mg or 1000 mg of anhydrous cyclophosphamide and sufficient sodium chloride to render the reconstituted solution isotonic. The vial should be reconstituted with a suitable volume of Water for Injection to produce a 20mg/ml solution. This solution can then be administered by slow intravenous bolus injection or further diluted for infusion. The dose should be reduced in renal impairment: for GFR 10-50ml/min reduce dose by 25%; for GFR <10 the dose should be reduced by 50%.

Side-effects: Haemorrhagic cystitis, mucositis, nausea and vomiting, and hypoglycaemia and hyperglycaemia may occur.

ATRA (Vesanoid™ - Roche Products)
The most common adverse effect of ATRA has been headaches of mild to moderate severity. Younger (paediatric) patients appear to be more sensitive to this particular effect. Bone pain, occasionally requiring analgesic treatment, has also been observed. Biochemical abnormality of liver function has occasionally been reported, specifically raised transaminases, alkaline phosphatase and bilirubin, but these are reversible on stopping the drug.

The most serious adverse event has been a syndrome characterised by fever, respiratory distress and episodic hypotension, usually in association with leucocytosis (now known as "Differentiation Syndrome"). The onset of this syndrome has usually been in the first 1-2 weeks of drug treatment. Should this occur the ATRA should be stopped and steroids commenced as detailed in section 20.8.1 above.

Some cases are reported to respond well to high-dose corticosteroid therapy (dexamethasone 10 mg i.v. 12 hourly for 3 or more days).

Prolonged ATRA treatment may cause dryness of the skin. ATRA is also believed to be highly teratogenic and advice regarding contraception should be given as appropriate.

Clofarabine (Evoltra™ Genzyme Inc)
Clofarabine is formulated at a concentration of 1mg/ml in sodium chloride (9mg/ml), (USP), and water for injection, USP, qs to 1 ml. Clofarabine is supplied in one vial size: a 20-mL clear, glass vial with gray stopper and blue flip off seal. The 20-mL vial contains 20 mL (20 mg) of solution with a pH range of 4.5 to 7.5. The solution is sterile, clear and practically colourless, is preservative-free, and is free from foreign matter.

Dose, Administration and Storage:
Vials containing undiluted Clofarabine for injection should be stored at controlled room temperature at 25°C (77°F); excursions permitted to 15-30°C (59-86°F).). Shelf-life studies of intact vials are currently ongoing. Clofarabine for injection should be filtered through a sterile 0.2μm syringe filter and then further diluted with 5% dextrose injection USP or European Pharmacopoeia (EP) (D5W) or 0.9% sodium chloride injection USP or EP (normal saline [NS]) prior to IVI. The resulting admixture may be stored at room temperature, but must be used within 72 hours of preparation. Clofarabine will be administered IV over 60 minutes daily for 5 consecutive days and repeated every 28 to 42 days. If patients develop somnolence (with or without dystonia), the infusion time may be increased to 120 minutes. To prevent drug incompatibilities, no other medications should be administered through the same IV line.
Patients should not receive Clofarabine until a normal serum creatinine has been confirmed for the day of dosing.

Arsenic Trioxide (Trisenox™ – Cephalon Inc.)
Trisenox is 1mg/ml concentrate for solution for infusion (arsenic trioxide). It is presented as a sterile, clear, aqueous solution in a single–use 10ml ampoule. ATO is a trivalent inorganic arsenical. The active substance is a white crystalline powder that is very poorly soluble in water.

Trisenox must be diluted with 100-250 ml of glucose (5%) injection or sodium chloride 9mg/ml (0.9%) injection immediately after withdrawal from the ampoule and must not be mixed with or concomitantly administered in the same intravenous line with other medicinal products.

Aseptic technique must be strictly observed throughout the handling of Trisenox since no preservation is present.

After dilution in intravenous solutions, Trisenox is chemically and physically stable for 24 hours at 15-30^{o} C and 48 hours at refrigerated temperatures (2-8ºC). From a microbiological point of view, the product must be used immediately. If not used immediately in-use storage times and conditions prior to use are the responsibility the user and would normally not be longer than 24 hours at 2-8ºC, unless dilution has taken place in controlled and validated aseptic conditions.

Trisenox is given as a slow infusion over 1-2 hours daily until bone marrow remission is achieved. The daily infusions should be given on an inpatient basis at the beginning of induction therapy, followed, when the acute symptoms of APL have resolved and the patient's condition is stable, by outpatient administration for the remaining induction and consolidation treatment period.

APPENDIX C: Procedures For Bone Marrow Transplantation

Pre-transplant investigations
Centres will wish to perform their own pre-transplant investigations but the following are strongly recommended because they may reveal possible contraindications for proceeding with marrow-ablative therapy.

1. Bone marrow aspiration to confirm remission (ABSOLUTELY ESSENTIAL)
2. Chest x-ray
3. ECG
4. MUGA scan or Echocardiogram
5. Lung function studies

Pre-graft ablative therapy with TBI and cyclophosphamide
The patient should receive allopurinol 300 mg/day for at least two days before the cyclophosphamide. One of the most distressing and dose-limiting side-effects of cyclophosphamide is haemorrhagic cystitis. This may be prevented by MESNA, a compound that inactivates toxic metabolites of cyclophosphamide in the bladder. Patients should also receive intensive hydration during the giving of cyclophosphamide and TBI.

Cyclophosphamide

Dosage
Cyclophosphamide is administered at a dose of 60 mg/kg for each of 2 successive days (use lean body weight for obese patients). It is dissolved in 250 ml of 5% glucose and administered over 60 min. Following the cyclophosphamide a clear 24 hours should elapse before TBI commences. The marrow is thawed and reinfused within 24 hours of completing TBI whether the TBI was given by single or multiple fractions.

MESNA
During cyclophosphamide administration MESNA is given in 4 divided doses by i.v. push at time 0 (time of commencement of cyclophosphamide), time +3 hours, and +6 and +9 hours. Each dose of MESNA is 40% of the total dose of cyclophosphamide, i.e. the total MESNA dose is 160% of the total cyclophosphamide dose. Each individual dose of MESNA must be prescribed separately and the time of administration clearly noted. The hydration regimen (up to 3l/m^2/day), unless used with MESNA, is itself insufficient to prevent cystitis.

Diuresis
Adequate urine flow must be maintained before and following cyclophosphamide administration to prevent urate nephropathy and haemorrhagic cystitis. All patients should receive i.v. fluids at twice the maintenance rate beginning at 6-12 hours before the cyclophosphamide dose. This will ensure adequate hydration.

Total body irradiation
TBI procedures cannot be completely standardized throughout the UK because of constraints of machine characteristics and availability. It is recognised that many schedules in use at present are effective and safe, but the adoption of a limited number for this study is recommended to make it possible to evaluate the significance of fractionation and lung shielding for control of leukaemia and normal tissue toxicity. This study should not obscure in any way the primary aims of the trial.

Single fraction TBI
- No lung shielding
- 1050 cGy if the dose rate is less than 5 cGy per minute.
- 950 cGy if the dose rate is 5-10 cGy per minute.
- 750 cGy if the dose rate is more than 10 cGy per minute.

Fractionated TBI

- 1440 cGy in 8 fractions over 4 days, 180 cGy per fraction.

Treatment will be given using a linear accelerator or cobalt unit operating at the SSD/FSD which gives an adequate, or the largest available, field size. The whole body dose should be defined as the maximum dose to the lung measured by thermoluminescent dosimetry or diodes over 20 minutes for single fraction treatments and for one whole fraction for fractionated treatments. Patient separations will be taken at, and calculation of dose made for, the following sites:

Lung
Abdomen (at umbilicus)
Pelvis

Additional measurements can be made at the discretion of the participating clinician. No lung shielding will be used and the prescribed dose will be that to the lung. Compensators may be used to give homogenous whole body dose if required: doses will then be measured under compensators. Depth dose data, built up depth and beam flatness must be determined by phantom measurement at the extended treatment distance. A central review of machine operating data and calculated doses will be undertaken.

Note: For patients with initial CNS involvement, additional cranial irradiation (3 x 200 cGy over 3 to 5 days) will be given before TBI using lateral fields encompassing the whole brain down to C2 and including the orbit with shielding of the lens. Additional radiotherapy will not be given to sites of initial bulk disease unless there is persistent extra-medullary disease in one site only which is not thought to be a contra-indication to transplantation. A dose of 1000 cGy in 5 fractions will then be given before TBI.

If you are unable to use TBI ablation please contact one of the transplant coordinators about possible alternatives.

Sedation and anti-nausea

Combinations of metoclopramide (20 mg i.v.), lorazepam (1-3 mg i.v.), ondansetron (8 mg i.v.) or other 5HT antagonists and dexamethasone (10 mg i.v.) may be used.

Prevention of infection

Specific prophylactic measures are not laid down and procedures may vary slightly from centre to centre. Infection prophylaxis is of great importance because of the difficulties in diagnosing and treating infection in immunocompromised patients.

Infusion of marrow

The marrow should be infused intravenously through a normal giving set. This may be at any time up to 24 hours following the TBI. Toxicity of the marrow infusion includes volume overload, pulmonary emboli and allergic reactions.

Other supportive care

Red cell or platelet transfusions will be necessary in the period following the graft. It is recommended that platelets be given if the peripheral platelet count is less than 10×10^9/L. All blood products, including platelets, must be irradiated to at least 2500 cGy post transplant. CMV negative recipients should receive CMV negative blood products whenever possible.

GVHD

Prophylaxis and treatment of graft versus host disease following allo-SCT should follow the practice of the individual transplant centre.

APPENDIX D: Derivation of a risk index for younger adults

This appendix gives brief details of the derivation of a risk index for younger adults, which will be used in AML17 to identify patients suitable to enter the "pick-a-winner" design. The work has been published (Burnett et al, *Blood* 2006;108;11:10a (Abstract 18)). It can be viewed as a companion index to the previously developed "Wheatley index" for elderly patients with AML (Wheatley et al. Blood 2005;106;11:199a (Abstract 674)).

AML is a heterogeneous disease, and prognosis, particularly in younger patients, varies considerably. Traditionally risk group stratification in MRC AML trials has been based on cytogenetics and response to the first course of chemotherapy, but this approach does not take into account variables such as age, white cell count, and performance status that are known to be prognostic.

As a result, data from the MRC AML10 and AML12 trials (recruiting some 5,400 patients between 1988 and 2002) were used to construct an index for survival following complete remission. Because of the design of AML17, where patients with APL are given separate treatment, these patients were excluded from the analyses. Additionally, all children were excluded.

The analysis concentrated on clinical parameters which were likely to be available following the end of the first course of chemotherapy. (For example, in view of the fact that FLT3 ITD status is only known for a minority of AML10,12 patients, and that FLT3 ITD +ve patients will in any event enter a CEP-701 randomisation, ITD status and other laboratory markers were not included as candidates for the model).

Using Cox regression, a forward selection model was derived for overall survival from remission, with the following candidate variables:

- Age
- WBC
- Performance status
- Sex
- de Novo/Secondary
- Cytogenetics (Using Grimwade classification favourable/intermediate/adverse)
- Platelets
- BM blasts
- Response after course 1 (CR/PR/NR)
- Height
- Weight

The level of significance to enter the model was set at p=0.05.

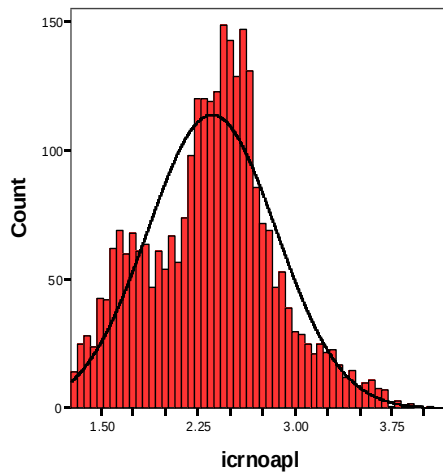
In order of entry to the model, the variables which make up the index are:

Variable	Estimate	χ^2	p-value
Cytogenetics	0.65082	102.7	<0.0001
Age	0.01325	29.16	<0.0001
Status post C1	0.19529	18.50	<0.0001
WBC	0.00169	11.92	0.0006
Male sex	0.16994	8.01	0.005
Secondary	0.22131	4.03	0.04

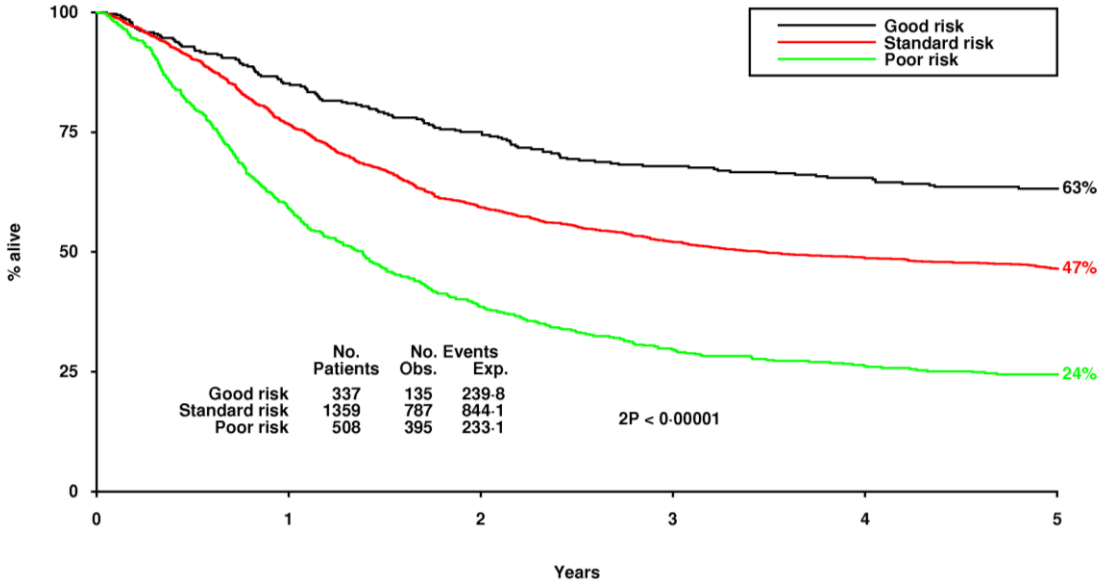
The index is therefore:

0.01325*age (in years) + **0.16994***sex (1=male, 0=female) + **0.22131***diagnosis (1=de novo, 2 secondary) + **0.65082***cytogenetics (1=favourable, 2=intermediate, 3 adverse) + **0.19529***status post C1 (1=CR, 2=PR, 3=NR) + **0.00169*** WBC ($x10^9$/l)

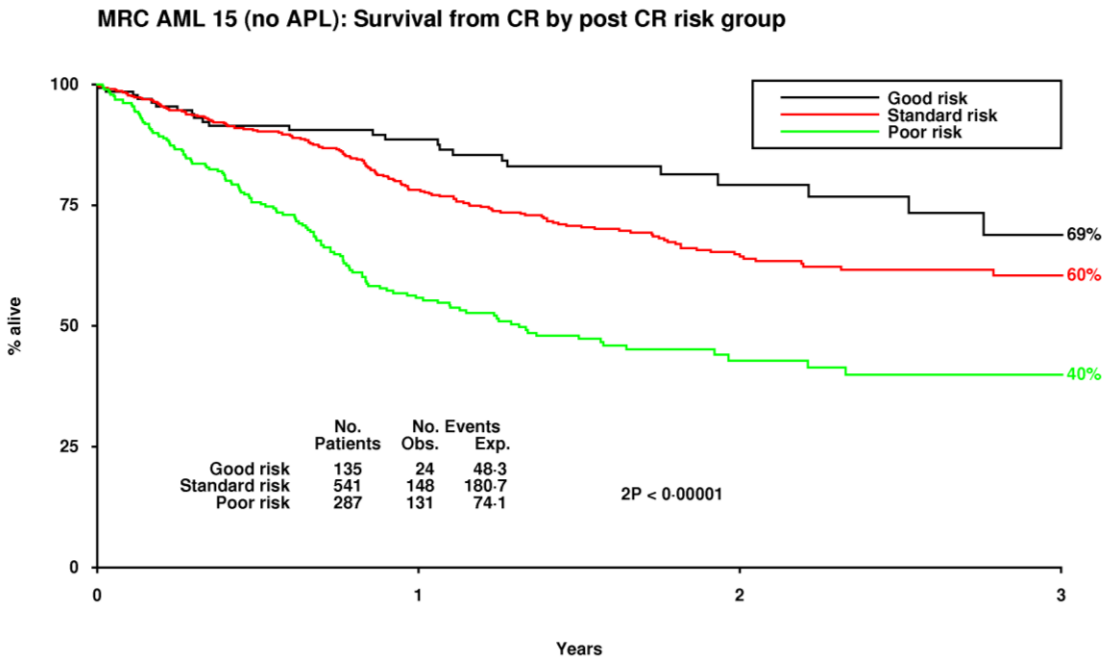
and the distribution of patients in AML10,12 by index is:



Taking into account the apparent bimodality of the curve, patients with an index of 2 or below were deemed good risk, and the data were arbitrarily divided at the 75th centile between standard and poor risk. Survival from CR in AML10,12 according to the risk groups was as follows:



The index was validated on data from AML15:



One important feature of the new risk classification is that the number of poor risk patients has increased. Compared to the old MRC risk classification, the new approach identifies a number of patients who have poor prognosis for reasons other than their cytogenetics:

	MRC Good	MRC Standard	MRC Poor	Total
New good	309	28	0	337
New standard	51	1289	42	1382
New poor	2	274	353	629
Total	362	1591	395	2348

APPENDIX E: Supportive Care Recommendations For Acute Promyelocytic Leukaemia

(Extract from the BCSH Guideline on the Management of acute myeloid leukaemia in adults)

- All-*trans* retinoic acid (ATRA) should be started as soon as the diagnosis is suspected (grade A; evidence level Ib).
- Leucopheresis should be avoided in high count patients (grade B; evidence level III).
- During induction, platelet count should be maintained at >50 × 10^9/l, together with fresh frozen plasma (FFP) and cryoprecipitate to normalize the activated partial thromboplastin time (APTT) and fibrinogen levels (grade B; evidence level IIb).
- ATRA syndrome should be treated promptly with dexamethasone 10 mg twice daily i.v., until the symptoms resolve (grade C; evidence level IV).
- Diagnostic work-up should include documentation of underlying *PML-RARA* fusion (grade B; evidence level IIa).
- Patients should undergo molecular monitoring after treatment to guide further therapy (grade B; evidence level IIa).
- In case of disease relapse, ATRA should not be used as single agent therapy because of the significant possibility of acquired secondary resistance, and arsenic trioxide (ATO) should only be used in patients with confirmed *PML-RARA* positive APL (grade B; evidence level IIa).

Coagulopathy: A major cause of treatment failure is induction death as a result of haemorrhage, which reflects to varying degree DIC excessive fibrinolysis and proteolysis. Patients with higher presenting WBC (i.e. >10 × 10^9/l) are at highest risk of haemorrhagic death. Patients with very high presenting leucocyte counts should not undergo leucopheresis, which may precipitate fatal exacerbation of the coagulopathy. High rates of induction death have also been observed when low-dose chemotherapy was used to attempt to reduce WBC in the first instance . Evidence to date suggests that patients with high presenting WBC are best commenced on ATRA and anthracycline-based induction therapy. Haemorrhagic deaths may be reduced by rigorous monitoring of the coagulation profile and administration of appropriate replacement therapy until morphological CR has been attained. APTT, prothrombin time, thrombin time, fibrinogen level and platelet count should be checked at least twice daily during the early stages of treatment. Coagulation times should be kept within the normal range using FFP as replacement. Fibrinogen levels may be low due to DIC and cryoprecipitate should be given as replacement aiming for a level of approximately 2 g/l. Elevated levels of fibrinogen should be avoided because of the increased risk of thrombosis associated with APL, which may be further exacerbated by ATRA. The platelet count should ideally be maintained above 50 × 10^9/l until morphological remission has been confirmed. Clinical studies have not established proven benefit for use of heparin or anti-fibrinolytic agents as a means of decreasing induction death rates in APL and their routine use is not recommended. Indeed, anti-fibrinolytic agents when combined with ATRA could potentially increase the inherent risk of thrombotic complications. Nevertheless, anti-fibrinolytic agents could be contemplated in situations of life-threatening haemorrhage in the presence of normal coagulation assays. Recombinant activated Factor VII has also been used in the context of potentially fatal haemorrhage

Differentiation syndrome: (see Section 19.8.1)