

**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 patients aged less than 60 years, whether adults or children, and also to patients aged 60 years or over for whom intensive therapy is considered appropriate. Children are defined as patients under 16 years. 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/m² or 90mg/m² 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. Children will receive two courses of high dose Ara-C in consolidation.**

After course 1 of treatment, patients will be segregated based on their molecular-genetic characteristics, and a validated risk score. Patients who have a FLT3 mutation will be randomised to receive the **FLT3 inhibitor CEP-701 or placebo** after course 1 and each subsequent chemotherapy course. Patients who are at high risk of relapse based on the AML Risk Score will be eligible for an allogeneic stem cell transplant if a donor is available, and/or enter a study of a novel combination. 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. Children who are high risk, , will be allocated to FLAG-Ida before proceeding to transplant.

Adult patients who have **Core Binding Factor (CBF) leukaemias** ie favourable risk disease will be randomised only to the 3 versus 4 comparison; children will receive two courses of High Dose Ara-C. The rest of the adult patients will be randomised to receive, or not, **mTOR inhibitor, Everolimus (RAD001) in combination with chemotherapy beginning after course 2.** The Everolimus randomisation is not available for children.

For adult patients only 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.**

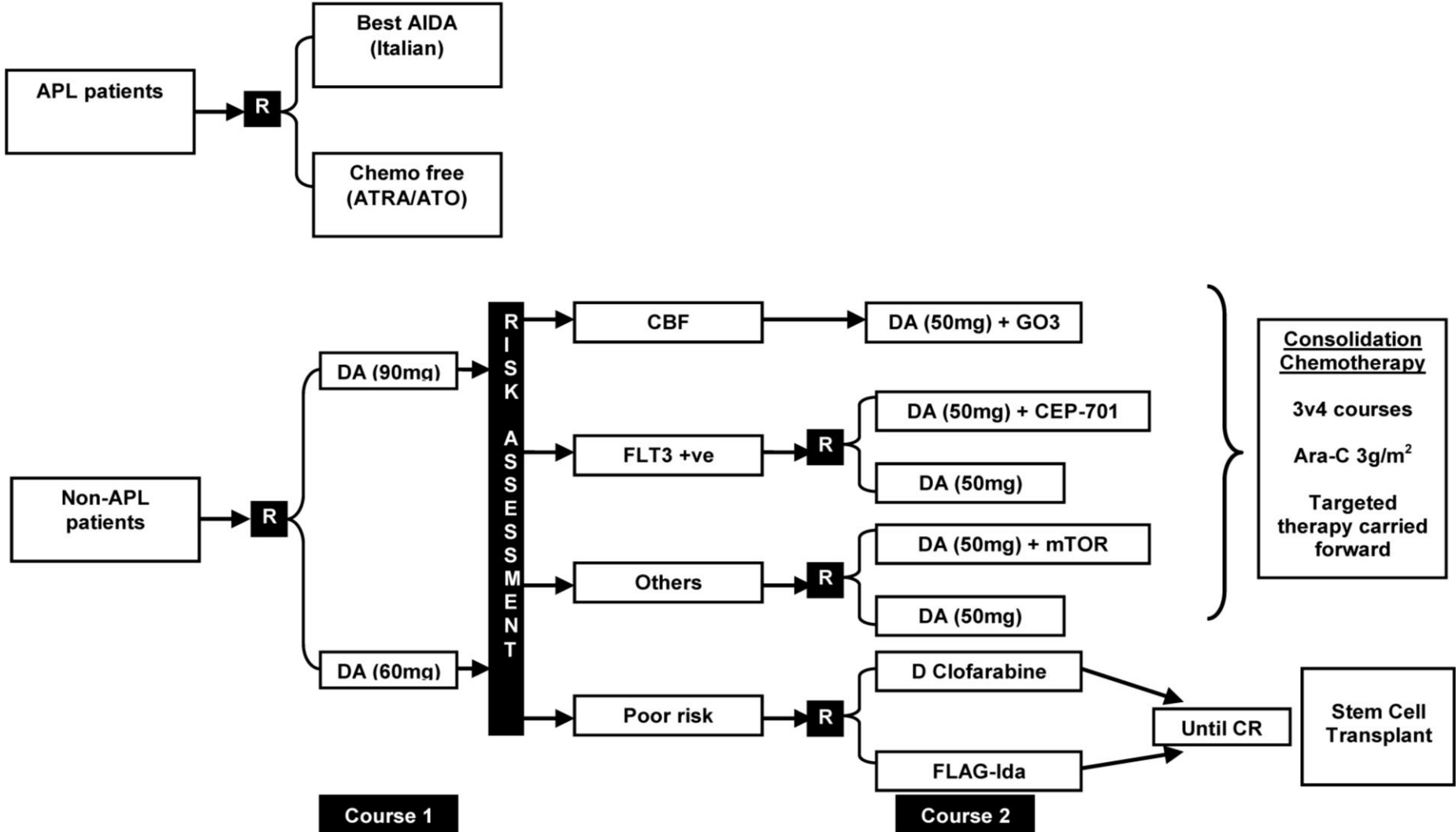
At diagnosis, material will be sent to reference labs for molecular and immunophenotypic characterisation and the identification of markers of minimal residual disease (MRD) detection. The predictive value of these markers will be validated in the early part of the trial, and the clinical impact of this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

There are about 700 cases of AML aged 0-59 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.

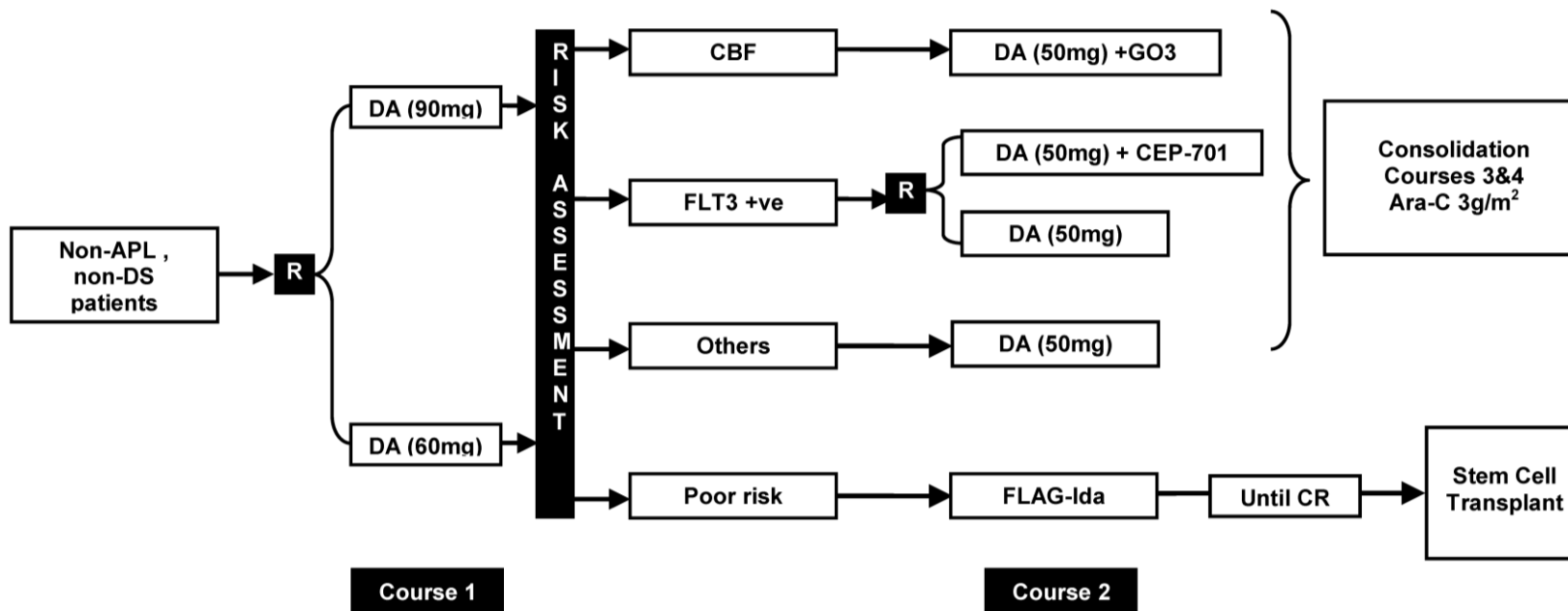
This protocol is intended to describe a trial conducted by the AML Working Group of the National Cancer Research Institute (NCRI) Haematological Oncology Study Group in Acute Myeloid Leukaemia and high risk Myelodysplastic Syndrome in adults and children under the sponsorship of Cardiff University. It 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



Flow chart for children (i.e. patients under 16 years) :



Children with APL or Down Syndrome are not eligible for the AML17 trial

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

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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.
- To assess the value of the **FLT3 inhibitor CEP-701** for patients with a FLT3 mutation
- To assess the value of **mTOR inhibition** in patients who lack a FLT3 mutation, and who are not high risk, and who do not have Core Binding Factor Leukaemia
- 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 Objectives for children with non-APL and High Risk Myelodysplastic Syndrome:

- To compare **D(90)A** with **D(60)A** in course 1.
- To assess the value of the FLT3 inhibitor CEP-701 for patients with a FLT3 mutation.
- In high risk patients, to assess the value of allogeneic stem cell transplantation following FLAG-Ida therapy
- To assess the clinical value of minimal residual disease monitoring.

Children with APL or Down's syndrome are not eligible for AML17

2.4 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).

2.5 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 the blood level of anti-FLT3 activity and the extent of dephosphorylation of the FLT3 receptor with response for patients allocated to receive FLT3 inhibition therapy
- To assess the level of plasma mTOR activity in relation to clinical outcome.
- 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 in adults and children.
- B. Consolidation phase: for patients who are not high risk two versus one further treatment courses of high dose Ara-C (two arms)(children receive 2 courses) FLT3 inhibition for patients with FLT 3 mutations: one randomisation (two arms).
- C. For high risk adult patients standard therapy (FLAG-Ida) vs D/Clofarabine (two arms)
- D. mTOR inhibition for adults only (two arms)

APL:

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

3.2 AML (other than APL)

There are six randomised comparisons for adults within the trial:

- At diagnosis:
 - i) D(90)A versus D(60)A (two comparisons)
- End of Course 1
 - ii) FLT3 inhibitor (CEP-701) versus placebo, for FLT3 mutation positive patients
 - iii) FLAG-Ida versus D/Clofarabine for high risk score cases.
 - iv) mTOR inhibition for non-CBF Leukaemias
- After Course 2
 - 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, the role of allogeneic SCT of either Standard or Reduced intensity will be assessed by means of a genetic randomisation (i.e. donor versus no donor comparison), and by transplant given versus not given.

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 10), the FLT3 mutation, Should be known, allowing randomisation to the FLT3 inhibitor or not. 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).
 - i) Patients with a FLT3 mutation can then be randomised to start FLT3 inhibition or not for four courses after *each course* of chemotherapy (Sections 4.1.3 and 11.3). NB in children it is required that liver function tests must be within 3 X the upper limit of locally determined limit.
 - ii) Patients who have a high risk score will enter the comparison of Daunorubicin/Clofarabine versus FLAG-Ida (Section 11.5)
 - iii) Core Binding Factor Leukaemias will receive mylotarg 3mg/m² 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.
 - iv) Other patients who are not involved in the options (i) to (iii), will be randomised to receive or not the mTOR inhibitor (Everolimus/RAD001) for 3 courses or not (Section 11.6).
 - v) All patients except the High Risk Index 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 16).
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.

NB Consolidation for children will comprise 2 courses of High Dose Ara-C (See Section 14)

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 $>10 \times 10^9/l$ are at a slightly higher risk of relapse and should receive Mylotarg ($6\text{mg}/\text{m}^2$) 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 20.5.4)
4. After 55 to 60 days assess remission status (see Section 20).
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 16)

Children with APL are not eligible for randomisation in AML17.

3.4 In Children (who do not have APL or Down syndrome):

There are four randomised comparisons within the trial.

- At diagnosis:
- i) D(90)A D(60)A in course 1. (two arms). Each arm will receive D(50)A as course 2.
By the end of the first course of induction chemotherapy (day 10), the FLT3 mutation status will be available to enable randomisation to CEP-701 or placebo. On recovery of counts, cytogenetics and molecular screening (Core Binding Factor), and Risk Index status of each non-APL patient will be available. (This risk index will be provided by the online service which must be used.)
 - ii) Patients with a FLT3 mutation can then be randomised to start FLT3 inhibition or placebo for four courses after *each course* of chemotherapy (Sections 4.1.3 and 11.3)
- In consolidation
- iii) Two courses of High Dose Ara-C in patients who are not poor risk.
 - iv) Patients with high risk disease should be treated with FLAG-Ida with a view to going to allogeneic stem cell transplant which will be assessed on a donor vs no-donor and transplant given versus not basis.
 - v) Patients may enter a randomisation to be MRD monitored or no (section16)

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 in the flow diagram at the front of the protocol.

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/m² versus 6mg/m²). In the next phase of AML 17 two dose levels of daunorubicin 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/m²) is optimal. In a randomised comparison in patients <60 years the ECOG Group compared a 90mg/m² X 3 with 45mg/m² 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/m². 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 children, the high risk group identifies patients at a high risk of relapse. It is clear from the nearly 8000 patients entered into the MRC AML10, 12 and 15 trials that there has been no improvement in survival for high risk patients, however defined, for the last 20 years. The AML17 trial, therefore, compares a novel combination (Daunorubicin/ Clofarabine) with FLAG-Ida, in adults, with a view to proceeding to allogeneic transplantation.

FLT3 Inhibition

A number of prognostic factors have been identified for CR and relapse. Among the adverse prognostic factors is the *fms*-like tyrosine kinase 3 (FLT3) activating mutation. These mutations spontaneously initiate ligand-independent autophosphorylation of the receptor, stimulating proliferation of AML cells. Two types of FLT3 activating mutations have been identified in patients: an internal tandem duplication (ITD) and a point mutation, usually at aspartate 835. The presence of FLT3/ITD mutations has been shown to be associated with a decreased remission induction rate and poorer outcome in paediatric AML and a higher rate of relapse and poorer overall survival in adult AML⁽⁷⁻¹²⁾.

Studies in animals suggest that inhibition of mutated FLT3 improves response to chemotherapy and/or overall survival^(13,14). CEP-701 (Lestaurtinib) is a potent FLT3 inhibitor and induces a cytotoxic-like effect on both FLT3/ITD transfected cells and primary leukaemic myeloblasts in patients with AML with the FLT3/ITD mutation. In a mouse model of FLT3/ITD leukaemia, treatment with CEP-701 significantly prolonged survival. In a recently completed Phase II clinical trial in patients with relapsed disease, CEP-701 at doses of 60 and 80 mg bd was associated with transient decreases in the number of peripheral AML myeloblasts⁽¹⁴⁾. A similar response was seen in a UK Phase II study in untreated older patients⁽¹⁵⁾. A pharmacokinetic /pharmacodynamic analysis indicated that this anti-leukaemic activity required a high degree of inhibition of the target kinase, FLT3⁽¹⁶⁾. *In vitro* studies have shown that AML cells that survive chemotherapy treatments

remain responsive to CEP-701 ([Levis, personal communication, 2003](#)). There is *in vitro* data to suggest that combining CEP-701 with Ara-C has a synergistic effect⁽¹⁵⁾. This has led to an ongoing randomised study in relapsed AML which compares high dose Ara-C with and without sequential CEP-701. In a preliminary analysis performed after the first 49 patients, 11 of 24 patients who had the combination arm achieved a CR or CRi compared with 6 of 25 in the chemotherapy only arm (P Brown, personal communication). Similar preliminary data is also emerging from the combination of another FLT3 inhibitor (PKC412) in relapsed disease⁽¹⁸⁾. An objective of the AML17 trial is to determine whether CEP-701, given in sequence with standard chemotherapy in first line, can reduce the risk of relapse and improve survival in patients who have a FLT3 mutation.

Background information on the non-clinical pharmacology and pharmacokinetics, toxicology, and clinical experience both in healthy subjects and patients with cancer is given in Appendix C. Of potential clinical relevance is the theoretical interaction with azole antifungal agents which use CYP3A4 in metabolism, with the potential effect of increasing blood levels of CEP-701. The extent to which this happens and whether it is clinically relevant is not known. As part of the assessment of CEP-701 in this trial, blood levels of free CEP-701 and azole blood levels will be measured on day 14 of each course of CEP-701 treatment.

The role of stem cell transplantation in FLT3 mutant patients is controversial. The MRC database indicates that FLT3 of itself is not an indication for transplantation. There will be emerging evidence on this issues and investigators will be provided with updated information periodically so that they can make an informed decision about what course of action to take. This issue is also complicated by the interaction that FLT3 mutation has with NPM1 mutation, which tends to negate the impact of FLT3.

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 CBF β -MYH11 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⁽¹⁹⁾, 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/m²) on day 1 of course 2. The new inhibitors are intended to be available to trial entrants if they relapse or are designated to be at high risk due to persistence of minimal residual disease.

Other Patients

Approximately 60% of all non-APL patients have neither a FLT3 mutation nor Core Binding Factor Leukaemia. Approximately half of these adult patients (and 10% of children) 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 G. 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 eg FLT3 status. FLT3 has been excluded from the score to be used in AML17 because such patients are being assessed in the FLT3 inhibitor part of AML17.

For the purposes of the AML17 trial patients who have a risk score of greater than 2.667, who do not have a FLT3 mutation or Core Binding Factor Leukaemia 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 NCR1 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.

It is uncommon for children to be high risk as defined by the risk score. However such children will be allocated to FLAG-Ida treatment with a view to proceeding to allogeneic stem cell transplant if a donor is identified.

m TOR Inhibition

Constitutive activation of the PI3K/AKT pathway has been demonstrated in 90% of AML samples where it has been shown to be central to the survival of AML blasts but not of normal CD34+ cells^(18,19). The pathway is of particular relevance to AML as it is also activated by FLT3. The serine/threonine kinase mTOR is downstream of PI3K/AKT and can be inhibited by mTOR inhibitors including Sirolimus and its analogue Everolimus (RAD001, Novartis). Studies in NOD/SCID mice have indicated that mTOR may regulate a critical cell survival pathway in AML stem cells⁽²¹⁾ and Sirolimus may have the potential to eliminate leukaemia-initiating stem cells without eliminating normal haematopoietic stem cells. Sirolimus strongly inhibits the growth of AML cell lines *in vitro* and dephosphorylation of downstream effectors of mTOR in a Sirolimus-sensitive manner has been demonstrated in 23 AML cases⁽²²⁾. It has also been shown that increased survival, proliferation and leukaemic transformation of cell lines by FLT3/ITD is mediated by AKT and mTOR and can be inhibited by Sirolimus at therapeutically achievable concentrations⁽²³⁾. In an unrandomised clinical trial, Sirolimus was administered as a single agent to 9 relapsed, refractory or poor-risk AML patients for 28 days at doses used for renal transplant recipients. At day 28, partial responses had occurred in 4 patients whilst one had stable disease and 4 had progressed⁽²⁴⁾. In an ongoing trial at Nottingham University Hospitals, 11 elderly patients with primary and relapsed AML have been treated with the combination of low dose Ara-C and Sirolimus. Following a single 28-day course of treatment, of the 7 patients eligible for analysis, one had achieved a CR, 4 a PR, one marrow was profoundly hypocellular and one patient was a non-responder. Patients in this trial reliably maintained trough Sirolimus levels of 8-16 ng/ml, which are consistent with the published concentrations required to inhibit AML cell growth *in vitro*⁽²⁵⁾. The feasibility of combining mTOR inhibition (Sirolimus) with intensive chemotherapy has also been assessed in AML patients in conjunction with the more intensive MEC (Mitoxantrone, Etoposide and Cytarabine) chemotherapy regimen in a phase I dose escalation study in which standard renal transplant doses were well tolerated and did not increase the non-haematologic toxicity of MEC chemotherapy with a median time to ANC recovery of 27 days⁽²⁶⁾. This provides a rationale for examining the addition of an mTOR inhibitor to allocated chemotherapy in the AML17 trial.

Temsirolimus (CCI-779, Wyeth Research) has been evaluated in a number of solid tumours and has been recently approved by the FDA for the indication of advanced renal carcinoma. It is most reliably dosed by a single intravenous infusion weekly. RAD001 (Everolimus-Novartis) has shown

pre-activity against numerous cell lines and in vivo models. It has also had extensive pre-clinical assessment in combination with several chemotherapeutic agents. In human studies on several hundred patients a steady state AUC can be achieved by daily or weekly oral administration with a half life of approximately 30 hours. A number of studies of RAD001 in combination with chemotherapy have been carried out. As (Certican^R) it is approved for prophylaxis of organ rejection in adults receiving allogeneic renal or cardiac transplants.

RAD001 (Everolimus) has been in clinical development since 1996 as an immunosuppressant, associated with cyclosporin and glucocorticoids, for the prevention of rejection in patients undergoing solid organ transplantation. In this context mTOR inhibition opposes interleukin-stimulated proliferation of activated T-lymphocytes. An antiproliferative effect on immuno-competent cells is also the rationale for investigation of the drug's activity in autoimmune diseases. The drug is also being investigated for use in the inhibition of initial proliferation after coronary angioplasty through its incorporation into drug-eluting stents.

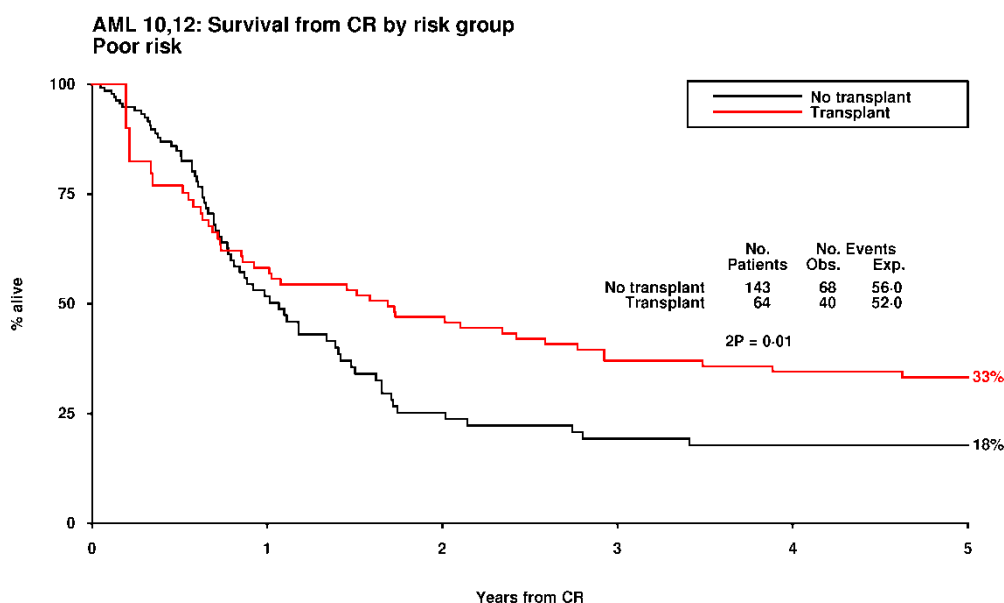
Pre-clinical investigations have demonstrated that RAD001 is a potent inhibitor of the proliferation of a range of human tumour cell lines in-vitro and inhibits tumour growth in-vivo in both xenografted, syngeneic and orthotopic animal models. Studies have also demonstrated the drug's inhibition of endothelial proliferation and its antiangiogenic activity. Experiments show the potential for combining RAD001 with other anticancer agents including paclitaxel, doxorubicin, cisplatin, carboplatin, gemcitabine, radiotherapy, imatinib, EGFR and VEGF inhibitors, and letrozole.

Clinical trials of RAD001 in oncology patients are ongoing since 2002 and include Phase I dose escalating studies as single agent, Phase II/III studies of RAD001 in indications where mTOR is known to be active, Phase I/II studies in combination with other anti-cancer agents and correlative investigations in the search for potential biomarkers. No children have been exposed to RAD001, so this option will not be available for children.

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)⁽²⁸⁻³⁰⁾. At the molecular level, APL blasts are characterised by a specific chromosomal translocation t(15;17) resulting in a hybrid *PML/RAR α* gene which is readily identified by reverse-transcriptase polymerase chain reaction (RT-PCR)⁽³¹⁻³⁴⁾. In addition to its diagnostic relevance, detection of the *PML/RAR α* 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^(35,36)). As a consequence, the achievement of molecular remission is nowadays universally considered as a therapeutic objective in this disease⁽³⁵⁾. 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^(86,97). 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/RAR α* 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^(35,36).

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⁽⁴⁰⁻⁵²⁾. 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⁽⁴⁰⁾. 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⁽⁵²⁾. 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 $>10 \times 10^9/L$ had significantly higher relapse risk⁽⁵¹⁾. 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^(52,53). 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⁽⁵³⁾. In line with these findings, most studies nowadays include risk-adapted approaches in which treatment intensification is based on initial WBC counts⁽⁴⁹⁾.

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^(54,55).

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⁽⁵⁶⁻⁶⁵⁾ 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 $\mu\text{mol/L}$) ATO induces apoptosis *in vitro*, through induction of caspases 2 and 3, while at lower concentrations (0.1 to 0.5 $\mu\text{mol/L}$) it induces partial differentiation of leukaemic promyelocytes through PML/RAR α degradation; furthermore, ATO is known to inhibit angiogenesis via down-regulation of vascular endothelial growth factor (VEGF)⁽⁶⁵⁻⁶⁸⁾. 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⁽⁶⁹⁾. 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⁽⁷⁰⁻⁷²⁾. 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⁽⁷²⁾. 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⁽⁶⁹⁻⁷¹⁾. 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^(56,57), 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⁽⁵⁸⁾, then in an expanded multicentre trial for patients relapsed after ATRA⁽⁶⁰⁾. 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⁽⁵⁸⁾. A CR rate of 86% was reported subsequently in the US multicentre study⁽⁶⁰⁾. 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%^(59,61-64).

Arsenic Trioxide in Combination

In addition to trials in which ATO was used as 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⁽⁷³⁾. No significant additional toxicity was reported in this or in other studies which analysed the effect of ATRA and ATO combination^(63,73,74). 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⁽⁷³⁻⁷⁷⁾. 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⁽⁷³⁻⁷⁷⁾. 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⁽⁷⁶⁾. 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⁽⁸⁰⁾ 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^(81, 82). 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⁽⁸³⁾. 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, CBF β -MYH11 and PML-RAR α 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, CEBP α and RAS which in some studies have been shown to have prognostic value (reviewed⁸⁴) 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⁽⁸⁵⁾. 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 20 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).
- Adult patients with acute promyelocytic leukaemia (APL) are eligible and should be entered into the randomisations specifically for APL (see Section 20).
- 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 be 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 the NCRI AML16 trial for older or less fit patients).**

CHILDREN WITH APL AND DOWN SYNDROME AND AML ARE NOT ELIGIBLE FOR AML 17

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 22 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. The relevant information and consents 1A should be used for children.** Further consent documents will be used at each randomisation point. **For APL patients see section 20 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. **For children use forms 2A.** 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 including children 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.
- For children under 10 use the Play Performance Scale (see Appendix F)
- 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 Professor Harrison will coordinate cytogenetics in children.

8.3.2 FLT3 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 mutation status of patients to determine eligibility for the FLT3 inhibition randomisation. 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 16).

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 mutation. 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 by the end of the first course of chemotherapy to enable eligible patients to be randomised between FLT3 inhibitor or placebo.

Laboratories for FLT3 Mutation Analysis and Molecular Screening:

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

Department of Haematology, University Hospital of Wales, Cardiff
(Dr P White)

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.
For children 2 ml of bone marrow and 8 and 8ml of blood in EDTA (2ml for infants)

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
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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) and to Prof Yin or Prof Grimwade for detection of molecular markers (see section 11.4 & 20.10 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 16. 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).

Molecular Inhibition (Form D) - return for patients allocated to receive either FLT3 or mTOR inhibitor

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

For patients allocated to FLT3 or mTOR inhibition will be sent periodic forms to assess compliance and toxicity. Adverse event forms can be downloaded from the website. Investigators will be able to report event via the website, but should retain a hard copy in the Study Site file and the patients' notes.

8.5 Health Economics

Basic information on resource usage will be collected in the data forms B to G 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, the presence of a FLT3 mutation, a high risk score, 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 11.5).

9.1 D(90)A schedule

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

Cytosine Arabinoside 100 mg/m² 12-hourly by i.v. push on days 1-10 inclusive (20 doses).

Daunorubicin 90 mg/m² 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/m² 12-hourly by i.v. push on days 1-8 inclusive (16 doses).

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

Cytosine Arabinoside 100 mg/m² 12-hourly by i.v. push on days 1-10 inclusive (20 doses).

Course 2 **DA 3+8**

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

Cytosine Arabinoside 100 mg/m² 12-hourly by i.v. push on days 1-8 inclusive (16 doses).

NB: Some sites have an established practice of giving Daunorubicin in a longer infusion time. Up to four hours will be permissible. All drug doses should be reduced by 25% in children aged less than one year or weighing less than 10kgs.

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.

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 Yin (for CBF leukaemias) or Prof Grimwade (other molecular targets) for molecular assessment of MRD (see Sections 8.3.4, 11.4 and 20.10 for addresses). Rarely patients with a FLT3 mutation will emerge as also having favourable cytogenetics. Such patients should continue on the FLT3 inhibitor allocated therapy. Favourable risk patients should also be randomised to receive 3mg/m² of Mylotarg if they have not received it in course 1.

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 10⁹/l and platelet count of at least 100 x 10⁹/l

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

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. ADDITIONAL TREATMENTS

11.1 Additional treatments

Immediately after the completion of chemotherapy patients become eligible for additional or alternative treatment. Within 10 days of entering the trial the reference labs will have defined the FLT3 mutation status. If a mutation is found the site Principal Investigator (PI) and research nurses will be informed thus enabling the patient to enter the FLT3 Inhibitor randomisation (Section 11.3). 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 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 11.5). Patients who are in none of these categories are eligible for the addition of mTOR inhibition (Section 11.6), which will start after course 2. The 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 mutation).

Patients in these randomisations will be randomised in a 2:1 fashion, so that there is a two out of three chance of receiving either mTOR or FLT3 inhibitor or Daunorubicin/Clofarabine in the high risk option. The FLT3 patients will receive active drug or placebo.

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.

11.2 Patient Information and Consent

Additional consent will be required for the additional treatments:

- For patients eligible for FLT3 Inhibition use **Patient Information Sheet 4 and Consent Form 4**
- For patients eligible for the high risk treatments use **Patient Information Sheet 5 and Consent Form 5**
- For patients eligible for mTOR inhibition use **Patient Information Sheet 6 and Consent Form 6**

11.3 FLT3 INHIBITION

Patients who have a confirmed FLT3 mutation are eligible to enter the FLT-3 inhibition randomisation. **Patient Information Form 4 and Consent Form 4** should be used. Patients should be randomised immediately following the end of the first course of chemotherapy. Patients will be randomised to receive CEP-701 in a 2:1 fashion and will commence treatment **2 days** after the completion of each course of chemotherapy and should continue until *two days before the commencement of the next chemotherapy course*, or for a maximum of 28 days after the end of the chemotherapy course. CEP-701 should not be administered concurrently with other chemotherapy. Patients allocated to inhibitor are intended to receive CEP-701 for four courses, each of up to 28 days in duration, between allocated chemotherapy. It is expected that CEP-701 will be administered at home, and investigators must ensure that the patient has received adequate instruction in reconstituting the drug. An instruction sheet is given in Appendix D and is available on the trial website (<http://AML17.cardiff.ac.uk>)

The CEP-701 randomisation should take place as soon as the FLT3 status is known, so that drug supplies can be put in place and so that treatment can start 2 days after completion of the allocated chemotherapy. Investigators are advised to discuss the possibility of randomisation to CEP-701 with the patient before initial entry to the trial, and before mutation status are known. Randomisation should take place irrespective of disease response.

To randomise a patient, (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> NB: children require to have liver function tests within 3X the upper limit of normal at the time of randomisation

Before treatment allocation is given, investigators will need to provide:

- The name and hospital of the randomising clinician
- The AML17 trial number (or full name and date of birth)
- The approved laboratory where mutation analysis was done
- Confirmation of the patient's FLT3/ITD mutation status
- Date of completion of the first course of chemotherapy
- Whether or not the patient is receiving prophylactic azole anti-fungal treatment.
- Confirmation in children that the liver function tests are within 3X the upper limit of normal

Prior to commencing CEP701 treatment, 10ml of blood (in Lithium Heparin) should be sent to Dr P White in the Cardiff laboratory

11.3.1 CEP-701 TREATMENT

Patients who enter this randomisation will receive active drug or placebo in a 2:1 randomisation. Patients randomised to chemotherapy plus sequential CEP-701 will receive a dose of **80mg bd** starting 2days after the last administration of each course of induction

chemotherapy. **Note: because of a likely interaction with azole antifungal agents, where the patient is to be treated with azoles, the initial CEP-701 dose should be 40mg bd, for the first 7 days of each course of treatment to avoid gastrointestinal symptoms, if tolerated the dose can be continued at 60mg bd. If it emerges that the patient cannot tolerate the 60mg dose they should continue on 40mg. Investigators may have to continue to adjust the dose based on the patient's symptoms. If a patient is on the 80mg dose and requires dose reduction this should be to 60mg in the first instance.**

Children can be dosed on one of two schedules based on the wide range of body weight. Children who will not be on azole therapy can receive 62.6mg/m² (if body surface area is <1.3m²) or a flat dose of 80mg (if body surface area is >1.3m²) whichever is lower. If patients are on azole therapy these doses should be reduced by 50%. If the selected dose is well tolerated the dose may be increased by 25% in subsequent administrations.

Under no circumstances should CEP-701 be co administered with any course of chemotherapy treatment.

Treatment or placebo will be given daily for **up to 28 days** or until 2 days before the next course of chemotherapy, whichever is sooner. When subsequent courses of chemotherapy are given, treatment with CEP-701/placebo must stop 2days before the first administration of the next course of chemotherapy, because elevated CEP-701 plasma concentrations may interfere with the effect of chemotherapy. Treatment with CEP-701/placebo will recommence 2days after the last administration of chemotherapy in the subsequent courses. In patients who are allocated to receive one course of consolidation treatment (see section 12 for details) the fourth course of CEP-701/placebo should be commenced 7 days after the completion of the 28 days of CEP-701/placebo given after consolidation course 1.

Patients randomised to receive chemotherapy plus sequential CEP-701/placebo who have not achieved complete remission after the second course of chemotherapy will discontinue the study and will be considered to have failed treatment. After patients have completed two courses of induction treatment those in CR will be expected to enter the consolidation randomisation as described in sections 12 and 13.

Information about unblinding SUSAR's in patients randomised to the CEP 701/Placebo trial arm can be located in section 22.1.5 of the protocol.

11.3.2 Dose Adjustment

In patients who are **not receiving azole anti-fungals** the dose of CEP-701 may be reduced to 60mg bd at any time for any patient receiving CEP-701 if the 80mg bd dosage is not well tolerated; a return to 80mg bd is permitted if tolerance improves. The dose may be increased to 100 mg bd under the following circumstances:

- Where patients have tolerated CEP-701 treatment at 80 mg bd well and have met the criteria for a second course of chemotherapy. (CEP-701 may be administered at 100 mg bd following completion of the second course of chemotherapy.)
- Where patients have tolerated CEP-701 treatment at 80 mg bd well and have achieved at least a PR, CEP-701 may be administered at 100 mg bd after the marrow assessment),

In both cases, the dosage of CEP-701 treatment must be reduced to 80 mg bd (and if necessary 60 mg bd) in the event of poor tolerability.

In patients who **are receiving azole anti-fungals** the dose of CEP-701 should commence at 40mg bd for the first 7 days of each treatment course. If well tolerated the dose can be increased thereafter to 60mg bd. If the 60mg dose is well tolerated the 80mg dose can be

attempted, but if the 60mg dose is not well tolerated the patient should continue at the 40mg dose.

Investigators are requested to record the sequence of dosing administered on the appropriate data collection form (Form D).

The dose can be increased to 100 mg bd under the following circumstances:

- Where patients have tolerated CEP-701 treatment at 80 mg bd well and have met the criteria for a second course of chemotherapy. (CEP-701 may be administered at 100mgbd following completion of the second course of chemotherapy.)
- Where patients have tolerated CEP-701 treatment at 80 mg bd well and have achieved at least a PR, CEP-701 may be administered at 100mgbd after the marrow assessment.)

The most likely side effect associated with CEP-701 treatment is nausea, so patients should routinely be given precautionary anti-emetic treatment.

CEP-701 is supplied in 100 ml glass bottles as a clear yellow oral solution at a concentration of 25mg/ml in polysorbate 80NF (10ml) and propylene glycol USP (10ml). Prior to administration, CEP-701 should be diluted in fruit juice. The following juices are approved for use to administer CEP-701:

- **grape**
- **pineapple**
- **apple**
- **V8® 100% vegetable juice**
- **orange juice (pulp-free)**

During the study, patients will receive multi-dose bottles, each containing 100ml of CEP-701 solution, from the investigator. Patients must be formally assessed on or near day 14 of CEP-701 treatment, and issued with further supplies as required. At each visit, patients should return all used and unused bottles to monitor dosage and compliance. Appendix D gives the dosing regimen.

11.3.3 CEP701 Day 14 assessment visit

Patients who are being treated with CEP-701 should be reviewed on or as near as possible to day 14 from the start of each course of CEP-701 treatment (if day 14 falls on a Friday, please delay until the following Monday). They should be evaluated for drug tolerance and a dose adjustment made if required. Patients should be asked to delay taking their morning CEP701 dose on the day of assessment until blood sampling has been performed. At each day 14 assessment, up to 20ml of blood should be collected (standard lithium heparin container without separator gel) and sent to Dr P White at the Cardiff laboratory, using the Royal Mail collection service provided, for the assessment of trough free CEP-701 level, FLT3 inhibitory activity and azole blood levels. Please complete the online CEP701 Day 14 assessment questionnaire which can be found on the AML17 website at the time of each Day 14 assessment.

In children the serum lipase, amylase and liver function should be checked weekly during CEP-701 treatment in course 1 and fortnightly subsequently. If a patient develops significant abnormality the case should be discussed with a trial co-ordinator (see section 14 for contact details).

11.4 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/m² 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 Manchester 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. This information can be provided on a commercial basis by arrangement with the Manchester lab contact details below:

Laboratory for Core Binding Factor Leukaemia Molecular Monitoring:

Dr Abida Awan
Molecular Diagnostics Centre
2nd Floor CADET and MDC Building
Central Manchester University Hospitals NHS
Foundation Trust
Manchester Royal Infirmary
Oxford Road
Manchester
M13 9WL
Tel: 0161 276 4137
Fax: 0161 276 4814
Email: abida.Anwan @cmmc.nhs.uk

Prof J A L Yin
Department of Haematology
Manchester Royal Infirmary
Oxford Road
Manchester
M13 9WL
Tel: 0161 276 4802 (direct to Sec)
Fax: 0161 276 4814
Email: jyin@labmed.cmht.nwest.nhs.uk

It is recognised that a small number of Core Binding Factor Leukaemias will already have entered the FLT3 inhibitor randomisation before the cytogenetic information was available. These patients should continue in the FLT3 assessment and should continue to receive the inhibitor medication. It is possible (but unlikely) that the cytogenetic definition of CBF leukaemia may become available before the FLT3 mutation status. These patients should enter the FLT3 randomisation.

11.5 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 internet system will allocate the risk category and indicate what treatment options are available.

N.B. Treatment of patients with Core Binding Factor leukaemias or who have a FLT3 mutation, 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

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/m² daily by 30-minute i.v infusion on days 2-6 inclusive (5 doses).

Cytosine Arabinoside 2 g/m² 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/m² 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/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 3 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.

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 1x10⁹/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/m² 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.

Children who are assessed to be high risk will enter the FLAG-Ida arm only.

11.6 mTOR Inhibition - Everolimus

Adults who do not have Core Binding Factor Leukaemia, a FLT3 mutation, or a High Risk Score are eligible to enter a randomisation to receive or not the mTOR inhibitor **Everolimus** (in a 2:1 ratio). This will be given as a starting daily oral dose of 5mgs starting 2 days after the end on each chemotherapy course starting after course 2 for up to 28 days or until 2 days before the next course is initiated, whichever is shorter. If patients are allocated to receive a total of 3 courses of chemotherapy in total they should have a third course of Everolimus after a break of one week, and the treatment should continue for 28 days. Patients allocated to Everolimus should have a formal assessment on day 14 of each course of Everolimus treatment. This aim of this assessment is to assess treatment compliance/ whether the daily dose can be increased to 10mgs/ and to obtain a pre-dose peripheral blood sample for associate pharmaco-dynamic studies. (investigators will be provided with a day 14 assessment form for this purpose) Patients entering the mTOR randomisation should use **Patient Information Sheet 6 and Consent Sheet 6**

Patients are requested to provide a 10ml sample of peripheral blood immediately before each treatment course and on the day 14 assessment visit of each course. Reminders will be sent to investigators where this is required.

Prior to commencement of Everolimus, 10ml of blood (in EDTA) should be sent to Dr P White at the Cardiff laboratory

The side effect profile of Everolimus is described in Appendix B. It should be noted that hyperlipidaemia and hyperglycaemia may occur and these levels should be monitored after each course, ie immediately before the next chemotherapy course.

11.6.1 Dose Adjustment

If patients have side effects which are thought to be due to the administration of Everolimus, the subsequent dose can be reduced by 50%. If this happens on the 5mg/day dose a reduction to alternate days should be implemented, if it occurs on the 10mg dose the patient should return to a 5mg daily dose.. If these dose reductions are not tolerated, subsequent doses should be omitted, but investigators are encouraged to deliver the prescribed dose. If a patient is tolerating the 10mg dose after course 1, they should initiate subsequent courses of Everolimus at the 10mg dose.

11.6.2 Everolimus Day 14 assessment visit

Patients who are being treated with Everolimus should be reviewed on or as near as possible to day 14 from the start of each course of Everolimus treatment (if day 14 falls on a Friday, please delay until the following Monday). They should be evaluated for drug tolerance and a dose adjustment made if required. Patients should be asked to delay taking their morning Everolimus dose on the day of assessment until blood sampling has been performed. At each day 14 assessment, 20ml of blood should be collected (in EDTA) and sent to Dr P White at the Cardiff laboratory, using the Royal Mail collection service provided. Please complete the online/hard copy Everolimus day 14 assessment questionnaire which can be found on the AML17 website at the time of each day 14 assessment.

11.7 Progression Through Induction Therapy

FLT3 mutation status should be available by the end of the first course of chemotherapy, (see Section 10). After recovery from course 1 and assessment of response, the risk score can be provided for individual patients who are not already in the CEP-701 randomisation or with 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 11.5. 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 $1.0 \times 10^9/l$ neutrophils and $100 \times 10^9/l$ 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. Patients who have been allocated to receive an inhibitor should continue to receive the inhibitor irrespective of which consolidation chemotherapy arm is allocated

12. CONSOLIDATION RANDOMISATION

Note: For this randomisation **Patient Information Sheet 7** and **Consent Form 7** should be used. **Note: the consolidation options for children (patients < 16 years of age) is different and is set out in Section 13.2**

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. Patients who have already been allocated to receive FLT3 or mTOR inhibition will continue with that treatment as well.

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).
- Whether already enrolled in the FLT3 or mTOR inhibitor randomisations.

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/m² 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/m² 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 1.0x10⁹/l neutrophils and 100x10⁹/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.

Patients who have entered the FLT3 inhibitor or mTOR randomisation, and who have been allocated CEP-701 or Everolimus respectively, should receive the drug after courses 3 and 4 as described in sections 11.3 and 11.6.

13.2 Consolidation for Children

Patients under 16 years of age (children) will enter consolidation after they have received and recovered from course 2. They will receive two courses of **High Dose Ara-C**.

Course 3

High Dose Ara-C (3.0 g/m²)

Cytosine Arabinoside 3.0 g/m² 12-hourly by 4 hour i.v. infusion on days 1, 3 and 5 (6 doses).

Course 4

High Dose Ara-C (3.0 g/m²)

Cytosine Arabinoside 3.0 g/m² 12-hourly by 4 hour i.v. infusion on days 1, 3 and 5 (6 doses).

Note: Prednisolone (0.5% Predsol) eye drops should be used during each course of high-dose Ara-C, and be continued for 5 days after the course finishes.

In children under 1 year the Ara-C dose should be calculated on body weight ie the 3.0g/m² will be calculated as 100mg/kg, and all other drug doses should be reduced by 25% in children less than one year or weighing less than 10kgs.

It is preferable to commence course 4 after count recovery (Neutrophils to 1.0 x10⁹/l; platelets to 100 x 10⁹/l).

14 SUMMARY OF MODIFICATIONS FOR CHILDREN

There are a number of differences between the treatment of adults and children within the AML17 protocol. These are indicated in the relevant sections and are summarised below.

14.1. Eligibility:

Children with acute myeloid leukaemia and high risk myelodysplastic syndrome other than those with:

- APL
- Down syndrome AML

14.2. Treatment Variations for Children

At diagnosis: D(90)A versus D(60)A (two arms)

The two induction treatment arms will therefore be:

Arm A D(90)A in course 1, followed by D(50)A as course 2

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

End of Course 1 FLT3 inhibitor (CEP-701) versus placebo for FLT3 mutation positive patients.

By the end of the first course of induction chemotherapy (day 10), the FLT3 mutation status will be available to enable randomisation to CEP-701 or placebo. Patients with a FLT3 mutation can be randomised to start FLT3 inhibition or placebo for four courses after *each course* of chemotherapy (Sections 4.1.3 and 11.3). **NB:** in children it is required that liver function tests must be within 3 x the upper limit of locally determined limit. The serum lipase, amylase and liver function should be checked weekly during CEP-701 treatment in course 1 and fortnightly subsequently. If a patient develops significant abnormality the case should be discussed with a trial co-ordinator.

On recovery of counts, cytogenetics and molecular screening (Core Binding Factor), and Risk Index status of each patient will be available (this risk index will be provided by the online service which must be used.)

Core Binding Factor Leukaemias will receive mylotarg (3mg/m²) on day 1 of course 2 Occasional patients with CBF leukaemia who are also FLT3 positive are eligible for the CEP-701 randomisation.

All patients except the High Risk patients will receive a second induction treatment course with D(50)A.

Patients who have a High Risk Index will receive FLAG/Ida as Course 2.

In consolidation

All patients except the High Risk patients will receive two courses of High Dose Ara-C.

High Risk Index patients, who are not proceeding to SCT, may receive up to three courses of FLAG-Ida and those proceeding to SCT may receive two courses of FLAG-Ida.

Allogeneic stem cell transplant which will be assessed on a donor vs no-donor and transplant given versus not basis.

Patients may enter a randomisation to be MRD monitored (section16)

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.

14.3 Paediatric Management Group

Clinical Coordinator

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Cytogenetics Coordination:

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14.4 Central Nervous System Prophylaxis and Treatment in Children

A lumbar puncture should be performed at the time of diagnosis in all children. CNS disease is defined by the presence of $>5 \times 10^6/l$ leukaemic blasts in a CSF cytospin preparation.

14.4.1 No CNS disease

If there is no evidence of CNS disease at diagnosis patients should receive a total of two courses of "triple" Intrathecal chemotherapy, one after each of the first two courses of chemotherapy.

Triple Therapy

AGE (years)	METHOTREXATE	CYTARABINE	HYDROCORTISONE
<1	5mg	15mg	5mg
1	7.5mg	20mg	7.5mg
2	10mg	25mg	10mg
3+	12.5mg	30mg	12.5mg

14.4.2 CNS disease at diagnosis

If CNS disease is present at diagnosis, patients should receive two courses of "triple" intrathecal therapy (as in Section 14.3.1 above) each week until the CNS is clear plus two further courses. A minimum of six courses should be given in a period of three weeks following diagnosis.

This intensive phase is followed by monthly courses of the same “triple” therapy until after the final course of systemic chemotherapy has been completed, and then two further courses of triple therapy which should be given at monthly intervals. Only children who fail to clear disease with intrathecal therapy should be considered for cranial radiotherapy.

14.5 Stem Cell Transplant

SCT will follow the recommendation of the CCLG BMT Subcommittee (copy available from Paediatric Co-ordinator).

14.5.1 Dosing Guidance

- Etoposide should be administered as a 4 hour infusion.
- Idarubicin should be administered as a 1 hour infusion.
- G-CSF where used, should be given intravenously.

14.5.2 Summary of chemotherapy reductions for children under one year of age or less than 10 kg

DA	All doses should be reduced by 25%
FLAG-Ida	All drug doses should be reduced by 25% except for GCSF, which should not be reduced
HD Ara-C 3gms/m²	Calculate Ara-C dose from patient weight - 100mg/kg per dose
CEP-701	NOT ON concomitant azole anti-fungal prophylaxis starting dose of 62.5mg bd ON concomitant azole anti-fungal prophylaxis starting dose of 31.25mg bd

14.6 CEP-701

Children with a body surface area $\geq 1.3\text{m}^2$ will receive CEP-701 / placebo according to the adult treatment schedule i.e. a starting dose of 80mg bd. Those receiving concomitant azole anti-fungal prophylaxis will start at 50 % of this dose i.e. 40mg bd.

Children with a body surface area $< 1.3\text{m}^2$ will receive CEP-701 / placebo at the modified paediatric dose of 62.5mg/m². Those receiving concomitant azole anti-fungal prophylaxis will start at 50% of this dose i.e. 31.25mg/m².

If well-tolerated by the patient, the dose of CEP-701 / placebo may be increased provided a minimum of 7 days has elapsed on the previous dose. Dosing may be increased in increments of 20mg bd for children with a body surface area $\geq 1.3\text{m}^2$ or by 15.6mg/m² bd for children with a body surface area $< 1.3\text{m}^2$

The maximum dose of CEP-701 / placebo is 100mg bd for children with a body surface area $\geq 1.3\text{m}^2$ or 78mg/m² bd for children with a body surface area $< 1.3\text{m}^2$.

Children entering the FLT3 randomisation are expected to be infrequent and most centres will enter only one or two patients.

Treatment with CEP-701 should begin 2 days after the completion of each course of chemotherapy and should continue until 2 days before the commencement of the next course, or for a maximum

of 28 days after the end of the previous course of chemotherapy. It is important to discontinue CEP-701 two days before the start of a subsequent chemotherapy course because elevated CEP-701 plasma concentrations may interfere with the effect of chemotherapy.

Under no circumstances should CEP-701 be co-administered with any course of chemotherapy treatment.

CEP-701 should be diluted with fruit juice prior to administration. See main AML17 protocol for further information about administration, supply and day 14 assessments required with each course of treatment. CEP-701 may be administered via a nasogastric tube if necessary, when it should be diluted in fruit juice as if taken orally. Undiluted CEP-701 would predictably increase the likelihood of nausea and vomiting.

The serum lipase, amylase and liver function should be checked weekly during CEP-701 treatment after Course 1 and every two weeks subsequently.

The most likely side-effect associated with CEP-701 treatment is nausea, so patients should routinely be given a prophylactic antiemetic. Information about unblinding SUSARs in patients randomised to the CEP-701/placebo trial arm can be located in Section 22.1.5 of the main AML17 protocol.

15 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. 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). [For children aged less than 2 years, conditioning should be with Busulphan and Cyclophosphamide] (See Appendix E).
2. Patients 35-44 years can receive a conventional allogeneic transplant or a reduced intensity allograft depending on investigator or patient choice.
3. Patients ≥45 years should receive a "mini" allograft.
4. Children should be transplanted according to CCLB BMT Group recommendations.

15.1 Conventional 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 4×10^6 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. Children intended for SCT will have a conventional allograft following CCLG Protocols (Section 14).

15.2 Reduced Intensity Allograft

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.

Transplant centres initially may wish to choose one of two reduced intensity protocols:

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)

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

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 E) should be completed via the web-based system.

16 ARRANGEMENTS FOR MOLECULAR SCREENING AND MINIMAL RESIDUAL DISEASE MONITORING

16.1 Molecular Screening

All diagnostic material will be collected into the AML cell bank at the UCL (Professor R Gale) or Cardiff (Dr P White) 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 mutation assessment to enable entry into the FLT3 inhibitor randomisation. Samples will also be assessed for in vitro sensitivity to CEP-701.

Laboratory Contacts:

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Department of Haematology
Cardiff University School of Medicine
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Prof R Gale
Cancer Institute, Department of Haematology
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16.2 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). For patients known to have CBF leukaemia post induction samples can be sent to Professor Yin in Manchester. who will provide monitoring on a commercial basis.. 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 Manchester for CBF leukaemia, 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

AML1-ETO/CBFB-MYH11

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Other Molecular Targets

Prof David Grimwade
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Samples from children for molecular screening and MRD monitoring should be sent to their designated reference laboratory (Paul Virgo, Bristol)

16.3 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 16.2).. 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 3 years following consolidation, to establish the most appropriate monitoring schedule for any given target.. Monitoring of Core Binding Factor Leukaemia will be available from the reference lab in Manchester, but is not being evaluated in the clinical evaluation of disease monitoring as described in section 16.5. 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

16.4 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:

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Division of Infection and Immunity
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Mr Paul Virgo
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16.5 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.

17 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.

18 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 $\geq 38^{\circ}\text{C}$ two hours apart, or a single reading $\geq 39^{\circ}\text{C}$
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 5 $\mu\text{g}/\text{kg}$ 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.

19 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.

The approach to CNS prophylaxis is different for children and is described in Section 14.3.

Section D:

ACUTE PROMYELOCYTIC LEUKAEMIA

20 ACUTE PROMYELOCYTIC LEUKAEMIA

20.1 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 20.10 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 16) with the aim of identifying patients failing first line therapy who require additional therapy in first CR (see Section 20.9) 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**.

20.2 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.

20.3 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 (\leq 51 μ mol/L)
- Serum creatinine \leq 3.0 mg/dL (\leq 260 μ mol/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.

20.4 Arm A. - AIDA Treatment

20.4.1 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).

20.4.2 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 >1.5x10⁹/L and platelets >100x10⁹/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/m²/d by short (20 minute) intravenous infusion on days 1, 2, 3, 4.

ATRA, 45 mg/m²/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/m²/d as 30 minute intravenous infusion on days 1, 2, 3, 4, and 5.

ATRA, 45 mg/m²/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/m²/d as short (20 minute) intravenous infusion only on day 1.

ATRA, 45 mg/m²/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.

20.5 ARM B: ATRA with Arsenic Trioxide

20.5.1 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 (As₂O₃=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.

20.5.2 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.

20.5.3 Patients with High White Counts at Diagnosis

Patients who present with a peripheral white cell count of >10x10⁹/l have a higher chance of developing differentiation syndrome if allocated to the ATRA plus Arsenic treatment. These patients should receive Mylotarg 3mg/m² on day 1 of treatment and on day 4 if the white count has not fallen below 10x 10⁹/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.

20.5.4 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).

20.6 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.

20.7 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.

20.8 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

20.8.1 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 hyperleukocytosis (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.

20.8.2 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.

20.8.3 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 hepatotoxicity 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.

20.9 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 20.10). 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.

20.10 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

20.11 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

21 STATISTICAL CONSIDERATIONS

21.1 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

For the randomisations to targeted therapy, approximately 90% of patients (i.e. 2450 patients) will commence their second course of treatment. Data from previous MRC AML trials have been used to determine the likely recruitment to each question. It is anticipated that at least 300 patients will enter the CEP-701 randomisation where two thirds of the patients will receive CEP-701. Together with the approximately 200 patients who will be randomised (in a 1:1 fashion) as part of AML15, this gives a total of at least 500 patients in this comparison. . Data from the AML10,12 trials show that five-year DFS in this group is about 33%; so the meta-analysis of AML15 and AML17 should have 80% power to detect a difference in DFS of 12% (from 33% to 45%).

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%. It is likely that other new therapies suitable for this group of patients will become available during the course of the trial and 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.

Assuming that around 15% of non-APL patients will have a core binding factor leukaemia, there remains a group of approximately 900 patients who do not fall into one of the predefined categories (CBF, FLT-3 positive, poor risk), and are therefore eligible for randomisation between mTOR inhibition and not. Assuming a 70% uptake of this randomisation, this means that there should be in excess of 600 patients who will be randomised in a 2:1 ratio between mTOR inhibitor and not. With 600 patients recruited there will be 85% power to detect a 12.5% improvement in disease-free survival (from 50% to 62.5% at 5 years). Again a primary endpoint of DFS is chosen because of the option for patients who relapse to enter the "poor risk" randomisation.

Of the patients who are not considered to be "poor risk", at least 80% should enter CR and therefore eligible for the consolidation randomisations. This equates to around 1600 patients. Even if only two-thirds of such patients are randomised to the consolidation questions (roughly equivalent to the rate in AML15), there will be at least 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. At this point the future direction of the MRD project will be assessed based on the preliminary results, and the treatment randomisation powered in line with the results found in the first stage.

21.2 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

* 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.

survival for all randomisations except the APL randomisation (see below), or those to mTOR or FLT-3 inhibition, where the primary outcome is DFS. 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 Group; simultaneous adjustment for more than one stratification variable will be by means of logistic or Cox regression analysis.

22. 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.

22.1 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.

22.1.1 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**, which does not resolve to grade 2 or less within 7 days
- Development of any grade 4 non-haematological toxicity (excluding alopecia)
- Development of neutropenia ($<1.0 \times 10^9/L$) or thrombocytopenia ($<50 \times 10^9/L$) for longer than 42 days after the end of chemotherapy in the absence of significant disease in the bone marrow ($>5\%$ blasts)
- 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

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

- 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
- 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

22.1.2 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.

22.1.3 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 02920742289

22.1.4 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.

22.1.5 Unblinding SUSAR's in patients randomised to the CEP 701/Placebo trial arm.

All sites will be provided with Code break envelopes once their patient has been randomised to the CEP 701/Placebo arm of the trial. These are normally held by the site pharmacy department.

The CEP701/Placebo arm of the study should only be unblinded for a valid medical or safety reason, for example, a serious adverse where it is necessary for the PI or treating healthcare professional to know which treatment the patients has been receiving to ensure the participant can receive appropriate safety measures. In this event the research team should contact the Trials office at 029 2184 7928 (office hours) or 029 20747747 (outside office hours, this is the hospital switchboard telephone number who has a direct number for the CI or his delegate).

22.1.6 Enhanced Pharmacovigilance

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 pharmacovigilance officer or her nominee will seek information of any treatment adverse effects or compliance difficulties.

22.1.7 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(CBF α)/ETO)	9896/3
Acute myeloid leukaemia with abnormal bone marrow eosinophils Inv(16)(p13q22) or t(16;16)(p13;q22); (CBF β /MYH11)	9871/3
Acute Promyelocytic leukaemia (AML with t(15;17)(q22;q12-21), (PML/RAR α) and variants.	9866/3
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 dysrhythmias. Subcutaneous extravasation may cause severe tissue necrosis.

Centres may have an established practice of administering Daunorubicin 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.

Etoposide (VP16-213) (Vepesid™ - Bristol-Myers Pharmaceuticals)

Etoposide is available as a solution in ampoules containing 100 mg in 5 ml. The appropriate dose should be diluted in sodium chloride 0.9% for infusion. The company recommend that the infusion solution concentration does not exceed 0.25 mg/ml of etoposide.

Give over at least 30 minutes as hypotension may be produced by excessively rapid infusion.

For hepatic dysfunction with bilirubin of 26-51µmol/L reduce dose by 50%. At higher bilirubin levels the decision to administer is a clinical one. For renal dysfunction dose reduction should be: Creatinine Clearance (Cr Cl) of 60ml/min the dose should be 85%; Cr Cl of 45ml/min reduce dose by 20%; for a Cr Cl of 30ml/min reduce dose by 25%.

Side effects include tissue necrosis if extravasation should occur, nausea, mucositis and alopecia. Anaphylactic-like reactions have been reported rarely and have responded to stopping the infusion and the administration of an antihistamine and hydrocortisone.

Gemtuzumab Ozogamicin — Mylotarg™ (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 (2-8°C).

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

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/m²/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 (EndoxanaTM – 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 (VesanoidTM - 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 (EvoltraTM 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°C and 48 hours at refrigerated temperatures (2-8°C). 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 2-8°C, 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.

CEP-701 (Cephalon Inc.)

The study drug should be stored, under adequate security, in the pharmacy at the study centre at a controlled room temperature of 20°C to 25°C (68°F to 77°F), protected from light, until taken by the study patients or until returned to Cephalon or its designated agent. The study drug is stable for at least 30 months from the day of manufacture if stored in amber bottles, protected from light, at a controlled room temperature below 25°C. Further information on CEP-701 is provided in Appendix C.

RAD001 (Everolimus Novartis)

RAD001 has been given as monotherapy to nearly 1000 patients with advanced cancer and over 100 healthy volunteers. Phase I studies established safety at 10mgs oral daily or 50mgs oral weekly. The most frequent side effects which occurred in 15% of patients was stomatitis and fatigue. Some patients also developed hyperglycaemia. Some patients developed a rash or erythema. Biomarker analysis indicated efficacy with both daily weekly dosing schedules.

Human Pharmacokinetics and Metabolism

General Clinical Pharmacokinetics

Absorption: Following oral administration in patients with advanced solid tumours, RAD001 is rapidly absorbed with a median time to peak concentration of 1-2 hours post dose. The steady-

state AUC_{0-} is dose-proportional over the dose range of 5 to 70mg in the weekly regimen and 5 to 10mg in the daily dosing regimen. C_{max} is dose-proportional between 5 and 10mg for both the weekly and daily regimens. At doses of 20mg/week and higher, the increase in C_{max} is less than dose-proportional. The coefficient of variation between patients is approximately 50%. Similar exposure-dose relationship was also noted in kidney transplant patients in a single-dose escalation study. The extent of RAD001 absorption in renal transplant patients (n=4), when RAD001 was administered as a solid dispersion in capsule concomitantly with cyclosporine, was estimated to be about 11% or higher based on the amount of radio-labelled RAD001 present in blood at t_{max} .

RAD001 is a substrate and weak inhibitor of P-glycoprotein.

Food Effects: When RAD001 was administered as six 0.25-mg dispersible tablets in healthy subjects, a high-fat breakfast reduced C_{max} by 50% and delayed the median t_{max} by 2 hours. However, the high-fat breakfast had no apparent effects on the AUC and $t_{1/2}$. The RAD001 blood concentrations in the post-absorption phase between 4 to 72 hours post-dose were similar when RAD001 was administered with food or without food.

When RAD001 was administered as two 1-mg Certican MF tablets in healthy subjects, a high-fat breakfast reduced C_{max} by 60% and delayed the median t_{max} by 1.3h. There was a 16% reduction in AUC. However, the RAD001 blood concentrations in the post-absorption phase between 4 to 72 hours post-dose were similar when RAD001 was administered with food or without food.

Thus, food can potentially reduce the C_{max}/C_{min} ratio of RAD001 at steady-state in the daily dosing regimen.

Distribution: The distribution of RAD001 between human blood cells and plasma was concentration-dependent over the range of 5 to 5000ng/mL. The proportion of RAD001 confined to plasma is approximately 20% at the concentration observed in cancer patients given 10mg/day of RAD001 whereas it increased from 17 to 73% over the wide concentration range (5-5000ng/mL). Plasma protein binding is approximately 74% in healthy subjects as well as patients with moderate hepatic impairment.

Metabolism: RAD001 is extensively metabolized in the liver. It is a substrate of CYP3A4. The main metabolic pathways identified in humans were mono-hydroxylations and O-dealkylations.

Two main metabolites were formed by hydrolysis of the cyclic lactone. The parent drug, RAD001 was the main circulating component in blood. None of the main metabolites contributes significantly to the pharmacological activity of RAD001.

Excretion: Elimination is essentially that of RAD001 metabolites in the bile. Elimination half-life in cancer patients averaged 30 hours, which is similar to that in healthy subjects. After a single dose of [^{14}C]RAD001 in renal transplant patients, the majority (80%) of radioactivity was recovered in the faeces, only a minor amount (5%) was excreted in the urine over the 10-day collection period. Parent drug was not detected in urine and faeces.

Contraindications, possible risks and adverse events: RAD001 should not be administered to patients with a known hypersensitivity to RAD001 (Everolimus) or other rapamycins (sirolimus, temsirolimus) or to its excipients.

Interactions with other medications: RAD001 is a substrate of CYP3A4, and a substrate and moderate inhibitor of P-glycoprotein. Pharmacokinetic interaction with drugs which modify CYP3A4 enzymes have been reported. Drugs in this category frequently used in oncology patients include the azole antimycotics (itraconazole, ketoconazole) and erythromycin which are CYP3A4-inhibitors and the anti-epileptic drugs phenytoin and Carbamazepine which are CYP3A4 inducers.

Co-administration with strong CYP3A inhibitors (eg ketoconazole, itraconazole, ritonavir), and strong inducers (eg rifampin, rifabutin) should be used with caution.

Co-administration with substrates, inducers, or inhibitors of P-glycoprotein should be avoided if possible, or used subject to caution (eg increased frequency of safety monitoring, temporary interruption of RAD001).

Grapefruit and grapefruit juice affect cytochrome P450 and –glycoprotein activity and should therefore be avoided. Lists of clinically relevant inhibitors and inducers can be found in the Investigator Brochure.

Special patient populations

Hepatic impairment: The oral clearance (CL/F) of RAD001 in patients with mild-moderate hepatic impairment (Child-Pugh Class A or B) was approximately half of that in subjects with normal liver function. For patients with their serum bilirubin $>34 \mu\text{mol/L}$ ($>2 \text{ mg/L}$), serum albumin $<35\text{g/L}$ ($<3.5 \text{ g/dL}$), and INR > 1.3 (>4 second prolongation of prothrombin time), the dose of RAD001 should be reduced to one-half of the dose for patients with normal liver function. Patients with severe liver function impairment should not be entered into clinical trials.

Renal impairment: Renal impairment has little or no impact on the pharmacokinetics of RAD001.

Elderly: No dose adjustment is required for advanced age.

Ethnicity: Pharmacokinetic characteristics are not notably different between Caucasian and Japanese subjects whereas in Black transplant patients pharmacokinetic studies have shown an average 20% higher clearance. In oncology studies, dose modification for reason of ethnicity is not considered necessary given the apparent broad window for dose effect in the Phase 1 pharmacodynamic studies.

Use in Pregnancy and lactation: RAD001 must not be administered to pregnant women or to women who are breast feeding. Negativity of a recent pregnancy test is a required selection criterion for women of child-bearing potential.

Effective contraception must be practiced by patients for the duration of studies.

Studies in animals have shown reproductive toxicity effects including embryo/foetotoxicity. The potential risk for humans is unknown. Limited data are available concerning exposure to RAD001 in pregnancy in transplantation patients. Out of six women exposed to RAD001 immediately before and/or shortly after becoming pregnant, 4 delivered healthy babies, 2 terminated their pregnancies voluntarily, one had a spontaneous rupture of the membranes after 30 weeks gestation (the baby died 3 days after vaginal delivery). Two cases of paternal exposure were recorded, each resulting in healthy babies. The information provided by these data are insufficient to draw a meaningful conclusion on the effects of RAD001 exposure during pregnancy or lactation. In oncology studies, conception should be prevented during treatment with RAD001 and the drug must not be administered to pregnant women.

It is not known whether RAD001 is excreted in human milk. In animal studies, RAD001 and/or its metabolites were readily transferred into the milk of lactating rats. Therefore women who are taking RAD001 should not breast feed.

Oral anticoagulants such as warfarin are CYP2C9 substrates and, as such, no interaction with RAD001 is expected. However, drug-drug interaction studies between macrolide antibiotics and warfarin have produced missed outcomes and the disparity in these findings has led to the conclusion that multiple factors may alter the clearance of warfarin. The Coadministration of RAD001 and oral anticoagulants is possible but should be subject to verification of coagulation (INR) once steady state is reached (after one week's treatment).

RAD001 and oral contraceptives: Preclinical and clinical data have shown RAD001 to have CYP3A4 inhibitory activity rather than induction activity, induction or metabolism of contraceptive

hormones by RAD001 is unlikely. Consequently, administration of RAD001 should not reduce the efficacy of oral contraceptives

APPENDIX C Background Information on CEP-701

Name and Description of Investigational Product

CEP-701 is an orally bioavailable receptor-tyrosine kinase inhibitor. The compound is a chemically synthesized derivative of K-252a, a fermentation product of *Nonomurea longicatena*, and belongs to a class identified as indolocarbazole alkaloids. CEP-701 drug product is supplied in a carrier of polysorbate 80 NF and propylene glycol USP, 25 mg CEP-701/ml, in 100ml glass bottles. A more detailed description of the treatment is provided in Section 11.3.

Findings From Nonclinical and Clinical Studies

Nonclinical Pharmacology

CEP-701 exhibits most potency in the inhibition of the neurotrophin receptor Trk A (tropomyosin receptor kinase) (concentration at which 50% of the enzyme is inhibited [IC_{50}] 3 nM) and the fms-like tyrosine kinase, FLT3 (IC_{50} 3 nM). In addition, CEP-701 can be classed as a moderately potent inhibitor of vascular endothelial growth factor receptors (VEGFR) 1 to 3 (IC_{50} 37 to 76 nM), mixed lineage kinases 1 to 3 (IC_{50} 11 to 71 nM), and c-Jun N-terminal kinases $1\beta 1$ (IC_{50} 81 nM), $2\alpha 2$ (IC_{50} 113 nM), and $3\alpha 1$ (IC_{50} 31 nM). It displays weaker inhibitory activity against platelet-derived growth factor receptor β (IC_{50} 216 nM), rat brain protein kinase C (IC_{50} 226 nM), and fibroblast growth factor receptor (IC_{50} 420 nM). CEP-701 does not show appreciable inhibition of epithelial growth factor receptor or the β -insulin receptor kinase. When added to intact cells under plasma-free conditions, CEP-701 inhibits TrkA and FLT3 with IC_{50} of 25 and 1 to 2 nM, respectively.

The inhibition of Trks by CEP-701 may be of value as therapy for both prostate cancer and paediatric neuroblastoma, where this family of tyrosine-receptor kinases is believed to play a role in tumour cell survival and/or invasive growth. Treatment of rats bearing the Dunning H rat prostate cancer with CEP-701 caused a significant regression of established tumours, (Cephalon data on file). CEP-701 was also shown to inhibit the growth of DU145, PC3, and CWR22Rv1 human androgen-independent prostate cancer xenografts in nude mice.

Studies with human neuroblastoma cell lines have been performed with CEP-751, a close analog of CEP-701 that is metabolized to CEP-701 in vivo. Treatment of nude mice bearing such xenografts with CEP-751 resulted in a significant inhibition of tumour growth.

Nonclinical Pharmacokinetics

Pharmacokinetic parameters for intravenous (iv) CEP-701 are similar in rats and dogs, with a plasma clearance of 0.8 l/hr.kg, a volume of distribution of approximately 1.4 l/kg, and an elimination half-life ($t_{1/2}$) of 1.2 hours. Orally administered CEP-701 is rapidly absorbed in both rats and dogs, with peak plasma concentrations occurring within 2 to 4 hours of dosing. In dogs, the absolute oral bioavailability of CEP-701 from a vehicle composed of polysorbate 80 and propylene glycol is 39%. The oral bioavailability of CEP-701 from solution formulations in dogs is more than 10-fold higher than in rats.

In vitro, CEP-701 binds with high affinity to human alpha-1-acid glycoprotein (hAGP), resulting in an estimated unbound, biologically active fraction of less than 1% in human plasma. The binding appears saturable. In an intact cell assay, hAGP (1 mg/ml) shifted the IC_{50} value of CEP-701 for TrkA 750-fold, from 8 nM to 6000 nM. In the same assay, 2 samples of human plasma caused 250- and 500-fold shifts in this IC_{50} value. The significance of these findings with respect to the clinical activity of CEP-701 is not known; however; they most probably explain the need to attain micromolar plasma concentrations of CEP-701 in order to observe biological effects in patients.

CEP-701 is primarily metabolised via hydroxylation and glucuronidation. Results from an in vitro study with cDNA-expressed human cytochrome P450 enzymes (CYP) indicate that CYP3A4, CYP1A2, and CYP2B6 may be involved in the metabolism of CEP-701. CEP-701 inhibits several CYP isoforms in human liver microsomal preparations, with K_i values below 5 μ M (approximately 2 μ g/mL) for CYP1A2, CYP2C9, and CYP3A4; and 10 μ M (approximately 4 μ g/mL) for CYP2C19. The potential for CEP-701 to inhibit these CYP isoforms in vivo has not been assessed. Caution

regarding concomitant administration of drugs that are substrates for these CYP isoforms, and especially those with narrow therapeutic indices, would be prudent.

Nonclinical Toxicology

The toxicity of CEP-701 has been evaluated in single-dose oral administration studies in mice, rats, and dogs (table C1). In addition, repeated-dose oral administration studies have been completed in rats (6 months) and dogs (9 months). In the single-dose studies, the lethal dose at which 10% of mice died after experiencing adverse events (LD₁₀) was 73 mg/kg, and an LD₅₀ of 103 mg/kg was determined in rats. A maximum tolerated dose (MTD) of 100 mg/kg was determined in dogs.

Table C1: Summary of Key Toxicology Parameters

Species	Parameter	Dosage (mg/kg/day)	Dosage (mg/m ² /day)	Human dosage ^a (mg/day)
Mouse	LD ₁₀	73	219	379
Rat	LD ₅₀	103	618	1069
Dog	Acute MTD	100	2000	3460
Rat	6-month MTD	20	120	208
Dog	9-month MTD	5	100	173

^a Extrapolated data, assuming a body surface area of 1.73 m².

LD₁₀ = the dose at which 10% of the species experienced adverse events that resulted in death.

LD₅₀ = the dose at which 50% of the species experienced adverse events that resulted in death.

MTD = maximum tolerated dose.

In a 6-month study, rats received CEP-701 by oral gavage at 0.8, 4, or 20 mg/kg/day. There was no compound-related mortality. A reduction in body weight gain was observed in all dose groups relative to the deionised water control group, including the CEP-701 vehicle group. However, the most prominent reduction in body weight gain (9% to 11% relative to vehicle) was noted in the 20-mg/kg/day CEP-701 dose group. No CEP-701-related changes were noted in ophthalmology, haematology, serum chemistry, or urinalysis data. Changes interpreted to be related to administration of CEP-701 were detected microscopically in the epithelium of the anorectal junction, caecum, extrahepatic bile duct, and the main pancreatic duct. Microscopic changes were described as “epithelial cell alteration” (bile duct, pancreatic duct, and caecum) and inflammation (anorectal junction). Treatment-related changes in these epithelia were subtle, did not occur in all treated animals/sections of affected groups, and, in some sites, were morphologically similar to microscopic changes that occurred in some control animals. No treatment-related changes were found histologically in rats following a 6-week recovery period. On the basis of the results of this 6-month oral toxicity study, 20 mg/kg/day of CEP-701 was identified at the MTD in rats.

In a 9-month study, dogs received CEP-701 by oral gavage at dosages of 0.2, 1, and 5 mg/kg/day. There was no compound-related mortality or morbidity. An increased incidence of emesis was noted in both male and female dogs in the 5.0 mg/kg/day dose group relative to dogs in both control groups. In general, increased salivation was noted in male and female dogs only in the 5.0 mg/kg dose group. There were no other compound-related clinical observations. There were no compound-related changes noted in body weights, food consumption, ophthalmology, haematology, serum chemistry, urinalysis, electrocardiograms, or organ weight data. Changes interpreted to be related to CEP-701 were detected microscopically in the mucosa of the anorectal junction of both male and female dogs in the 5.0 mg/kg/day dose group. Many dogs from the other groups, including the deionised water and vehicle control groups, also had evidence of inflammation within the anorectal mucosa. However, in the high-dose group, the inflammation was more severe and there was evidence of hyperplastic changes within deeper layers of the anorectal epithelium. In addition, the high-dose group had a shift in cell populations that included large numbers of neutrophils, especially in the squamous mucosa of the anal area. There were no new treatment-related histologic changes following the 9-week recovery period. Basal cell hyperplasia

also resolved, indicating that the chronic irritation with CEP-701 administration had subsided. On the basis of the results of this 9-month oral toxicity study, 5 mg/kg/day of CEP-701 was identified as the MTD in dogs.

In addition to these findings, it is noted that a dosage of 15 mg/kg/day in dogs was found to be associated with significant toxicity when administered for 28 days. Clinical signs included hunched posture, decreased activity, weight loss, vomiting, and increased salivation. Faecal occult blood was increased in these animals, and gastrointestinal haemorrhage was noted in some males. Four of the 10 dogs dosed at 15 mg/kg/day were euthanised *in extremis* prior to the completion of the 28-day study. Terminal serum chemistry findings in these animals were consistent with cholestasis, and hepatic inflammation and necrosis. Treatment-associated lesions were observed throughout the gastrointestinal tract as well as in the liver, gallbladder, bile duct, prostate, and thymus. The day-28 toxicokinetics revealed a maximum plasma concentration (C_{max}) of 385 ng/ml in males and 606 ng/ml in females, and an area under the plasma concentration-time curve from time zero to 24 hours (AUC_{0-24}) of 1365 and 733 ng-hr/ml, respectively.

A battery of *in vitro* and *in vivo* tests for genotoxicity has been conducted with CEP-701. CEP-701 was negative in the bacterial point (Ames) assay and the *in vivo* aneuploidy/micronucleus assay. CEP-701 was positive in the mouse lymphoma mutagenesis assay (–S9 5.0 µg/ml, +S9 2.5 µg/ml), and also caused increased percentages of polyploidy (–S9 ≥ 0.4 µg/ml) and endo-reduplicated cells (+S9 ≥ 5.0 µg/ml) in the Chinese hamster ovary cell chromosome aberration assay. In a chromosome aberration assay with human peripheral blood lymphocytes (HPBL), CEP-701 caused an increase in the number of polyploid cells (–S9 ≥ 0.38 µg/ml, +S9 ≥ 2.0 µg/ml). In a separate study with HPBLs, CEP-701 was able to induce micronuclei (–S9 0.74 µg/ml, +S9 1.4 µg/ml). Subsequent investigation into the mechanism of action via the application of a pain-centromeric DNA probe indicated that micronuclei were generated via an aneugenic mechanism.

Ancillary pharmacology studies of CEP-701 suggest little potential for interaction with the cardiovascular and gastrointestinal smooth muscle systems. Effects on renal excretion and interactions with the central nervous system were apparent only at oral doses of 100 mg/kg or above.

Clinical Experience

Healthy Subjects

The safety and pharmacokinetics of single doses of oral CEP-701 from 3 to 20 mg were evaluated in healthy subjects (study M97-792). Nine subjects were treated at each dose; 6 received active drug and 3 received a placebo. C_{max} of CEP-701 ranged from 114 to 1041 ng/ml, mean AUC from 929 to 7889 ng-h/ml, and mean apparent $t_{1/2}$ from 6.8 to 9.2 hours. There was no significant departure from dose proportionality. In the second stage of the study, in which 12 subjects received 10 mg CEP-701 under fasting and non-fasting conditions, the time to maximum plasma concentration (t_{max}) was lengthened from 1.8 hours to 3.7 hours ($p < 0.001$) and C_{max} reduced from 403 to 294 ng/mL ($p = 0.002$) when CEP-701 was taken with food. CEP-701 was well tolerated and treatment-emergent adverse events were mild in nature (headache, sweating, constipation). No clinically significant abnormal haematology, chemistry, urinalysis, or stool laboratory values were observed.

In a separate study (study M99-067), the tolerability of oral CEP-701 vehicle (polysorbate 80 and propylene glycol, USP) was assessed in 24 healthy subjects who were administered twice-daily volumes of 3.2 to 9.6 ml of the vehicle diluted to 30 ml in apple juice for 14 days. Results indicated that the vehicle alone was well tolerated at volumes used in clinical studies.

Patients with Cancer

The safety and/or efficacy of CEP-701 in cancer patients has been explored in 5 Phase 1 or Phase 2 studies. In a pilot study, CEP-701 was administered preoperatively for 5 days at 40 mg bd to 9 men with localized prostate cancer who had elected to have radical prostatectomy (study M99-108). In 5 patients who completed the study, the mean plasma concentration of CEP-701 was 427.9 ng/ml immediately prior to surgery, and the mean prostate tissue concentration was 97.1 ng/g, indicating reasonable tissue penetration.

The MTD and dose-limiting toxicities (DLTs) of repeated doses of CEP-701 were defined in a study of patients with advanced solid tumors (study M98-909). A total of 30 patients were enrolled at twice-daily doses of 5 mg (n=3), 10 mg (n=3), 20 mg (n=3), 40 mg (n=13), 80 mg (n=7), and 120 mg (n=1). The majority of patients (67%) received a single 28-day cycle of CEP-701. However, 7 patients received study drug for at least 3 months, including 3 patients who were treated for more than 6 months. One patient received 13 cycles of treatment. The most frequently reported adverse events were nausea (63%), diarrhoea (47%), anorexia (37%), asthenia (30%), constipation (27%), and vomiting (27%). The incidence of adverse events tended to be greater in patients who received at least 40 mg bd. Most of these events were intermittent, but lasted several days.

Dose-limiting toxicities were reported for 1 patient at 80 mg bd (grade 3 nausea) and 1 patient at 120 mg bd (grade 3 hypotension). The formal definition of MTD was not met for either of these dosages, but treatment-related adverse events, especially of the gastrointestinal system, made CEP-701 poorly tolerated at 80 mg and above. After expanding the MTD group (40 mg bd) to 13 patients, DLTs were reported in 2 patients at this dosage (grade 3 anorexia, nausea, dyspepsia and grade 3 asthenia). Thus, the MTD of CEP-701 without antiemetic prophylaxis was considered to be 40 mg bd. However, in view of the intermittent nature of many of the gastrointestinal events, the tolerability of higher dosages (60 and 80 mg bd) is considered worthy of further study when CEP-701 is given with appropriate antiemetic support.

The pharmacokinetics of CEP-701 in patients with cancer were found to be similar to those in healthy subjects. CEP-701 was rapidly absorbed, with mean t_{max} values ranging from 0.8 to 2.7 hours on days 1 and 28 across all dosages. At 40 mg bd, the day-28 mean C_{max} was 3973 ng/mL, and the mean AUC_{0-12} was 26630 ng·hr/mL. At 80 mg bd, the day-28 mean C_{max} was 12117 ng/mL, and the mean AUC_{0-12} was 114857 ng·hr/mL. Plots of dose-normalized CEP-701 AUC_{0-12} on days 1 and 28 indicated greater than dose-proportional increases in AUC_{0-12} after multiple doses. As a result there was greater accumulation of CEP-701 with bd administration at higher doses, with mean accumulation factors of 0.9 at 5 mg, 1.7 at 10 and 20 mg, 3.0 at 40 mg, and 4.1 at 80 mg.

CEP-701 did not produce an objective tumour response in any patient. The median duration of treatment was 5 weeks. Three patients had stable disease for more than 6 months and 1 of the patients with small cell lung cancer was stable for almost a year.

In study M99-051, a 40-mg bd dosage of CEP-701 was shown to be ineffective in maintaining or lowering prostate specific antigen concentrations in patients with hormone-refractory prostate cancer. Eight-week trough plasma concentrations of 1538 ng/ml (n=37) were similar to plasma concentrations observed in earlier studies. Nausea and diarrhoea were the most common adverse events, observed in 42% and 30% of patients, respectively. Severe gastrointestinal events (nausea, vomiting, abdominal pain, diarrhoea) considered possibly or probably related to CEP-701 were recorded for 7 of the 159 patients enrolled in the study. In addition, there were 4 reports of moderate to severe gastrointestinal hemorrhage, of which 1 event was considered possibly related and the other 3 unrelated to study drug.

Preliminary findings are available from 2 recently completed studies. In the first study (study C0701a/102/ON/US), 19 patients with advanced pancreatic cancer received CEP-701 at 20 and 40 mg bd in combination with a standard regimen of gemcitabine. The study was closed early because of lack of efficacy. Preliminary assessment of safety data indicated that the combination

of CEP-701 with gemcitabine did not result in significant additive toxicity. A single serious adverse event of acute renal failure was considered possibly related to CEP-701. The second study (study C0701a/202/ ON/US) explored the activity of CEP-701 in 18 patients with refractory or relapsed AML with activating mutations of the receptor tyrosine kinase FLT3. The starting dosage of CEP-701 was raised in this study from 40 mg bd to 60 mg bd after the first 4 patients showed no response and a cell-based ex vivo assay of plasma indicated that the inhibition of the FLT3 target may have been suboptimal. Fourteen patients were subsequently enrolled at 60 mg bd, and the dosage for 3 patients was increased to 80 mg bd after approximately 1 month of treatment. The higher dosages of 60 and 80 mg bd appeared to be relatively well tolerated in this group of patients. At a dosage of 60 mg bd, ex vivo assay indicated that a high degree of inhibition (>90%) of the FLT3 target was maintained over the 12-hour interval between doses. Transient decreases in the number of peripheral blast counts and the recovery of elements of the normal peripheral cell population were documented in several patients. Serious adverse events of gastrointestinal haemorrhage, fatigue and congestive heart failure in 1 patient each were considered possibly related to CEP-701. These events were also considered possibly related to the patients' disease and/or prior treatment.

Known and Potential Risks and Benefits to Human Subjects

The principal treatment-related side effects of CEP-701 are gastrointestinal in nature (nausea, diarrhoea, anorexia, constipation, and vomiting). Asthenia has also been reported. Severe events are relatively uncommon, and clinical experience suggests that the nausea and vomiting should respond to 5-HT₃ receptor antagonist antiemetics. These effects are consistent with the gastrointestinal signs and symptoms observed in repeat-dose toxicology studies in animals. More severe findings of cholestasis and hepatic inflammation that were observed in the 28-day dog toxicology study have not been manifested in the clinical program to date. Gastrointestinal hemorrhage has been reported in a small number of patients. It is not known to what extent these hemorrhagic events are related to CEP-701 treatment.

The benefits of CEP-701 to patients remain to be determined. Transient decreases in peripheral blast count have been observed in some patients with refractory AML treated with CEP-701. A scientific rationale, based on in vitro studies and animal cancer model data, exists to support the potential benefit of inhibiting FLT3 in patients with AML and of inhibiting Trks in patients with prostate cancer and neuroblastoma.

Additional information regarding risks to human subjects may be found in the Investigator's Brochure.

APPENDIX D Instructions to the Patient/Caregiver for Administering CEP-701 25mg/ml/Placebo oral solution.

Trial: AML 172007-003798-16

General Instructions

- Store at room temperature. Protect from light. Keep used and unused bottles in original box. Keep out of the reach of children. For Oral Administration Only.
- You will initially be started on a dose of 80mg (which represents 3.2ml on your oral syringe) twice a day. This dose may subsequently vary as your treatment continues.
- Each supply of CEP-701/Placebo will be fully labelled with the dose you are to take.
- **Return all used and unused** bottles together with the oral syringe and dosing cup in the original box at each study visit.
- CEP-701/Placebo may be taken with food. If you vomit after a dose, do not repeat this dose. You should take your next dose at the regular scheduled time.
- Administer this study drug in the morning and in the evening approximately every 12 hours (there must be at least 8 hours between doses).
- You have been supplied with one syringe and one dosing cup for each bottle which can be washed in warm water and re-used for as long as you are using that bottle.
- See your physician or hospital pharmacist if you have any questions.

Dosing of CEP-701 (25mg/ml)/Placebo oral solution 100ml bottle.

- Each bottle contains 100ml of CEP-701 (25mg/ml)/Placebo'
- Remove the Child Resistant cap from the bottle.
- On the oral syringe an adhesive line indicates the dose mark.
- Insert the 5ml oral syringe into the adapter **DO NOT USE EXCESSIVE FORCE** as this could push the adapter into the bottle.
- There is no need to remove the bottle adapter between doses.
- Carefully turn the bottle and withdraw the prescribed amount of study drug (as indicated on the labelled bottle and the adhesive line on the oral syringe) from the bottle into the syringe by pulling down on the syringe plunger as you have been shown at the hospital.
- Turn the bottle to the upright position and carefully remove the oral syringe from the bottle. Leave the adapter in the bottle.
- Place the cap back onto the bottle and twist to engage the Child Resistant mechanism. If the Child Resistant mechanism does not engage, remove the cap and repeat. It is important to keep the cap and bottle threads clean of solution.

Preparation of Diluted CEP-701/Placebo & Juice solution and administration.

1. Place 30ml of juice into a plastic dosing cup then add the entire contents of the oral syringe. The following juices are approved for use for the administration of the study drug: grape, pineapple, apple and orange juice (pulp free).

CEP-701/Placebo should not be taken with grapefruit juice or drinks containing grapefruit juice.

2. Stir the solution until well mixed.
3. Drink the diluted solution immediately after mixing or within 1 hour of mixing with juice otherwise discard.
4. Rinse the dosing cup 3 times with 30ml of juice and drink the juice after each rinse.

Syringe Cleaning Instructions

- Disassemble syringe after use. Rinse syringe components and dosing cup with warm water for 30 seconds. Dry and reassemble syringe. Store syringe and dosing cup in the original box for next dose.

APPENDIX E 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/m²/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 $10 \times 10^9/L$. 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 F WHO Play Performance Scale For Children Aged 0-9 Years

- 100 Fully active, normal.
- 90 Minor restrictions in physically strenuous activity.
- 80 Active but tires more quickly.
- 70 Both greater restriction of, and less time spent, in active play.
- 60 Up and about but minimal active play; keeps busy with quieter activities.
- 50 Gets dressed but lies around most of the day; no active play; able to participate in quiet play and activities.
- 40 Mostly in bed; participates in quiet play and activities.
- 30 In bed; needs assistance even for quiet play.
- 20 Often sleeping; play entirely limited to very passive activities.
- 10. No play; does not get out of bed; unresponsive.

APPENDIX G: 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 $p=0.05$.

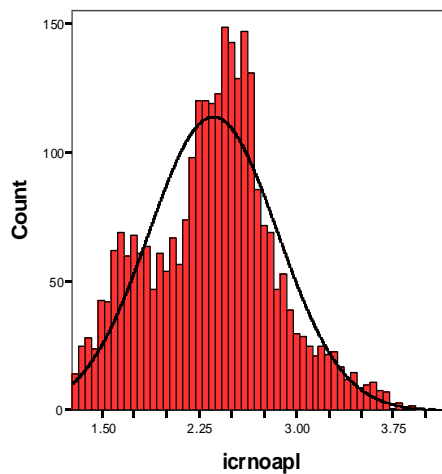
In order of entry to the model, the variables which make up the index are:

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

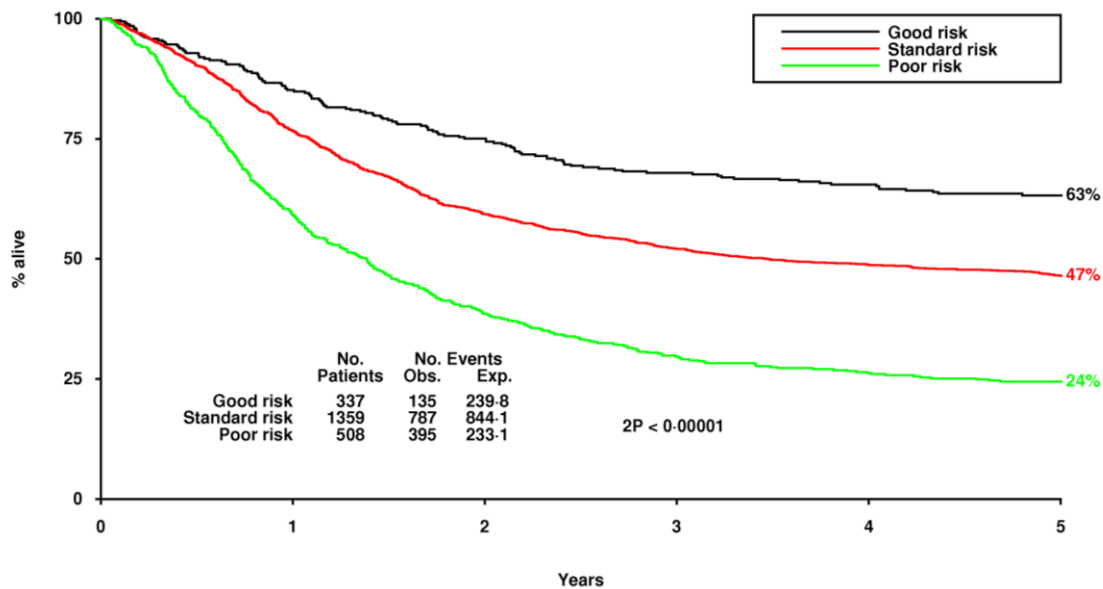
The index is therefore:

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

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

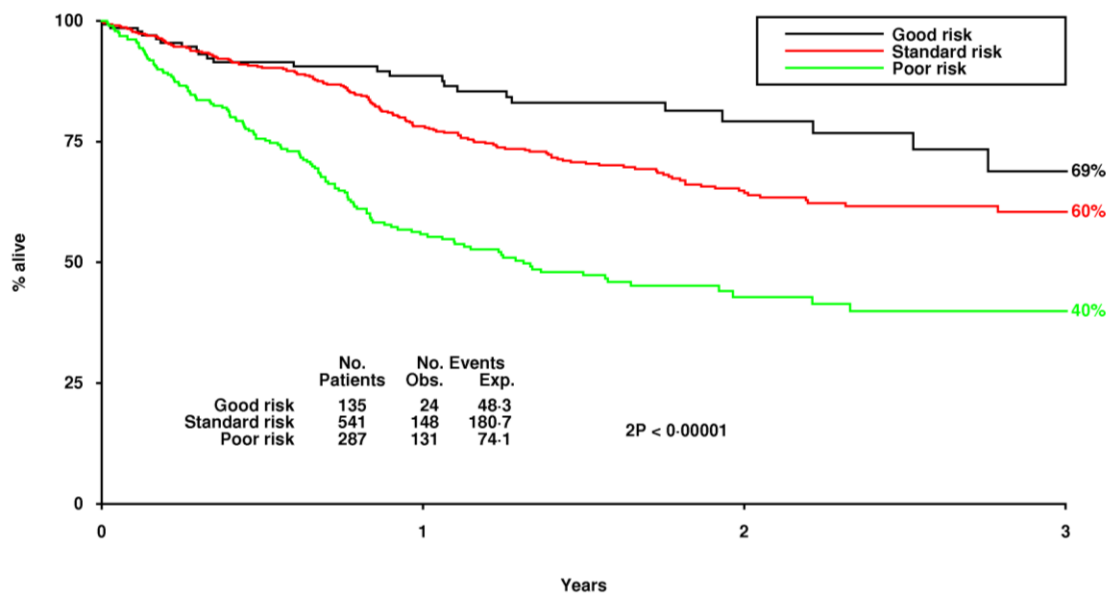


Taking into account the apparent bimodality of the curve, patients with an index of 2 or below were deemed good risk, and the data were arbitrarily divided at the 75th centile between standard and poor risk. Survival from CR in AML10,12 according to the risk groups was as follows:



The index was validated on data from AML15:

MRC AML 15 (no APL): Survival from CR by post CR risk group



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 H:

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 $>50 \times 10^9/l$, 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. $>10 \times 10^9/l$) 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 (Vahdat et al, 1994). 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 $50 \times 10^9/l$ 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 (Alimoghaddam K et al, 2006).

Differentiation syndrome: (see Section 20.8.1)