PROTOCOL FOR PATIENTS AGED UNDER 60
(Trial Reference ISRCTN55675535)

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

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

Patients with APL can enter the trial and will be allocated to the Italian **AIDA** anthracycline plus ATRA based chemotherapy.

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.

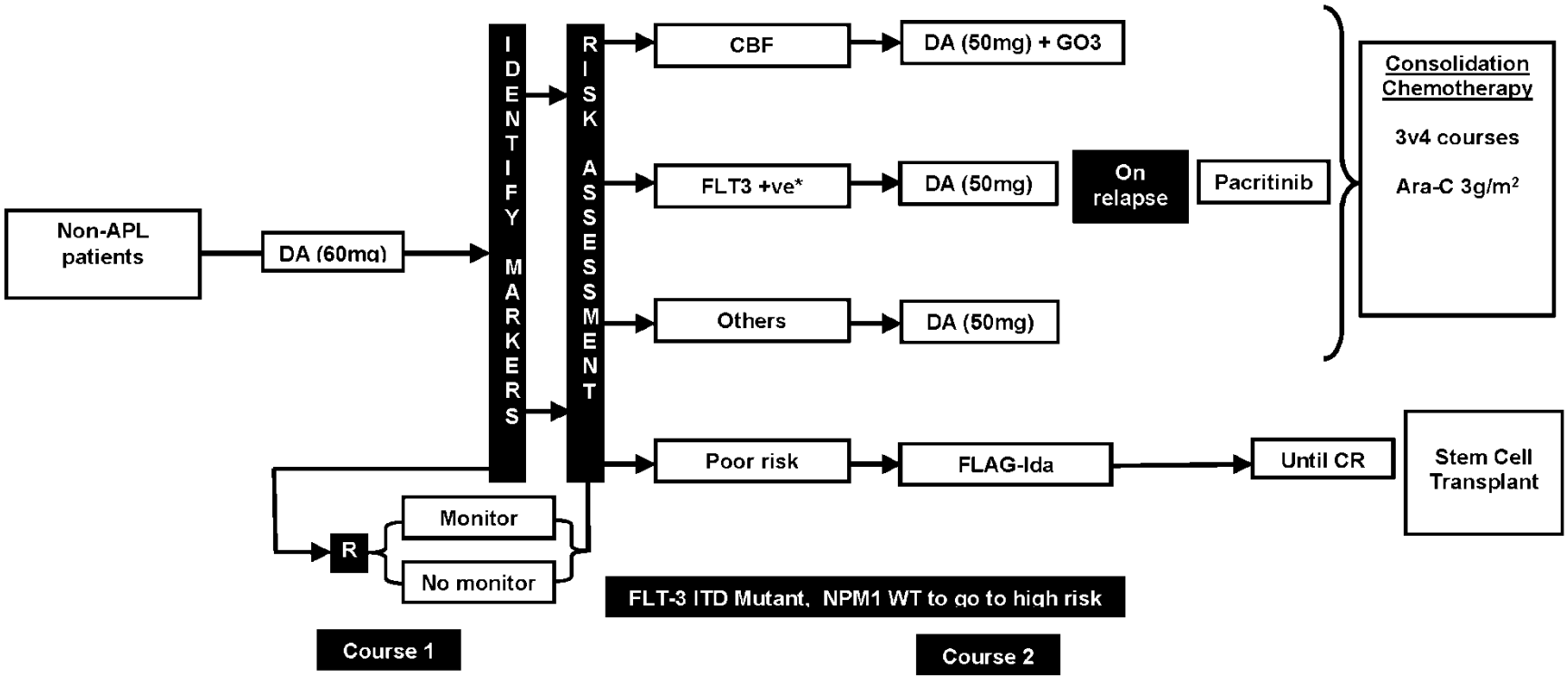
Patients with a FLT3 mutation who enter the trial who relapse will be able to be treated with the tyrosine kinase inhibitor, **Pacritinib**.

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

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

Clinicians are required to read the whole protocol before commencing treatment

Flow chart for adult patients



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

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RANDOMISATION, ADMINISTRATION AND FOLLOW-UP

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

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

Contents

1 ETHICAL CONSIDERATIONS

The AML17 Trial Protocol has been approved by the Wales Multicentre Research Ethics Committee (NRES), now called Wales REC 3. 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 a total of **three versus four courses** of treatment in total, comparing one versus two courses of **HD-Ara-C** in consolidation.
- In high risk patients, to 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 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).

2.3 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 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: treatment with DA.

B. Consolidation phase: for patients who are not high risk two versus one further treatment courses of high dose Ara-C (two arms)

C. For patients with a FLT3 mutation who have relapsed, treatment with Pacritinib

APL: Patients with APL can enter the trial. They will be allocated to AIDA therapy, which can be accompanied by molecular monitoring as considered appropriate.

3.2 AML (other than APL)

There are two randomised comparisons for adults within the trial:

End of Course 1	i) Patients will be invited to enter a randomisation between minimal residual disease monitoring or no monitoring.
After Course 2	**ii)** 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

Additionally the trial management system will inform investigators of which intermediate risk patients should be considered for myeloablative or Reduced Intensity Transplant.

Patients with a FLT3 mutation who relapse can be treated with Pacritinib

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

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

1. At diagnosis in adults: D(60)A as induction chemotherapy.

   One Course of D(60)A followed by a course of D(50)A

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

   i) Patients who have a high risk score and FLT+/NPM1c- genotype should FLAG-Ida (Section 11.3)

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

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

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

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

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

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

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

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

There are two randomised comparisons within the trial.

In consolidation
i) Two courses of High Dose Ara-C in patients who are not poor risk.

ii) Patients may enter a randomisation to be MRD monitored or not (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 out weigh 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 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).

4.2 Interventions Based on Molecular Genetic Characteristics and Risk Score
The genetic and molecular heterogeneity of AML is well known[(2)]. 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[3]. The important effect when compared with the cytogenetic risk definition is to move approximately one sixth of the patients who were previously standard risk into the high risk category and to move about one tenth of previously poor risk patients into the standard risk group. The net effect is that 27% of patients in AML10, & 12 are now defined as high risk compared with 17% previously. When we examine the role of transplantation on the new high risk group, Mantel-Byar analysis shows a significant survival advantage, although in the light of possible selection biases this result needs to be interpreted cautiously. , In a recent review of the accumulating data from our database there is emerging evidence that, whereas to date the role of transplantation in patients with the high risk FLT3+/NPM1c- genotype was uncertain, there is now evidence that this subgroup also benefit from a myeloablative stem cell transplant. The AML17 trial, will therefore, recommend FLAG-Ida with a view to proceeding to allogeneic transplantation.

4.3 Core Binding Factor Leukaemias

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

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

4.3.1 Other Patients

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

4.4 High Risk Score

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

For the purposes of the AML17 trial patients who have a risk score of greater than 2.667 or the FLT3+/NPM1c- genotype will be designated as high risk with a predicted survival at 5 years of 24% (based on AML10, 12). This will comprise approximately 30% of all patients who enter CR. Retrospective information indicates that this group of patients may have an improved survival following transplant (33%

vs 20%), so at the present level of knowledge a stem cell transplant from a sibling or unrelated matched donor may well be indicated. It is expected that a donor (sibling or unrelated) will be found for most patients.

4.5 Pacritinib

Pacritinib is a third generation tyrosine kinase inhibitor with potent FLT3 and JAK1 inhibitory activity. The JAK1 inhibitory activity has focussed its development in JAK mutation associated disease such as myelofibrosis.

Pacritinib (SB1518) is a novel JAK2-FLT3 inhibitor that has demonstrated promising antitumour activity in 2 mouse models of human malignancies and 4 clinical studies to date. Two additional clinical studies have characterized pharmacokinetics (PK) of pacritinib and effect of food on pacritinib. Preclinical toxicology and clinical studies have established the oral dose. The indications of interest primarily include (a) polycythemia vera, essential thrombocythemia and idiopathic myelofibrosis, all of which are myeloproliferative disorders with a high frequency of a JAK2V617F mutation; (b) certain leukaemias and lymphomas where other forms of JAK aberrations have been reported; and (c) acute myeloid leukaemia (AML), where FLT3 inhibitors have shown preliminary clinical promise. Pacritinib is believed to be active whether or not the JAK2V617F mutation is present, but is a potent inhibitor of JAK2 and FLT3 kinase activities (IC_{50} = 23 nM and 22 nM, respectively) and of cellular proliferation in human leukaemia and lymphoma cell lines selected for their dependence on either of the target kinases (cellular IC_{50} ranges from 0.03 to 0.46 μM). With respect to other second generation FLT3 inhibitors this is more potent based on nM sensitivity. Consistent with these activities, exposure to pacritinib resulted in the reduction of phos-JAK2, phos-STAT3 or phos-STAT5 in the relevant cell lines. Unlike some JAK2 inhibitors, pacritinib does not inhibit JAK1.

The antitumor activity of pacritinib has been demonstrated in two tumour models driven by FLT3 or JAK2 mutations. In nude mice bearing MV4-11, an FLT3-dependent AML cell line, pacritinib treatment caused a dose-dependent inhibition of tumour growth, with complete regression at the highest dose tested (100 mg/kg). In nude mice bearing BaF3-JAK2V617F, a JAK2-dependent leukaemia, untreated mice developed the hallmark symptoms of myeloproliferative disease, including spleen/liver hyperplasia and severe leukocytosis. Pacritinib treatment (300 mg/kg daily) alleviated clinical symptoms including leukocytosis and splenomegaly. The higher dosage required to achieve comparable therapeutic effects in the BaF3 model relative to the MV4-11 model (300 mg/kg vs 100 mg/kg daily) was consistent with the antiproliferative effects of pacritinib on these cells in vitro (IC_{50}=461 nM and 32 nM, respectively).

Cardiovascular safety testing in conscious dogs showed no treatment-related changes in cardiac electrophysiology (P, R-R, PQ, QRS, QTR, and QTc intervals), heart rate, respiratory rate, body temperature, blood pressure or activity, following a single oral dose of 30 mg/kg, despite gastrointestinal effects (vomiting) that were observed clinically. In the enzyme screen, pacritinib was shown to bind to hERG at an IC_{50} of 3.51 μM.

4.5.1 Clinical Experience

Pacritinib has been studied in 6 clinical trials to date: a phase 1/2 study in advanced myeloid malignancies (SB1518-2007-001), a phase 1 study in advanced lymphoid malignancies (SB1518-2007-002), a phase 1/2 study in chronic idiopathic myelofibrosis (SB1518-2008-003), a phase 2 study in advanced lymphoid malignancies (SB-2010-005), and two healthy volunteer trials to establish PK profile (SB1518-2010-004) and evaluate food effect (SB1518-2010-006) on pacritinib absorption.

SB1518-2007-001 was an open label phase 1/2 study in patients with advanced myeloid malignancies. In the phase 1 portion, cohorts of 3 to 6 patients received one of a series of escalating doses of pacritinib

ranging from 100 to 600 mg per day for 25 days. A total of 43 patients were enrolled and treated at 7 dose levels. DLTs included grade 3 QTc prolongation (150 mg), grade 3 diarrhoea (300 mg) and grade 3 diarrhoea (500 mg), nausea and vomiting (600 mg) and grade 2 dizziness, blurred vision, unsteadiness and worsened performance status (600 mg). Based on these findings, the 500-mg dose level was determined to be the MTD. The most commonly reported treatment emergent AEs (>10%) in phase 1 were: diarrhoea, nausea, vomiting, fatigue, peripheral oedema, pyrexia, constipation, anaemia, dyspnoea, asthenia, abdominal distension, thrombocytopenia, epistaxis, back pain, abdominal pain, hyperuricaemia, dizziness, insomnia, night sweats, cardiac murmur, hyperbilirubinaemia, pain in extremity, neutropenia, chills, anorexia, dehydration, cough, petechiae, arthralgia, and skin lesions.

AML is also a potential target for pacritinib. Although JAK2V617F is a rare finding in de novo AML, STAT3 activation is common [5]. Since STAT proteins are phosphorylated and activated by JAKs, the finding of frequent STAT activation in AML suggests the involvement of JAK2 extrinsic regulators and other proteins in the JAK-STAT pathway. In addition, JAK-STAT represents one alternate pathway by which leukaemic cells escape FLT3 inhibition. In vitro studies show that FLT3 inhibitors upregulate the JAK-STAT pathway and that JAK2 inhibition may overcome resistance to FLT3 inhibition, suggesting that co-inhibition may improve outcomes in AML [6].
As an inhibitor of FLT3, pacritinib has potential application for the treatment of leukaemia. A family of Class III receptor tyrosine kinases (RTK), including c-Fms, c-Kit, fms-like receptor tyrosine kinase 3 (FLT3), and platelet-derived growth factor receptors (PDGFR α and β) are important in the maintenance, growth, and development of both haematopoietic and non-haematopoietic cells[7]. Overexpression and activating mutations of these RTKs are involved in the pathophysiology of diverse human cancers from both solid and haematologic origins[8].

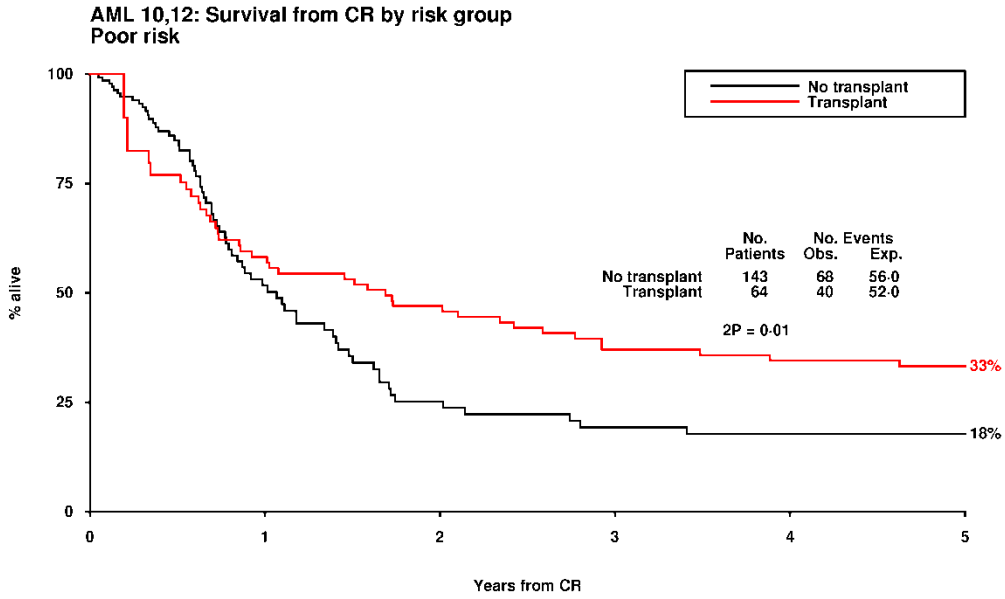
Pacritinib is also a potent inhibitor of FLT3 (IC_{50}=22 nM) and its drug resistant D835Y mutant (IC_{50}=6 nM). However, pacritinib differs from other FLT3 compounds that have been studied in clinical trials as it has no activity against most of the other class III RTKs [9]. Because of its potency and specificity, pacritinib may offer a unique therapeutic application in AML.

The effects of pacritinib were studied in primary blast cells expanded from PBMCs of patients with AML. Pacritinib inhibited the viability of blast cells with a mean IC_{50} of 0.47 $\pm$ 0.32 47 d the cytometric analyses verified that the expanded cells were predominantly leukemic stem cells, specifically CD38the viability of blast cells with a mean IC-Kit, fms-like receptor tyrosine kinase 3 (FLT3), and platelet-derived growth facThis provides a rationale to test pacritinib in high risk FLT3 mutated patients.

4.6 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. While the risk score confirms that only high risk patients benefit from a myeloablative transplant (from a sibling or matched unrelated donor), recent data shown that intermediate risk patients who have a FLT3+/NPM1- also benefit. Recent analysis of the Reduced Intensity Transplant(RIC) data within the trial database in patients 40 to 60 years indicates that a RIC is beneficial but only from a matched sibling donor.

4.7 Acute Promyelocytic Leukaemia (APL)

In the first years of the AML17 Trial the question in APL patients is a comparison of the “standard” which was established in the AML15 trial, which was AIDA, with the novel “chemo-free” combination of Arsenic Trioxide and ATRA. Accrual to this randomisation is complete and the analysis will be undertaken when enough events accrue. For the remainder of the AML17 trial APL patients can enter the trial and receive AIDA treatment[10]. They will be eligible for molecular monitoring guidance.

4.8 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[11,12].

A more universal target, is the leukaemia specific immunophenotype which can be established in over 90% of cases[13]. 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. 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 three reference labs which have been established for AML16.

4.8.1 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.

6 REFERENCES

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Section C:
NON – APL AML and HIGH RISK MYELODYSPLASTIC SYNDROME

7 INCLUSION AND EXCLUSION CRITERIA

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

7.1 Inclusion Criteria Non APL Leukaemia

Patients are eligible for the AML17 trial if:

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

- They are considered suitable for intensive chemotherapy.

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

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

- Women of child-bearing potential (ie women who are pre-menopausal or not surgically sterile) must use acceptable contraceptive methods (abstinence. Intrauterine device (IUD) and must have a negative pregnancy test within 2 weeks of trial entry. Pregnant or nursing patients are excluded. Male patients with partners of childbearing potential must agree to use effective contraception during the study period and a period of 3 months after the last dose of study drug.

- They have given written informed consent.

7.2 Exclusion criteria

Patients are not eligible for the AML17 trial if:

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

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

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

- They have a concurrent active malignancy.

- They are pregnant or lactating.

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

8 PROCEDURES FOR ENTRY INTO THE TRIAL AND DATA RECORDING

8.1 Centre Registration

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

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

8.1.1 Patient Recruitment

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

8.2 Randomisation

There are four points in the trial for which contact must be made with the Haematology Clinical Trials Unit (HCTU). Patients fulfilling the criteria for entry into the trial (see Section 7) should be entered into the first randomisation by telephoning the HCTU in Cardiff (tel: 029 2184 7909). 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 Induction

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

Induction chemotherapy allocation will be given once the required patient details have been supplied. Patients will be allocated to induction chemotherapy treatment as follows:.

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

8.2.2 Information required at first randomisation

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

8.3 Diagnostic material

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

8.3.1 Cytogenetics

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

8.3.2 FLT3/NPM1c- Mutation Status and Molecular Screening

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

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

It is essential that a sample is sent to a designated laboratory for the identification of patients with a FLT3/NPM1c- mutations. These laboratories will pass samples on to the laboratories designated

for MRD monitoring. It is intended that investigators will have the results of FLT3 assays within approximately two weeks of the end of the first course of chemotherapy.

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

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

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

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

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

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

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

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

8.3.3 Immunophenotyping

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

Laboratories for Immunophenotypic Characterisation and Monitoring:

Dr Sylvie Freeman
Clinical Immunology
Division of Infection and Immunity
University of Birmingham
P.O. Box 1894
Vincent Drive
Edgbaston
Birmingham, B15 2SZ
Tel: 0121 4158759 Mob: 07884310528
Fax: 01214143069
s.freeman@bham.ac.uk

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

Dr Paul White
Department of Haematology
University Hospital of Wales
Heath Park, Cardiff
CF14 4XN
Tel: 029 20742370
Fax : 029 20745084
e-mail: whitepc@cardiff.ac.uk

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

8.3.4 Follow-up Material

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

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

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

8.4 Data recording

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

http://AML17.cardiff.ac.uk

A user password will be supplied to investigators on receipt of the letters of local R&D approval, and centre registration information (see Section 8.1).

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

Web based data collection forms should be completed as follows:

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

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

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

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

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

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

Long-term follow-up – all randomised patients will be followed up for life or until they withdraw consent to be part of the trial, irrespective of whether they complete all trial treatments. This information will normally be collected on annual follow-up forms, which are provided to the clinician that randomised the patient.

8.5 Health Economics

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

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

9 INDUCTION CHEMOTHERAPY: Courses 1 and 2

The induction schedule comprises two courses of allocated chemotherapy. Remission status will be determined after each course. After Course 1, the additional or alternative treatments will be decided as patients are characterised as having Core Binding Factor leukaemia, a high risk score, a FLT3/NPM1c-genotype or none of these. The additional interventions are described in section 11 of the protocol. If a patient is not in complete remission after course 2, they may enter the high risk randomisation (section 11.5).

9.1 D(60)A schedule

Course 1 **DA 3+10**

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

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

Course 2 **DA 3+8**

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

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

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

10 ASSESSMENT OF RESPONSE

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

In order to achieve a subsidiary aim of the trial (i.e. assessing the relevance of residual cytogenetic or molecular existence of disease in morphological CR) investigators should also request cytogenetic analysis on this sample. In addition a paired marrow and blood sample should be sent to the relevant reference immunophenotyping laboratory and a separate paired sample to Prof Grimwade for molecular assessment of MRD (see Sections 8.3.4, 11.4 and 20.10 for addresses). Favourable risk patients should also be randomised to receive 3mg/m^2 of Mylotarg ion day 1 of course 2..

10.1 Definitions of Complete Remission, Partial Remission and Resistant Disease

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

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

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

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

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

11 SUBSEQUENT TREATMENTS

11.1 Subsequent Treatments

After recovery of blood counts and marrow assessment of response additional information will be available. Patients with Core Binding Factor Leukaemias will be identified and sufficient information will be available to calculate the individual patient's risk score. The investigator should ascertain the risk score which is only calculated and provided by the internet data system which will inform investigators if the patient has a high risk score or not , and what treatment options the patient is eligible for. (for high risk see Section 11.3 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+/NPM1c- genotype).

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 CORE BINDING FACTOR LEUKAEMIA

Patients who have Core Binding Factor Leukaemia, t(8;21)/AML1-ETO and inv(16)/t(16;16)/CBFB-MYH11, will continue with the chemotherapy as allocated for course 2. **They will receive mylotarg 3mg/m^2 on day 1 of course 2** . Samples of bone marrow and blood at time of routine assessment for remission may be sent to the molecular laboratory in Manchester who can provide information on the patient's molecular response on a commercial basis. However Core Binding Factor Leukaemia minimal residual disease monitoring is **not part of the formal assessment of the value of minimal residual disease monitoring versus no monitoring** being evaluated in the trial.

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

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11.3 HIGH RISK SCORE PATIENTS.

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

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

Adult patients who are defined as high risk will enter the high risk treatment randomisation with the expectation that they should proceed to transplantation. Patients who relapse are eligible for this high risk randomisation with a view of going to transplant. Patients with a FLT3 mutation who relapse can enter the pacritinib option (see Section 16,2). For these patients **Form F** should be used.

The standard arm in this patient group is FLAG-Ida. The treatment is:

Up to three courses of FLAG-Ida [standard arm]

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

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

G-CSF [Lenograstim 263μg (1 vial)] s.c. daily days 1-7 inclusive (7 doses).
Idarubicin 8 mg/m^2 i.v. daily on days 4, 5 and 6 (3 doses).

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

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**.** For the preparative

schedule for reduced intensity allografts see section 14**.** However it is recognised that it takes time for the arrangements for transplant to be made, and that there will be a number of patients for who a donor cannot be identified. Therefore patients should continue with the allocated treatment courses until the transplant can be delivered. It is recommended that patients in whom a reduced intensity allograft is intended should receive a minimum of two high risk treatment courses.

11.4 Progression Through Induction Therapy

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

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

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

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

12 CONSOLIDATION RANDOMISATION

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

12.1 Randomisation Options for Adults:

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

12.2 Timing of Consolidation Randomisation

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

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

12.3 Information Required at Consolidation Randomisation

Before carrying out the consolidation randomisation please make sure that:

a) The patient is in complete remission

b) The patient's risk group is known.

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

For randomisation: (i) telephone HCTU (tel: 029 2184 7909) during office hours (09:00 to 17:00 hrs, Monday to Friday); or (ii) use the 24 hour internet randomisation available at:
http://AML17.cardiff.ac.uk

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

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

13 CONSOLIDATION CHEMOTHERAPY: Courses 3 and 4

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

13.1 Consolidation

Course 3 **High Dose Ara-C**

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

Course 4 **High Dose Ara-C**

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

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

NB. Patients over 60 years who enter the trials who are due to receive high dose ara-C should receive it at a dose level of 1.5g/m^2

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

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

14 STEM CELL TRANSPLANTATION

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

1. Patients <35 years should receive a conventional allogeneic transplant with Cyclophosphamide and Total Body Irradiation (8 x 180cGy fractions).

2. Patients 35-40 years can receive a conventional allogeneic transplant or a reduced intensity allograft (RIC) depending on investigator or patient choice.

3. Patients ≥40 years should receive a Reduced Intensity allograft (RIC).

14.1 Conventional Myelo-Ablative Allogeneic Transplantation

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

14.2 Reduced Intensity Allograft Schedules

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

14.2.1 Reduced Intensity Protocols for Patients with Intermediate Risk Disease

a) FBC Protocol:

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

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

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

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

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

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

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

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

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

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

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

Day -12 to -9:	**Intravenous chemotherapy**
Day -12 to -9:	Fludarabine 30 mg/m²/d
Day -12 to -9:	Cytarabine 2 g/m²/d
Day -12 to -9:	Amsacrine 100 mg/m²/d
Day -8 to -5:	Rest day
Day -4 to -2**:**	**Conditioning** :

IV Busulphan total doses 8 mg/kg
IV Fludarabine total dose 60 mg/m²

Day -4: IV Bu at 3.2 mg/kg/d in 3 hours
Day -3: IV Bu at 3.2 mg/kg/ in 3 hours
Day -2: IV Bu at 1.6 mg/kg/d in 3 hours
Day -3 to-2: IV Flu 30 mg/m²/d once daily over 1 hour

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

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

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

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

15 ARRANGEMENTS FOR MOLECULAR SCREENING AND MINIMAL RESIDUAL DISEASE MONITORING

15.1 Molecular Screening

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

These labs will undertake the FLT3/NPM1c mutation assessment.

Laboratory Contacts:

Dr P White
Department of Haematology
Cardiff University School of Medicine
Heath Park
Cardiff
CF14 4XN
Tel: 029 20744524
Fax: 29 2074 4655 029 20744655

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

15.2 Minimal Residual Disease Monitoring

A major question to be addressed in the AML17 trial is the **clinical value** of disease monitoring using molecular and immunophenotypic approaches. The referred sample to UCL or Cardiff will identify patients who are candidates for molecular monitoring of MRD. In patients consenting to MRD assessment **(using PIS and consent form 8**), **paired marrow and blood samples** should be sent following each course of chemotherapy. Post-induction samples from all patients should be sent to Yvonne Morgan at Guy's Hospital to assess molecular response (in addition a separate paired marrow and blood sample should be sent to the designated immunophenotyping lab, see Sections 8 and 10). After induction, investigators will be informed if a patient has a relevant marker and subsequent samples should be sent to Professor Grimwade in London for other molecular markers, and to the designated immunophenotyping laboratory. **If patients are randomised to "no monitor" they should not be monitored and no samples should be obtained. If it intended to undertake any monitoring local (which is not recommended or justified) patients should not enter the monitor vs no monitor randomisation**. . Investigators will receive requests for further follow up samples in relevant patients who have given consent to be monitored (PIS and consent form 8).

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

Molecular Targets:

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

Address for Samples:

Dr Yvonne Morgan,
Molecular Oncology Diagnostics Unit
GSTS Pathology,
4th Floor, Southwark Wing,
Guy's Hospital,
Great Maze Pond,
London SE1 9RT
Tel +44 207188 7188 x 51060

15.3 Frequency of Molecular Monitoring

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

15.4 Monitoring by Immunophenotyping

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

Laboratories for Immunophenotypic Characterisation and Monitoring:

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

Mr Paul Virgo
Department of Immunology
Southmead Hospital
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Tel: 0117 959 6306
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Dr Paul White
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CF14 4XN
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Fax : 029 2074 5084
e-mail: whitepc@cardiff.ac.uk

15.5 Assessment of the Clinical Value of Minimal Residual Disease Monitoring

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

In order to meet the objectives of phase 3, patients will be asked to consent to be randomised to be **monitored or not to be monitored**. Patients allocated to the monitored arm will be required to agree to regular blood & marrow tests. All patients who are randomised i.e both the monitored and not monitored groups will be expected to complete a periodic Quality of Life assessment (see below) and to complete a short resource usage questionnaire when they visit the clinic to collect information 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.

If a patient is randomised to the “no monitor” arm no additional samples should be sent or taken: this randomisation is not appropriate for centres which perform their own local monitoring.

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 6 weeks, 18 weeks, 24 weeks, 30 weeks, 9 months, 12 months, 15 months and 18 months following the start of course 3 of chemotherapy. **It is imperative that this data is collected at the correct time points, and the Quality of Life questionnaire will need to be completed if the patient relapses or is to receive a transplant.**

From the start of course 3, patients will be asked to complete a short resource use questionnaire together with the research team at each centre to assess the use of other resources since their last clinic visit. A patient diary will be available for patients to use as an aide memoire.

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

16 MANAGEMENT OF PATIENTS WHO RELAPSE or are REFRACTORY

16.1 Non- FLT3 Mutated Patients

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 FLAG-Ida as for high risk patients, with a view to progressing to stem cell transplant (section 11.35). 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.

16.2 Relapsed FLT3 Mutated Patients

Patients with a FLT3 mutation diagnosed within the AML17 or AML15 trials who relapse, with or without prior stem cell transplantation, can be treated with **pacritinib.** On a precautionary basis the following requirements apply:

i) Pregnant or lactating females should be excluded
ii) Both males and females should agree to use effective methods of birth control.
iii) A sample of the marrow on which relapse was diagnosed should be sent to the molecular lab in Cardiff. (see section 8.3.2)

16.2.1 Treatment Schedule

Patients will commence on a **daily dose** of 400mg which will be given as **200mg bid.** A formal assessment will take place on or around day 14 (where day 1 was the first day of pacritinib treatment). The formal assessment will include examination and a history and recording of any side effects and blood samples for FBC/liver and renal function and ECG. Based on this assessment the daily dose may be increased to 600mg /day i.e.**300mg bid.** Should any side effects which have been attributed to pacritinib occur, but are minor or have resolved, the patient has the option to continue on the 200mg bid daily dose for a further 14 days until the next assessment.
A marrow assessment should be undertaken on the day 14 visit if there is evidence (e.g. on peripheral blood counts) of a response.

There will be a second formal assessment on **day 28** when the same enquiries /examination and blood tests will be undertaken, but this time a **compulsory marrow** to assess response will be undertaken. In addition a **10ml peripheral blood sample should be sent** to the Cardiff lab for biomarker assessment.

16.2.2 Introduction of Chemotherapy.

It is hoped that peripheral blood and marrow responses may be seen from the use of monotherapy. However investigators are free to introduce chemotherapy when it is felt to be in the patient's interests. The chemotherapy will be at the investigators discretion, but patients will be eligible for the high risk treatment options of the AML17 trial. For patients who have not relapsed post- transplant should proceed to transplant if this is a feasible option. Patients are intended to continue on pacritinib for 12 months. If chemotherapy or transplant has been deployed, pacritinib should be discontinued during the chemotherapy and re-commenced 2 days after the end of therapy. Post-transplant administration of pacritinib is permitted and can be re-introduced at the discretion of the transplant team. Patients who have completed 12 months of pacritinib, and who are considered to be benefiting, will be able to continue for an additional 6 months on a compassionate basis.

16.2.3 Side Effects & Management.

Pacritinib has been implicated in causing reversible **myelosuppression** which will be expected in relapsed AML patients. It is therefore important to record full blood counts on a regular basis. If myelosuppression happens, and if the patients have previously improved counts or are on maintenance, dose interruption or reduction may be required and investigators should discuss with the Chief Investigator(s).

Gastro-intestinal symptoms have been the most common side effects seen in clinical trials so far. It is recommended that **prophylactic loperamide** is used. Any effect on **Liver or Renal** function is likely to be dose related. Both should be monitored regularly and dose interruption considered, but should be discussed with the Chief Investigator(s). Patients should be warned about the possibility of more general symptoms: fatigue, peripheral fluid accumulation, unexplained fever and rash.

As for all adverse events in the trial, patients on pacritinib should be included in the adverse event reporting requirements (section 21) and will be monitored by the trial office by the enhanced pharmaco-vigilance procedures.

16.2.4 Supply of Pacritinib

As soon as an eligible patient has consented, investigators should contact HCTU will arrange for the first 2 months of supply to be provided to the site.This will usually take 48 hours to deliver. If a decision is made to dose escalate at any time, HCTU must be informed to enable additional supplies to be dispatched.

After the first 2 months investigators must activate the required supplies for each subsequent 2-month period. Maintenance supplies will be arranged on a 60 day supply basis. **It is the responsibility of the treating site to contact HCTU for continued supply.**

17 SUPPORTIVE CARE

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

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

1. Venous access via Hickman-type catheter
2. Control of nausea and vomiting
3. Mouth care
4. Prophylactic gut decontamination (if considered appropriate)
5. Antifungal prophylaxis
6. Response to a significant pyrexia — i.e. two readings of ≥38°C two hours apart, or a single reading ≥39°C
7. Antibiotic treatment of febrile episodes — including antibiotic choice(s) and monitoring, duration of therapy, and the treatment of non-response
8. G-CSF therapy [Lenograstim 263 µg (1 vial) s.c. daily in adults] 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 Fludarabine or Stem Cell Transplant.

18 CNS TREATMENT

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

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

Section D:
ACUTE PROMYELOCYTIC LEUKAEMIA

19 ACUTE PROMYELOCYTIC LEUKAEMIA

19.1 APL

Patients will enter this part of the protocol at diagnosis with de novo or secondary acute promyelocytic leukaemia (APL) recognised morphologically as FAB-M3/M3v. Treatment with ATRA and supportive care for coagulopathy should be started as soon as the diagnosis is suspected, without awaiting results of cytogenetic/FISH/PCR analyses (see BCSH AML guideline (Appendix H). Diagnostic bone marrow (4mls in EDTA) and peripheral blood (30mls in EDTA) from all patients with suspected APL should be sent to Prof David Grimwade at Guy's Hospital (see Section 19.10 for address). Arrangements can be made for rapid confirmation of presence of PML-RARA fusion by PML immunofluorescence testing by contacting Prof David Grimwade, whose laboratory is also responsible for MRD testing. Confirmation of the molecular lesion is important because cases lacking the PML-RARA fusion will be under treated. Patients who enter the APL part of this trial will be monitored for minimal residual disease (MRD) with the aim of identifying patients failing first line therapy who require additional therapy in first CR. Patients entering this part of the trail should use **Patient Information Sheet 3 and Consent Form 3.**

19.2 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 < 2.0 mg/dL (≤51 umol/L)
- Serum creatinine < 3.0 mg/dL (< 260 µmol/L)
- Women of child-bearing potential (ie women who are pre-menopausal or not surgically sterile) must use acceptable contraceptive methods (abstinence. Intrauterine device (IUD) and must have a negative pregnancy test within 2 weeks of trial entry. Pregnant or nursing patients are excluded. Sexually active men must also use acceptable contraceptive methods

Exclusion criteria:

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

19.3 AIDA Treatment

19.3.1 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

19.3.2 Consolidation Therapy

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

First consolidation cycle:

Idarubicin 5 mg/m$_2$/d by short (20 minute) intravenous infusion on days 1, 2, 3, 4

ATRA, 45 mg/m$_2$/d, will be administered orally in two equally divided doses and rounded to the nearest 10 mg increment, given from day 1 to day 15

Second consolidation cycle:

Mitoxantrone 10 mg/m$_2$/d as 30 minute intravenous infusion on days 1, 2, 3, 4, and 5.

ATRA 45 mg/m$_2$/d will be administered orally in two equally divided doses and rounded to the nearest 10 mg increment, given from day 1 to day 15

Third consolidation cycle:

Idarubicin 12 mg/m$_2$/d as short (20 minute) intravenous infusion only on day 1.

ATRA 45 mg/m$_2$/d will be administered orally in two equally divided doses and rounded to the nearest 10 mg increment, given from day 1 to day 15

Marrow samples will be collected around day 60 (i.e. following course 1 in patients requiring prolonged ATRA to achieve CR, or following course 2 in those with earlier documentation of CR) and on regeneration following each consolidation course for testing by real-time quantitative PCR (RQ-PCR) by the reference laboratory for assessment of molecular remission. Patients who do not achieve molecular

remission by the end of the 3rd consolidation cycle will be considered as molecular resistant and will go off study. Marrow samples collected at earlier time points are used to measure disease response and provide early indication of patients at risk of failing first line therapy.

19.4 Differentiation (ATRA) Syndrome Patients with High White Counts at Diagnosis

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

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19.4.1 Treatment Modification

During induction treatment, ATRA may be temporarily discontinued in the presence of one of the following complications: Differentiation syndrome, pseudotumour cerebri, hepatotoxicity.

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:

- 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.

19.4.2 Pseudotumour Cerebri

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

19.4.3 Hepatotoxicity

This is defined as: an increase in serum bilirubin, AST/ALT, or alkaline phosphatase >5 times the normal upper level. This requires a temporary suspension of the ATRA. If hepatoxicity persists following discontinuation of ATRA, The Idarubicin doses should not be changed on the AIDA arm.
As soon as the symptoms and the patient‘s clinical condition improves, treatment with ATRA will be resumed at 50% of the previous dose during the first 4 days after the disappearance of retinoic acid syndrome, amelioration of pseudotumour cerebri or when serum bilirubin, AST/ALT or alkaline phosphatase are reduced to <4 times the normal upper level. Thereafter, in absence of worsening of the previous toxicity, ATRA should be resumed at full dosage.
In case of reappearance of signs and symptoms of ATRA toxicity, the drug must be discontinued indefinitely during induction therapy.

19.5 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. .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.

Section E:
STATISTICS & TRIAL GOVERNANCE

20 STATISTICAL CONSIDERATIONS

20.1 Patient numbers

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

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

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

In the pacritinib non-randomised pilot, it is anticipated that upwards of 60 relapsing patients will be identified during the course of the remaining life of the trial. The second remission rate of patients with a FLT-3 mutation who relapse is historically 30% from AML15; thus with 60 patients it will be possible to estimate the second remission rate with pacritinib with a standard error of 6%. There will also be greater than 80% power to distinguish between an unfavourable second remission rate of 25% and a promising second remission rate of 40% using a single stage Fleming approach.

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

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

20.2 Data analysis

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

The main analyses will be based on the intention to treat - i.e. **all** patients believed to be eligible at the time of randomisation will be included in the analysis, irrespective of protocol compliance, early death, etc. Comparisons of randomised treatments will be made using the log-rank test for time to event outcomes; and the Mantel-Haenszel test for dichotomous outcomes. Resource usage data will be compared using Wilcoxon rank-sum tests or t-tests as appropriate. The primary outcome is survival for all randomisations 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.

21 TRIAL GOVERNANCE AND ADVERSE EVENT REPORTING

Cardiff University is the Trial Sponsor and has delegated certain responsibilities to participating sites. These define the responsibilities of the Principal Investigator on each site. The trial will be conducted in compliance with the MRC Guidelines for Good Clinical Practice in Clinical Trials copies of which are available from the MRC or the Trial Office. In the use of unlicensed drugs the trial is conducted under a CTA issued by the MHRA which requires the investigators to report Serious Adverse Events (SAEs) as described in below. The trial will be monitored by an independent Data Monitoring and Ethics Committee.
Patients have been informed that they can withdraw consent at any time. A distinction should be made between not entering a randomisation, who will continue to be followed up, and those who withdraw consent, who cannot be followed up.

21.1 Adverse Event Reporting

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

* 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.

21.1.1 Definitions:

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

- Development of a non-haematological toxicity of grade 3 as defined in the NCI Common Toxicity Criteria Version 3**, which does not resolve to grade 2 or less within 7 days
- Development of any grade 4 non-haematological toxicity (excluding alopecia) (this includes any life threatening event)
- Development of neutropenia (<1.0 x 109/L) or thrombocytopenia (<50 x 109/L) for longer than 42 days after the end of chemotherapy in the absence of significant disease in the bone marrow (>5% blasts)
- Events which are not related to AML or its treatment which result in hospitalisation or prolongation of hospitalisation.
- Any event which results in persistent or significant disability or incapacity
- Any event which results in a congenital abnormality or birth defect
- Death from any cause including persistent or progressive disease
- Other Medically important event*

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

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

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

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

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

21.1.2 Causality

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

21.1.3 Collection of Data

Preliminary discussion of the event may take place with a clinical co-ordinator. SAEs should be recorded on the Adverse Event Form which is available on the trial website, and sent to the Trial Office in Cardiff. SAE Fax Number 02920742289

21.1.4 Time of Report

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

21.1.5 Enhanced Pharmaco-Vigilance

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

21.1.6 Reporting to the Regulatory Authorities

The Chief Investigator or his nominee will review and record all SAEs. He will be responsible for reporting the events to the MHRA, REC, 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 as per regulatory requirements..

APPENDIX A WHO Histological Classification of Acute Myeloid Leukaemias

	ICD Code
Acute myeloid leukaemia with recurrent genetic abnormalities	
Acute myeloid leukaemia with t(8;21)(q22;q22); (AML1(CBFα)/ETO)	9896/3
Acute myeloid leukaemia with abnormal bone marrow eosinophils Inv(16)(p13q22) or t(16;16)(p13;q22); (CBFß/MYHII)	9871/3
Acute Promyelocytic leukaemia (AML with t(15;17)(q22;q12-21), (PML/RARα) 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. For hepatic dysfunction with a bilirubin 20-50 µmol/L reduce by 25%; for bilirubin >50 µmol/L reduce by 50%. In patients with renal impairment dose reduction should take place: Serum Creatinine 105-265µmol/L, reduce dose by 25%; Serum Creatinine >265µmol/L reduce dose by 50%.

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

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

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

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

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

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

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

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

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

Administration
DO NOT ADMINISTER AS AN INTRAVENOUS PUSH OR BOLUS

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

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

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

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

Cautions

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

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

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

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

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

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

Idarubicin (Zavedos™ - Pharmacia)

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

For administration the vial contents should be dissolved in water for injection to give a solution of 1mg/ml. The resultant solution should be administered intravenously into the side arm of a freely running intravenous infusion of 0.9% sodium chloride over 5 to 10 minutes.
In cases with hepatic dysfunction dose reduction is required: bilirubin 21 – 34umol/L reduce the dose by 50%.Greater rises contraindicate the administration. For renal impairment with a serum creatinine 100 – 175umol/L reduce the dose to 50%.Administration at higher creatinine levels is a clinical decision.

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

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

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

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

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

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

Collection of Autologous and Allogeneic Stem Cells:

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

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

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

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

Cyclophosphamide (Endoxana™ – 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.

PACRITINIB (Cell Therapeutics, Inc)

Pacritinib is supplied as hard gelatin capsules with gray bodies and red caps. Capsules contain 100 mg pacritinib (free base) and the following inactive ingredients: microcrystalline cellulose NF, polyethylene glycol 8000 (PEG 8000) NF, and magnesium stearate NF. The capsule gelatin is bovine/porcine-derived.

Pharmacies at investigational sites will receive bottles containing either 30 capsules packaged in 60 mL HDPE bottles or 120 capsules packaged in 200 mL HDPE bottles. Both packaging sizes have child-resistant closures.

Drug product should be stored in the pharmacy, hospital, clinic, or warehouse at controlled room temperature, 20° to 25°C (68° to 77°F), with excursions allowed between 15° to 30°C (59° to 86°F). Patients should be instructed that storage temperatures in the home should be below 30°C (86°F).

Based on the adverse effects observed in toxicity studies, as well as data from phase 1and 2 studies in patients with advanced myelofibrosis, AML, and lymphoma, the investigator is advised to observe the following precautions:

- **Hematologic**: Reversible myelosuppression; leukopenia, neutropenia, thrombocytopenia, and anemia have been observed; these events are common in hematologic malignancies and may not be related to pacritinib administration. Patients participating in the pacritinib component of the AML17 trial will be monitored frequently for myelosuppression. Provisions for interruption of treatment and dose reduction in the event of myelosuppression are outlined in section 16.2.3 of the protocol.
- **Gastrointestinal**: Treatment with pacritinib is associated with dose-related diarrhoea and nausea. Vomiting, abdominal pain, abdominal distension, anorexia, and constipation have also been reported to be related to pacritinib administration. Antiemetic and antidiarrhoeal medications should be prescribed prophylactically to control symptoms. Fluid and electrolytes should be replaced as needed to prevent dehydration. Pre-existing nausea, vomiting, and diarrhoea should be adequately controlled before beginning therapy. Patients with significant GI symptoms despite optimal supportive care may have study drug interrupted or have the dose of study drug reduced per protocol.
- **Hepatic**: Animal studies suggest that treatment may cause dose-related hepatotoxicity. There has been little evidence of hepatotoxicity in clinical trials to date. Significant increases in aminotransferases have been observed infrequently and have not been accompanied by concomitant increases in bilirubin. No instances of hepatic dysfunction meeting Hy's Law criteria have been observed. Hepatic function (AST/SGPT, ALT/SGOT, alkaline phosphatase, total bilirubin) will continue to be assessed at frequent intervals during treatment with pacritinib.

- **Renal**: Animal studies suggest that treatment may cause dose-related renal toxicity. To date, little impact on renal function has been observed in human clinical trials. Assessment of renal function (creatinine, BUN, sodium, potassium) will be undertaken frequently in patients participating in clinical trials of pacritinib.
- **General**: Additional adverse effects commonly reported during pacritinib administration include fatigue, asthenia, peripheral edema, pyrexia, and rash. Patients participating in AML 17 will be monitored closely for these adverse effects.

No reproductive or developmental toxicity studies have been performed; therefore, pregnant and lactating patients will not be eligible for treatment with pacritinib. If fertile, both males and females must agree to use effective birth control. Women of childbearing potential must use highly effective methods (defined as those resulting in a failure rate of <1% per year when used consistently and correctly) for the duration of study treatment and for 12 months after last dose of study drug. The contraceptive methods considered highly effective are intrauterine devices and hormonal contraceptives (contraceptive pills, implants, transdermal patches, hormonal vaginal devices, or injections with prolonged release).

No drug-drug interaction studies have been conducted with pacritinib. In vitro studies indicate that pacritinib has no significant potential to inhibit or induce CYP450 isozymes and has no significant involvement with p-glycoprotein mediated transport. Pacritinib is believed to be metabolised by CYP3A4. Therefore, caution is advised when considering the co-administration of potent inhibitors of CYP3A4 in conjunction with pacritinib.

Overdosage
There is no specific antidote to pacritinib. Patients participating in clinical trials have received doses of up to 600 mg daily. If overdose is suspected, administration of study drug should be stopped and general supportive measures instituted. The risk of overdose is minimized by the use of a standard dose and written dosing instructions for patients.

Minimizing Risks
The risks to patients participating in clinical studies with pacritinib will be minimized by:

Close monitoring of AEs and laboratory results, as specified by the study-specific protocol, by trained and experienced study personnel who are aware of the observed and potential risks of pacritinib therapy

Protocol-specified drug interruption and dose reduction for patients who develop significant reversible toxicities

The exclusion of patients who are pregnant, including precautions for the prevention of pregnancy in treated patients, and discontinuation of pacritinib in any patient who becomes pregnant or whose partner becomes pregnant in the course of treatment

APPENDIX C Procedures For Bone Marrow Transplantation

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

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

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

Cyclophosphamide

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

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

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

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

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

- 950 cGy if the dose rate is 5-10 cGy per minute.
- 750 cGy if the dose rate is more than 10 cGy per minute.

Fractionated TBI

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

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

Lung
Abdomen (at umbilicus)
Pelvis

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

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

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

Sedation and anti-nausea

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

Prevention of infection

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

Infusion of marrow

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

Other supportive care

Red cell or platelet transfusions will be necessary in the period following the graft. It is recommended that platelets be given if the peripheral platelet count is less than 10×10^9/L. All blood products,

including platelets, must be irradiated to at least 2500 cGy post-transplant. CMV negative recipients should receive CMV negative blood products whenever possible.

GVHD

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

APPENDIX D: Derivation of a risk index for younger adults

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

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

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

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

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

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

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

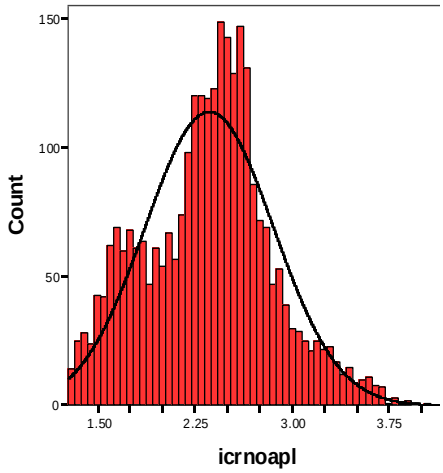
In order of entry to the model, the variables which make up the index are:

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

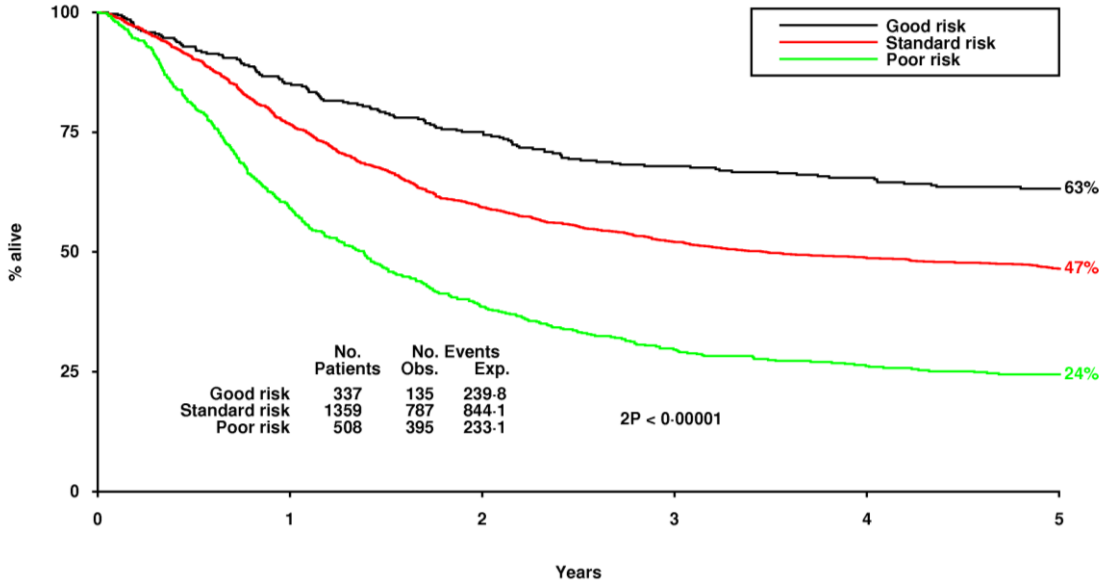
The index is therefore:

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

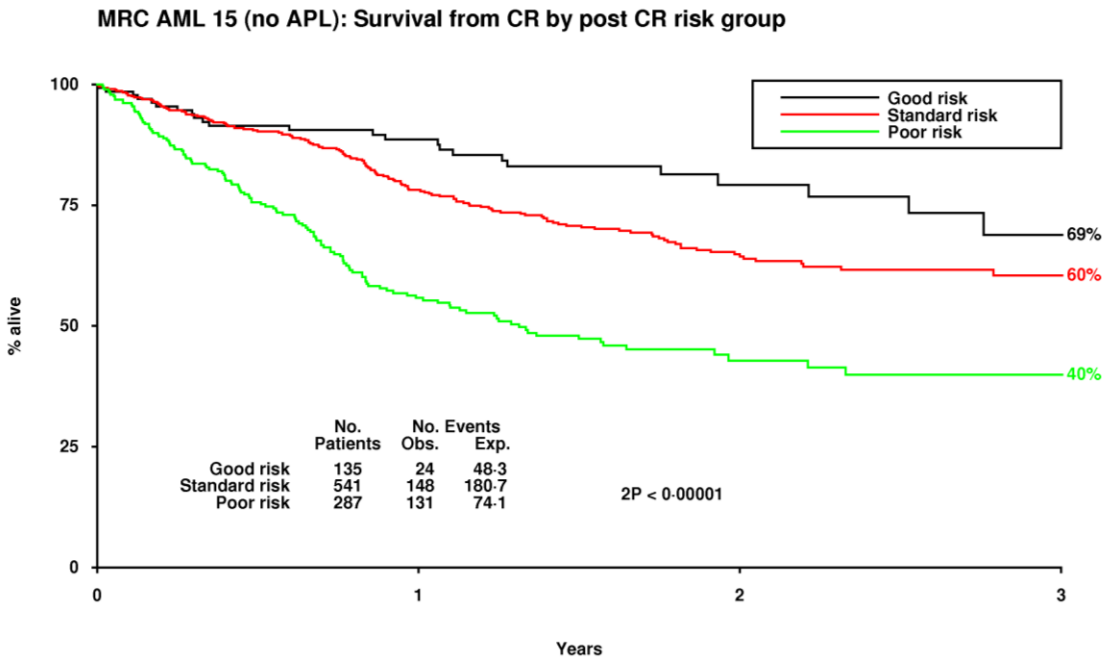
and the distribution of patients in AML10,12 by index is:



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



The index was validated on data from AML15:



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

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