

# AML 16

NATIONAL CANCER RESEARCH  
INSTITUTE ACUTE MYELOID  
LEUKAEMIA AND HIGH RISK MDS  
TRIAL 16

**A PROGRAMME OF DEVELOPMENT FOR OLDER PATIENTS WITH ACUTE  
MYELOID LEUKAEMIA AND HIGH RISK MYELOYDYSPLASTIC SYNDROME  
(Trial Reference ISRCTN 11036523)**

The AML16 Trial will evaluate several relevant therapeutic questions in Acute Myeloid Leukaemia (AML), as defined by the WHO, and High Risk Myelodysplastic Syndrome. The trial is primarily designed for patients over 60 years, but younger patients who may not be considered suitable for the concurrent MRC AML Trial for younger patients may also enter. Approximately 2000 patients will be recruited.

The Programme is in two parts. For patients who are considered fit for an intensive approach to treatment, a randomisation will compare the standard DA regimen (**Daunorubicin/Ara-C**) with DClo (**Daunorubicin/Clofarabine**). In addition, the role of **Mylotarg** in combination with these treatments in the first induction course will be evaluated. Patients who achieve complete remission(CR) or partial remission(PR) after course one will receive course 2 and will then be randomised to **one or two further courses** and will be eligible for a **non-intensive allogeneic stem cell transplant** if a suitable HLA matched donor is available. Patients who fail to achieve a CR or PR after course 1 and are in CR after course 2 will receive course 3. Patients who do not have a donor will be randomised to maintenance with **Azacitidine** or not.

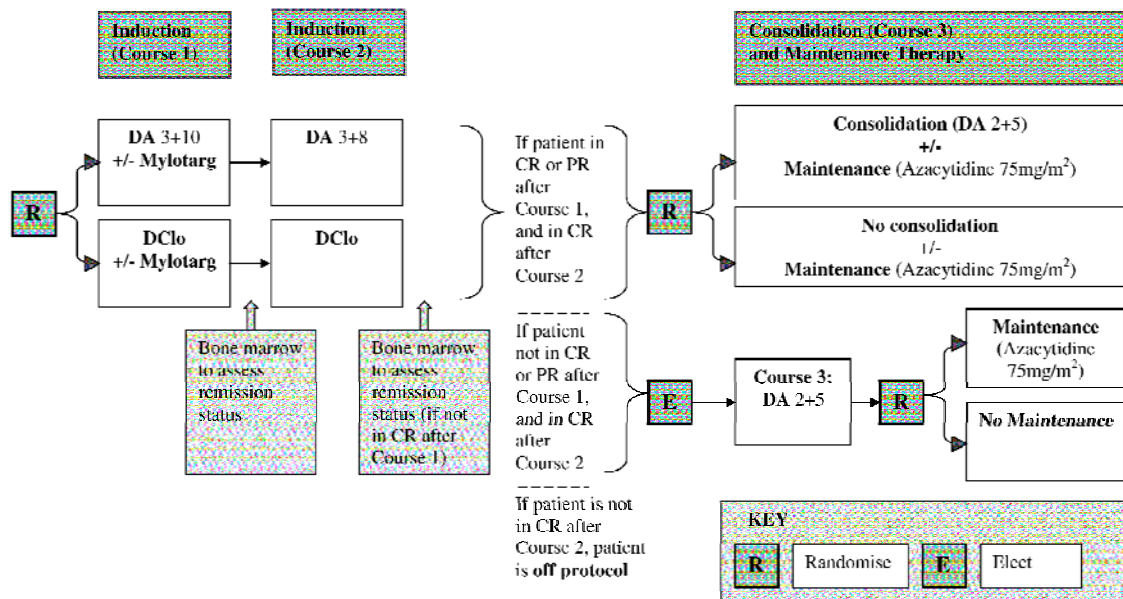
Patients who are not considered fit for an intensive treatment approach will be randomised between an established approach to non-intensive treatment, namely **Low Dose Ara-C** versus one of three novel treatments, which are **Low Dose Ara-C combined with Mylotarg**, **Low dose Clofarabine** and **Low Dose Ara-C combined with Arsenic Trioxide**.

There are about 2000 cases of AML each year in adults aged over 60 years in the British Isles alone. About 270 patients over 60 years annually enter national trials, which offer an intensive treatment approach. It is expected that a similar number of patients can be recruited to the non-intensive treatment options of this trial.

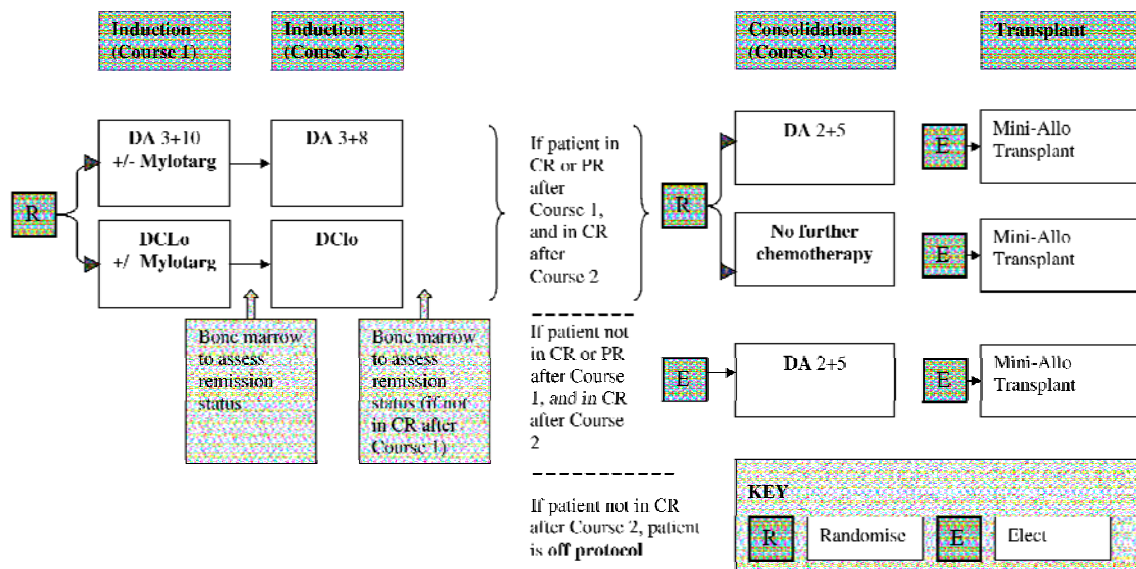
This protocol describes a collaborative trial in acute myeloid leukaemia primarily for patients over 60 years, which is being undertaken by the NCRI Haematological Oncology Study Group under the sponsorship of Cardiff University, and provides information about procedures for the entry, treatment and follow-up of patients. It is not intended that this protocol should be used as an aide-memoire or guide for the treatment of other patients. Every care has been taken in its drafting, but corrections or amendments may be necessary. Before entering patients into the trial, clinicians must ensure that the trial protocol has received clearance from their Local Research Ethics Committee and that they conform to the host institution's Research Governance procedures. 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 asked to read the whole protocol before commencing  
treatment**

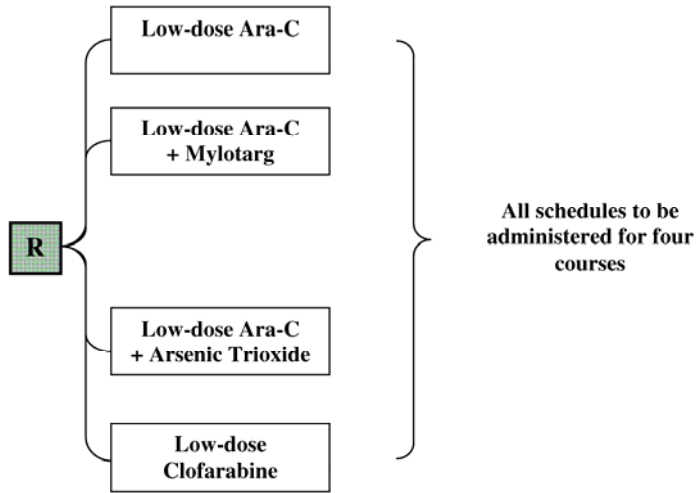
## Flow Chart 1: Intensive treatment for patients not scheduled for mini-allo transplant



## Flow Chart 2: Intensive treatment for patients scheduled for mini-allo transplant



### Flow Chart 3: Non-intensive treatment



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**Fax: 0121 687 2313**

### **Telephone randomisation availability:**

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

### **24 hour internet randomisation and data entry:**

**<https://www.trials.bham.ac.uk/aml16>**



## **1 ETHICAL CONSIDERATIONS**

The AML16 Programme has been approved by COREC and must also be approved by the Local Research Ethics Committee (LREC) and conform with local Research Governance procedures at each centre before patients are entered. A copy of a centre's LREC approval and site specific assessment must be lodged with the Trial Office at BCTU before entry of patients can commence at that centre. Centres are required to go through a registration process with the Trial Office before recruitment is started and to confirm acceptance of the terms of sponsorship required by 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. All patients will be followed up annually for life.

The AML16 trial programme 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).

## **2 OBJECTIVES**

The AML16 trial programme is available to any patient who has primary or secondary AML as defined by the WHO Classification (Appendix A) (excluding Acute Promyelocytic Leukaemia), or high risk Myelodysplastic Syndrome (i.e. > 10% marrow blasts) who is not considered suitable for the current NCRI trial for younger patients (MRC AML 15). The programme has two separate parts:

- For patients who are considered fit for an intensive chemotherapy approach to treatment.
- For patients who are not considered fit for an intensive approach to treatment.

The objectives for each of these components are summarised below.

### **2.1 Therapeutic questions for patients considered fit for intensive treatment:**

- To compare two induction schedules (DA and DClo).
- To assess the value of Mylotarg during induction when used in combination with DA or DClo in course 1.
- To compare a total of two versus three courses of treatment in patients who achieve at least Partial Remission (<15% blasts) after induction course 1.
- To compare the use of Demethylation maintenance treatment with Azacytidine with no maintenance.
- To assess the value of Reduced Intensity Allogeneic Stem Cell Transplantation as consolidation for patients with matched donors.

### **2.2 Therapeutic questions for patients not considered fit for intensive treatment:**

To compare Low Dose Ara-C versus available novel approaches:

- Low Dose Ara-C with Mylotarg
- Low Dose Ara-C with Arsenic Trioxide
- Low Dose Clofarabine

During the course of the Programme other novel therapies are expected to become available, and will be considered for inclusion in this comparison.

Although not part of the AML16 trial randomised assessment, encouraging responses have been seen in a small number of patients with with monosomy 7 chromosome abnormality, alone or in combination, in patients using Azacytidine treatment. Such patients are eligible to enter a separate unrandomised phase 2 study. Details can be obtained by contacting Professor G Mufti (phone: 0207 346 3080).

## 2.3 Endpoints

The main endpoints for the therapeutic questions in patients **considered fit** for intensive treatment for each comparison will be:

- Overall survival
- Complete remission (CR) achievement and reasons for failure (for induction questions)
- Duration of remission, relapse rates and deaths in first CR
- Toxicity, both haematological and non-haematological
- Supportive care requirements (and other aspects of health economics)

The main endpoints of the comparisons for patients **not considered fit** for intensive treatment will be:

- Overall survival
- Complete remission (CR) achievement and reasons for failure (for induction questions)
- Duration of remission, relapse rates and deaths in first CR

- Toxicity, both haematological and non-haematological
- Supportive care requirements (and other aspects of health economics)

## **2.4 Subsidiary objectives**

Patients in both the intensive and non-intensive parts of the trial will be assessed for fitness by means of developing and prospectively validating a “Frailty Index”, with a view to correlating the score with choice of intensive or non-intensive therapy and response to treatment.

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 presence of a cytogenetic abnormality in the bone marrow of patients in morphological remission
- The relevance of the molecular detection of FLT3 and RAS mutation, genetic signature and resistance protein status to response to treatment
- Evaluation of methods of minimal residual disease (MRD) monitoring
- To assess gene methylation status in relationship to treatment with maintenance Azacytidine
- To store diagnostic tissue for future research in the AML Cell Bank

## **3 TRIAL DESIGN**

AML16 is a programme of development of treatment primarily for older patients with AML and high risk Myelodysplastic Syndrome (MDS) which has two parts. For patients considered fit for an intensive approach it offers a randomised controlled

Phase III trial which uses a factorial design for maximum efficiency to evaluate two novel induction options followed by a maintenance option. For patients not considered fit for an intensive approach to treatment there will be the option to enter a randomised Phase II comparison of standard therapy versus one of three novel treatments. In the event of any of the novel treatments appearing superior on preliminary analyses, the comparison will continue in a Phase III design.

For patients **considered fit** for intensive treatment:

- A. Induction phase: Two randomisations (four arms in total)
- B. Three courses versus two courses of induction/consolidation therapy in patients who are in CR and have achieved at least a PR after course 1.
- C. Maintenance: One randomisation
- D. Consolidation phase: Reduced Intensity transplantation.

For patients **not considered fit** for intensive treatment:

- A. Treatment plan: Standard treatment randomised against one of three novel treatments.

### **3.1 For patients considered fit for intensive treatment:**

There are three randomised comparisons within the trial:

At diagnosis:

- (i) **DA** versus **DClo**
- (ii) **Mylotarg** versus not in course 1

As consolidation:

- (i) **Three** courses versus **two** courses of total induction/consolidation therapy (for patients in CR achieving at least a PR after course 1)
- (ii) **Non-intensive allogeneic stem cell transplant** for patients with donors

As maintenance: (i) **Azacytidine** or not for one year

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

1. At diagnosis: randomise between DA and DClo as induction therapy, and also randomise to Mylotarg versus not. Before commencing the allocated treatment each patient should have a Frailty Score assessment when available as part of an associated study.

The four induction treatment arms will therefore be:

- Arm A Two courses of DA (with no Mylotarg)
- Arm B Two courses of DA (with Mylotarg in course 1)
- Arm C Two courses of DClo (with no Mylotarg)
- Arm D Two courses of DClo (with Mylotarg in course 1)

Clinicians must undertake the chemotherapy randomisation, DA vs DClo. Together with the Mylotarg randomisation unless they are ineligible for Mylotarg.

2. After recovery from course 1, assess bone marrow response. All patients will receive course 2 which will be the same chemotherapy (without mylotarg) as in course 1.
3. After Course 2, assess remission status in patients who have not been confirmed to be in remission after course 1. Patients who have achieved a marrow response (CR or PR) after course 1 will be randomised to have one more course or not and at the same time to Azacytidine maintenance or not. Patients who do not achieve at least a PR with course 1 will continue in the trial, but should receive two further courses. After the third course of treatment, if in complete remission, patients will be randomised to maintenance with Azacytidine or not. Patients for whom a

matched donor has been identified and for whom a stem cell transplant is intended, should receive the allocated chemotherapy and should not be randomised for maintenance with Azacytidine.

4. After the patient has entered CR and received the second or third treatment course as appropriate, they should receive one of the post induction options available in the trial:
  - (i) Non-intensive allogeneic stem cell transplantation
  - (ii) Maintenance with Azacytidine for 1 year versus no maintenance

**NB** Marrow should be assessed for remission status, MRD status if eligible, and methylation status if entering the Azacytidine randomisation.

### **3.2 For patients not considered fit for intensive treatment:**

Patients will be randomised to standard treatment, Low Dose Ara-C, versus one of three alternative novel treatment approaches. The available treatment arms are thus:

Arm E: Low Dose Ara-C

Arm F: Low Dose Ara-C plus Mylotarg

Arm G: Low Dose Clofarabine

Arm H: Low Dose Ara-C plus Arsenic Trioxide

Patients are expected to enter all randomisations for which they are eligible and which are currently available. For each of these options the treatment plan is for **four courses** to be given. Marrow response should be assessed before each course until complete remission is established.

## 4 BACKGROUND

Acute Myeloid Leukaemia is a heterogeneous disease with respect to morphology, immunophenotype, molecular abnormalities, cytogenetics, gene expression signature and treatment outcome. Treatment choice and outcome is substantially decided by age. Prognostic factors which determine poorer outcome are proportionately over-represented in patients over 60 years and co-morbidity limits the ability to deliver intensive and potentially curative chemotherapy<sup>(1)</sup>. But even when it is delivered the outcome is not satisfactory.

In the sequential trials conducted by the MRC (now NCRI) Adult Leukaemia Working Party over the last 30 years there has been significant improvement in survival in patients under 60 years of age (Figure 1) largely due to delivery of more intensive chemotherapy assisted by better supportive care.

Figure 1. Survival in MRC AML trials: patients aged 15-59

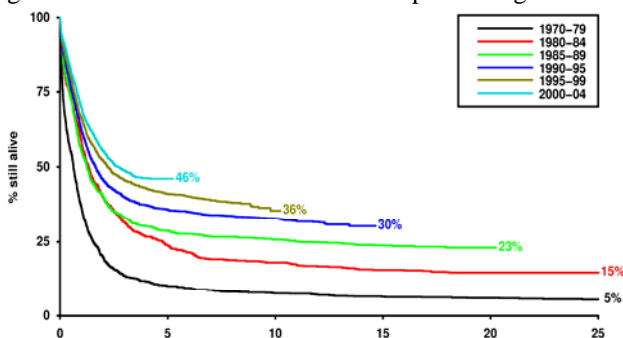
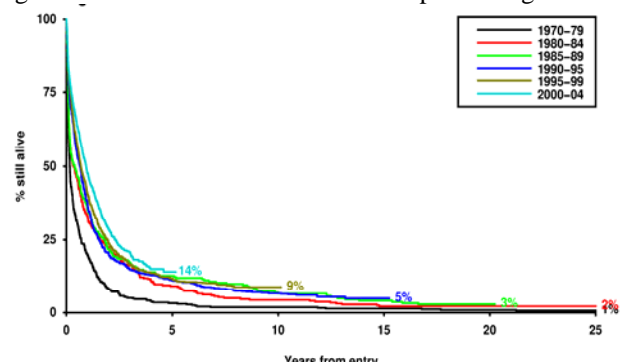


Figure 2. Survival in MRC AML trials: patients aged 60+

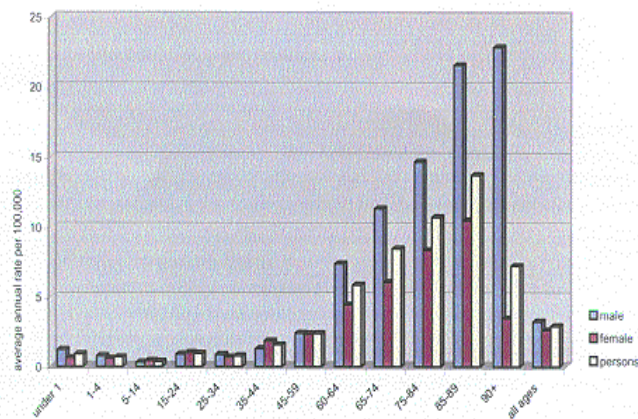


In older patients who have been treated with intensive chemotherapy over the same period, there is little evidence of any improvement in survival (Figure 2). This raises the important issue that the current strategy of intensifying chemotherapy by increasing doses of existing drugs is ineffective and may indeed shorten life for some older patients. In the last 15 years, our group has undertaken 4960 randomisations in older patients to treatments using conventional chemotherapy options. Ten questions have been addressed but the rate of complete remission has not improved beyond 60% and the overall survival has not improved. In only one comparison was a significant difference in survival shown.



An additional important issue is that, since the median age of this disease is 65 years, there is a large number of patients who cannot be offered, or who are considered unsuitable for, conventional intensive treatment strategies. As the general population lives longer, the number of patients in this age group will increase (Figure 3). Therefore, there is now an urgent need to find new treatments for these patients who are traditionally not catered for in most trials.

Figure 3. Incidence of AML by age



A further key point in reflecting on our progress, and that of other collaborative groups, in this area is whether our traditional approach to trials in this age group makes optimal use of the patients available. The motivation behind the AML 16 Programme of Development is that more progress could be made by including a randomised Phase II evaluation stage within the overall treatment strategy for this patient group. We have already been pursuing this approach within a limited number of centres with a view to identifying new treatments at a very early stage of their development. As a result, there is a number of novel agents available within this trial. At this stage in the development of AML treatment in older patients, we propose that it will be more productive to include the identification of new approaches which can thereafter be taken forward by us within this trial, or by others.

One reason for adopting this approach is that the improved understanding of the biology of the disease is beginning to make new agents available such as molecularly targeted treatment. This trial is designed in such a way to enable

several agents, which may become available, to be assessed over the life of the trial. If there is evidence of potentially meaningful benefit from the planned randomised Phase II comparison, the comparison can be extended to a fully powered Phase III evaluation.

Finally there is the issue that, since intensive treatment may well be shortening life for some, which patients should be treated with an intensive approach and who should not. We have found that traditional parameters such as Performance Score and age are relatively insensitive for this purpose. Within this trial, in an associated study, we will develop, and then prospectively validate, a “Frailty Index” to try to improve precision.

In summary this programme approach is needed now because: 1) current treatments are unsatisfactory, 2) there has been very little improvement in the last 15 years, 3) a new strategic approach is needed to include a preliminary assessment of promising new agents, 4) a number of novel agents is available to us, 5) treatments for patients not considered fit for an intensive approach need to be developed, 6) a more useful objective measure of who would benefit from an intensive approach has important social and economic implications, 7) our group has one of the largest networks in the world and should be capable of delivering the required recruitment, 8) the previous national trial (AML14) has closed.

## **5 JUSTIFICATION OF TREATMENT OPTIONS**

### **5.1 Intensive Approach**

#### **5.1.1 Daunorubicin and Ara-C**

Currently available treatment with a combination of Daunorubicin (D) and Ara-C (A) has achieved a remission rate of approximately 60% in patients over 60 years who were considered fit for intensive treatment. However almost 90% of these patients relapse within 3 years<sup>(2)</sup>. In previous MRC trials alternative anthracyclines, and

higher doses of Ara-C were not superior to the standard DA combination. Thioguanine was formerly included as a third drug, but since it became unavailable in the UK there has been no deterioration in rates of remission. We will therefore take the DA forward as the standard treatment arm in standard doses in AML16.

### 5.1.2 Clofarabine

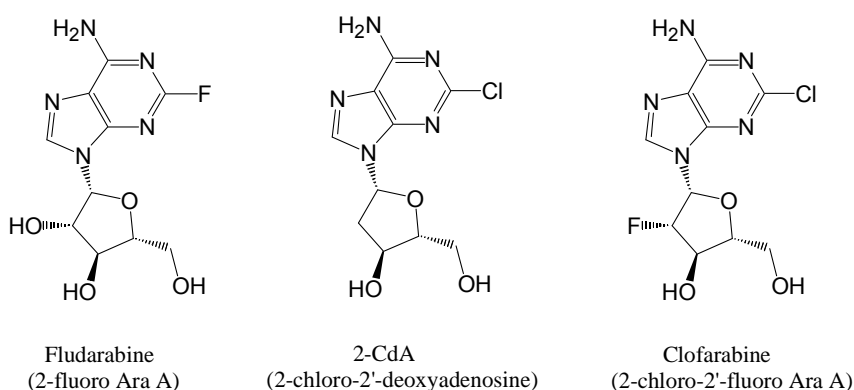
Clofarabine ([2-chloro-9-(2-deoxy-2-fluoro-D-arabinofuranosyl)adenine]; Cl-F-ara-A; CAFdA) is a second-generation purine nucleoside analogue which has been designed as a hybrid molecule to overcome the limitations and incorporate the best qualities of both Fludarabine (F-ara-A) and cladribine (CdA, 2-CdA), both of which are currently used for the treatment of haematological malignancies<sup>(3)</sup>. Because Clofarabine has a chloro group at the 2-position of adenine, its chemical structure is more closely related to CdA than to F-ara-A. Halogenation at the 2-position of adenine renders this class of compounds resistant to cellular degradation by the enzyme adenine deaminase. Substitution of a fluorine at the C-2' position of the arabinofuranosyl moiety of Clofarabine increases its stability in gastric acid and decreases its susceptibility to phosphorolytic cleavage by the bacterial enzyme *Escherichia coli* purine nucleoside phosphorylase in the gastrointestinal tract both of which may lead to enhanced oral bioavailability. It is probable that during the course of this trial that the oral formulation of Clofarabine will become available, and so provision for that is made in this protocol.

Like most other deaminase-resistant nucleoside analogues (see Figure 4), Clofarabine requires intracellular phosphorylation by deoxycytidine kinase (dCK) to its active triphosphate form for cytotoxic and therapeutic activity. The cellular distribution and substrate specificity of the nucleoside-activating enzyme dCK are highly species specific. The activity of dCK has been reported to be 10-fold greater in human bone marrow than in mice. Thus, the toxicity of these nucleoside analogues is qualitatively the same among the various species, but the maximum tolerated dose (MTD) and dose level required to produce toxicity in a particular target organ may vary greatly.

Clofarabine is a more efficient substrate for dCK exceeding CdA and the natural substrate deoxycytidine<sup>(4)</sup>. Similar to the other purine nucleosides, Clofarabine potently inhibits DNA synthesis by inhibiting both DNA polymerase and ribonucleotide reductase<sup>(5)</sup>. Unique to Clofarabine and CdA is the demonstrated ability to disrupt mitochondrial integrity that results in the release of pro-apoptotic proteins-cytochrome C and apoptosis-inducing factor<sup>(6)</sup>. The latter activity may be a factor in the cytotoxic effects of Clofarabine towards non-dividing lymphocytes.

The precise mechanism of Clofarabine, CdA, and F-ara-A on dividing and non-dividing cells is unknown. In dividing cells, the incorporation of the phosphorylated form of the halogenated-nucleosides into DNA appears to be an important part of their activity in arresting cell division. Inhibition of DNA polymerase and ribonucleotide reductase has been associated with their mechanism of cytotoxicity.

Figure 4: Structure of 2 Nucleoside Analogues and Clofarabine



### 5.1.2.1 Clinical Studies of Clofarabine

A Phase I dose finding study in 121 patients with high risk acute leukaemia established a MTD of 40 mg/m<sup>2</sup> /day for 5 days every 4 weeks, in adults and 55mg/m<sup>2</sup> in children<sup>(7)</sup>. Ten percent of these high risk patients showed a response. Clinical activity was noted through a fairly wide dose range (4mg/m<sup>2</sup>/day to 55mg/m<sup>2</sup>/day), with no clear-cut dose response above 11.25mg/m<sup>2</sup>/day. All but 1 patient reported adverse events during the study. Patients in the 40 mg/m<sup>2</sup>/day and 55mg/m<sup>2</sup>/day dose cohorts experienced more drug-related adverse events than

patients in the other dose cohorts. The most frequently reported drug-related toxicities (i.e., those that occurred in >10% of the study population) included nausea, infection, neuro-cortical dysfunction (primarily fatigue), neuro-motor dysfunction (primarily asthenia), fever in the absence of infection, neuro-headache, vomiting, skin abnormalities, pulmonary and musculoskeletal events (primarily muscle aches and joint pain), diarrhoea, haemorrhage, and stomatitis. Biochemical toxicity, as indicated by alterations in liver function tests, increased with ascending doses, reaching a maximum at 40mg/m<sup>2</sup>/day and 55mg/m<sup>2</sup>/day.

In order to assess the tolerability and efficacy of Clofarabine in untreated patients not considered fit for intensive chemotherapy, the NCRI AML Working Group conducted a non-randomised Phase II study in 30 patients with a median age of 72 years. In an effort to avoid liver toxicity which had occurred in 25% of patients in the phase 2 study in relapsed AML,<sup>(8)</sup> the dose administered was reduced to 30mg/m<sup>2</sup>/day for 5 days. The overall complete marrow response rate in 30 untreated patients of median age 69 years was 56%<sup>(9)</sup>. Grade 3 or 4 liver toxicity was seen in 4 patients but was transient. One patient had a skin reaction, but the treatment was well tolerated at this dose level. However this dose was still associated with significant myelosuppression with the median recovery of neutrophils to 1.0 x 10<sup>9</sup> /l and platelets to 100 x 10<sup>9</sup> /l being 24 and 25 days respectively. As an extension to this study a small number of patients were treated at a daily dose of 20mg/m<sup>2</sup>. Remissions were seen at this dose.

The aim within the AML16 trial intensive treatment option is to assess the value of Clofarabine (daily dose of 15 to 30mg/m<sup>2</sup>) in combination with Daunorubicin against standard care. A cohort of patients has been treated at 4 Clofarabine doses with full toxicity assessment undertaken, to confirm the safety. This has resulted in a study dose of 20mg/m<sup>2</sup> being chosen for evaluation in the AML16 Trial.

### **5.1.3 Mylotarg (Gemtuzumab Ozogamicin)**

Mylotarg (Gemtuzumab Ozogamicin)-GO, is the first antibody directed chemotherapy in AML. It targets via the CD-33 epitope which is frequently

expressed on AML blasts, by combining a humanised anti-CD-33 monoclonal antibody to which is coupled the anti-tumour antibiotic Calicheamicin. In Phase I and II studies in relapsed AML, this agent was able to achieve remission in 25-30% of patients<sup>(10,11,12)</sup> and it was licensed in the United States for the treatment of relapsed AML in patients over 60 years. The NCRI AML Working Group undertook a feasibility study of combining Mylotarg with full dose intensive chemotherapy in younger patients as first line treatment<sup>(13)</sup>. This established the feasibility of this approach but only when the Mylotarg dose was reduced to 3mg/m<sup>2</sup>. It was further established that Mylotarg could be given with high dose Ara-C (3g/m<sup>2</sup>). Some studies have given older patients with poor performance score Mylotarg in full dose (9mg/m<sup>2</sup> or 6mg/m<sup>2</sup>) as single agent as initial remission induction treatment. Results from these Phase II studies suggest that a remission rate of 25% is achieved. The HOVON 43 trial in older patients gives full dose Mylotarg as monthly maintenance after chemotherapy induction. The current MRC AML15 trial is evaluating the value of combining Mylotarg with intensive induction and/or consolidation chemotherapy in younger patients under 60 years. With 1000 patients now randomised this is a feasible approach with an overall CR rate of 87% in randomised patients. Over 130 patients who entered the Mylotarg randomisation in the AML15 trial were over 60 years. The remission rate in these patients is 82%, which is superior to what would be expected in patients over 60 years of age. This has led to the decision to assess Mylotarg in a dose of 3mg/m<sup>2</sup> prospectively in the first course of both of the induction arms in AML16 in a 2 x 2 factorial design.

#### **5.1.4 Number of Treatment Courses**

While there are emerging data in younger patients as to how many courses of chemotherapy are optimal, relatively limited information is available in older patients. There is an emerging view that there may be little to gain from more than two courses of intensive chemotherapy<sup>(14,15)</sup>. In a recent unpublished non-randomised study there was no difference in the outcome for patients who received post induction treatment and those who did not<sup>(16)</sup>. Since the question has never been addressed in a prospective manner this trial will randomise patients who have responded (CR or PR) to the first course of chemotherapy, and who are confirmed to be in CR after course 2, to two versus three course of chemotherapy.

Patients who have failed to achieve at least a PR with course one will not be randomised, but will be allocated to receive three courses. In the NCRI AML14 trial 66% of patients who achieved CR achieved it with the first course. Of the 16% of patients who achieved PR with course 1, half achieved CR with course 2. Of the 16% of patients who achieved less than a PR with course 1, half achieved a CR with course 2. There was no difference in survival in patients who achieved CR and PR after course 1, but patients who achieved less than a PR after course 1 and entered CR after course 2 survived less well. Patients who have achieved a CR after course 2, and for whom no stem cell transplant is planned will be randomised to demethylation or not, as maintenance.

### **5.1.5 Non- Intensive Allogeneic Stem Cell Transplant**

There is now clear evidence that allogeneic stem cell transplant is feasible in older patients when non-intensive conditioning is used<sup>(17)</sup>. Full donor chimaerism is reliably established but there remains a risk of both infectious and immunological sequelae (graft versus host reaction). Much less is established about the value of this approach in controlling disease. It was initially thought unlikely to be of value in acute myeloid leukaemia because the immunological graft versus leukaemia effect took several weeks to establish. However there is increasing experience accumulating that this approach may offer a level of disease control which is similar to that of a conventional allograft<sup>(18)</sup>.

AML16 will be one of the first studies to prospectively evaluate the contribution that non-intensive allograft can make as an approach to consolidation in any disease setting. The evaluation will be on the basis of whether a donor is available or not, in situations where tissue typing is undertaken, i.e. a donor versus no donor comparison.

### **5.1.6 Maintenance Treatment with Azacytidine**

In general most studies, including our previous MRC AML11 Trial, have failed to show an advantage for maintenance treatment. Where a benefit has been demonstrated in older patients, it has usually been relatively modest. Epigenetic

therapy represents a new approach to control disease. Azacytidine is not a new drug and was evaluated several years ago in conventional myelosuppressive therapy. Interest has been re-kindled in recent years by the possibility of using this agent, and similar agents, at low dose to facilitate gene activation to enable completion of cell differentiation<sup>(19,20)</sup>.

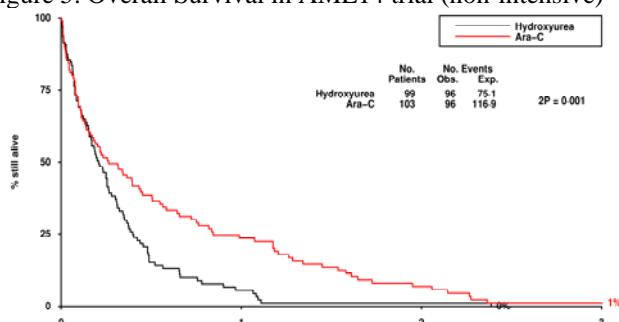
The clinical potential was demonstrated in a number of non-randomised studies and in a randomised trial conducted by the CALGB in Myelodysplastic Syndromes,<sup>(21)</sup> where haematological responses were demonstrated and there was a significantly lower risk of progression to AML and superior survival. Responses were not limited to the low risk subtypes of MDS. Twenty-three percent of patients achieved a CR or PR, demonstrating the efficacy even in high risk disease. Using this approach as a maintenance strategy is novel.

## 5.2 Non-Intensive Approach

### 5.2.1 Low Dose Ara-C

A substantial majority of patients diagnosed with AML or high risk MDS are elderly and either decline, or are not considered fit for, intensive treatment. Until recently, there was no established treatment for these patients. As part of the NCRI/LRF AML14 trial, low dose Ara-C was compared with Hydroxyurea. The trial was closed early because low dose Ara-C was significantly superior. Although an 18% remission rate was observed the overall survival was still poor at 5 months<sup>(22)</sup> (Figure 5).

Figure 5. Overall Survival in AML14 trial (non-intensive)





### **5.2.2 Mylotarg (Gemtuzumab Ozogamicin)**

Mylotarg is the only treatment licensed in the United States for relapsed AML in patients over 60 years. The registration studies using a dose of 9mg/m<sup>2</sup> as a single agent and achieved a marrow remission rate of 34% with acceptable tolerability. A current Gimema Study Group trial is evaluating its use as a single agent for first line treatment in older patients who are not considered fit for a chemotherapy based treatment. These studies illustrate that the drug can safely be given to older patients. The reason for considering it as an option in the non-intensive choices of AML16 is that we initiated a randomised Phase II evaluation of Low Dose Ara-C versus Low Dose Ara-C plus Mylotarg in a standard dose of 5mg as an amendment to the AML14 trial. This was intended to demonstrate whether or not the combination was feasible, in the knowledge that that amendment would not recruit sufficient patients to provide reliable evidence on efficacy. This experience with 100 patients randomised has not been associated with any reported serious adverse events, and is therefore considered feasible. This component of AML16 represents a continuation of this comparison.

### **5.2.3 Clofarabine**

Our recent experience with Clofarabine in patients not considered fit for intensive chemotherapy provided encouraging responses as described in section 5.1.2. However treatment at 30 mg/m<sup>2</sup> was associated with a significant duration of neutropenia and thrombocytopenia. Subsequently 7 patients have been treated with a dose of 20 mg/m<sup>2</sup>, 4 of whom are known to have entered CR. There therefore appears to be encouraging activity at this lower dose level. In AML 16 the lower dose will be compared with Low Dose Ara-C, with the provision for dose reduction if the same degree of myelosuppression is seen as in the higher dose.

### **5.2.4 Arsenic Trioxide**

Arsenic Trioxide (Trisenox<sup>TM</sup>) has proved to be a highly effective agent in inducing morphological and molecular remission in Acute Promyelocytic Leukaemia<sup>(28,29)</sup>.

Some responses have also been observed in myelodysplastic syndromes with good tolerability in older patients<sup>(30)</sup>. It has several potential modes of action including inhibition of angiogenesis, mitochondria membrane depolarisation with activation of apoptosis, direct activation of apoptosis and disruption of the interaction with the marrow microenvironment<sup>(31,32,33)</sup>. In a small study there was little activity when used as monotherapy<sup>(34)</sup>, although some modest effects were observed when it was combined with Ascorbic acid<sup>(35)</sup>. In a recent non-randomised Phase II study in untreated AML and MDS in combination with low dose Ara-C a complete remission rate of 38% was observed in 37 patients with AML and 25% in patients with high risk MDS<sup>(36)</sup>. This suggests the possibility that the efficacy of “standard” treatment with low dose Ara-C could be enhanced by the addition of Arsenic Trioxide. The tolerability of the combination in older patients who were not considered fit for an intensive approach was generally acceptable. The main side effects were neutropenic sepsis and febrile neutropenia, some fluid retention (16%), non-symptomatic QT/QTc prolongation (42%) and transient elevation of liver function tests (5-11%).

The AML 16 trial will thus include an option to compare low dose Ara-C with low dose Ara-C combined with Arsenic Trioxide.

### **5.3 Frailty Index**

While there is evidence that older patients who are considered fit for intensive treatment tend to benefit from that approach, there is no clarity about the definition of fitness for intensive therapy. For this reason it is difficult to be sure that trials in older patients recruit comparable patients. Traditionally, treatment decisions are influenced by the patient’s age and a crude measure of performance, whether formally as an entry criteria or informally influencing the doctor treating the patient. While age might be a surrogate for better outcome for treatment it does not in itself, at an individual level, identify patients who will or will not benefit from an intensive treatment approach. Underlying the decision to offer, or not, an intensive approach to treatment there are a number of other, more specific characteristics of the patient. While this issue is well recognised there are hardly any studies in acute leukaemia which have focussed on this issue or tried to develop a more sensitive

“score” that guides the treatment choice. Most leukaemia studies have only identified disease characteristics which have prognostic significance and not those which are predictive of which approach to treatment is likely to be better.

Older patients have co-morbidities which tend to accumulate with greater age, but are not directly associated with age. It is also quite possible that more older patients will appear for treatment who have less co-morbidity irrespective of chronological age. There are a number of assessments available which have been developed in geriatric medicine to predict outcomes, to assess needs or to assess interventions. These include, but are not confined to, the Comorbidity Index<sup>(37)</sup>, Cumulative Index Rating Scale (Geriatrics)<sup>(38)</sup>, The Multilevel Assessment Questionnaire<sup>(39)</sup>, Linear Assessment Self Assessment<sup>(40)</sup>, MAX2<sup>(41)</sup>, Multidimensional Evaluation Scale<sup>(42)</sup>, Geriatric Depression Scale<sup>(43)</sup>, and the Mini-Mental State scale<sup>(44)</sup>. All of these have been validated in different aspects of geriatric medicine, some in the context of cancer, but none in acute leukaemia.

Each of these assessments has aspects that appear to be relevant to acute leukaemia in older patients. As a directly associated, but separate, study we propose to formulate, test and prospectively evaluate a “Frailty Index” with respect to predicting which patients may benefit, or indeed be harmed, by one or other of the treatment approaches in AML16, and to determine whether such an assessment provides more precision than currently available methods.

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## **6 INCLUSION AND EXCLUSION CRITERIA**

### **6.1 Inclusion Criteria**

Patients are eligible for the AML16 trial if:

- They have one of the forms of acute myeloid leukaemia, except Acute Promyelocytic 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 greater than 10% marrow blasts (RAEB-2).
- They should normally be over the age of 60, but patients under this age are eligible if they are not considered fit for the MRC AML15 trial.
- They have given written informed consent.

### **6.2 Exclusion criteria**

Patients are not eligible for the AML16 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).
- They have a concurrent active malignancy excluding basal cell carcinoma.
- They are pregnant or lactating.
- They have Acute Promyelocytic Leukaemia.
- Patients with abnormal liver function tests exceeding twice the local upper limit of normal are not eligible for the Mylotarg randomisations.
- Patients with a serum creatinine above the local upper limit of normal are not eligible for the Clofarabine randomisations in either the intensive or the non-intensive parts of the trial.

## 7 PROCEDURES FOR ENTRY INTO THE TRIAL AND DATA RECORDING

### 7.1 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, an individual at each participating institution is required to act as the Principal Investigator for the Institution. They will be asked to confirm: (1) that the trial will be conducted under the institution's research governance framework; (2) that they have received and have read the MRC guidelines for good clinical practice in clinical trials; (3) that they agree with the requirements of Cardiff University as the trial sponsor; (4) that the study has LREC approval; (5) that written consent will be obtained for each patient and a copy retained in the notes, and a copy sent to the Trial Office; (6) that they agree to report serious unexpected adverse events as set out in Section 17 of this protocol; (7) that they agree to participate in random audit if requested; (8) that they will report data in a timely fashion; (9) that material to be stored for research is obtained using the trial consent documentation.

For administrative reasons, investigators will also be asked to supply details of the location of their immunophenotyping, cytogenetic, molecular, genetic, pharmacy, tissue typing and transplant services, whether they wish to transmit data using the web based data collection system, 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, and relevant institutional normal ranges (bilirubin, AST, ALT and LDH) will be registered.

### 7.2 Patient Recruitment

Patients may be recruited only once a centre is fully registered (Section 7.1). Patients to whom it has been decided to offer an intensive treatment approach should be consented for entry into the trial using **Patient Information Sheet 1 and Consent Form 1**. Patients who will be offered a non-intensive approach should be consented using **Patient Information Sheet 2 and Consent Form 2**. Consent for

storage of excess diagnostic material should be obtained using **Patient Information Sheet 3 and Consent Form 3**.

### 7.3 Randomisation

There are two randomisation points in the intensive treatment option of the trial and one randomisation point in the non-intensive option, for which contact must be made with the Birmingham Clinical Trials Unit (BCTU). Patients fulfilling the criteria for entry into the trial (see Section 6) should be entered into the first randomisation by telephoning the BCTU in Birmingham (Tel: 0800 953 0274 from the UK, +44 121 687 2319 from outside the UK). Telephone randomisation is available Monday to Friday, 09.00–17.00; internet randomisation is available seven days a week at: <https://www.trials.bham.ac.uk/aml16>

### 7.4 First randomisation (Intensive)

For this randomisation **Patient Information Sheet 1 and Consent Form 1** should be used.

During the course of the trial certain randomisation options may not be available. Investigators will be informed in advance so that only relevant information is given to the patient during the consent procedure.

Treatment allocation will be given once the required patient details have been supplied. There are two randomisations available at this timepoint, namely:

**DA or DClo** (for details, see Section 9.1).

**Mylotarg or not** (for details, see Section 9.2 and Appendix B).

**Note:** It is expected that most patients will be entered into both randomisations. If a patient is not eligible to receive Mylotarg because of abnormal liver function they will be randomised to the chemotherapy options only. If they present with a white count in excess of  $30 \times 10^9/l$  they can either reduce it to this level with Hydroxycarbamide before starting trial treatment, or give Mylotarg on day 4 of chemotherapy..



The four available treatment arms are:

- Arm A Two courses of DA (with no Mylotarg)
- Arm B Two courses of DA (with Mylotarg in course 1)
- Arm C Two courses of DClo (with no Mylotarg)
- Arm D Two courses of DClo (with Mylotarg in course 1)

If a patient is randomised both between chemotherapy regimens and between Mylotarg versus not, they will be allocated to one of the four arms with a 25% chance of receiving any particular treatment arm.

If a patient is not randomised between Mylotarg or not they will be allocated between Arm A versus Arm C 50% chance of receiving each one. If a patient has an elevated serum creatinine they cannot enter the Clofarabine randomisation, but can be randomised instead between Arms A and B.

**Note:** Patients will be expected to complete a “**Frailty Index**” **Assessment** prior to commencing therapy when this becomes available.

After a patient has recovered from course 1 and had a marrow assessment they will receive course 2 which will be the same chemotherapy as they were allocated in course one, but Mylotarg will not be given. After recovery from course 2, patients who have achieved at least a PR after course 1, and are in CR after course 2, will be eligible for the second randomisation in the intensive arm (see section 7.11)

## **7.5 First randomisation (Non-Intensive)**

For this randomisation **Patient Information Sheet 2 and Consent Form 2** should be used.

During the course of the trial certain arms may not be available. Investigators will be informed in advance so that only relevant information is given to the patient during the consent procedure.

Treatment allocation will be given once the required patient details have been supplied, and will be one of the following four treatments:

- E. Low Dose Ara-C (see Section 13.1 for details)
- F. Low Dose Ara-C plus Mylotarg (see Section 13.2 for details)
- G. Low Dose Clofarabine (see Section 13.3 for details)\_
- H. Low Dose Ara-C with Arsenic Trioxide (see Section 13.4 for details)

Clinicians will normally be expected to randomise patients between all the available options, in which there is a 25% of receiving any one of the four treatments. If a patient is not eligible for one of the treatments, he/she will be randomised between the options for which they are eligible.

During the course of the trial additional options may be introduced and/or existing options closed. Such changes will be achieved by means of protocol amendment.

#### **7.6 Information required at first randomisation**

- Is the patient to receive the Intensive or the Non-intensive treatment?
- 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 Myelodysplastic Syndrome
- Confirmation that the Frailty Index assessment, when available in a separate study, will be completed

- For patients entering a Mylotarg randomisation, confirmation that liver function tests are within twice the upper limit of normal and that the WBC is  $<30 \times 10^9/l$ .
- For patients entering a Clofarabine randomisation, confirmation that serum creatinine are within the normal range

For patients to be treated with a non-intensive approach, the investigator will be asked to complete a brief separate questionnaire to define the main reasons why the patient was not considered fit for intensive treatment. The investigator will also be asked to state whether, based on a preliminary assessment, a patient in the intensive treatment option is a potential candidate for a non-intensive stem cell allograft if a matched donor were to be identified. It is obviously difficult to make such judgements at diagnosis, and investigators will not be expected to stick with their initial evaluation, but this information is necessary to give an idea of the patient's possible course.

## **7.7 Diagnostic material**

One objective of the trial is to investigate the relevance of cytogenetic and molecular characteristics and the value of minimal residual disease detection. Diagnostic material is essential for these studies. It is of particular importance to define the cytogenetic abnormalities, and the molecular characteristics, of each patient as this may be relevant to the treatment strategy in the future. It is also intended to store excess diagnostic material for future research, but it is mandatory to obtain the patient's specific consent to do so. For this purpose **Patient information sheet 3 and Consent form 3** should be used.

## **7.8 Morphology**

Central morphological review will be provided by Dr David Swirsky as in previous MRC AML trials. Six unstained unfixed marrow slides should be sent at diagnosis (see page ii for address).

## 7.9 Immunophenotypic Characterisation and Molecular Genetics

A network of laboratories has been established to examine diagnostic material for aberrant immunophenotypes which may be useful for disease monitoring. Cells will also be processed in these laboratories for molecular characterisation and cell banking. Investigators will be supplied with a collection kit which should be sent to one of these reference laboratories using the enhanced Royal Mail delivery service using the account reference number provided with the collection kit.

### REFERENCE LABORATORIES FOR AML16 IMMUNOPHENOTYPING FOR MRD DETECTION:

Dr Sylvie Freeman  
Clinical Immunology  
Division of Infection and Immunity  
University of Birmingham  
P.O. Box 1894  
Vincent Drive  
Edgbaston  
Birmingham, B15 2SZ  
Tel: 01214158759 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 9596306  
Fax: 0177 959 6062  
E-mail: [Paul.Virgo@nbt.nhs.uk](mailto:Paul.Virgo@nbt.nhs.uk)

Mr Steve Couzens  
Department of Haematology  
University Hospital of Wales  
Heath Park, Cardiff  
CF14 4XN  
Tel: 02920742370  
Fax : 02920745084  
e-mail: [Couzenssj@cardiff.ac.uk](mailto:Couzenssj@cardiff.ac.uk)

Dr Christopher McNamara  
Haematology Department  
Royal Free Hospital  
Pond Street  
LONDON  
NW3 2QG  
Tel: Tel: 0207 794 0500  
Fax: 020 7830 2313  
[Christopher.McNamara@royalfree.nhs.uk](mailto:Christopher.McNamara@royalfree.nhs.uk)

During the course of the trial patients who have suitable expression types may be selected for sequential monitoring of residual disease.

Molecular definition is intended for all patients initially for characterisation of *FLT3* and *RAS* mutations or for other relevant mutations which may be identified during the course of the trial. Diagnostic material will also be used for studies of gene expression by DNA microarray and future research studies, for which patient informed consent must be obtained.

Samples at diagnosis for dispatch to the reference labs should be:

6 ml of bone marrow in a lithium heparin tube

*and*

20 ml of heparinised blood

## **7.10 Cytogenetics**

Cytogenetics should be carried out locally and reports sent directly from the cytogenetics laboratory to the BCTU. Cell pellets should be stored locally. If there are difficulties locally, central facilities will be provided by Mrs Yashma Patel at UCH — please indicate clearly on the samples that cytogenetic analysis is required.

## **7.11 Diagnostic Immunophenotyping**

Immunological definition is essential and should be carried out locally at the regional service — a copy of the report should be sent to BCTU with the "Notification of Entry" form. The local analysis is carried out for diagnostic purposes and is done in addition to material being sent to the reference laboratories for aberrant phenotype detection for the minimal residual disease assessment.

## 7.12 Follow-up Material

Investigators will be informed of patients who are of particular interest for subsequent assessments (e.g. patients in whom an aberrant phenotype is confirmed at diagnosis or demethylation studies on samples from patients who enter the demethylation treatment randomisation (see section 11.3 for details)) 2 to 4mls of marrow which is collected for remission status after each cycle should be sent to the designated lab. Investigators will receive a prompt from the trial office a few days before the sample is due.

## 7.13 Second randomisation (Intensive)

For this randomisation **Patient Information Form 4 and Consent Form 4** should be used.

After patients have received course 2 of the allocated treatment a bone marrow assessment of response will be carried out (see section 10). If the patient has achieved a CR or PR (ie less than 15% blasts) after course 1, and is in complete remission after course 2, they are eligible to be randomised between **another course of chemotherapy or not**. They will at the same time be randomised to **maintenance with Azacytidine or not**. Patients who fail to achieve at least a PR after course 1, but who are in CR after course 2, will receive a third course of treatment and then be randomised to Azacytidine or not. Patients who are intended to receive an allogeneic stem cell transplant will not be eligible for randomisation to Azacytidine.

If allocated to receive a third course of chemotherapy it should be **DA 2+5** (see Section 9.4).

## 8 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 institutional password, and complies with Data Protection Act standards. The system can be accessed on:

<https://www.trials.bham.ac.uk/aml16>

A user password will be supplied to investigators on receipt of the letter of LREC Approval, site specific assessment and centre registration information (see Section 7.1).

For investigators who do not wish to use the internet system, a patient record book will be available to download from the trial website: <http://www.aml16.bham.ac.uk>, and it can be sent to the consultant in charge of a patient's management on request to the Trial Office following entry.

Forms should be completed and either entered via the web-based system or returned to BCTU as follows:

### 8.1 For patients receiving intensive treatment:

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

**Two Course Report** (Form B) — return when the patient has received two courses of treatment, or at prior death (but not later than 2 months after completion of Course 2).

**Three Course Report** (Form C) — return when the patient has received a third course. This will apply to patients who failed to achieve at least a partial remission after course 1 who will receive and not be randomised to course 3. It will also apply to patients who were randomised to receive 3 courses.

**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).

**Maintenance Reports** (Form E&F) to be completed at six and 12 months from entering the maintenance randomisation.

**One Year Follow-up** (Form G) — return at one year after entry to the trial, or at death if the patient dies within 1 year of finishing therapy.

**Relapse** (Form H) — return at the completion of reinduction (and consolidation) therapy or at death (but not later than 4 months after relapse).

## **8.2 For patients receiving non-intensive treatment**

**Notification of Entry** (Form A1) — return when all the diagnostic data requested are available (but not later than 1 month after entry).

**Two Course Report** (Form B1) — return when the patient has received two courses of treatment, or at prior death (but not later than 2 months after completion of Course 2).

**Four Course Report** (Form C1) --- return when the patient has received courses 3 and 4 of the non-intensive treatment

**Six Month Follow-up** (Form D1) --- return at six months from trial entry, this is an important follow up date since it corresponds to the primary endpoint for this treatment option



**One Year Follow-up** (Form E1) — 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 F1) — return at the completion of re-induction (and consolidation) therapy or at death (but not later than 4 months after relapse).

Once a patient has been randomised, it is very important to have full and timely 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.

#### **8.4 Health Economics**

Basic information on resource use will be collected on all patients as part of the data forms outlined in Sections 8.1, 2.

#### **8.5 Frailty Index**

As a separate study, for which additional consent will be required, patients will have a multidimensional assessment carried out before the first randomisation. This will be designed to take about 20 to 30 minutes, and will have separate documentation.

### **9 INTENSIVE TREATMENT CHEMOTHERAPY SCHEDULE**

Each induction treatment arm comprises two courses of allocated chemotherapy. Remission status will be determined after each course. If after Course 1, the

patient has failed to respond, i.e. has more than 15% residual marrow blasts, they will receive 3 courses of treatment and will not be eligible for the 2 versus 3 randomisation. The local investigator may feel that such patients should not continue with further trial treatment, but follow-up data must continue to be collected on all patients who go off protocol.

Patients who present with high white cell counts ( $>30 \times 10^9/l$ ) and have been randomised to receive Mylotarg can either receive oral Hydroxyurea (up to 4 g/day) to reduce the count before starting treatment, or can delay the administration of Mylotarg until day 4 of the chemotherapy. Patients with high counts at diagnosis can be considered for treatment with Rasburicase to reduce the risk of tumour lysis.

### **9.1 DA schedule**

#### **Course 1 DA 3+10**

Daunorubicin  $50 \text{ mg/m}^2$  daily by i.v. infusion on days 1, 3 and 5 (3 doses).

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

#### **Course 2 DA 3+8**

Daunorubicin  $50 \text{ mg/m}^2$  daily by i.v. infusion on days 1, 3 and 5 (3 doses).

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

### **9.2 DClo schedule**

#### **Course 1 DClo**

Daunorubicin  $50 \text{ mg/m}^2$  daily by i.v. infusion on days 1, 3 and 5 (3 doses).

Clofarabine  $20 \text{ mg/m}^2$  by i.v. infusion over 1 hour daily on days 1 – 5 inclusive (5 doses over 5 days)

#### **Course 2 DClo**

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

Clofarabine 20mg/m<sup>2</sup> by i.v. infusion over 1 hour daily on days 1 – 5 (5 doses over 5 days).

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

During the course of the trial Clofarabine is likely to become available in an oral formulation, which can then be used instead of the parenteral formulation.

### 9.3 Mylotarg Therapy

Patients allocated to receive Mylotarg must not have a white count greater than 30 x 10<sup>9</sup>/l at the time of Mylotarg administration because of the risk of tumour lysis. Such patients should either have the WBC reduced with Hydroxycarbamide before commencing trial chemotherapy or have the administration of Mylotarg delayed until day 4 of the chemotherapy. Patients are only eligible to receive Mylotarg if the liver function tests do not exceed twice the upper limit of normal.

**Mylotarg will be given at a dose of 3 mg/m<sup>2</sup> on day 1 of Course 1.** Details of the premedication, and other procedures for Mylotarg administration, are set out in Appendix B.

### 9.4 Course 3

If a patient achieves a PR or CR after course 1 and is in CR after course 2 they will be eligible to be randomised to receive or not, a third course of chemotherapy. If a patient fails to achieve a PR or CR after course 1 but achieves a CR after course 2 they should receive a third course of treatment. When allocated, the third course will be given prior to entering the non-intensive transplant or maintenance options. The treatment for course 3 will be:

### **DA 2+5**

Daunorubicin 50 mg/m<sup>2</sup> daily by i.v. infusion on days 1 and 3 (2 doses).

Cytosine Arabinoside 100 mg/m<sup>2</sup> 12-hourly by i.v. push on days 1-5 inclusive (10 doses).

## **10 ASSESSMENT OF RESPONSE**

Response should be assessed 21 to 28 days from the end of each course until complete marrow remission is confirmed. If the marrow sample is too hypoplastic to evaluate it should be repeated 7 to 10 days later.

### **10.1 Definition of Complete Marrow Remission:**

- Cellularity of marrow should be at least 20% with evidence of trilineage regeneration.
- Less than 5% blasts
- No Auer rods
- No extra-medullary disease
- Evidence of peripheral blood count recovery

### **10.2 Definition of Partial Marrow Remission:**

Meets all the criteria for complete remission but marrow blasts are between 5 and 15%.

### **10.3 Resistant Disease:**

Patients who fail to have < 15% blasts in the marrow in response to course 1 have resistant disease. Such patients should receive the second course of treatment.

### **10.4 Refractory Disease:**

Patients will be considered to have refractory disease if they have failed to achieve a CR after course two. These patients will **not continue** in the treatment protocol but will continue to be followed up annually for life.

## **11 SECOND INTENSIVE TREATMENT RANDOMISATION: TO COURSE 3 and/or MAINTENANCE TREATMENT**

Patients who have achieved CR or PR after course 1, and are in CR after course 2, are eligible for randomisation to have **one further course of treatment or not**, and to **maintenance Azacytidine or not**. Patients who failed to achieve at least a PR after course 1 but who achieved a CR after course 2, will receive a third treatment course after which they are eligible to be randomised to maintenance Azacytidine or not.

Patients who are intended to receive a reduced intensity allograft should undergo the chemotherapy randomisation but are not eligible for the maintenance randomisation to Azacytidine or not.

**Patient Information Sheet 4 and Consent Form 4** should be used. Note that the patient is being asked to have a marrow assessment to confirm remission status and to agree to samples to be taken for methylation status.

Although randomisation will be carried out as close to the last course of chemotherapy 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.

For the 3 course/maintenance randomisation: (i) telephone the BCTU (tel: 0800 953 0274) during office hours (09:00 to 17:00 hrs, Monday to Friday); (ii) internet randomisation is available seven days a week at: <https://www.trials.bham.ac.uk/aml16>

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

- AML16 trial number (or full name and date of birth).
- Confirmation that the patient is in complete remission
- Remission status of the patient after course 1
- Whether the patient is, or is not scheduled for a Non-Intensive Stem Cell Transplant.
- That the patient has received either 2 or 3 courses of chemotherapy (depending on the randomisation options being entered).

### **11.1 Maintenance Treatment**

Patients who have been allocated to receive maintenance treatment will receive a five day course of Azacytidine every six weeks for nine courses. Methylation status for those allocated to treatment will be assessed at randomisation and after 18, 36 and 54 weeks.

#### **Maintenance Therapy**

Azacytidine 75 mg/m<sup>2</sup> subcutaneously daily for 5 days to be repeated at 6 week intervals for nine courses.

There may be a need to consider a dose reduction due to cytopenia during the proposed 54 weeks of treatment, but the first course should be given at the full dose and should commence when the peripheral neutrophil count reaches  $1.0 \times 10^9 /l$  and platelets reach  $80 \times 10^9 /l$ . Treatment should be preceded by the administration of an 5-HT3 receptor antagonist (e.g. ondansetron) approximately 30 minutes before Azacytidine. Patients may experience diarrhoea which should be treated symptomatically.

## **11.2 Dose Reduction Criteria**

If a patient experiences a **non-haematological toxicity** with an NCI CTC (National Cancer Institute Common Toxicity Criteria) grade 3 or 4 which represents a deterioration from the pre-dose level, the Azacytidine should be temporarily delayed until the toxicity grade returns to baseline level. If the grade 3 or 4 toxicity does not return to baseline within 21 days from onset, Azacytidine should be permanently discontinued. This eventuality should be reported as an adverse event to the Trial Office.

The NCI CTC definitions are available from the Trial Office or can be downloaded from the trial website (<http://www.aml16.bham.ac.uk>).

Patients who have not recovered the neutrophil and platelet counts to the pre-treatment level by the end of the 6 week interval should have the next course delayed. If the recovery then takes place within 14 days the next course can be given at full dose. If full recovery has not taken place and there is no evidence of relapse the next course can be given at a 50% dose reduction. Deliver the 9 planned total courses.

## **11.3 Assessment of Methylation Status**

Gene methylation status will be assessed at randomisation and after 18, 36 and 54 weeks of treatment. For this 10ml of peripheral blood should be sent to the reference lab (at Cardiff) listed on page ii of the protocol. Investigators will be sent

a reminder approximately 2 weeks before samples become due. Consent for these tests is be included in Patient Information and Consent Form 4.

## **12 NON-INTENSIVE ALLOGENEIC STEM CELL TRANSPLANT**

Patients who have an HLA matched donor available are eligible to receive a non-intensive allograft. Such patients should be discussed with the local transplant service as soon as a donor is identified so that arrangements can be made to medically assess the fitness of the donor and the patient. The precise protocol to be used in the AML16 trial will be prescribed and, as the field develops over the next five years, will be subject to changes in light of experience.

Transplant centres initially may choose one of two mini-allograft protocols:

### **FBC Protocol:**

Fludarabine	30 mg/m <sup>2</sup> /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 as VOD prophylaxis is optional)

### **UCL Protocol:**

Fludarabine	30 mg/m <sup>2</sup> /day	days -7 to -3 inclusive
Melphalan	140 mg/m <sup>2</sup>	on day -2
Campath 1H	20 mg/day	days -8 to -4 inclusive

Since patient and donor will require time to be counselled about the transplant option which may be delivered as early as course 3, investigators are encouraged to identify donor availability as soon as possible after diagnosis.



On completion of the transplant the completed "Transplant" form (Section D) should be returned to BCTU or entered via the web-based system.

### **13 NON – INTENSIVE TREATMENT SCHEDULE**

Patients not considered fit for intensive treatment are eligible to enter a randomised comparison of **Low Dose Ara-C** versus three novel treatments. These options are being evaluated in a randomised Phase II design with the primary endpoint being CR. Based on an interim analysis (see Section 17 for full details) a decision may be made to alter the comparison for the treatments that are showing promising results, to a Phase III design with overall survival as the primary endpoint, or to close those treatment options that do not appear to show any benefit. This design means that one or more of the treatment options are likely to become unavailable during the course of the trial. Similarly new options may be introduced to the Phase II design during the course of the trial. Investigators will be notified by the Trial Office about the status of treatment availability.

The novel treatments will be:

- (i) Low Dose Ara-C with Mylotarg.**
- (ii) Low Dose Ara-C with Arsenic Trioxide**
- (iii) Low Dose Clofarabine.**

#### **13.1 Low Dose Ara-C**

Patients randomised to receive low dose Ara-C will receive:

**Ara-C 20 mg bd by subcutaneous injection daily on days 1-10 (20 doses) to be repeated at 28 to 42 day intervals.**

In some patients it may be necessary to extend the intervals to up to 42 days. A minimum of **4 courses** should be administered. If it is considered appropriate,

further courses can be administered (with no limit to the number given). It is intended that low-dose Ara-C will be given in the community although the patient may need to attend as a day case to receive the first dose.

### **13.2 Low Dose Ara-C with Mylotarg**

Patients are only eligible to receive Mylotarg if the liver function tests do not exceed twice the upper limit of normal. Patients allocated to receive Mylotarg whose presenting WBC is greater than  $30 \times 10^9 / l$  should have the WBC reduced by Hydroxycarbamide before starting treatment.

Patients should not be given azole antifungal drugs until day 5 after the administration of Mylotarg.

Patients who are randomised to receive Low Dose Ara-C with Mylotarg should be given:

**Ara-C 20 mg bd by subcutaneous injection daily on days 1-10 (20 doses)**

*and*

**Mylotarg (Gemtuzumab Ozogamicin) 5 mg intravenously on *day 1* of Low Dose Ara-C treatment**

The treatment should be repeated at **28 to 42 day intervals for four courses**. Details of the premedication, and other procedures for Mylotarg administration, are set out in Appendix B. It is intended that the low-dose Ara-C will be given in the community although the patient will need to attend as a day case to receive the first dose and the Mylotarg on day 1.

### **13.3 Low Dose Clofarabine**

Patients who are randomised to Clofarabine should receive:

**Clofarabine 20 mg/m<sup>2</sup> by IV infusion over 1 hour, daily on days 1 to 5**

The treatment should be repeated at **28 to 42 day intervals for 4 courses**. The main side effect at higher doses has been myelosuppression, so if haemopoietic recovery has not recovered by 28 days from the completion of course 1, but has done so by day 42, the subsequent courses should be reduced to 15mg/m<sup>2</sup> daily for 5 days. Patients whose serum creatinine is above normal on any treatment day should omit that day's dose

During the course of the trial an oral formulation of Clofarabine may become available, and this can be used in place of the parenteral formulation. Investigators will be advised of the availability and dose of the oral formulation at that time.

#### **13.4 Low Dose Ara-C with Arsenic Trioxide**

Patients who enter this option will be randomly allocated to receive Arsenic Trioxide (Trisenox) with Low Dose Ara-C:

**Ara-C 20 mg bd by subcutaneous injection daily on days 1-10 (20 doses)**

**Arsenic Trioxide 0.25mg/kg on days 1 to 5 (5 doses) and on days 9 and 11 (giving 7 doses in total).**

Patients should have an ECG assessment before and up to twice weekly during treatment to ensure that the QT interval does not exceed 460ms. Drugs which can prolong the QT interval should be avoided (a list of such drugs is given on the website [www.torsades.org](http://www.torsades.org)). During therapy the serum potassium must be kept above 4mmol/l and the serum magnesium above 1.8mg/dl.

## 14 HEALTH ECONOMICS ASSESSMENT

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

## 15 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
5. Antifungal prophylaxis Response to a significant pyrexia — i.e. two readings of  $\geq 38^{\circ}\text{C}$  two hours apart, or a single reading  $\geq 39^{\circ}\text{C}$ .
6. Antibiotic treatment of febrile episodes — including antibiotic choice(s) and monitoring, duration of therapy, and the treatment of non-response.
7. G-CSF therapy [Lenograstim 263  $\mu\text{g}$  (1 vial) S.C. daily] may be given in case of prolonged neutropenia but it is not intended that it should be part of routine supportive care.
8. Irradiated blood products should be given to patients who receive Stem Cell Transplant.

## **16 RELAPSE**

Relapse will be diagnosed either on morphological or cytogenetic grounds. When observed relapse and its treatment should be documented. It is probable that patients who enter AML16 and relapse will not wish to receive further treatment, but for those who do and are considered suitable should be entered into the current NCRI high risk AML trial if available.

The "Relapse" form from the patient's AML16 record book should be completed giving details of the relapse, subsequent therapy and its outcome. This form should either be completed online or filled in and returned to BCTU when all the necessary data are available.

## **17 STATISTICAL CONSIDERATIONS**

### **17.1 Patient numbers**

The large improvements in survival of younger patients with AML observed over the last 40 years have, unfortunately, not been mirrored in older patients — in the intensive arm of AML14, survival at 5 years in patients aged 60 or over is only 15%, while even with low-dose Ara-C nearly all patients in the non-intensive arm have died within 3 years. Thus, it is unrealistic to expect any of the treatments being evaluated in AML16 to lead to improvements in survival of more than 10% to 15%, while smaller benefits would probably not be worthwhile given the likely costs of the new agents under investigation. In order to be able to detect or refute improvements of this order, large trials are needed. For example, to demonstrate (at a 2-tailed  $P=0.05$ ) a 67% proportional improvement in five-year survival from 15% on one treatment to 25% on the other requires approximately 550 patients (with 440 deaths) to have a 90% chance of detecting this difference.

There are approximately 2000 cases of AML in patients aged 60 or over diagnosed each year in the British Isles, and probably a similar number with high risk MDS. Some of these might be too old or unfit to be considered for any form of chemotherapy. The NCRI network of investigators has recruited 200 patients per annum for the AML11 and AML14 Trials which offered an intensive approach to treatment. It is therefore expected that at least 1250 patients will be available to the intensive option in the life of this trial.

It is estimated that there a similar number of patients can be recruited who are not considered fit for an intensive approach to treatment. Such patients are sometimes reluctant to enter clinical trials of any type, but we hope that the novel approach and the inclusion of new agents will encourage both physicians and patients to participate.

## **17.2 Intensive therapy**

It is estimated that about 250 patients will enter the intensive part of AML16 per annum, and that nearly half of these will subsequently achieve remission, complete the induction/consolidation therapy and be eligible for randomisation for maintenance treatment. Therefore, of the annual projected intake of patients up to 100 will be available for the maintenance randomisation.

Thus, if trial entry proceeds successfully for at least 5 years, over 1200 patients could be randomised for induction chemotherapy. With 800 patients randomised to each comparison, there will be 90% power to detect (at 2-tailed  $p=0.05$ ) a 10% absolute difference in 2 year survival (25% to 35%) between DA and DClo and between Mylotarg versus not. A larger number of patients entered at diagnosis is needed in order to obtain enough maintenance randomisations (see below), but once sufficient numbers are accrued to the initial induction questions, one or more new randomisations could be introduced (as in previous MRC AML trials, e.g. AML12).

In a 5 year accrual period, at least 500 patients could be randomised between Azacytidine maintenance versus not, giving 90% power to detect a 15% absolute difference in survival at 2 years (40% to 55%) between arms.

### **17.3 Non-intensive therapy**

The aim of the non-intensive options in the AML16 trial is to recruit at least 250 patients per annum. To detect a 10% absolute difference in 2 year survival from 10% with LD Ara-C to 20% with a novel therapy (at a 2-tailed p-value of 0.01 with 80% power) would require about 200 patients and 170 deaths per arm. Thus, for novel therapies that are taken forward for full-scale Phase III evaluation, the aim will be to accrue 200 patients to each arm. Not all patients will be submitted for randomisation between all available arms, and not all arms will be available at any one time, but with more than 1200 patients in total over a 5 year accrual period there will be sufficient numbers to achieve the target of 200 per arm for promising treatments.

This evaluation will take place in three stages. Recruitment will proceed until at least 50 patients have entered each comparative arm (Ara-C and novel therapy). This component will then be analysed using CR as endpoint. While this assessment is taking place, recruitment will continue. If the arm appears sufficiently promising, then recruitment will continue until 100 patients are in each arm. At this point, a similar analysis on CR will be undertaken. If, on the basis of examining the data from the first 100 patients in each arm, the novel treatment is sufficiently promising then recruitment will continue to 200 patients per arm as a Phase III study, with the trial endpoints will be changed to CR, relapse and overall survival. However, if at either of the earlier analysis points, the judgement is that the treatment is unlikely to hold promise, the comparison will be discontinued. To allow for the flexible trial design, where patients may enter either a full randomisation or any pairwise comparison with Ara-C, the trial will be analysed stratified by choice of comparison, using standard meta-analytic techniques.

The choice of CR as endpoint in the initial comparisons is driven by two considerations. First, patients in the non-intensive part of AML14 who failed to enter remission had very poor prognosis. Thus, it is reasonable to assume that a treatment is unlikely to be able to improve overall survival without also improving remission rates in this group. CR is also an endpoint for which data become available very quickly, so a decision on whether there is sufficient evidence of improved CR rates to persist with a given therapy can be made in an expeditious fashion.

The decision on whether to proceed with a comparison will depend on the experimental treatment meeting certain preset improvements in CR rate compared to the Ara-C arm of the trial. The precise choice of cut-off at the two interim monitoring points (50 and 100 patients per arm) will depend on the treatment being tested: for example, for an inexpensive, well-tolerated drug one would be likely to accept a smaller improvement than if the drug were expensive, toxic and difficult to administer. For each treatment, the Data Monitoring and Ethics Committee will be issued with a detailed monitoring plan, incorporating guidelines for deciding whether to stop or continue with a novel therapy. A possible scenario would be: assuming a CR rate of 15% with low dose Ara-C and aiming to identify new treatments that produce a CR rate of 30% or more, a minimum of 50 patients will be accrued to each novel therapy arm; if the improvement in CR rate in the novel therapy arm is less than 2.5% (15% to 17.5%), that arm will be closed (with a 7% chance of rejecting a treatment with a true CR rate of 30% or more); if the difference in CR rates exceeds 2.5%, the arm will continue to 100 patients. At this point, if the improvement in CR rate in the novel therapy arm is less than 7.5%, that arm will be closed (with a further 8% chance of rejecting a treatment with a true CR rate of at least 30%); if the improvement in CR rate exceeds 7.5%, the comparison will continue to the full 200 patient per arm trial. Under this scenario, the overall power to identify an effective new treatment will be about 85%, and all but 7% of totally ineffective treatments will be dropped after either 50 or 100 patients.



## 17.4 Data analysis

Interim analyses of the main endpoints will be supplied periodically, in strict confidence, to an independent Data Monitoring and Ethics Committee (DMEC). In the light of these interim analyses, the DMEC will advise the chairman of the Leukaemia Steering Committee if, in their view, the randomised comparisons in the trial have provided proof beyond reasonable doubt\* that one treatment is clearly indicated or clearly contraindicated. In addition to looking at safety and evidence of efficacy, given the high cost of the novel treatments used in AML16 the DMEC will also review randomisations for futility, thereby enabling resources to be saved if there is good evidence that a treatment is unlikely to be shown to be effective (and cost-effective) if more patients are recruited to that arm.

The main analyses will be performed using standard contingency table and log-rank methods 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. The randomisations — and subsidiary data analyses — will be stratified by age (<60, 60-64, 65-69, 70-74, 75+), performance status, white blood count (0-9.9, 10-49.9, 50-99.9, 100+) and type of disease (*de novo* AML, secondary AML, high risk MDS). The 2 course versus 3 course and maintenance randomisations in the intensive arm will also be stratified by initial allocations and by status after Course 1 (CR, PR, RD, etc). All 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).

In the non-intensive part of AML16, all comparisons of novel treatments will initially be with the standard arm (i.e. low-dose Ara-C). Because of the multiple comparisons, the level of statistical significance will be set at  $p=0.01$ . Analyses of

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\* 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.

the non-intensive randomisation will be stratified by the comparison the patient took part in (e.g. 2-way comparison, full 5-way comparison).

## **18 TRIAL GOVERNANCE AND ADVERSE EVENT REPORTING**

Investigators have obligations described in the MRC handbook “MRC Guidelines for Good Clinical Practice in Clinical Trials”. The Trial is sponsored by Cardiff University with defined responsibilities delegated to the Birmingham Clinical Trials Unit, and to the Principal Investigator on each site. The trial is authorised by a Clinical Trials Authorisation (CTA) issued by the MHRA. The trial protocol has been approved by Central Office Research Ethics Committee (COREC). The COREC approval requires that investigator site have a designated Principal Investigator and that participating institutions submit a Site Specific Agreement (SSA) to the MREC before a site can participate which is regarded by the Sponsor as an acceptance by the participating institution that the trial will be conducted under the local policies in compliance with the Research Governance Framework. Each participating institution will be required to complete a site registration with the Birmingham Clinical Trials Unit as described in section 6.1. The trial will be monitored by the MRC Trial Steering Committee and an independent Data Monitoring and Ethics Committee.

### **18.1 Adverse Event Reporting**

Principal Investigators on each participating institution have an obligation to report relevant Serious Unexpected Adverse Events (SAE) which occur in this trial to the Trial Office **in Cardiff** 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.

### **18.2 Definitions**

For the purpose of this trial a **Serious Unexpected Adverse Event (SUAE)** is defined as:

- development of a non-haematological toxicity of grade 3 as defined in the NCI Common Toxicity Criteria, which does not resolve to grade 2 or less within 7 days.
- development of any grade 4 non-haematological toxicity (excluding alopecia)
- development of neutropenia ( $<1.0 \times 10^9/l$ ) or thrombocytopenia ( $<50 \times 10^9/l$ ) for longer than 42 days after the end of chemotherapy in the absence of significant disease in the bone marrow ( $>5\%$  blasts).
- Any event which results in persistent or significant disability or incapacity.
- Any event which results in a congenital abnormality or birth defect.
- Death in the absence of persistent or progressive disease.

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

- Grade 4 haematological toxicity is an expected consequence of effective treatment, but this only requires 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.

### **18.3 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.

#### **18.4 Collection of Data**

Preliminary discussion of the event may take place with a trial 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**.

#### **18.5 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.

#### **18.6 Reporting to the Regulatory Authorities**

The Chief Investigator or his nominee will review and record all SUEs. He will be responsible for reporting the events to the MHRA, COREC, and the Trial Steering Committee and Data and Ethics Monitoring Committee according to the appropriate timelines. He will also report, where relevant, to the provider of the IMP (Investigational Medicinal Product) and produce periodic reports for all investigators to forward to their LREC.

#### **18.7 Adverse event Reporting for Unlicensed Combinations:**

Some unlicensed agents in this trial will be used in combinations. This will require additional pharmacovigilance arrangements which will be carried out by a vigilance officer based in Cardiff. This individual will communicate with investigators by phone discussion on a regular basis to monitor events, and will conduct periodic site visits. These site visits will be conducted in accordance with a Standard Operating Procedure which will be issued to sites 1 month in advance of any visit.

## APPENDIX A: WHO Histological Classification of Acute Myeloid Leukaemias

	ICD Code
Acute myeloid leukaemia with recurrent genetic abnormalities	
Acute myeloid leukaemia with t(8;21)(q22;q22); (AML1(CBF $\alpha$ )/ETO)	9896/3
Acute myeloid leukaemia with abnormal bone marrow eosinophils Inv(16)(p13q22) or t(16;16)(p13;q22); (CBF $\beta$ /MYH11)	9871/3
Acute Promyelocytic leukaemia (AML with t(15;17)(q22;q12-21), (PML/RAR $\alpha$ ) 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 AML16**

### **DAUNORUBICIN** (Cerubidin™ - May & Baker Ltd)

Daunorubicin is presented as a red powder in glass vials containing 20 mg with mannitol as a stabilising agent. The drug is reconstituted in sodium chloride 0.9% or water for injection. Following reconstitution, further dilution with sodium chloride 0.9% to a concentration of 1mg/ml is recommended. The resultant solution is given by a one hour infusion into a swiftly flowing drip. In children Daunorubicin should be administered as a 6 hour infusion. For hepatic dysfunction with a bilirubin 20 – 50 µmol/L reduce by 25%: bilirubin >50 µmol/l reduce by 50%. In patients with renal impairment dose reduction should take place: Serum Creatinine 105 – 265, reduce dose by 25%: Creatinine >265 reduce by 50%.

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

### **Ara-C** (Cytosine Arabinoside , 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 diluents 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 >34 µmol/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 Cytosar 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.

**MYLOTARG™** (Gemtuzumab Ozogamicin for Injection)(Wyeth Research)

Mylotarg is supplied as an amber glass vial containing 5mg of MYLOTARG lyophilised powder. This vial should be refrigerated (2-8°C).

**Preparation:**

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

Before administration, withdraw the desired volume from each vial and inject into a 100ml IV bag of 0.9% Sodium Chloride for Injection. Place the 100-ml 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



chlorphenamine), should be given before each infusion to reduce the incidence of a post-infusion symptom complex. Vital signs should be monitored during infusion and for four hours following infusion

### **Instructions for Use, Handling and for Disposal:**

Procedures for handling and disposal of cytotoxic drugs should be applied.

### **Cautions**

Hepatic Insufficiency: Patients with hepatic impairment will not be included in the clinical studies.

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. "intrapur lipid" (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 18. Other events which have been reported in at least 10% of recipients of single agent Mylotarg include fever, nausea, chills, vomiting, headache, dyspnoea, hypotension, hypertension, and hyperglycaemia. It is not necessary to report these events.

### **CLOFARABINE (Evoltra™; Bioenvision Limited)**

Clofarabine is a purine nucleoside anti-metabolite. It is formulated as a 1 mg/ml sterile concentrate solution for infusion. It is a clear, practically colourless solution with a pH of 4.5 to 7.5 and an osmolarity of 270 to 310 mOsm/l.

Clofarabine is supplied in 20 ml, Type I glass vials with bromobutyl rubber stopper, polypropylene flip-off cap and aluminium overseal. The vials contain 20 ml sterile concentrate and are packaged in boxes of 4 vials.

Each 20 ml vial contains 20 mg of clofarabine and 180 mg of sodium chloride. The latter is equivalent to 3.08 mmol (or 70.77 mg) of sodium and should be taken into consideration for patients on a controlled sodium diet.

### **Posology and method of administration**

The dose per protocol ( $\text{mg}/\text{m}^2$ ) is administered by intravenous infusion in 100 to 250ml of N saline over 1 hour daily for 5 consecutive days. Body surface area must be calculated using the actual height and weight of the patient before the start of each cycle.

Clofarabine 1 mg/ml concentrate for solution for infusion must be diluted prior to administration. It should be filtered through a sterile 0.2 micrometre syringe filter and then diluted with sodium chloride 9 mg/ml (0.9%) intravenous infusion as required. If the use of a 0.2 micrometre syringe filter is not feasible, the sterile concentrate should be pre-filtered with a 5 micrometre filter, diluted and then administered through a 0.22 micrometre in-line filter. The diluted sterile concentrate should be a clear, colourless solution. Visually inspect for particulate matter and discolouration prior to administration.

The recommended dosage should be administered by intravenous infusion. Clofarabine should not be mixed with or concomitantly administered using the same intravenous line as other medicinal products.

Patients with renal insufficiency: There is no experience in patients with renal insufficiency (serum creatinine  $\geq 2 \times \text{ULN}$  for age) and clofarabine is predominately

excreted via the kidneys. In AML16, clofarabine is contraindicated in patients with serum creatinine levels above the normal range.

Patients with hepatic impairment: There is no experience in patients with hepatic impairment (serum bilirubin > 1.5 x ULN plus AST and ALT > 5 x ULN) and the liver is a potential target organ for toxicity. Therefore, clofarabine is contraindicated in patients with severe hepatic impairment and should be used with caution in patients with mild to moderate hepatic impairment.

For detailed information on product, refer to Investigator Brochure.

### **Storage**

Vials should not be frozen. The diluted sterile concentrate is chemically and physically stable for 72 hours at 2 to 8°C and at room temperature. From a microbiological point of view, it should be used immediately. If not used immediately, in-use storage times and conditions prior to use are the responsibility of the user and would normally not be longer than 24 hours at 2 to 8°C unless dilution has taken place under controlled and validated aseptic conditions.

### **AZACYTIDINE (Vidaza™) (Pharmion Corporation)**

Azacytidine is a pyridine nucleoside analogue of Cytidine (C<sub>2</sub>H<sub>12</sub>N<sub>4</sub>O<sub>5</sub>) is supplied for injectable suspension as a lyophilized powder in 100mg single vials for single use which should be stored at 15° to 30° C. Procedures for the proper handling of chemotherapy should be applied. In this trial it will be used in a dose of 75mg/m<sup>2</sup> daily for 5 days by subcutaneous injection, which may be subject to dose reduction.

The main side effects are gastrointestinal and of myelosuppression. Patients should be pre-medicated with an anti-emetic (e.g. ondansetron).

Azacytidine is reconstituted in 4ml of water for injection. The diluent should be injected slowly into the vial which will then contain 25mg/ml. If a larger dose is

required the sc injection should be given in two sites. The suspension should be gently mixed and can be kept a room temperature for up to one hour or kept refrigerated at 4° to 8° C for up to 8 hours. After removal from refrigeration the suspension should be injected within 30 minutes. If more than one vial is required to achieve the dose the dose should be equally divided between two syringes and injected into two sites.

### **Cautions**

There is no experience of treating patients with pre-existing hepatic or renal dysfunction, so no recommendations are available, however such patients should be observed carefully after treatment. Azacytidine is contraindicated in patients with an allergy to mannitol.

### **ARSENIC TRIOXIDE (Trisenox™) (Cephalon Inc.)**

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

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

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

After dilution in intravenous solutions, Trisenox is chemically and physically stable for 24 hours at 15-30°C and 48 hours at refrigerated temperatures (2-8°C). From a microbiological point of view, the product must be used immediately. If not used immediately in-use storage times and conditions prior to use are the responsibility

the user and would normally not be longer than 24 hours at 2-8°C, unless dilution has taken place in controlled and validated aseptic conditions.

Trisenox is given as a slow infusion over 1-2 hours. The daily infusions may be given on an inpatient basis, but investigators should ensure that serum potassium and magnesium levels are within the normal range at the start of each treatment week. Trisenox should not be mixed with other medications.