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 MYELODYSPLASTC 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 two standard chemotherapy schedules DA (**Daunorubicin/Ara-C**) with ADE (**Daunorubicin/Ara-C/Etoposide**). In addition, the role of **all-trans-retinoic Acid (ATRA)** 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 a second course of the same treatment with the ATRA if allocated to do so, and will then be randomised to **one further course or not** 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 **Azacytidine** or not.

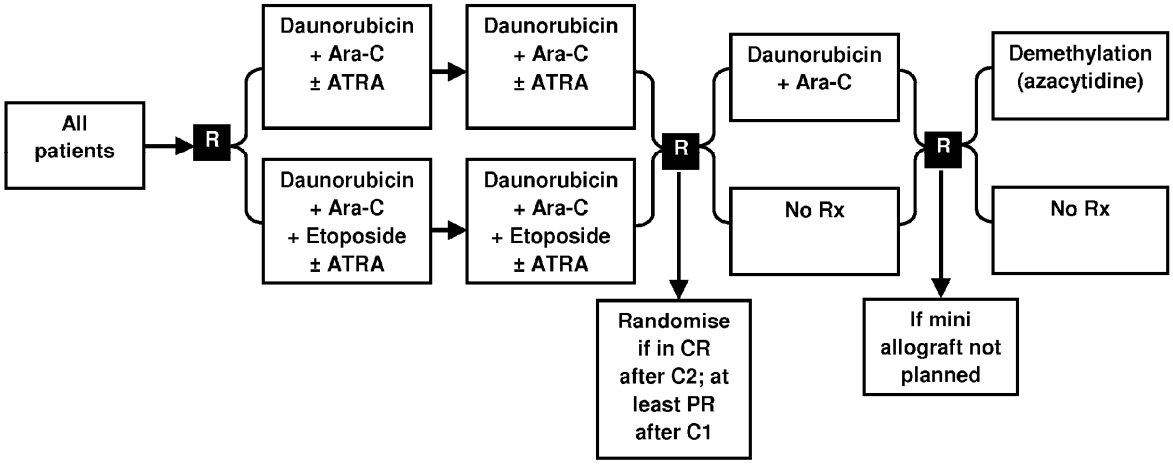
Patients who are not considered fit for an intensive treatment approach will be randomised between an established approach to non-intensive treatment, namely **Low Dose Ara-C** versus one of two novel treatments, which are **Low Dose Clofarabine or** Sapacitabine

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.

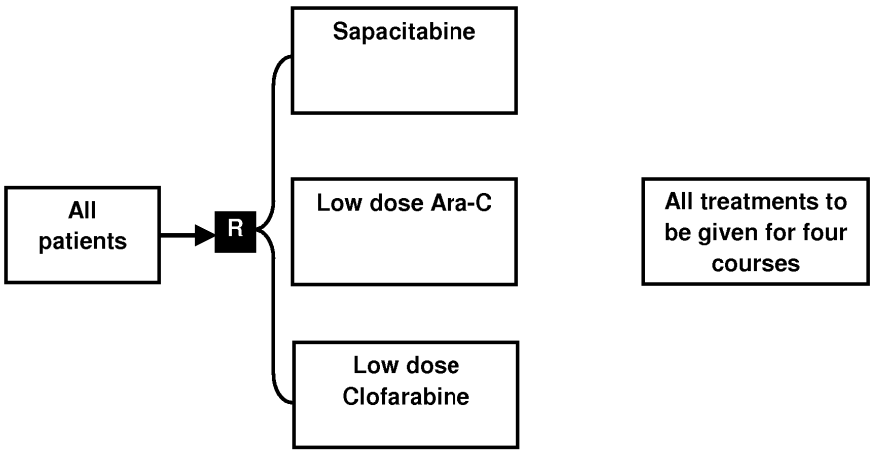
This protocol describes a collaborative trial in acute myeloid leukaemia primarily for patients over 60 years, which is being undertaken by the NCRI Haematological Oncology Study Group under the sponsorship of Cardiff University, and provides information about procedures for the entry, treatment and 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



Flow Chart 2: Non-intensive treatment



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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 the National Research Ethics Service (NRES) 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 17). 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 <u>considered fit</u> for intensive treatment:

- To compare two induction schedules (DA and ADE).
- To assess the value of ATRA during induction when used in combination with DA or ADE for the first 60 days.
- 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 <u>not considered fit</u> for intensive treatment:

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

- Low Dose Clofarabine.
- **Sapacitabine**

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

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 + CRi) 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 + CRi) achievement and reasons for failure (for induction questions).
- Duration of response (CR, CRi) 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. This will be based on co-morbidity assessment information collected at study entry.

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 molecular characteristics and 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. A total of 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 (Low Dose Ara-C) randomised against one of two novel treatments, Clofarabine or Sapacitabine.

3.1 For patients considered fit for intensive treatment:

There are three randomised comparisons within the trial:

At diagnosis:

(i) **DA** versus **ADE .**

(ii) **ATRA** versus no **ATRA** for 60 days .

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 ADE as induction therapy, and also randomise to ATRA versus not. Before commencing the allocated treatment each patient should have a Co-morbidity 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 ATRA)
Arm B	Two courses of DA (with ATRA for 60 days)
Arm C	Two courses of ADE (with no ATRA)
Arm D	Two courses of ADE (with ATRA for 60 days)

Clinicians must undertake the chemotherapy randomisation, DA vs ADE together with the ATRA randomisation. .

2. After recovery from course 1, assess bone marrow response. All patients will receive course 2 which will be the same chemotherapy with ATRA if allocated.

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 (that is three courses in total). 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 two alternative novel treatment approaches. The available treatment arms are thus:

Arm E: Low Dose Ara-C
Arm F: Low Dose Clofarabine
Arm G: Sapacitabine

Patients are expected to enter all randomisations for which they are eligible and which are currently available. For options E to G the treatment plan is for **four courses** to be given, it is permissible to give more courses if the patient is having benefit. A 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[1]. 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 (Figure1) largely due to delivery of more intensive chemotherapy assisted by better supportive care.

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

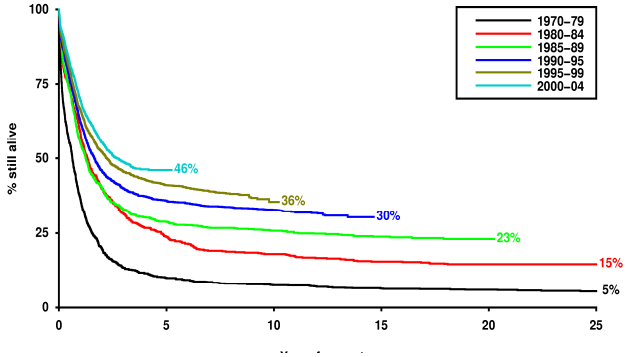
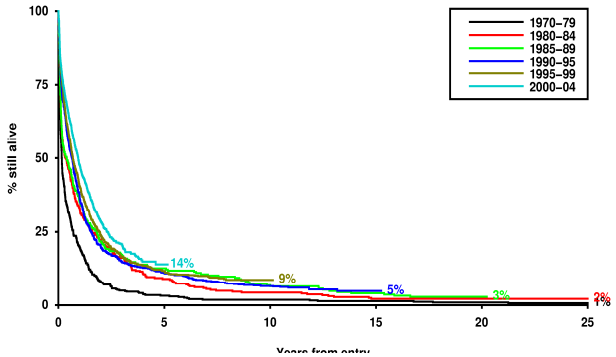


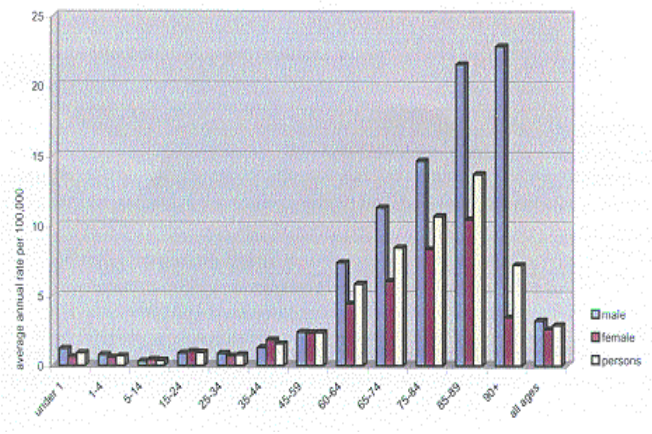
Figure 2. Survival in MRC AML trials: patients aged 60+



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 are 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 are 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, a "Frailty Index" based on co-morbidity information collected at diagnosis, 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.

5 JUSTIFICATION OF TREATMENT OPTIONS

5.1 Intensive Approach

5.1.1 Daunorubicin and Ara-C with or without Etoposide

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[(2)]. However almost 90% of these patients relapse within 3 years. 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. Previous MRC/NCRI studies (AML11) have not shown that the addition of etoposide was beneficial compared with thioguanine in combination with DA[3]. However the main issue that we wish to address is whether or not ATRA is beneficial in association with an Etoposide containing compared with an Etoposide non-containing schedule as inferred by a recent German study[4] Furthermore the ATRA benefit may be restricted to patients who have a NPM1 mutation[5]. The trial will therefore compare DA versus ADE each with or without ATRA treatment for the first 60 days. **It is essential that diagnostic material is sent to the referral lab for NPM1 mutation status.**

5.1.2 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[13,14 ,]. 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[15]. 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 the16% 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.

5.1.3 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[16]. 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[17].

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.

5.1.4 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[18,19].

The clinical potential was demonstrated in a number of non-randomised studies and in a randomised trial conducted by the CALGB in Myelodysplastic

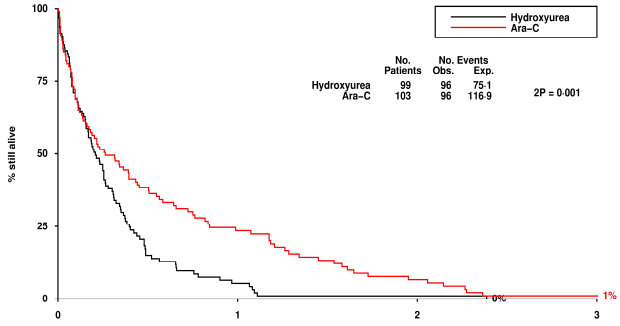
Syndromes,[20] where haematological responses were demonstrated and there was a significantly lower risk of progression to AML and superior survival. Responses were not limited to the low risk subtypes of MDS. Twenty-three percent of patients achieved a CR or PR, demonstrating the efficacy even in high 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[21] (Figure 5).

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



5.2.2 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[6]. Because Clofarabine has a chloro group at the 2-position of adenine (Figure 4), its chemical

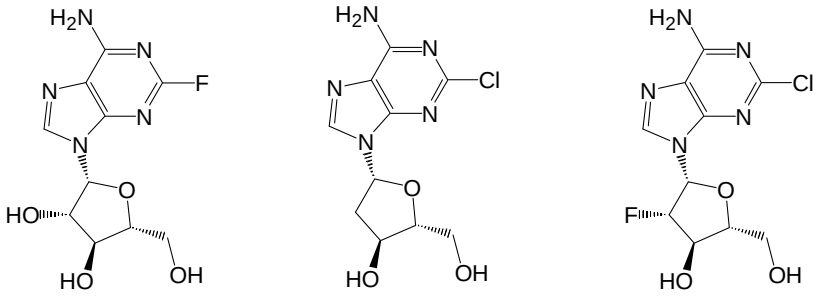
structure is more closely related to CdA than to F-ara-A. Halogenation at the 2-position of adenine renders this class of compounds resistant to cellular degradation by the enzyme adenine deaminase. Substitution of a fluorine at the C-2' position of the arabinofuranosyl moiety of Clofarabine increases 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[7]. Similar to the other purine nucleosides, Clofarabine potently inhibits DNA synthesis by inhibiting both DNA polymerase . and ribonucleotide reductase[8]. 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[9]. 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



Fludarabine
(2-fluoro Ara A)

2-CdA
(2-chloro-2'-deoxyadenosine)

Clofarabine
(2-chloro-2'-fluoro Ara A)

5.2.2.1 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^2 /day for 5 days every 4 weeks, in adults and 55mg/m^2 in children[10]. Ten percent of these high risk patients showed a response. Clinical activity was noted through a fairly wide dose range (4mg/m^2/day to 55mg/m^2/day), with no clear-cut dose response above 11.25mg/m^2/day. All but 1 patient reported adverse events during the study. Patients in the 40 mg/m^2/day and 55mg/m^2/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^2/day and 55mg/m^2/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,[11] the dose administered was reduced to

30mg/m^2/day for 5 days. The overall complete marrow response rate in 30 untreated patients of median age 69 years was 56%[11]. 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 10^9 /l and platelets to 100 x 10^9 /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^2. Remissions were seen at this dose. In a subsequent study a further 66 patients confirmed this experience[12]

Our recent experience with Clofarabine in patients not considered fit for intensive chemotherapy provided encouraging responses as described above. However treatment at 30 mg/m^2 was associated with a significant duration of neutropenia and thrombocytopenia. Subsequently, 7 patients have been treated with a dose of 20 mg/m^2, 4 of who 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.

5.2.2 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^2 was associated with a significant duration of neutropenia and thrombocytopenia. Subsequently, 7 patients have been treated with a dose of 20 mg/m^2, 4 of who 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.

5.2.3 Sapacitabine

Nucleoside analogues are prodrugs that are generally not active by themselves. Upon entering cells, nucleoside analogues are phosphorylated by nucleoside kinases and the phosphorylated metabolites are incorporated into DNA causing a pause in, or termination of, DNA synthesis. The close correlation between the degree of drug-induced cell death and the amount of incorporated analogue molecules in cellular DNA strongly suggests that the incorporation of these molecules into DNA is a key cytotoxic event.[22]

The clinical effectiveness of nucleoside analogues appears to be influenced by many factors including the substrate specificities of nucleoside kinases, the expression levels of kinases in tumor tissues, and the rate of metabolic elimination by inactivating enzymes.[22,23] Rationally designed nucleoside analogues with improved biochemical properties may be more effective antitumor agents.

Sapacitabine (also known as CS-682, CYC682) is a rationally designed 2'-deoxycytidine-type nucleoside analogue that can be administered orally. When compared with other nucleoside analogues, sapacitabine is unique in its ability to induce cell cycle arrest at the G2 phase and in causing single-strand DNA breakage that is irreparable by ligation.

Following oral administration, sapacitabine is converted to 1-(2-C-cyano-2-deoxy-β (-D-arabino-pentafuranosyl) cytosine (CNDAC) by amidases and esterases in the gut, plasma, and liver. CNDAC is further converted to CNDAC-mono phosphate by deoxycytidine kinase (dCK) and this is thought to be the rate-limiting step in the formation of CNDAC-triphosphate (CNDACTP), the most active metabolite in terms of cytotoxicity. CNDAC-phosphates are degraded by cytidine deaminase (CDA) and 5'nucleotidase. Both sapacitabine and CNDAC are active against a wide range of human cancer cell lines *in vitro* and animal models *in vivo*.

392 patients have received sapacitabine in 8 clinical studies. A total of 226 patients with MDS or AML in relapse were treated with different dose schedules. The predominant dose-limiting toxicity (DLT) was myelosuppression in solid tumor patients which consisted of neutropenia, febrile neutropenia, neutropenic sepsis, and thrombocytopenia. Myelosuppression was generally reversible after interruption of drug dosing. To date, the predominant DLTs reported in patients with advanced leukemias

or MDS were gastrointestinal toxicities which included abdominal pain/small bowel obstruction, diarrhea and neutropenic enteritis. Common non-hematological toxicities were fatigue, nausea, vomiting, diarrhea, constipation, anorexia, abdominal pain, fever, pneumonia, cough, dyspnea, dizziness, epistaxis, peripheral edema, alopecia and hypokalemia which were generally mild to moderate.

CYC682-05-04	Phase I (leukemias and MDS)	*b.i.d*. for 7 days every 3 weeks or *b.i.d.* x 3 days/week x 2 weeks every 3 weeks	325 mg *b.i.d.* x 7 days every 3 weeks or 425 mg *b.i.d.*x 3 days/week x 2 weeks every 3 weeks	Completed (n=48/47)
CYC682-06	Phase II (elderly AML/MDS)	Part 1: 200 mg *b.i.d*. x 7 days every 3-4 weeks (Arm A) 300 mg *b.i.d*. x 7 days every 3-4 weeks (Arm B) 400 mg *b.i.d*. x 3 days/week x 2 weeks every 3-4 weeks (Arm C) Part 2: 300 mg *b.i.d*. x 3 days/week x 2 weeks every 4 weeks	Not applicable	Ongoing Part 1: AML (n=107/105) MDS (n=63/60) Part 2: AML (n=14/14)

There were 11 deaths that were considered by the investigator to be definitely, probably or possibly related to the study drug, 3 occurred in patients with MDS (n= 65), and 8 occurred in patients with AML (n=161). Thirteen deaths were associated complications of myelosuppression and/or underlying disease and one death was associated with small bowel obstruction in a patient with a prior history of colitis and rectal infection.

These studies established that on balance, the 300mg orally b.i.d. schedule given for 3 consecutive days a week for 2 weeks every 3 or 4 weeks was the most acceptable schedule. In patient with a median age of 75 years, complete remissions were seen in 25% of patients, with an all cause 30 day mortality of 10%. The 12 month overall survival was 30% [(24)]

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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 AML17 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 a serum creatinine above the local upper limit of normal are not eligible for the Clofarabine randomisation in the non-intensive part 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 local Research Governance approval ; (5) that written consent will be obtained for each patient and a copy retained in the notes, and copies kept in the study material and also sent to the Trial Office in Birmingham; (6) that they agree to report serious unexpected adverse events as set out in Section 18 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 Clinical Cancer Trials Unit 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 ADE (for details, see Section 9.1& 9.2).

ATRA or not (for details, see Section 9.3 and Appendix B).

The four available treatment arms are:

Arm A Two courses of DA (with no ATRA)

Arm B Two courses of DA (with ATRA for 60 days)

Arm C Two courses of DA (with no ATRA)

Arm D Two courses of ADE (with ATRA for 60 days)

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

Note: Investigators will be expected to provide information for the frailty index before commencing treatment.
After a patient has recovered form 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. 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 7.13)

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 Clofarabine (see Section 13.2 for details).

G. Sapacitabine (see Section 13.3 for details).

Clinicians will normally be expected to randomise patients between all the available options, in which there is a 33 % of receiving any one of the three 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 co-morbidity information has been recorded,
- Confirmation that a sample has been sent for mutation assessment to the referral lab Cardiff **NB: establishing the NPM1 mutation status is an essential part of the induction evaluation (see Section 7.9 for details)**
- For patients entering a Clofarabine randomisation, confirmation that serum creatinine is 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

The morphological characterisation should be undertaken locally and patients defined within the WHO classification as set out in Appendix A.

7.9 Immunophenotypic Characterisation and Molecular Genetics

A network of laboratories has been established to examine diagnostic material for aberrant immunophenotypes (not routine immunophenotyping) 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 IMMUNOPHENTYPING 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
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BS10 5NB
Tel: 0117 9596306
Fax: 0177 959 6062
E-mail: 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

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 NPM1 mutations or for other relevant mutations which may be identified during the course of the trial. Diagnostic material may also be used for studies of gene expression by DNA microarray and future research studies, for which patient informed consent to storeage of excess material must be obtained using Patient Information and Consent Form 3..

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

2 - 4 ml of bone marrow in EDTA tube

and

10 ml of peripheral blood in EDTA tube

Please note that an additional 10ml of peripheral blood in EDTA should be sent to Cardiff for NPM1 analysis.

7.10 Cytogenetics

Cytogenetics should be carried out locally and reports sent directly from the cytogenetics laboratory to the Trial Office in Birmingham Cell pellets should be stored locally.

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 Trial Office in Birmingham 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 azacytidine treatment randomisation. 2 to 4mls of marrow which is collected for remission status after each cycle should be sent to the designated immunophenotyping lab for all patients treated intensively. Investigators will be informed of samples required for demethylation studies on patients who enter the azacytidine randomisation (see section 11 for details)),and will receive a prompt from the trial office a few days before the sample is due. For demethylation studies only peripheral blood is required

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 (i.e. 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 separately 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, but can enter the 2 vs 3 randomisation.

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 (Birmingham) 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 the 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 and 8.2.

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 the 3rd course 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 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 mg/m^2 daily by i.v. infusion on days 1, 3 and 5 (3 doses).

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

Course 2 **DA 3+8**

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

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

9.2 ADE schedule

Course 1 **ADE**

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

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

Etoposide 100 mg/m^2 daily by 1 hour i.v. infusion on days 1-5 inclusive (5 doses).

Course 2 **ADE**

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

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

Etoposide 100 mg/m^2 daily by 1 hour i.v. infusion on days 1-5 inclusive (5 doses).

9.3 ATRA Therapy

ATRA, 45 mg/m^2/day will be administered orally in two equally divided doses and rounded to the nearest 10 mg increment, given from day 1 to day 60, where day 1 is the first day of chemotherapy of course 1.

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^2 daily by i.v. infusion on days 1 and 3 (2 doses).

Cytosine Arabinoside 100 mg/m^2 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 Trial Office in Birmingham (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 courses 3, 6 and 9 - for this a 10ml blood sample should be sent to Paul White in Cardiff (see 11.3).

Maintenance Therapy

Azacytidine 75 mg/m^2 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 x 10^9 /l and platelets reach 80 x 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 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 3, 6 and 9 courses 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 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^2/day	days –9 to –5 inclusive
Busulphan	4 mg/kg/day	days –3 and –2
Campath 1H	20 mg/day/i.v.	days –5 to –1 inclusive

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

UCL Protocol:

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

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

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 two novel treatments. These options are being evaluated in a randomised Phase II design with the primary endpoint being CR or 12 month survival. 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 Clofarabine

(ii) Sapacitabine .

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 Clofarabine

Patients who are randomised to Clofarabine should receive:

Clofarabine 20 mg/m^2 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^2 daily for 5 days. Patients whose serum creatinine is above normal on any treatment day should omit that day's dose

.

13.3 Sapacitabine

Patients who are randomised to Sapacitabine should receive:

Sapacitabine 300mg orally b.i.d. for 3 consecutive days in week one and in week two. This comprises one course. This should be followed by a minimum of 4 weeks of no treatment since Sapacitabine may take longer to work than four courses.

Patients should receive **four courses** of treatment but may continue with further courses if they are experiencing benefit. Since sapacitabine is a new treatment patients will be monitored by communication with the trial site from the Cardiff office on a weekly basis after therapy has commenced for a period of four weeks.

For treatment on Day 8 of Cycle 1 and on Day 1 and 8 of all subsequent cycles, dosing will not start until clinically significant and drug-related non-hematologic toxicities have resolved to ≤ grade 1 or baseline. After recovery, a dose reduction of 50 mg b.i.d. will be instituted for grade 3-4 drug-related non-hematologic toxicities for the next treatment cycle or next week of dosing. For patients who have had a >25% decrease in bone marrow blasts, there needs to be a dose reduction of 50 mg b.i.d. for delay in recovering absolute neutrophils and platelets to baseline or best level on study beyond day 42.

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 ≥38℃ two hours apart, or a single reading ≥39℃.
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 µg (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 they 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, over the entire period of the trial approximately 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. A similar number will be available for the 2 versus 3 course randomisation, giving similar power to detect a 15% absolute survival benefit, equivalent to a hazard ratio of 0.65. In both randomisations ,the critical number events to obtain 90% power is 238.

In the induction question, during the planned remainder of trial recruitment approximately 600 new cases should be randomised. The main question posed by the Ulm group regarding the effectiveness of ATRA is that it is effective in patients with an NPM1 mutation, only when etoposide containing chemotherapy is given. Over the course of the recruitment period, approximately 150 cases of AML with NPM1 mutation will be recruited. The Ulm (AMLSG) study group found that in the 26 patients with NMP1 mutant/ITD wild type AML, 3-year survival with ATRA+ADE was 50%, compared to around 10% without ATRA (a hazard ratio of approximately 0.3). To have 90% power to see this sort of difference, only 28 patients per arm would need to be recruited. The hypothesis of the Ulm group is that this effect is only present in NPM1 positive patients and those treated with etoposide. As the trial currently stands, the factorial design enables this hypothesis to be tested using stratified analyses with standard heterogeneity tests. Overall, with 75 patients in each group (ATRA v no ATRA) one has 80% power to detect a hazard ratio of 0.5, with similar power to detect a qualitative interaction between ADE and DA.

The additional recruitment of NPM1 WT patients enables one further to see whether there is interaction between ATRA and NPM1 status. Using the same argument as for the ADE versus DA randomisation, 150 NPM1-WT patients would be sufficient to have 80% power to see the qualitative interaction posited. Thus, for NPM1 WT patients there remains the option to introduce a different induction question at a later date to avoid wasting patients.

The results from AML16 intensive will be meta-analysed together with the other trials, both positive (as in the Ulm study), and negative (e.g. AML12, AML-HR and the MD Anderson study).

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). For treatments where the proposed effect is to improve survival by inducing a greater number of remissions (e.g. Clofarabine), 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.

While the benefit derived from some treatments is expected to come from an increase in the number of durable remissions, some drugs, notably hypomethylating agents give the promise that survival may be prolonged even without the achievement of a complete remission. In this case, an interim analysis based on complete remission is inappropriate. Instead, a minimum of six months follow-up will be sought on each patient. At this point, the decision to stop or continue will be made on the basis of the hazard ratio for overall survival. A doubling of survival from 10% to 20% at two years equates to a hazard ratio of 0.70. Thus, for drugs such as sapacitabine, where remission is not viewed as a prerequisite for survival, after 50 patients per arm, the hazard ratio (drug versus Ara-C) must not exceed 1; and after 100 patients per arm it must not exceed 0.85 (i.e. half the required effect). Simulations show similar power under these requirements to the scenario for CR given above.

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

* 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.

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). For the revised induction interventions in protocol version7, outcome analyses will be carried out with stratification for NPM1 mutation status. 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 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 Trial Office in Birmingham, 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 the National Research Ethics Service (NRES). NRES approval requires that investigator sites have a designated Principal Investigator and that participating institutions submit a Site Specific Information (SSI) form 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 Trial Office in Birmingham as described in section 7.1. 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 (SUAE) 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. However all deaths should be reported.

18.6 Reporting to the Regulatory Authorities

The Chief Investigator or his nominee will review and record all SUAEs. He will be responsible for reporting the events to the MHRA, NRES, 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 Unlicenced Combinations:

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

APPENDIX B: Preparation, Administration and Toxicity of Drugs used in 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 20 mg/ml and 100 mg/ml solutions of cytarabine in a variety of vial sizes. It is recommended that before administration by intravenous bolus injection the hypertonic 100 mg/ml solution is further diluted in water for injection, sodium chloride 0.9%, or glucose 5% solution, to produce a solution of 20 mg/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.

ATRA (VesanoidTM - Roche Products)

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

The most serious adverse event has been a syndrome characterised by fever, respiratory distress and episodic hypotension, usually in association with leucocytosis (now known as "Differentiation Syndrome"), however in previous experience in non-APL cases suggests that this is rare. The onset of this syndrome has usually been in the first 1-2 weeks of drug treatment. Should this occur the ATRA should be stopped and steroids commenced. Some cases are reported to respond well to high-dose corticosteroid therapy (dexamethasone 10 mg i.v. 12 hourly for 3 or more days). Prolonged ATRA treatment may cause dryness of the skin. ATRA is also believed to be highly teratogenic and advice regarding contraception should be given as appropriate.

CLOFARABINE (Evoltra^{TM}; Genzyme Inc)

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 (mg/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 ≥ 2 x 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 – 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 – 8°C unless dilution has taken place under controlled and validated aseptic conditions.

AZACYTIDINE (Vidaza™) (Celgene)

Azacytidine is a pyridine nucleoside analogue of Cytidine ($C_2H_{12}N_4O_5$) is supplied for injectable suspension as a lyophilized powder in 100mg single vials for single use which should be stored at 15 – 30°C. Procedures for the proper handling of chemotherapy should be applied. In this trial it will be used in a dose of 75 mg/m^2 daily for 5 days by subcutaneous injection, which may be subject to dose reduction.

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

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

The suspension should be vigorously shaken and should be prepared immediately before use. The reconstituted suspension should be administered within 45 minutes. If elapsed time is greater than 45 minutes, the reconstituted suspension should be discarded appropriately and a new dose prepared. Alternatively, if the product needs to be reconstituted in advance of the administration, it must be placed in a refrigerator (2℃ to 8℃) immediately a fter reconstitution, and kept in the refrigerator for a maximum of 8 hours.

If the elapsed time in the refrigerator is greater than 8 hours, the suspension should be discarded appropriately and a new dose prepared. The syringe filled with reconstituted suspension should be allowed up to 30 minutes prior to administration to reach a temperature of approximately 20℃-25℃. If the elapsed time is longer than 30 minutes, the suspension should be discarded appropriately and a new dose prepared.

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.

SAPACITABINE (Cyclacel)

Two formulations of oral sapacitabine have been used in clinical trials. The initial formulation of sapacitabine (Sankyo formulation) was a granulated formulation using an amorphous form of the active pharmaceutical ingredient and standard pharmaceutical excipients. A new formulation (Cyclacel BL formulation, previously called Encap formulation) has been developed to improve stability and will be used for all current and future trials.

The Cyclacel BL formulation comprises liquid-filled capsules of a suspension of the crystalline Form B of the active pharmaceutical ingredient in miglyol 812N and is supplied as 25 mg, 50 mg and 75 mg strength capsules. Capsules are packaged in high-density polyethylene bottles, with low-density polyethylene snap-on tamper resistant closures. The capsules should be stored at room temperature (15-25^{o}C) in a closed container, protected from light in a secure, limited-access storage area. The 25, 50 and 75 mg capsules currently have a 60 -month retest date.

Sapacitabine (Cyclacel BL) Formulation Capsule (mg/capsule)

Ingredient	25 mg	50 mg	75 mg
Sapacitabine Form B	25 mg	50 mg	75 mg
Miglyol 812N Ph.Eur/GRAS	100 mg	200 mg	300 mg
White gelatin capsule with a gelatin band (comprising gelatin USP/Ph Eur and sterile water for irrigation)	Size 3	Size 2	Size 1
Banded white gelatin capsule (blue ink band on the capsule body and a blue ink band and a black ink band on the capsule cap) with a gelatin band (comprising gelatin USP/Ph Eur and sterile water for irrigation)	Not applicable	Size 2	Not applicable

Physical and Chemical Properties of Sapacitabine (crystalline B-form)

Test Item	Properties
Physical Appearance	White powder to off white crystalline solid
Molecular Formula	$C_{26}H_{42}N_4O_5$
Molecular Weight	490.64
Solubility	Practically insoluble in water and slightly soluble in most organic solvents.
Stability	Crystalline B-form – 4 years at room temperature (15-25^{o}C)
UV spectrum	λ_{max} = 297 to 301 nm, 248 to 252 nm
Related compounds (HPLC)	CNDAC: Not more than 1.5% Any other related compounds: Not more than 0.5% Total related compounds: Not more than 3.0%
Purity (anhydrous basis)	Not less than 95%

Effects on Cytochrome P450 Enzymes

Sapacitabine demonstrated a weak potential to be metabolized in vitro by the human recombinantly expressed isoforms CYP 2C9, CYP3A4 and CYP2C19 at an incubation concentration of 5□M. CNDAC demonstrated no appreciable potential to be metabolized in vitro by any of the recombinantly expressed isoforms CYP1A2, CYP2C9, CYP2C19, CYP2D6 or CYP3A4 at an incubation concentration of 5□M.[26]

Cytochrome P450 inhibition status

Neither sapacitabine nor CNDAC appreciably inhibited the in vitro metabolism of the recombinantly expressed isoforms CYP1A2, CYP2C9, CYP2C19, CYP2D6 or CYP3A4 at incubation concentrations up to 100□M.[26]

Cytochrome P450 induction status

Sapacitabine did not appreciably induce the expression levels of functional P450 proteins, CYP1A2, CYP3A4 or CYP2C9 in rat primary hepatocytes at an incubation concentration of 20□M. CNDAC demonstrated a mild induction potential of CYP1A2 and CYP2C9 in rat