AML 18	**NATIONAL CANCER RESEARCH INSTITUTE ACUTE MYELOID LEUKAEMIA AND HIGH RISK MDS TRIAL 18**

A TRIAL FOR OLDER PATIENTS WITH ACUTE MYELOID LEUKAEMIA AND HIGH RISK MYELODYSPLASTIC SYNDROME

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Protocol Version: 15.0

Signatories:

Approved By: Professor Nigel H Russell

Position: Chief Investigator

Signature: N. H. Russell

The AML18 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 considered fit for an intensive chemotherapeutic approach, but younger patients who may not be considered suitable for the concurrent NCRI AML19 Trial for younger patients may also enter if pre-approved by the study CI. Patients for whom intensive chemotherapy is not thought suitable may enter the concurrent NCRI trial of less intensive therapy. Patients will be added to the protocol until the induction question is fully recruited – we estimate this will require bringing the total number of patients in the trial to 2000.

For patients without either known adverse risk cytogenetics or AML secondary to previous MDS or therapy-related AMLa randomisation will compare a standard chemotherapy schedule DA (Daunorubicin/Ara-C) combined with 2 doses of Mylotarg (gemtuzumab ozogamicin, or GO) in course 1 against CPX-351. Patients will be randomised in a 2:1 ratio in favour of CPX-351. If a patient is ineligible for CPX-351 or if CPX-351 is unavailable during the trial for logistical reasons patients can enter and receive DA alone; they would be eligible for downstream randomisations including AC220 if eligible. If a patient is ineligible for mylotarg or if mylotarg is unavailable for logistical reasons patients will enter a randomisation of DA alone vs CPX-351. This randomisation is 2:1 in favour of CPX-351. Patients who achieve complete remission (CR) and who are MRD negative by flow cytometry after course one of DA with Mylotarg will receive one further course of DA, with a randomisation to receive, either a course of DA or intermediate dose Cytarabine (IDAC) as a third course. Patients who are MRD negative by flow cytometry after course one of CPX-351 will receive up to 2 further courses of CPX-351. Patients who fail to achieve a CR after course 1 of DA or who are MRD positive by flow cytometry or for whom MRD information is not available, are eligible to be randomised to compare DA with FLAG-Ida for up to two further courses of therapy. Patients who fail to achieve a CR after course 1 of CPX-351 or who are MRD positive by flow cytometry or for whom MRD information is not available are eligible to be randomised between a second course of standard dose CPX-351 versus a repeat of the course 1 schedule. Patients on the CPX-351 arm will also receive CPX-351 in course 3.

Following the outcome of course 1, patients who were allocated DA chemotherapy in course 1 and who have a *FLT3* mutation at trial entry will be randomised to receive or not further therapy with the 2nd generation *FLT3* inhibitor AC220. Patients randomised to AC220 will be allocated a maximum of 3 courses (short AC220) or 3 courses plus maintenance for 1 year (long AC220). Patients allocated CPX-351 are excluded from this randomisation.

Patients will be eligible for a non-intensive allogeneic stem cell transplant if a suitable HLA matched donor is available.

There are about 2000 cases of AML each year in adults aged over 60 years in the British Isles alone. About 350 patients aged over 60 years annually enter national trials offering an intensive treatment approach.

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 approval via the host institution's Research Governance procedures. During the trial, not all randomisation options will be open at all times and some additional options may be included by protocol amendment.

This trial is funded by Cancer Research UK, with additional limited financial support to cover IMP activities by Daiichi Sankyo, Jazz Pharmaceutical Inc, Janssen Pharmaceutical, Sunesis Pharmaceuticals and Chugai Pharmaceutical Co., Ltd.

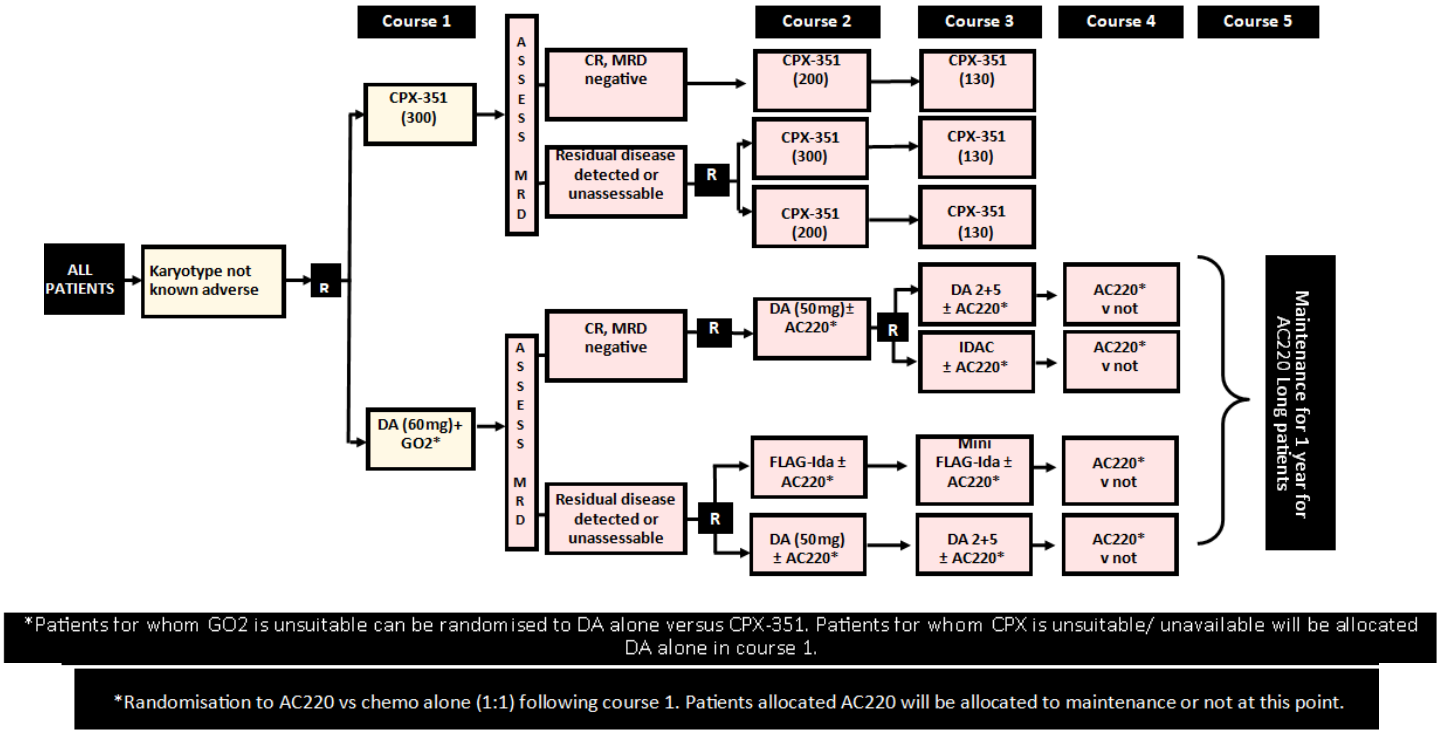
Clinicians are asked to read the whole protocol before commencing treatment

PROTOCOL

Study Title:	A Trial for Older Patients with Acute Myeloid Leukaemia and High Risk Myelodysplastic Syndrome
ISRCTN:	ISRCTN31682779
Protocol Number:	SPON1227-13
Indication(s):	Acute Myeloid Leukaemia and High Risk Myelodysplastic Syndrome
Development Phase:	Phase II/III
Sponsor:	Cardiff University Research and Commercial Division 30-36 Newport Road Cardiff UK CF24 0DE
Sponsor's Responsible Medical Officer:	Prof Nigel Russell
Date:	July 2022
Version:	Version 15.0

This study will be conducted according to the principles of Good Clinical Practice for non-commercial trials.

Trial Flow Chart 1



*Patients for whom GO2 is unsuitable can be randomised to DA alone versus CPX-351. Patients for whom CPX is unsuitable/ unavailable will be allocated DA alone in course 1.

*Randomisation to AC220 vs chemo alone (1:1) following course 1. Patients allocated AC220 will be allocated to maintenance or not at this point.

Patients for whom an HLA matched donor is available may proceed to transplant at any time.

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24 hour internet randomisation, response to course 1 and Cytogenetic response forms can be accessed via the website at:

https://trials.cardiff.ac.uk/aml-18

Please report all SAEs to the email address below. If email is not possible please fax to the number below.

Serious Adverse Event (SAE) email address:

CTR-Safety@cardiff.ac.uk

SAE Fax number:

0203 0432 376

1 ETHICAL CONSIDERATIONS

The AML18 Programme has been approved by the Research Ethics Service (RES) and must also conform with local Research Governance procedures at each centre before patients are entered into the trial at site. Centres are required to complete a registration process with CTR before recruitment is started and to confirm acceptance of the terms of sponsorship required by Cardiff University. For non- UK sites, evidence of national regulatory approvals must also be provided to CTR and Sponsor.

Centres are required to go through a registration process with the AML18 trial office in CTR 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, should remain within the study for the purposes of follow-up and data analysis unless they have withdrawn their consent. All patients who have not withdrawn consent will be followed up annually for life.

The AML18 trial programme will be conducted in accordance with Good Clinical Practice and the Declaration of Helsinki in line with the current legislation within the member states taking part in the trial.

The following Investigational Medicinal Products (IMPs) are being used and evaluated in the AML18 trial:

- Mylotarg (Gemtuzumab Ozogamicin) (licensed in the EU, commercial stock will be used)
- CPX-351 (Vyxeos) (licensed in the EU, Investigator Stock supplied for AML18)
- AC220 (Quizartinib) (unlicensed in the EU)

Fuller details on each of these IMPs and notes on preparation, administration and toxicity of drugs is provided in the current trial pharmacy manual.

Non-IMPs (nIMPs) are defined as the standard treatment options, as shown below:

- Daunorubicin
- Cytarabine (Ara-C)
- G-CSF
- Fludarabine
- Idarubicin

2 OBJECTIVES

The AML18 trial programme is available to any patient who is not considered suitable for the current NCRI trial for younger patients and is considered fit for intensive chemotherapy, and who has:

- De novo AML as defined by the WHO Classification (Appendix A) (excluding Acute Promyelocytic Leukaemia), or
- high risk Myelodysplastic Syndrome (i.e. > 10% marrow blasts).

The objectives are summarised below.

2.1 Therapeutic questions

1. Does CPX-351 given for 3 courses improve survival compared to the current standard of care of DA plus two doses of GO 3mg/m^2 (maximum 5 mg per dose) for older patients with AML without either known adverse risk cytogenetics or secondary AML?
2. Does the addition of either a short or long (maintenance) course of AC220 starting at course 2 after DA chemotherapy for patients with a *FLT3* mutation in the diagnostic sample improve outcomes?
3. Is MRD status following course 1 of clinical value? In particular, can outcomes be improved by intensifying treatment in patients who show evidence of residual disease following course 1 of treatment?

4. To compare a further course of DA versus intermediate-dose Cytarabine in patients who are in CR or CRi and MRD -ve after induction course 1 and have received a second course of DA induction
5. To assess the value of Reduced Intensity Allogeneic Stem Cell Transplantation as consolidation for patients with a matched sibling or matched unrelated donor.

2.2 Endpoints

The main endpoints for the therapeutic questions for each comparison will be:

- Overall survival.
- Event free survival (Events:- Death, relapse, resistant disease as measured by failure to achieve CR/CRi post course 2).
- Complete remission (CR + CRi) achievement and reasons for failure (for induction questions).
- Duration of remission, relapse, and deaths in first CR.
- Toxicity, both haematological and non-haematological.
- Supportive care requirements (and other aspects of health economics).

2.3 Subsidiary objectives

Blood and bone marrow will be required at diagnosis, post course 1 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.
- To store diagnostic tissue for future research in the AML Tissue Bank.
- To determine the predictive impact of the LSC17 gene signature on outcome of patients entering the trial.

3 TRIAL DESIGN

AML18 is a trial primarily for older patients with AML and high risk Myelodysplastic Syndrome (MDS). It offers a randomised, controlled, open-label Phase II/III trial, which uses a factorial design for maximum efficiency to evaluate two induction options followed by treatment with AC220 beyond course 1 (for patients with a *FLT3* mutation at trial entry), and dose intensification for patients without evidence of MRD negativity.

There are five randomised comparisons within the trial:

1. At diagnosis:

   For patients not known to have adverse risk cytogenetics or without AML secondary to previous MDS or therapy-related AML (t-AML)

   DA chemotherapy plus two doses of 3 mg/m^2 (maximum 5mg per dose) of Mylotarg versus CPX-351

   Patients ineligible for mylotarg can enter the randomisation but receive DA alone or CPX-351.

   Patients ineligible for CPX-351 will receive DA alone.

2. For patients who were allocated DA chemotherapy (with or without mylotarg) in course 1 but are not in CR or who are MRD +ve, or for whom MRD is not assessable post course 1

   DA versus FLAG-Ida

3. All patients at second course who have were allocated DA induction and who have a *FLT3* mutation in the diagnostic sample.

   AC220 versus no AC220 for a maximum of 3 cycles; then with or without maintenance for 1 year for patients allocated AC220

4. For patients who are in CR or CRi and MRD -ve post course1 and have completed 2 courses of DA

   DA versus intermediate dose Cytarabine (IDAC)

5. For patients who were allocated CPX-351 chemotherapy in course 1 but are not in CR or who are MRD +ve, or for whom MRD is not assessable post course 1

   Course 2 : CPX-351 100 units/m^2 x 3 doses (CPX-351 300) versus CPX-351 100 units/m^2 x 2 doses (CPX-351 200)

   Course 3: CPX-351 65 units/m^2 given on days 1 and 3 (CPX-351 130)

   The trial will also assess:

   - **Non-intensive allogeneic stem cell transplant** for patients with matched sibling or matched unrelated donors

Full details of the rationale for these comparisons and progress through the trial and treatments can be found in the relevant sections (Section 9) of the protocol, but are summarised below (and in the flow diagram at the front of the protocol):

1. At diagnosis for patients **not known** to have adverse risk cytogenetics or without AML secondary to previous MDS or therapy-related AML (t-AML randomise between DA plus Mylotarg 3mg/m^2 x 2 doses (maximum 5mg per dose) versus CPX-351 (300).

   The two induction treatment arms will therefore be:

   Arm A. DA with Mylotarg 3 mg/m^2 x 2 doses (maximum 5mg per dose) on days 1 and 4

   Arm B. CPX-351 100 units/m^2 on days 1, 3 and 5 (CPX-351 300)

   Patients ineligible for mylotarg can enter the randomisation but receive DA alone or CPX-351.

   Patients ineligible for CPX-351 will receive DA alone.

2. After recovery from course 1, assess bone marrow response morphologically and send sample for MRD assessment to the central laboratory. A bone marrow should normally be done by day 21 to day 28 post course 1. Subsequent courses of treatment should normally start when blood counts have regenerated to >1.0 x 10^9/L neutrophils and >80 x 10^9/L platelets unless CR has not been achieved.

3. All patients who are not in CR or who are in CR but MRD +ve or unassessable following course 1 of **DA**, (this does not include patients who were allocated CPX-351 in course 1) can be randomised between:

   Arm C. Two courses of DA

   Arm D. One course of FLAG-Ida (mini FLAG-Ida if 70+ years) followed by one course of mini-FLAG-Ida

   Patients who have completed 2 courses of DA+/- Mylotarg and who are in CR or CRi and MRD –ve will be randomised to

   Arm E. One further course of DA or one course of intermediate dose Cytarabine.

4. All patients who are not in CR or who are in CR but MRD +ve or unassessable following course 1 of **CPX-351** will be randomised to

   Arm F. One course of CPX-351 100 units/m^2 given on days 1,3 and 5 (CPX-351 300) followed by one course of CPX-351, 65 units/m^2 given on days 1 and 3 (CPX-351 130)

Arm G. One course of CPX-351 100 units/m^2 given on days 1 and 3 (CPX-351 200) followed by one course of CPX-351 65 units/m^2 given on days 1 and 3 (CPX-351 130).

Patients who are in CR or CRi and who are MRD –ve following course 1 of CPX-351 will receive a further 2 courses:

(i) CPX-351 100 units/m^2 given on days 1 and 3 (CPX-351 200) followed by

(ii) CPX-351 65 units/m^2 given on days 1 and 3 (CPX-351 130).

Centres will be informed of the post Course 1 MRD assessment by email enabling them to consider further randomisation for eligible patients.

5. Following recovery from course 1 patients who have a *FLT3* mutation detected in the diagnostic sample and who were allocated **DA +/- Mylotarg** in induction, irrespective of MRD status, can enter the randomised comparison of:

AC220 versus no AC220;
Patients allocated AC220 maintenance (long AC220) or no maintenance (short AC220). Patients allocated AC220 maintenance will receive 3 cycles of AC220 before starting their 12 courses of maintenance.

Randomisation availability

It is expected that patients should normally be randomised between all available treatment options which are available and for which they are eligible. To discuss other eventualities, please contact the Trials Office.

Please note: the statistical power calculations differ with each randomisation, so recruitment to some randomisations may be completed before others. This will mean that a randomised component of the trial may close or be changed before completion of the trial as a whole. In platform trials such as this, individual components might require alteration in the light of trial monitoring or other experiences.

For these or other reasons, not all of the randomisations will be available at all times. When such circumstances arise investigators will be informed, in order that patients are aware of the current status of the trial when information is presented to them.

3.1 Risk assessment

A Trial Risk Assessment has been completed to identify the potential hazards associated with the trial and to assess the likelihood of those hazards occurring and resulting in harm. This risk assessment has been completed in accordance with the MRC/DH/MHRA Joint project guidance document 'Risk-adapted approaches to the management of Clinical Trials of Investigational Medicinal Products' and includes:

- The known and potential risks and benefits to human subjects
- How high the risk is compared to normal standard practice
- How the risk will be mitigated

This trial has been categorised as a TYPE B, where the level of risk is somewhat higher than the risk of standard medical care. A copy of the trial risk assessment may be requested from the Trial Manager. The trial risk assessment is used to determine the intensity and focus of monitoring activity

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 (Figure 1) largely due to delivery of more intensive chemotherapy assisted by better supportive care.

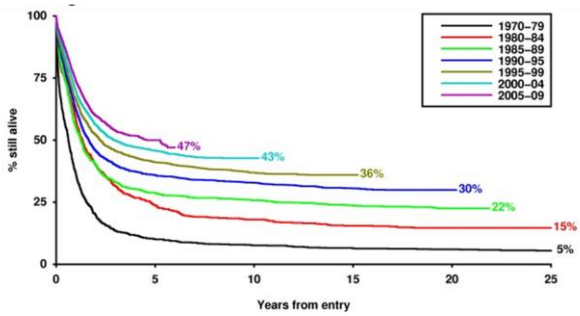


Figure 1. Survival in MRC AML trials: patients aged 15-59

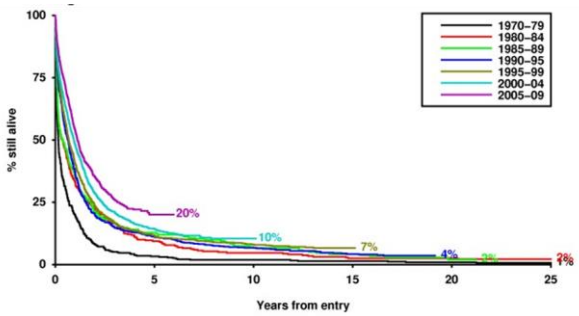


Figure2. Survival in MRC AML trials: patients aged 60+

AML18 trial is the replacement trial for the AML16 trial for patients >60 years with Acute Myeloblastic Leukaemia (AML) or high risk Myelodysplastic Syndrome (MDS) with >10% blasts who are considered fit to receive intensive chemotherapy. Progress in the treatment of older AML patients has been slow and treatment outcomes remain poor compared to younger patients because of lower CR rate and a higher risk of relapse reflecting drug resistance.

In the design of AML18 we have incorporated positive findings from AML16 including the survival advantage for patients receiving Mylotarg in induction for patients with good and intermediate risk cytogenetics and the importance of MRD detection in determining prognosis. At the same time we wish to add new molecules, to try to improve outcome.

Figure 2: *Incidence of Acute Myeloid Leukaemia*

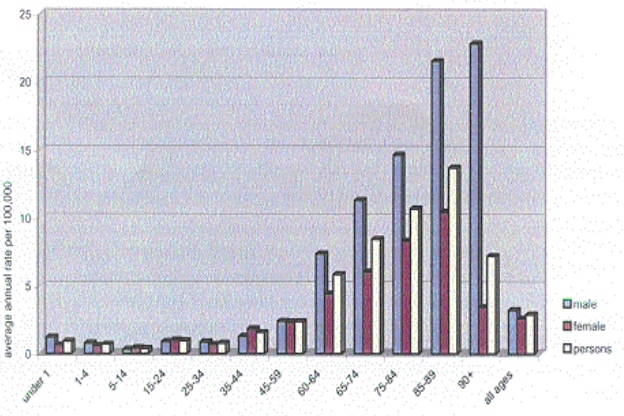


Figure 3. Incidence of AML by age

The two induction questions will be combined in an efficient factorial design which will also allow any possible interactions to be investigated.

5 JUSTIFICATION OF TREATMENT OPTIONS

5.1 Daunorubicin and Ara-C with Mylotarg versus CPX-351

Currently available treatment with a combination of Daunorubicin (D) and Ara-C(A) has achieved a remission rate of over 60% in patients over 60 years considered fit for intensive treatment[2].

However three quarters of these patients relapse within 3 years. In AML16, we studied the addition of Mylotarg at the same dose to two different induction therapies, Daunorubicin/Ara-C (DA) and Daunorubicin/Clofarabine (DClo). The results from AML16 have been important in the development of AML18. A key result was that a single dose of Mylotarg (3mg/m^2) significantly improved overall survival in older patients (20% v 15% at 4 years HR 0.82 (0.72-1.0), p=0.05) due to a reduction in relapse risk (Figure 4)[2]. This was not associated with any increased toxicity. This survival benefit was seen when Mylotarg was combined with either DA or DClo and represents one of very few interventions to improve survival in older patients with AML.

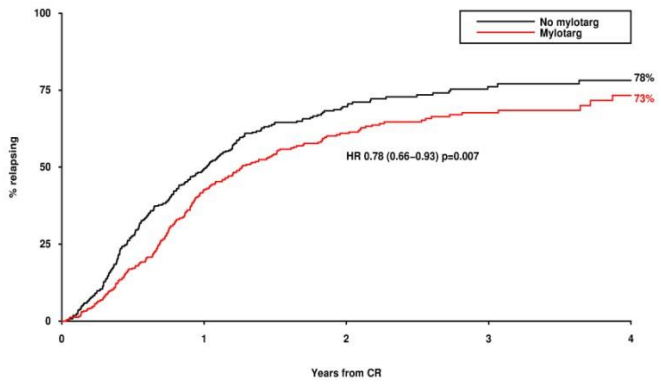


Figure 4: Reduction in relapse risk with treatment using Mylotarg

The first part of AML18 compared one with two doses of Mylotarg; with no evidence of excess harm from 2 doses and with indirect evidence from the individual patient data meta-analysis that fractionation gave a higher point estimate of effect on relapse [3]; as a consequence two doses of Mylotarg with DA has been selected as the standard arm in AML18.

CPX-351 is a liposomal formulation of a fixed combination of the antineoplastic drugs cytarabine and daunorubicin. The two drugs are in a 5:1 molar ratio within the liposome, a ratio shown in vitro to maximize anti-tumour efficacy across multiple leukaemic and solid tumour cell lines and in animal model studies to be

consistently more efficacious than conventional free drug treatment. The two drugs are present inside the liposome in a 5:1 molar ratio. A randomised phase 2 study (204) (n=126) was carried out in newly diagnosed older patients (60-75 years) versus the traditional "3+7" schedule. At 1 year there was a trend for better disease free and overall survival in the CPX-351 arm, which was achieved with acceptable safety as seen by a superior 60 day mortality (4.7% vs 14.6%), in spite of more greater myelosuppression resulting in increased febrile neutropenia (63.5% vs 51.2%). In exploratory subgroup analysis it appeared that the benefit was suggested in patients with secondary disease. In a randomised phase 2 study (CLTR0308-205) (n=120) in relapsed patients aged 18-65 years, it was again found that patients with unfavourable disease were the likely beneficiaries with a 12 month survival advantage (30% vs 10%).

More recently 300 patients 60-75 years of age with untreated secondary AML were randomised 1:1 to CPX-351 (100units/m^2, days 1, 3, 5) or 7+3 (cytarabine 100 mg/m^2/day x 7 days, daunorubicin 60 mg/m^2 days 1, 2, 3) induction therapy. After minimum follow-up of 13.7 months final analysis began.CPX-351 treatment resulted in superior overall survival (HR=0.69; P=0.005; median OS 9.56 vs. 5.95 months), EFS (HR=0.74; P=0.021), and CR+CRi response (47.7% vs. 33.3%; P=0.016). 60-day mortality favoured CPX-351 (13.7% vs. 21.2%).

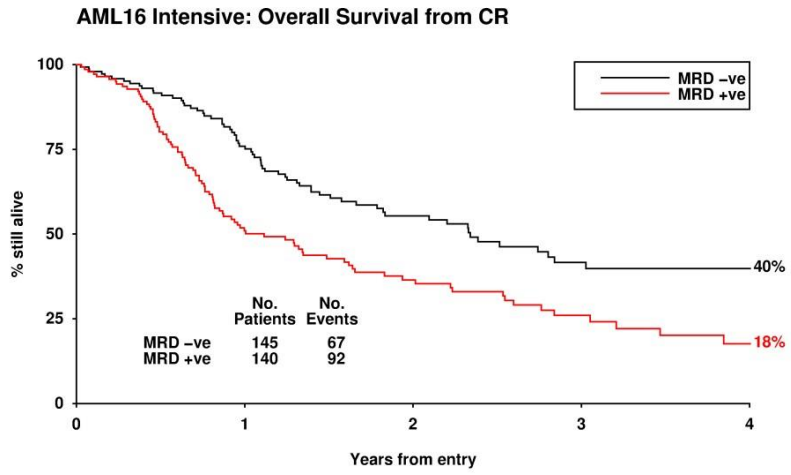
These results suggest that CPX-351 could become the standard of care for older patients with secondary AML and it is now licensed in this setting. The question is now whether CPX is superior to standard chemotherapy in older patients with denovo AML. In AML18 we propose to randomise CPX-351 against DA+GO2 in the general older AML and high risk MDS population without known adverse risk cytogenetics or AML secondary to previous MDS or therapy-related AML (t-AML). Patients randomised to CPX-351 will receive up to 3 courses of CPX-351.

5.2 Allocation of Treatment based on post course 1 MRD Assessment

An important result from AML16 was the finding that flow cytometry for minimal residual disease (MRD) could identify a poor risk group of patients[6]. In an updated analysis, a Leukaemia Associated Phenotype (LAP) could be identified in 90% of 892 analysed patients in whom an adequate pre- treatment sample was received. 286 patients in remission were evaluable for MRD after induction course 1 of whom 49% were MRD positive (MRD +ve). Patients who were MRD

+ve in their remission bone marrow at this time point had a significantly greater increased risk of disease relapse compared to patients who were MRD negative. The 3 year cumulative incidence of relapse was 83% for MRD
+ve patients vs. 71% for MRD- patients, with survival of 26% vs 42% (Figure 5). Adjusted analysis (allowing for age, sex, white blood cell (WBC) count, cytogenetics, secondary disease and performance status) showed MRD positivity after course 1 is highly prognostic for relapse; 1.95 (1.34-2.84) p=0.0004, and overall survival; HR 1.96 (1.39-2.76) p<.0001.

Figure 5: Survival by post course 1 MRD assessment



These results suggest that flow cytometric analysis of MRD after course 1 could be used to stratify for further treatment by identifying patients who have an extremely poor outcome with standard therapy. Therefore in AML 18 patients who were allocated DA (+/- mylotarg) and are not MRD –ve (either not in CR or MRD+ve/unknown) after course 1 will be randomised to continue treatment with DA or to receive alternative chemotherapy with FLAG-Ida (the best anti-leukaemic treatment of AML 15)

Patients who were allocated CPX-351 and are not in CR or are MRD positive CR post course 1 will enter a second randomisation between a standard second course of CPX-351 (CPX-351 200: 100units/m^2 on days 1 and 3) or a repeat of the of the course 1 CPX-351 dose schedule (CPX-351 300: 100units/m^2 on days 1, 3 and 5) followed by MRD assessment.

The question being asked in this post course 1 randomisation for MRD +ve patients is: '*does an intensification of treatment improve the rate of achievement of MRD negativity post course 2 and improve relapse free, and ultimately overall*

survival?'. In AML18 we will also evaluate the prognostic significance of leukaemic stem cell (LSC) MRD analysis, to ask the question whether this enhances conventional flow MRD prognostication.

5.3 Number of Treatment Courses

The question as to how many courses of chemotherapy are optimal in older patients was studied in the NCRI AML16 trial by randomising patients who had responded (CR or PR) to 2 versus 3 course of DA in total. Updated results from this randomisation showed no difference in 5-year survival by consolidation randomisation (consolidation 25% vs not 22% HR 0.92 (0.75-1.12) p= 0.4) overall however in the ~50% of patients who achieved CR with course 1 and who were MRD –ve there was a strong trend for improved OS (36% vs 26%) for recipients of consolidation (p=0.09). The question of optimal consolidation in this group of patients is however not certain. In AML16 a 2+5 schedule of DA was used whereas intermediate dose Cytarabine has been used by other groups. A third course of DA also increases anthracycline exposure which may have additional long term adverse cardiac effects in older patients. In AML18 it is proposed to randomise those patients who are MRD-ve post course 1 to DA 2+5 versus intermediate dose Cytarabine (1 gmm^2 daily x 5 days).

Patients who have not achieved a CR or who are not MRD -ve post course 1 are not eligible for this randomisation, which will take place following course 2.

5.4 Treatment of patients who do not achieve remission or with MRD+ve/ MRD unknown disease

The choice of FLAG-Ida is based upon the results of MRC AML15 trial which included 237 patients >60 years. In this trial 2 cycles of the FLAG-Ida induction regimen offered the best anti-leukaemic therapy when compared to 2 cycles of standard schedules, viz, DA or ADE induction, in all age groups including the over 60s . This combination also had more haematological toxicity and it was found to be difficult to deliver further planned consolidation chemotherapy beyond course 2 in patients of all ages. However recent analysis of AML15 results has shown that just 2 courses of FLAG-Ida chemotherapy (i.e. without consolidation) was equivalent to 2 courses of DA plus 2 further courses of consolidation chemotherapy due to a reduction in relapse[5]. In AML15 the CR rate with FLAG-Ida was 77% in patients aged 60+ yrs. (compared with the standard rate expected of 65% achieved in AML16) so we propose to randomise FLAG-Ida against DA in this poor risk group with the second course of FLAG-Ida being at a reduced dose (mini FLAG-Ida). Patients aged ≥70 years would receive mini-FLAG-Ida for both courses to reduce myelotoxicity.

For patients randomised to CPX-351 the standard course 2 schedule is given at a dose of 100 units/m^2 for 2 doses. In AML 18 patients who were allocated CPX-351 and are not in CR or are MRD positive CR post course 1 will enter a second randomisation between a standard second course of CPX-351 (CPX-351 200: 100units/m^2 on days 1 and 3) or a repeat of the of the course 1 CPX-351 dose schedule (CPX-351 300: 100units/m^2 on days 1, 3 and 5) followed by MRD assessment. On completion of course 2 patients in both arms will receive a third course of CPX-351, 65 units/m^2 on days 1 and 3 (CPX-351 130).

5.5 AC220 in patients with a *FLT3* mutation

Based upon the results of the AML18 Pilot Study, we propose to test the addition of AC220 to be given with courses 2 and 3 of chemotherapy, and as fourth course, or not. Patients will be randomised in a 1:1 fashion between no AC220 and chemotherapy plus AC220 long (maintenance) or short. Patients randomised to maintenance therapy will receive AC220 for up to 1 year. This randomisation is now only available for patients with a confirmed *FLT3* mutation in the diagnostic sample as determined by the Cardiff University lab.

5.6.1 AC220

AC220 is a novel second-generation Class III RTK inhibitor with potent and highly efficacious *FLT3* activity in vitro and in vivo. Toxicology studies with AC220 in rats, dogs, and monkeys up to 90-days in duration have shown that the principle toxicological target organs were the bone marrow and lymphoid organs in rat, dog, and monkey. These findings are consistent with the presumed FLT-3 and c-KIT kinase inhibition mechanism of action of the drug. In addition, toxicological effects were observed in the kidney (all species), the liver (dog only) and the ovary, vagina, and testes (rat only). Target-organ toxicity appears to be dose-, time-, and species-dependent, but reversible following a 28-day reversal period, except for the dog-specific liver toxicity. Bioavailability and exposure were good across species, ranging from 16% in monkeys to 40% in dogs. Plasma protein binding with AC220 was high (>99%) in all 5 species examined (mouse, rat, dog, monkey, and human) and the compound has been shown to penetrate the brain poorly. In vitro YP450 studies demonstrate that AC220 is neither an inhibitor nor inducer of major human CYP isoforms. Evidence from rat toxico-kinetic studies indicate that AC220 does not accumulate nor does it induce its own metabolism. A major pharmacologically active metabolite, termed AC886, has been identified in the plasma of rat, dog, monkey, and humans. A cardiovascular safety pharmacology study in cynomolgus monkeys demonstrates that single oral doses of AC220 result in prolonged QTc interval at ≥ 10 mg/kg doses and biologically significant increased systolic blood pressure at ≥ 30 mg/kg doses.

It should be noted that increases in blood pressure were not observed in the Phase 1 dose-escalation study, Protocol AC220-CP0001; henceforth called Study CP0001. No QTc interval prolongation was evident in monkeys dosed with 3 mg/kg AC220. The prolonged QTc interval is presumably not related to inhibition of the IKr ion channel as evidenced by negative human ether-a-go-go-related gene (hERG) assays conducted for AC220 and the major metabolite, AC886 (11%-15% IKr channel inhibition at 10 μM for AC220 and < 10% at 10 μM for AC886). There were no apparent electrocardiogram (ECG) abnormalities in the dog (28-day and 90-day) or monkey (28-day) general toxicology studies. In addition, there were no apparent toxicologically-relevant AC220-related heart microscopic changes in the rat, dog, or monkey general toxicology studies. At steady-state, there is approximately a 1.5-fold exposure margin of AC220+AC886 between monkeys

dosed with 30 mg/kg and humans dosed with 200 mg/day.

5.6.2 Clinical Results

Interim results from Study CP0001[8] have shown that AC220 has been well tolerated in the 76 treated patients, thus far. The majority of adverse events (AEs) observed in the study were those associated with the underlying disease. In addition, the safety profile was similar between patients receiving continuous dosing (28 days continuous dosing as 1 cycle) and those on the intermittent schedule (14 days dosing and 14 days rest as 1 cycle). In spite of limitations of the Phase 1 design, e.g. limited evaluation of response and the potentially suboptimal intermittent dosing schedule, encouraging preliminary evidence of clinical activity has been observed in the first 76 patients treated with AC220. The overall response (complete remission [CR] + partial remission [PR]) observed in all AC220-treated patients was 32% (24/76). Responses were defined per modified Cheson criteria.

A phase 2 study was conducted to assess the efficacy and safety of AC220 monotherapy in *FLT3-ITD* positive (+) and *FLT3-ITD* negative (-) patients with a total of 333 patients included in two cohorts. Cohort 1 included patients aged 60 years with AML relapsed in <1 year or refractory to 1st-line chemotherapy. A total of 134 patients were included aged 60 years+.

Data through 31 January 2012 from 134 patients in this cohort were recently presented[9]. These patients included 92 (69%) who were *FLT3* ITD(+), 41 (31%) who were *FLT3-ITD*(-), and 1 (1%) whose *FLT3-ITD* status was unknown. Half the 92 *FLT3-ITD*(+) patients and 46% of the 41 *FLT3-ITD*(-) patients were male. The *FLT3-ITD*(+) patients had a median age of 70 years (range 54 to 85 years), and the *FLT3-ITD*(-) patients had a median age of 69 years (range 60 to 78 years). Patients received AC220 at a starting dose of 90 mg/day (females) or 135 mg/day (males and 1 female), and were treated continuously during 28-day cycles.

The composite complete remission (CRc) rate included complete remission (CR), complete remission with incomplete platelet recovery (CRp), and complete remission with incomplete haematologic recovery (CRi). For *FLT3-ITD*(+) patients the CRc rate was 54% (0 CR, 3% CRp, and 51% CRi), with a median duration of response of 12.7 weeks and median overall survival of 25.3 weeks. Of those refractory to their last AML therapy, 39% achieved a CRc with AC220. For *FLT3-ITD*(-) patients the CRc rate was 32% (2% CR, 2% CRp, and 27% CRi), with a median duration of response of 22.1 weeks and median overall survival of 19.0 weeks. Of those refractory to their last AML therapy, 44% achieved a CRc. The final data from this Phase 2 study for elderly relapsed/refractory AML patients with few other available therapy options confirm the high degree of activity of AC220 in *FLT3-ITD*(+) patients and also suggest activity in *FLT3-ITD*(-).

A second cohort of 137 patients aged >18 yrs. with AML relapsed or refractory to 2nd-line, salvage chemotherapy or relapsed after haematopoietic stem cell transplantation (HSCT) included 99 (72%) who were *FLT3-ITD*(+) and 38 (28%) who were *FLT3-ITD*(-). The *FLT3-ITD*(+) patients had a median age of 50 years (range 19 to 77 years), and the *FLT3-ITD*(-) patients had a median age of 55 years (range 30 to 73 years). Patients received AC220 at a starting dose of 90 mg/day (females) or 135 mg/day (males), and were treated continuously during 28-day cycles.

The composite complete remission (CRc) rate included complete remission (CR), complete remission with incomplete platelet recovery (CRp), and complete remission with incomplete haematologic recovery (CRi). For *FLT3-ITD*(+) patients the CRc rate was 44% (4% CR, 0 CRp, and 40% CRi), with a median duration of response of 11.3 weeks and median overall survival of 23.1 weeks. Of those refractory to their last AML therapy, 47% achieved a CRc with AC220. For

FLT3-ITD(-) patients the CRc rate was 34% (3% CR, 3% CRp, and 29% CRi), with a median duration of response of 5.0 weeks and median overall survival of 25.6 weeks. Of those refractory to their last AML therapy, 31% achieved a CRc. The final data from this Phase 2 study confirm the high degree of activity of AC220 in *FLT3-ITD*(+) and *FLT3-ITD*(-) AML patients relapsed/refractory to 2nd-line treatment or HSCT. These data represent the highest level of single agent activity observed to date for *FLT3*-targeted therapy[10].

5.6.3 Toxicity

The dose-limiting toxicity (DLT) as per protocol in Study CP0001 was National Cancer Institute (NCI) Common Terminology Criteria for Adverse Events (CTCAE) version 3 (v3) Grade 3 QTcF (corrected QTc by Fridericia's correction factor) interval prolongation at the 300-mg dose of AC220 administered continuously, and considered to be possibly drug related. It should be noted that this study did not exclude high risk cardiac patients, including those with abnormal baseline QTcF prolongation, nor did it exclude the administration of QT/QTc-prolonging concomitant medications, and patients were sometimes in a state of electrolyte imbalance with low serum potassium and/or magnesium levels. These patients with Grade 3 QTcF interval prolongation have been asymptomatic, with no evidence of arrhythmias and have had reversal of QTcF prolongation when AC220 dosing was interrupted. The ECGs were originally analysed by the sites, however they were subsequently re-read using a centralised digital analysis system employed by a central ECG laboratory (EResearch Technology in Philadelphia, PA) and a formal analysis was performed. This determined that the ECG data in Study CP0001 did not demonstrate any clear signal of any clinically important effect on heart rate, PR (atrio-ventricular conduction) or QRS interval duration (depolarisation) or morphology.

The primary finding in this first-in-human study with limited cardiac safety data was that AC220 appears to have a marked effect on cardiac repolarisation, which is best defined by the Fridericia's corrected QT data (QTcF). The time-averaged mean change from baseline for QTcF duration for the intermittent dose groups showed no QTcF effect for the 12 to 60-mg dose groups. Starting with 90-mg dose, however, the QTcF interval change from baseline was> 20 ms and tended to

increase with dose, up to 30 to 38 ms for the 300 to 450mg doses. For the continuous treatment dose groups, the QTcF interval change from baseline for 200-mg was 26 ms and for 300-mg was 54 ms. During the first 14 days of dosing, the intermittent and continuous treatment dose groups at 200 mg and 300 mg experienced the same exposure to AC220, and therefore, the data over this time period may be considered together. Taken together, the QTcF interval change from baseline for the 200-mg dose was 22 ms to 26 ms, and for the 300-mg dose was 38 ms to 54 ms, and the incidence of new cases (not seen at baseline) of Grade 3 QTcF interval prolongation (> 500 ms) was 9.5% (2/21 patients) at 200-mg dose and 30% (3/10 patients) at 300-mg dose. This demonstrates a clear dose-related and marked QTcF interval change at the ≥ 200- mg dose level. The QTcB (Bazett's correction factor) results were comparable. The onset of increase to > 500 ms QTcF occurred as early as 2 hours after the first dose and by Cycle 1 Day 8 of dosing in the cases that were observed.

Additional observations were made in women and patients of > 62 years, as is usual in these settings. The pharmacokinetic-pharmacodynamic (PK-PD) relationship for parent and metabolite was also indicative that AC220 caused a positive QTc response of a magnitude that was observed by the ECG data. Hence the apparent marked effect of AC220 on cardiac repolarisation is likely to be observed in this and further studies.

The usual precautions of cardiac exclusion criteria, avoidance of QT/QTc-prolonging concomitant drugs (where possible, and with the exception of azole antifungals and quinoline antibiotics, and other antimicrobials used as standard of care to prevent or treat infections in the study population), attention to electrolyte levels and careful evaluation of ECG data (using central ECG reading and oversight by a core ECG laboratory) during the study with clear rules for interruption of dosing with either discontinuation of dosing or dose reduction subsequently, will be implemented to safeguard the patients in this study. Introduction during the study of new concomitant medications that may predispose or cause QT/QTc prolongation will be carefully monitored. Safety will be reviewed continuously.

In the first part of AML18 we assessed the combination of 40mg of AC220 with chemotherapy in all patients irrespective of *FLT3* mutation status. We now have advice from the IDMC based upon an interim analysis that it is unlikely that patients

without a *FLT3 mutation* will benefit from AC220. Therefore, only patients with a *FLT3 mutation* in the diagnostic sample (this includes either FLT3-ITD or FLT3-TKD point mutation) will enter the AC220 randomisation if they were allocated DA+/- mylotarg in course 1. Patients allocated CPX-351 are excluded from this randomisation.

5.7 Non-Intensive Allogeneic Stem Cell Transplant

AML16 was one of the first studies to prospectively evaluate the contribution that non-intensive allograft can make to AML treatment in older patients. 963 patients were aged 60-70 years inclusive and did not have favorable risk cytogenetics. Of these 144 patients received a RIC transplant in first remission (sibling n=51, MUD n=93) with median follow-up for survival from CR of 46.3 months. 85% of patients had a Haemopoietic cell transplant specific co-morbidity index (HCT-CI) of <3. Comparisons of allogeneic transplant in 1st remission versus not were carried out using Mantel-Byar analysis. Among the 144 allografts, 93 had intermediate risk cytogenetics, 19 adverse risk, and 32 were unknown. In transplanted patients, survival from transplant was 36% at 5 years, and while the survival for sibling allografts (42%) was better than that for MUDs (37%) this did not reach statistical significance (p=0.2). In analyses adjusted for Wheatley risk group there was no significant difference in outcome between sibling and MUDs (HR 1.27 (0.80-2.03) p=0.3). When comparing RIC versus chemotherapy survival was significantly improved (35% vs 20%, HR 0.75 (0.61-0.93) p=0.006) by RIC transplant. When stratified by Wheatley risk group into good, standard and poor risk there was consistent benefit for RIC (HR 0.76 (0.61-0.94), p=0.008). (11) In AML16 we have previously reported that post course 1 MRD status was the most important independent prognostic factor for relapse and survival, however, we could not find any evidence of an interaction with treatment outcome following RIC and all groups appeared to benefit independent of their post course 1 MRD status including those patients who were MRD +ve or had failed to achieve a CR with their first course of chemotherapy. These findings suggest that patients aged between 60 and 70 years with a co-morbidity score of ≤3 should be considered for RIC transplant if they have a matched sibling or unrelated donor.

5.8 References

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6 INCLUSION AND EXCLUSION CRITERIA

6.1 Inclusion Criteria

Patients are eligible for the AML18 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 AML (including patients with AML with morphological MRC who do not have a history of MDS or known MDS-related cytogenetics) or high risk Myelodysplastic Syndrome, defined as greater than 10% marrow blasts (RAEB-2). Patients with a prior history of a myeloproliferative neoplasm may be included provided they meet none of the exclusion criteria.
- Patients should normally be over the age of 60, but patients under this age are eligible if they are not considered eligible for the MRC AML19 trial. Please contact the trial team for further information
- They have given written informed consent
- Serum creatinine ≤ 1.5 × ULN (upper limit of normal)
- Sexually mature males must agree to use an adequate and medically accepted method of contraception throughout the study if them or their sexual partners are women of childbearing potential (WOCBP). These measures must be in place for 6 months for patients receiving CPX-351 and 7 months (females) or 4 months (males) for Mylotarg – more comprehensive guidance is included in the current Summary of Product Characteristics (SPC) for these agents. Men should be advised to not father a child while receiving trial treatment. Similarly women must agree to adequate contraceptive measures and avoid becoming pregnant while on protocol treatment. In the event of pregnancy at any point during the trial, the IMPs should be immediately stopped and the Trial Team should be contacted and pregnancy reporting procedures followed
- ECOG Performance Status of 0-2

Please note for the AC220 intervention: specific electrolyte and cardiac criteria (see page 33) must be met. Only patients with a confirmed *FLT3* mutation by the trial laboratories in the diagnostic sample are eligible for the AC220 randomisation.

6.2 Exclusion criteria

Patients are not eligible for the AML18 trial if:

- Patients that are known to either have adverse risk cytogenetics as defined by Grimwade (appendix D) or that cytogenetic changes that meet the WHO definition of AML-MRC (appendix A)
- They have therapy –related AML (t-AML) with documented history of prior cytotoxic therapy or radiotherapy
- They have a documented history of CMMoL prior to transformation to AML
- They have a documented history of MDS prior to transformation AML
- 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
- Known infection with human immunodeficiency virus (HIV)
- Patients with prior cumulative anthracycline exposure (from prior treatment of a non AML cancer) of greater than 300 mg/m^2 daunorubicin (or equivalent).
- History of myocardial infarction (MI), unstable angina, cerebrovascular accident, or transient ischemic attack (CVA/TIA) within 3 months before entry

Mylotarg Specific Exclusion Criteria:

Specific exclusion criteria for the Mylotarg Arm

- Pre-existing liver impairment with known cirrhosis
- Total bilirubin > 2.0 x the upper limit of normal (ULN)
- Aspartate aminotransferase (AST) > 2.5 x ULN
- Alanine aminotransferase (ALT) > 2.5 x ULN

CPX-351 Specific Exclusion Criteria:

Specific exclusion criteria for CPX-351 treatment

- Hypersensitivity to cytarabine, daunorubicin or liposomal products
- History of Wilson's disease or other copper-metabolism disorder

In addition patients are not eligible for the **AC220 randomisation** if they have:

Cardiovascular System Exclusion Criteria:

Known serious cardiac illness or medical conditions, including but not limited to:

I. Clinically unstable cardiac disease, including unstable atrial fibrillation, symptomatic bradycardia, unstable congestive heart failure, active myocardial ischemia, or indwelling temporary pacemaker

II. Ventricular tachycardia or a supraventricular tachycardia that requires treatment with a Class Ia antiarrhythmic drug (e.g., quinidine, procainamide, disopyramide) or Class III antiarrhythmic drug (e.g., sotalol, amiodarone, dofetilide). Use of other antiarrhythmic drugs is permitted.

III. Use of medications that have been linked to the occurrence of torsades de pointes (see Appendix B for the list of such medications)

IV. Second- or third-degree atrioventricular (AV) block unless treated with a permanent pacemaker

V. Complete left bundle branch block (LBBB)

VI. History of long QT Syndrome or a family member with this condition

VII. Serum potassium, magnesium, and calcium levels outside the laboratory's reference range

VIII. QTc >450 ms (average of triplicate ECG recordings); a consistent method of QTc calculation **must** be used for each patient's QTc measurements. QTcF (Fridericia's formula) is preferred. Please see the trial website for QTcF calculator.

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. 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. The following must be completed and returned to the Sponsor prior to the centre being open to recruitment:

- R&D/local approval (confirmation of capacity and capability)
- Site added to the CTA
- Signed Site Agreement (including signed PI declaration)
- Trial-specific initiation, involving key site personnel
- Site delegation log
- A green light email should be received from CTR prior to recruiting patients

For administrative reasons, investigators will also be asked to supply

- Details of the location of their immunophenotyping, cytogenetic, molecular, genetic and pharmacy services
- Investigator contact e-mail addresses

In addition a limited amount of biochemical data will be collected and, as part of the Centre Registration process, relevant institutional normal ranges (bilirubin, AST and/or ALT and creatinine) 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 1a and Consent Form 1a**. Patients for whom a non-intensive approach is considered appropriate should be entered into the NCRI LI-1 Trial or its successor. Consent for storage of excess diagnostic material should be obtained using **Patient Information Sheet 2 and Consent Form 2.**

A GDPR information sheet should also be given to any new patients.

7.3 Randomisation

There are five randomisations in the trial which occur at entry, post course 1 and post course 2:

- one at entry between DA + two doses of Mylotarg and CPX-351
- three following course 1 when MRD status is known
    - one is for no CR or MRD +ve patients who were allocated DA(+/- Mylotarg) as course 1
    - one is for no CR or MRD +ve patients who were allocated CPX-351 as course 1
    - one is for patients with a *FLT3* mutation in the diagnostic sample as determined by the Cardiff University lab, and who were allocated DA (+/- mylotarg) as course 1 and fulfil the eligibility criteria for the AC220 randomisation
- A fifth randomisation follows course 2 for MRD -ve patients who are in CR/Cri post course 1 who have been allocated DA chemotherapy between DA and IDAC.

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.

The randomisations will be carried out using a secure computer system at the AML18 Trial Office, CTR, Cardiff University. Patients fulfilling the criteria for entry into the trial (see Section 6) should be entered into the first randomisation by using internet randomisation. Internet randomisation is available 24 hours a day, seven days a week via:

https://trials.cardiff.ac.uk/aml-18

If a site is unable to randomise a patient via the AML18 website they should contact the trials office (Page 9).

7.4. First randomisation

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

Treatment allocation will be given once the required patient details have been supplied. There is one randomisation available at this time point, namely:

DA plus Mylotarg 2 doses or CPX-351 (for details, see Section 9.1 & 9.2). This is a 2:1 randomisation in favour of CPX-351. Patients with known adverse risk cytogenetics are excluded from this randomisation. However if treatment is urgent investigators do not have to wait for cytogenetic results before entering a patient into the trial. If a patient is later found to have adverse risk cytogenetics they may remain on trial.

The two available treatment arms are:

Arm A **One course of DA with two doses of Mylotarg**

Arm B **One course of CPX-351**

If a patient is ineligible for CPX-351 or if CPX-351 is unavailable during the trial for logistical reasons patients can enter the trial and receive DA alone in course 1; they would be eligible for downstream randomisations including AC220 if eligible.

If a patient is ineligible for mylotarg (for example, they do not fulfil the hepatic criteria) or if mylotarg is unavailable for logistical reasons patients will enter a randomisation of DA alone vs CPX-351. This randomisation is 2:1 in favour of CPX-351.

After a patient has recovered from course 1 of chemotherapy, and undergone a marrow assessment for morphology and MRD assessment, they will be allocated to the MRD negative or MRD +ve/unknown arm. This latter group of patients are eligible to enter the dose intensification randomisation.

After recovery from course 1 all patients who were allocated DA chemotherapy (with or without mylotarg), irrespective of MRD status and who have a *FLT3* mutation in the diagnostic sample as determined by the Cardiff University lab, will also be eligible for the randomisation with AC220 subject to appropriate criteria.

The post course 1 randomisation for AC220 only applies to those patients allocated DA induction (with or without Mylotarg), NOT to patients allocated CPX-351 who are excluded.

7.5 Information required at first randomisation

- Centre and name of consultant in charge of management.
- Patient's name (family name and given name).
- Sex.
- Date of birth.
- NHS number.
- ECOG 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 will be sent for characterisation of a Leukaemia associated phenotype (LAP) to a central lab and for mutation assessment including assessment of the presence of a *FLT3* mutation to the referral lab Cardiff University.
- Cytogenetics (if known).

NB: establishing the LAP is an essential part of the induction evaluation (see section 7.6).

Investigators will be supplied with a postage box for sending samples. Ideally, these samples should be sent to arrive before the weekend if at all possible, particularly for post-treatment samples. Please do not refrigerate samples – they should be stored at room temperature.

7.6 Diagnostic Material

A number of diagnostic procedures are required. Morphology, diagnostic immunophenotyping and cytogenetics should be carried out locally at the regional service – a copy of the immunophenotyping report should be sent to AML18 Trial Office with the "Notification of Entry" form. One objective of the trial is to investigate the value of therapeutic interventions based upon minimal residual disease detection following the first course of induction therapy. Diagnostic material is essential for these studies and should be sent to the reference lab in University of Birmingham. (Please refer to sample flow chart document for further guidance).

In addition a diagnostic sample should be sent to the Cardiff University lab for molecular characterisation including *FLT3* assessment and storage for future research. This sample will also be used for the analysis of the LSC17 gene signature to be performed in collaboration with the Princess Margret Cancer Centre, Toronto, Canada and NGS sample analysis. No specific consent is required beyond **Patient Information Sheet 1a** for the referred minimal residual disease assessment, but samples for storage must have the patient's consent using **Patient information sheet 2 and Consent form 2.** This consent documentation should be kept in the Site study file – confirmation of whether the patient has consented for their samples to be stored for future research will be captured during the randomisation process.

7.6.1 Morphology

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

7.6.2 Immunophenotypic Characterisation and Cytogenetics Immunological definition is essential and should be carried out locally at the regional service —

a copy of the report should be sent to AML18 Trial Office 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.

Cytogenetics should be carried out locally. The regional cytogenetic lab will automatically be informed when a new patient is randomised. They will enter the result directly on to the trial database as well as returning the result to the investigator.

7.6.3 Reference Laboratories for Immunophenotyping for Minimal Residual Disease (MRD) Detection

Samples from each trial patient should be sent to the reference immunophenotypic MRD laboratory below.

AML Trial Immunophenotyping (c/o Steve Dix) Clinical Immunology Service
College of Medical & Dental Sciences University of Birmingham
Vincent Drive Edgbaston B15 2TT

7.6.4 Molecular Characterisation and Storage

Molecular characterisation is essential for all patients, initially for characterisation of *FLT3* and *NPM1* mutations and for other relevant mutations which may be identified during the course of the trial. Excess diagnostic material will be stored for future leukaemia research, for which it is essential that patient informed consent to storage of excess material must be obtained using **Patient Information Sheet and Consent Form 2.**

7.6.5 Dispatch of Samples to Reference Labs

Investigators will be provided with sample packaging to send samples to the reference laboratory. Samples at diagnosis for dispatch to the reference lab should be:

i) **Diagnostic Samples For Minimal Residual Disease**
2 - 4 ml of bone marrow in EDTA tube

and

20-30 ml of peripheral blood in EDTA tube

ii) **For Molecular Characterisation and Storage 2 - 4 ml of bone marrow in EDTA**

and

20 - 30 ml of peripheral blood in EDTA tube

The samples should be dispatched via the Royal Mail service in the packaging provided. (Please refer to the sample flow chart for detail).

7.7 Follow-up Material Post Course 1

For Minimal Residual Disease assessment after course 1 a sample of the marrow being done to assess remission status should be sent to the reference immunophenotyping lab in University of Birmingham:

2 - 4 ml of bone marrow in EDTA tube

At the same time, investigators should enter directly to the web-based system, whether the patient is in morphological remission or not. Investigators will be informed whether the patient is MRD positive, negative or unknown post course 1.

Patients who are in the high risk arm should have a bone marrow for morphology and **MRD assessment post course 2.** The sample for dispatch to the reference lab (University of Birmingham) should be:

2 - 4 ml of bone marrow in EDTA tube

7.8 Cytogenetics

Cytogenetics should be carried out locally and reports entered into the AML18 database.

7.9 Eligibility for Second Randomisation

Eligibility for the Second Randomisation depends on the disease status following course 1. Patients who are not in CR or CRi morphologically after course 1 are eligible for the dose intensification randomisation irrespective of MRD. For all other patients, for whom an LAP has been identified, eligibility for randomisations post course 1, including the addition of AC220 to treatment will be contingent on the receipt of a sample by the MRD laboratory. Based upon the results of the MRD assessment, centres will be informed by email, normally within 5 working days, which pathway is appropriate.

7.10 Second randomisation for patients who are not MRD -ve following course 1 of DA chemotherapy (+/- Mylotarg)

For this randomisation **Patient Information Form 3a and Consent Form 3a** should be used. After patients have received course 1 of the allocated treatment a bone marrow assessment for morphology and MRD assessment will be carried out. If the patient is either not in CR/CRi (see section 10.1 for definition) after course 1 or is in CR but MRD positive, or if MRD information is not available, they are eligible for the dose intensification randomisation between DA and FLAG-Ida if they were allocated DA chemotherapy.

7.11 Randomisation for AC220 in *patients with a FLT3 mutation*

For patients with a *FLT3* mutation (confirmed via email following analysis in the Cardiff University lab of the sample taken at trial entry) after recovery from course 1 of DA chemotherapy (+/- Mylotarg) and prior to starting course 2.
For this randomisation **Patient Information Form 4 and Consent Form 4** should be used.

7.12 Randomisation for MRD -ve Patients who were allocated DA chemotherapy

Patients in the MRD -ve arm who have recovered from course 2 are eligible to be randomised to either DA or intermediate dose cytarabine (IDAC) as the third chemotherapy course. For this randomisation **Patient Information Form 5 and Consent Form 5** should be used.

7.13 Second Treatment Randomisation for MRD +ve/unknown patients following course 1 of CPX-351

Patients who have failed to achieve CR/ CRi after course 1 or who are MRD +ve or unasessable after course 1, are eligible for randomisation into the high risk arm to have intensified treatment. Those who were allocated CPX-351 can be randomised to receive one of two different schedules of CPX-351. **Patient Information Sheet 3b and Consent Form 3b** should be used. (Note that the patient is being asked to have a marrow assessment to confirm remission and MRD status).

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

- AML18 trial number (date of birth)
- Remission status and MRD status of the patient after course 1

8 DATA RECORDING

The web-based system for patient randomisation can be accessed via:

https://trials.cardiff.ac.uk/aml-18

This is a secure encrypted system accessed by an institutional password, and complies with General Data Protection Regulations (GDPR). This system is also used to record the participant's response to course 1 data and cytogenetic data. A user password will be supplied to investigators on receipt of a signed agreement, R&D/local approval, site training and centre registration information (see Section 7.1).

All other CRF's should be completed and returned to AML18 Trial Office at:

AML18 Trial Office
Centre for Trials Research (CTR)
College of Biomedical & Life Sciences
Cardiff University
6th Floor, Neuadd Meirionnydd
Heath Park
Cardiff
CF14 4YS
Fax: 029 2068 7501

Or sent via secure email to the AML18 inbox:

Aml18@cardiff.ac.uk

8.1 Data Submission

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

First Course Report — return when the patient has received one course of treatment, or at prior death (but not later than 2 months after completion of Course 1).

Second Course Report— return when the patient has received course 2 of treatment, or at prior death (but not later than 2 months after completion of Course 2).

Third Course Report— return when the patient has received course 3 of treatment, or at prior death (but not later than 2 months after completion of Course 3).

Fourth Course Report (If Applicable) - return when the patient has received course 4 of treatment.

Fifth Course Report (Vosa/Dec Patients Only) – return when the patient has received 5 courses of treatment, or at prior death (but not later than 2 months after completion of Course 5). **(This randomisation is now closed).**

AC220 Maintenance Forms (If Applicable) – return when the patient has completed each monthly cycle of AC220.

Decitabine Maintenance Forms (If Applicable) – return at 6 month intervals.

Transplant Form (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). Note: if the trials office have been informed that a transplant has been undertaken, a transplant form will be requested.

One Year Follow-up— return at one year after entry to the trial, or at death if the patient

dies within 1 year of finishing therapy. All randomised patients will be followed up for life or until they withdraw consent to be part of the trial, irrespective of whether they complete all trial treatments. This information will normally be collected at the annual follow up.

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

Patient Withdrawal Form– Return in the event of patient withdrawal within 1 month of date of withdrawal.

Annual Follow Up Form – Return within 1 month

8.2 Health Economics

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

8.3 Quality of Life

Quality of Life will be undertaken at baseline, prior to trial treatment, 1 month, 3 months, 6 months and 12 months. The instruments used will be the QLQ-C30 including HADS score, which has been validated in acute leukaemia, and the EuroQoL EQ-5D.

Electronic form entry

The forms listed below can only be completed electronically via the AML18 Website:

- Cytogenetics Form
- Response to course 1

Please note: it is the responsibility of the site to ensure that these forms are completed and returned.

8.4 Source Data

Source Data is defined as “All information in original records and certified copies of original records of clinical findings, observations or other activities in a clinical trial necessary for the reconstruction and evaluation of the trial. Source data are contained

in source documents." There is only one set of source data at any one time for any data element, this will be held locally at either the site (if clinical in nature) or AML18 laboratories (if related to sample analysis) and will be made available to monitors as required, as per Site Agreement/ Material Transfer Agreement.

Trial Data	**Source Location**
Informed consent	Signed PIS kept by site
GP letters	Kept by site
Medical History related to AML	Patient notes
Demographics	Patient notes
Inclusion/exclusion criteria	Patient notes
Blood and bone marrow analysis	Lab analysis report
Molecular genetics (FLT3 and NPM1)	Lab analysis report
Immunophenotyping	Lab analysis report
Cytogenetics	Lab analysis report
AEs/SAEs	AE in patient notes
QoL forms	Questionnaire filled by patient
Death	Patient notes
Withdrawal	Patient notes
IMP compliance	Pharmacy file

8.5 End of Study Definition

The treatment phase will be followed by a follow-up period which will continue until the required number of events have been confirmed. Salvage therapies may improve survival of some patient groups so survival is assessed for analysis on an ongoing basis.

It is accepted that in a phase III trial, some patients may be lost to follow-up – therefore, it is possible not all patients will be followed until death. The end of the trial is defined as the date of data capture which allows all comparisons to be analysed as described in the statistical section (section 15).

When the end of trial has been reached, Sponsor will notify the regulatory authorities within 90 days (or within 15 days if the trial is terminated early).

9 CHEMOTHERAPY SCHEDULE

Randomisation takes place at patient entry between two induction treatment arms.

- DA with Mylotarg (GO) delivered at 3mg/m^2 (maximum 5 mg per dose) on days 1 and 4 of chemotherapy, randomised against
- CPX-351 on days 1, 3 and 5

Randomisation is in a 2:1 ratio in favour of CPX-351.

If a patient is ineligible for CPX-351 or if CPX-351 is temporarily unavailable during the trial, patients can enter the trial and receive DA alone in course 1; they would be eligible for downstream randomisations including AC220 if eligible. If a patient is ineligible for mylotarg (for example, they do not fulfil the hepatic criteria) or if mylotarg is unavailable for logistical reasons patients will enter a randomisation of DA alone vs CPX-351. This randomisation is 2:1 in favour of CPX-351.

Remission status will be determined after course 1 by morphology and by flow cytometric MRD assessment. If after Course 1, the patient is not MRD negative and been allocated DA +/- Mylotarg or CPX-351, the patient is eligible for the dose intensification randomisation. Patients who were allocated DA chemotherapy (with or without Mylotarg) and have a *FLT3 mutation* in the diagnostic sample are eligible for the randomisation to receive AC220 or not providing that they meet the entry criteria.

Patients with high counts at diagnosis can be considered for treatment with Rasburicase to reduce the risk of tumour lysis.

In general it is recommended to deliver chemotherapy dosing according to full surface area based on actual body weight, but if in doubt please discuss with one of the Clinical co-ordinators.

Treatment Schedule for patients not known to have adverse risk Cytogenetics or AML secondary to previous MDS or therapy-related AML (t-AML)

9.1 DA plus Mylotarg 2 doses schedule

Course 1

Daunorubicin 60 mg/m^2 daily by IV 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)

Mylotarg (GO) 3mg/m^2 (maximum 5 mg per dose) on days 1 and 4 of DA chemotherapy

If the patient is ineligible to receive Mylotarg the DA schedule is the same as above

9.2 CPX-351 schedule

Course 1

CPX-351(300) 100 units/m^2 is administered by intravenous infusion over 90 minutes on days 1, 3 and 5 inclusive for course 1 of CPX-351 (this is equivalent to 132 mg/m^2 of daunorubicin).

9.3 Administration of Mylotarg

Patients allocated to receive Mylotarg must not have a white count greater than 30 x 10^9/L because of the risk of tumour lysis. Such patients should have the WBC reduced with Hydroxycarbamide before commencing trial chemotherapy. Patients are only eligible to receive Mylotarg if the liver function tests are within the specified range.

Mylotarg will be given at a dose of 3mg/m^2 (maximum 5 mg per dose) on days 1 and 4 of Course 1. If the WBC is not responsive to Hydroxycarbamide patients may still enter the Mylotarg randomisation and start DA with delayed administration of Mylotarg on days 4 and 7. In all patients, liver function tests must continue to meet the entry criteria for the second dose to be administered. Further details of the premedication, and other procedures for Mylotarg administration, are set out in the current pharmacy manual.

CPX-351 will be provided to sites directly from St Mary's Pharmacy Unit in Cardiff, who will be responsible for labelling, QP releasing and distributing the drug.

9.4 Courses 2 and 3 for MRD -ve patients

For patients allocated DA (+/- Mylotarg) in Course 1

If a patient is in CR or CRi after course 1 and is MRD –ve by flow cytometry they will have DA as course 2. However, at this point patients found to have a *FLT3* mutation in the diagnostic sample by the Cardiff University lab and who meet the cardiac eligibility criteria will be randomised to receive AC220 or not (Section 9.6). Following recovery from course 2, patients in the MRD -ve arm will be randomised between a further 5-day cycle of DA or a cycle of intermediate dose cytarabine (IDAC) as the third chemotherapy course.

Any previously-allocated AC220 will be given alongside chemotherapy. The allocated AC220 will additionally be given as monotherapy for course 4.

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)

Course 3:

DA 2+5 schedule

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)

Intermediate dose Cytarabine (IDAC) schedule

Cytosine Arabinoside 1g/m^2 daily by 4 hour infusion on days 1- 5 inclusive (5 doses)

For patients allocated CPX-351 in Course 1 and are MRD-ve

Course 2. CPX-351(200)

CPX-351 is administered by intravenous infusion over 90 minutes at a dose of 100 units/m^2 on days 1 and 3 only (CPX-351 200)

Course 3 CPX-351(130)

For the 3rd course CPX-351 will be administered by intravenous infusion over 90 minutes at a dose of 65 unit/m^2 on days 1 and 3 only (CPX-351 130)

If sufficient count recovery is not achieved within 42 days, post course 1, investigators may consider an adjustment to the CPX dosing in course 2, in line with the current Vyxeos SPC. Any such adjustment should be discussed with the Chief Investigator or the clinical coordinators before commencing treatment in subsequent courses.

9.5 Courses 2 and 3 in MRD +ve/unknown Patients

For patients allocated DA (+/- Mylotarg) in Course 1

If a patient is not in CR or CRi after course 1 or is MRD +ve/unknown by flow cytometry they will be eligible to be randomised in a 1:1 fashion between DA and FLAG-Ida (or mini FLAG-Ida if 70 years or older) .

This randomisation will take place before course 2 commences. Patients found to have a *FLT3* mutation in the diagnostic sample by the Cardiff University lab and who meet the cardiac eligibility criteria will be simultaneously entered into the randomisation to receive AC220 or not (Sections 9.7).

9.5.1 FLAG-Ida and mini FLAG-Ida

Course 2: for patients aged 60-69 years

Fludarabine 30 mg/m^2 daily i.v. on days 2-6 inclusive (5 doses)

Cytosine Arabinoside 1g/m^2 daily over 4 hours, starting 4 hours after Fludarabine on days 2-6 inclusive (5 doses). **Please note that this dose of Ara-C is lower than that used in AML17.**

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

Course 2: Mini FLAG-Ida for patients aged 70+ years

Fludarabine 25 mg/m^2 daily i.v. on days 2-5 inclusive (4 doses)

Cytosine Arabinoside 1 g/m^2 daily over 4 hours, starting 4 hours after Fludarabine on days 2-5 inclusive (4 doses)

G-CSF [Lenograstim* 263 µg (1 vial)] s.c. daily days 1-6 inclusive (6 doses).

Idarubicin 5 mg/m^2 i.v. daily on days 3, 4 and 5 (3 doses)

Course 3: Mini FLAG-Ida (for all patients)

Fludarabine 25 mg/m^2 daily i.v. on days 2-5 inclusive (4 doses)

Cytosine Arabinoside 1 g/m^2 daily over 4 hours, starting 4 hours after Fludarabine on days 2-5 inclusive (4 doses)

G-CSF [Lenograstim* 263 µg (1 vial)] s.c. daily days 1-6 inclusive (6 doses).

Idarubicin 5 mg/m^2 i.v. daily on days 3, 4 and 5 (3 doses)

[1] G-CSF: Lenograstim is recommended in this regime but is not obligatory

9.5.2 DA Therapy

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)

Course 3: 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)

9.5.3 For patients allocated CPX-351 in Course 1 and who are MRD+ve/unknown

If a patient is not in CR or CRi after course 1 or is MRD +ve/unknown by flow cytometry they will be eligible to be randomised in a 1:1 fashion between CPX-351 given on days 1, 3 and 5 (3 doses) and CPX-351 given on days 1 and 3 (2 doses). This randomisation will take place before course 2.

If allocated CPX-351 (300) Course 2:

CPX-351 100 units/m^2 is administered by intravenous infusion over 90 minutes on days 1, 3 and 5 (CPX-351 300).

If allocated CPX-351 (200) Course 2:

CPX-351 is administered by intravenous infusion over 90 minutes at a dose of 100 units/m^2 on days 1 and 3 only (CPX-351 200)

Course 3. CPX-351 (130)

For all patients the 3rd course CPX-351 will be administered by intravenous infusion over 90 minutes at a dose of 65 unit/m^2 on days 1 and 3 only (CPX-351 130)

During trial treatment safety data will be assessed through laboratory and haematology testing and recorded in the patient CRFs.

Lab testing will consist of:

- Blood chemistry: sodium, potassium, calcium, phosphate, magnesium, creatinine, total protein, albumin, ALT (SGPT) and/or AST (SGOT), alkaline phosphatase, LDH, bilirubin, uric acid, glucose and amylase

Haematology testing will consist of:

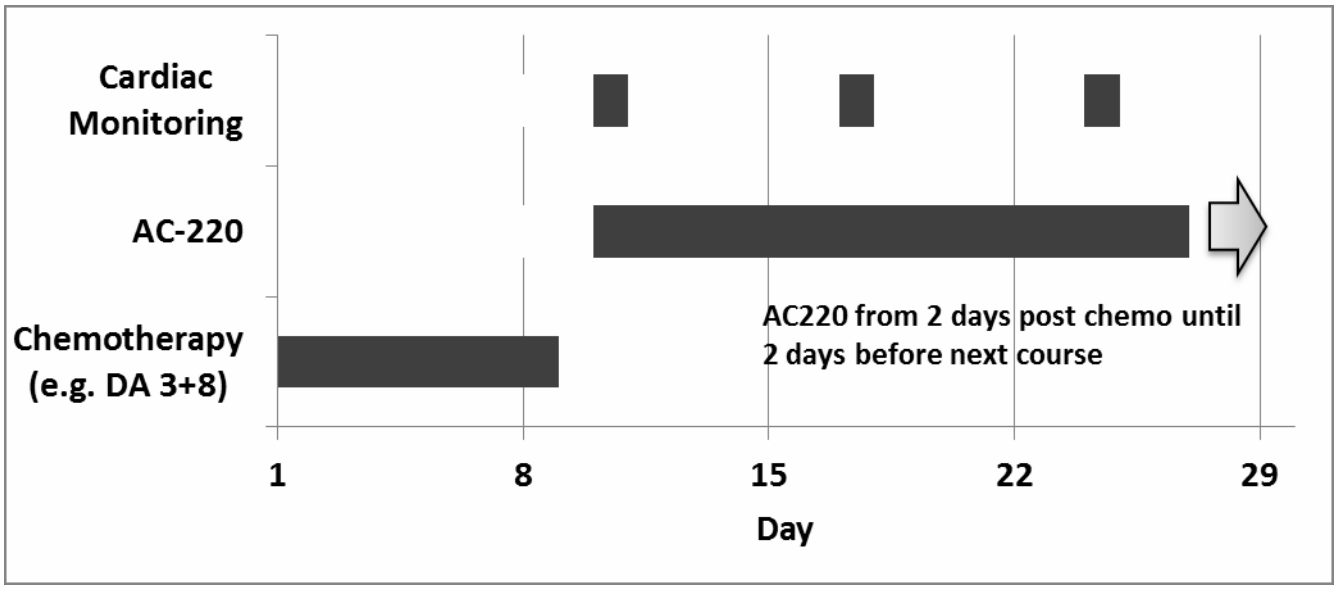
- Haemoglobin, erythrocyte count, MCV, leukocyte count, differentials, absolute neutrophil count, lymphocytes, and platelets
- Coagulation: PT, APTT

9.6 AC220 Treatment Schedule

The randomisation to AC220 or not will take place immediately before course 2 of treatment for **patients who were allocated DA induction +/- Mylotarg and who have a *FLT3* mutation confirmed by the Cardiff University lab following diagnosis**, irrespective of residual disease status. Patients allocated to receive AC220 will be randomised in a 1:1 fashion to AC220 or no AC220 with a 1:1 randomisation in patients drawing AC220 between short and long therapy**.** AC220 will commence at least 2 days after the end of each course of chemotherapy at a dose of 40mg per day.

Each patient will receive or be instructed to take AC220 as 2 x 20mg tablets in the morning, with or without food for a maximum of 14 days after cycles 2 and 3 of chemotherapy, and discontinued so as to have a minimum gap of 48 hours prior to the next cycle of chemotherapy. Patients that have not taken the dose in the morning have until midnight to take the dose or that dose is considered missed. Should a patient vomit after taking their dose, no replacement should be taken that day. **Before commencing each course of AC220, the electrolyte levels and ECG criteria should comply with the AC220 eligibility criteria, i.e. electrolytes in normal range and QTc interval not >450ms.**

These electrolyte and ECG criteria should also be met on each of the formal review days (8 and 14) as set out in section 9.6.4.



A similar procedure will be carried out after each subsequent chemotherapy course.

In addition, patients will receive one 28-day course of AC220 after completing their final course of chemotherapy (course 4 as shown on the flowchart) and their neutrophil count has recovered to >1.0 x 10^9/L and platelets to >50 x 10^9/L.

Following course 4, patients allocated maintenance therapy (long AC220) will take AC220 at a dose of 40mg once daily for 12 cycles lasting 28 days.

AC220 Schedule

Short AC220	ECGs Required
Course 1 for 14 days	Day 1, 8 and 14 (pre-dose, +2, +4 hours)
Course 2 for 14 days	Day 1, 8 and 14 (pre-dose, +2, +4 hours)
Course 3 for 28 days	Day 1, 8, 14 and 21 (pre-dose, +2, +4 hours)

Long AC220	ECGs Required
Course 1 for 14 days	Day 1, 8 and 14 (pre-dose, +2, +4 hours)
Course 2 for 14 days	Day 1, 8 and 14 (pre-dose, +2, +4 hours)
Course 3 for 28 days	Day 1, 8, 14 and 21 (pre-dose, +2, +4 hours)

Then proceed to Long (Maintenance) of 12 cycles lasting for 28 days each. ECG's required day 1 pre-dose for each maintenance cycle.

Patients can start taking AC220 as maintenance when their neutrophil count has risen to >1.0 x 10^9/L and platelets >50 x 10^9/L. Patients who are randomised to long AC220 and are proceeding to RIC transplant are eligible to continue AC220 28 days post- transplant, providing their neutrophil count has recovered to >1.0 x 10^9/L and platelets to >50 x 10^9/L. AC220 should be stopped 14 days prior to transplant.

9.6.1 Dose Interruption/Modification

AC220 dosing will be interrupted for up to 14 days if the absolute value of the QTcF interval increases to >500 ms or increases > 60 ms above baseline defined as the day 1 pre-treatment QTcF average of each cycle (see details in Section 9.7.6). Patients with Grade 3 or 4 non-haematological toxicity will have AC220 interrupted in certain circumstances (see details in Section 9.7.6).

9.6.2 Concomitant Medications

In the case of a recorded adverse event on patients receiving AC220, the history of concomitant medications leading up to and co-incident with the adverse event must be recorded.

9.6.3 Prohibited Therapies

Medications associated with QT/QTc prolongation and CYP3A4 inhibitors are prohibited and are detailed in Appendix B. Exceptions are made for antibiotics and

anti-fungals, and other antimicrobials that are used as standard of care for the prevention or treatment of infections. Patients enrolled in this study should not be taking or begin taking medications known to prolong the QT/QTc interval. Antacid agents may be taken, but not within 8 hours before or 4 hours after dosing with AC220.

Use of concomitant drugs that prolong QT/QTc interval or are CYP3A4 inhibitors are prohibited, with the exception of those listed below, if clinically indicated:

- antibiotics;
- antifungals;
- other antimicrobials that are used as standard of care to prevent or treat infections;
- other such drugs that are considered absolutely essential for the care of the patient.

Administration must be fully documented in the CRF (recommended washout of 5 half-lives, prior to the patient‘s first dose with AC220).

9.6.4 Safety Evaluations

Patients will be monitored for adverse events (AEs) and will undergo safety testing at defined intervals - prior to the study start, and there will be formal cardiac safety assessments on day 1, 8 and 14 after the commencement of each treatment course of AC220, by the Principal Investigator or his/her nominee. These days could be flexible at weekends. Toxicity will be assessed using the CTCAE version 3 instrument. For patients on maintenance AC220 the cardiac assessments will be done on day 1 of each cycle.

9.6.5 Biomarker Sampling for AC220

For patients allocated to AC220 a 10ml blood sample should be sent in the EDTA sample tube supplied on day 14 of each of the first 3 courses. For patients on the long AC220 randomisation this sample should also be taken following course 2, 4 and 6. The sample should be sent to:

AML Trial Samples/ Amanda Gilkes
Department of Haematology
7th Floor,
University Hospital of Wales,
Heath Park,
Cardiff,
CF14 4XN
Tel: 029 2074 4524
E-mail: Gilkes@cardiff.ac.uk

9.6.6 Guidelines for Electrocardiogram Monitoring and Dose Modification

Formal cardiological monitoring will be undertaken on days 1, 8 and 14 of each of the first 2 courses of AC220 treatment. Course 3 monitoring will be undertaken on days 1, 8, 14 and 21. ECGs will be performed pre-first dose and at +2 and +4 hours of each course. The 12-lead ECGs will be recorded in triplicate (3 separate ECGs at least 3 minutes apart per time point). Please see Appendix C for details of the cardiac monitoring schedule. The best point estimate for QTcF duration at any time point will be calculated based on the average of all the ECGs obtained around that time point. Because AC220 has been shown in Study CP0001 in AML patients to cause QTcF prolongation, and this was the DLT, the use of concomitant drugs that prolong QT/QTc interval or are CYP3A4 inhibitors is prohibited with the exception of antibiotics and antifungals, and other antimicrobials that are used as standard of care to prevent or treat infections, and other such drugs that are considered absolutely essential for the care of the patient. Introduction during the study of a new concomitant medication that is a CYP3A4 inhibitor or that may cause QTc prolongation, or an increase in dosage of such a concomitant medication, should be done only if necessary (lack of any alternatives for the identified medical condition). The use of such medications must be approved by the Chief Investigator and the medication, dose, and its indication must be documented in the CRF.

Electrocardiograms will be reviewed by a suitably qualified physician at the investigational site in order to make immediate decisions with respect to patient safety. When an ECG abnormality of concern is suspected (e.g., QTcF >500 ms), the ECG should be repeated in triplicate within 2 hours on that day and re-analysed.

A limited number of skilled readers should analyse the patient‘s ECGs where baseline and on-treatment ECGs should be analysed (using all 12 leads superimposed) by the same reader if possible. The QTcF duration will be used to make all decisions based on QT criteria. The analysis of QT data is complicated by the fact that the QT interval is highly correlated with heart rate. Because of this correlation, formulae are routinely used to obtain a corrected value, denoted QTc, which is independent of heart rate. This QTc interval is intended to represent the QT interval at a standardised heart rate. Several correction formulas have been proposed in the literature. For this analysis, the QT interval will be corrected for heart rate by Fridericia‘s formula, QTcF, defined as: QTcF = QT/(RR)0.33, where RR interval is inversely proportional to heart rate (approximately, RR = 60/heart rate).

Please go to the AML18 website to use the AML18 QTcF calculator

https://trials.cardiff.ac.uk/aml-18/

If the absolute value of the QTcF interval increases to >500 ms or a rise above baseline of >60 ms, the patient will be monitored with another ECG in triplicate within 2 hours of the QTcF value being >500 ms on the same day. If that ECG also shows that the QTcF is still >500 ms or greater than 60ms above baseline, this will be considered a DLT (and reported as an SAE), and the AC220 dose will be interrupted for up to 14 days until the QTc interval returns to within 30 ms of the baseline value. Every effort should be made to keep the levels of potassium, magnesium and calcium within the institute‘s normal ranges.

Concomitant medication that may cause QT/QTc prolongation or inhibit CYP3A4 should be reviewed and discontinued, if considered appropriate by the Investigator. If there is further QTc prolongation reaching Grade 3 when dosing is re-introduced the patient will be taken off the study drug and not retreated with AC220.

If any prolonged QTcF interval is associated with a clinically significant arrhythmia (such as torsades de pointes [TdP], ventricular tachycardia, or prolonged QTcF interval Grade 4), appropriate treatment and ECG monitoring should be initiated immediately and AC220 must be discontinued permanently.

All confirmed Grade ≥ 3 QTcF interval prolongation (increase of >60 ms+) will be reported as an SAE. If clinically significant other ECG changes from baseline (pre-

dose, Day 1) are noted, the changes will be documented as AEs on the Adverse-Event page of the CRF. Clinical significance will be defined as any variation in ECG, which has medical relevance that could result in an alteration in medical care. The Investigator must continue to monitor the patient until the parameter returns to ≤Grade 1 or until the Investigator determines that follow-up is no longer medically necessary.

For patients on maintenance therapy with AC220, monitoring will be on day 1 only (pre-dose only) unless a concomitant medication that may cause QT/QTc prolongation or inhibit CYP3A4 has been started when monitoring should revert to cycle 1 monitoring.

9.6.7 Other Non-haematological Toxicities and Dose Modification

Patients with any other Grade 3 or 4 non-haematological toxicity that is possibly, probably or definitely related to AC220 and persisting for > 48 hours without resolution to Grade ≤ 2 will have dosing of AC220 interrupted (or sooner at the discretion of the investigator if considered necessary for patient safety) with the exception of those Grade 3 or 4 non-haematological toxicities that are associated with AML and its treatment. These include Grade 3 anorexia, nausea, vomiting or diarrhoea and irregularities or imbalances in electrolytes (Na, K, Cl, bicarbonate, etc.) that are controlled with appropriate therapies or Grade 3 neutropenic fever (with or without infection) or infection. If the AC220-related Grade 3 non-haematological toxicity reverses to Grade ≤ 1 on or before 14 days following interrupting the study drug, then the patient may resume therapy if considered tobe safe and in the best interest of the patient by the Investigator with the agreement of the CI. Patients on maintenance AC220 can have their treatment interrupted for a maximum of 14 days per 28 day cycle. Patients with non-haematological study drug-related Grade 4 will not be re-dosed with AC220 and will discontinue the study.

9.7 Management of overdose/ underdose

In the event of a dosing error in regard to any drug described within AML18, any further treatment should be withheld and the AML18 Trial Manager should be notified via a dosing error form being completed and submitted within 24 hours of site becoming aware. Treatment should not recommence until advice has been obtained from the

Sponsor, CI and drug companies as required. This does not need to be reported as an adverse event but if the patient experiences an SAE that the local PI considers may be causally related to the overdose then this must be clearly stated on the SAE form submitted to the CTR PV team.

If a patient is underdosed due to reasons not related to toxicity then a dosing error form should be completed. If doses are stopped prematurely due to toxicity then this is captured on the Course CRF.

10 ASSESSMENT OF RESPONSE

Response should be assessed 14 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 to confirm remission status.

10.1 Definition of Complete Remission (CR in marrow)

- The post course 1 or 2 bone marrow is regenerating normal haemopoietic cells and contains <5% blast cells by morphology in an aspirate sample with at least 200 nucleated cells. Additionally, there is an absolute neutrophil count of more than 1.0x 10^9/L and platelet count of at least 100 x 10^9/L within 14 days of marrow assessment. No extra-medullary disease.
- Patients must be platelet transfusion independent.

10.2 Definition of CRi:

Fulfilling all criteria for CR except for residual neutropenia (1.0x 10^9/L) or thrombocytopenia (100 x 10^9/L) for more than 14 days after marrow assessment. Patients must be transfusion independent.

10.3 Definition of Partial Remission (PR):

The post course 1 bone marrow is regenerating normal haemopoietic cells and blast count has reduced by at least half, to a value between 5 and 15% leukaemic cells. These patients will be eligible to enter the dose intensification randomisation.

10.4 Resistant Disease (RD):

The post course 1 bone marrow shows persistent AML not meeting the criteria for PR, and the patient survives at least 7 days beyond end of course. These patients will be eligible to enter the dose intensification randomisation.

10.5 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.

10.6 Patient Withdrawal:

Participants have the right to withdraw consent for participation in any aspect of the trial at any time. The participants care will not be affected at any time by declining to participate or withdrawing from the trial.

If a participant initially consents but subsequently withdraws from the trial, clear distinction must be made as to what aspect of the trial the participant is withdrawing from. These aspects could be:

1. Withdrawal of Trial Treatment/ Intervention
2. Withdrawal from Quality of Life questionnaires
3. Withdrawal from Samples (Blood, bone marrow)
4. Withdrawal of Consent from trial activity and further data
5. Withdrawal of permission to use any previously collected data/ samples

The withdrawal of participant consent shall not affect the trial activities already carried out and the use of data/samples collected prior to participant withdrawal. The use of the data/samples collected prior to withdrawal of consent is based on informed consent before its withdrawal.

Furthermore, it is important to collect safety data ongoing at the time of withdrawal, especially if the participant withdraws because of a safety event.

In all cases participants who consent and subsequently withdraw should complete a withdrawal form or the withdrawal form should be completed on the participant's behalf by the researcher/clinician based on information provided by the participant. This withdrawal form should be sent to the CTR. Any queries relating to potential withdrawal of a participant should be forwarded to the AML18 trial team.

If a patient specifically withdraws their permission to use data already collected, data will be kept but not used in any future analysis or reports. Lab staff will be contacted to request destruction of any stored samples but sample data may be kept but not used in any future reports or analysis.

10.7 Lost to Follow Up

Participants who cease to attend trial visits prior to the end of the follow-up period, or for whom remote follow-up is unsuccessful, will be classed as lost to follow up if we do not have confirmation of death.

Every effort will be made to obtain follow-up information on these participants, unless they have completely withdrawn from the trial. Participants who are not present for a scheduled visit will be contacted by their local research team by telephone or letter. If they are contactable, the local research team will ask them to make an appointment to be seen at the next available clinic. If the participant declines or cannot be contacted, the local research team will inform their GP and will aim to complete the remote follow-up as per the study schedule. The minimum information we will aim to collect is date of death and SAE data.

If the participant is alive but not compliant with trial medication or the approved visit schedule they will be withdrawn from trial treatment/ intervention. The participant has not explicitly removed their consent, and both previously collected data/samples and data obtained from public records such as NHS flagging can still be used.

11 REDUCED INTENSITY ALLOGENEIC STEM CELL TRANSPLANT

Patients who have an HLA matched donor available, either a sibling or suitably matched unrelated donor (defined as no more than a single allele mismatch by high resolution typing at HLA A,B,C or DRB1), are eligible to receive a reduced intensity (RIC) 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 AML18 trial will not be prescribed because as the field develops over the next five years, will be subject to changes in light of experience. Patients should have an HSCT co-morbidity index of not >3.

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 should be returned to AML18 Trial Office or entered via the web-based system.

12 HEALTH ECONOMICS ASSESSMENT AND QUALITY OF LIFE

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/antifungals. This will be collected in the CRF.

Quality of Life is a key assessment of treatment in this patient population. This will be undertaken at baseline (within the first 7 days on trial entry), 1 month, 3 months, 6 months and 12 months. The instruments used will be the QLQ-C30 including HADS score, which has been validated in acute leukaemia, and the EuroQoL EQ- 5D. Patients should be able to undertake the assessment themselves, and the trial team at each centre is asked to facilitate this and to return the responses to the Trials Office in the pre-paid envelope. Assessment may be due at time points when the patient is at home, in which case the form will be sent to the Site trial team who are asked to pass them on if the patient is alive.

13 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. Antibiotic prophylaxis for neutropenic fever — including antibiotic choice(s) and monitoring, duration of therapy, and the treatment of non-response
6. Antiviral prophylaxis is recommended
7. G-CSF therapy [Lenograstim[2] 263 µg (1 vial) S.C. daily] should be given in case of prolonged neutropenia and should be routinely used following FLAG- Ida

chemotherapy

8. Irradiated blood products should be given to patients who receive FLAG-Ida chemotherapy or Stem Cell Transplant.

14 RELAPSE

Relapse will be diagnosed either on morphological or cytogenetic grounds. When observed relapse and its treatment should be documented.

The Relapse form 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 the Trial Office when all the necessary data are available. Where possible any excess material from the relapse marrow samples should be sent, provided the patient has agreed by completion of Information Sheet and Consent Form 2.

Excess material should be sent to:

AML Trial samples/ Amanda Gilkes
Department of Haematology
7th floor
University Hospital of Wales
Heath Park, Cardiff
CF14 4XN
Tel: 029 2074 4524
Fax: 029 2074 4655
E-mail: gilkes@cardiff.ac.uk

15 STATISTICAL CONSIDERATIONS

15.1 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 patients treated intensively with Mylotarg in AML16, survival at 5 years in patients aged 60 or over is only 15%.Thus, it is unrealistic to expect any of the treatments being evaluated in AML18 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.

Induction

Daunorubicin/Ara-C plus Mylotarg given on days 1 and 4 of course 1 will be compared with CPX-351 and the initial intention of this comparison was to accrue an expected 600 patients in approximately 2 years and analyse for five-year survival. 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, would have required approximately 440 deaths (events) to have a 90% chance of detecting this difference. Based upon a baseline 5-year survival of 20% as seen in AML16 with the DA+GO regimen, 700 patients randomised 2:1 between CPX-351 and DA+GO will be sufficient to detect a 10% improvement in survival from 20% to 30% (HR 0.75) with >85% power at p<0.05 (requiring 441 events), or 80% power to see an improvement of 8.75% (HR 0.77, requiring 504 events).

However, given the impact on recruitment during the COVID pandemic in 2020 and 2021, the trial team propose to move to Event-Free Survival (EFS) as the primary endpoint, which incorporates relapse, or resistant disease (to 2 courses of treatment) as defined events, alongside death. EFS is a well-established endpoint in AML trials including the pivotal trial that led to the approval of mylotarg by the FDA and EMA[4]. Most failures of treatment are in the form of resistant disease, and these events typically emerge within 3-4 months of treatment initiation.

Another important consideration are the randomized comparisons regarding salvage therapy which are incorporated into the trial design. A growing number of effective

interventions in AML, including intensification of treatment, are now available and will potentially bias or dilute the frontline treatment effect in a pure survival-based analysis. Amending the primary endpoint to EFS will require a minimum of 506 patients randomized in a 2:1 fashion between CPX-351 and DA+GO; this sample will be sufficient to detect a 10% improvement in five-year event-free survival from19% to 29% (HR: 0.75; 376 events) with 80% power and a 5% significance level. It is considered this will allow a timely analysis of the comparison, focused on the efficacy of the induction treatment, and based on a realistic assessment of accrual.

Following course 1

Patients with a *FLT3*- mutation will be randomised in a 1:1 fashion between chemotherapy and AC220. Based upon advice from the independent data monitoring committee it was considered unlikely that patients who do not harbour the mutation would derive any benefit from AC220 therapy, and this option has therefore been closed following the recruitment of 506 patients of whom 22% have a confirmed *FLT3* mutation. Going forward, the results for the *FLT3*-wild type and *FLT3*-mutant groups will be reported separately; additionally, there will be a stratified analysis pre- and post-amendment to see whether the effect of AC220 on *FLT3*-mutated patients is consistent. Based upon a similar mutation rate in the following 700 randomised patients, and a similar uptake for the randomisation, the comparison should recruit a total of approximately 200 patients. Based upon published data from AML16, 3-year survival in the control arm of this comparison should be approximately 20%. There will therefore be sufficient power to provide indicative evidence of effectiveness, as a randomised phase 2 trial, with 80% power at p=0.2 one-sided to detect a hazard ratio of 0.75 (requiring 138 events, and 186 patients, and equating to a 10% improvement in survival from 20% to 30%). Given the lack of survival plateau in this group of patients, should the 3-year survival in the control arm be better than 20%, then longer follow-up will be performed until the required number of events is seen.

For patients allocated AC220, there is a further randomisation between short and long therapy. Given the reduced number of patients within the *FLT3* mutated group alone, this comparison is purely exploratory, and will be used in determining whether AC220 should be carried forward to a successor trial, and which regimens.

One finding from AML16 was that MRD following course 1 was highly prognostic, with

MRD associated with increased relapse, and worse survival. This population will be randomised 1:1 between DA (for two courses) and FLAG-Ida. Based upon AML16, approximately 200/1600 patients will die before the end of course 1. Of the remaining 1400 patients, about 900 will have detectable or unknown residual disease. Figures from AML16 indicate 3-year survival in this group will be about 12.5%. With significance at p=0.025 to allow for multiple comparisons, with 400 patients in each comparison (200 per arm) with 312 events there will be approximately 90% power to detect an absolute improvement in survival from 12.5% to 25% (hazard ratio 0.67) for each novel therapy. There will be no direct comparison of novel therapies; rather, they will be compared against control. For patients in the CPX-351 arm the course 2 No CR/ MRD positive randomisation (CPX-351 300 v CPX-351 200) would expect to randomise 240 patients which with PFS as the primary endpoint will allow us to detect with 80% power an increase of survival from 12% to 24% (HR 0.67).

Results from AML16 indicate that 2 courses of therapy for patients without residual disease following course 1 is likely to be suboptimal. For this reason, the original 2 vs 3 course randomisation in this population has been discontinued. Instead, attention focusses on the optimal third course for these patients. Of the original 500 patients expected to be MRD –ve following course 1, going forward approximately 450 patients will be eligible for this comparison. Based upon a control group survival of 38% in this group (as seen in AML16), even if only 2/3 of patients entered this randomisation there would be 80% power to detect a hazard ratio of 0.64, with 160 events, representing a 16% absolute improvement in survival.

15.2 Data Analysis

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

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

The primary outcome is overall survival for all randomisations. All stratification variables used at randomisation will be used in analyses: In addition, any analyses of treatment effectiveness will be stratified by other treatments, cytogenetic risk group, and any relevant molecular markers (including, but not limited to *FLT3*-ITD, *FLT3*-TKD and NPM1 status). All stratified analyses will assume that there may be some quantitative differences in the size of any treatment effects in these different strata, but that there is unlikely to be any qualitative difference (i.e. harm in one group, benefit in another). Interactions between treatments and presenting characteristics, or between treatments, will be tested using standard techniques developed by the Early Breast Cancer Trialists Collaborative Group.

16 TRIAL GOVERNANCE AND ADVERSE EVENT REPORTING

The Trial is sponsored by Cardiff University with defined responsibilities delegated to CTR, 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 Research Ethics Service (RES) Wales REC 3. RES approval requires that investigator sites have a designated Principal Investigator and that participating institutions receive R&D approval via Capability & Capacity confirmation. This 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 AML18 Trial Office, CTR as described in section 7.1. The trial will be monitored by an independent Data Monitoring Committee.

The trial is being sponsored by Cardiff University with responsibilities delegated to the CTR. The CTR shall be responsible for ensuring that the trial is performed in accordance with the following:

- The Medicines for Human Use (Clinical Trials) Regulations 2004 (SI2004/1031) and subsequent amendments.
- Conditions and principles of Good Clinical Practice.
- Declaration of Helsinki (1996)
- UK Policy Framework for Health and Social Care Research.
- The General Data Protection Regulation 2016.
- The Human Tissue Act 2004.
- Other regulatory requirements as appropriate.

16.1 Adverse Event Reporting

Principal Investigators at each participating institution have an obligation to report relevant Serious Adverse Events (SUSARs, SARs and SAEs) that occur in this trial to the CTR safety team within **24 hours** of becoming aware of the event.

Reports should be emailed to the CTR Safety Team at the address below:

Serious Adverse Event (SAE) email address:
CTR-Safety@cardiff.ac.uk
SAE Fax number:
0203 0432 376

It is recognised that adverse events that 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. Within the trial, any event that occurs within 30 days of the patient's treatment, and meets the criteria laid out in Section 16.2 of the protocol, should be reported as an SAE. Beyond this period, any event which is felt to be causally linked to the medication received on the trial, and meets the criteria laid out in the protocol, should also be reported. Also, any patient deaths in CR require reporting as an SAE as per section 16.2 of the protocol.

16.2 Definitions

For the purpose of this trial a **Serious Adverse Event (SAE)** 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 or 5 non-haematological toxicity (excluding alopecia).
- Development of neutropenia (<1.0 x 10^9/L) or thrombocytopenia (<50 x 10^9/L) for longer than 42 days after the end of chemotherapy treatment in the absence of significant disease in the bone marrow (<5% blasts). Please send bone marrow reports with the SAE.
- Any event which is unrelated to the expected consequences of AML or its treatment which results in hospital admission or prolongation. 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.

- If the absolute value of the QTcF interval increases to >500 ms or there is a rise above baseline of >60 ms, the patient will be monitored with another ECG in triplicate within 2 hours of the QTcF value being >500 ms on the same day. If that ECG also shows that the QTcF is still >500 ms or greater than 60ms above baseline, this will be considered a DLT and a reportable SAE.

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

- 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
- Deaths from persistent or progressive disease.

Please refer to AML18 SAE reporting guidance for further information.

16.3 Safety Assessments

16.3.1 Causality

Investigators will be asked to record their opinion as to whether the SAE as defined above was related to the study medication. It is accepted that a causality assessment is not always available at the time of the initial report – however, this opinion should be provided prior to day 7 to ensure regulatory reporting timelines are met.

16.3.2 Expectedness

The Chief Investigator (or another delegated appropriately qualified individual) will assess each SAR to perform the assessment of expectedness.

The expectedness assessment should be made with reference to the current Reference Safety Information (RSI) for each IMP. Expectedness decisions must be based purely on the content of the RSI; other factors such as the participant population and participant history should not be taken into account. Expectedness is not related to what is an anticipated event within a particular disease.

SARs which add significant information on specificity or severity of a known, already documented adverse event constitute unexpected events. For example, an event more specific or more severe than that described in the RSI is considered unexpected.

The table below lists the RSI's that should be referenced:

IMP	RSI to be used for expectedness assessment	Relevant Section to be used for expectedness assessment
Mylotarg	Summary of Product Characteristics Manufacturer: Pfizer Limited	Section 4.8
AC220	Investigator brochure Manufacturer: Daiichi Sankyo	Section 6.14
CPX-351 (Vyxeos)	Summary of Product Characteristics Manufacturer: Jazz Pharmaceuticals	Section 4.8

Vosaroxin*	Investigator brochure Manufacturer: Sunesis Pharmaceuticals	Appendix 1
Decitabine*	Summary of Product Characteristics Manufacturer: Janssen Research and Development	Section 4.8
Cladribine*	Summary of Product Characteristics Manufacturer: Lipomed	Section 4.8

* These IMPS are now closed but RSI remains in place for any potential SARs.

Reference Safety Information (RSI) on any CTR trial will be reviewed regularly according to CTR procedures. The CTR will consider the above assessments and report events as per Sponsor requirements.

16.4 Collection of Data

Preliminary discussion of the event may take place with a Clinical Co-ordinator or Chief Investigator. SAEs should be recorded on the Serious Adverse Event Form which is available on the trial website, and emailed to the CTR Safety Team. Details of adverse events (including SAEs) should be recorded in the patient's notes at the Study Site. Relevant concomitant medications, including details of the dose and duration for the period of 14 days before, and until conclusion of, the event should be recorded in the patient's record. For SUSARs, the Chief Investigator will require to review the clinical documented record of the event and relevant concomitant medication history for each event. For SAEs the Chief Investigator may wish to review information as for a SUSAR, but will not routinely do so. All SAEs will be followed up until resolution.

16.5 Time of Report

All SAEs should be reported to the CTR ***within 24 hours***.

16.6 Reporting to the Regulatory Authorities

Cardiff University is undertaking the duties of trial Sponsor and has delegated CTR the responsibility for reporting SUSARs and other SARs to the regulatory authorities (MHRA and relevant ethics committees) and to drug companies as follows:

SUSARs which are fatal or life-threatening must be reported to the MHRA and REC within 7 calendar days of receipt at the CTR. If report is incomplete then additional follow-up information should be reported within a further 8 calendar days of submitting the initial report.

SUSARs that are not fatal or life-threatening must be reported to the MHRA and REC

within 15 days of receipt at the CTR. Any additional, relevant information must be reported within a further 15 days.

16.7 Protocol/ GCP non-compliance

The Principal Investigator should report any non-compliance to the trial protocol or the conditions and principles of Good Clinical Practice to the CTR in writing as soon as they become aware of it.

17 PUBLICATION POLICY

The Sponsor will inform investigators and funders as results from the randomised questions in the trial become publicly available.

The Trial Management Group includes input from Patient and Public Involvement (PPI) representatives who will provide guidance as to how to best disseminate results to investigators, their teams, the patients and the public.

18 DATA PROTECTION

The CTR will act to preserve participant confidentiality and will not disclose or reproduce any information by which participants could be identified, except where specific consent is obtained. Data will be stored in a secure manner and will be registered in accordance with the General Data Protection Regulation 2016. Patients will always be identified using their unique trial identification number, and data will be stored alongside the patient's initials and date of birth. While the trial is open the samples remain under the custodianship of the Trial Management Group, which includes representation from Sponsor. This includes collection of NHS number (or equivalent – e.g. CHI number in Scotland).

19 ARCHIVING

The TMF and TSF containing essential documents will be archived at an approved external storage facility as per trial site agreement. The CTR will archive the TMF and TSFs on behalf of the Sponsor. The Principal Investigator is responsible for archival of the ISF at site which should be retained for at least 15 years after the conclusion or early termination of the trial on approval from Sponsor. Essential documents pertaining to the trial shall not be destroyed without permission from the Sponsor.

APPENDIX A: WHO Histological Classification of Acute Myeloid Leukaemias

	ICD-0-3 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	9861/3
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: Prohibited Medications for AC220

'AC220 may cause QTc prolongation therefore use of medications associated with QTc prolongation or Torsade de Pointes will be avoided or used with caution. The decision whether or not such a medication used will be taken in the context of patient's medical history and current QTc values. A up to date list of these medications can be found on the Credible Meds website https://www.crediblemeds.org/.

Use of ondansetron has been linked to QTc prolongation and the occurrence of torsades de pointes.

Note: Exceptions are made if the Investigator believes that beginning therapy with a potentially QTc-prolonging medication is vital to an individual patient's care while on study in this case it is recommended that risks and benefits are discussed with the patient and documented in the notes and QTc interval is measured regularly..

Potential QT/QTc Prolonging Drugs:

Drugs with **known risk** of TdP (These drugs prolong the QT interval AND are clearly associated with a known risk of TdP, even when taken as recommended)	
Aclarubicin	Ibogaine
Amiodarone	Ibutilide
Anagrelide	Levofloxacin
Arsenic trioxide	Levomepromazine (Methotrimeprazine)
Astemizole	Levomethadyl acetate
Azithromycin	Levosulpiride
Bepridil	Meglumine antimoniate
Cesium Chloride	Mesoridazine
Chloroquine	Methadone
Chlorpromazine	Mobocertinib
Chlorprothixene	Moxifloxacin
Cilostazol	Nifekalant
Ciprofloxacin	Ondansetron
Cisapride	Oxaliplatin
Citalopram	Papaverine HCl (Intracoronary)
Clarithromycin	Pentamidine
Cocaine	Pimozide
Disopyramide	Probucol
Dofetilide	Procainamide
Domperidone	Propofol
Donepezil	Quinidine
Dronedarone	Roxithromycin
Droperidol	Sertindole
Erythromycin	Sevoflurane
Escitalopram	Sotalol
Flecainide	Sparfloxacin
Fluconazole	Sulpiride
Gatifloxacin	Sultopride

Grepafloxacin	Terfenadine
Halofantrine	Terlipressin
Haloperidol	Terodiline
Hydroquinidine (Dihydroquinidine)	Thioridazine
Hydroxychloroquine	Vandetanib
Drugs with **possible risk** of TdP (These drugs can cause QT prolongation BUT currently lack evidence for a risk of TdP when taken as recommended.)	
Abarelix	Lurasidone
Alfuzosin	Maprotiline
Alimemazine (Trimeprazine)	Melperone
Apalutamide	Mianserin
Apomorphine	Midostaurin
Aripiprazole	Mifepristone
Artemether/Lumefantrine	Mirabegron
Artenimol/piperaquine	Mirtazapine
Asenapine	Necitumumab
Atomoxetine	Nicardipine
Bedaquiline	Nilotinib
Bendamustine	Norfloxacin
Betrixaban	Nortriptyline
Bicalutamide	Nusinersen
Bortezomib	Ofloxacin
Bosutinib	Oliceridine
Buprenorphine	Osilodrostat
Cabozantinib	Osimertinib
Capecitabine	Oxytocin
Carbetocin	Ozanimod
Ceritinib	Paliperidone
Clofazimine	Palonosetron
Clotiapine	Panobinostat
Clozapine	Pasireotide
Cobimetinib	Pazopanib
Crizotinib	Perflutren lipid microspheres
Cyamemazine (Cyamepromazine)	Perphenazine
Dabrafenib	Pilsicainide
Dasatinib	Pimavanserin
Degarelix	Pipamperone
Delamanid	Pitolisant (Tiprolisant)
Desipramine	Ponesimod
Deutetrabenazine	Pretomanid
Dexmedetomidine	Primaquine phosphate
Dextromethorphan/Quinidine	Promethazine
Dolasetron	Prothipendyl
Efavirenz	Relugolix
Eliglustat	Remimazolam
Encorafenib	Ribociclib
Entrectinib	Rilpivirine
Epirubicin	Romidepsin
Eribulin mesylate	Rucaparib
Ezogabine (Retigabine)	Saquinavir
Felbamate	Selpercatinib

Fingolimod	Siponimod
Fluorouracil (5-FU)	Sorafenib
Flupentixol	Sunitinib
Gemifloxacin	Tacrolimus
Gilteritinib	Tamoxifen
Glasdegib	Tazemetostat
Granisetron	Telavancin
Hydrocodone - ER	Telithromycin
Iloperidone	Tetrabenazine
Imipramine (Melipramine)	Tiapride
Inotuzumab ozogamicin	Tipiracil/Trifluridine
Isradipine	Tizanidine
Ivosidenib	Tolterodine
Ketanserin	Toremifene
Lacidipine	Tramadol
Lapatinib	Trimipramine
Lefamulin	Tropisetron
Lenvatinib	Valbenazine
Leuprolide (Leuprorelin)	Vardenafil
Levetiracetam	Vemurafenib
Levomethadone (levamethadone)	Venlafaxine
Linezolid	Voclosporin
Lithium	Vorinostat
Lofexidine	Zotepine
Lopinavir/Ritonavir	Zuclopenthixol (Zuclopentixol)
Lumateperone	
Drugs with **conditional risk** of TdP (These drugs are associated with TdP BUT only under certain conditions of their use (e.g. excessive dose, in patients with conditions such as hypokalemia, or when taken with interacting drugs) OR by creating conditions that facilitate or induce TdP (e.g. by inhibiting metabolism of a QT-prolonging drug or by causing an electrolyte disturbance that induces TdP).	
Abiraterone	Ivabradine
Amantadine	Ketoconazole
Amisulpride	Lansoprazole
Amitriptyline	Loperamide
Amphotericin B	Metoclopramide
Amsacrine (Acridinyl anisidide)	Metolazone
Atazanavir	Metronidazole
Bendroflumethiazide (Bendrofluazide)	Nelfinavir
Chloral hydrate	Olanzapine
Cimetidine	Omeprazole
Clomipramine	Pantoprazole
Diltiazem	Paroxetine
Diphenhydramine	Piperacillin/Tazobactam
Doxepin	Posaconazole
Eperisone	Propafenone
Esomeprazole	Quetiapine
Famotidine	Quinine sulfate
Fluoxetine	Ranolazine
Fluvoxamine	Risperidone
Furosemide (frusemide)	Sertraline
Galantamine	Solifenacin

Garenoxacin	Telaprevir
Hydrochlorothiazide	Torsemide (Torasemide)
Hydroxyzine	Trazodone
Indapamide	Voriconazole
Itraconazole	Ziprasidone
Drugs to be avoided by congenital Long QT patients (Drugs to Avoid in Congenital Long QT Syndrome (CLQTS) - These drugs pose a high risk of TdP for patients with cLQTS and include all those in the above three categories (KR, PR & CR) PLUS additional drugs that do not prolong the QT interval per se but which have a Special Risk (SR) because of their other actions.)	
Albuterol (salbutamol)	Lisdexamfetamine
Amphetamine (Amfetamine)	Metaproterenol (Orciprenaline)
Arformoterol	Methamphetamine (Metamfetamine)
Benzphetamine	Methylphenidate
Dexmethylphenidate	Midodrine
Dextroamphetamine (Dexamfetamine)	Norepinephrine
Diethylpropion (amfepramone)	Olodaterol
Dobutamine	Oxymetazoline
Dopamine	Phendimetrazine
Droxidopa	Phentermine
Ephedrine	Phenylephrine
Epinephrine (Adrenaline)	Phenylpropanolamine
Fenfluramine	Pseudoephedrine
Fenoterol	Ritodrine
Fluticasone And Salmeterol	Salmeterol
Formoterol	Sibutramine
Indacaterol	Sulfamethoxazole and Trimethoprim
Isoproterenol (isoprenaline)	Terbutaline
Levalbuterol (Levsalbutamol)	Vilanterol/fluticasone furoate
Levomethamphetamine (Levmetamfetamine)	Xylometazoline (Xylomethazoline)

Woosley RL, HeiseCW , Gallo T, Tate J, Woosley D and Romero KA, www.CredibleMeds.org, QTdrugs List, [16/11/2021], AZCERT, Inc. 1457 E. Desert Garden Dr., Tucson, AZ 85718

Common Inhibitors of CYP3A4:

HIV Protease Inhibitors
Indinavir
Nelfinavir
Ritonavir
Saquinavir

Food/Juice
Grapefruit Juice

Others
Amiodarone
Cimetidine
Clarithromycin
Erythromycin

Fluoextine
Fluvoxamine
Itraconazole
Ketoconazole
Mibefradil
Nefazodone
Troleandomycin
Verapamil

To be used with caution

Phenytoin
Voriconazole

APPENDIX C: Assessment Schedule for Patients

	Baseline	**Prior** to Course 2	**Post** Course 2	
Obtain Informed Consent	X			
Review exclusion/inclusion criteria	X	X (AC220 criteria)		
Medical history	X			
Performance status	X			
Vital Signs	X			
Haematology	X	X		
Bone marrow aspirate	X	X	X (high risk patients post c1 only)	
Peripheral blood sample	X			
Blood Chemistry	X	X		
Randomisation between DA + Mylotarg (GO2) and CPX-351	X			
ECG (triplicate)		X (AC220 criteria)		
Assessment of MRD and randomisation to AC220 or not (*FLT3* mutated patients only) (see below)	X	X	X (MRD assessment in high risk patients post course 1 only	
MRD positive/unknown patients : randomisation between DAand FLAG-Ida		X		
MRD –ve patients: randomisation to DA vs IDAC			x	

Patients Randomised to Receive AC220												
	Course 2				Course 3				Course 4			
	Day 1 (Post Chemo + 2 days)	Day 8	Day 14		Day 1 (Post Chemo + 2 days)	Day 8	Day 14		Day 1	Day 8	Day 14	Day 21
AC220	X (for 14 days)				X (for 14 days)				X (for 28 days)			
ECG (triplicate)	X Pre-dose +2hrs +4hrs	X Pre-dose +2hrs +4hrs	X Pre- dose +2hrs +4hrs		X Pre-dose +2hrs,+4 hrs	X Pre-dose +2hrs +4hrs	X Pre- dose +2hrs +4hrs		X Pre- dose +2hrs +4hrs	X Pre-dose +2hrs +4hrs	X Pre- dose +2hrs +4hrs	X Pre- dose +2hrs +4hrs
Biomarker Sample			X				X				X	

<u>Please Note</u>: AC220 must be discontinued 48 hours prior to the start of each course of chemotherapy

Long (Maintenance) AC220

	Cycle 1 - 12	
	Day 1	Timepoint
AC220	X for 28 days for each 12 cycles	
ECG (triplicate)	X (Pre-dose)	
Biomarker sample		2, 4 & 6 months

APPENDIX D: 2010 Grimwade Cytogenetic Classification

Cytogenetic abnormality	Comments
Favourable	
t(15;17)(q22;q21)	
t(8;21)(q22;q22)	Irrespective of additional cytogenetic abnormalities*
inv(16)(p13q22)/t(16;16)(p13;q22)	
Intermediate	
Entities not classified as favourable or adverse	
Adverse	
abn(3q) [excluding t(3;5)(q21~25;q31~35)],	
inv(3)(q21q26)/t(3;3),	
add(5q), del(5q), --5,	
--7, add(7q)/del(7q),	Excluding cases with favourable karyotype†
t(6;11)(q27;q23),	
t(10;11)(p11~13;q23),	
t(11q23)[excluding t(9;11)(p21~22;q23) and t(11 ;19)(q23 ;p13)]	
t(9 ;22)(q34 ;q11),	
--17/abn(17p),	
Complex (≥ 4 unrelated abnormalities)	

*All favourable-risk abnormalities.
†All adverse-risk abnormalities.

APPENDIX E: IMP Provision Schedule

IMP	SUPPLIER	DISTRIBUTOR
Mylotarg	Pfizer	Hospital stock
AC220	Daiichi Sankyo (provided free of charge)	St Mary's Pharmaceutical Unit (SMPU)
CPX-351	Jazz Pharmaceutical Inc (supplied free of charge)	St Mary's Pharmaceutical Unit (SMPU)

- Following randomisation, provision of all initial IMPs will be arranged by the trials office.
- Subsequent treatment courses for individual patients should follow the 'ordering flowchart' process as detailed in the pharmacy manual. Sites should only order IMPs using the current request forms provided in the pharmacy file.
- Any queries regarding supply, storage, handling or destruction of IMP should be directed to the Trial Manager (AML18@cardiff.ac.uk).
- Deliveries to UK sites are usually made within 2 working days of collection from the distribution facility.
- Deliveries from SMPU are via a temperature-controlled supply chain, so no temperature logs are included with the deliveries.
- QP release certification is included with each delivery.

APPENDIX F: List of Abbreviations

AE: **a**dverse **e**vents
AML: **a**cute **m**yeloid **l**eukaemia
ANC: **a**bsolute **n**eutrophil **c**ount
ALT: **al**anine amino**t**ransferase
AST: **as**partate amino**t**ransferase
AV: **a**trio**v**entricular
CML: **c**hronic **m**yeloid **l**eukaemia
CNS: **c**entral **n**ervous **s**ystem
CR: **c**omplete **r**emission
CRc: **c**omposite **c**omplete **r**emission
CRi: **c**omplete **r**emission with **i**ncomplete haematological recovery
CRp: **c**omplete **r**emission with **i**ncomplete platelet recovery
CSF: **c**erebro-**s**pinal **f**luid
CTA: **C**linical **T**rials **A**uthorisation
CTR: **C**entre for **T**rials **R**esearch
CTCAE: **C**ommon **T**erminology **C**riteria for **A**dverse **E**vents
DA: **d**aunorubicin + cytarabine (**Ara-C**)
DClo: **d**aunorubicin + **clo**farabine
DLT: **d**ose **l**imiting **t**oxicity
ECG: **e**lectro**c**ardio**g**ram
EUDRACT: **Eu**ropean **C**linical **T**rials **D**atabase
FLAG-Ida: **fl**udarabine _+ cytarabine (**Ara-C**) + **G**-CSF + **ida**rubicin
FLT3: **f**ms-**l**ike **t**yrosine kinase 3
GCP: **G**ood **C**linical **P**ractice
GO: **g**emtuzumab **o**zogamicin (mylotarg)
GVHD: **g**raft **v**ersus **h**ost **d**isease
HADS: **h**ospital **a**nxiety and **d**epression **s**cale
HCT-CI: **h**aemopoietic **c**ell **t**ransplant **c**o-morbidity **i**ndex
HIV: **H**uman **I**mmunodeficiency **V**irus
HRA: **H**ealth **R**esearch **A**uthority
HSCT: **h**aematopoietic **s**tem **c**ell **t**ransplantation
IB: Investigator brochure
IDAC: **i**ntermediate **d**ose **c**ytarabine
IDMC: **i**ndependent **d**ata **m**onitoring **c**ommittee
IMP: **i**nvestigational **m**edicinal **p**roduct
LAP: **l**eukaemia **a**ssociated **p**henotype
LBBB: **l**eft **b**undle **b**ranch **b**lock
LSC: **l**eukaemic **s**tem **c**ell
LVEF: **l**eft **v**entricular **e**jection **f**raction
MDS: **m**yelo**d**ysplastic **s**yndrome
MHRA: **M**edicine and **H**ealthcare Products **R**egulatory **A**gency
MRC: **M**edical **R**esearch **C**ouncil
MREC: **M**edical **R**esearch and **E**thics **C**ommittee
MRD: **m**inimal **r**esidual **d**isease
MUGA: **mu**ltiple **ga**ted acquisition scan
NCRI: **N**ational **C**ancer **R**esearch **I**nstitute
NIMP:Non Investigational MedicinalProduct
NHS: **N**ational **H**ealth **S**ervice, UK
NRES: **N**ational **R**esearch **E**thics **S**ervice
OS: **O**verall **S**urvival
PML: **p**ro**m**yelocytic **l**eukaemia
PML-RARA: **p**ro**m**yelocytic **l**eukaemia (PML), **r**etinoic **a**cid **r**eceptor **a**lpha (RARA)

PK-PD: **p**harmaco**k**inetic-**p**harmaco**d**ynamic
PPI: **P**atient and **P**ublic **I**nvolvement
PR: **p**artical **r**emission
QoL: **Q**uality **o**f **L**ife
R&D: **r**esearch and **d**evelopment
REC: **R**esearch **E**thics **C**ommittee
RES: **R**esearch **E**thics **S**ervice
RIC: **r**educed **i**ntensity **c**onditioning
SAE: **S**erious **A**dverse **E**vent
SPC: **s**ummary of **p**roduct **c**haracteristics
SSI: **s**ite **s**pecific **i**nformation
SUSAR: **S**uspected **u**nexpected **s**erious **a**dverse **r**eaction
TdP: **t**orsades **d**e **p**ointes
ULN: **u**pper **l**evel of **n**ormal
WBC: **w**hole **b**lood **c**ount
WCC: **w**hite **c**ell **c**ount
WHO: **W**orld **H**ealth **O**rganisation
WOCBP: **W**omen **o**f **c**hild-**b**earing **p**otential