Optimising Therapy in FLT3-mutated Acute Myeloid Leukaemia

PROTOCOL V2.0 12 DECEMBER 2025

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SIGNATURE PAGE

The undersigned confirm that the following protocol has been agreed and accepted and that the Chief Investigator agrees to conduct the trial in compliance with the approved protocol and will adhere to the principles outlined in the relevant trial regulations, GCP guidelines, and CTR's SOPs.

I agree to ensure that the confidential information contained in this document will not be used for any other purpose other than the evaluation or conduct of the clinical investigation without the prior written consent of the Sponsor

I also confirm that I will make the findings of the trial publicly available through publication or other dissemination tools without any unnecessary delay and that an honest accurate and transparent account of the trial will be given; and that any discrepancies from the trial as planned in this protocol will be explained.

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General Information This protocol describes the Optimise-FLT3 clinical trial, and provides information about the procedures for entering participants into the trial. The protocol should not be used as a guide, or as an aide-memoire for the treatment of other patients. Every care has been taken in drafting this protocol; however, corrections or amendments may be necessary. These will be circulated to the known Investigators in the trial. Problems relating to the trial should be referred, in the first instance, to CTR.

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This protocol has been developed by the Optimise-FLT3 Trial Management Group (TMG) on behalf of the UK (formerly NCRI)AML Working group.

For **all queries** please contact the Optimise-FLT3 team through the main trial email address. Any clinical queries will be directed through the Trial Manager to either the Chief Investigator or a Co-Investigators

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Where the adverse event meets one of the serious categories, an SAE form should be completed by the responsible clinician and submitted to CTR Safety within 24 hours of becoming aware of the event (See section 15 for more details).

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List of Abbreviations

AE	Adverse Event
AML	Acute Myeloid Leukaemia
ALT	Alanine Aminotransferase
APL	Acute Promyelocytic Leukaemia
AST	Aspartate Aminotransferase
AR	Adverse Reaction
BM	Bone Marrow
CA	Competent Authority
CF	Consent Form
CI	Chief Investigator
CIR	Cumulative Incidence of Relapse
CML	Chronic Myeloid Leukaemia
CR	Complete Remission
CRh	Complete remission with partial haematological recovery
CRi	Complete remission with incomplete count recovery
CRUK	Cancer Research UK
CTIMP	Clinical Trial of Investigational Medicinal Product
CTIS	Clinical Trials Information System
CTR	Centre for Trials Research
CTU	Clinical Trials Unit
CU	Cardiff University
DA	Daunorubicin and Cytarabine (Ara-C)
DSUR	Development Safety Update Report
eCRF	Electronic case report form
ECOG	Eastern Cooperative Oncology Group
EDTA	Ethylenediaminetetraacetic acid
EFS	Event Free Survival
ELN	European Leukaemia Network
FISH	Fluorescence In Situ Hybridization
FLAG-Ida	Fludarabine, Ara-C, G-CSF, Idarubicin
FLT3	FMS-like Tyrosine Kinase 3
GCP	Good Clinical Practice
G-CSF	Granulocyte Colony Stimulating Factor
GDPR	General Data Protection Regulations

GO	Gemtuzumab Ozogamicin (Mylotarg)
HIDAC	High Dose Cytarabine
HIV	Human Immunodeficiency Virus
HR	Hazard Ratio
HSCT	Haematopoietic Stem Cell Transplant
HTA	Human Tissue Act
IC	Informed consent
IDMC	Independent Data Monitoring Committee
IMP	Investigational Medicinal Product
ISF	Investigator Site File
ISRCTN	International Standard Randomised Controlled Trial Number
ITD	Internal tandem duplication
MedDRA	Medical Dictionary for Regulatory Activities
MHRA	Medicine and Healthcare products Regulatory Agency
MLFS	Morphological Leukaemia Free State
MRD	Measurable Residual Disease
MUGA	Multiple Gated Acquisition Scan
NCRI	National Cancer Research Institute
NGS	Next Generation Sequencing
NPM1	Nucleophosmin 1
PAG	Patient Advisory Group
PB	Peripheral Blood
PI	Principal Investigator
PIC	Participant Identification Centre
PIS	Participant Information Sheet
QA	Quality Assurance
QL (QoL)	Quality of Life
R&D	Research and Development
RCT	Randomised Controlled Trial
REC	Research Ethics Committee
RIC	Reduced Intensity Conditioning
RNA	Ribonucleic Acid
RT-qPCR	Reverse Transcriptase Quantitative Polymerase Chain Reaction
SAE	Serious Adverse Event
SAR	Serious Adverse Reaction
SCT	Stem Cell Transplant

SmPC	Summary Product Characteristics
SOP	Standard Operating Procedure
SUSAR	Suspected Unexpected Serious Adverse Reactions
TKD	Tyrosine kinase domain
TMF	Trial Master File
TMG	Trial Management Group
TSC	Trial Steering Committee
WBC	White blood cell counts
WHO	World Health Organisation
WOCBP	Woman of Child Bearing Potential
UK	United Kingdom
UKCRC	United Kingdom Clinical Research Collaboration
ULN	Upper Limit of Normal

Amendment History

The following amendments and/or administrative changes have been made to this protocol since the implementation of the first approved version.

Amendment No.	Protocol version no.	Date issued	Summary of changes made since previous version
	1.3	4 Feb 2025	Minor typographical errors were updated in: • Study Arm A • Treatment durations were updated in Treatment Schedule of Events in Appendix A
	2.0		Inclusion criteria updated to require ALT and/OR AST, whereas previously both were required. Clarifications were made to the dosing schedules. Patients updated to participants where appropriate. Administrative corrections. Clarification added for situations when PIS2 is used, and recording missed doses.

1 Synopsis

Short title	Optimising Therapy in *FLT3*-mutated Acute Myeloid Leukaemia
Acronym	Optimise-FLT3
Clinical phase	Phase II/III
Funder and ref.	Cancer Research UK
Trial design	Optimise-FLT3 is a phase II/III randomised three-arm, multi-stage, controlled trial comparing two experimental regimens, DA-GO-Mido and FLAG-Ida-GO-Mido, against the current standard of care, DA-Mido.
Trial participants	Adults with newly diagnosed *FLT3*-mutated AML who are considered suitable for intensive therapy with curative intent
Planned sample size	390
Planned number of sites	80 sites, with additional 8-10 sites internationally
Inclusion criteria	1) Diagnosis of AML 2) Age ≥16yrs (no specified upper age limit) 3) Considered fit for intensive AML therapy by the treating physician 4) Confirmed *FLT3* ITD or TKD mutation (or *FLT3* status unknown but requires urgent therapy - see below*) 5) Serum creatinine less than or equal to 1.5 x ULN (upper limit of normal) 6) Serum Alanine Aminotransferase (ALT) and/ OR Aspartate Aminotransferase (AST) less than or equal to 2.5 x ULN and bilirubin less than or equal to 2 x ULN 7) A negative pregnancy test within 2 weeks prior to trial entry in WOCBP (to be repeated throughout the trial prior to each course of protocol treatment and at the end of consolidation and maintenance). 8) Sexually mature males and females must agree to use an adequate and medically accepted method of contraception throughout the study and for 6 months following treatment if they or their sexual partners are women of childbearing potential (WOCBP) 9) WHO/ECOG performance status 0-2 10) Written informed consent **FLT3*-mutated AML is associated with proliferative disease features such as hyperleukocytosis (high white blood cell count) and may present as a medical emergency. It is important that the full clinical spectrum of *FLT3*-mutated AML is represented in Optimise-FLT3, including hyper-proliferative cases. Should the treating physician feel that the safety of an individual participant could be compromised by delaying therapy while awaiting *FLT3* genotyping, they may, on discussion with the study team, proceed with study entry (using PIS2), randomisation and treatment provided all other eligibility criteria are met. Any participants who enter the trial and are subsequently found to have wild type *FLT3* will still be considered evaluable for safety/toxicity analysis but will be replaced with additional FLT3-mutated cases in order to maintain statistical power for the clinical efficacy endpoints and equipoise between the trial arms. Once *FLT3*-mutated AML is confirmed, participants consented using PIS2 do not need to be re-consented with PIS1.

Exclusion criteria	• Receipt of any previous therapy for AML or any antecedent haematological condition (the use of oral hydroxycarbamide to control white blood cell count is permitted) • Other active malignancy requiring treatment • Patients who are pregnant or lactating • Uncontrolled infection with Human Immunodeficiency Virus (HIV) or Hepatitis B or C. Patients with known chronic infections who are receiving or have completed therapy and have recent documented negative viral PCR tests are not excluded • Blast transformation of chronic myeloid leukaemia (CML) • Contraindications to any of the IMP as per the SmPC
Treatment duration	Recruitment is expected to last 3 years 8 months
Follow-up duration	Participants will be followed up annually until the trials unit notifies otherwise. It is anticipated the required number of events will be achieved after the last participant enrolled has been in follow-up for 2 years however, depending on event rate, this may continue beyond 2 years.
Planned trial period	7 years
Primary objective	• To compare Event Free Survival (EFS) between trial arms
Secondary objectives	To compare the following between trial arms: • Incidence of complete remission (CR, CRh and CRi in ELN2022) within 2 courses • Death within 30 and 60 days from randomisation • Overall survival time • Time to haematological relapse • Incidence of MRD negativity after cycle 2 by RT-qPCR (for *NPM1*^{mut}) or flow cytometry • MRD levels after cycle 1 and 2. • Time to MRD relapse • Cumulative incidence of grade 3 and 4 toxicity • Cumulative resource use including hospital admission days, blood product usage and days on intravenous antibiotics and antifungals • Rate and timing of allogeneic stem cell transplant • Health related quality of life
Tertiary/Exploratory objectives	The number and percentage of participants who have bone marrow *FLT3* ITD NGS MRD negativity after two cycles of therapy
Primary outcomes	• Event-free survival (EFS) time

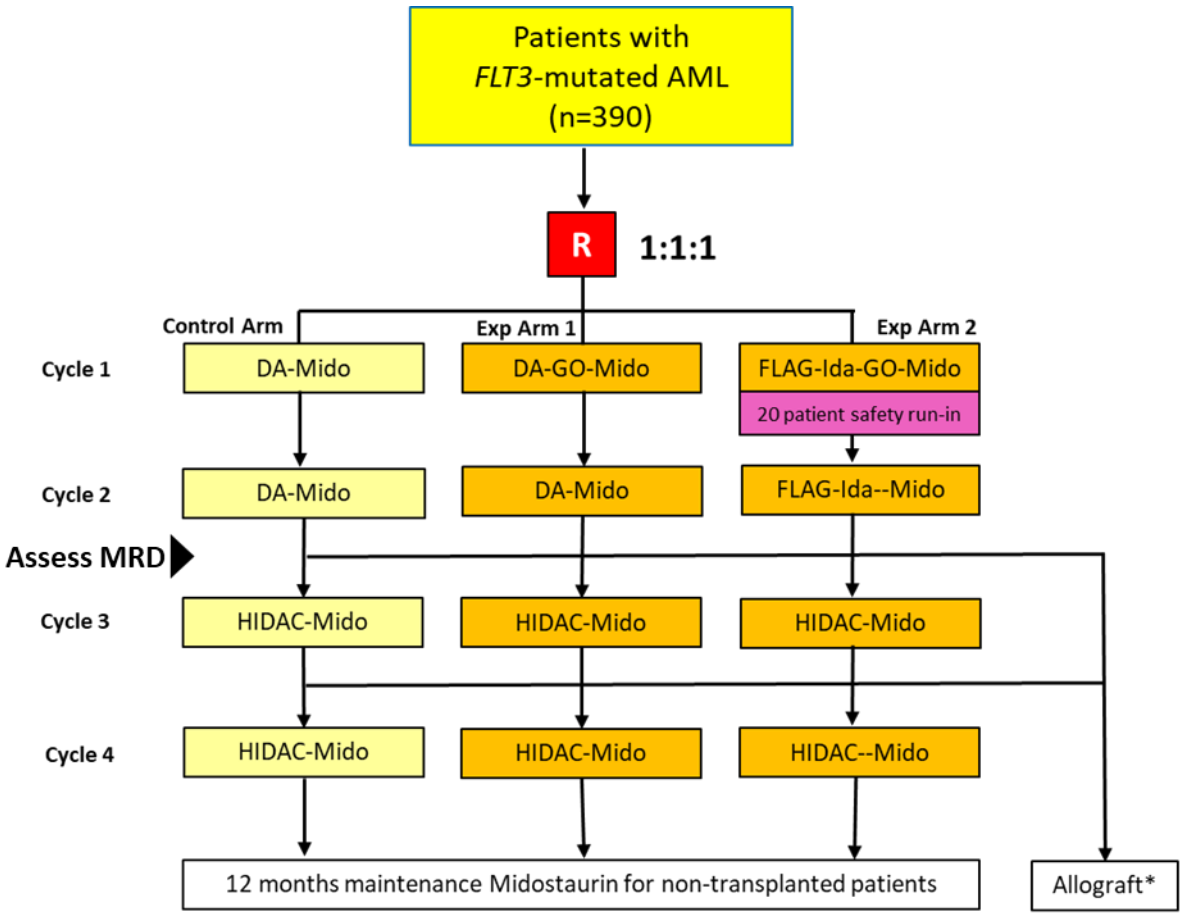
	Specified EFS events will include: • death from any cause • failure to achieve CR, CRh or CRi after two chemotherapy cycles • MRD relapse, as defined by the European Leukaemia Network[23]. • frank relapse Participants who are event free at the end of follow-up will be censored at the date of the most recent documented blood or bone marrow test that shows parameters consistent with ongoing disease response.
Secondary outcomes	• Incidence of complete remission (CR, CRh and CRi by ELN2022) within 2 cycles • Number of deaths within 30 and 60 days from randomisation • Overall survival time, measured in days from the date of randomisation until the date of death from any cause. Participants still alive at the end of follow-up will be censored at the date last seen in clinic (telephone confirmation will be acceptable). • Time to haematological relapse, measured from the date of documentation of 1st CR, CRi or CRh until the date of frank relapse. Participants who have not relapsed at the end of follow-up will be censored at the date of last documented blood or bone marrow test that shows parameters consistent with ongoing disease response. • The number and percentage of participants with MRD negativity after cycle 2 by RT-qPCR (for NPM1^{mut}) or flow cytometry • Time to MRD relapse for participants with a monitored MRD marker, measured from the date of first molecular complete remission, until the date of MRD relapse (as defined by the ELN2022 [23]). Participants who are MRD negative will be censored at the date of last MRD assessment. • Cumulative incidence of grade 3 and 4 toxicity over the duration of follow-up. The worst grade of toxicity will be reported. • Cumulative resource use including hospital admission days, blood product usage and days on intravenous antibiotics and antifungals • Rate and timing of allogeneic stem cell transplant Health related quality of life assessed during treatment and over 2 years of post-treatment follow-up
Tertiary/Exploratory outcomes	The number and percentage of participants who have bone marrow *FLT3* ITD NGS MRD negativity after two cycles of therapy
	Study arm 1: DA + Midostaurin Therapy will consist of:

Treatment Schedules	Course 1: DA60 3+10 + Midostaurin. Daunorubicin IV 60mg/m^2 OD on D1,3,5. Cytarabine IV 100mg/m^2 BD on D1-10 Midostaurin PO 50mg BD on D11-24 Course 2: DA50 3+8 + Midostaurin. Daunorubicin IV 50mg/m^2 OD on D1,3,5. Cytarabine IV 100mg/m^2 y BD on D1-8 Midostaurin PO 50mg BD on D9-22 Courses 3-4: Cytarabine IV 3g/m^2 BD on D1,3,5 (1.5g/m^2 BD on D1,3,5 in participants 60-69yrs, 1g/m^2 OD on D1-5 in participants aged ≥70yrs)) Midostaurin PO 50mg BD on D6-19 **Study arm 2: DA + GO + Midostaurin** Therapy will consist of: Course 1: DA60 3+10 + GO + Midostaurin. Daunorubicin IV 60mg/m^2 OD on D1,3,5. Cytarabine IV 100mg/m^2 BD on D1-10 Gemtuzumab Ozogamicin (GO) IV 3mg/m^2 (capped at the contents of one 5 mg vial) on D1 + 4, delayed to D4 + 7 if WBC ≥ 30x10^9/L Midostaurin PO 50mg BD on D11-24 Course 2: DA50 3+8 + Midostaurin. Daunorubicin IV 50mg/m^2 OD on D1,3,5. Cytarabine IV 100mg/m^2 BD on D1-8 Midostaurin PO 50mg BD on D9-22 Courses 3-4: Cytarabine IV 3g/m^2 BD on D1,3,5 (1.5g/m^2 BD on D1,3,5in participants 60-69yrs, 1g/m^2 OD on D1-5 in participants aged ≥70yrs)) Midostaurin PO 50mg BD on D6-19 **Study arm 3: FLAG-Ida + GO + Midostaurin** Therapy will consist of: Course 1: FLAG-Ida + GO + Midostaurin Fludarabine IV 30mg/m^2 OD on D2-6. Cytarabine IV 2g/m^2 OD on D2-6 (1g/m^2 in participants ≥60yrs) G-CSF S/C 263µg (or equivalent 300µg) OD on D1-7. Idarubicin IV 8mg/m^2 OD on D4-6. Gemtuzumab Ozogamicin IV 3mg/m^2 OD on D2 (capped at the contents of one 5mg vial and delayed to D5 if WBC ≥ 30x10^9/l). Midostaurin PO 50mg BD on D7-20. Course 2: FLAG-Ida + Midostaurin Fludarabine IV 30mg/m^2 OD on D2-6.

	Cytarabine IV 2g/m^2 OD on D2-6 (1g/m^2 in participants ≥60yrs) G-CSF S/C 263µg (or equivalent 300µg) OD on D1-7. Idarubicin IV 8mg/m^2 OD on D4-5. Midostaurin PO 50mg BD on D7-20. Course 3-4: Cytarabine IV 3g/m^2 BD on D1,3,5 (1.5g/m^2 BD on D1,3,5 in participants 60-69yrs, 1g/m^2 OD on D1-5 in participants aged ≥70yrs) Midostaurin PO 50mg BD on D6-19 Maintenance On completion of chemotherapy, all participants who are not proceeding to allogeneic stem cell transplant in first remission will receive maintenance Midostaurin PO 50mg twice daily, for 12 x 28-day cycles.

3 Trial summary & schema

3.1 Trial schema



24 months follow-up, including 3-monthly bone marrow qPCR MRD assessment for patients with *FLT3+/NPM1+*

***Allogeneic haematopoietic stem cell transplant in 1st complete remission**

Recommended for:
- *FLT3+/NPM1+* patients with PB NPM1 qPCR+ post cycle 2
- *FLT3+/NPM1-* patients with baseline *FLT3* mutation level of ≥ 5%
- *NPM1-* patients allocated to flow MRD monitoring who are flow MRD positive (>0.1%) by central testing
- Patients with ELN 2022 adverse risk disease or NUP98 rearrangement.

Generally:
- Myeloablative conditioning following cycle 2 where practical for patients ≤40-45yrs
- Reduced intensity conditioning (RIC) following cycle 3 for patients aged ≥40-45yrs

3.2 Trial lay summary

Acute myeloid leukaemia (AML) is an aggressive blood cancer and is the commonest form of acute leukaemia in adults, affecting more than 3000 people per year in the UK, the majority of who will die from the disease. Younger and fitter participants can have treatment aiming to cure the disease with cycles of intensive chemotherapy followed for some participants by stem cell transplantation (also

called bone marrow transplantation). In recent years, survival rates have gradually increased following improvements to chemotherapy, transplantation, better general care measures and the addition of various new targeted drugs for patients in specific AML sub-groups.

This study focusses on a subgroup of AML with mutations in the *FLT3* gene. These mutations are found in about one-third of AML patients. These patients have worse overall outcomes due to increased rates of early disease relapse. Clinical trials in recent years have identified several promising strategies to improve outcomes for patients with *FLT3* AML including 1) using an intensified 3-drug chemotherapy protocol called FLAG-Ida 2) adding a drug called midostaurin to inactivate FLT3 and 3) adding a chemotherapy linked-antibody called Gemtuzumab Ozogamicin (also called GO or Mylotarg) but these approaches have not yet been combined in a single trial.

In the Optimise-FLT3 trial we want to establish the best way to treat *FLT3*-AML. Currently the standard treatment is standard intensity chemotherapy (called DA) combined with midostaurin (the FLT3 inactivating drug). We will compare this standard treatment with two new combinations. One is standard intensity chemotherapy combined both midostaurin and GO; this combination has already undergone pilot testing in the AML19 study and was safe and appeared very effective. The second new combination is intensified chemotherapy (FLAG-Ida) combined with midostaurin and GO. Pilot testing of the safety of this combination is built into this study.

Over 4 years, 390 newly diagnosed patients at hospitals around the UK and in partner countries who decide to enrol in the study (after reading written information from and having a discussion with their local haematology teams) will be allocated, at random, to receive one of the three treatment schedules. They will receive up to 4 cycles of intensive chemotherapy treatment, including stem cell transplant in selected cases; during this time the response of the leukaemia to treatment will be measured by standard tests of the blood and bone marrow to see whether patient outcomes are improved in terms of increased survival, reduction in rates of relapse and reduction in the need for stem cell transplant. At the same time, we will monitor for any increased toxicity (side effects) associated with the intensified treatment schedules.

Results of the trial will be provided to participating participants and their families, presented at international conferences and published in scientific journals. If the trial finds that one of the treatment arms is clearly superior to the others, these results will be used to engage with health bodies to make the treatment with the best results available for future patients across the NHS.

4 Background to Optimise-FLT3

4.1 Justification of treatment options

Acute myeloid leukaemia – intensive therapy

Acute myeloid leukaemia (AML) is an aggressive haematological malignancy diagnosed in 3-4,000 individuals in the UK each year, a majority of whom continue to die from the disease[1]. In fitter patients treated with curative intent, the mainstay of treatment remains intensive chemotherapy followed, in selected cases, by allogeneic stem cell transplantation (SCT)[2,3]. This is associated with substantial

short- and long-term morbidity, mortality and impact on quality of life, placing a disproportionately large burden on healthcare services, with estimated financial costs between £100,000-£400,000 per patient[4-7].

In recent years substantial progress has been made via a series of large randomised UK-based studies (NCRI AML15 to AML19) which have evaluated changes to chemotherapy schedules and the addition of novel targeted therapies, leading to incremental improvements in patient outcomes[8]. Several recently approved therapies have demonstrated efficacy that is restricted to cytogenetic or molecular subgroups of patients, meaning that approved treatment algorithms have become genetically stratified and increasingly complex[2]. Consequently, a single randomised study, with common control arm, for all patients with newly diagnosed AML is no longer considered viable.

Optimising frontline therapy for patients with *FLT3*-mutated AML

Optimise-FLT3 will concentrate exclusively on the large subgroup of patients with activating somatic mutations in the gene encoding the FLT3 receptor tyrosine kinase (*FLT3*) where considerable unmet need remains. These mutations are present in one-third of AML patients[9-11] and are associated with a high risk of early relapse, occurring in 40-50% of intensively treated patients[12]. In recent years, separate studies have identified beneficial therapeutic strategies in this group, namely: 1) intensification of the chemotherapy backbone, 2) addition of FLT3 kinase inhibition and 3) incorporation of the anti-CD33 immunoconjugate Gemtuzumab Ozogamicin (GO). These approaches (detailed individually below) have not yet been optimally combined. Optimise-FLT3 seeks to improve clinical outcomes by defining the best combination of these strategies, while simultaneously evaluating the clinical utility of highly sensitive and specific next generation sequencing (NGS) based methods to track the *FLT3* mutation during treatment for detection of measurable residual disease (MRD).

1. **Intensification of the chemotherapy backbone**

The NCRI AML17 study previously demonstrated that dose intensification of daunorubicin (90mg/m^2 vs 60mg/m^2) within the standard daunorubicin-cytarabine (DA) induction chemotherapy schedule was effective only in patients with a *FLT3* mutation (*FLT3*^{mut})[13]. The first part of the recent NCRI AML19 study (funded by CRUK) compared DAG0 with an intensified induction chemotherapy schedule (FLAG-Ida), each combined with GO. Although there was no difference in overall survival (OS) in the whole cohort, in patients with *FLT3*^{mut}, the intensified schedule (FLAG-Ida-GO) improved 3-year overall survival by 10% from 54% to 64% **(Fig.1)** compared with DA-GO. Cumulative incidence of relapse (CIR) was reduced from 44% to 28%. No FLT3 inhibitors were used. No increase was seen in day 30 or day 60 mortality with FLAG-Ida-GO[15]**.**

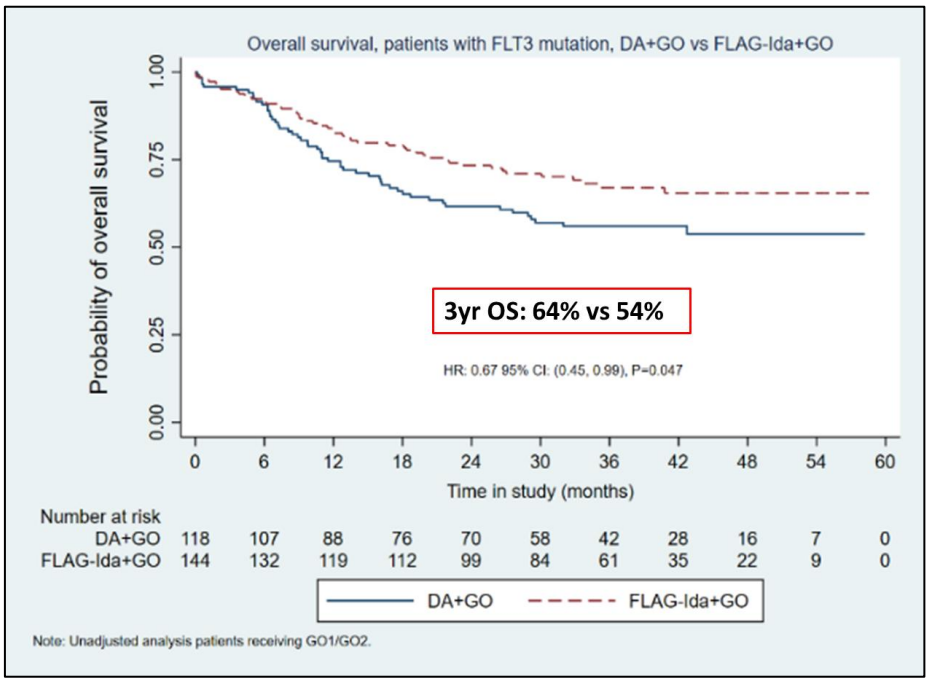


Figure 1. Intensified induction chemotherapy with FLAG-Ida-GO resulted in improved overall survival in the UK NCRI AML19 study, in the absence of FLT3 inhibitors.

Approximately half of *FLT3*^{mut} patients also harbour mutations in the gene encoding nucleophosmin (*NPM1*). In AML19, these patients with co-mutated *FLT3* and *NPM1* had a particularly marked benefit from FLAG-Ida-GO (hazard ratio, HR, for death 0.32, **Fig. 2**).

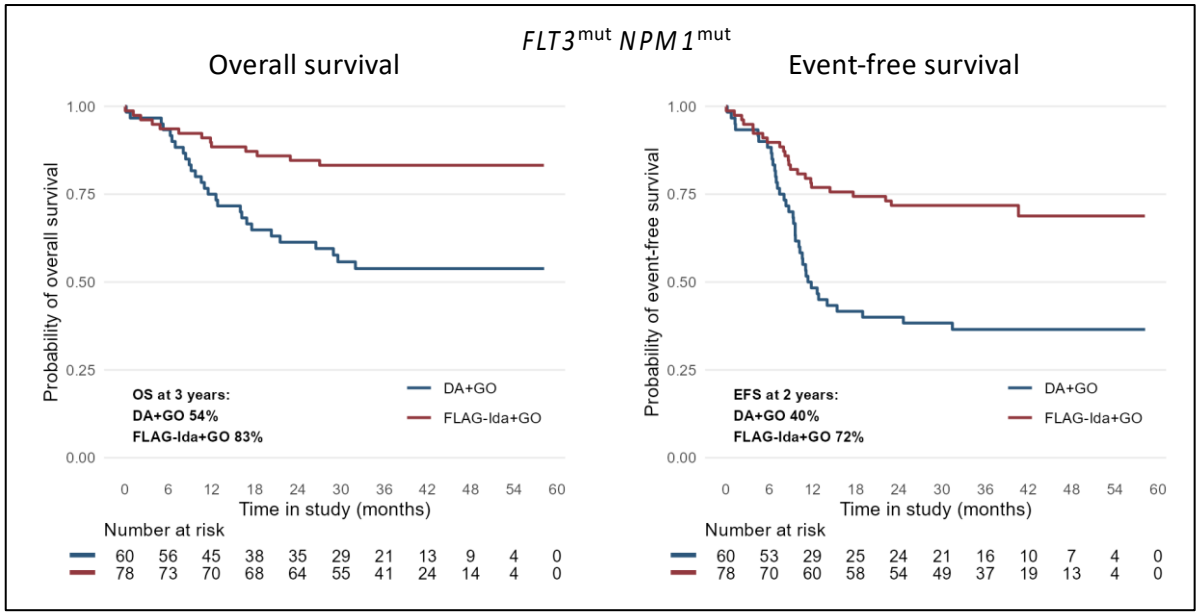


Figure 2. Overall (left panel) and event-free survival (right panel) in AML19 according to chemotherapy backbone for patients with both *NPM1* and *FLT3* mutations.

Additionally, *NPM1* mutation is a well-established leukaemia-specific target for monitoring MRD using reverse-transcription quantitative PCR (RT-qPCR). The NCRI AML17 study demonstrated that patients achieving *NPM1* MRD negativity in the peripheral blood (PB) after 2 cycles of induction chemotherapy (PB PC2 MRD–) had a greatly reduced risk of relapse and death, this outweighing traditional clinical and molecular risk factors[15]. Consequently, *NPM1* MRD was established as a standard of care test in partnership with the NHS Genomic Laboratory Hubs and is now used routinely to identify patients who may safely avoid SCT in first complete remission (CR1), sparing significant risk of transplant related mortality, short- and long-term morbidity as well as avoiding large financial costs associated with transplant.

In AML19, patients treated with FLAG-Ida-GO had faster, deeper clearance of *NPM1* MRD than those receiving DA-GO **(Fig.3**) and there was a 12% increase (from 76 to 88%) in patients testing PB PC2 MRD negative, thus reducing requirement for allogeneic SCT in CR1 by half (from 24% to 12%). The improvement in MRD negativity was greater in patients with *FLT3* co-mutation (15% increase from 68% to 83%) again consistent with an ~50% reduction in requirement for SCT in this group.

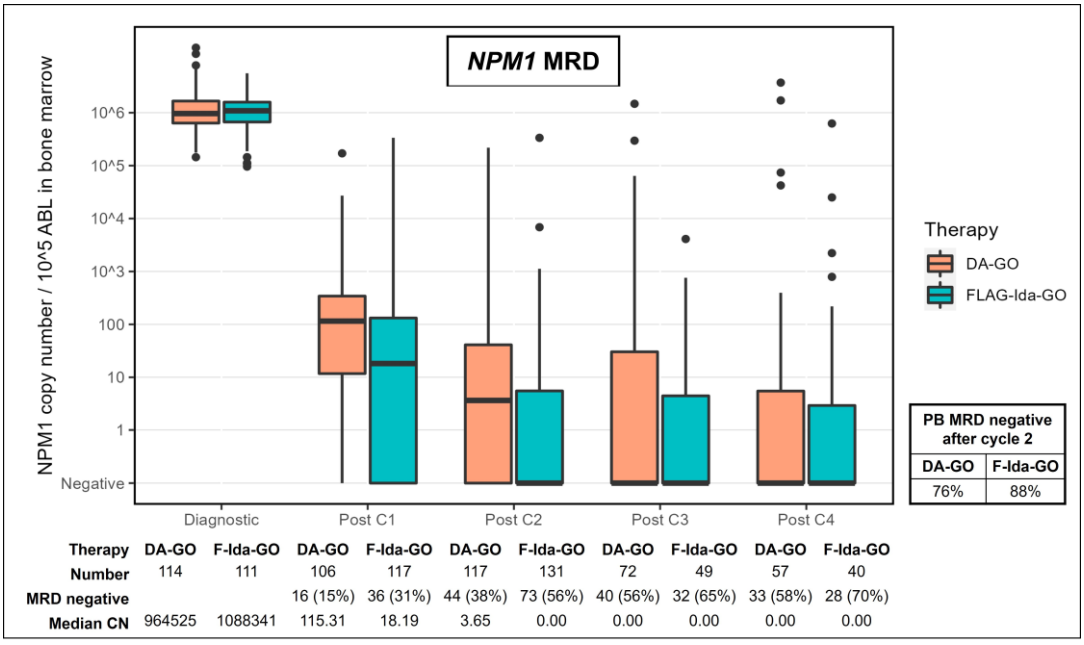


Figure 3. *NPM1* MRD clearance in NCRI AML19 according to treatment (DA-GO vs FLAG-Ida-GO).

Importantly, benefits from FLAG-Ida-GO were seen both in patients testing PB PC2 MRD positive *and* negative, indicating that although half as many patients received a transplant, their risk of death was nevertheless reduced by more than half compared to patients receiving DA-GO **(Fig.4).**

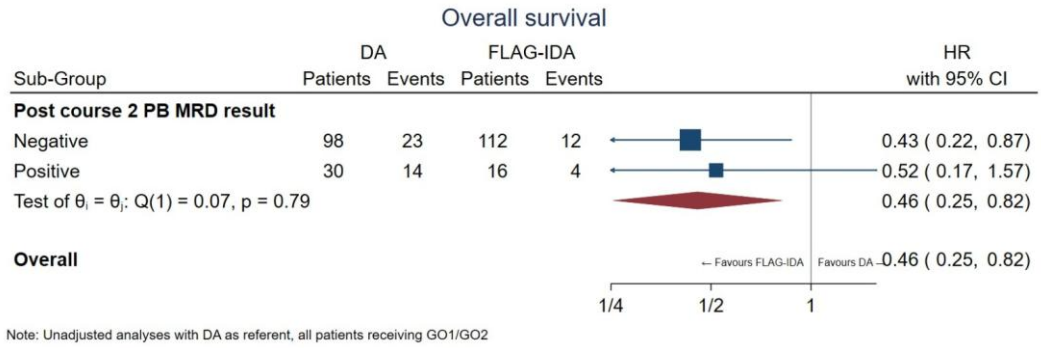


Figure 4. Forest plot demonstrating improved survival in NCRI AML19 participants testing PB PC2 MRD– despite a reduction in the number of stem cell transplants performed.

2. Addition of FLT3 kinase inhibition

The RATIFY study evaluated the addition of the oral kinase inhibitor midostaurin to standard induction chemotherapy (DA, without GO) in participants with newly diagnosed *FLT3*^{mut} AML. The addition of midostaurin increased 3yr OS by 7% (from 47% to 54%), 5yr OS was increased by the same amount (from 43% to 50%)[16]. 3yr cumulative incidence of relapse (CIR) was reduced by 10% (from 55% to 45%)[12]. Based on these results, midostaurin is now approved for routine use in the NHS for patients with *FLT3*^{mut} AML outside the setting of clinical trials but cannot yet be permissibly combined either with intensified chemotherapy schedules such as FLAG-Ida or given together with GO in routine treatment. DA with midostaurin (DA-Mido) currently represents 'standard of care' therapy for *FLT3*^{mut} AML in the NHS and beyond[2].

3. Addition of Gemtuzumab Ozogamicin (GO)

Subgroup analyses from two randomised studies evaluating the addition of GO to standard intensity induction chemotherapy performed by the French ALFA group[17] and the US Children's Oncology Group[18] suggested that patients with *FLT3*^{mut} AML benefit from GO. This is also supported by the comparable 3yr OS rates in patients receiving DA-GO without midostaurin in AML19 (54%)[14] and those receiving DA-Mido in RATIFY (54%)[16]. Combining midostaurin and GO is thus also an attractive strategy to increase survival rates and warrants further investigation, although we note that a benefit of GO in patients with *FLT3* mutation was not observed in a subgroup analysis of the AMLSG 1610 study[19], which used an intensified chemotherapy backbone. Relatively few patients with *FLT3* mutation were enrolled in that study, however, underlining the need for a formal randomised comparison in this subgroup.

To investigate the safety and preliminary efficacy of combining intensive chemotherapy with both midostaurin and GO, the latter part of AML19 included a single-arm pilot of the triplet combination

DA-GO-Mido (termed 'Midotarg'). A total of 77 participants were treated. The combination was well tolerated, with no 60-day mortality, no excess toxicity and an encouraging overall response rate (complete remission with or without count recovery, CR+ CRi) of 88% after one cycle[20]. The safety of the DA-GO-Mido combination is also supported by two other small studies, performed by the German Study Alliance Leukaemia (SAL) co-operative group[21] (n=11 reported) and a US co-operative group[22] (n=8 reported), both confirming the safety and feasibility of delivering the triplet; there were no treatment-related deaths in the first 30 days in either study. In AML19, for the subset of participants with *NPM1* mutation, greater clearance of MRD was observed in a non-randomised comparison of DA-GO-Mido with *FLT3*^{mut} participants who had received DA-GO (without Mido) in the earlier stages of AML19 **(Fig. 5).** The number of participants testing PB PC2 MRD– was increased by 12% (from 68% to 80%) again implying a substantial reduction in the proportion of participants requiring allogeneic SCT in first remission[20].

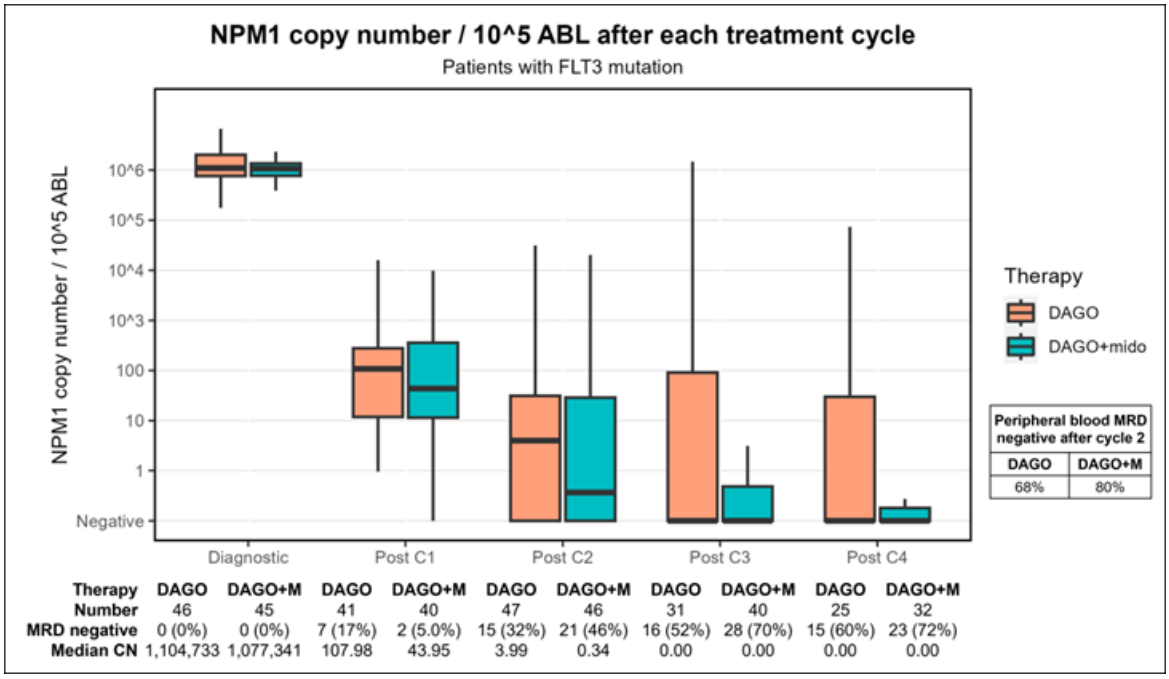


Figure 5. *NPM1* MRD clearance in AML19 participants receiving the DA-GO -Mido, compared with historical *FLT3* mutated participants treated with DA-GO earlier in the trial

4.2 Clinical aims of Optimise-FLT3

Optimise-FLT3 will build on the AML19 'Midotarg' pilot findings through a randomised efficacy comparison of DA-Mido (current standard of care) with DA-GO-Mido. Given the significantly improved outcomes seen in AML19 for *FLT3*^{mut} participants receiving intensified induction however, FLAG-Ida-GO may potentially represent the new standard of care chemotherapy backbone for *FLT3*^{mut} AML. No group has yet investigated the combination of FLAG-Ida-GO with midostaurin which will form the second experimental arm of Optimise-FLT3. The trial will include an internal safety review of the FLAG-

Ida-GO-Mido combination for the first 20 participants randomised to this arm; if safety is confirmed, 3-way efficacy randomisation between DA-Mido, DA-GO-Mido and FLAG-Ida-GO-Mido will continue.

4.3 Exploratory laboratory aims of Optimise-FLT3: Ultrasensitive *FLT3*-NGS for MRD monitoring

Ultrasensitive *FLT3*-NGS for MRD monitoring

Until recently, technical and biological issues have precluded the use of *FLT3* mutations as targets for molecular MRD monitoring; current guidelines recommend monitoring co-occurring molecular lesions, principally mutations of *NPM1*[23]. These, together with other fusion genes that have been validated for MRD monitoring within previous NCRI AML studies, are present in ~70% of patients with *FLT3*-mutated AML **(Fig.6**). In Optimise-FLT3, these previously established PCR markers together with flow cytometric MRD (allowing cover of the remaining 30% of patents who lack a molecular marker) will be used for routine monitoring and clinical decision making. However, because of preliminary evidence that monitoring the *FLT3* mutation itself using ultrasensitive NGS techniques is technically feasible and may provide additive prognostic information we will, in parallel, evaluate the utility of *FLT3* NGS MRD to better stratify patients and serve as an early efficacy endpoint.

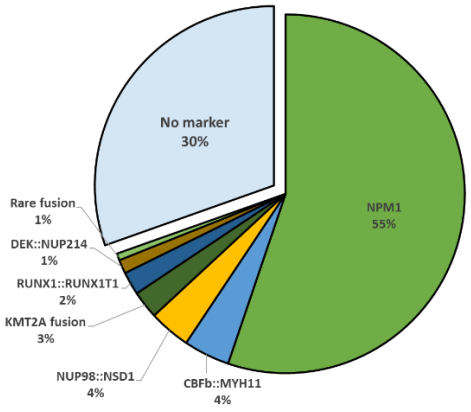


Figure 6. Molecular lesions suitable for MRD monitoring were identified in 70% of patients with a *FLT3* mutation in AML19 (n=481). Unpublished data (Dillon).

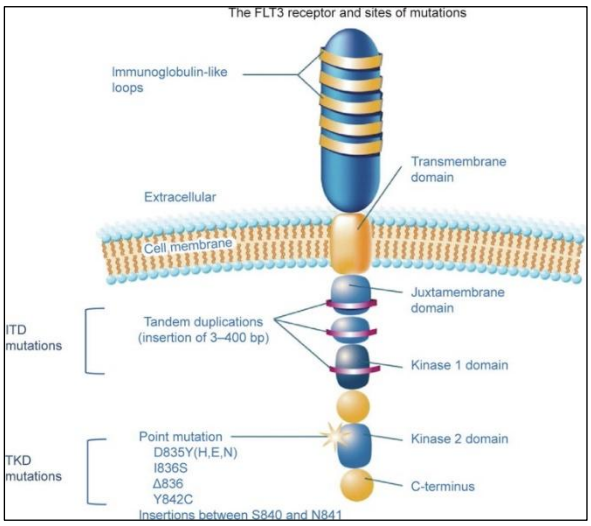


Figure 7. Schematic depiction of the FLT3 receptor showing regions affected by Internal Tandem Duplication (ITD) and Tyrosine Kinase Domain (TKD) mutations. Figure from Pemmaraju et al[24]

The two commonest types of *FLT3* mutation are internal tandem duplications (ITD) most often in the juxtamembrane domain and point mutations in the tyrosine kinase domain (TKD) (**Fig.7**). ITD mutations are more frequent, accounting for ~80% of *FLT3* mutations, and these associate with a poorer prognosis[25,26]. While it is relatively straightforward to detect TKD mutations with high sensitivity using NGS or digital PCR, until recently it was difficult to detect ITD mutations at very low levels. Advances in NGS technology and bioinformatics now make it feasible to detect *FLT3* ITD with very high specificity and sensitivity (up to $1:10^{-5}$)[27] allowing this to be used for MRD monitoring. Recently published retrospective studies applying these techniques to samples taken after chemotherapy[28,29] or before SCT[30,31] demonstrate that *FLT3* ITD NGS MRD positivity is highly predictive of relapse. These studies also show that *FLT3* ITD NGS MRD appears to provide additional prognostic information to that provided by both *NPM1* and flow cytometric MRD. This assay therefore has the potential to better stratify patients, for example with regards to selection for SCT in CR1, but it currently remains unclear how this information should be incorporated into treatment algorithms. Current practice for patients with *FLT3* mutated AML in the UK is informed by the baseline molecular profile and MRD response. For patients with an *NPM1* co-mutation, transplant decision is based on the post cycle 2 peripheral blood *NPM1* MRD status assessed by RT-qPCR; those testing MRD negative receive consolidation chemotherapy only. However, ~30% of these patients subsequently relapse[16] and *FLT3* ITD NGS MRD may be able to better identify those at increased risk. For patients lacking an *NPM1* co-mutation, those with a baseline *FLT3* ITD with an allelic ratio >0.05 are currently routinely referred for CR1 transplantation if eligible but a subset of these patients who do not ultimately go on to receive a transplant still become long-term survivors. *FLT3* ITD NGS MRD may therefore identify a subgroup of patients within the $NPM1^{neg}FLT3^{ITD+}$ genotype who are at low risk of relapse and may not require upfront transplant.

At this relatively early stage it remains unclear whether *FLT3* ITD NGS MRD will, in future, be used alongside existing MRD techniques to add precision, or whether it can replace them altogether for the purposes of risk stratification, potentially simplifying MRD testing algorithms and allowing greater international standardisation. Optimise-FLT3 will provide an ideal platform to prospectively and comprehensively evaluate these issues.

4.4 Potential clinical impacts of Optimise-FLT3

Improved survival We anticipate that both DA-GO-Mido and FLAG-Ida-GO-Mido will improve outcomes compared to the current standard of care (DA-Mido) in terms of OS and EFS. If the 7% absolute difference in OS seen in RATIFY is reproduced in Optimise-FLT3, we anticipate a 5yr OS of approximately 70% with FLAG-Ida-GO-Mido and 60% with DA-GO-Mido compared with 50% with DA-Mido. Such a finding could lead to changes in national and international treatment algorithms for $FLT3^{mut}$ AML. Applying a conservative estimate of 500 patients per year in the UK with $FLT3^{mut}$ AML fit enough to receive FLAG-Ida-GO-Mido, this would translate to 100 fewer leukaemia deaths per year. It is important to acknowledge that increased toxicity of FLAG-Ida-GO-Mido *could* offset some of the efficacy gains, reducing differences in OS between FLAG-Ida-GO-Mido and DA-GO-Mido. If this were the case, DA-GO-Mido could potentially replace DA-Mido as the new standard of care; in this situation an improvement in 5yr OS to 60% would translate into approximately 50 fewer leukaemia related deaths per year in the UK.

Reduced requirement for stem cell transplantation Patients with *NPM1* co-mutation (present in just over half of *FLT3*^{mut} patients) are currently selected for SCT in first remission based on their *NPM1* PB PC2 MRD results. In AML19, FLAG-Ida-GO reduced PC2 MRD positivity by 15% (compared with DA-GO) and the addition of Mido to DA-GO reduced MRD positivity by 12% (compared with DA-GO only). We conservatively estimate that FLAG-Ida-GO-Mido will reduce MRD positivity by 18% (from 30% to 12%) compared to the current standard of care. If confirmed, UK transplant numbers could be reduced by ~45/year, sparing substantial morbidity and mortality risk to patients and saving ~£5m/year. Our exploratory *FLT3* ITD MRD assay may serve to highlight further sub-groups of patients who might safely avoid first remission SCT in future.

Reduced relapse Incidence of relapse of *FLT3*^{mut} AML with the current standard of care DA-Mido remains high (3y CIR 45%[12]), and patients with relapsed disease continue to have a very poor outlook despite recent approval of the second generation FLT3 inhibitor Gilteritinib, based on results of the ADMIRAL study[32]. Second-line response rates remain poor (CR + CRi 34%) and most patients die soon after relapse (2y OS 19%). Patients not achieving second remission experience inferior quality of life (transfusion dependency and recurrent infections) and the treatment is very expensive (>£170,256 per year in drug costs alone*). In AML19, FLAG-Ida-GO reduced the CIR from 44% to 28% [15]; although CIR for DA+GO-Mido is not yet established we expect that both experimental combinations will substantially reduce relapse risk.

5 Trial objectives/endpoints and outcome measures

The AML outcome measures proposed by the European Leukaemia Network (2022) will be used[2].

5.1 Primary objective

- To compare Event Free Survival (EFS) between trial arms

5.2 Secondary objectives

To compare the following between trial arms:

- Incidence of complete remission (CR, CRh and CRi in ELN2022) within 2 courses
- Death within 30 and 60 days from randomisation
- Overall survival time
- Time to haematological relapse
- Incidence of MRD negativity after cycle 2 by RT-qPCR (for *NPM1*^{mut}) or flow cytometry
- MRD levels after cycle 1 and 2
- Time to MRD relapse
- Cumulative incidence of grade 3 and 4 toxicity
- Cumulative resource use including hospital admission days, blood product usage and days on intravenous antibiotics and antifungals
- Rate and timing of allogeneic stem cell transplant
- Health related quality of life

5.3 Primary outcome measure(s)

- Event-free survival (EFS) time

Specified EFS events will include:

- death from any cause
- failure to achieve CR, CRh or CRi after two chemotherapy cycles
- MRD relapse, as defined by the European Leukaemia Network[23].
- frank relapse

Participants who are event free at the end of follow-up will be censored at the date of the most recent documented blood or bone marrow test that shows parameters consistent with ongoing disease response

5.4 Secondary outcome measure(s)

- Incidence of complete remission (CR, CRh and CRi by ELN2022) within 2 cycles
- Number of deaths within 30 and 60 days from randomisation
- Overall survival time, measured in days from the date of randomisation until the date of death. Participants still alive at the end of follow-up will be censored at the date last seen in clinic (telephone confirmation will be acceptable).
- Time to haematological relapse, measured from the date of documentation of 1st CR, CRi or CRh until the date of frank relapse. Participants who have not relapsed at the end of follow-up will be censored at the date of last documented blood or bone marrow test that shows parameters consistent with ongoing disease response.
- The number and percentage of participants with MRD negativity after cycle 2 by RT-qPCR (for NPM1^{mut}) or flow cytometry
- Time to MRD relapse for participants with a monitored MRD marker, measured from the date of first molecular complete remission, until the date of MRD relapse (as defined by the ELN2022 [23]). Participants who are MRD negative will be censored at the date of last MRD assessment.
- Cumulative incidence of grade 3 and 4 toxicity over the duration of follow-up. The worst grade of toxicity will be reported.
- Cumulative resource use including hospital admission days, blood product usage and days on intravenous antibiotics and antifungals
- Rates of allogeneic stem cell transplant

Health related quality of life assessed during treatment and over 2 years of post-treatment follow-up

5.5 Separate exploratory/translational objectives and endpoints

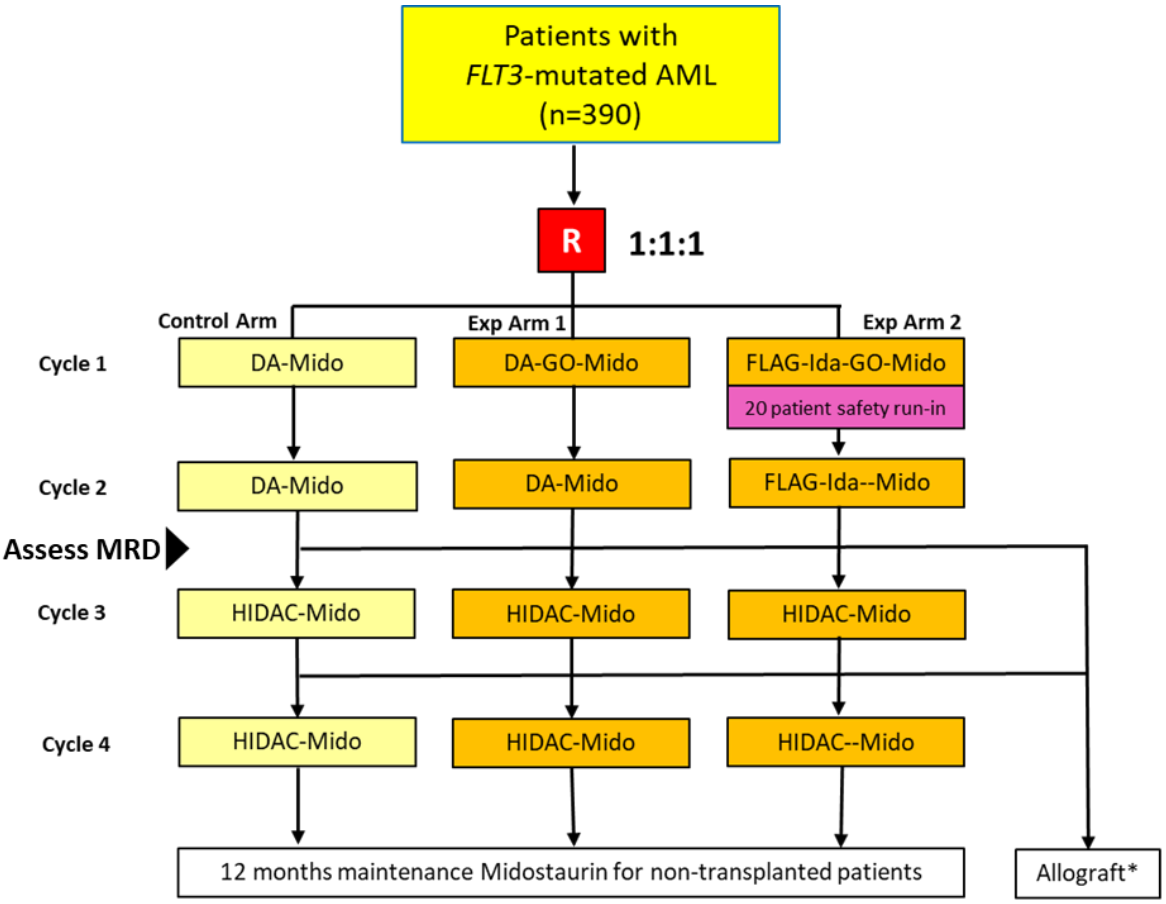
- The number and percentage of participants who have bone marrow *FLT3* ITD NGS MRD negativity after two cycles of therapy

6 Trial design and setting

Optimise-FLT3 is a phase II/III randomised three-arm, multi-stage, controlled trial comparing two experimental regimens, DA-GO-Mido and FLAG-Ida-GO-Mido, against the current standard of care, DA-Mido. The study incorporates an initial pilot phase for FLAG-Ida-GO-Mido with a safety review by the independent data monitoring committee (IDMC) planned after 10 and 20 participants have been treated. The study population is adults with newly diagnosed *FLT3*^{mut} AML who are considered suitable for intensive therapy with curative intent. Randomisation will be stratified according to *NPM1* co-mutation status, by subtype of *FLT3* mutation (TKD, ITD) and by age (below or above 60yrs) and may be further stratified if other important subgroups emerge during the course of the trial.

Stage 1 is the phase II part of the trial which will establish whether either of the experimental arms demonstrate an efficacy signal based on EFS with a target hazard ratio (HR) of 0.64. 236 participants will be recruited by the end of stage 1 at month 34 when interim analysis will determine whether both experimental arms should continue and whether either of the interventions should be restricted according to *NPM1* co-mutation status. Stage 2 is a phase III trial which will aim to confirm the efficacy signal in EFS with a target HR of 0.64. 390 participants will be recruited in total by the end of stage 2. It is expected that recruitment will take 3 years 8 months to complete. Following completion of scheduled treatment, participants will be followed up 6-monthly until 2 years after the final participant completes therapy.

At trial entry participants will be randomised (1:1:1) between the three treatment arms (**Fig. 8)**, balancing randomisation by *FLT3* mutation type, *NPM1* status and age. In each arm, participants will receive two cycles of induction chemotherapy and will then undergo MRD assessment (by RT-qPCR if *NPM1* is co-mutated or by flow cytometry if *NPM1* is unmutated). Participants testing MRD positive, and those with baseline *FLT3* ITD >5% with unmutated *NPM1* will be recommended for allogeneic haematopoietic stem cell transplant. The remaining participants will receive consolidation chemotherapy with two cycles of high dose cytarabine (HIDAC) and midostaurin, followed by maintenance treatment with midostaurin monotherapy for 12 months. The conditioning intensity and timing of allograft will be decided by the treating physician but those undergoing reduced intensity conditioning will be recommended to receive three cycles of chemotherapy before transplantation.



24 months follow-up, including 3-monthly bone marrow qPCR MRD assessment for patients with *FLT3+/NPM1+*

***Allogeneic haematopoietic stem cell transplant in 1st complete remission**

Recommended for:
- *FLT3+/NPM1+* patients with PB NPM1 qPCR+ post cycle 2
- *FLT3+/NPM1-* patients with baseline *FLT3* mutation level of ≥ 5%
- *NPM1-* patients allocated to flow MRD monitoring who are flow MRD positive (>0.1%) by central testing
- Patients with ELN 2022 adverse risk disease or NUP98 rearrangement.

Generally:
- Myeloablative conditioning following cycle 2 where practical for patients ≤40-45yrs
- Reduced intensity conditioning (RIC) following cycle 3 for patients aged ≥40-45yrs

Figure 8. Optimise-FLT3 Trial schema

As with previous frontline UK AML Working Group studies (AML15 through to 19) we plan to open Optimise-FLT3 at the majority of UK adult haematology centres that provide intensive AML therapy, approximately 80 sites; this includes regional allogeneic stem cell transplant sites and large / medium-sized district general hospitals. Both types of haematology centre are integral to the UK AML trials network and including them is necessary for this study to be representative of 'real world' practice. Optimise-FLT3 is also planned to open in Denmark and New Zealand; these countries have traditionally

recruited strongly to NCRI AML studies and will add a further 8-10 hospital sites, giving an anticipated total of around 90 sites internationally.

This document will be considered the master protocol and a group specific appendix will be drafted with non-UK centres which will incorporate country specific requirements.

6.1 Local investigator responsibilities

The investigator will ensure that data are recorded on the eCRFs. The Investigator ensures the accuracy, completeness, legibility, and timeliness of the data recorded and of the provision of answers to data queries according to the Clinical Study Agreement. Once data cleaning is complete and only at the end of the trial, data will be returned to sites in line with CTR SOP returning data to trial sites (SOP/007/12). A copy of the completed eCRFs will be archived at the study site in accordance with the CTR closure and archiving requirements. Safety concerns should be discussed with the trial team immediately upon occurrence or awareness to determine if the participant should continue or discontinue study treatment. All screening evaluations must be completed and reviewed to confirm that potential participants meet all eligibility criteria. The investigator will maintain a screening log to record details of all participants screened and to confirm eligibility or record reasons for screening failure, as applicable. Procedures conducted as part of the participant's routine clinical management (e.g. blood count and imaging assessments) and obtained before signing of the ICF may be utilised for screening or baseline purposes, provided the procedures met the protocol-specified criteria.

6.2 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 minimised/managed

This trial has been categorised as a Type C where the level of risk is /markedly 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 (see section 25.1).

This clinical trial is to be conducted in compliance with the protocol, the EU Clinical Trial Regulation 536/2014 and Good Clinical Practice.

7 Site and Investigator selection

This trial will be carried out at participating sites within the UK, Denmark and New Zealand. All sites who are interested in participating in the trial will be required to complete a registration form to confirm that they have adequate resources and experience to conduct the trial.

Before any site can begin recruitment a Principal Investigator at each site must be identified. The following documents must be in place and copies sent to the Optimise-FLT3 Trial email account (*optimise-flt3@cardiff.ac.uk*):

- Confirmation of Capacity and Capability (C&C) from R&D department following sharing of local information pack.
- Favourable opinion of host care organisation/PI from Main Ethics committee
- A signed Trial Agreement
- Current Curriculum Vitae and GCP training certificate of the Principal Investigator (PI)
- Completed Site Delegation Log and Roles and Responsibilities document
- Full contact details for all host care organisation personnel involved, indicating preferred contact
- A copy of the most recent approved version of the Participant Information Sheet(s) and Consent Form(s) on host care organisation headed paper
- A copy of the most recent approved GP letter on host care organisation headed paper
- A copy of the most recent Pregnancy Information Sheet(s) and Consent Form(s) on host care organisation headed paper
- A set of laboratory normal ranges and laboratory certification/accreditation from the host care organisation laboratory being used for analyses
- Signed Source Data Verification Form

Upon receipt of all the above documents, the Trial Manager will send written confirmation to the Principal Investigator detailing that the centre is now ready to recruit participants into the trial. This letter/email must be filed in each site's Site File. Along with the written confirmation, the site should receive a trial pack holding all the documents required to recruit into the Trial.

Occasionally during the trial, amendments may be made to the trial documentation listed above. CTR will issue the site with the latest version of the documents as soon as they become available. It is the responsibility of the CTR to ensure that they obtain local R&D approval for the new documents.

Site initiation will be by teleconference.

8 Participant selection

Participants are eligible for the trial if they meet all of the following inclusion criteria and none of the exclusion criteria apply. All queries about participant eligibility should be directed to the Trial Manager before randomisation/registration.

8.1 Inclusion criteria

Participants are eligible for the Optimise-FLT3 trial if they fulfil all of the criteria below:

1. Diagnosis of AML
2. Age ≥16yrs (no specified upper age limit)
3. Considered fit for intensive AML therapy by the treating physician
4. Confirmed *FLT3* ITD or TKD mutation (or *FLT3* status unknown but requires urgent therapy - see below*)
5. Serum creatinine less than or equal to 1.5 x ULN (upper limit of normal)
6. Serum Alanine Aminotransferase (ALT) **OR** Aspartate Aminotransferase (AST) less than or equal to 2.5 x ULN and bilirubin less than or equal to 2 x ULN
7. A negative pregnancy test within 2 weeks prior to trial entry in WOCBP (to be repeated throughout the trial prior to each course of protocol treatment and at the end of consolidation and maintenance)
8. Sexually mature males and females must agree to use an adequate and medically accepted method of contraception throughout the study and for 6 months following treatment if they or their sexual partners are women of childbearing potential (WOCBP)
9. WHO/ECOG performance status 0-2
10. Written informed consent

**FLT3*-mutated AML is associated with proliferative disease features such as hyperleukocytosis (high white blood cell count) and may present as a medical emergency. It is important that the full clinical spectrum of *FLT3*-mutated AML is represented in Optimise-FLT3, including hyper-proliferative cases. Should the treating physician feel that safety of an individual participant could be compromised by delaying therapy while awaiting *FLT3* genotyping, they may, on discussion with the study team, proceed with study entry (using PIS2), randomisation and treatment provided all other eligibility criteria are met. Any participants who enter the trial and are subsequently found to have wild type *FLT3* will be considered evaluable for safety/toxicity analysis but will be replaced with additional *FLT3*-mutated cases to maintain statistical power for the clinical efficacy endpoints and equipoise between arms. Once *FLT3*-mutated AML is confirmed, participants consented using PIS2 do not need to be re-consented with PIS1.

8.2 Exclusion criteria

Patients will be ineligible for Optimise-FLT3 trial if any of the criteria below apply:

1. Receipt of any previous therapy for AML or any antecedent haematological condition (the use of oral hydroxycarbamide to control white blood cell count is permitted)

2. Other active malignancy requiring treatment
3. Patients who are pregnant or lactating
4. Uncontrolled infection with Human Immunodeficiency Virus (HIV) or Hepatitis B or C. Patients with known chronic infections who are receiving or have completed therapy and have recent documented negative viral PCR tests are not excluded
5. Blast transformation of chronic myeloid leukaemia (CML)
6. Contraindications to any of the IMPs as per the SmPC

9 Recruitment, screening and registration

9.1 Participant identification

Diagnosis is undertaken by a trained haemato-oncologist, based upon the result of blood or bone marrow from a local haematology laboratory. Participants are eligible for the trial if they meet all of the inclusion criteria and none of the exclusion criteria apply. All queries about participant eligibility should be directed to the Trial Manager before enrolment. Protocol waivers are not permitted.

Once a patient has been identified as potentially eligible to participate, the opportunity to take part in the trial will be discussed with the patient and they will be given a copy of the relevant PIS and CF. The patient will be given adequate time to consider the trial and given the opportunity to ask further questions. The PI or another delegated medically qualified doctor must confirm the eligibility of a patient in the patient's medical notes prior to enrolment.

9.2 Screening logs

A screening log of all patients who were considered for potential participation in the trial will be kept by each site. This log will record whether the person was considered eligible based on their medical notes, whether they were approached, and whether they gave consent to participate. When at site, logs may contain identifiable information, but this must be redacted prior to being sent to the CTR. The redacted screening log should be sent to the optimise-flt3@cardiff.ac.uk email address quarterly.

9.3 Informed consent

The participant's written informed consent must be obtained using the trial Consent Form (ICF1), which follows the main Participant Information Sheet (PIS1). The participant should be given sufficient time after the initial invitation to participate before being asked to sign the Consent Form. Informed consent must be obtained prior to the participant undergoing procedures that are specifically for the purposes of the trial. Consent may be taken by the Principal Investigator or delegated personnel that are GCP-trained and suitably clinically qualified and experienced at the participating centres.

As detailed in section 8.1, for participants entering the trial with 'hyper-proliferative AML' and high WBC for whom urgent treatment cannot be delayed for the results of *FLT3* mutation testing, PIS2/ICF2 should be used for trial entry and consent.

Please note, only when written informed consent has been obtained from the participant and they have been randomised into the trial can they be considered a trial participant.

Participants should always be asked to sign a consent form. One copy should be given to the participant but the original copy should be kept in the investigator site file and a further copy should be kept with participant's hospital notes.

The right of the participant to refuse to participate in the trial without giving reasons must be respected. After the participant has entered the trial, the investigator must remain free to give alternative treatment to that specified in the protocol, at any stage, if he/she feels it to be in the best interest of the participant. However, the reason for doing so should be recorded and the participant will remain within the trial for the purpose of follow up and data analysis according to the treatment option to which he/she has been allocated. Similarly, the participant must remain free to withdraw at any time from the protocol treatment without giving reasons and without prejudicing his/her further treatment.

9.4 Randomisation

The trial is a randomised phase II/III trial. Randomisation will be performed using a computer-implemented minimisation algorithm by the CTR.

At trial entry participants will be randomised (1:1:1) between the three treatment arms, balancing randomisation by *FLT3* mutation type, *NPM1* status and age, and this information must be available at the point of randomisation (unless the participant is entering as a medical emergency using PIS/ICF2, in which case *FLT3* and/or *NPM1* mutation may be recorded as to be confirmed).

Back up randomisation system is in place and can be used in the event that the randomisation system is not available. Please contact the Optimise Trial Team for further information.

10 Trial Treatments

10.1 Details of IMPs to be evaluated in Optimise-FLT3

For the purposes of the protocol, the Investigational Medicinal Products (IMPs) are defined by the sponsor as:

- Gemtuzumab Ozogamicin (Mylotarg)
- Midostaurin (when this is used within the trial as a component of DA-Mido, DA-Mido+GO, FLAG-Ida-GO-Mido regimens)
- Daunorubicin (as a component of both DA-Mido or DA-Mido+GO)
- Cytarabine (when this is used as a component of DA-Mido, DA-Mido+GO and FLAG-Ida-GO-Mido)

- The other individual components of FLAG-Ida (as part of FLAG-Ida-GO-Mido) are also defined as IMPs:
    - Fludarabine
    - Idarubicin
    - G-CSF

When given within the standard treatment schedules described below, these agents are defined as Non-IMPs (nIMPs):

- Cytarabine (when used in consolidation cycles [cycles 3 & 4])
- Midostaurin (when used in consolidation [cycles 3 & 4] and in maintenance stages)

Full details of IMPs and nIMPs are provided in Appendix C.

10.2 Treatment supply and storage

IMPs and nIMPs should be stored as per the Optimise-FLT3 pharmacy manual and relevant IB or SmPC. Sites should source their own Mylotarg, Midostaurin, Daunorubicin, Cytarabine, Fludarabine, Idarubicin and G-CSF local hospital stock.

10.3 Notes on Safety Monitoring of IMPs in Optimise-FLT3

Safety monitoring of participants in the trial should be conducted in line with the recommendations of the SmPCs for all the IMPs listed in section 10.1, and in line with expected standard of care for participants receiving intensive AML therapy (also see guidance in Section 13.1).

Cytarabine, Daunorubicin, Fludarabine and Idarubicin should only be administered under the supervision of physicians experienced in the use of cytotoxic therapy. Prior to, and during each course of intensive chemotherapy, careful monitoring of full blood count, renal and hepatic function will be performed at the local clinical team's discretion, this generally being carried out a minimum of three times per week during administration of chemotherapy and at least twice weekly during periods of outpatient monitoring of post-chemotherapy aplasia (also see guidance in Sections 12.1.5-6 and schedule of events).

Cardiotoxicity is a risk of anthracycline treatment. Cardiac function should be assessed before participants undergo treatment with daunorubicin or idarubicin and should be monitored throughout therapy to minimise the risk of incurring severe cardiac impairment. As per standard of care, a baseline cardiac evaluation either with echocardiography or multiple gated acquisition (MUGA) scan is recommended, especially in participants with baseline risk factors for increased cardiotoxicity (including older participants, those history of cardiac disease or hypertension, prior exposure to mediastinal radiotherapy or other cardiotoxic drugs).

In relation to fludarabine: transfusion-associated graft versus host disease has been observed after transfusion of non-irradiated blood in fludarabine treated participants; to minimise risk, participants receiving fludarabine (study arm 3) should receive irradiated blood only. Use of prophylaxis against pneumocytis jirovecii pneumonia (PJP) should also be considered in these participants.

Participants receiving high doses of cytarabine should be observed for neuropathy/neurotoxicity since dose adjustments may be needed to avoid irreversible neurological disorders.

Specific guidance on issues pertaining to safe administration of Gemtuzumab Ozogamicin (Mylotarg) and Midostaurin are given in the following sections (10.3.1 and 10.3.2).

10.3.1 Notes on Gemtuzumab Ozogamicin (Mylotarg) administration (study arms 2 & 3)

Participants allocated to receive Gemtuzumab Ozogamicin (Mylotarg) must not have a white count greater than 30 x 10^9/L at the time of Mylotarg administration because of the risk of tumour lysis. Such participants should either have their WBC reduced with Hydroxycarbamide before commencing trial chemotherapy or have the administration of Mylotarg delayed until day 4 of the chemotherapy course. If the first dose is delayed to day 4 and the participant is scheduled to receive 2 doses of Mylotarg then the second dose should be given on day 7.

(Please note that in the FLAG-Ida schedule the 'first day of chemotherapy' is actually day 2 as G-CSF is given alone on day 1. With FLAG-Ida, Mylotarg is administered on day 2 or delayed until day 5 if the day 2 WBC exceeds 30 x 10^9/l).

Participants with AST or ALT more than 2.5 times the local upper limit of normal or bilirubin more than twice upper limit of normal, should not receive Mylotarg.

Mylotarg will be given at a dose of 3mg /m^2, capped at the contents of one 5 mg vial for participants with BSA above 1.67m^2.

Details of the premedication, and other procedures for Mylotarg administration, are set out in Appendix C.

10.3.2 Notes on Midostaurin treatment administration (all study arms)

Midostaurin should not be administered concurrently with other chemotherapy. It should be taken orally, twice daily at approximately 12-hour intervals. The capsules should be taken with food. Capsules should be swallowed whole with a glass of water. Prophylactic anti-emetics should be administered in accordance with local medical practice as per participant tolerance. In the event of any missed doses of Midostaurin, please consult the trials office for advice.

Caution is required when concomitantly prescribing Midostaurin with strong CYP3A4 inhibitory drugs such as, but not limited to, antifungals/ azoles (e.g. ketoconazole, itraconazole, posaconazole), certain antivirals (e.g. ritonavir), macrolide antibiotics (e.g. clarithromycin), St John's Wort and nefadozone because they can increase the plasma concentrations of Midostaurin. Where possible, alternative

medical products that do not strongly inhibit CYP3A4 should be considered. In situations where satisfactory therapeutic alternatives do not exist, participants should be closely monitored for Midostaurin-related toxicity.

Caution is warranted in participants at risk of QTc prolongation (e.g. due to concomitant medicines and/or electrolyte disturbance). Interval assessment of QTc by ECG should be considered if Midostaurin is taken concurrently with medical products that can prolong QT interval, with the following recommendations in the event of QTc prolongation:

- QTc interval >470msec and ≤500msec: decrease Midostaurin to 50mg once daily for the remainder of the cycle. Resume Midostaurin at the initial dose in the next cycle provided the QTc improves to ≤470msec at the start of that cycle (otherwise continue at 50mg once daily)

- QTc interval >500msec: withhold Midostaurin for the reminder of the cycle. If QTc improves to ≤470msec just prior to the next cycle, resume Midostaurin at the initial dose. If QTc interval has not improved in time for start of the next cycle, do not administer Midostaurin. Midostaurin may be held for as many cycles as necessary until QTc improves.

In participants receiving haematopoietic stem cell transplant, Midostaurin should be discontinued 48 hours prior to the start of conditioning and it should not be restarted post-transplant.

10.4 Notes on IMP dosing used in Optimise-FLT3

The chemotherapy schedules used in Optimise-FLT3 build on the findings of the preceding NCRI AML15, 17, 18 and 19 studies (see also protocol section 4.1 'Justification of treatment options'), within which extensive safety and efficacy data were accrued in justification of the DA and FLAG-Ida 'backbone' induction chemotherapy doses to be used in study Arms 1-3 (detailed in section 10.5).

Daunorubicin, used at an induction dose of 60mg/m^2 (3 doses given on alternate days), was established as the standard of care dose within the DA schedule, and the dose to be used within the 'DA60' backbone of future trials following the AML17 study; when prospectively compared with a higher 90mg/m^2 dose, lower 60-day mortality was demonstrated (5% vs 10%), with no overall difference in complete remission rate[34]. 'DA60' was safely combined with Gemtuzumab Ozogamicin (used as a single dose of 3mg/m^2 and a fractionated dose of 2 x 3mg/m^2) in the subsequent NCRI AML19 study[15].

Although the SmPC for Fludarabine refers to its use as monotherapy in the treatment of chronic lymphocytic leukaemia, there is now very extensive international published experience of its use as part of the 'FLAG-Ida' combination in AML (combined with Cytarabine, Idarubicin and G-CSF) dating back >30 years, in both relapsed/refractory disease and frontline intensive therapy settings [15,35,36,37,38]. The doses of all of the individual drug components of FLAG-Ida to be used in Optimise-FLT3, including the protocol-recommended dose modifications for participants aged >60yrs are now well established following the preceding MRC/NCRI AML15, 18 and 19 studies and are also used routinely within standard of care relapse protocols. In the AML19 study, FLAG-Ida was safely combined with Gemtuzumab Ozogamicin at the doses which will be used in Optimise-FLT3[15].

'High dose cytarabine' although not defined as an IMP within the study, has formed a cornerstone of AML consolidation regimens for >30yrs. Older participants are more susceptible to toxicity, particularly in terms of myelosuppression and neurotoxicity and the age-dependent dose reductions for participants aged >60yrs to be used in Optimise-FLT3 reflect those used in recent studies and recommended by recent guidelines[15,2].

The doses of Midostaurin to be used in Optimise-FLT3, both following induction and consolidation chemotherapy, and when used as maintenance therapy reflect those used in the RATIFY licensing study and those recommended in the Midostaurin SmPC[12].

10.5 Induction chemotherapy: Courses 1 and 2

Each induction schedule (arms 1-3) comprises two courses of allocated chemotherapy, both followed by 14 days of oral midostaurin. Remission status will be determined after each course (see section 12.2).

10.5.1 Study arm 1: DA + Midostaurin

Please note that there are minor differences between this schedule and that used within the RATIFY trial protocol[16]. These reflect adaptation to standard UK practice and have been necessary to ensure direct comparability with the DA + GO + Midostaurin schedule that was piloted within the preceding AML19 trial[20].

Course 1: DA 3+10 + Midostaurin

Daunorubicin 60mg/m^2 by intravenous infusion over 1 hour on days 1,3 and 5 (3 doses)

Cytarabine (Ara-C) 100mg/m^2 twelve-hour by slow intravenous bolus on days 1-10 (20 doses)

Midostaurin PO 50mg twice daily, D11-24 (14 days) (see section 10.3.2)

After course 1, if the participant is in CR, CRh or CRi and the counts have regenerated to ≥1.0 x 10^9/l neutrophils and ≥80 x 10^9/l platelets, course 2 can commence:

Course 2: DA 3+8 + Midostaurin

Daunorubicin 50mg/m^2 by intravenous infusion over 1 hour on days 1,3 and 5 (3 doses)

Cytarabine (Ara-C) 100mg/m^2 twelve-hourly by slow intravenous bolus on days 1-8 (16 doses)

Midostaurin PO 50mg twice daily, D9-22 (14 days)

10.5.2 Study arm 2: DA + GO + Midostaurin

This schedule was piloted in the second part of AML19 and was shown to be safe with initial evidence of efficacy[20]. GO is not given with the second or subsequent courses because of evidence from the

AML15 trial which used a 2-way factorial randomisation to evaluate GO in induction and consolidation; this showed a survival benefit for GO in induction but not in consolidation[41].

Course 1: DA 3+10 + GO + Midostaurin

Daunorubicin 60mg/m^2 by intravenous infusion over 1 hour on days 1,3 and 5 (3 doses)

Cytarabine 100mg/m^2 12-hourly by slow intravenous bolus on days 1-10 (20 doses)

Gemtuzumab Ozogamicin (Mylotarg) 3mg/m^2 by intravenous infusion over 2 hours (capped at the contents of one 5mg vial) on days 1 and 4, delayed to days 4 and 7 if WBC ≥ 30x10^9/L (section 10.3.1)

Midostaurin PO 50mg twice daily, D11-24(see section 10.3.2)

After course 1, if the participant is in CR, CRh or CRi and the counts have regenerated to ≥1.0 x 10^9/l neutrophils and ≥80 x 10^9/l platelets, course 2 can commence

Course 2: DA 3+8 + Midostaurin

Daunorubicin 50mg/m^2 by intravenous infusion over 1 hour on days 1,3 and 5 (3 doses)

Cytarabine 100mg/m^2 12-hourly by intravenous bolus on days 1-8 (16 doses)

Midostaurin PO 50mg twice daily, D9-22

10.5.3 Study arm 3: FLAG-Ida + GO + Midostaurin

Course 1: FLAG-Ida + GO + Midostaurin

Fludarabine 30mg/m^2 daily by intravenous infusion over 30 minutes on days 2-6 (5 doses)

Cytarabine 2g/m^2 daily by intravenous infusion over 4 hours, starting 4hrs after fludarabine on days 2-6 (5 doses) (reduced to 1g/m^2 in participants ≥60yrs)

G-CSF – Filgrastim/filgrastim biosimilar 300µg daily by subcutaneous injection on days 1-7 (7 doses (other G-CSF analogues, including lenograstim may be used with adequate dosage adjustments, in line with local practice).

Idarubicin 8mg/m^2 daily slow intravenous bolus on days 4-6 (3 doses)

Gemtuzumab Ozogamicin (Mylotarg) 3mg/m^2 intravenous infusion over 2 hours on day 2 (capped at the contents of one 5mg vial) and delayed to D5 if WBC ≥ 30x10^9/l (see below - section 10.3.1)
Midostaurin PO 50mg twice daily, D7-20 (14 days) (see section 10.3.2)

**For participants receiving FLAG-Ida course 1 who have a WCC ≥30 x 10^9/L it is advised to omit G-CSF until the WCC has fallen to <30 x 10^9/l.*

After course 1, if the participant is in CR, CRh or CRi and the counts have regenerated to ≥1.0 x 10^9/l neutrophils and ≥80 x 10^9/l platelets, course 2 can commence

Course 2: FLAG-Ida + Midostaurin

Fludarabine 30mg/m^2 daily by intravenous infusion over 30 minutes on days 2-6 (5 doses)

Cytarabine 2g/m^2 daily by intravenous infusion over 4 hours, starting 4hrs after fludarabine on days 2-6 (5 doses) (reduced to 1g/m^2 in participants ≥60yrs)

G-CSF – Filgrastim/filgrastim biosimilar 300µg daily by subcutaneous injection on days 1-7 (7 doses (other G-CSF analogues, including lenograstim may be used with adequate dosage adjustments, in line with local practice).

Idarubicin 8mg/m^2 daily slow intravenous bolus on days 4-5 (2 doses)

Midostaurin PO 50mg twice daily, D7-20 (14 days)

Enhanced safety arrangements for study arm 3

The FLAG-Ida-GO-Mido schedule has not yet been piloted, therefore this arm includes a safety run-in phase where the first 20 participants will undergo enhanced pharmacovigilance. The IDMC will review safety data after 10 and 20 participants have been treated. They will particularly focus on:

- Increased haematological toxicity (delayed count recovery following courses 1 and 2)
- Infective complications
- Evidence of increased 60-day mortality

If these or other concerning toxicities are observed, the study team will act in close liaison with the IDMC. Contingencies to modify the arm 3 treatment schedule will depend on when toxicity is observed (post course 1 vs course 2) and whether it is age group-related (younger vs older participants).

In the event that arm 3 requires modification, the IDMC will advise on subsequent monitoring requirements; at a minimum, a further 20 participants will be subject to enhanced pharmacovigilance and, should the issues not be resolved then the trial arm will be stopped, the trial then switching to a 2-arm design.

10.6 Stem Cell Transplantation in Optimise-FLT3

10.6.1 Indications for transplant

The four main groups of participants for whom allogeneic haematopoietic stem cell transplant (HSCT) from a suitably matched sibling or alternative donor in first remission is *recommended* are:

1) Participants with FLT3-ITD mutation (without NPM1 co-mutation) where the baseline FLT3 allelic ratio is >0.05 or percentage is >5%.
2) Participants with *NPM1* mutation who test peripheral blood *NPM1* qPCR positive after course 2.
3) Participants with other ELN 2022 intermediate risk disease initially allocated to flow MRD monitoring (i.e *NPM1* unmutated) who are flow MRD positive (>0.1%) by central testing (or testing at a trial-approved local flow MRD laboratory) after course 2.
4) Participants with ELN 2022 adverse risk disease or *NUP98* rearrangement.

Other disease scenarios will undoubtedly arise where the local team may wish to pursue HSCT in first remission. The Optimise-*FLT3* clinical coordinators will be happy to provide clinical advice on decision making around transplant in individual participants. Ultimately the decision as to whether to transplant in first remission (or not) will be made by local teams in consultation with participants and (if applicable) with their regional transplant centre.

10.6.2 Timing and approach to transplant

Since participant and donor will require time to be counselled about transplant options which may be delivered as early as course 3, investigators are encouraged to identify donor availability as soon as possible after diagnosis. It is recognised that it takes time for arrangements for transplant to be made, and that there will be a number of participants for whom a donor cannot be readily identified. Participants should continue with their allocated Optimise-FLT3 treatment courses until the transplant can be delivered.

The choice between myeloablative and reduced intensity conditioning (RIC) approaches is multi-faceted and will be made by local teams and transplant centres. Generally, allogeneic HSCT with myeloablative conditioning will be offered to participants aged ≤40-45yrs, this following course 2 of trial chemotherapy where practical. Participants aged ≥40-45yrs will generally receive reduced intensity conditioning, usually following a first cycle of cytarabine/midostaurin consolidation chemotherapy (course 3).

Following transplant, participants should **not** receive maintenance midostaurin therapy. It is recognised, however, that variations in practice currently exist around the use of other tyrosine kinase inhibitors in post-allograft maintenance therapy; in Optimise-FLT3 this will be left at the discretion of individual investigators and transplant centres.

10.7 Consolidation chemotherapy (all study arms)

After course 2, when participants in remission have regenerated to ≥1.0 x 10^9/l neutrophils and ≥80 x 10^9/l platelets, they should proceed with cytarabine/midostaurin consolidation treatment, i.e.. Courses 3 and 4.

For participants who are not in complete remission (CR, CRi, CRh) after course 2, treatment will be deemed to have failed. These participants will not continue with Optimise-FLT3 protocol treatment and should continue to be treated at the local investigator's discretion.

10.7.1 Courses 3 and 4: Cytarabine + Midostaurin (all study arms)

- **Cytarabine 3g/m²** IV over 4 hours every 12 hours on days 1,3 and 5 (6 doses) *(* see below for dose reductions applying to participants aged ≥60yrs)*
- Midostaurin **50m**g orally **twice daily, D6-19**

Cytarabine Dose Reductions by Age:

- Age 60-69 years:
  - Cytarabine **1.5 g/m²** IV **every 12 hours** on **Days 1, 3, and 5 (6 doses)**
- Age ≥70 years:
  - Cytarabine **1 g/m²** IV **once daily** on **Days 1–5 (5 doses)**

It is also permissible to deliver these high dose cytarabine schedules on three consecutive days i.e. day 1,2,3 instead of 1,3,5. That applies to both the 3g schedule here and also the 1.5g schedule. This does not apply to the 1g schedule.

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

After course 3, if the participant is in CR, CRh or CRi and the counts have regenerated to ≥1.0 x 10^9/l neutrophils and ≥80 x 10^9/l platelets, they should proceed with course 4.

10.8 Midostaurin maintenance therapy (all study arms: non-transplant participants only)

Following blood count recovery to 1.0 x 10^9/L neutrophils and 80 x 10^9/L platelets following the final course of chemotherapy, participants should recommence midostaurin at a dose of 50mg PO twice daily. In the event of delayed count recovery, discussion with the clinical coordinators may be required. Maintenance midostaurin should be continued for 12 continuous 28-day cycles (48 weeks in total) and stopped in the event of relapse.

In participants receiving haematopoietic stem cell transplant, midostaurin should be discontinued 48 hours prior to the start of conditioning. Midostaurin should not be restarted following transplant.

During maintenance Midostaurin therapy, investigators should bear in mind the following guidance on safety monitoring (as per Midostaurin SmPC):

- Caution should continue to be applied for participants receiving concomitant therapy with strong CYP3A4 inhibitory drugs, and interval QTc monitoring by ECG considered (see section 10.3.2 for recommended dose adjustments)
- In the event of Grade 4 neutropenia (ANC <0.5x10^9/l) Midostaurin should be interrupted until ANC ≥1.0x10^9/l then resumed at 50mg twice daily. If neutropenia (ANC <1.0x10^9/l) persists for >2 weeks, Midostaurin should be permanently discontinued
- Persistent grade 1-2 toxicity that participants deem unacceptable may prompt an interruption for as many as 28 days

10.9 Management of overdose

A medication error is any unintentional error in the prescribing, dispensing or administration of a medicinal product that may cause or lead to inappropriate medication use or participant harm. In the event of an overdose/underdose of any drug* provided within Optimise-FLT3, any further treatment should be withheld and the Optimise-FLT3 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 participant experiences an SAE that the local PI considers may be causally related to the overdose/underdose then this must be clearly stated on the SAE form submitted to the CTR PV team. Please see Section 15 for more information on reporting SAEs.

If a participant 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.

*Please note that missed Midostaurin doses do not need to be individually reported on the dosing error form, but must be captured on the eCRF.

10.10 Prohibited medications and interaction with other drugs

Please refer to current SmPCs/ IBs and the information in Appendix C, which may not be exhaustive. In general, concomitant medications and therapies deemed necessary for the supportive care and safety of the participant are allowed provided their use is documented in the participant records.

10.11 Accountability procedures

CTR will provide template participant level accountability logs to participating trial sites, in order to record accountability of IMP dispensed and destroyed. Accountability logs may be requested by Sponsor. Accountability logs for nIMPs will not be provided to participating sites or collected by Sponsor. Please refer to relevant Optimise-FLT3 Pharmacy Manual for further details.

11 Participant sample management (diagnostic and MRD samples)

The following section provides guidance on expected diagnostic tests expected to be performed as part of AML diagnosis and on the trial pathways for central laboratory and MRD samples. Please also refer to flowchart in Appendix B.

11.1 Samples to be taken at diagnosis

11.1.1 Standard of care diagnostic samples

A full diagnostic AML workup is essential as this informs treatment and monitoring within the trial.

In Optimise-FLT3 this workup should be performed in your usual standard-of-care laboratory, therefore it is important for each site to be clear which laboratory tests and which samples are required.

The following tests are mandatory:

- Rapid screening for *FLT3* ITD, TKD and *NPM1* mutations
- G-banded karyotype and relevant FISH according to local laboratory practice
- Next generation sequencing panel according to local laboratory practice
- CD33 status of AML blasts (positive / heterogenous /negative by local flow cytometry laboratory)

The following tests are recommended for participants who have *FLT3* ITD and are *NPM1* negative:

- RNA sequencing panel
- FISH for *NUP98* rearrangements if an RNA sequencing panel cannot be performed

11.1.2 Trial specific diagnostic samples

In addition to the local diagnostic workup the following trial specific samples are required:

Sample banking and molecular baseline

5ml BM in EDTA and 20ml PB in EDTA to be sent to:

Amanda Gilkes, Department of Haematology, Room 167, 7th Floor, A-B Link, School of Medicine, Cardiff University, Heath Park, Cardiff, CF14 4XN.

Email: Haem-Trial-Samples@cardiff.ac.uk and SparyLK@cardiff.ac.uk

Flow MRD baseline

Unless the participant is already known to have a *NPM1* mutation, 10ml PB or up to 5ml BM in EDTA should be sent same day as taken to:

AML Flow Cytometric MRD, Clinical Immunology Service, College of Medical & Dental Sciences, University of Birmingham, Vincent Drive, Birmingham, B15 2TT, UK.

AML Flow Cytometric MRD, Clinical Immunology Service, 3rd Floor No. 1 Birmingham Health Innovation Campus (BHIC), Aston Webb Boulevard, Selly Oak, Birmingham, B29 6SJ
Laboratory Contact: Nick McCarthy, Clinical Scientist, N.I.McCarthy@bham.ac.uk, Sylvie Freeman, s.freeman@bham.ac.uk

Individual trial centres will be permitted, following initial coordination with CTR and the Birmingham flow MRD laboratory to perform diagnostic / baseline testing for flow cytometric MRD targets locally according to ELN Guidelines (ref). In these circumstances, blood does not need to be sent to the central laboratory, and the local laboratory will provide results to enter into the trial database (standardised reporting database form).

11.2 Monitoring samples (See Sample Flow Chart – Appendix B)

Once the results of genetic standard-of-care testing are available, each participant will be allocated to either a flow or molecular MRD monitoring strategy. You will be notified of this by email. Usually, you will only have to send samples to one of these laboratories. However, sometimes a rare PCR molecular marker could be identified. In these cases, samples should be sent for flow monitoring after cycle 1 and cycle 2 and you will be informed by email with further instructions on required molecular monitoring.

11.2.1 Participants allocated to flow MRD monitoring

All participants without an *NPM1* mutation or core binding factor (*CBF*) rearrangement will be initially monitored by flow cytometric MRD after treatment courses 1 and 2. If a rare molecular PCR target is subsequently identified, the participant will also be allocated to molecular surveillance MRD monitoring.

Time points: on regeneration after courses 1 and 2, pre-transplant if transplant is planned.
(surveillance post-transplant is as per standard of care by transplant centre)

3-5ml BM in EDTA (first pull) to be sent to:

AML Flow Cytometric MRD, Clinical Immunology Service, 3rd Floor No. 1 Birmingham Health Innovation Campus (BHIC), Aston Webb Boulevard, Selly Oak, Birmingham, B29 6SJ
Laboratory Contact: Nick McCarthy, Clinical Scientist, N.I.McCarthy@bham.ac.uk
Sylvie Freeman, s.freeman@bham.ac.uk.

For New Zealand and Denmark sites please refer to Lab manual and site specific appendix.

11.2.2 Participants allocated to molecular MRD monitoring

This will include participants with *NPM1* mutation and core binding factor AML, who are monitored only using molecular MRD. This will also include participants with a rare molecular marker, who are initially monitored by flow cytometry but will also have molecular monitoring once this has been identified.

Time points: on regeneration after each course of chemotherapy, then every 3 months for 2 years, unless notified otherwise by the laboratory or there is further treatment, transplant or relapse. This is consistent with UK standard-of-care and guidelines from the European Leukaemia Network.

If the participant has a transplant, samples should be sent pre-transplant, and then at 30, 60 and 100-days post-transplant and then every 3 months for a further 2 years. If a participant relapses, samples should be sent prior to starting treatment, after each course of treatment (if any is received) and then every 3 months for a further 2 years.

5ml BM in EDTA and 20ml PB in EDTA to be sent to:

Dr Richard Dillon, Molecular Oncology Diagnostics Unit, 4th Floor, Southwark Wing, Guy's Hospital, London, SE1 9RT. Email: richard.dillon@kcl.ac.uk

For New Zealand/international sites please refer to Lab manual.

11.3 Sample charges

There are various charges associated with monitoring samples in the Optimise-FLT3 trial, summarised as follows:

- AML participants with a molecular marker (*NPM1* mutation and fusion genes): £160 per time point. For sites in England, this will be covered through agreements between the GLHs and no invoices will be issued. For other sites, invoices will be issued on an annual basis.
- AML participants with no molecular MRD marker (i.e. for Flow MRD): £150 per time point (diagnostic sample will be charged for).

11.4 End of Trial Arrangements

At the end of the trial, where participants have provided consent for storage of excess material for research, any remaining samples will be transferred to a facility with a HTA Licence such as the research tissue banks at Cardiff University and Guy's Hospital. Samples may be used for ethically approved research by commercial companies and researchers across UK and abroad and will be held in line with requirements of the Human Tissue Act (2004). Where participants have not provided consent for sample storage, any remaining material will be destroyed at the end of the trial.

12 Study Procedures and Assessments

The procedures to be conducted at trial visits for each study subject are presented in tabular format in Appendix A and described in the text below.

12. 1 Participant assessments

12.1.1 Full medical history

To be taken at the initial screening / registration assessment to ensure that the participant satisfies all trial eligibility requirements. This should include recording past medical history including history and treatment of past and intercurrent diseases, documentation of any concomitant medications and any family history of cancer.

12.1.2 Physical examination and vital signs

Participants will receive a physical examination and measurement of vital signs (temperature, blood pressure and pulse) at screening/registration, and within 3 days of commencing treatment courses 1-4 and maintenance therapy. This will be performed for safety reasons and this information will not be routinely collected within the eCRF.

During inpatient stays, clinical examination will be carried out as part of routine clinical care daily, or more often if the participant is acutely unwell.

12.1.3 WHO/ECOG performance status

Assessment of performance status will be carried out and recorded at screening / registration, before each course of therapy (+/- 3 days), then at the 3, 6, 12 and 24 months follow up timepoints (see appendix D)

12.1.4 Height/weight and body surface area

Weight will be measured at screening/registration and repeated within 3 days of the start of each treatment course in order to calculate body surface area (BSA) to enable accurate prescription of each cycle of therapy. Methods used to calculation BSA will be as per local practice.

Height measurement need only be performed at baseline to inform future BSA calculations.

12.1.5 Haematology

A full blood count will be performed at baseline/screening; white blood cell, neutrophil and platelet counts will be collected in the eCRF. A blood count should also be performed for safety reasons within 3 days prior to starting each course of therapy (including maintenance therapy); these values will not be routinely collected in the eCRF.

Throughout therapy, full blood counts will be carried out at the local clinical team's discretion as part of the routine management of AML. These tests will usually be carried out a minimum of three times weekly during inpatient stays and at least twice weekly during haematology day unit / outpatient monitoring of periods of post-chemotherapy aplasia and will inform the need for blood product replacement and allow sites to provide information on the kinetics of bone marrow recovery (duration and depth of neutropenia / thrombocytopenia) and for formal disease response assessment reporting purposes.

12.1.6 Biochemistry

Biochemistry assessment will include sodium, potassium, urea, creatinine, calcium, phosphate, uric acid magnesium, albumin, total protein, bilirubin, ALP, ALT and/or AST.

Biochemical assessment will be performed at baseline/screening, with serum creatinine, bilirubin, ALT and/or AST being collected in the eCRF and used to confirm trial eligibility. Biochemical assessment should also be performed for safety reasons within 3 days prior to starting each course of therapy (including maintenance therapy); these values will not be routinely collected in the eCRF.

Throughout therapy, biochemistry assessments will be carried out at the local clinical team's discretion as part of the routine management of AML.

12.1.7 Pregnancy test

A urine pregnancy test should be performed for all women of childbearing potential at screening/registration, within 7 days prior to starting each course of study treatment and following completion of consolidation and maintenance treatment

Female subjects of childbearing potential must have a negative pregnancy test and must agree to use an effective form of contraception during the trial and for at least 6 months after all treatment has finished. If a female trial subject is found to be pregnant this must be reported to Cardiff CTR; further information can be found in section 16.

Male trial subjects must agree to use a highly effective form of contraception during the trial and for at least 6 months after all treatment has finished. To align with SmPCs, this should be increased for gemtuzumab ozogamicin and idarubicin to 7 and 6.5 months, respectively. If a partner of a male trial subject is found to be pregnant during this period, this must be reported to Cardiff CTR; further information can be found in section 16.

12.1.8 Virology

Testing for hepatitis B, C and HIV will be performed during screening. Participants with chronic infections may enrol if their last two tests for viral load have been negative. Specialist pharmacy advice may be required to manage drug-drug interactions, particularly with azole antifungals, in this situation.

12.1.9 Bone marrow aspirate / trephine

A bone marrow aspirate and trephine sample will be taken during the diagnostic / screening process: samples will be taken for local morphology, histopathology and flow cytometry and for full baseline genetic evaluation (as detailed in section 11.1.1). Where possible, participants should not be subjected to multiple bone marrow sampling procedures at the point of AML diagnosis; if the bone marrow is being performed as part of standard of care diagnostic assessment prior to the participant being formally consented for Optimise-FLT3, central samples may be sent to the trial laboratories (as detailed in section 11.1.2) in order to avoid repeat procedures (provided this is within the 28-day screening window).

Further bone marrow aspirates will be taken at the end of chemotherapy cycles 1-4 once counts have recovered to determine morphological response assessment (see section 12.2); at these time points, central samples will be sent for either flow MRD or molecular MRD monitoring depending on the strategy allocated to individual participants (see section 11.2.1-11.2.2) – please refer to the sample

flowchart. A trephine will only be required during follow-up if counts have not recovered by day 42 of the corresponding course of treatment, to confirm a morphological leukaemia free state (MLFS).

For details of MRD surveillance bone marrow samples required during years 1 and 2 of follow-up please refer to section 11.2.2 and the schedule of events table.

12.1.10 Quality of life

Quality of life (QoL) will be assessed at baseline (during screening / registration) and then at the following timepoints:

- Within 2 weeks from recovery from treatment course 2
- Within 2 weeks from recovery from treatment course 4
- Pre and post maintenance
- 3 months from end of treatment* (+/- 4 weeks)
- 6 months from end of treatment* (+/- 4 weeks)
- 12 months from end of treatment* (+/- 4 weeks)
- 24 months from end of treatment* (+/- 4 weeks)

(* For purposes of QoL assessments, 'end of treatment' is defined as the point of recovery from final cycle of intensive chemotherapy or day 0 of allogeneic stem cell transplant)

The following questionnaires will be used:

- EORTC QLQ-C30: a validated questionnaire developed to assess the quality of life of cancer participants. It consists of 30 items, each scored on a numbered scale, relating to experiences in the past week (see Appendix E)
- EQ-5D-5L: a standardised instrument for measuring generic health status. Quality of life is measured on a 5-component scale including mobility, self-care, usual activities, pain/discomfort and anxiety/depression. Participants are also asked to rate their health on a scale of 1-100 (see Appendix F)

Prior to commencing the baseline QOL assessment, a member of the local research team should discuss the questionnaires with the participant and answer any questions they might have.

12.1.11 Recording adverse events

Details of adverse events will be collected in the eCRF following courses 1-4 of chemotherapy treatment and graded according to Common Terminology Criteria for Adverse Events (CTCAE) version 5.0 (see Appendix G and Section 15)

12.1.12 Recording supportive care requirements and treatment compliance

Details of supportive care requirements including blood product usage (red cell and platelet transfusions administered), number of days in hospital and number of days of IV antibiotic and anti-fungal treatment will be collected following courses 1-4 of chemotherapy and recorded in the eCRF.

Details of chemotherapy / midostaurin doses received (including details of reasons for any missed doses) will also be collected following courses 1-4 of chemotherapy and recorded in the eCRF. Details of compliance with midostaurin maintenance treatment will be collected following completion of maintenance therapy.

12.2 Assessment of Response

Response will be formally assessed following each of the four scheduled courses of intensive chemotherapy.

A bone marrow aspirate to assess remission status should be taken upon count recovery; this will generally be taken 18-21 days after the end of chemotherapy administration (exclusive of midostaurin). If the bone marrow is of adequate cellularity for the assessment of haematopoiesis, the participant's remission status should be ascertained (see 13.2.1-13.2.2). If the marrow is hypoplastic and assessment of remission status is not possible, a repeat marrow should be performed after a further 7-10 days and remission status assessed.

If counts have not recovered by day 42 from the start of chemotherapy course, both aspirate and trephine biopsy should be obtained to confirm whether there is a 'morphological leukaemia free state'.

12.2.1 Response and relapse definitions[2]

- **Morphological complete remission (CR):** the bone marrow is regenerating normal haemopoietic cells and contains <5% blasts with neutrophil count ≥1.0 x 10^9/l and platelet count ≥100 x 10^9/l, no Auer rods or extramedullary disease
- **Morphological complete remission with partial haematological recovery (CRh):** the bone marrow is regenerating normal haemopoietic cells and contains <5% bone marrow blasts with neutrophil count ≥0.5 x 10^9/l and platelet count ≥50 x 10^9/l, no Auer rods or extramedullary disease
- **Morphological complete remission with incomplete count recovery (CRi):** the bone marrow is regenerating normal haemopoietic cells and contains <5% bone marrow blasts but there is residual neutropenia (neutrophil count <1.0 x 10^9/l) and/or thrombocytopenia (platelet count <100 x 10^9/l), no Auer rods or extramedullary disease and CRh criteria are not met.
- **CR, CRh, or CRi without MRD (CR_{MRD-}, CRh_{MRD-}, CRi_{MRD-}):** Fulfilling the definitions above, plus, absence of detectable measurable residual disease by either flow cytometry or RT-qPCR (or both, if both have been measured) in technically adequate samples taken on two separate occasions at

least four weeks apart. The date of the first negative sample is then taken as the date of MRD negative response.

- **Morphological leukaemia free state (MLFS):** <5% bone marrow blasts, no Auer rods or extramedullary disease, but not meeting criteria for CR or CRi (ie. hypocellular marrow appearances, confirmed by trephine biopsy).
- **Morphological partial response (PR):** meeting all the above criteria for CR, but bone marrow contains 5-25% blasts, and there has been a decrease from pre-treatment bone marrow blast percentage by at least 50%.
- **Resistant disease (RD):** the bone marrow shows persistent AML, failing to meet the any of the response definitions listed above.
- **Morphological relapse:** ≥5% blasts in the blood or bone marrow or (re-) emergence of extramedullary disease in a participant with a previously documented CR, CRh, CRi or MLFS.
- **MRD relapse**: conversion from CR_{MRD-}, CRh_{MRD-}, CRi_{MRD} to MRD positivity, or increase in MRD by 1 log_{10}, measured by either central flow cytometry or RT-qPCR, in all cases confirmed in a second sample, without evidence of morphological relapse[23].

12.3 Follow-up

The survival and remission status of each participant will be requested from the randomising clinician which will continue for two years after the last participant completes protocol treatment. Upon a report of death, the randomising site must provide a cause and date of death.

It is accepted that participants may have their treatment transferred to another participating site. Participants can be transferred within the Optimise-FLT3 database and it will be the new site's responsibility to obtain, complete and submit follow up information.

13 Supportive Care and CNS Treatment

13.1 Supportive Care

The remission induction and consolidation phases of therapy within Optimise-FLT3 are intensive and are associated with appreciable risks of infection and haemorrhage. The care of participants will make stringent demands on supportive care. Some information regarding aspects of supportive care will be collected in the participant records (see section 13.1.12).

It is expected that participants will follow established local supportive care protocols. Policies relating to the following aspects of supportive care should be decided in advance to ensure that treatment-related complications are minimised.

- Venous access via Hickman-type catheter or equivalent
- Prophylaxis / control of nausea and vomiting
- Mouth care

- Management and prophylaxis of tumour lysis syndrome
- Steroid eye drops for cytarabine (see section 11.6.1)
- Prophylactic gut decontamination (if considered appropriate)
- Antifungal prophylaxis and stewardship. A minimum 5-day washout period is recommended between the administration of Mylotarg and azole anti-fungals, during which time alternative prophylaxis may be given according to local protocols.
- Response to significant pyrexia, i.e. two readings of ≥38◦C two hours apart or a single reading of ≥39◦C
- Antibiotic treatment of febrile episodes, including antibiotic selection, monitoring, duration of therapy and treatment of non-response
- G-CSF therapy – Filgrastim/filgrastim biosimilar 300µg daily by subcutaneous injection may be given in cases of prolonged neutropenia, particularly following FLAG-Ida chemotherapy and following confirmation of disease remission (other G-CSF analogues, including lenograstim may be used with adequate dosage adjustments, in line with local practice).
- Irradiated blood products should be given to participants who receive fludarabine or HSCT
- Investigators are required to ensure that advice is given to participants on the conservation of sperm/and or ova as per the SmPC of the IMPs used in this trial which may lead to permanent damage to fertility. This is most relevant to those who may wish to conceive after the trial treatment is completed.

13.2 Central nervous system (CNS) disease and treatment

Participants who present with CNS disease are not specifically excluded from Optimise-FLT3 and may be entered into the trial and randomised at the same points as participants without CNS involvement. If a participant presents with physical signs suggesting CNS disease, an intrathecal injection of cytarabine (50mg) should be given when the diagnostic lumbar puncture is performed. If blast cells are identified in the CNS sample, a series of intrathecal injections with cytarabine should be given on 3 days of each week until CSF samples are clear. This may need to be modified if the platelet count is very low or coagulation is abnormal. Thereafter, treatment should be repeated at intervals of approximately 2 weeks until consolidation treatment has been completed.

The routine administration of treatment to the CNS is not recommended for participants where there is no evidence of CNS disease at diagnosis. Routine CNS investigation at diagnosis for participants without CNS symptoms is not recommended.

14 Withdrawal & lost to follow-up

14.1 Withdrawal

Participants have the right to withdraw consent for participation in any aspect of the trial at any time. The participant's 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 QoL questionnaires
3. Withdrawal from further sample collection (Blood, bone marrow)
4. Withdrawal from follow-up assessments
5. Withdrawal of Consent to all of the above
6. Withdrawal from any previous samples to be used
7. Withdrawal of consent for sample banking

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. There is specific guidance on this contained in the Participant Information Sheet but briefly:

If a participant wishes to stop taking part in the trial completely, they will need to be seen one last time for an assessment and tests. If the participant is suffering a serious reaction to the trial treatment when they decide to stop, you will need to continue to collect information about them for as long as the reaction lasts.

A participant may withdraw or be withdrawn from trial treatment for the following reasons:

- Intolerance of trial medication
- Withdrawal of consent for treatment by the participant
- Any alteration in the participants condition which justifies the discontinuation of the treatment in the Investigator's opinion
- Non-compliance (for individual participants, the local investigators are encouraged to discuss the level and impact of non-compliance with the Optimise-FLT3 team before withdrawal decisions are taken)

In all instances participants who consent and subsequently withdraw should complete a withdrawal form (see Withdrawal Form in trial pack) or the withdrawal form should be completed on the participant's behalf by the researcher/clinician based on information provided by the participant. The withdrawal form should be retained in your local site file and does not need to be sent to the CTR. Any queries relating to potential withdrawal of a participant should be forwarded to optimise-flt3@cardiff.ac.uk.

14.2 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 annotated 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 they may be withdrawn from trial medication after discussion with the Optimise-FLT3 clinical coordinators. If the participant is not compliant with the approved visit schedule, every effort will be made to collect data as close to the visit schedule as possible, and the participant will only be withdrawn from data collection as a final resort, with their data collected up until the point of withdrawal being used for analysis.

15 Trial Governance and Adverse Event Reporting

Investigators have trial governance and adverse event reporting obligations as described in GCP guidelines. The local principal investigator is responsible for ensuring that all site staff involved in this trial are familiar with the content of this section. All adverse events including serious adverse events are to be recorded and reported from the date of consent.

Optimise-FLT3 is sponsored by Cardiff University and managed the CU CTR with defined responsibilities delegated to the PI at each site. The trial is authorised in the UK by a Clinical Trials Authorisation (CTA) issued by the MHRA, and by the relevant competent authorities in other participating countries. The trial protocol has been approved by the National Research Ethics Service and its conduct, including safety data, will be monitored by an Independent Data Monitoring Committee (IDMC).

All SAEs must be reported immediately (and within 24 hours of knowledge of the event) by the PI at the participating site to the CTR Pharmacovigilance and Safety Specialist unless the SAE is specified as not requiring immediate reporting (see section 15.3). This includes SAEs related to both IMPs and to non-Investigational Medicinal Products (nIMPs).

15. 1 Adverse Event Reporting

The PI at each participating institution has an obligation to report relevant serious adverse events (SAEs) that occur in this trial to CU CTR, in a timely manner (see below).

It is recognised that adverse events that may be life-threatening may be a normal consequence of AML of its effective treatment, and many clinical changes in the participant's condition are expected as they progress through therapy. Within Optimise-FLT3, events that occur at any point from time of signature of informed consent, throughout the treatment period, up to and including 28 days after their last dose protocol treatment, and meet the criteria laid out below (sections 15.2 and 15.3) 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. Serious adverse reactions (such as long term side effects of trial treatment under investigation) should continue to be reported until the end of follow up as defined in the protocol.

Adverse events should be graded using the NCI Common Terminology Criteria for Adverse Events (CTCAE) Version 5.0 (**Appendix G)**.

15.2 Definitions

Term	Definition
Adverse Event (AE)	Any untoward medical occurrence in a participant or clinical trial participant administered a medicinal product which is not necessarily caused by or related to that product
Adverse Reaction (AR)	Any untoward and unintended response in a clinical trial participant to an investigational medicinal product which is related to any dose administered to that participant
Serious Adverse Event (SAE)	Any adverse event that - • Results in death • Is life-threatening* • Requires hospitalisation or prolongation of existing hospitalisation** • Results in persistent or significant disability or incapacity • Consists of a congenital anomaly or birth defect • Other medically important condition***
Serious Adverse Reactions (SARs)	Any SAE occurring in a clinical trial participant for which there is a reasonable possibility that it is **related** to the IMP at any dose administered.
Suspected Unexpected Serious Adverse Reactions (SUSARs)	A SAR, the nature and severity of which is not consistent with the Reference Safety Information (RSI) for the IMP.

Table 1. Definitions of Adverse events

***Note:** The term 'life-threatening' in the definition of serious refers to an event in which the trial participant was at risk of death at the time of the event or it is suspected that use or continued use of the product would result in the subject's death; it does not refer to an event which hypothetically might have caused death if it were more severe.

**** Note:** Hospitalisation is defined as an inpatient admission, regardless of the length of stay, even if the hospitalisation is a precautionary measure for continued observation. Pre-planned hospitalisation e.g. for pre-existing conditions which have not worsened, or elective procedures, does not constitute an SAE.

***** Note:** other events that may not result in death, are not life-threatening, or do not require hospitalisation, may be considered as an SAE when, based upon appropriate medical judgement, the event may jeopardise the participant and may require medical or surgical intervention to prevent one of the outcomes listed above.

15.3 Optimise-FLT3 Specific SAE Reporting requirements

In addition to the items defined as Serious Adverse events in the table 1 above, the events specified below should be reported as SAEs unless fitting the exemptions that are given in each case.

Grade 5: Events resulting in death
EXCEPT: Where the primary cause of death is persistent or progressive AML

Grade 4 Life Threatening and/or disabling non-haematological toxicity of any duration
INCLUDING: Neutropenic Sepsis / Neutropenic Fever at Grade 4 (any duration)

Grade 3: Severe non-haematological toxicities lasting ≥ 7 days*
INCLUDING: Neutropenic Fever / Febrile Neutropenia persisting for ≥ 7 days *This includes:* - *Infection (documented clinically or microbiologically) with Grade 3 or 4 neutrophils* - *Fever with Grade 3 or 4 neutrophils in the absence of documented infection - classified as febrile neutropenia*

Grade 3/4: Severe haematological toxicity* lasting ≥ 42 days from the day after completion of chemotherapy (excluding Midostaurin), with bone marrow blasts less than 5%

Hospitalisation or prolonged hospitalisation
EXCEPT: If the cause of hospitalisation is an expected consequence of AML or its treatment** - *Neutropenic fever/ febrile neutropenia G3 or lower <7 days AND its expected consequences leading to hospitalisation does not need to be reported as an SAE (unless meeting other SAE criteria e.g. G3≥7 days or G4/ 5)*

Development of Veno-occlusive Liver Disease (VOD) of any grade or duration

Event which results in cancer

*A list of adverse events that may be classified as 'haematological toxicities' is provided in **Appendix G** (any other events are considered non-haematological and should be reported accordingly)

** A list of events that may be considered as 'expected consequence of AML and its treatment' is provided in **Appendix G**. These events do not require reporting as SAEs unless they meet other SAE reporting criteria (e.g. grade 3 non-haematological toxicity persisting ≥7 days, or grade 4/5 toxicity of any duration).

Participants may enter the trial with pre-existing conditions which meet the criteria set out above, but it is only the development of these toxicities after entering the trial which should be reported.

Please note:

- The initial day of event reaching the reportable grade should be considered day 0.
- If the grade fluctuates, this is not reportable unless it meets the criteria required for SAE reporting (e.g. non-haematological G3 maintained ≥7 days, or G3/4 neutropenia lasting ≥42 days from end of chemotherapy. Fluctuation between G3/4 neutropenia would not prevent the event being reported if it lasted ≥42 days in total, and the worst grade would be noted on the SAE form).
- 'Chemotherapy' does not include targeted molecular therapy (Midostaurin).
- Only Grade 5 events considered to be the primary cause of death need to be reported as SAEs.

15.4 Causality assessment

The causal relationship of the SAE will be assessed by both the PI (or other delegated medically qualified doctor) at the reporting site and the CI/PV teams for IMPs, other trial treatments (nIMPs) and procedures according to the table 2 below.

IMPs: Mylotarg, Midostaurin (courses 1 + 2), Daunorubicin, Cytarabine (courses 1 + 2), individual components of FLAG-Ida: Fludarabine, Idarubicin, G-CSF **nIMPs:** Cytarabine (in consolidation courses 3+4), Midostaurin (in consolidation courses 3 +4 and in maintenance)

Relationship	**Description**	**Reasonable possibility that the SAE may have been caused by the IMP/nIMP?**
Unrelated	There is no evidence of any causal relationship with the trial/intervention	No
Unlikely	There is little evidence to suggest there is a causal relationship with the trial/intervention (e.g. the event did not occur within a reasonable time after administration of the trial medication). There is another reasonable explanation for the event (e.g. the participant's clinical condition, other concomitant treatment).	No

Possible	There is some evidence to suggest a causal relationship with the trial/intervention (e.g. because the event occurs within a reasonable time after administration of the trial medication). However, the influence of other factors may have contributed to the event (e.g. the participant's clinical condition, other concomitant treatments).	Yes
Probable	There is evidence to suggest a causal relationship and the influence of other factors is unlikely.	Yes
Definite	There is clear evidence to suggest a causal relationship and other possible contributing factors can be ruled out.	Yes

Table 2. Table for causality assessment

The causality assessment given by the Principal Investigator (or delegate) cannot be downgraded by the Chief Investigator (or delegate), and in the case of disagreement both opinions will be provided.

15.5 Assessment of 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 whether the event is listed in 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. Fatal and life-threatening (LT) SARs should not be considered expected (unless explicitly stated in the RSI and approved by the NCA). For example, an event more specific or more severe than that described in the RSI is considered unexpected.

The CTR will evaluate expectedness on events reported as serious, to determine whether or not the case qualifies for expedited reporting, using the approved RSI in section 4.8 of the SmPC for:

- Gemtuzumab Ozogamicin (Mylotarg)
- Midostaurin (when this is used within the trial as a component of DA-Mido, DA-Mido+GO, FLAG-Ida-GO-Mido regimens) (Course 1 and 2)
- Daunorubicin (as a component of both DA-Mido or DA-Mido+GO) (Course 1 and 2)
- Cytarabine (when this is used as a component of DA-Mido, DA-Mido+GO and FLAG-Ida-GO-Mido) (Course 1 and 2)
- Fludarabine
- Idarubicin
- G-CSF

Details of the relevant RSIs to be used for expectedness will be detailed in the trials Safety Management Plan, and updated accordingly to reflect any changes to the approved RSIs.

Reference Safety Information (RSI) on any CTR trial will be reviewed regularly according to CTR procedures.

15.6 Reporting procedures

15.6.1 Participating Site Responsibilities

The PI (or delegated medically qualified doctor from the trial team) should sign and date the SAE CRF to acknowledge that he/she has performed the seriousness and causality assessments. Investigators should also report SAEs to their own health boards or trust in accordance with local practice.

A completed SAE form for all events requiring immediate reporting should be submitted via email to the CTR within 24 hours of knowledge of the event. A separate form must be used to report each event, irrespective of whether or not the events had the same date of onset.

Sites are reminded that all AEs including SAEs are to be recorded and reported from the date of consent.

The participant will be identified only by trial number, date of birth or year of birth and initials. The participant's name (or any other personal identifiers) should not be used on any correspondence.

It is also required that sites respond to and clarify any queries raised on any reported SAEs and report any additional information as and when it becomes available through to the resolution of the event. Additionally, CTR/pharmaceutical companies may request additional information relating to any SAEs/SARs and the site should provide as much information as is available to them in order to resolve these queries.

Serious Adverse Event (SAE) email address:

CTR-Safety@Cardiff.ac.uk

SAE Fax number:

0203 0432 376

An SAE form should contain at least the minimum information:

- Full participant trial number
- An Adverse Event / Adverse Reaction
- IMP or trial intervention
- A completed assessment of the seriousness, and causality as performed by the PI (or another appropriately medically qualified doctor registered on the delegation log)

If any of these details are missing, the site will be contacted and the information must be provided by the site to the CTR within 24 hours.

All other AEs should be reported on the CRF following the CRF procedure described in Section 15.

15.6.2 CTR responsibilities

Following the initial report, all SAEs should be followed up to resolution wherever possible, and further information may be requested by the CTR. Follow up information must be provided on a new SAE form.

The CTR should continue reporting SAEs until 28 days after the participant receives their last dose of the protocol treatment. Serious adverse reactions should continue to be reported until the end of follow up.

Once an SAE is received at the CTR, it will be evaluated by staff at the CTR and sent to the Chief Investigator (or their delegate) for an assessment of expectedness.

Investigator reports of suspected SARs will be reviewed immediately and those that are identified as SUSARs are reported to the MHRA and Main Ethics Committee.

15.7 SUSAR reporting

Cardiff University is undertaking the duties of trial Sponsor and has delegated to the CTR the responsibility for reporting SUSARs and other SARs to the regulatory authorities (NCAs and relevant ethics committees) 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.

SUSARs that are not fatal or life-threatening must be reported to the MHRA and REC within 15 days of receipt at the CTR.

If a report is incomplete then additional follow-up information should be reported within a further 8 calendar days of submitting the initial report, for all fatal and non-fatal, life-threatening and non-life-threatening

Any additional, relevant information must be reported within above timescales.

N.B. There is no requirement for the CTR to report SUSARs to nIMPs to the MHRA except in the following instances:

- If the adverse reaction is suspected to be linked to an interaction between a nIMP and IMP, and is serious and unexpected, CTR should report as a SUSAR due to the interaction with the IMP.
- If a SUSAR is suspected and might be linked to either a nIMP or an IMP and cannot be attributed to only one of these.
- If the adverse reaction due to the nIMP is likely to affect the safety of trial subjects then CTR should report it to the MHRA and REC in accordance with the relevant Standard Operating Procedure for reporting Urgent Safety Measures.

15.8 Safety Reports

A list of all SARs (expected and unexpected) will be reported annually to the MHRA REC and trial sponsor in the form of a Development Safety Update Report (DSUR). This report must be submitted within 60 days of the anniversary of the MHRA CTA approval date.

The CTR will report a list of all SARs (expected and unexpected) and any other safety recommendations to all PIs annually throughout the course of the trial. This frequency may be reviewed and amended as necessary. This reporting will be done via the Investigator safety report (ISR).

15.9 Enhanced Pharmacovigilance

The FLAG-Ida-GO-Mido schedule has not yet been piloted. As this is a novel combination, this will require enhanced pharmacovigilance. Safety data will be collected on a weekly basis following commencement of FLAG-Ida-GO-Midostaurin. CTR will communicate with sites on a regular basis to ensure timely data completion.

15.10 Urgent Safety Measures (USMs)

An urgent safety measure is an action that the Sponsor, Chief Investigator or Principal Investigator may carry out in order to protect the subjects of a trial against any immediate hazard to their health or safety. Any urgent safety measure relating to this trial must be notified to the MHRA and Research Ethics Committee immediately by telephone, and in any event within 3 days in writing, that such a measure has been taken. USMs reported to the CTR will be handled according to CTR processes.

16 Contraception and pregnancy

16.1 Contraception

FLAG-ida, Mylotarg and Midostaurin in this trial have a demonstrated or suspected human teratogenicity/fetotoxicity.

Women of Child Bearing Potential (WOCBP) are defined as fertile, following menarche and until becoming post-menopausal unless permanently sterile. Permanent sterilisation methods include hysterectomy, bilateral salpingectomy and bilateral oophorectomy. A postmenopausal state is defined as no menses for 12 months without an alternative medical cause. A high follicle stimulating hormone (FSH) level in the postmenopausal range may be used to confirm a postmenopausal state in women not using hormonal contraception or hormonal replacement therapy (HRT). However, in the absence of 12 months of amenorrhea, confirmation with more than one FSH measurement is required.

(WOCBP) entering into this trial must agree to use a highly effective method of contraception preferably with low user dependency for at least six months after the last dose of IMP. To align with SmPCs, this should be increased for gemtuzumab ozogamicin and idarubicin to 7 and 6.5 months,

respectively. A highly effective method of contraception is considered as having a failure rate of less than 1% per. Some acceptable contraception methods are listed below;

- combined (estrogen and progestogen containing) hormonal contraception associated with inhibition of ovulation:
    - oral
    - intravaginal
    - transdermal
- progestogen-only hormonal contraception associated with inhibition of ovulation
    - oral
    - injectable
    - implantable*
- intrauterine device (IUD)*
- intrauterine hormone-releasing system (IUS)*
- bilateral tubal occlusion*
- vasectomised partner*
- sexual abstinence defined as refraining from heterosexual intercourse during the entire period of risk associated with the trial treatments.

N.B. periodic abstinence (calendar, symptothermal, post-ovulation methods), withdrawal (coitus interruptus), spermicides only, and lactational amenorrhoea method (LAM) are not acceptable methods of contraception.

**These contraception methods are considered to be low user dependency.*

Male participants with a WOCBP partner should use condom during treatment and at least until six months after the last dose of IMPs. For a non-pregnant WOCBP partner, contraception recommendations should also be considered.

16.2 Pregnancy reporting whilst participating in the trial

Pregnancy, or the pregnancy of a partner occurring whilst participating in the trial, is not considered an SAE, however, a congenital anomaly or birth defect is. Other cases (e.g. termination of pregnancy without information on congenital malformation, and reports of pregnancy exposure without outcome data) should not normally be reported as such. When pregnancy occurs in a trial, either in a female participant or the female partner of a male participant, this should be followed up until at least the end of pregnancy, whether that is a live birth, abortion etc. Without follow-up of the pregnancy,

it would not be possible for the CTR to know if a congenital anomaly or birth defect occurred, and therefore if there was an SAE that must be included in the safety evaluation of the IMP. In the event of a participant or partner becoming pregnant, a Pregnancy Information Sheet will be provided and information on a pregnancy in a trial participant will be captured on the CTR Pregnancy Report Form supplied to sites by the CTR. If a participant becomes pregnant during the trial, they should be withdrawn from trial treatment and discuss continuing treatment options with their local clinical team who may seek advice from the chief investigator If the participant is willing, and consents to do so, they can continue to provide follow up data for the remainder of the trial.

Sites should report pregnancy occurring within SAE reporting periods stipulated in the trial protocol (for example, some trial protocols may state that SAEs should be reported during the trial treatment period and up to 30 days after the last date of treatment, this timeline would also apply to the reporting of pregnancies). Congenital anomalies or birth defects are considered an SAE and so these events must also be reported to the CTR on a trial-specific SAE form. Congenital anomalies or birth defects related to the IMP and unexpected with respect to the IMP Reference Safety Information (RSI) must be submitted by the CTR within expedited SUSAR time frames (7 or 15 days) to the MHRA, relevant REC and the drug manufacturer of the IMP (to comply with any contractual agreement).

17 Statistical considerations

17.1 Randomisation

At trial entry participants will be randomised (1:1:1) between the three treatment arms, balancing randomisation by FLT3 mutation type, NPM1 status and age.

17.2 Sample size

The trial is powered on EFS as the primary outcome. In the RATIFY trial, the median EFS in the DA-Midostaurin arm was 8 months[16], but the definition of EFS in that study included CRi as an event. The AMLSG-1610 trial[40], a single arm evaluation of DA-Midostaurin combination therapy in 440 participants, used our EFS definition and, like Optimise-FLT3 also included participants in the 60-70 age range; participants receiving DA-Midostaurin in AMLSG-1610 had a median EFS of 13.6 months and a 2yr EFS of 41%. Our rationale for expected effect size in Optimse-FLT3 is based on evidence from *FLT3*-mutated participants in the AML19 trial[14]. We calculated 2yr EFS for the existing AML19 arms, DA-GO2 (45%) and FLAG-Ida-GO (60%), assuming that adding midostaurin will add a further ~10% EFS, then we may expect an HR of about 0.67 for the DA-GO-Mido group and 0.4 for the FLAG-Ida-GO-Mido group (compared to 41% EFS in AMLSG-1610.

The nstage MAMS program in Stata was used to calculate the sample size for a 3-arm, 2-stage trial with a time to event outcome of EFS in both stages. The parameters in stage 1 are based on a phase 2 screening design, with an expected median EFS of 13.6 months in the control arm to calculate the number of events needed, assuming a relaxed one-sided type 1 error of 30%, power 90%, 1:1:1

allocation ratio, hazard ratio 0.64, and a recruitment rate of 8 per month. This yields a total sample size of 236 participants for stage 1, with 96 events needed.

In stage 2, the parameters were selected to reflect a phase 3 design, with one-sided type 1 error of 2.75%, power 85%, hazard ratio 0.64 and a recruitment rate of 10 per month, with an anticipated stop to recruitment at month 44, and non-binding stopping rules. This yields a final sample size of 381, with 258 events needed in total. The AML1610 trial showed a tail off in event rate at two years and, to allow for potential recruitment delays, we anticipate that we will reach the required number of events by month 68, when all active participants have had 2 years follow-up. The sample size has been rounded up to 390 participants to account for 2.5% expected loss to follow-up (based on previous NCRI AML trials in this patient group).

If, after stage 1, FLAG-Ida-GO-Mido is dropped from the randomisation of the *NPM1* non-mutated subgroup (see 17.1.1), we would still have an estimated 104 evaluable participants in this arm, and depending on follow-up we should still have 85% power to detect a meaningful difference of around HR 0.62 to 0.64 in this arm.

17.3 Missing, unused & spurious data

There will be no data imputation for missing data in the primary endpoints. Imputation methods may be proposed for purposes of sensitivity analysis—imputation methods for missing data in the primary endpoint and secondary endpoints will be fully documented in the Statistical Analysis Plan (SAP).

Time to event data will be censored at the date the participant last had a blood count performed if no EFS event is recorded during the trial, or date last seen (or successfully contacted by telephone) for overall survival.

17.4 Procedures for reporting deviation(s) from the original SAP

Major deviations from the original SAP which deviate from the plan stated in the protocol will be submitted as substantial amendments where applicable and recorded in subsequent versions of the Protocol and SAP. Any deviation(s) from the final statistical plan will be described and justification given in the final report.

17.5 Termination of the trial

Decisions on the termination of the trial are the responsibility of the TSC with guidance from the IDMC. The adaptive MAMS design will provide the option to terminate the trial after the stage 1 interim analysis if neither of the two experimental arms meet the critical hazard ratio. However, the stopping-rules in the MAMS design are non-binding and there is no obligation for the IDMC to recommend termination if they consider that there is a benefit to continuing.

17.6 Inclusion in analysis

Under the treatment policy strategy, all participants randomised into the trial will be included in the primary analysis of EFS, regardless of whether they received their allocated treatment. Participants with no follow-up data will be censored at day 1. Intercurrent events of treatment discontinuation, change to planned treatment and stem cell transplant will not affect the primary analysis. All participants who received at least one dose of trial treatment will be included in the safety analysis.

17.7 Main analysis

The primary estimand targets the effect of FLAG-Ida-GO-Midostaurin and DA-GO-Midostaurin compared to DA-Midostaurin in adult participants with newly diagnosed FLT3 mutated AML. EFS is defined as the time from randomisation until death from any cause, failure to achieve CR, CRh or CRi after two chemotherapy cycles, MRD relapse, and frank relapse. The event date for participants that fail to achieve CRi is assumed to be the date of randomisation. Participants not evaluable for the initial response assessment will be censored on day 1. Participants known to be alive and achieving a response will be censored on the date of the last blood test showing them to be relapse free. Participants will be assessed for EFS until two years after the last randomisation. Intercurrent events such as treatment discontinuation change to planned treatment and stem cell transplants will be ignored under the treatment policy strategy.

The analysis of the primary outcome (EFS) will take place using the Cox proportional hazards model after the required number of events is recorded in each stage. A point estimate of the HR will be calculated, (adjusting for ITD and, *NPM1* status at randomisation and age) with a one sided 70% upper confidence interval in stage 1 and a one-sided 97.25% upper confidence interval in stage 2; both these confidence intervals should exclude 1 in order to reject the null hypothesis. A Kaplan-Meier curve will also be plotted for EFS.

Secondary outcomes will be analysed by trial arm and will include the following:

- Incidence of complete remission (CR, CRh and CRi by ELN2022) within 2 cycles, this will be presented as a percentage and 95% confidence interval.
- Number and percentage of deaths within 30 and 60 days from randomisation, with 95% confidence interval.
- Overall survival time, measured in days from the date of randomisation until the date of death from any cause, this will be presented as median and interquartile range.
- Time to haematological relapse, measured from the date of documentation of 1st CR, CRi or CRh until the date of frank relapse, this will be presented as median and interquartile range.
- The number and percentage of participants with MRD negativity after cycle 2 by RT-qPCR (for NPM1^{mut}) or flow cytometry, this will be presented with 95% confidence intervals.
- Time to MRD relapse for participants with a monitored MRD marker, measured from the date of first molecular complete remission, until the date of MRD relapse (as defined by the ELN2022 [23]). This will be presented by median and interquartile range.

- Cumulative incidence of grade 3 and 4 toxicity over the duration of follow-up, this will be presented as a percentage and 95% confidence interval.
- Cumulative resource use including hospital admission days, blood product usage and days on intravenous antibiotics and antifungals, this will be presented as a percentage for each resource use and 95% confidence interval.
- Rates of allogeneic stem cell transplant, this will be presented as the percentage of participants with 95% confidence interval. Additionally, the number of days from randomisation to transplant will be reported.
- Health related quality of life assessed during treatment and over 2 years of post-treatment follow-up, this will be summarised for each QoL domain for each timepoint, and will be tabulated with median scores and interquartile ranges, and will also be graphically represented to show change of scores over time.

Kaplan-Meier curves will be plotted for all time to event outcomes.

17.8 Sub-group & interim analysis

At approximately 30-34 months (depending on recruitment and event rate), an interim analysis will be performed (using Cox proportional hazards) following the recording of 96 events, (randomisation will continue) and if the observed HR is 0.879 or less for each comparison, then one or both arms will proceed to stage 2

At the stage 1 interim, if the HR for the FLAG-Ida-GO-Mido exceeds the critical HR, there will also be a subgroup analysis of the *NPM1* mutated and non-mutated subgroups. If a benefit of FLAG-Ida-GO-Mido is only apparent in the mutated group, the IDMC will have the discretion to drop the non-mutated subgroup from this randomisation.

Please see section 19 for details of the translational analysis plan.

18 Data Management

Trial data	***Source Data***				
	eCRF	*Participant medical notes*	*Pharmacy File*	*Questionnaire*	*SAE form*
Medical History	*X*				
Concurrent Medications		*X*			
Serious Adverse events					*X*
Adverse Events	*X*				
Physical examination	*X*				
Vital signs	*X*				

WHO/ ECOG performance status	*X*				
Height, Weight, BSA	*X*				
Laboratory tests	*X*				
Pregnancy tests	*X*				
Bone Marrow Aspirate/Biopsy	*X*				
Randomisation	*X*				
Investigational product dispensing	*X*				
Remission Assessment	*X*				
Stem Cell Transplant (if applicable)	*X*				
Quality of life assessments	*X*				
Treatment compliance	*X*				
Supportive care requirements		*X*			

Table 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 time for any data element, as defined in site source data agreement.

Source data (Table 4) include, but are not limited to, hospital records (from which medical history and previous and concurrent medication may be summarised into the CRF), clinical and office charts, laboratory and pharmacy records, Quality of life questionnaires, radiographs, and correspondence. CRF entries will be considered source data if the CRF is the site of the original recording (e.g., there is no other written or electronic record of data). All documents will be stored safely in confidential conditions. Sites will retain all original source of data from these investigations for future reference. On all trial-specific documents, other than the signed consent form, the participant will be referred to by the trial participant ID, not by name.

18.1 Data collection

Participant randomisation and data collection will take place via electronic online databases. Further information can be found in the Optimise-FLT3 site manual.

18.2 Completion of CRFs

Data should be submitted via the web-based system. This is a secure encrypted system accessed by an institutional password, and complies with General Data Protection Regulations (GDPR) and Data Protection Act 2018 standards. The system can be accessed on:

https://optimise.ctr.cardiff.ac.uk/

A unique username and password will be supplied to investigators upon completion of all processes required prior to opening including training on the data collection tools and completed delegation log.

Forms that may impact trial procedure and therefore should be completed as quickly as possible include:

- Medical History form (completed by site staff)
- Cytogenetics form (completed by local cytogeneticist)
- Response to course forms (completed by site staff)
- *FLT3*/*NPM1*, Sequential Monitoring and Flow MRD forms (completed by Optimise-FLT3 laboratories)

For further information on required data, guidance on how to complete forms, and additional information on when forms should be completed and by whom- please contact to the AML19 team.

If missing or illogical data are identified, a data query will be raised on a data clarification form. The data clarification form will be sent to the relevant participating site. The site shall be requested to respond to the data query on the data clarification form and update the database.

All answered data queries and corrections should be signed off and dated by a delegated member of staff at the relevant participating site. The completed data clarification form should be returned to the CTR

The CTR will send reminders for any overdue data. It is the site's responsibility to submit complete and accurate data in timely manner.

19 Translational research – evaluation of FLT3 ITD NGS MRD testing

We will evaluate whether MRD testing using the *FLT3* ITD mutation as a target can improve selection of participants for upfront SCT and provide improved relapse prediction over current standard testing. This will inform whether *FLT3* ITD NGS should be adopted by the NHS Genomic Medicine Service (GMS), influencing future treatment algorithms and clinical trial development.

Advances in NGS technology and bioinformatics now allow the detection of *FLT3* ITD mutations with very high specificity and sensitivity[27,43] allowing this to be used as an MRD marker. Selection of participants with *FLT3* ITD for SCT in CR1 is currently controversial, particularly in participants with the genotype *NPM1*^{mut} *FLT3*$^{ITD+}$. Here practice varies by centre and country; while many advocate that all

such participants receive upfront SCT, current UK practice is to use *NPM1* MRD to identify participants at highest risk of relapse who are more likely to benefit from SCT. For these participants, we will investigate whether *FLT3* ITD NGS MRD can allow more accurate risk stratification and therefore more rational treatment allocation in future.

In AML17, we showed that the detection of *NPM1* mutant transcripts in PB after two cycles of chemotherapy (PB PC2+) is associated with a very high risk of relapse (86% at 3 years, rising to 92% in participants with *FLT3* ITD at baseline). In the UK these participants are now routinely directed towards CR1 SCT[16]. However, relapse also occurs in ~35% of participants testing PB PC2 *NPM1* MRD negative **(Fig.9**) and BM *NPM1* MRD status at this time point does not improve prognostic discrimination[16]. Our primary hypothesis is that *FLT3* ITD MRD testing will identify a subset of PB PC2 *NPM1* MRD negative participants at much higher risk of relapse, which may justify upfront SCT.

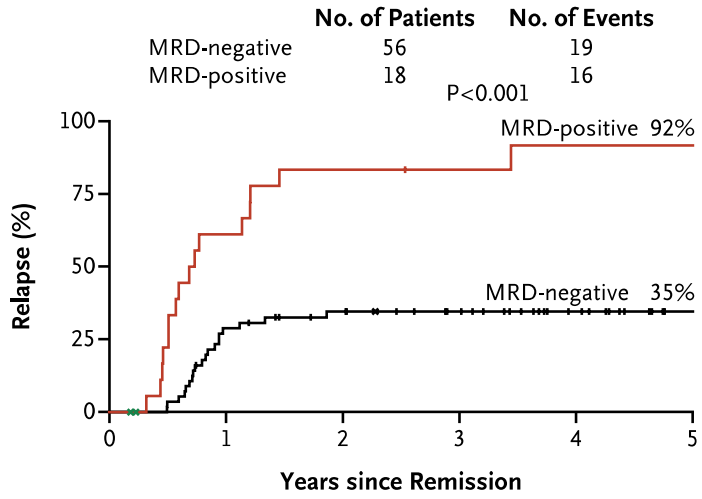


Figure 9. Risk of relapse in participants with *FLT3*-ITD mutation according to *NPM1* MRD status in AML17. Reproduced from Ivey A *et al*, NEJM 2016[15]

Four studies have now shown that *FLT3* ITD MRD is strongly associated with relapse and may add additional prognostic power to that provided by *NPM1* MRD. At post-induction time points, the HOVON co-operative group (n=161) showed that participants testing MRD positive for both *FLT3* and *NPM1* had a 2yr OS of 22% vs 45% in those testing positive in the *NPM1* assay only[28]. In AMLSG 1610 (n=142) participants testing *FLT3* ITD NGS MRD positive after cycle 2 had a 4yr OS of 42% vs 70% in those testing negative, again with evidence of additional prognostic discrimination compared to the *NPM1* assay[29]. These data are supported by two additional studies in the pre-transplant setting. The US NIH "Pre-Measure" study (n=266 $FLT3^{mut}NPM1^{mut}$) showed a 2y CIR of 75% in participants testing MRD positive for both *FLT3* and *NPM1* mutations compared with 53% in those testing *NPM1* MRD positive only and 23% in those testing negative in both assays[30]. Finally, in a pooled analysis of pre-transplant samples in UK NCRI AML17 and the Australian ALLG studies, participants testing both *FLT3* ITD NGS and *NPM1* MRD positive prior to SCT had a 2yr OS of 20% vs 65% in participants testing

positive in the *NPM1* assay alone[31]. The *FLT3* ITD NGS MRD assay therefore appears to identify a population of participants at particularly high risk of relapse. However, although highly compelling, these data cannot yet be used for clinical decision making; some were gathered prior to the incorporation of FLT3 inhibitors alongside frontline chemotherapy and, crucially, there has not yet been a direct comprehensive and adequately powered prospective comparison with current standard-of-care MRD testing and outcomes with and without transplant.

We will also evaluate the utility of *FLT3* ITD MRD in participants with baseline *FLT3* ITD without *NPM1* co-mutation as an exploratory objective. In the UK, based on historical data obtained before the advent of *FLT3* inhibitors[42], all such participants with a baseline *FLT3* allelic ratio >0.05 are currently directed to CR1 SCT. We will therefore evaluate the outcomes of participants in this group who do not undergo CR1 SCT (due to fitness, lack of donor or other reasons) according to their *FLT3* ITD MRD status aiming to identify participants that achieve a good outcome in the absence of CR1 SCT.

For this work, we propose to use the modified getITD assay (**Fig.10**), laboratory validation of which has already been completed[27,30]. Using a sequencing depth of 1-2 million reads per participant, specificity and sensitivity of 100% for detection to $1:10^{-4}$ can be achieved. We plan to perform a detailed comprehensive prospective clinical validation, initially focussing on the bone marrow samples collected after cycle 2 from all participants with a baseline *FLT3* ITD mutation. Genomic DNA will be extracted from excess material stored after standard of care MRD testing has been completed and the getITD assay will be performed in batches to minimise costs, since results will not be used to influence treatment within the study. Results will be correlated with standard of care MRD testing and clinical outcomes. The main clinical outcome measures of interest are cumulative incidence of relapse and overall survival. We will also analyse the effect of SCT in CR1 according to *FLT3* ITD MRD status using time-dependent analyses as described below. Samples from all other standard MRD timepoints will be stored and available for additional exploratory analyses if required. These will depend on the results of our initial analyses and published findings from other groups but could include analysis of the effect of the *FLT3* ITD MRD at the end of treatment, prior to transplant and at molecular relapse. In order for this assay to be ready for implementation within the NHS GMS if clinical utility is demonstrated, we have already initiated work to ensure inter-laboratory concordance in partnership with UK NEQAS who have already performed a pilot *FLT3* ITD NGS MRD EQA exercise in March 2023 with initial results from the 8 participating laboratories indicating high levels of inter-laboratory concordance. We have also engaged with the NHSE GMS to discuss the potential addition of *FLT3* ITD MRD to the national test directory.

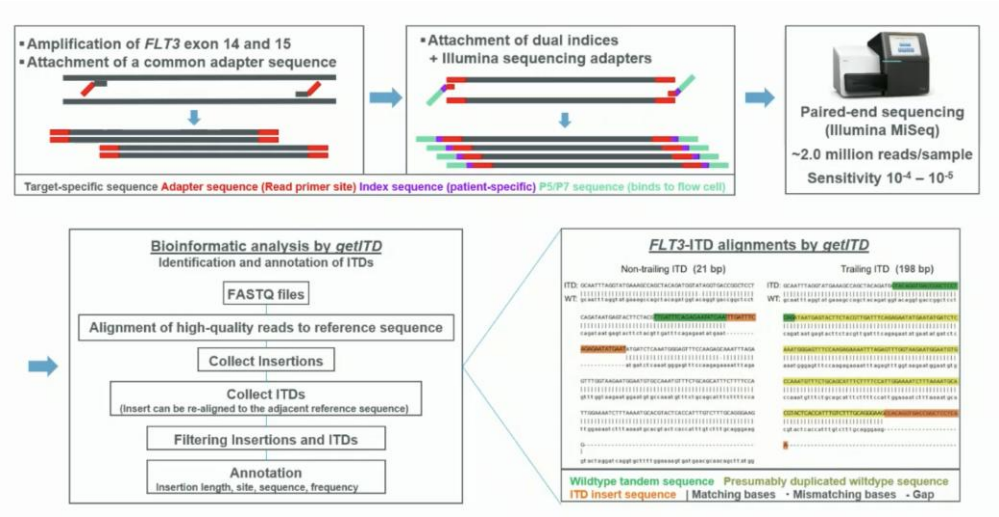


Figure 10. *FLT3* ITD MRD detection using the GetITD workflow (reproduced from references [27,30]).

Our primary hypothesis is that *FLT3* ITD MRD testing applied to the BM aspirate taken after the second cycle of chemotherapy will identify participants who are PB PC2 *NPM1* MRD negative but who are at an elevated risk of relapse. Of 390 participants, we expect approximately 48% to have the genotype *NPM1*^{mut} *FLT3*$^{ITD+}$ based on rates observed in AML19. Of these we expect up to 20% to be PB PC2 *NPM1* MRD positive (i.e. already high risk). Allowing for 10% dropout due to induction death, induction failure, withdrawal from trial and sampling errors, we estimate that evaluable PC2 bone marrow aspirate samples will be available from 134 PB PC2 *NPM1*MRD negative participants for this analysis. We assume that 25% of these will test *FLT3* ITD MRD positive based on published studies showing positivity rates of 22-36%[28-30]. With α=0.05, 134 samples will provide 90% power to detect a clinically relevant 33% absolute difference in cumulative incidence of relapse (i.e. 60% vs 27% in those testing *FLT3* ITD MRD positive and negative, respectively).

Our exploratory hypothesis is that *FLT3* ITD MRD can also inform selection for transplant in participants with *FLT3* ITD (with baseline allelic ratio >0.05) but no *NPM1* mutation. We estimate that this genotype will account for ~31% of trial participants and ~30% of these will not undergo transplant due to fitness, donor availability, physician decision or participant choice, based on rates observed in AML19. This leaves us with ~36 samples from those not undergoing transplantation. We assume that 33% of the PC2 bone marrow aspirate samples from these participants will test *FLT3*-ITD MRD positive, therefore 36 samples will provide 83% power to detect an absolute difference in cumulative incidence of relapse of 40% (i.e. 70% vs 30% in those testing *FLT3* ITD MRD positive and negative, respectively, based on published data from the HOVON study[26]) using one-sided α=0.10. In a further exploratory analysis, we will evaluate the effect of CR1 transplant on overall survival in this genotype considering transplant as a time-dependent variable using the Mantel Byar method. The analysis will be stratified by *FLT3* ITD MRD status to identify whether there is preliminary evidence of an interaction between MRD status and benefit from transplant in this group.

20 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.

21 End of Trial definition

The treatment phase will be followed by a follow-up period which will continue for two years after the last participant completes protocol treatment.

The end of the trial is defined as the date of final data capture to meet the trial endpoints. In this case, end of trial is defined as last participant last visit, this will be determined when sufficient events have been reached and all sites will be notified when this is the case.

The Sponsor will notify the MHRA and main REC of the end of the trial within 90 days of its completion or within 15 days if the trial is terminated early.

22 Archiving

The TMF and TSF containing essential records 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 on approval from Sponsor and documents should be retained for 25 years after the conclusion or early termination of the trial on approval from the Sponsor. Essential records pertaining to the trial shall not be destroyed without permission from the Sponsor.

23 Regulatory Considerations

23.1 CTA

This trial has Clinical Trials Authorisation (CTA) from the UK Competent Authority: MHRA.

Regulatory approval will be sought for international sites as per local requirements.

23.2 Ethical and governance approval

This protocol has approval from a Research Ethics Committee (REC) that is legally “recognised” by the United Kingdom Ethics Committee Authority for review and approval.

This trial protocol will be submitted through the relevant permission system for global governance review dependant on the location of the lead site e.g. HCRW/HRA.

Approval will be obtained from the host care organisation who will consider local governance requirements and site feasibility. The Research Governance approval of the host care organisation must be obtained before recruitment of participants within that host care organisation.

Ethics approval will be sought for international sites as per local requirements.

23.3 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 and The Data Protection Act 2018. The data custodian and the translational sample custodian for this trial is the Cardiff University.

23.4 Indemnity

- Non-negligent harm: This trial is an academic, investigator-led and designed trial, coordinated by the CTR. The Chief Investigator, local Investigators and coordinating centre do not hold insurance against claims for compensation for injury caused by participation in a clinical trial and they cannot offer any indemnity. The Association of the British Pharmaceutical Industry (ABPI) guidelines will not apply.

- Negligent harm: Where studies are carried out in a hospital, the hospital continues to have a duty of care to a participant being treated within the hospital, whether or not the participant is participating in this trial. Cardiff University does not accept liability for any breach in the other hospital's duty of care, or any negligence on the part of employees of hospitals. This applies whether the hospital is an NHS Trust or not. The Sponsor shall indemnify the site against claims arising from the negligent acts and/or omissions of the Sponsor or its employees in connection with the Clinical Trial (including the design of the Protocol to the extent that the Protocol was designed solely by the Sponsor and the Site has adhered to the approved version of the Protocol) save to the extent that any such claim is the result of negligence on the part of the Site or its employees.

All participants will be recruited at NHS sites and therefore the NHS indemnity scheme/NHS professional indemnity will apply with respect to claims arising from harm to participants at site management organisations.

23.5 Trial sponsorship

Cardiff University will act as Sponsor for trial. Delegated responsibilities will be assigned to the sites taking part in this trial.

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 Data Protection Act 2018.
- The Human Tissue Act 2004.
- Other regulatory requirements as appropriate.

The trial will be conducted in compliance with the protocol, the EU regulation and Good Clinical Practice as required by the regulations.

The Sponsor has/will be delegating certain responsibilities to Cardiff University (CTR), the Chief Investigators, Principal Investigators, host sites and other stakeholder organisations as appropriate in accordance with the relevant agreement that is informed by regulation and trial type.

23.6 Funding

Optimise-FLT3 is funded by Cancer Research UK.

24 Trial management

24.1 Trial Management Group (TMG)

The role of the TMG is to monitor all aspects of the day-to-day conduct and progress of the trial, ensure that the protocol is adhered to and take appropriate action to safeguard participants and the quality of the trial itself. The Optimise-FLT3 TMG will meet regularly, the frequency of meetings may potentially varying depending on the stage of the trial. The Trial Management Group will include individuals responsible for the day-to-day management of the trial, including the Chief Investigator, Statistician, Trial Manager, Data Manager and Patient Representatives.

The TMG will operate in accordance with a trial specific charter. TMG members will be required to sign up to the remit and conditions as set out in the TMG Charter.

24.2 Trial Steering Committee (TSC)

The role of the independent steering committee is to provide oversight of the conduct of the trial. This includes oversight of the practical aspects of the study and ensuring that the study continues to be run in a way which is both safe for the participants and provides appropriate safety and efficacy data to the sponsor and investigators. The TSC will have oversight of participant recruitment, data completeness, losses to follow-up and deviations from the protocol. In discharging its safety role, the Steering Committee will work in conjunction with the Independent Data Monitoring Board that will

also be established for the trial (see below). Membership will include an independent clinician, independent statistician, independent specialist with expertise in sample analysis, a representative from the sponsor and at least one patient advocate. Selected members of the TMG, including the chief investigator, trial statistician and co-investigators will report to the TSC.

The Steering Committee will have ultimate responsibility for the trial and will assume primacy over the Independent Data Monitoring Committee or chief investigator. The Steering Committee can prematurely terminate the trial. The sponsor and chief investigator will agree, in writing prior to the start of the study, to the Charter of the Steering Committee.

TSC members will be required to sign up to the remit and conditions as set out in the TSC Charter. The TSC will meet shortly before commencement of the trial and then 6-monthly thereafter.

24.3 Independent Data Monitoring Committee (IDMC)

The role of the Independent Data Monitoring Committee is to safeguard the interests of the trial's participants and to monitor the data collected in the trials. Data analyses will be provided in confidence to the IDMC, which will be asked to give advice on whether the accumulated data, together with the results from other relevant research, justifies the continuing recruitment of further participants.

The Independent Data Monitoring Committee may recommend modifications to the data management and monitoring procedures in the trial or to the trial protocol. Every effort will be made to reach a consensus within the Independent Data Monitoring Committee and with the chief investigator. In case of disagreement on recommended modifications, the Steering Committee will decide. The sponsor and principal investigator will agree, in writing prior to the start of the study, to the charter of the Independent Data Monitoring Committee. IDMC members will be required to sign up to the remit and conditions as set out in the IDMC Charter.

25 Quality Control and Assurance

25.1 Monitoring

The clinical trial risk assessment has been used to determine the intensity and focus of central and on-site monitoring activity in the Optimise-FLT3 trial. Higher monitoring levels will be employed and are fully documented in the trial monitoring plan.

Investigators should agree to allow trial related monitoring, including audits and regulatory inspections, by providing direct access to source data/documents as required. Participant consent for this will be obtained. Where electronic health records (EHR) are being used, trial teams and monitors should check EHR process early in trial and periodically thereafter.

Findings generated from on-site and central monitoring will be shared with the Sponsor, CI, PI & local R&D.

25.2 Audits & inspections

The trial is participant to inspection by relevant regulatory bodies, as outlined in the informed consent form. The trial may also be participant to inspection and audit by Cardiff University under their remit as Sponsor.

The CI or PI organisations/institution(s) will permit trial-related monitoring, audits, REC/ IRB review, and regulatory inspection(s), providing direct access to source data / documents.

The site must inform the CTR of any MHRA inspections.

26 Public Involvement and Engagement

Public and patient involvement has provided input into the design of the study and patient facing materials and a dedicated patient advisory group (PAG) has been formed to provide input throughout the trial and members of the PAG will serve on the TMG and TSC groups throughout the trial.

27 Publication policy

Results of the trial will be submitted for publication in a peer reviewed journal. The manuscript will be prepared by the TMG and authorship will be determined by mutual agreement. A lay summary of the results will be published on the CRUK website.

Any secondary publications and presentations prepared by investigators must be reviewed by the TMG. Manuscripts must be submitted to the TMG in a timely fashion, in advance of being submitted for publication, to allow time for review and resolution of any outstanding issues. Authors will acknowledge that the trial was performed with the support of Cardiff University and funded by CRUK.

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29 Appendices

Appendix A: Schedule of Participant Assessments

		Induction				Consolidation				Maintenance		Follow-up					
	Screening/ Registration	Pre-course 1	Post-course 1	Pre-course 2	Post-course 2	Pre-course 3	Post-course 3	Pre-course 4	Post-course 4	Pre-maintenance	Post-maintenance	3 months post end of intensive therapy	6 months post end of intensive therapy	12 months post end of intensive therapy	24 months post end of intensive therapy	6 monthly follow-up	At relapse
Informed consent	X																
Medical history	X																
Physical examination	X	X		X		X		X		X							
Vital signs (1)		X		X		X		X		X							
WHO/ECOG performance status	X	X		X		X		X		X		X	X	X	X		
Height, weight and BSA (2)		X		X		X		X									
Full blood count (3)	X	X	X	X	X	X	X	X	X	X		X	X	X	X		
Biochemistry profile	X	X		X		X		X		X							
Virology (HBV, HCV, HIV)	X																
Pregnancy test (if applicable)	X	X		X		X		X	X	X	X						
Bone marrow aspirate (+/- trephine) and blood sample	X (4)		X (5)		X (5)		X (5)		X (5)			X (5)	X (6)	X (6)	X (6)		X
Randomisation	X																
Prescription for IMPs		X		X													
Assessment of remission status			X		X		X		X			X	X	X	X	X	X
Quality of life assessments (7)	X				X				X	X	X	X	X	X	X		

Assessment of treatment compliance			X		X		X		X		X					
Assessment of supportive care requirements (8)			X		X		X		X							
CRF/eCRF completion including data transfer and query resolution	X		X		X		X		X		X	X	X	X	X	X	X
Review/reporting of participant AEs/SAEs			X	X	X	X	X	X	X	X	X	X	X	X	X		
Echocardiogram/ MUGA scan (9)		X															
ECG (10)		X		X		X		X		X							

(1) Blood pressure, temperature, pulse

(2) Height only required at baseline assessment. Body surface area (BSA) to be calculated as per local practice.

(3) During each cycle of therapy, Haematology and Biochemistry assessments will be performed as per standard of care requirements for participants receiving intensive chemotherapy, including regular assessments of FBC, renal and liver chemistry and monitoring of participants at risk of tumour lysis (see guidance in protocol sections 10.2, 12.1 and 13.1)

(4) Baseline BM assessment includes aspirate for local morphology, flow cytometry and diagnostic genetic evaluation (see section 12.1.1). Trephine performed for local histopathology as per standard of care. Samples to be sent for central baseline flow MRD analysis and sample banking (see section 12.1.2).

(5) Perform upon count recovery (where applicable). If counts do not recover, perform on day 42 (+/- 3 days). BM includes aspirate slides for local morphological remission assessment and aspirate sample (+/- blood sample) for central MRD monitoring (see section 12.2 – dependant on whether participant has been allocated molecular or flow MRD monitoring). Repeat if sample inadequate. Trephine only performed at this point for evidence of morphological leukaemia free state in cases with delayed count recovery. Disease status must be confirmed before continuing treatment.

(6) Surveillance bone marrow aspirates every 3 months for 2 years (+3, 6, 9, 12, 15, 18, 21 and 24 months following completion of intensive chemotherapy) for participants with mutated NPM1, CBF or other fusion genes that have been identified as amenable to qPCR surveillance (for allograft participants with these genotypes, a molecular sample should also be taken pre-allograft and at 30, 60 and 100 days post allograft before reverting to the same 3-monthly surveillance schedule)

(7) By completion of EORTC QLQ30 and EQ-5D-3L questionnaires

(8) Including number of days in hospital, number of red cell and platelet units transfused, days of intravenous antibiotics, days of anti-fungal therapy

(9) Pre-chemotherapy cardiac assessment is recommended, as per standard of care, for participants who have baseline risk factors for increased cardiotoxicity (see protocol section 10.3)

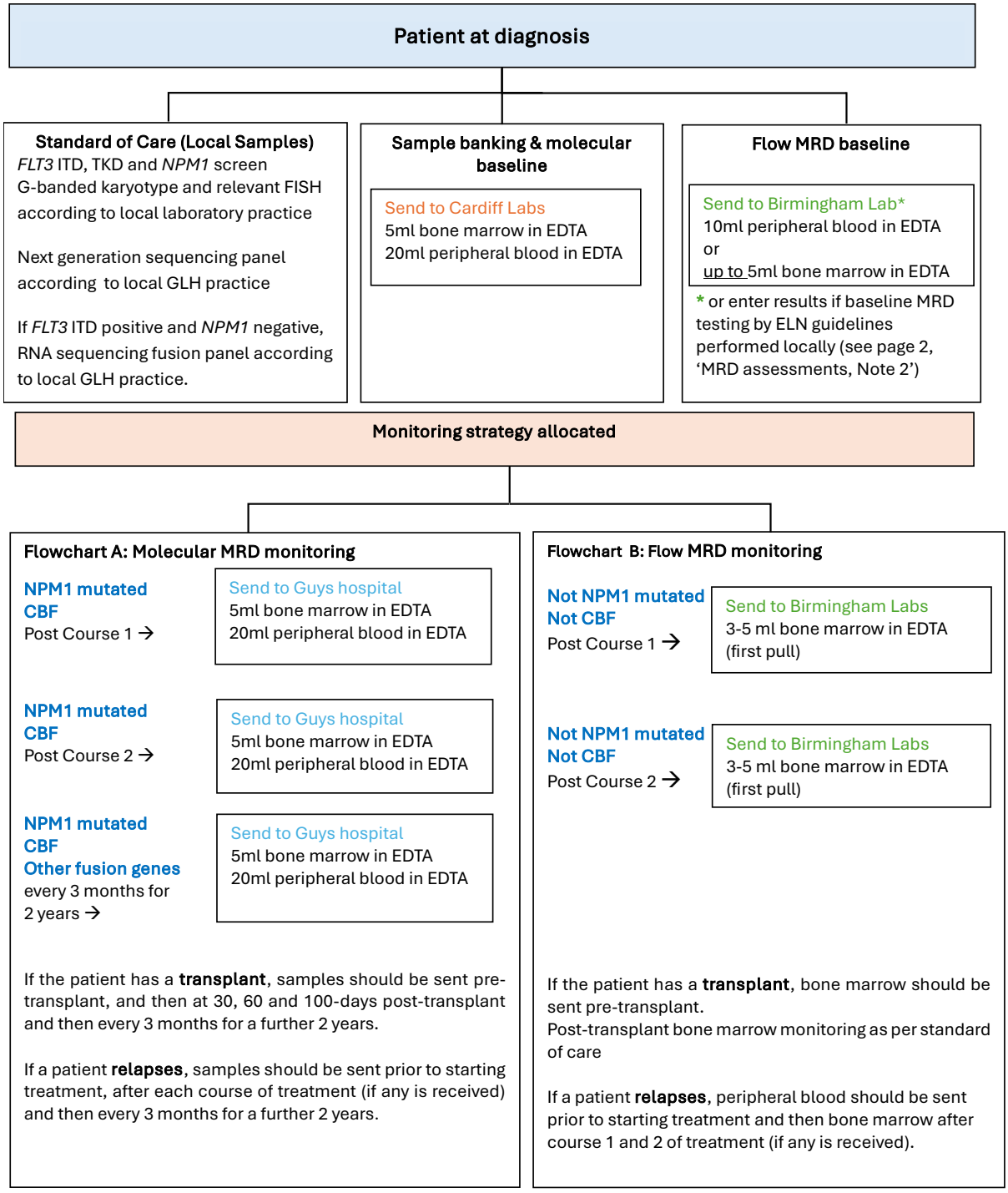
(10) For participants receiving concomitant CYP3A4 inhibitory drugs, (as per standard of care) consider assessment of QTc prior to commencing Midostaurin and at intervals during Midostaurin administration (see protocol sections 10.3.2 and 10.8)

Treatment Schedule of Events

	Cycle 1																									Cycle 2																									Cycle 3 & 4*			
	D1	D2	D3	D4	D5	D6	D7	D8	D9	D10	D11	D12	D13	D14	D15	D16	D17	D18	D19	D20	D21	D22	D23	D24	D25-D28	D1	D2	D3	D4	D5	D6	D7	D8	D9	D10	D11	D12	D13	D14	D15	D16	D17	D18	D19	D20	D21	D22	D23-D28	D1	D3	D5	D6-19
Treatment arm 1 – DA - Mido																																																					
Dauno	x		x		x																					x		x		x																							
Cytarabine	x	x	x	x	x	x	x	x	x	x																x	x	x	x	x	x	x	x																x	x	x	
Mido											x	x	x	x	x	x	x	x	x	x	x	x	x	x											x	x	x	x	x	x	x	x	x	x	x	x	x					x
Treatment arm 2 - DA + GO + Midostaurin																																																				
Dauno	x		x		x																					x		x		x																							
Cytarabine	x	x	x	x	x	x	x	x	x	x																x	x	x	x	x	x	x	x																x	x	x	
GO	x			x																																																
Mido											x	x	x	x	x	x	x	x	x	x	x	x	x	x											x	x	x	x	x	x	x	x	x	x	x	x	x					x
Treatment arm 3 - FLAG-Ida + GO + Midostaurin																																																				
Fludarabine		x	x	x	x	x																					x	x	x	x	x																						
Cytarabine		x	x	x	x	x																					x	x	x	x	x																				x	x	x	
GO		X																																																	
G-CSF	x	x	x	x	x	x	x																			x	x	x	x	x	x	x																				
Idarubicin				x	x	x																							x	x																						
Mido							x	x	x	x	x	x	x	x	x	x	x	x	x	x	x										x	x	x	x	x	x	x	x	x	x	x	x	x					x

* For cycles 3 and 4, the cytarabine D1,3,5 schedule may also be given as D1,2,3 and midostaurin would then be given D4-17.

Appendix B: Laboratory Sample Flow Chart and Information



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MRD Labs

Birmingham Flow Lab

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Richard Dillon (richarddillon@nhs.net)

MRD Assessments

Patient will be allocated to appropriate MRD monitoring strategy consistent with UK standard-of-care and guidelines from the European Leukemia Network (ELN) following results of standard-of-care genetic testing (including RNA sequencing fusion panels). MRD results will be reported back as per standard of care. MRD monitoring incurs a standard charge per sample.

Notes:

1) All patients without an *NPM1* mutation or CBF rearrangement will be monitored by flow cytometric MRD after first and second cycle. If a suitable molecular PCR target is available, patient will then be allocated to PCR MRD or PCR + Flow MRD.

2) If diagnostic /baseline testing for flow cytometric MRD targets according to ELN guidelines performed locally (after initial coordination with trial office / Birmingham), blood does not need to be sent but laboratory to provide results to enter into trial database. Standardised reporting form provided.

3) Centralised flow cytometric MRD (University of Birmingham lab) will allow parallel evaluation of NGS FLT3 ITD MRD on a single bone marrow sample and results can be used by local flow laboratories to facilitate uniform flow cytometric MRD results and reporting between UK laboratories.

Sample Processing

Samples should usually be taken from Monday to Thursday where possible, and sent as early as possible on the day taken using Royal Mail Special Delivery (guaranteed next day service). No processing is required.

Samples should be packaged in a secure container with an internal tube holder, according to local practice.

Tubes should be clearly labelled with trial ID, initials, date of birth, date of sample and sample type (PB or BM).

Sample forms should be sent with each sample.

Where it is not possible to send samples on the same day, these should be kept overnight at room temperature and sent the following morning.

Where sampling on a Friday is unavoidable the sample should be sent by Special Delivery to arrive by Monday am.

Appendix C: Preparation, Administration and Toxicity of Drugs

This appendix is intended as a **guide** for the administration and toxicity management of the IMPs and NIMPs used as part of the OPTIMISE-FLT3 trial.

Drugs used may be generic or biosimilar brands as per local hospital policy. Drug dose banding is permitted according to local practice. Those centres with a firm local policy which differs in administration detail (but not dose or frequency) from the information provided below may follow their local guidelines.

Body Surface area should be calculated according to the Dubois formula prior to each treatment cycle:

$$\textit{Body Surface Area } (m^2) = (\textit{participant weight in kg})^{0.425} \times (\textit{participant height in cm})^{0.725} \times 0.007184$$

Assessment of renal function should be made prior to each treatment cycle using the Cockcroft-Gault creatinine clearance formula:

CrCl = [140 – Age (years)] x Weight (kg) / Plasma creatinine (µmol/L)

For males, multiply above equation by 1.23
For females, multiply above equation by 1.04
Use adjusted body weight in obesity (i.e. if participant's weight is > 15% over IBW or BMI > 25)

DAUNORUBICIN

Formulation	Vial containing a red lyophilised powder for intravenous administration following reconstitution in Water for Injections and dilution with sodium chloride 0.9%. Each vial contains 21.4 mg daunorubicin hydrochloride (equivalent to 20 mg as base).
Storage	Daunorubicin vials should be stored below 25° C and protected from light. After reconstitution Daunorubicin should be stored at 2 - 8° C, protected from light.
Administration	The contents of a vial should be reconstituted with 4ml of Water for Injection giving a concentration of 5 mg per ml. Following reconstitution, the calculated dose of Daunorubicin should be further diluted with sodium chloride 0.9% to give a final concentration of 1 mg per ml. The resultant solution is given by intravenous infusion over one-hour. Daunorubicin is extremely irritating to tissues. It should be administered through a large vein and the infusion kept free flowing.

Side effects	Include nausea, alopecia, chronic and acute cardiac failure and dysrhythmias (please note SmPC advice regarding cumulative anthracycline dosing). Subcutaneous extravasation may cause severe tissue necrosis (careful administration and monitoring is recommended)
Toxicity Management	Hepatic Impairment: Bilirubin 20-50 µmol/L: 75% of the original dose. Bilirubin > 50 µmol/L: 50% of the original dose Renal Impairment: CrCl 30-50 ml/min: 75% of the original dose. CrCl < 30 ml/min: 50% of the original dose

Cytarabine

Formulation	Vial containing clear, colourless solution for injection
Storage	Do not store above 25° C. Keep container in the outer carton, to protect from light. After reconstitution – as per local practice
Administration	The calculated dose of cytarabine can be diluted with dextrose 5% or sodium chloride 0.9% The resultant solution is given by slow intravenous bolus
Side effects	Nausea, diarrhoea, oral ulceration, hepatic dysfunction. A cytarabine syndrome is also recognised in which patients suffer from fever, myalgia, bone pain, occasional chest pains, maculopapular rash, malaise and conjunctivitis (please note advice regarding steroid eye drop prophylaxis for cytarabine doses >1g/m^2) and malaise. It usually occurs 6 to 12 hours following administration.
Toxicity Management	Hepatic impairment: Mild and moderate: no need for dose adjustment is expected Severe: consider 25-50% of the original dose and increase if tolerated Renal impairment - Dose reduction **only** required with cytarabine doses >1g/m^2 CrCl ≥ 60 ml/min: no dose adjustment is needed CrCl 31-59 ml/min: 50% of the original dose CrCl < 30 ml/min: not recommended

Fludarabine

Formulation	Vial containing white lyophilised powder for intravenous administration following reconstitution with water for injection and diluted with sodium chloride 0.9%
Storage	Store in original container After reconstitution fludarabine should be stored at 2 - 8° C
Administration	The contents of each vial should be reconstituted with 2 ml water for injection giving a concentration of 25mg per ml. Following reconstitution, the calculated dose of fludarabine may be diluted in 100 ml sodium chloride 0.9%. The resultant solution is given by intravenous infusion over 30 minutes.
Side effects	Include nausea, vomiting, alopecia, cough, fever, fatigue, weakness, diarrhoea. Fludarabine is a prolonged inhibitor of T-cells and has been associated with the development of transfusional GVHD (please note advice with regards to Irradiated blood products) and pneumocystis pneumonia. CNS side-effects have been rarely described (agitation, confusion, visual disturbance).
Toxicity Management	Hepatic Impairment: no need for dose adjustment is expected. Renal Impairment: CrCl > 70 ml/min: no dose adjustment is needed CrCl 30-70 ml/min: 80% of the original dose CrCl < 30 ml/min: not recommended

Idarubicin

Formulation	Vial containing orange-red, clear solution for injection
Storage	Store and transport refrigerated (2° C - 8° C). Keep the vial in the outer carton to protect from light After reconstitution – as per local practice
Administration	The calculated dose of idarubicin should be drawn from the vial and administered by a slow intravenous bolus over 5- 10minutes via the tubing of a freely running intravenous infusion of 0.9% sodium chloride
Side effects	Red discoloration of urine for 2 to 3 days after administration. Alopecia. Nausea and vomiting. Elevation of liver enzymes may occur. Cardiotoxicity, manifested by cardiac failure, arrhythmias or cardiomyopathies, either during therapy or

	several weeks later (please note SmPC advice regarding cumulative anthracycline dosing).
Toxicity Management	Hepatic Impairment: Bilirubin 45 - 86 µmol/L: 50% of the original dose Bilirubin > 86 µmol/L: not recommended Renal Impairment: CrCl ≥ 30 ml /min: no need for dose adjustment is expected CrCl < 30 ml/min: consider 67% of the original dose

G-CSF (Human Granulocyte Colony-Stimulating Factor): advice given based upon the filgrastim biosimilar, Zarzio. Please consult the relevant SmPC for alternative biosimilars or analogues.

Formulation	Pre-filled syringe containing clear, colourless to slightly yellowish solution
Storage	Store and transport refrigerated (2° C to 8° C). Keep the pre-filled syringe in the outer carton in order to protect from light.
Administration	Filgrastim may be given as a daily subcutaneous injection. The needle safety guard covers the needle after injection to prevent needle stick injury. This does not affect normal operation of the syringe. Depress the plunger slowly and evenly until the entire dose has been given and the plunger cannot be depressed any further. While maintaining pressure on the plunger, remove the syringe from the patient. The needle safety guard will cover the needle when releasing the plunger.
Side effects	Thrombocytopenia, Anaemia, Headache, Diarrhoea, Vomiting, Nausea, Alopecia, Musculoskeletal pain, Fatigue, Mucosal inflammation, Pyrexia.

Gemtuzumab Ozogamicin

Formulation	Amber glass vial containing white to off-white lyophilised powder for intravenous administration following reconstitution in Water for Injections and dilution with sodium chloride 0.9%
Storage	Store in a refrigerator (2° C-8° C). Do not freeze. Store the vial in the original carton to protect from light. Following reconstitution - Store the prepared solution in a refrigerator (2° C– 8° C) and protect from light.

	Any temperature deviations should be reported to the CTR if it impacts on the doses or scheduling of patient treatment.
Administration	The contents of each vial should be reconstituted with 5 ml water for injection. Gently swirl each vial to dissolve the drug taking care not to bubble the contents to make 1mg/ml solution. Each vial should be inspected to ensure complete dissolution and for particulates. Following reconstitution, the calculated dose of gemtuzumab ozogamicin should be diluted with sodium chloride 9 mg/mL (0.9%) solution for injection to a final concentration between 0.075 mg/mL to 0.234 mg/mL The resultant solution is given by intravenous infusion over 2 hours via an IV line equipped with an in-line, low protein-binding 0.2 micron polythersulphone (PES) filter To reduce the incidence of infusion reactions, premedication with a corticosteroid, antihistamine, and paracetamol is recommended 1 hour prior to dosing. Monitor for vital signs prior to, during and for 4 hours post administration.
Side effects	Veno-occlusive disease (VOD)/sinusoidal obstruction syndrome (SOS), tumour lysis syndrome, infusion related reaction (see advice regarding premedication), chills, haemorrhage, pyrexia, fatigue, abdominal pain. Due to the risk of VOD, liver function tests, hepatomegaly (which may be painful), rapid weight gain, and ascites should be closely monitored before each dose
Toxicity Management	Hepatic Impairment: no need for dose adjustment is expected. Renal Impairment: no need for dose adjustment is expected.

Midostaurin (Rydapt)

Formulation	Rydapt® 25 mg soft capsules
Storage	Store in the original container to protect from moisture.
Administration	Midostaurin capsules should be swallowed whole with a glass of water with/after food. The capsules should not be opened, crushed or chewed to ensure dosing and avoid the unpleasant taste of the capsule content. If a dose is missed or vomiting occurs, no additional dose is required, and the next dose should be taken at the scheduled time.

Side effects	Includes febrile neutropenia, nausea, exfoliative dermatitis, vomiting, headache, petechiae and pyrexia. Less common side effects include deranged LFTs, elevated serum lipase, hyperglycaemia, hypotension. QT prolongation (Interval assessments of QT by ECG should be considered if Midostaurin is taken concurrently with medicinal products that can prolong QT interval), pulmonary toxicity (pneumonitis/ILD)
Toxicity Management	Hepatic Impairment: Mild and moderate: no dose adjustment is needed Severe: not recommended Renal Impairment: no need for dose adjustment is expected.

Appendix D: WHO/ECOG Performance Scale

Grade	Explanation of Activity
0	Fully active, able to carry on all pre-disease performance without restriction.
1	Restricted in physically strenuous activity, but ambulatory and able to carry out work of a light or sedentary nature, e.g. light house work, office work.
2	Ambulatory and capable of all selfcare but unable to carry out any work activities. Up and about more than 50% of waking hours.
3	Capable of only limited selfcare. Confined to bed or chair for >50% of waking hours.
4	Completely disabled. Cannot carry out any selfcare. Totally confined to bed or chair.
5	Dead.

Appendix E: EORTC QLQ-C30 questionnaire

EORTC QLQ-C30 (version 3)

We are interested in some things about you and your health. Please answer all of the questions yourself by circling the number that best applies to you. There are no "right" or "wrong" answers. The information that you provide will remain strictly confidential.

Please fill in your initials: ____
Your birthdate (Day, Month, Year): ____
Today's date (Day, Month, Year): 31 ____

	Not at All	A Little	Quite a Bit	Very Much
1. Do you have any trouble doing strenuous activities, like carrying a heavy shopping bag or a suitcase?	1	2	3	4
2. Do you have any trouble taking a long walk?	1	2	3	4
3. Do you have any trouble taking a short walk outside of the house?	1	2	3	4
4. Do you need to stay in bed or a chair during the day?	1	2	3	4
5. Do you need help with eating, dressing, washing yourself or using the toilet?	1	2	3	4

During the past week:	Not at All	A Little	Quite a Bit	Very Much
6. Were you limited in doing either your work or other daily activities?	1	2	3	4
7. Were you limited in pursuing your hobbies or other leisure time activities?	1	2	3	4
8. Were you short of breath?	1	2	3	4
9. Have you had pain?	1	2	3	4
10. Did you need to rest?	1	2	3	4
11. Have you had trouble sleeping?	1	2	3	4
12. Have you felt weak?	1	2	3	4
13. Have you lacked appetite?	1	2	3	4
14. Have you felt nauseated?	1	2	3	4
15. Have you vomited?	1	2	3	4
16. Have you been constipated?	1	2	3	4

During the past week:	Not at All	A Little	Quite a Bit	Very Much
17. Have you had diarrhea?	1	2	3	4
18. Were you tired?	1	2	3	4
19. Did pain interfere with your daily activities?	1	2	3	4
20. Have you had difficulty in concentrating on things, like reading a newspaper or watching television?	1	2	3	4
21. Did you feel tense?	1	2	3	4
22. Did you worry?	1	2	3	4
23. Did you feel irritable?	1	2	3	4
24. Did you feel depressed?	1	2	3	4
25. Have you had difficulty remembering things?	1	2	3	4
26. Has your physical condition or medical treatment interfered with your family life?	1	2	3	4
27. Has your physical condition or medical treatment interfered with your social activities?	1	2	3	4
28. Has your physical condition or medical treatment caused you financial difficulties?	1	2	3	4

For the following questions please circle the number between 1 and 7 that best applies to you

29. How would you rate your overall health during the past week?

1	2	3	4	5	6	7
Very poor						Excellent

30. How would you rate your overall quality of life during the past week?

1	2	3	4	5	6	7
Very poor						Excellent

Appendix F: EuroQoL EQ-5D-5L questionnaire

EQ-5D-5L

Under each heading, please tick the ONE box that best describes your health TODAY.

MOBILITY

I have no problems in walking about ❑
I have slight problems in walking about ❑
I have moderate problems in walking about ❑
I have severe problems in walking about ❑
I am unable to walk about ❑

SELF-CARE

I have no problems washing or dressing myself ❑
I have slight problems washing or dressing myself ❑
I have moderate problems washing or dressing myself ❑
I have severe problems washing or dressing myself ❑
I am unable to wash or dress myself ❑

USUAL ACTIVITIES *(e.g. work, study, housework, family or leisure activities)*

I have no problems doing my usual activities ❑
I have slight problems doing my usual activities ❑
I have moderate problems doing my usual activities ❑
I have severe problems doing my usual activities ❑
I am unable to do my usual activities ❑

PAIN / DISCOMFORT

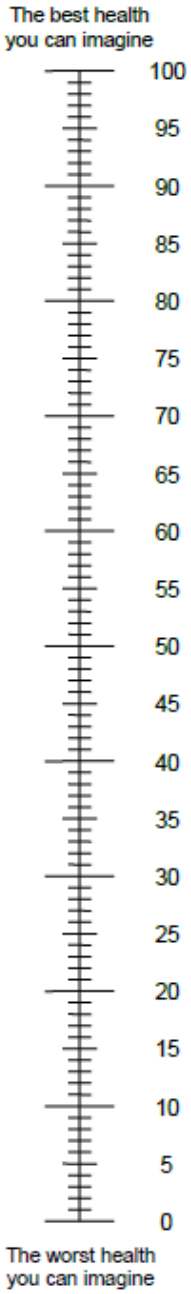
I have no pain or discomfort ❑
I have slight pain or discomfort ❑
I have moderate pain or discomfort ❑
I have severe pain or discomfort ❑
I have extreme pain or discomfort ❑

ANXIETY / DEPRESSION

I am not anxious or depressed ❑
I am slightly anxious or depressed ❑
I am moderately anxious or depressed ❑
I am severely anxious or depressed ❑
I am extremely anxious or depressed ❑

- We would like to know how good or bad your health is TODAY.
- This scale is numbered from 0 to 100.
- 100 means the best health you can imagine.
  0 means the worst health you can imagine.
- Please mark an X on the scale to indicate how your health is TODAY.
- Now, write the number you marked on the scale in the box below.

YOUR HEALTH TODAY = ☐



Appendix G: Common Terminology Criteria for Adverse Events (NCI CTC)

Toxicities will be recorded according to the Common Terminology Criteria for Adverse Events (CTCAE) version 5.0 (November 2017). The full CTCAE document is available on the National Cancer Institute (NCI) website. The following address was correct when this version of the protocol was approved.

https://ctep.cancer.gov/protocoldevelopment/electronic_applications/docs/ctcae_v5_quick_reference_5x7.pdf

The following adverse events are to be considered as haematological toxicities (any other events are considered non-haematological and should be reported accordingly):

- Anaemia
- Bone marrow hypocellularity
- CD4 count reduced
- Granulocytopenia
- Haemoglobin reduced
- Haemolysis
- Iron overload
- Leukopenia
- Lymphopenia
- Myelodysplasia
- Neutropenia
- Pancytopenia
- Thrombocytopenia

The following events are to be considered **expected** toxicities of AML and/or its treatment and do NOT require reporting as SAEs UNLESS they meet other SAE reporting criteria (e.g. grade 3 non-haematological toxicities persisting > 7 days, or grade 4 toxicities of any duration, or grade 5 toxicities):

- Alopecia (hair loss)
- Bruising or bleeding easily
- Chills
- Diaphoresis (sweating)
- Diarrhoea
- Dyspnoea (breathlessness)
- Fatigue/tiredness
- Fever
- Headache
- Hyperglycaemia (high levels of blood glucose

- Hyperliasaemia (increased serum lipase, also known as hyperlipidaemia)
- Hyperuricaemia (excess uric acid in blood)
- Hypocalcaemia (low calcium levels in blood)
- Hyponatraemia (low sodium levels in blood)
- Hypotension/ Hypertension (blood pressure changes)
- Line infections (e.g. vascular access infections)
  Infection
- Loss of appetite / weight loss
- Lymphadenopathy (swollen glands)
- Mucositis (swollen mouth/ gums)
- Musculoskeletal pain / arthralgia / arthritis (bone/ joint pain)
- Nausea and vomiting
- Neutropenia / Thrombocytopenia <42 days from end of chemotherapy administration
- Neutropenia / Thrombocytopenia ≥42 days from end chemo with blasts >5%
- Non-haematological toxicities G3 <7 days
- Oedema
- Pallor (pale skin)
- Parotitis (swollen parotid gland)
- Petechiae (red/ purple spots on skin)
- Skin rashes

Sites are reminded that all adverse events including serious adverse events are to be recorded and reported from the date of consent.

Appendix H: List of web based collection forms

- Notification of Entry form (completed by site staff)
- Cytogenetics form (completed by local cytogeneticist)
- Response to course forms (completed by site staff)
- *FLT3*/ *NPM1*, Sequential Monitoring and Flow MRD forms (completed by Optimise-FLT3 laboratories)
- Course Forms (1, 2, 3, 4)
- One Year Follow up Form
- Withdrawal Form
- Transplant Form
- Transplant (One Year) Form
- Notification of Relapse Form
- Molecular Relapse Form
- Haematological Relapse Form
- Patient Events Form
- Notification of death form

Optimise-FLT3 Protocol V2.0 12.12.25 Clean

Final Audit Report 2026-02-13

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By:	Christie Heiberg (HeibergC@cardiff.ac.uk)
Status:	Signed
Transaction ID:	CBJCHBCAABAAn7HuMOOr9L2RcflXqpSvR-F63N_KdJBg

"Optimise-FLT3 Protocol V2.0 12.12.25 Clean" History

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