MyeChild 01

International Randomised Phase III Clinical Trial in Children with Acute Myeloid Leukaemia -

Incorporating an Embedded Dose Finding Study for Gemtuzumab Ozogamicin in Combination with Induction Chemotherapy

Protocol v2.0

25th January 2018

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SIGNATURE PAGE

MyeChild 01 Trial Protocol

This protocol has been approved by:

Name:	Professor Brenda Gibson	**Trial Role:**	Chief Investigator
Signature:	_____	**Date:**	DD / MON / YYYY

This protocol describes the MyeChild 01 trial and provides information about procedures for patients taking part in the MyeChild 01 trial. The protocol should not be used as a guide for treatment of patients not taking part in the MyeChild 01 trial.

AMENDMENTS

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

Amendment number	Date of amendment	Protocol version number	Type of amendment	Summary of amendment
1.0	25-Jan-2018	2.0	Substantial	Additional of Australia and New Zealand NCC and collaborator details Trial Schema amended to include patients that are MRD negative post course 1, and then MRD positive post course 2. Clarification that for patients on the dose finding study who aren't count recovered by day 45 post course 1 or 2, blood count should be taken at day 45 to confirm/rule out haematological dose limiting toxicity. Clarification that where Randomisation 1 is not available, patients may be registered to receive the standard induction chemotherapy arm Clarification that patients can enter R1 but not be required to receive one dose of gemtuzumab ozogamicin (when found to be safe). Addition of patients with NPM1 mutation undergoing monitoring by both flow and molecular MRD methods for risk group assignment purposes. Inclusion criteria clarified for treatment with gemtuzumab ozogamicin not as part of the dose finding study Removal of inclusion criterion 'Karnofsky or Lansky

				performance score of ≥50' from gemtuzumab ozogamicin inclusion criteria Clarification of risk group assignment for patients with extramedullary disease. Clarification of CNS directed treatment (section 9.1) Minor clarifications throughout to make protocol clearer regarding the treatment with gemtuzumab ozogamicin outside of the dose finding study Amended timing of starting next course of chemotherapy (for non-dose finding patients) to count recovery to neutrophils 0.75 x 10^9/L and platelets 75 x 10^9/L. Clarification on bone marrow aspirate timings in case of delayed count recovery, and amend timing of bone marrow aspirate post course 1 and 2 to be no later than day 35 from the start of the course. Addition of relapse definition Updated definition of VOD Clarification of gemtuzumab ozogamicin PK sample timing for the dose finding study. Clarification of AE reporting for dose finding study and all other part of the protocol. Addition of a new optional pharmacogenomic sub-study (section 21.7 and appendix 10). This involves an extra saliva or buccal swab sample for consenting patients. Time points for analysis of outcome measures added

				Appendix 4 removed Minor clarifcations throughout

TRIAL SYNOPSIS

Title

International randomised phase III clinical trial in children with acute myeloid leukaemia (AML) incorporating an embedded dose finding study for gemtuzumab ozogamicin in combination with induction chemotherapy.

Trial Design

An international randomised phase III clinical trial incorporating an embedded dose finding study.

Primary Objectives

In newly diagnosed AML and high risk myelodysplastic syndrome (MDS) (>10% blasts in the bone marrow):

Non-randomised

To establish the optimum tolerated number of 3 mg/m^2 doses of gemtuzumab ozogamicin (up to a maximum of 3 doses) that can be delivered safely in combination with cytarabine plus mitoxantrone or liposomal daunorubicin in induction

Randomised

1. To compare mitoxantrone (anthracenedione) & cytarabine with liposomal daunorubicin (anthracycline) & cytarabine as induction therapy.
2. To compare a single dose of gemtuzumab ozogamicin 3 mg/m^2 with the optimum tolerated number of doses of gemtuzumab ozogamicin (identified by the dose-finding study) when combined with induction chemotherapy.
3. To compare two consolidation regimens: high dose cytarabine (HD Ara-C) and fludarabine & cytarabine (FLA) in standard risk patients.
4. To compare the toxicity and efficacy of two haemopoietic stem cell transplant (HSCT) conditioning regimens of different intensity: conventional myeloablative conditioning (MAC) with busulfan/cyclophosphamide and reduced intensity conditioning (RIC) with fludarabine/busulfan.

Secondary Objectives

1. To compare the predictive value of flow and molecular MRD monitoring for relapse risk.
2. To evaluate a number of prognostic factors with a view to defining a Risk Score for children and adolescents with AML

Exploratory Objectives

Exploratory objectives for each sub study are stated in the respective Appendix.

Outcome Measures

Primary Outcome Measures

Gemtuzumab Ozogamicin Dose Finding Study

- The incidence of dose limiting toxicities (DLTs)

Randomisation 1: Induction Randomisation (R1)

- Event-free survival (EFS) from date of randomisation 1 (R1)

Randomisation 2: Gemtuzumab Ozogamicin Randomisation (R2)

- EFS from date of randomisation 2 (R2)

Randomisation 3: Consolidation Randomisation (R3)

- Relapse-free survival (RFS) from date of randomisation 3 (R3)

Randomisation 4: HSCT Conditioning Randomisation (R4)

- Early treatment related adverse events (AEs)
- RFS from date of randomisation 4 (R4)

Secondary outcome measures

Gemtuzumab Ozogamicin Dose Finding Study

- The nature, incidence and severity of AEs.
- Response measured by bone marrow morphology and MRD assessment
- Pharmacokinetic (PK) parameters of gemtuzumab ozogamicin including clearance (CL) and volume of distribution (Vd)

All randomisations

- Complete Remission (CR) (R1 and R2 only)
- Reasons for failure to achieve CR (R1 and R2 only)
- Cumulative incidence of relapse (CIR)
- Death in CR (DCR)
- EFS
- Overall Survival (OS)
- Incidence of toxicities
- Incidence of cardiotoxicity (R1, R2 and R4 only)
- Incidence of bilirubin of grade 3 or higher (R2 and R4 only)
- Incidence of veno-occlusive disease (R2 and R4 only)
- MRD clearance after course 1 and course 2 and MRD negativity post-therapy (R1 and R2 only)
- Time to haematological recovery
- Days in hospital after each course of treatment
- Incidence of mixed chimerism at day 100 post-transplant (R4 only)
- Treatment Related Mortality (TRM) (R4 only)
- Gonadal function at 1 year post-transplant and end of study follow up (R4 only)

Exploratory outcome measures

Sub-study outcomes are stated for each sub-study in the respective Appendix.

Patient Population

Children and young adults up to their 18th birthday with newly diagnosed AML, high risk MDS or isolated myeloid sarcoma (MS).

Sample Size

The target recruitment for the trial is up to 700 patients.

Main Inclusion and Exclusion Criteria

Inclusion Criteria:

- Newly diagnosed AML, high risk MDS (greater than 10% blasts in the bone marrow), or isolated MS (either de novo or secondary)
- Age <18 years
- No prior chemotherapy or biological therapy for AML/high risk MDS/isolated MS other than that permitted in the protocol
- Normal cardiac function: fractional shortening ≥ 28%, or ejection fraction ≥ 55%

- Fit for protocol chemotherapy
- Written informed consent

Exclusion criteria:

- Acute promyelocytic leukaemia (APL)
- Myeloid leukaemia of Down Syndrome (ML DS)
- Blast crisis of chronic myeloid leukaemia (CML)

Trial Duration

The trial will recruit for approximately 5-6 years, and all patients will be followed up for a minimum of 1 year.

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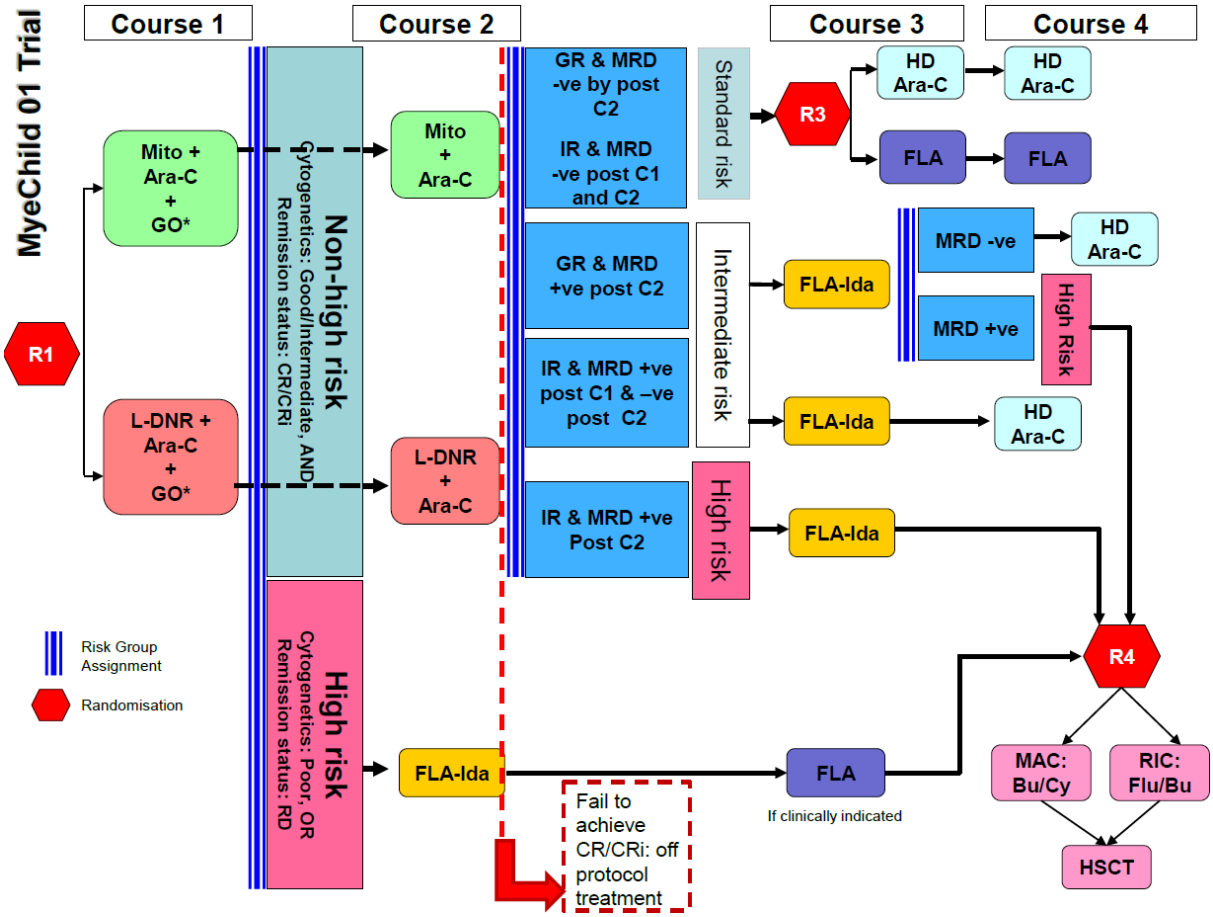
Trial Schema

Figure 1: Trial schema prior to the opening of R2.

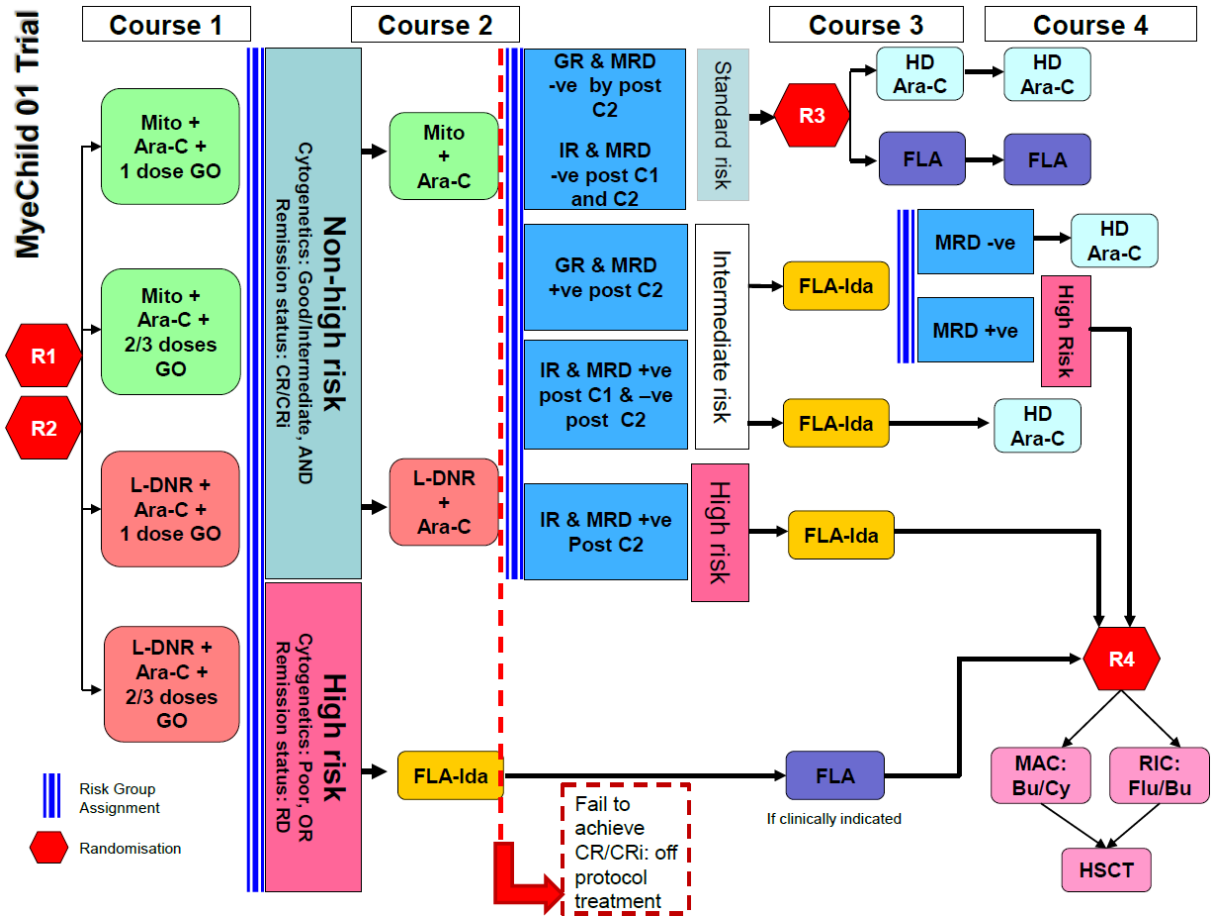


*Patients will only receive GO with induction chemotherapy as part of the embedded gemtuzumab ozogamicin dose finding study (restricted centres), or after the first dose finding cohort has been completed and it has been shown that one dose of GO is safe when given in combination with induction chemotherapy.

Note: For information on patients with extramedullary disease but no marrow involvement, please refer to section 12.2

Ara-C: Cytarabine
Bu/Cy: Busulfan & cyclophosphamide
CR: Complete remission
CRi: Complete remission with incomplete blood count recovery
FLA: Fludarabine & cytarabine
FLA-Ida: Fludarabine, cytarabine & idarubicin
Flu/Bu: Fludarabine & busulfan
GO: Gemtuzumab ozogamicin
GR: Good risk cytogenetics/molecular genetics
HD-Ara-C: High dose cytarabine
HSCT: Haemopoietic stem cell transplant
IR: Intermediate risk cytogenetics
L-DNR: Liposomal daunorubicin
MAC: Myeloablative conditioning
Mito: Mitoxantrone
MRD: Minimal residual disease
R1: Randomisation 1: Induction randomisation
R3: Randomisation 3: Consolidation randomisation
R4: Randomisation 4: Haemopoietic stem cell transplant conditioning randomisation
RIC: Reduced intensity conditioning
RD: Resistant disease

Figure 2: Trial schema following the opening of R2



Note: For information on patients with extramedullary disease but no marrow involvement, please refer to section 12.2

Ara-C: Cytarabine
Bu/Cy: Busulfan & cyclophosphamide
CR: Complete remission
CRi: Complete remission with incomplete blood count recovery
FLA: Fludarabine & cytarabine
FLA-Ida: Fludarabine, cytarabine & idarubicin
Flu/Bu: Fludarabine & busulfan
GO: Gemtuzumab ozogamicin
GR: Good risk cytogenetics/molecular genetics
HD-Ara-C: High dose cytarabine
HSCT: Haemopoietic stem cell transplant
IR: Intermediate risk cytogenetics
L-DNR: Liposomal daunorubicin
MAC: Myeloablative conditioning
Mito: Mitoxantrone
MRD: Minimal residual disease
R1: Randomisation 1: Induction randomisation
R2: Randomisation 2: Gemtuzumab ozogamicin randomisation
R3: Randomisation 3: Consolidation randomisation
R4: Randomisation 4: Haemopoietic stem cell transplant conditioning randomisation
RIC: Reduced intensity conditioning
RD: Resistant disease

Schedule of Events

The following schedule of events includes assessments and investigations which are standard of care and not all data will be collected in the Case Report Form (CRF).

Table 1: Schedule of events

Procedure	Diagnosis	Course 1	Pre-course 2-4 of treatment (as applicable)	End of Treatment	Relapse (any time)
Informed consent[1]	X				
Medical history	X		X		X
Physical examination	X		X	X	X
Karnofsky/Lansky performance status	X		X	X	X
Height, weight and body surface area (BSA)[2]	X		X	X	X
Blood count and biochemistry	X		X	X	X
Blood count for DLT assessment[3]		X Post course 1	X Post course 2		
Coagulation screen	X				
Urine pregnancy test[4]	X	X[5]			
Vital signs		X[6]			
Lumbar puncture diagnostic (local cerebral spinal fluid (CSF) processing)[7]	X		X (only course 2)		
Intrathecal chemotherapy	X		X		
Bone marrow aspirate [8, 9, 10, 11]	X		X	X	X
Peripheral blood sample[8, 9]	X		X	X	X
Buccal swab for transcriptome study (optional study)	One sample to be taken at any time				
Saliva or buccal swab for pharmacogenomic study (optional study)		X Post course 1 only			
Cytogenetic/Fluorescence in situ hybridisation (FISH) testing[12]	X				
Peripheral blood samples for liposomal daunorubicin & mitoxantrone PK sub-study[13]		X			
Peripheral blood samples for gemtuzumab ozogamicin PK sub-study[14]		X			
Echocardiogram	X		X[15]	X	
Tissue typing (recommended)	X				

Monitoring and recording adverse reactions		<<continuous throughout study>>		
Gemtuzumab ozogamicin dose limiting toxicity assessment[16]		<<From randomisation to day 45 post course 2>>		

1. Prior to any trial specific assessments and prior to each randomisation
2. Height to be measured at diagnosis only. BSA calculated according to national policy. See national pharmacy manual
3. Patients on the Dose Finding Study should have a blood count performed at day 45 post course 1 and post course 2 for DLT assessment if not count recovered before day 45.
4. In females of child bearing potential
5. In patients receiving gemtuzumab ozogamicin only, pregnancy test should be repeated prior to administration of first dose of gemtuzumab ozogamicin
6. Patient monitoring should include temperature, respiratory rate, heart rate and blood pressure and should be continuous throughout administration of gemtuzumab ozogamicin and for 4 hours following infusion
7. Intrathecal chemotherapy to be given at the same time as the diagnostic lumbar puncture
8. Bone marrow and peripheral blood morphology will be performed locally
9. Bone marrow and peripheral blood samples for centralised MRD monitoring should be forwarded directly to the MRD laboratories, see the national MyeChild 01 laboratory manual
10. Where the diagnostic bone marrow aspirate yields a dry tap, a trephine biopsy should be carried out
11. For consenting patients, a bone marrow sample should be sent for leukaemic stem cell (LSC) monitoring after each course of chemotherapy and for transcriptome sequencing studies at diagnosis only.
12. Cytogenetic/FISH testing to be carried out locally and/or according to local practice
13. For consenting patients only, during course 1. Multiple samples, see section 21.6
14. For consenting patients registered to the embedded gemtuzumab ozogamicin dose finding study only. Multiple samples, see section 21.5
15. Prior to courses containing liposomal daunorubicin, mitoxantrone or idarubicin only
16. For patients taking part in the gemtuzumab ozogamicin dose finding study

ABBREVIATIONS

ADA	Anti Drug Antibodies
ADE	Cytarabine, Daunorubicin and Etoposide
AE	Adverse Event
ALFA	Acute Leukemia French Association
ALP	Alkaline Phosphatase
ALT	Alanine Transaminase
AMH	Anti-Mullerian Hormone
AML	Acute Myeloid Leukaemia
ANC	Absolute Neutrophil Count
APL	Acute Promyelocytic Leukaemia
ARA-C	Cytarabine
ARDS	Acute Respiratory Distress Syndrome
AST	Aspartate Transaminase
ATG	Anti-thymocyte Globulin
AUC	Area Under the Curve
BFM	Berlin-Frankfurt-Munster
BM	Bone Marrow
BNFc	British National Formulary for Children
BSA	Body Surface Area
CB	Cord Blood
CBF	Core Binding Factor
CI	Confidence Interval
CIR	Cumulative Incidence of Relapse
CL	Clearance
CNS	Central Nervous System
COG	Children's Oncology Group
CR	Complete Remission
CRCTU	Cancer Research UK Clinical Trials Unit, Birmingham
CRp	Complete Remission Without Complete Recovery of Platelets
CRUK	Cancer Research UK
CRF	Case Report Form
CRi	Complete Remission with Incomplete Blood Count Recovery
CSA	Ciclosporin
CSF	Cerebral Spinal Fluid
CTCAE	Common Terminology Criteria for Adverse Events
DCR	Death in Complete Remission
DFS	Disease Free Survival
DLI	Donor Lymphocyte Infusion
DLT	Dose Limiting Toxicity
DMC	Data Monitoring Committee
DSUR	Development Safety Update Report
ELAM	Enfant Leucemie Aigue Myeloblastique
EFS	Event-Free Survival
eRDC	Electronic Remote Data Capture
FAB	French American British
FISH	Fluorescence in situ hybridisation
FLA	Fludarabine and Cytarabine
FLA-Ida	Fludarabine, Cytarabine, Idarubicin
FLAG-Ida	Fludarabine, Cytarabine, Idarubicin and Granulocyte-Colony Stimulating Factor
FSH	Follicle-Stimulating Hormone
GCP	Good Clinical Practice
G-CSF	Granulocyte-Colony Stimulating Factor
GGT	Gamma-glutamyl Transferase
GI	Gastrointestinal
GO	Gemtuzumab Ozogamicin
GOELAMS	Groupe Ouest Est d'Etudes des Leucémies et Autres Maladies du Sang
GvHD	Graft-versus-Host Disease
HD Ara-C	High Dose Cytarabine
HEPA	High-Efficiency Particulate Arrestance
HLA	Human Leukocyte Antigen
HR	Hazard Ratio
HSCT	Haemopoietic Stem Cell Transplant
I-BFM	International Berlin-Frankfurt-Munster
ICF	Informed Consent Form
IMP	Investigational Medicinal Product

IPD	Individual Patient Data
ISF	Investigator Site File
ITD	Internal Tandem Duplication
ITT	Intention to Treat
IV	Intravenous
LAIP	Leukaemia Associated Aberrant Immunophenotypes
LH	Luteinizing Hormone
LSC	Leukaemic Stem Cell
LVFS	Left Ventricular Fractional Shortening
MAC	Myeloablative Conditioning
MACE	Amsacrine, Cytarabine, Etoposide
MIDAC	Mitoxantrone and Intermediate Dose Cytarabine
MA	Mitoxantrone and Cytarabine
MAE	Mitoxantrone, Cytarabine and Etoposide
MDF	Multiparameter/Multidimensional Flow Cytometry
MDS	Myelodysplastic Syndrome
MFD	Matched Family Donor
MHRA	Medicines and Healthcare Products Regulatory Agency
ML DS	Myeloid Leukaemia of Down Syndrome
MMF	Mycophenolate Mofetil
MMFD	Mismatched Family Donor
MMUCB	Mismatched Unrelated Cord Blood
MMUD	Mismatched Unrelated Donor
MRC	Medical Research Council
MRD	Minimal Residual Disease
MS	Myeloid Sarcoma
MSD	Matched sibling donor
MUD	Matched Unrelated Donor
NCC	National Coordinating Centre
NCI	National Cancer Institute
NIH	National Institutes of Health
NOPHO	Nordic Paediatric Haematology Oncology Group
OS	Overall Survival
PBSC	Peripheral Blood Stem Cells
PCP	Pneumocystis jirovecii Pneumonitis
PCR	Polymerase Chain Reaction
PIS	Patient Information Sheet
PK	Pharmacokinetic
RD	Resistant Disease
REC	Research Ethics Committee
RIC	Reduced Intensity Conditioning
RR	Relapse Rate
RT-qPCR	Real-Time Quantitative Polymerase Chain Reaction
SAE	Serious Adverse Event
SAR	Serious Adverse Reaction
SCT	Stem Cell Transplant
SPC	Summary of Product Characteristics
SUSAR	Suspected Unexpected Serious Adverse Reaction
TMG	Trial Management Group
TNC	Total nucleated cell
TRM	Treatment Related Mortality
TSC	Trial Steering Committee
UK	United Kingdom
Vd	Volume of Distribution
VOD	Veno-occlusive Disease
WCC	White cell count

1. BACKGROUND AND RATIONALE

1.1 Background

1.1.1 Acute myeloid leukaemia (AML) in children

AML is a rare disease in children and teenagers (70 cases per annum in children less than 16 years in the UK), but is a significant cause of childhood cancer mortality. This trial is an International collaboration and will recruit patients with newly diagnosed AML, high risk myelodysplastic syndrome (MDS) defined as greater than 10% blasts in the bone marrow, and isolated myeloid sarcoma (MS) up to their 18th birthday. Children with myeloid leukaemia of Down syndrome (ML DS) and acute promyelocytic leukaemia (APL) are excluded. The expected number of patients recruited per year is 120-150 or up to 700 cases in 5-6 years and is sufficient to address the four randomised questions proposed.

All major national groups report similar outcomes for childhood AML: overall survival (OS) 65-70%, event-free survival (EFS) 50-60% and cumulative incidence of relapse (CIR) 35-40% (consensus of the International Berlin Frankfurt Munster (I-BFM) AML group, 2011). Whilst advances in supportive care and better salvage therapy after relapse have led to a moderate improvement in OS, the CIR remains unacceptably high with relapsed disease the commonest cause of death. This study plans to build on experience gained from previous UK, French and international trials and to test a number of strategies with the potential to improve outcome: 1) intensive anthracycline or anthracenedione therapy combined with cytarabine in induction, 2) induction intensification with a higher dose of gemtuzumab ozogamicin, 3) assessment of fludarabine, a purine analogue, in consolidation and 4) evaluation of reduced intensity conditioning (RIC) in allogeneic haemopoietic stem cell transplantation (HSCT) in 1st complete remission (CR1). In addition, risk group stratification will direct therapy and will include cytogenetic/molecular genetic characteristics, morphological response to induction therapy and minimal residual disease (MRD) assessment of treatment response. Different MRD methodologies will be studied for their predictive value. The treatment choices and risk stratification are now discussed with relevance to previous UK and French studies and the literature.

1.1.2 Results from UK Medical Research Council (MRC) AML studies

MRC AML10 (1988-1995: recruited 359 children) confirmed the graft-versus-leukaemia (GVL) effect of allogeneic HSCT (allo-HSCT) and provided the data for a risk stratification based on cytogenetics and molecular genetic characteristics and response to treatment[1, 2]. Children with a matched sibling donor (MSD) were eligible for allo-HSCT following four courses of intensive chemotherapy. A significant reduction in CIR for MSD HSCT did not translate into a significant advantage in OS because of a high transplant related mortality (by donor vs. no donor analysis: CIR allo-HSCT vs. no allo-HSCT: at 5 years 30% vs. 45%, p=.0.02; OS at 10 years 68% vs. 59%, p=0.3; transplant related mortality 15% for those who received a HSCT). I-BFM reported a similar lack of benefit for allo–HSCT in CR1 and these data influenced the UK approach to HSCT in CR1 for the next decade and longer. The transplant related mortality for both related and unrelated allo-HSCT is now low (5-10%), allowing the allo-HSCT associated reduction in CIR the potential to improve outcome in patients at risk of relapse.

MRC AML12 (1995-2002: recruited 564 children) identified a benefit in relapse free survival (RFS) and CIR for mitoxantrone compared to daunorubicin but found no advantage for 5 rather than 4 courses of chemotherapy. Mitoxantrone (12 mg/m^2 x 3) was compared with daunorubicin (50 mg/m^2 x3) in 504 children; both drugs given with cytarabine and etoposide (MAE vs ADE) in induction courses 1 and 2. Disease free survival (DFS) was 63% vs. 55%, p=0.03, and CIR 32% vs. 39%, p=0.05 for MAE vs ADE respectively; but no difference in OS p=0.2. Two hundred and seventy children were randomised to receive either two or three courses of consolidation therapy with anthracycline and cytarabine. There was no benefit for a fifth course of chemotherapy CIR 37% vs. 37%, p=1.0, OS 74% vs. 74%, p=1.0 [3].

MRC AML15 (2004-2009: recruited 199 children) identified, in the trial as a whole including adults, a benefit for CIR for FLAG-Ida (fludarabine, cytarabine, granulocyte-colony stimulating factor (G-CSF) and idarubicin) in induction and found non-anthracycline consolidation (high dose cytarabine (HD Ara-C) 18 g/m^2) not to be inferior to anthracycline heavy consolidation with amsacrine, cytarabine and etoposide (MACE) followed by mitoxantrone and cytarabine (MidAC) in patients with good and intermediate risk cytogenetics, but not poor risk cytogenetics. Children were randomised

to ADE or FLAG-Ida in induction, and to MACE/MidAC or HD Ara-C x 2 (18 g/m^2) in consolidation and finally to a fifth course (HD Ara-C) or not. The 8 year OS/CIR for children (112 randomised) for FLAG-Ida vs. ADE was 71% v 67% (p=0.8) and 28% vs. 38% (p=0.4) respectively. The reduction in the CIR in favour of FLAG-Ida was highly significant (p <0.001) when the trial as a whole (n=3251) was considered. Children with core binding factor (CBF) leukaemias and those with intermediate risk cytogenetics had an OS of 96% vs. 93% (p=0.7) and 75% vs. 64% (p=0.4) respectively from 2nd randomisation for HD Ara-C vs. MACE/MidAC. The number of children with poor risk cytogenetics was too small to be evaluable, but MACE/MidAC was superior in adults with poor risk cytogenetics. A fifth course provided no benefit[4].

1.1.3 Results from French LAME (Leucemie Aigue Myeloblastique Enfant) studies

LAME 89/91 (1988-1998 recruited 309 children) introduced mitoxantrone as the standard induction anthracenedione based on its presumed reduced cardiotoxicity compared to daunorubicin and LAME has retained mitoxantrone in induction for over 25 years. LAME 89/91 demonstrated that mitoxantrone at doses as high as 60 mg/m^2 and up to 84 mg/m^2 in induction was associated with acceptable acute toxicity and very low cardiac toxicity. Patients received an induction regimen of mitoxantrone (12 mg/m^2/d for 5 days) with cytarabine and those with >20% blasts in the bone marrow at day 20 received an additional 2 days of mitoxantrone (12 mg/m^2/d) with cytarabine (reinforcement). The cumulative dose of anthracycline/daunorubicin equivalence was 460 mg/m^2, rising to 580 mg/m^2 in those patients receiving reinforcement[5, 6]. Despite this high anthracycline exposure, the induction death rate from toxicity was low (2.3%). A subgroup analysis of combined paediatric and adult French trials reported no benefit for HSCT in CR1 for patients with CBF leukaemia[7, 8].

ELAM 02 (Enfant Leucemie Aigue Myeloblastique 02) **(2005-2012: recruited 441)** confirmed the efficacy and good tolerability of mitoxantrone as induction therapy with improvement in outcome attributed to a higher dose of cytarabine in consolidation and better management of allo-HSCT. Patients all received mitoxantrone 12 mg/m^2 x 5 in induction with cytarabine 200 mg/m^2/d x 7d. The 5 year OS was 71% with EFS of 57% (personal communication and ASH 2014). All patients with good and intermediate risk cytogenetics with a matched family donor (MFD), with the exception of those children with t(8;21),were candidates for HSCT after 1 to 2 courses of consolidation, whilst children with poor risk cytogenetics were eligible for HSCT with an unrelated Human Leukocyte Antigen (HLA) identical donor. 119 children underwent allogeneic HSCT in CR1. The OS for transplanted patients was 76% and DFS 70%. Four out of 441 had grade 3/4 early cardiotoxicity. For both LAME 89/91 and ELAM02 the cumulative incidence of clinical cardiotoxicity at 10 years is 3% (personal communication).

1.1.4 Gemtuzumab ozogamicin

Gemtuzumab ozogamicin is an anti-CD33 antibody linked to the anti-tumour antibiotic calicheamicin. After internalisation and intracellular release gemtuzumab ozogamicin delivery is targeted to CD33–expressing leukaemia cells. More than 80% of cases of AML express CD33.

1.1.4.1 Gemtuzumab ozogamicin safety studies

I-BFM Relapsed AML 2001/02, a phase II study of 30 children with refractory AML at 1st relapse, or a 2nd relapse of AML, reported a response rate of 37% in children who had received single agent gemtuzumab ozogamicin 7.5 mg/m^2 on day 1 and 14[9]. Toxicity was acceptable. A similar response rate of 26% was reported for adult patients with first relapse of AML following single agent gemtuzumab ozogamicin 9 mg/m^2 on days 1 and 14 with acceptable toxicity[10]. A number of studies have now shown that gemtuzumab ozogamicin 3 mg/m^2 can be safely combined with intensive induction chemotherapy in children and in adults. Gemtuzumab ozogamicin 3 mg/m^2 had an acceptable safety profile when given with ADE and FLAG-Ida in MRC AML15 [11] and mitoxantrone and cytarabine (MA) in Children's Oncology Group (COG) AAML00P2[12]. AAML03P1, a pilot study of 350 children, combined ADE with gemtuzumab ozogamicin 3 mg/m^2 in induction and reported an induction mortality rate of 1.5%[13]. Fractionated gemtuzumab ozogamicin 3 mg/m^2 on days 1, 4, and 7 (total of 9 mg/m^2) was given to 17 children with refractory/relapsed AML in combination with cytarabine 100 mg/m^2/d for 7 days. Seven patients also received gemtuzumab ozogamicin-based consolidation. The response rate, including CR or CR without complete recovery of platelets (CRp), was 35%, but rose to 53% after consolidation. The

toxicity was acceptable and mainly haematological with no sinusoidal obstructive syndrome/veno-occlusive disease (VOD) in heavily pre-treated patients[14].

1.1.4.2 Gemtuzumab ozogamicin paediatric efficacy studies

COG AAML 0531 Study showed that gemtuzumab ozogamicin can be safely added to induction chemotherapy with a significant reduction in CIR and improvement in EFS. 1070 patients with *de novo* AML aged 0-29 years were randomly assigned gemtuzumab ozogamicin 3 mg/m^2 or not combined with ADE in induction (day 6) and MA (MiDAC) in consolidation (day 7) (4th course). Risk stratified therapy allowed allo-HSCT (MSD or matched unrelated donor (MUD)) for high risk patients (defined as monosomy 7, monosomy 5/5q-, high allelic ratio >0.4 FLT 3 internal tandem duplication (ITD), >15% blasts by morphology after course 1 of induction therapy) and MSD only for patients with intermediate risk cytogenetics. Induction mortality was 2% in each arm. Gemtuzumab ozogamicin was associated with a significantly better EFS (53% vs. 47%; hazard ratio (HR) 0.83, 95% confidence interval (CI) 0.70-0.99; p=0.04), although OS was not significantly improved (HR 0.91, 95% CI 0.74-1.13) and there was no difference in CR rate. The CIR was significantly reduced by the addition of gemtuzumab ozogamicin (32.8% v 41.3 %; HR 0.73; p<0.006) . However increased toxicity was seen with a regimen of gemtuzumab ozogamicin 3 mg/m^2 in induction and consolidation (4th course). This could largely be attributed to an excess of infectious deaths in low risk patients (defined as those with CBF leukaemias) in the gemtuzumab ozogamicin arm after course 4 and 5 (8 vs. 2 deaths; p 0.02) and was associated with prolonged neutropenia (14% vs. 7%; p=0.01) following the administration of gemtuzumab ozogamicin in consolidation. The incidence of life- threatening VOD and VOD of any degree was similar in patients with and without gemtuzumab ozogamicin.

The CIR for low risk, intermediate risk (who received HSCT), intermediate risk (HSCT censored) and high risk (who received HSCT) patients (as defined in this study) with and without gemtuzumab ozogamicin was 20% vs. 30%, 24% vs. 38%, 44% vs. 47% and 27% vs. 44.8% and for OS 85% vs. 86%, 84% vs. 73%, 67% vs. 68% and 68% vs. 49% respectively. The interpretation of the benefit of gemtuzumab ozogamicin by risk group in COG AAML 0531 is complicated by the intervention of HSCT and significant toxicity in low risk patients. However, the apparent benefit in high risk patients not demonstrated in previous trials warrants further study.

Nordic Paediatric Haematology Oncology Group (NOPHO) –AML 2004 randomised 120 children with AML, who did not undergo HSCT, to receive gemtuzumab ozogamicin (5 mg/m^2/dose) not earlier than 4 weeks after completion of all consolidation therapy, with a second dose administered at least 3 weeks later. There were no statistically significant differences in EFS or OS at 5 years for those receiving gemtuzumab ozogamicin compared with no further therapy (55% vs. 51%, and 74% vs. 80%, respectively)[15].

1.1.4.3 Gemtuzumab ozogamicin adult efficacy studies

Not all studies have reported benefit for gemtuzumab ozogamicin, and lack of benefit and concerns of increased toxicity in the SWOG-0106 study [16] led to the Federal Drug Administration (FDA) withdrawing approval. This occurred prior to the publication of both AML 15 and ALFA-0701. An individual patient data (IPD) meta-analysis of five predominantly adult randomised trials (MRC/NCRI AML 15 and 16 trials, ALFA-0701 trial, SWOG-0106 study and Groupe Ouest Est d'Etudes des Leucémies et Autres Maladies du Sang (GOELAMS) AML 20061R trial) with the common aim of augmenting induction chemotherapy by the addition of gemtuzumab ozogamicin sought to determine whether the totality of the evidence demonstrates benefit for gemtuzumab ozogamicin, and/or in which specific subgroups. The addition of gemtuzumab ozogamicin did not improve the remission rate (p=0.3), but did improve the OS (p=0.01), due to a significant reduction in CIR (p=0.00006), leading to significantly improved survival from remission (p=0.001). There was a highly significant benefit for patients with good risk (p=0.001) and intermediate risk (p=0.007) cytogenetics, but no benefit for patients with poor risk cytogenetics (p=0.7). The investigators concluded that gemtuzumab ozogamicin given with course 1 of induction chemotherapy shows a significant benefit in survival which more than outweighs any possible increase in early mortality. There was a suggestion of a slightly higher early mortality (30 day mortality p=0.08) which was greater in patients given higher doses of gemtuzumab ozogamicin[17].

MRC AML15 and ALFA-0701 (Acute Leukemia French Association) both reported similar results; a benefit in survival for patients with good and intermediate risk cytogenetics, but not for patients with poor risk cytogenetics[11].These studies used very different doses and scheduling. MRC 15

randomly assigned patients to receive or not to receive a single dose of gemtuzumab ozogamicin (3 mg/m^2) on day 1 of induction chemotherapy and on day 1 of course 3 in consolidation. The French ALFA-0701 randomly assigned patients (50-70 years of age) to receive or not to receive gemtuzumab ozogamicin (3 mg/m^2) on days 1, 4 and 7 (3-3-3 regimen) of induction chemotherapy and on day 1 of the first and second consolidation courses. The addition of gemtuzumab ozogamicin was well tolerated with no significant increase in toxicity in either study. Haematological toxicity, particularly persistent thrombocytopenia, was more common in patients who received gemtuzumab ozogamicin but there was no increase in the risk of death. MRC AML15 reported no benefit for gemtuzumab ozogamicin in consolidation and this finding combined with the increased toxicity observed with the late administration of gemtuzumab ozogamicin in COG AAML 0531 suggests that gemtuzumab ozogamicin may be best restricted to induction therapy. ALFA-0701 data supports the view that fractionated doses of 3 mg/m^2 gemtuzumab ozogamicin may allow the safe delivery of higher cumulative doses of gemtuzumab ozogamicin.

1.2 Trial Rationale

This trial will test strategies in both induction and consolidation for their value in improving survival by reducing the CIR without significantly increasing toxicity. The study will ask four randomised questions and will incorporate an embedded dose finding study for gemtuzumab ozogamicin. All of the trial treatment regimens have been widely used across Europe or internationally.

1.2.1 Rationale for Randomisation 1: Induction randomisation (R1)

R1 will compare two different induction regimens: mitoxantrone and cytarabine (standard arm) vs liposomal daunorubicin and cytarabine for anti-leukaemic efficacy and toxicity.

Anti-leukaemic efficacy and anthracycline dose intensity: MRC AML12 identified a benefit in DFS (p=0.03) and CIR (p=0.05), but no difference in OS (p=0.2) for mitoxantrone (MAE) compared to daunorubicin (ADE). AML15 reported FLAG-Ida to result in a lower CIR than ADE: whole trial highly significant (p<0.001): children 28% vs. 38% (p=0.4). There was no difference in OS (p=0.8) for children. Idarubicin is associated with greater toxicity and for that reason will be reserved for high-risk patients. Mitoxantrone is therefore the preferred UK anthracenedione based on its probable anti-leukaemic benefit over daunorubicin and acceptable toxicity. It is the standard induction anthracenedione in France, where it has been used since 1989 with acceptable toxicity.

Increasing the anthracycline dose in induction has been reported to improve outcome in adult AML[18] and it seems reasonable to assume that this benefit of anthracycline intensification can be extrapolated to children, although not proven. Liposomal daunorubicin was primarily developed to allow anthracycline dose escalation without compromising cardiac function. It is emerging as the induction anthracycline of choice in paediatric AML across Europe because of its potential to allow the use of higher anthracycline doses with limited acute toxicity, although its superior anti-leukaemic efficacy and lack of late cardiotoxicity have not been proven.

BFM AML 2004 trial compared idarubucin 12 mg/m^2 x 3 with liposomal daunorubicin 80 mg/m^2 x 3; both given with cytarabine and etoposide. Liposomal daunorubicin was comparable to, but not superior to, idarubicin (5yr EFS 59% v 53%, p=0.25; TRM 2/257 v 10/264, p=0.04).

Toxicity profiles and cardiotoxicity: The cumulative anthracycline dose in the BFM AML 2004 study was 350/450 mg/m^2 or 410/510 mg/m^2 for standard/high-risk patients in the idarubicin or liposomal daunorubicin arms respectively (daunorubicin equivalence based on a conversion factor of 5:1). Liposomal daunorubicin was associated with less acute treatment related toxicity despite the use of a higher anthracycline dose[19]. There was no difference in acute cardiotoxicity between the treatments and the cumulative incidence of cardiomyopathy at a 5 year median was 0.7% (n=1) for liposomal daunorubicin and 1.8% (n=3) for idarubicin. There are no data on later cardiotoxicity. AML-BFM 2012 trial takes forward liposomal daunorubicin in induction for de novo AML.

The French AML group have used mitoxantrone 12 mg/m^2 x 5 (300 mg/m^2 daunorubicin equivalence) since 1989 [5]. In their earlier study, LAME 89/91, the cumulative dose of anthracycline equivalence was 460 mg/m^2, rising to 580 mg/m^2 in some patients. Despite this high anthracycline exposure, the induction death rate from toxicity was low (2.3%). ELAM 02 reported a similar low induction death rate reflecting acceptable acute toxicity. 4/441 (0.9%) patients in ELAM 02 had grade 3/4 early cardiotoxicity. The combined cumulative incidence of late clinical cardiotoxicity for LAME 89/91 and ELAM02 is 3% at 10 years. Long term follow up data from the MRC AML12 trial

suggests that mitoxantrone may be associated with less late cardiotoxicity than daunorubicin (personal communication).

In this study the dose of liposomal daunorubicin and mitoxantrone in course 1 and 2 will be equivalent when converted to daunorubicin equivalence based on a conversion factor of 5:1 (daunorubicin equivalence 420 mg/m^2) and these drugs will be compared for their anti-leukaemic efficacy and toxicity profile.

1.2.2 Rationale for the gemtuzumab ozogamicin dose finding study and randomisation 2: gemtuzumab ozogamicin randomisation (R2)

Gemtuzumab ozogamicin is a promising new drug in paediatric AML. Cardiac myocytes do not express CD33 (the target of gemtuzumab ozogamicin) and gemtuzumab ozogamicin may enable treatment intensification without increasing cardiotoxicity.

1.2.2.1 Gemtuzumab ozogamicin dose finding study

In children with AML gemtuzumab ozogamicin has mainly been given as a single dose of 3 mg/m^2 when combined with induction chemotherapy, but the most effective dose, schedule and timing of administration remains uncertain. This study will include an embedded dose finding study to determine the maximum number of doses of gemtuzumab ozogamicin which can safely be combined with the intensive induction chemotherapy in this protocol.

1.2.2.2 Rationale for fractionated dosing

R2 will randomise one single dose of gemtuzumab ozogamicin 3 mg/m^2 against the maximum number of doses (either 2 or 3) of gemtuzumab ozogamicin 3 mg/m^2 identified in the dose finding study. The studies which have reported benefit for gemtuzumab ozogamicin (AML15, ALFA and COG AAML0531) have employed different doses and scheduling, but have provided useful information[11, 20]. A single dose of gemtuzumab ozogamicin 3 mg/m^2 given in combination with induction chemotherapy appears to have an acceptable safety profile and gemtuzumab ozogamicin at this dose saturates about 80% of CD33 antigens on the cell surface. Three fractionated doses of gemtuzumab ozogamicin 3 mg/m^2 delivers a total of 9 mg/m^2 (maximum tolerated dose for a single agent dose of gemtuzumab ozogamicin). After the first dose of gemtuzumab ozogamicin there is rapid re-expression of CD33 antigens on the cell surface to which subsequent doses of gemtuzumab ozogamicin bind, thereby enhancing the CD33 internalization process and intracellular accumulation of drug. Therefore, a fractionated dosing schedule delivers a high total dose of 9 mg/m^2 while lowering the peak gemtuzumab ozogamicin blood levels and minimising off target toxicity. Fractionated gemtuzumab ozogamicin 3 mg/m^2 on day 1, 4, 7 has been given to children with refractory/relapsed AML in combination with cytarabine with acceptable toxicity[14]. Single doses higher than 6 mg/m^2 exceed saturation of CD 33 targets on CD 33 positive AML cells and increase off target toxicities such as hepatotoxicity and thrombocytopenia.

1.2.2.3 Rationale for scheduling

Different studies have given the first dose of gemtuzumab ozogamicin on different days of induction. AAML 0531 gave the first dose of gemtuzumab ozogamicin on day 6. ALFA 0701 gave gemtuzumab ozogamicin on days 1, 4 and 7 i.e. 72 hour interval between doses. Gemtuzumab ozogamicin should not be given to patients with a white cell count (WCC) >30 x10^9/L because of concerns of tumour lysis. Forty percent of children eligible for MyeChild 01 are expected to have a WCC >30 x 10^9/L at diagnosis and by this criterion would not be eligible for gemtuzumab ozogamicin. Therefore day 4 has been chosen for the first dose of gemtuzumab ozogamicin to allow cyto-reduction and a WCC <30 x 10^9/L before gemtuzumab ozogamicin is administered. If the WCC remains high after 4 days of chemotherapy, gemtuzumab ozogamicin is unlikely to result in tumour lysis. Therefore gemtuzumab ozogamicin 3 mg/m^2 as a single dose will be given on day 4. If two doses are tolerable, these will be given on days 4 and 7. If three doses are tolerable, these will be given on days 4, 7, 10.

1.2.2.4 Rationale for restricting gemtuzumab ozogamicin administration to induction therapy

No benefit was found in AML15 for gemtuzumab ozogamicin in consolidation and increased toxicity was seen in COG AAML 0531 with a regimen of gemtuzumab ozogamicin 3 mg/m^2 in induction and consolidation (course 4), which was at least in part attributable to prolonged neutropenia after gemtuzumab ozogamicin in consolidation. Therefore, MyeChild 01 will restrict gemtuzumab ozogamicin to induction.

1.2.2.5 Rationale for administration to all risk groups

Gemtuzumab ozogamicin has been reported to be most effective for patients with good risk (p=0.001) and intermediate risk (p=0.007) cytogenetics, but not poor risk cytogenetics (p= 0.7) [17]. However, all patients will receive gemtuzumab ozogamicin as their cytogenetics will not be known by day 4 when the first dose will be given.

1.2.2.6 Rationale for the absence of a no gemtuzumab ozogamicin control arm

Whilst it is appreciated that the results from the COG AAML 0531 study require longer follow up, the consensus view of specialists in both paediatric and adult haemato-oncology, is that the benefit of gemtuzumab ozogamicin is proven [14, 20, 21] and that a study limited to assessing the benefit of gemtuzumab ozogamicin (gemtuzumab ozogamicin vs no gemtuzumab ozogamicin) would be a lost opportunity to improve the outcome for children with AML, which at present is only 70%. Therefore the control arm in the first course of induction phase will be mitoxantrone, cytarabine, and gemtuzumab ozogamicin on day 4.

1.2.3 Rationale for randomisation 3: consolidation randomisation (R3)

The current standard UK and French consolidation of high dose cytarabine will be compared with fludarabine and cytarabine (FLA). Patients with good and intermediate risk cytogenetics who become MRD negative early in treatment (after course 1 for those with intermediate risk cytogenetics and after course 2 for those with good risk cytogenetics) will receive non–anthracycline based consolidation because they will already have received a cumulative anthracycline dose of 420 mg/m^2 in courses 1 and 2. FLA is an effective non-anthracycline containing regimen used in relapsed AML. I-BFM Relapsed AML 2001/02 study randomly assigned 394 patients with relapsed or primary refractory non APL AML to fludarabine, cytarabine and G-CSF (FLAG) or to FLAG plus liposomal daunorubicin in the first re-induction course. The second course of chemotherapy was with FLA alone. Patients then proceeded to HSCT. The corresponding CR rates were 59% and 69% (p=0.07) and OS at 4 years 36% v 40%, p=0.54 for FLAG v FLAG plus liposomal daunorubicin respectively[22]. A regimen effective in relapsed disease, which might be considered more resistant to treatment than newly diagnosed AML, may have greater anti-leukaemic efficacy than high dose cytarabine alone as consolidation therapy in children with newly diagnosed AML.

There were too few children with poor risk cytogenetics in AML15 to make any meaningful comparison between high dose cytarabine and anthracycline based consolidation, but adult data suggests that high dose cytarabine is inferior to anthracycline based consolidation in these patients. Patients with poor risk cytogenetics will not be eligible for this randomisation but will receive anthracycline based consolidation and HSCT.

1.2.4 Rationale for randomisation 4: HSCT conditioning randomisation (R4)

Whilst HSCT has generally resulted in a significant reduction in CIR, this has not always translated into a significant OS advantage because of a high transplant related mortality. A low transplant related mortality for both related and unrelated allo-HSCT may allow the reduction in CIR associated with allo-HSCT the potential to improve EFS in patients at significant risk of relapse. The standard myeloablative conditioning (MAC) busulfan/cyclophosphamide regimen will be evaluated against a RIC (fludarabine/busulfan) regimen. This will allow comparisons of outcome, toxicity, post HSCT MRD, chimerism and immune reconstitution. The objective of this comparison is to test whether a RIC regimen is associated with less treatment related toxicity compared to a full MAC regimen, without increasing the relapse risk. Transplantation with conventional MAC is often associated with severe acute organ toxicity [23] and late effects (e.g. cardiotoxicity, delayed puberty/infertility and secondary malignancy)[24]. Transplantation with RIC enables reliable engraftment with less acute toxicity in adult patients [25] whilst AML RIC regimens utilise highly immunosuppressive but less

myeloablative chemotherapy to achieve donor stem cell engraftment as a platform for an immune-mediated GVL effect to eliminate residual leukaemic cells. This approach could potentially be of major clinical benefit to children with AML undergoing HSCT by reducing the short- and long-term toxicity associated with conditioning chemotherapy. The key question to be answered is whether this strategy can achieve equivalent disease control with reduced toxicity. Despite the fact that RIC regimens have been widely used for 15 years, to date there have been no significant prospective randomised studies comparing outcomes of RIC vs MAC transplantation in patients with AML and this will be the first major study to address this important issue.

Regimen-related toxicity and CIR in patients randomised to receive the current standard-of-care busulfan/cylophosphamide MAC regimen vs a fludarabine/busulfan based RIC regimen will be compared. Busulfan based conditioning will be used in both arms in light of recent data indicating that intravenous ((IV) busulfan improves CIR in patients with AML transplanted in CR1[26]. While a number of RIC regimens are available, this regimen has been chosen because it achieves high level myeloid engraftment with remarkably low toxicity in children with non-malignant disorders[27].

Children identified as high risk based on poor risk cytogenetics at presentation, or those with good or intermediate risk cytogenetics who qualify for HSCT based on MRD positivity, will be eligible for this randomisation.

1.2.5 Rationale for risk group stratification

Patients will initially be stratified by cytogenetic and molecular characteristics and response to the first course of induction chemotherapy assessed by morphology and MRD measurement.

1.2.5.1 Rationale for cytogenetic and molecular risk group stratification

The cytogenetic risk group stratification for MyeChild 01 acknowledges a number of key publications in the field. A study [28] of the combined analysis of the cytogenetic data from children (n=729) treated on MRC AML10 and 12 confirmed the favourable prognosis for CBF leukaemias: t(8;21)(q22;q22) and inv(16)(p13q22). The outcome for all patients with 11q23 abnormalities was intermediate with no difference observed for those with t(9;11)(p21~22;q23). Rearrangements of the *MLL* gene at 11q23 were the most frequent abnormalities (16%), particularly in infants (50%). Data from an international study of 756 childhood AML patients with *MLL* rearrangements reported a poor outcome for patients with the translocations t(4;11)(q21;q23), t(6;11)(q27;q23), t(10;11)(p12;q23) and t(10;11)(p11.2;q23) [29]. A favourable outcome was reported for patients with t(1;11)(q21;q23), but numbers were small. Therefore all patients with *MLL* rearrangements other than those earlier specified as poor risk are classified as intermediate risk. The adverse outcome in patients with monosomy 7, abnormalities of 5q, t(6;9)(p23;q34)/DEK-NUP214 and t(9;22) (q34;q11) was confirmed. In contrast to adults, abnormalities of 3q and complex karyotypes, in the absence of good risk or poor risk features, were not associated with a significantly adverse outcome in children. The variable outcome for 3q abnormalities may be explained by age related differences in the incidence of 3q abnormalities with a poorer prognosis being specifically dependent on the EVI1 expression status and/or the presence of the inv(3)(q21q26/t(3;3)(q21;q26). Thus the stratification to poor risk specifies abnormalities involving 3q26. The presence of 12p abnormalities predicted a poor outcome; a finding confirmed by the I-BFM [30]. More recently rare cryptic chromosomal abnormalities have been described, which confer a poor outcome. These include: t(5;11)(q35;p15.5)/NUP98-NSD1[31-33], t(7;12)(q36;p13)/MNX1-ETV6 [34, 35] and inv(16)(p13.3q24.3)/CBFA2T3-GLIS2[36, 37]. NUP98-NSD1 tends to be associated with FLT3-ITD, to occur in older patients and result in refractoriness to current chemotherapy, but can potentially be salvaged by allo-HSCT[31]. MNX1-ETV6 is usually cryptic, mainly but not exclusively seen in infants, and is often accompanied by a deletion of the long arm of chromosome 7. CBFA2T3-GLIS2 was originally associated with the AML M7 French American British (FAB) type, but it has recently been observed in all FAB types. Mutations in CEBPA (4.5%) and NPM1 (8%) of paediatric AML respectively are associated with a normal karyotype and a favourable outcome; the latter only in the absence of FLT3-ITD[38, 39]. FLT3-ITD increases in incidence with age and is associated with a poor outcome in the absence of favourable risk cytogenetic features[40].

Table 2: The cytogenetic and molecular risk group assignment for MyeChild 01, expected incidence and estimated number of cases

Paediatric AML cytogenetic risk groups	Approximate incidence in whole trial	Expected number
Good risk		
t(8;21)(q22;q22)/*RUNX1-RUNX1T1**	12%	90
inv(16)(p13q22)/t(16;16)(p13;q22)/*CBFB-MYH11**	6%	45
Double mutation of *CEBPA* without *FLT3*-ITD	5%	40
Mutation of *NPM1* without *FLT3*-ITD	5%	40
Intermediate risk		
t(9;11)(p21;q23)/*MLL-MLLT3* /	11%	85
t(11;19)(q23;p13.3)/*MLL-MLLT1*		
Other MLL rearrangements not classified as poor risk		
All other abnormalities which are neither good or poor risk	25%	190
Poor risk		
inv(3)(q21q26)/t(3;3)(q21;q26)/abn(3q26)	~1%	<10
-5/del(5q)	~1%	<10
-7	4%	30
t(6;9)(p23;q34)/*DEK-NUP214*	~1%	<10
t(9;22)(q34;q11)/*BCR-ABL1*	~1%	<10
12p abnormalities	2-4%	15-30
t(6;11)(q27;q23)/*MLL-MLLT4*	5%	38
t(4;11)(q21;q23)/*MLL-AFF1*		
t(10;11)(p11-p14;q23)/*MLL-MLLT10*		
t(5;11)(q35;p15.5)/*NUP98-NSD1*	<5%	<40
t(7;12)(q36;p13)/*MNX1-ETV6*	<1%	<10
inv(16)(p13.3q24.3)/*CBFA2T3-GLIS2*	<2%	<15
FLT3-ITD without NPM1 or CBF	10%	75

* CBF leukaemias

1.2.5.2 Rationale for defining of CR by flow cytometry

Two large paediatric studies in AML have demonstrated morphological assessment of response to the first course of induction chemotherapy to be of low sensitivity and poor specificity [41, 42]. Both have shown multiparameter/multidimensional flow cytometry (MDF) using aberrant expression of surface antigens on leukaemic blasts to be more predictive of outcome. COG reported 24% of 188 patients in complete morphological remission at the end of their first course of induction therapy (EOI1) to have MDF detectable disease at a level of 0.1% or greater. Patients in CR with residual disease by MDF had a CIR of 60% at 3 years compared with that of 29% in patients without RD ($p<0.001$) and an OS of 56% vs 80% ($p=0.002$) respectively. Nineteen percent of 180 patients in morphological CR after course 2 had evidence of RD by MDF at a level of >0.1% or greater and a CIR at 3 years from EOI2 of 67% compared to 30% in those without RD ($p<0.001$). MDF was available for 27 of 42 patients who failed to achieve morphological CR (>5% blasts). All 7 (26%) patients who were RD negative are long term survivors, whilst 20 (74%) who were RD positive had a 3 year OS of 35% ($p=0.005$). In a multivariate analysis, including cytogenetic and molecular risk factors, RD by MDF was an independent predictor of relapse ($p<0.001$), confirming the superior predictive value for outcome of MDF over morphology. In MyeChild 01 morphological remission status will be confirmed by flow MRD or an alternative method if flow is not informative.

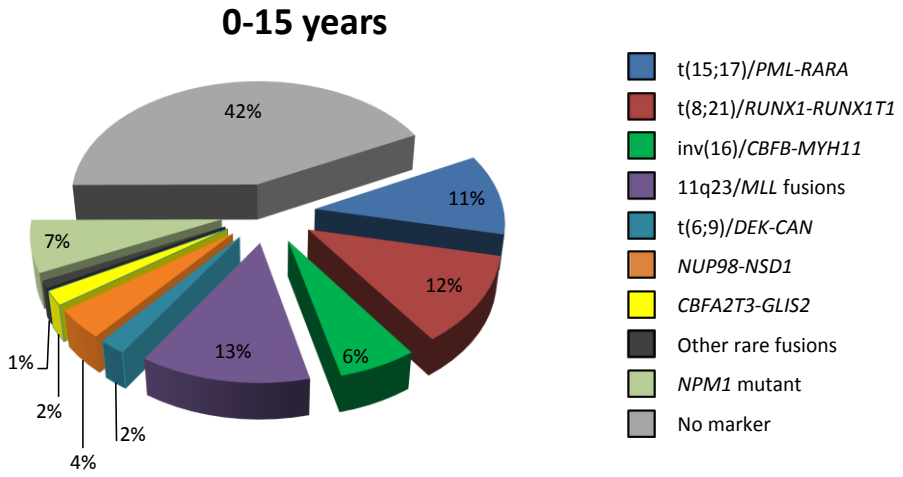
1.2.5.3 Rationale for MRD monitoring by flow cytometry and molecular methodologies

Neither the French nor the UK have previously incorporated MRD assessment in risk stratification for paediatric AML, but multiparameter flow cytometry detecting leukaemia associated aberrant immunophenotypes (LAIP) and real-time quantitative polymerase chain reaction (RT-qPCR) in patients with a leukaemia-specific molecular marker have shown themselves in a number of studies to be independently predictive of outcome[43].

The ability of MDF, irrespective of methodology, to define absolute risk remains limited, with nearly a quarter of patients without measurable RD at a level of 0.1% after course 1 relapsing and a similar sized cohort of patients with documented RD at a level of 0.1% or greater remaining relapse free long term. MDF may be most informative in patients with intermediate risk cytogenetics. The prevalence of RD by MDF at the EOI1 varies by molecular subtype, suggesting heterogeneity of the rate of regression of leukaemic clones, and in this setting molecular monitoring may be more predictive than RD assessment by MDF.

Approximately 60% of paediatric AML should have an informative molecular marker (Figure 3). MRD based on amplification of leukaemia-specific molecular targets [44, 45] affords a sensitivity ranging between 1 in 10^3 and 1 in 10^7, with maximal sensitivity dictated by the relative level of expression of the MRD target in leukaemic blasts[46]. Large adult studies have consistently shown that relapse can be reliably predicted by 1) persistently high MRD levels following frontline therapy or 2) by a rising trend in transcripts after an initial molecular response. Molecular MRD assessment has been shown to provide a more reliable and powerful predictor of relapse risk compared to the diagnostic mutational profile [47, 48]. ALFA and GOELAMS groups in 198 CBF AML patients showed that patients failing to achieve a 3-log reduction in leukaemic transcript level following 2 courses of chemotherapy were at significantly increased risk of subsequent relapse. These findings were extended by Zhu and colleagues, who reported that the poor outcome of patients who: 1) failed to achieve a 3-log reduction in *RUNX1-RUNX1T1* transcripts by the end of the second consolidation; or 2) developed early molecular relapse (within 6 months), might be improved by allo-HSCT in CR1 [47].

Figure 3: Approximately 60% of children have an informative leukaemia-specific molecular marker[43]



In this study treatment will be allocated by risk group assignment using 1) cytogenetic/molecular characteristics and 2) response to treatment assessed by morphology and MRD measurement. MRD will be assessed by flow cytometry or transcript levels (molecular monitoring) for informative patients measured after each course of chemotherapy. Initially, flow MRD will be used to direct treatment but it is anticipated that between 10-15% of children will either not have a marker, or not have a marker of sufficient sensitivity, for flow monitoring. These patients will be monitored by a molecular marker, if an informative molecular marker is present. Patients with CBF leukaemias will be monitored by both flow and transcript levels and any discrepancy between results reviewed centrally. Patients with neither a flow nor a molecular marker will have their treatment assigned by cytogenetic/molecular genetic risk group. The MRD discriminatory level for flow monitoring will be 0.1% and for molecular monitoring a transcript level reduction of 3 logs. This may be amended as further information on the comparative sensitivity and predictive value of MRD by different methodologies becomes available and the data will be under continuing scrutiny for superiority of one or other methodology in paediatric AML as a whole and within subgroups. This will be the responsibility of the Trial Management Group (TMG).

1.2.6 Rationale for treatment allocation

Treatment in MyeChild 01 will be allocated by cytogenetic/molecular characteristics and response to treatment assessed by morphology and MRD measurement after each course of treatment.

1.2.6.1 High risk patients

Cytogenetics/molecular genetics and response, serially assessed after each course of treatment, will identify patients considered to be at high risk of relapse, who may benefit from HSCT. These patients will receive treatment intensification with fludarabine, cytarabine and idarubicin (FLA-Ida) prior to HSCT with the aim of reducing their leukaemic burden. The following patients will be classified as high risk:

1. All patients with poor risk cytogenetics/molecular genetics. MRD status in patients with poor risk cytogenetics will not influence the decision to proceed to HSCT based on preliminary data from adult studies and AAML 03P1 which suggests limited discriminatory value for flow MRD in patients with poor risk cytogenetics.

2. Patients with intermediate risk cytogenetics/molecular genetics who fail to achieve confirmed CR or CR with incomplete blood count recovery (CRi) after course 1. Patients considered not to be in morphological CR/CRi after course 1 but with a MRD flow level <5%, will not be classified as high risk, because of the recognised poor sensitivity and specificity of morphological assessment post course 1. In the absence of a flow marker for assessment of CR/CRi, an

informative molecular marker will be used, and in the absence of an informative molecular marker, fluorescence in situ hybridisation (FISH) assessment.

3. Patients with intermediate risk cytogenetics/molecular genetics and a MRD level of >0.1% by flow after course 2 are considered at higher risk of relapse. The decision to intensify treatment and proceed to HSCT for patients with persistent MRD is based on the observation from COG AAML03P1 that patients with no RD at the end of treatment, but with previously documented RD, remain at high risk of relapse and poor outcome, suggesting that intervention beyond clearance of RD is required for improved outcome[28, 29, 41] .

4. Patients with good risk cytogenetics/molecular genetics and a MRD level of >0.1% by flow and a decrease in transcript levels <3-log with rising transcript levels after course 3, despite treatment intensification, are at higher risk of relapse. However stable or falling transcript levels may be an indication for further chemotherapy rather than HSCT. The lack of statistical significance for MRD observed in patients with good risk cytogenetics in the AAML03P1 study favours a monitoring /chemotherapy approach rather than early intervention with HSCT. All patients with good risk cytogenetics who are MRD positive after course 3 will be discussed with the Clinical Coordinators.

1.2.6.2 Non-high risk patients

Non-high risk patients will not be eligible for HSCT but may have their treatment intensified to reduce their leukaemic burden, achieve MRD negativity and avoid HSCT. These patients will be further divided into two broad treatment groups: standard and intermediate.

Standard risk:

These patients are those at the lowest risk of relapse. These patients will not receive treatment intensification. They will be eligible for R3 and to receive non anthracycline consolidation to limit their anthracycline exposure. These patients will have good risk cytogenetics/molecular genetics and have a MRD level of <0.1% by flow after course 2, or will have intermediate risk cytogenetics/molecular genetics and have a MRD level of <0.1% by flow after course 1 and course 2. The justification for the use of non anthracycline consolidation is based on AML15 data and has previously been explained.

Intermediate risk:
These patients are those at intermediate risk of relapse. These patients will not be eligible for any subsequent randomisation within the trial but will follow a pathway of treatment intensification to reduce their leukaemic burden, achieve MRD negativity and avoid HSCT.

Patients with intermediate risk cytogenetics/molecular genetics with a MRD level of >0.1% by flow after course 1, which falls to <0.1% after course 2, will have their 3rd course of treatment intensified with FLA-Ida and continue with further chemotherapy, and will not proceed to HSCT.

Similarly, patients with good risk cytogenetics/molecular genetics with a MRD level of >0.1% by flow after course 2, which falls to <0.1% after intensification of therapy in course 3, will continue with further chemotherapy and will not be considered for HSCT.

Patients who are not informative by flow, but who have an informative molecular marker will be monitored molecularly. Those who achieve >3-log reduction in leukaemic transcripts will be considered to have achieved MRD negativity, but in addition to the absolute log reduction, the trend in the level of leukaemic transcripts will be considered.

Patients who have their treatment intensified with FLA-Ida will receive the highest cumulative dose of anthracycline. FLA-Ida will increase the cumulative anthracycline dose by 120 mg/m^2 to 540 mg/m^2 based on a 5:1 conversion factor. This cumulative dose is similar to that delivered to high risk patients receiving liposomal daunorubicin on BFM AML 2004 and slow responding patients on LAME 89/91 who received mitoxantrone. Both these studies reported low rates of cardiotoxicity. There is little evidence base for the conversion factor of 5:1 for mitoxantrone. Both liposomal daunorubicin and mitoxantrone are believed to be less cardiotoxic than daunorubicin at equivalent

doses. Patients treated on this study will have an echocardiogram performed at 5 and 10 years post treatment to document the incidence of late cardiotoxicity.

1.2.7 Rationale for inclusion of patients with non-central nervous system (CNS) extramedullary disease

Extramedullary disease can occur at a number of sites including skin, soft tissues and gingival infiltrates. It may be present at the time of diagnosis of AML or may rarely antedate this by weeks to months, although virtually all patients will progress to AML. Extramedullary disease is classified into MS and leukaemia cutis.

Isolated MS occurs with an incidence of around 5% [49] and is most often seen in the CBF leukaemias, whilst leukaemia cutis has an incidence of 1-3% and is usually associated with MLL gene rearrangements [50].

Sites of extramedullary disease should be fully assessed and every effort made to obtain adequate biopsy specimens to carry out cytogenetics, FISH and molecular diagnostics to allow risk stratification. Bone marrow examination should be carried out and should include molecular analysis to detect low level disease. Available evidence suggests that extramedullary disease should be risk stratified by the same criteria as AML without extramedullary disease [51].

Recent studies have shown that 18F-PET CT may be useful in the assessment of isolated MS and extramedullary leukaemia [52]. It was positive in 90% of known extramedullary lesions and picked up additional lesions in a further 60% of patients studied. It has also been used to follow response to treatment in both isolated MS and marrow disease [53], [54]. The use of 18F-PET CT in assessment and follow up is at the discretion of the treating clinician.

Extramedullary AML should be treated systemically as de novo AML. Delayed or reduced initial treatment has been demonstrated to lead to a worse outcome[51]. Local radiotherapy has not been proven to be of benefit although it may be considered in organ threatening compressive lesions.

Any residual lesions persisting after the second cycle of treatment should be fully reassessed and should be imaged and biopsied to document disease activity. Patients who fail to clear disease at extramedullary sites after 2 courses of treatment are considered at high risk of relapse and will be treated accordingly.

2. OBJECTIVES AND OUTCOME MEASURES

2.1 Objectives

2.1.1 Primary objectives

In newly diagnosed AML, high risk MDS (>10% blasts in the bone marrow) and isolated myeloid sarcoma:

Non-randomised

To establish the optimum tolerated number of 3 mg/m^2 doses of gemtuzumab ozogamicin (up to a maximum of 3 doses) that can be safely delivered in combination with cytarabine plus mitoxantrone or liposomal daunorubicin in induction.

Randomised

1. To compare mitoxantrone (anthracenedione) & cytarabine with liposomal daunorubicin (anthracycline) & cytarabine as induction therapy.
2. To compare a single dose of gemtuzumab ozogamicin 3 mg/m^2 with the optimum tolerated number of doses of gemtuzumab ozogamicin (identified by the dose-finding study) when combined with induction chemotherapy.
3. To compare two consolidation regimens: high dose cytarabine (HD Ara-C) and fludarabine & cytarabine (FLA) in standard risk patients.

4. To compare the toxicity and efficacy of two HSCT conditioning regimens of different intensity: conventional MAC with busulfan/cyclophosphamide and RIC with fludarabine/busulfan.

2.1.2 Secondary objectives

1. To compare the predictive value of flow and molecular MRD monitoring for relapse risk.
2. To evaluate a number of prognostic factors with a view to defining a Risk Score for children and adolescents with AML.

2.1.3 Exploratory objectives

Exploratory objectives for each sub study are stated in the respective Appendix.

2.2 Outcome Measures

2.2.1 Primary outcome measures

Gemtuzumab Ozogamicin Dose Finding Study

- The incidence of dose limiting toxicities (DLTs)

Randomisation 1: Induction Randomisation (R1)

- EFS from date of randomisation 1(R1)

Randomisation 2: Gemtuzumab Ozogamicin Randomisation (R2)

- EFS from date of randomisation 2 (R2)

Randomisation 3: Consolidation Randomisation (R3)

- RFS from date of randomisation 3 (R3)

Randomisation 4: HSCT Conditioning Randomisation (R4)

- Early treatment related adverse events (AEs)
- RFS from date of randomisation 4 (R4)

2.2.2 Secondary outcome measures

Gemtuzumab Ozogamicin Dose Finding Study

- The nature, incidence and severity of AEs
- Responses measured by bone marrow assessment using morphology and MRD assessment between day 21-45 post day 1 of induction therapy.
- Serum Pharmacokinetic (PK) parameters of gemtuzumab ozogamicin : CL and Vd

All randomisations

- CR (R1 and R2 only)
- Reasons for failure to achieve CR (R1 and R2 only)
- CIR
- Death in CR (DCR)
- EFS
- OS
- Incidence of toxicities
- Incidence of cardiotoxicity (R1, R2 and R4 only)
- Incidence of bilirubin of grade 3 or higher (R2 and R4 only)
- Incidence of VOD (R2 and R4 only)

- MRD clearance after course 1 and course 2 and MRD negativity post-therapy (R1 and R2 only)
- Time to haematological recovery
- Days in hospital after each course of treatment
- Incidence of mixed chimerism at day 100 post-transplant (R4 only)
- TRM (R4 only)
- Gonadal function at 1 year post-transplant and end of study follow up (R4 only)

2.2.3 Exploratory outcome measures

Sub-study outcomes are stated for each sub-study in the respective Appendix.

3. TRIAL DESIGN

MyeChild 01 is an international randomised phase III clinical trial with an embedded gemtuzumab ozogamicin dose finding study.

3.1 Design of the gemtuzumab ozogamicin embedded dose finding study

The dose finding study aims to identify the optimum number of doses of gemtuzumab ozogamicin 3 mg/m^2 (up to a maximum of 3 doses) which can be combined safely with liposomal daunorubicin or mitoxantrone in induction therapy..

Initially, all centres will open R1 but the gemtuzumgemab ozogamicin dose finding study will be restricted to centres with experience in conducting early phase studies in children and can offer full supportive care.

All trial patients will have their induction treatment assigned by randomisation (R1). Where R1 is not available, patients may still enter the trial and will be registered to receive the standard treatment arm (Arm A).

Two gemtuzumab ozogamicin dose finding studies (a major and minor) will run in parallel and will recruit two distinct age groups; patients ≥12 months of age and infants aged between ≥12 weeks and <12 months. Infants aged <12 weeks will not be eligible for the dose finding study.

Infants ≥28 days and <12 weeks will only receive gemtuzumab ozogamicin after all of the data from cohort 1 of the minor infant dose finding study has been reviewed by the Data Monitoring Committee (DMC), and a single dose of 3 mg/m^2 in combination with induction chemotherapy is judged to be safe in this age group. Only a single dose gemtuzumab ozogamicin (3 mg/m^2) will be administered in this age group because of concerns of potential toxicity. Infants <28 days will not receive gemtuzumab ozogamicin due to a lack of safety data in this age group.

All patients registered in the gemtuzumab ozogamicin dose finding study who have commenced therapy with gemtuzumab ozogamicin will be evaluable for DLT assessment. Haematological and non-haematological DLTs will be evaluated from the date of trial entry (R1) through to count recovery after course 2 of chemotherapy or day 45 from the start of course 2.

Failure to recover a peripheral count by day 45 from the start of course 1, if due to leukaemic infiltration, will render the patient non-evaluable for haematological DLT. These patients will however be evaluable for non-haematological DLT.

3.1.1 Major dose finding study for patients ≥ 12 months

A total of 10 patients will be recruited to the 1st dose cohort and each will receive a single dose of gemtuzumab ozogamicin 3 mg/m^2 on day 4 of course 1 of induction chemotherapy. The DMC will review and assess the safety data as defined in the protocol and this will inform the decision to roll recruitment to the next dose cohort (cohort 2).

A total of 20 patients will be recruited to cohort 2, and each will receive two doses of gemtuzumab ozogamicin 3 mg/m^2, one on day 4 and one on day 7 of course 1 of induction chemotherapy. Safety assessment and the criteria for progressing to the next dose cohort (cohort 3) will follow the practice described above.

If the decision is made to recruit a third and final dose cohort (cohort 3), a further 20 patients will be recruited who will each receive three doses of gemtuzumab ozogamicin 3 mg/m^2, the first on day 4, the second on day 7 and the third dose on day 10 of course 1 of induction chemotherapy. The DMC will review and assess the safety data as defined in the protocol.

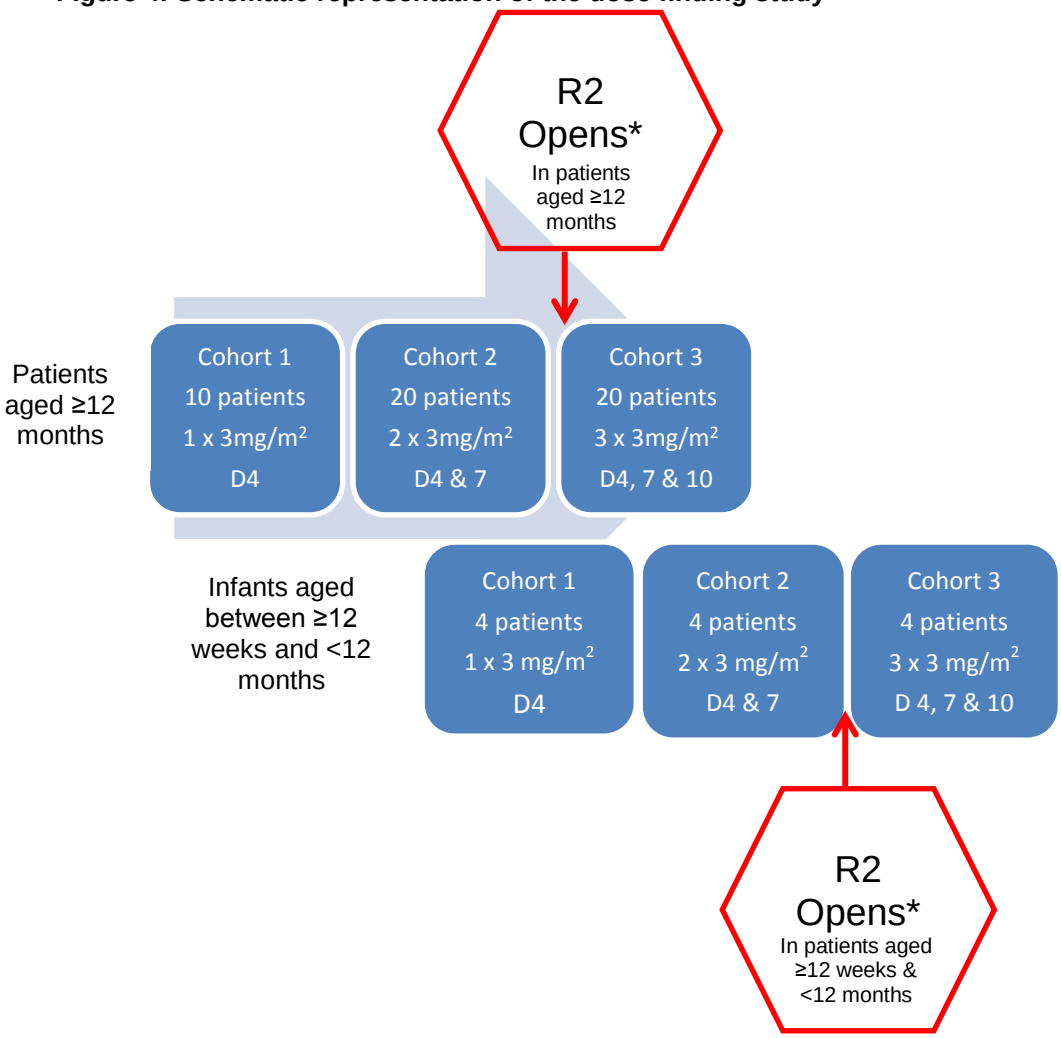
3.1.2 Minor dose finding study for infants aged ≥12 weeks and <12 months

The minor dose finding study for gemtuzumab ozogamicin in infants aged between ≥12 weeks and <12 months will not open until cohort 1 of the major dose finding study has been completed and the data reviewed by the DMC. If the DMC confirms the safety of 3 mg/m^2 dose of gemtuzumab ozogamicin in cohort 1 of the major dose finding study, a separate cohort of infants aged between ≥12 weeks and <12 months will open to recruitment. A total of 4 patients will be recruited to each cohort. Recruitment of infant cohorts will lag one dose cohort behind recruitment to the major dose finding study. Each cohort in the minor study will only open after safety has been established in the full cohort of the major study receiving that number of doses.

After one dose has been approved for use in patients ≥12 months of age by the DMC, patients who are not treated in a centre participating in the major dose finding study, are ≥12 months of age and are eligible to receive gemtuzumab ozogamicin, can receive a single dose of gemtuzumab ozogamicin on day 4 of course 1 of induction chemotherapy. Similarly, after one dose has been approved for use in infants aged between ≥12 weeks and <12 months of age by the DMC, patients who are not treated in a centre participating in the minor dose finding study, are ≥12 weeks and <12 months and are eligible to receive gemtuzumab ozogamicin, can receive a single dose of gemtuzumab ozogamicin on day 4 of course 1 of induction chemotherapy.

Patients recruited at centres participating in both the major and minor dose finding studies are eligible to receive a single dose of gemtuzumab ozogamicin on day 4 of course 1 of induction chemotherapy during any pauses in recruitment to the next dose finding cohort after the safety of one dose of gemtuzumab ozogamicin has been confirmed by the DMC in their respective age group.

Figure 4: Schematic representation of the dose finding study



* If the dose finding study shows that 2 x 3 mg/m^2 is safe to be carried forward to the R2 randomisation, R2 will open following full review of cohort 2 data and will compare 1 x 3 mg/m^2 with 2 x 3 mg/m^2.

3.2 Design of the Randomised Trial

Randomisation 1 (R1) will compare two different induction chemotherapy regimens. The two induction regimens being compared are mitoxantrone & cytarabine vs liposomal daunorubicin & cytarabine.

Randomisation 2 (R2) will open after the data from the first and second dose cohorts of gemtuzumab ozogamicin have been evaluated for dose limiting toxicities. R2 will initially compare 1 dose (1 x 3 mg/m^2) with 2 doses (2 x 3 mg/m^2) of gemtuzumab ozogamicin. If after review of the safety data from cohort 3, the DMC recommends 3 doses of gemtuzumab ozogamicin as the optimum tolerated number of doses (3 x 3 mg/m^2), R2 will be amended to compare 1 dose (1 x 3 mg/m^2) with 3 doses (3 x 3 mg/m^2) of gemtuzumab ozogamicin. R2 aims to identify the optimum number of fractionated doses of gemtuzumab ozogamicin when given with induction chemotherapy. This randomisation may open later in infants aged between ≥12 weeks and <12 months than in patients ≥12months.

Patients will undergo risk assessment after course 1 and course 2 of treatment (section 7), which will assign their risk group. They may be eligible for further randomisations.

Randomisation 3 (R3) will compare two different consolidation regimens in standard risk patients. The two consolidation regimens being compared are fludarabine & cytarabine (FLA) vs high-dose cytarabine (HD Ara-C).

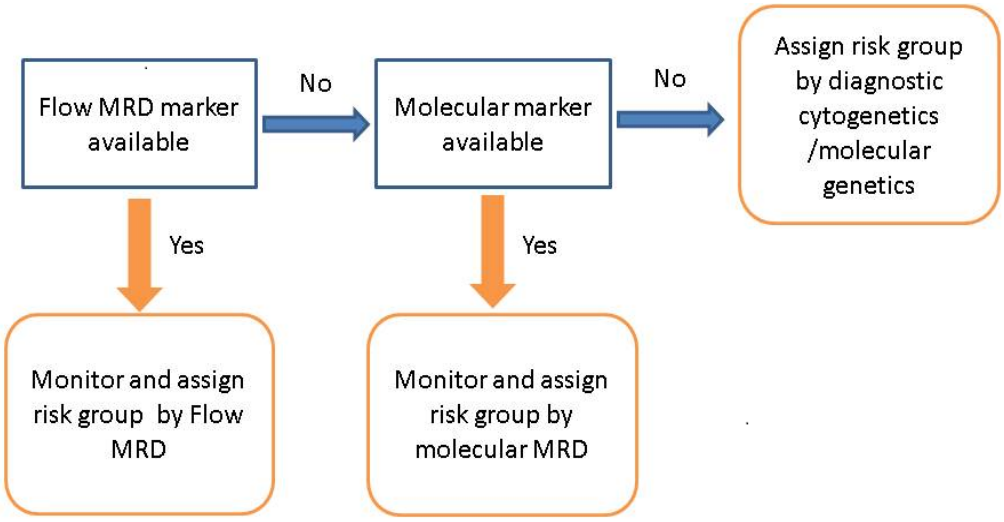
Randomisation 4 (R4) will compare two different conditioning regimens of different intensity in high risk patients: MAC (busulfan/cyclophosphamide) and RIC (fludarabine/busulfan).

Not all randomisations will be open at all times. If R1 is not available, patients may still enter the trial and be registered to receive the standard treatment arm (Arm A). In the event that any other randomisation is not available, patients can take part in all other available randomisations/treatments within the trial. Countries may choose not to participate in certain randomisations, but will still be able to take part in the trial as a whole.

Treatment will be assigned by risk stratification of patients based on cytogenetics and molecular genetics, remission status post course 1 and MRD response. Patients with poor risk cytogenetics (see Table 2) will be classified as high risk by cytogenetics alone. Patients with intermediate risk cytogenetics will be classified high risk if they fail to achieve confirmed CR /CRi after course 1.

Treatment for patients with good risk and intermediate risk cytogenetics and molecular genetics who achieve CR or CRi post-course 1 will be stratified by subsequent response assessed by MRD clearance. The MRD methodology for assessing response is shown in figure 5. The exception to this will be patients with CBF leukaemias and those with an NPM1 mutation, who will undergo monitoring by both flow MRD and molecular MRD methods and discrepant results will be reviewed centrally. Where patients do not have an informative flow or molecular marker, their risk group for treatment assignment will be based on their diagnostic cytogenetic risk group.

Figure 5: Methodology for MRD monitoring for risk group assignment



3.2.1 Risk group definitions

For the purpose of this trial the following risk group definitions will apply:

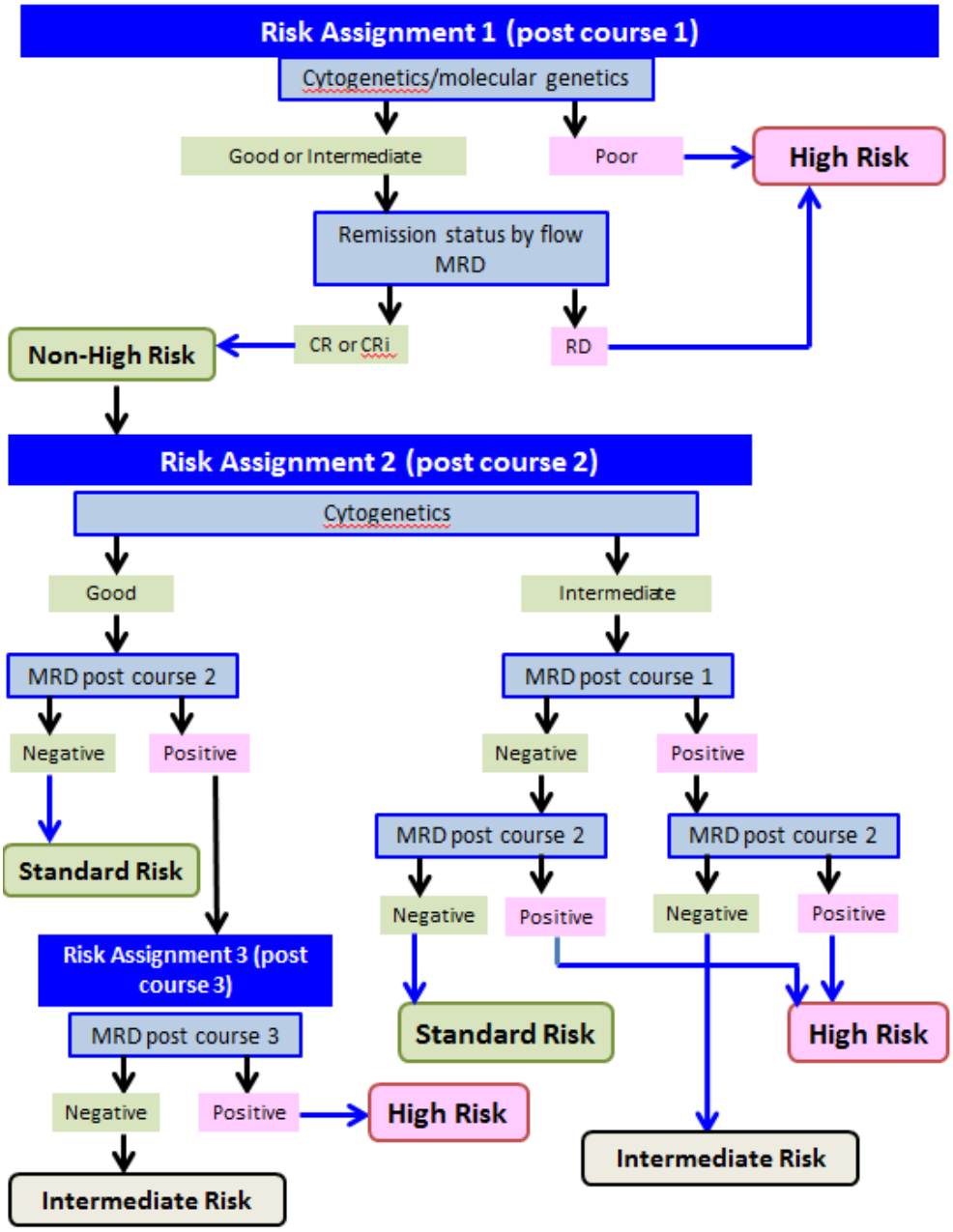
- Cytogenetic/molecular risk will be defined as good, intermediate and poor risk (see Table 2).
- Risk groups incorporating cytogenetics, molecular abnormalities **and** response will be defined as standard, intermediate and high risk.

Based on response criteria patients may change risk group as they proceed through treatment.

Patients with neither an informative marker for flow or molecular MRD monitoring will be assigned treatment according to their cytogenetic/molecular risk group

- Patients with good risk cytogenetics /molecular genetics will be classified as standard risk
- Patients with intermediate risk cytogenetics will be classified as intermediate risk.

Figure 6: Risk group assignment flow chart



CR: complete remission

CRi: complete remission with incomplete blood count recovery

RD: resistant disease

4. ELIGIBILITY

4.1 Inclusion Criteria

4.1.1 Inclusion criteria for trial entry and R1 randomisation

- Diagnosis of AML/high risk MDS (>10% blasts in the bone marrow)/isolated MS (either de novo or secondary)
- Age <18 years at trial entry
- No prior chemotherapy or biological therapy for AML/high risk MDS/isolated MS other than that permitted in the protocol
- Normal cardiac function defined as fractional shortening ≥28% or ejection fraction ≥55%
- Fit for protocol chemotherapy
- Documented negative pregnancy test for female patients of childbearing potential
- Patient agrees to use effective contraception (patients of child bearing potential)
- Written informed consent from the patient and/or parent/legal guardian

Patients with reproductive potential must agree to use effective contraception during the period of therapy. Both men and women of childbearing potential should be advised to use effective contraception to avoid pregnancy up to 12 months after the last dose of study treatment. Effective contraceptive methods include hormonal and barrier contraception etc.

4.1.2 Inclusion criteria for participation in the gemtuzumab ozogamicin dose finding study:

Centres must be formally activated in order to be take part in the embedded dose escalation study. Please contact the trial office for further information.

- Patient meets the inclusion criteria for trial entry (section 4.1.1)
- Age:
  - ≥12 months for the major dose finding study
  - ≥ 12 weeks and <12 months for the minor dose finding study
- Normal renal function defined as calculated creatinine clearance ≥90ml/min/1.73m^2
- Normal hepatic function defined as total bilirubin ≤2.5 upper limit of normal (ULN) for age unless it is caused by leukaemic involvement or Gilbert's syndrome or similar disorder
- ALT or AST ≤10 x ULN for age
- Written informed consent from the patient and/or parent/legal guardian

4.1.3 Inclusion criteria for treatment with gemtuzumab ozogamicin for patients not participating in the gemtuzumab ozogamicin dose finding study

Centres must be formally activated to be able to deliver treatment with gemtuzumab ozogamicin. Please contact the Trial Office for further information.

- Patient meets the inclusion criteria for trial entry (section 4.1.1)
- Age:
  - ≥12 months
  - ≥ 12 weeks (once age group approved to receive one dose gemtuzumab ozogamicin outside of the dose finding study)
- Normal renal function, defined as calculated creatinine clearance ≥90 ml/min/1.73m^2
- Normal hepatic function, defined as total bilirubin ≤2.5 upper limit of normal (ULN) for age and not due to leukaemic involvement or Gilbert's syndrome or similar disorder
- ALT or AST ≤10 x ULN for age
- Written informed consent from the patient and/or parent/legal guardian

4.1.4 Inclusion criteria for participation in R2 (once open to randomisation)

- Patient meets the inclusion criteria for trial entry (section 4.1.1)
- Age ≥12 months
-
- Normal renal function defined as calculated creatinine clearance ≥90ml/min/1.73m^2

- Normal hepatic function defined as total bilirubin ≤2.5 upper limit of normal (ULN) for age and not due to leukaemic involvement or Gilbert's syndrome or similar disorder
- ALT or AST ≤10 x ULN for age
- Written informed consent from the patient and/or parent/legal guardian

4.1.5 Inclusion criteria for participation in R3

- Patient meets the inclusion criteria for trial entry (section 4.1.1)
- Induction treatment as per MyeChild 01 protocol or treated with 2 courses of mitoxantrone & cytarabine off trial
- MRD response (performed in MyeChild 01 centralised laboratories, see national MyeChild 01 Laboratory Manual):
  - Patients with good risk cytogenetics/molecular genetics and a MRD level <0.1% by flow after course 2, or a decrease in transcript levels of >3 logs after course 2 for those with an informative molecular marker, but without an informative marker of sufficient sensitivity for flow MRD monitoring
    or
  - Patients with intermediate risk cytogenetics/molecular genetics with a MRD level <0.1% by flow after course 1 and course 2, or a decrease in transcript levels of >3 logs after course 1 and course 2 for those with an informative molecular marker, but without an informative marker of sufficient sensitivity for flow MRD monitoring
- Written informed consent from the patient and/or parent/legal guardian

4.1.6 Inclusion criteria for participation in R4

- Patient meets the inclusion criteria for trial entry (section 4.1.1)
- Induction treatment as per MyeChild 01 protocol or treated with 1 or 2 courses of mitoxantrone & cytarabine ± treatment intensification with FLA-Ida off trial
- Patient is in CR or CRi defined as <5% blasts confirmed by flow cytometry/ molecular/FISH in a bone marrow aspirate taken within 6 weeks prior to randomisation to R4
- Patient meets one of the following criteria and is a candidate for HSCT as per the protocol:
  - High risk after course 1 (all patients with poor risk cytogenetics and patients with intermediate risk cytogenetics who fail to achieve CR/CRi)
  - Intermediate risk cytogenetics with MRD >0.1% after course 1 and 2 measured by flow. If no flow MRD marker of sufficient sensitivity is identified, a molecular MRD marker with a sensitivity of >0.1% may be used
  - Good risk cytogenetics with flow MRD >0.1% or a decrease in molecular MRD of <3 logs or rising transcript levels after course 3 despite treatment intensification (FLA-Ida) and after discussion with the Clinical Co-ordinators
- Availability of a 9-10/10 HLA matched family or unrelated donor or 5-8/8 matched cord blood unit with an adequate cell dose as defined by the protocol section 17.1
- Written informed consent from the patient and/or parent/legal guardian

4.2 Exclusion Criteria

4.2.1 Exclusion criteria for all randomisations

- Acute Promyelocytic Leukaemia
- Myeloid Leukaemia of Down Syndrome
- Blast crisis of chronic myeloid leukaemia
- Relapsed or refractory AML
- Bone marrow failure syndromes
- Prior anthracycline exposure which would inhibit the delivery of study anthracyclines
- Concurrent treatment or administration of any other experimental drug or with any other biological therapy for AML/high risk MDS/isolated MS
- Pregnant or lactating females

5. SCREENING AND CONSENT

5.1 Screening

Investigators will be expected to maintain a screening log of all potential study participants. This log will contain limited information about the potential participant and will include the date and outcome of the screening process.

The Investigator will provide trial information to patients and/or parents/legal guardians of children who are considered to meet the study eligibility criteria. This information should be sufficient to allow patients/parents/legal guardians to make an informed decision about participation. If informed consent is obtained the Investigator will conduct a full screening evaluation to ensure that the patient meets all inclusion and exclusion criteria (see section 4).

Note that assessments conducted as standard of care do not require informed consent and may be provided as screening data, if conducted within an appropriate interval before randomisation (i.e. within 72 hours).
The following procedures must be performed prior to randomisation to R1 to confirm eligibility:

- Echocardiogram
- Urine pregnancy test for female patients of child bearing potential
- Bone marrow aspiration for local morphology, immunophenotyping, cytogenetics and FISH, as detailed in the national MyeChild 01 Laboratory Manual. Laboratories not able to perform the full list of cytogenetic testing should refer to reference service laboratories in keeping with local practice.
- A trephine biopsy is not essential, but should be carried out if the bone marrow aspirate yields a dry tap

The diagnosis of AML should be made on a bone marrow aspirate. A trephine biopsy is required if an adequate sample cannot be obtained by aspiration. If the patient's clinical condition precludes a bone marrow aspirate then mandatory tests may be performed on peripheral blood.

The following procedures should be performed prior to trial entry for patients receiving gemtuzumab ozogamicin to confirm eligibility:

- Blood tests to include bilirubin, ALT or AST and calculation of creatinine clearance

Trial entry may be at R1, R3 or R4. For patients entering the trial at R1, the patients' diagnostic workup should be performed according to local practice prior to starting treatment and should include the following assessments and procedures:

- Medical history and physical examination
- Height*, weight and body surface area (BSA) measured and calculated in accordance with national guidance
- Assessment of performance status (Karnofsky score for patients aged >16 years or Lansky score for patients aged ≤16 years)
- Full blood count
- Coagulation screen
- Biochemistry to include bilirubin, amylase, alkaline phosphatase (ALK Phos), ALT or AST and urate
- Bone marrow samples:
    - for centralised molecular diagnostics and flow and molecular MRD assessment
    - for leukaemic stem cell (LSC) monitoring and transcriptome sequencing studies (optional consent required)

To avoid the need for repeat sampling, investigators may collect and send bone marrow samples for central investigations at the same time as samples are taken for local diagnostic tests. Patients must consent to the additional samples on a standard NHS consent form.

- Peripheral blood sample for centralised molecular MRD assessment
  To avoid the need for repeat sampling, investigators may collect and send blood samples for central investigations at the same time as samples are taken for local diagnostic tests. Patients must consent to the additional sample on a standard NHS consent.
- Lumbar puncture for cell count and cytospin to be performed at the same time as therapeutic intrathecal chemotherapy is administered
- Echocardiogram
- Urine pregnancy test for all female patients of child bearing potential in patients receiving gemtuzumab ozogamicin. This should be performed prior to administration of gemtuzumab ozogamicin i.e. day 4.
- Recommended: tissue typing of patient at diagnosis to aid donor search if patient goes on to HSCT

** where clinically possible.*

Isolated MS

Patients with isolated MS, defined as AML at an extramedullary site without detectable marrow disease by morphology, flow cytometry, or cytogenetics/FISH, should have the following assessments at diagnosis in addition to those listed above:

- Biopsy with cytogenetics /FISH/molecular genetic analysis
- Cross sectional imaging as appropriate (see section 1.2.7)

If cytogenetics/FISH fails a repeat biopsy for genetic analysis should be considered. If a repeat biopsy is difficult for clinical or technical reasons, the need should be discussed with the clinical coordinators.

Details on sample collection are given in section 21.

5.2 Diagnostic Samples

Please refer to your national MyeChild 01 Laboratory Manual for further details.

5.2.1 Cytogenetics and molecular screening

Diagnostic cytogenetics will be performed locally, wherever possible. Local laboratories not able to identify all of the cytogenetic abnormalities, particularly cryptic rearrangements (listed below), or perform the FISH studies as listed in the national MyeChild 01 Laboratory Manual, should refer samples to reference laboratories in keeping with local practice. Diagnostic molecular screening as listed in the national MyeChild 01 Laboratory Manual will be centralised.

A copy of the reports of all diagnostic and relapse cytogenetics, FISH and molecular testing will be collected centrally. The local cytogenetic result should be available within one week of receipt of the sample. Results from the molecular screening should be available within one week of central receipt of the cytogenetic result.

Cryptic rearrangements:

- t(5;11)(q35;p15.5)*/NUP98-NSD1*
- t(7;12)(q36;p13)*/MNX1-ETV6*
- inv(16)(p13.3q24.3)*/CBFA2T3-GLIS2*

5.2.2 Identification of LAIP to allow MRD assessment by flow cytometry

2 ml bone marrow (ideally from the first pull) should be taken at the same time as the diagnostic aspirate. In the event of a dry tap please send 3 ml peripheral blood.

For further details, including where to send the sample, refer to the national MyeChild 01 Laboratory Manual.

5.2.3 Identification of informative molecular markers for molecular MRD assessment

5-10 ml peripheral blood and 2-5 ml bone marrow should be taken at the same time as the diagnostic aspirate.

For further details, including where to send the sample, refer to the national MyeChild 01 Laboratory Manual.

5.2.4 Genetic and functional LSC monitoring

Optional consent is required for participation in this study. 2 ml bone marrow should be taken at the same time as the diagnostic aspirate. For further details, including where to send the sample, refer to the national MyeChild 01 Laboratory Manual.

5.2.5 Transcriptome sequencing

Optional consent is required for participation in this study. 2 ml bone marrow should be taken at the same time as the diagnostic aspirate.
In addition, a buccal swab is required for this study, which can be taken at any time during treatment.

For further details, including where to send the samples, refer to the national MyeChild 01 Laboratory Manual.

5.3 Informed Consent

It is the responsibility of the Investigator, or person to whom the Investigator delegates the responsibility, to obtain written informed consent for each patient/parent prior to performing any trial related procedure in compliance with national regulations. Where this responsibility has been delegated, this must be explicitly stated on a Site Signature and Delegation Log (or country specific equivalent). Country specific Parent/Patient Information Sheets (PIS) are provided.

Investigators must ensure that they adequately explain the trial aims, trial treatment, anticipated benefits and potential hazards of taking part in the trial to the patient/parent. The Investigator should make it absolutely clear that the patient/parent is completely free to refuse to take part or withdraw from the trial at any time. The patient/parent should be given adequate time to read the PIS and to discuss their participation with others out with the site research team, if they wish to do so. However, because of the acute nature of AML, the available time may be less than 24 hours. The patient/parent must be given an opportunity to ask questions which should be answered to their satisfaction.

The trial includes both children and young adults and written consent/assent will be obtained from the patient whenever it is possible to do so (as appropriate to age and national legislation). There is a section on the Parent Informed Consent Form (ICF) where assent can be obtained. For children who are not able to read or write, the clinician will explain the study and obtain verbal assent.

If the patient or parent/legal guardian agrees to participate in the trial they should be asked to sign and date the current version of the applicable Trial Entry ICF. The Investigator or delegate where appropriate, must sign and date the form. A copy of the ICF should be given to the patient or parent/legal guardian, a copy should be filed in the patient's medical records, and the original placed in the Investigator Site File (ISF) or country specific equivalent (henceforth referred to as ISF). When the patient has been entered into the trial, the patient's trial number should be entered on the ICF maintained in the ISF. If allowed by country specific legislation/guidance and if the patient has given explicit assent a copy of the signed ICF should be sent in the post to the relevant National Coordinating Centre (NCC) for review. Where national guidelines do not permit transfer of ICFs outside of the treating organisation, consent will be monitored by the relevant NCC at site visits.

Details of the informed assent discussions should be recorded in the patient's medical records. This should include date of, and information on, the initial discussion, the date consent was obtained, the

trial name and the version number of the PIS and ICF. Throughout the trial, the patient and/or parent/legal guardian should have the opportunity to ask questions about the trial and any new information that may be relevant to the patient's continued participation should be shared with them in a timely manner. On occasion it may be necessary to re-consent the patient in which case the process above should be followed and the patient's right to withdraw from the trial respected.

Electronic copies of the PIS and ICF are available from the relevant NCC and should be printed or photocopied onto the headed paper of the local institution, where required by country specific legislation/guidance.

Details of all patients approached about the trial should be recorded on a screening and enrolment log.

With the patient's and/or parent's/legal guardian's consent, their primary physician should be informed of their trial participation. A General/Medical Practitioner Letter is provided electronically for this purpose, but it is anticipated that this letter will be translated and adapted in accordance with national practices.

Note: Additional informed consent must be obtained prior to randomisation into any of the subsequent protocol randomisations (R3 and R4) on the Subsequent Randomisations ICF, and registration to the gemtuzumab ozogamicin dose finding study on the Dose Finding Study ICF.

6. TRIAL ENTRY

Patients may be entered into the trial by a treatment site once the relevant NCC has confirmed that all regulatory requirements have been met by the site and the site has been formally activated for randomisation by the UK Coordinating Centre.

It is anticipated that most patients will enter the trial at R1. Once informed consent has been obtained, patients can be entered into the trial and will be randomised to R1. Randomisation must be performed prior to the commencement of trial treatment. For details of what treatment is allowed prior to trial entry see section 9.

Patients who are either not eligible to receive or choose not to be treated with gemtuzumab ozogamicin may still participate in the R1 component of the study.

Patients who do not consent to trial entry (R1) will not be eligible to take part in the gemtuzumab ozogamicin dose finding study; however, they may be able to enter the trial at the subsequent randomisations (R3, R4) if they meet the eligibility criteria.

Not all randomisations will be open at all times. If R1 is not available, patients may still enter the trial and be registered to receive the standard treatment arm (Arm A). In the event that any other randomisation is not available, patients can take part in all other available randomisations/treatments within the trial. Countries may choose not to participate in certain randomisations, but will still be able to take part in the trial as a whole.

6.1 Randomisation and Registration

6.1.1 Randomisation

Randomisation into the trial should be performed by sites using the MyeChild 01 online Remote Data Capture (eRDC) system which has been developed by the Cancer Research UK Clinical Trials Unit (CRCTU), University of Birmingham (UK Coordinating Centre).

https://www.cancertrials.bham.ac.uk/MyeChild01Live

Informed consent must be obtained prior to randomisation/registration as described in section 5.

In order to randomise a patient, the appropriate Eligibility Checklist must be completed followed by the appropriate Randomisation Form. A separate gemtuzumab ozogamicin Eligibility Checklist must be completed for all patients who will receive gemtuzumab ozogamicin.

The patient's unique Trial Number will be assigned at trial entry and will remain the same throughout the trial. At each randomisation, the patient's allocated treatment will be provided to the investigator. A copy of each randomisation report should be printed out and filed in the ISF, and the randomised treatment allocated should be documented in the patient's medical records.

In the event of a problem with the online system, the appropriate paper Eligibility Checklist and Randomisation Form should be completed. These details can be phoned through to the UK Coordinating Centre using the numbers below:

RANDOMISATION
(09:00 to 17:00 UK Time, Monday to Friday)
☏ +44 (0)121 415 1049 or +44 (0)121 414 9819

The Trial Number will be used to identify the patient and should be recorded on all further correspondence with the relevant NCC. The Trial Number should also be documented on the original signed ICF filed in the ISF.

Patients will be randomised to treatments based on minimisation algorithms. All randomisations will be minimised by their age at randomisation (<12 months; ≥1 year and <2 years; ≥2 years and <10 years; ≥10 years), diagnosis (AML; high risk MDS; Isolated MS) and type of disease (De novo; secondary).

In R1, patients will be randomised 1:1 to receive either mitoxantrone with cytarabine or liposomal daunorubicin with cytarabine. Patients in this randomisation will also be minimised by their WCC (<100 x 10^9 /l; ≥100 x 10^9 /l) and the number of doses of gemtuzumab ozogamicin assigned (non-randomised 1 dose; dose finding 2 doses; dose finding 3 doses; randomised to 1 dose via R2; randomised to 2 doses via R2; randomised to 3 doses via R2; no gemtuzumab ozogamicin allocated).

In R2, patients will be randomised 1:1 to receive either 1 dose of gemtuzumab ozogamicin or the optimum tolerated dose of gemtuzumab ozogamicin. Patients in this randomisation will also be minimised by WCC (<100 x 10^9/L; ≥100 x 10^9/L) and the allocated treatment in R1 (mitoxantrone and cytarabine; liposomal daunorubicin and cytarabine;).

In R3, patients will be randomised 1:1 to receive either high dose cytarabine or fludarabine & cytarabine.
In R4, patients will be randomised 1:1 to receive either busulfan/cyclophosphamide MAC or fludarabine/busulfan RIC.

Patients in both R3 and R4 will also be minimised by their treatment allocation in R1 (mitoxantrone and cytarabine; liposomal daunorubicin and cytarabine; mitoxantrone and cytarabine off trial) and the number of doses of gemtuzumab ozogamicin assigned (non-randomised 1 dose; dose finding 2 doses; dose finding 3 doses; randomised to 1 dose via R2; randomised to 2 doses via R2; randomised to 3 doses via R2; no gemtuzumab ozogamicin allocated). Patients in R4 will also be minimised by donor type (related; unrelated; cord).

6.1.2 Registration to the gemtuzumab ozogamicin dose finding study

Only sites activated for the gemtuzumab ozogamicin dose finding study can register patients into this embedded study using the eRDC system.

Patients will be randomised to R1 at the same time as registration to the dose finding study and a unique Trial Number assigned. Informed consent must be obtained prior to registration to the dose finding study. In order to register a patient, an eligibility checklist must be completed followed by the Dose Finding Study Registration Form. A copy of the registration report should be printed and filed in the ISF and participation should be documented in the patient's medical records.

In the event of any problems with the online registration, a paper Eligibility Checklist and Dose Finding Study Registration Form should be completed. These details can be phoned through to the UK coordinating centre using the contact details above.

7. RISK GROUP ASSIGNMENT

Diagnostic cytogenetics/FISH analysis and morphological assessment after course 1 will be performed locally. If confirmatory FISH assessment post course 1 is required, this can be performed locally. Flow and molecular MRD monitoring will be performed centrally. Response assessment will be centralised and the responsibility of the clinical coordinators. The Investigator will be informed of the risk group assigned and allocated treatment.

For further detail on the risk group assignment please see Appendix 1 – Risk Group Stratification.

7.1 Risk Group Assignment 1 (Post Course 1)

On count recovery after course 1 of induction, all patients will undergo a central assessment to assign a clinical risk group: risk group assignment 1. This will include assessment of the diagnostic cytogenetics, FISH, molecular analysis and remission status post course 1 (morphology and MRD result). Patients will be stratified into two risk groups and assigned treatment accordingly:

- Non-high risk
- High risk

Patients for whom there is no cytogenetic, molecular or FISH result at diagnosis will be grouped with patients who have intermediate risk cytogenetics.

Patients with extramedullary disease will undergo risk group assignment based on any available results from biopsy and/or imaging.

7.1.1 High risk

Patients classified as high risk after course 1 will have treatment intensification prior to HSCT and may be eligible for R4. Whilst they will be subject to ongoing MRD monitoring, this will not affect their risk group assignment. Patients classified as high risk after course 1 are those patients with poor risk cytogenetics/molecular genetics and those patients with intermediate or good risk cytogenetics/molecular genetics who fail to achieve confirmed CR or CRi after course 1.

7.1.2 Non-high risk

Patients classified as non-high risk after course 1 will receive course 2 of randomised induction therapy. They will be subject to ongoing MRD monitoring, which will inform their subsequent risk group assignment.

7.2 Risk Group Assignment 2 (Post Course 2)

On count recovery after course 2, non-high risk patients will undergo a second central assessment: risk group assignment 2 which will assign them to one of three clinical risk groups: standard; intermediate or high risk, based on cytogenetic/molecular characteristics and MRD results post course 1 and post course 2. Patients with no flow or molecular marker will have their risk group assignment based on their diagnostic cytogenetic risk group.

MRD assessment will be by flow MRD. In the absence of an informative or sufficiently sensitive flow MRD marker, MRD will be monitored molecularly in those patients with an informative molecular MRD marker. The exception will be patients with CBF leukaemia and patients with a NPM1 mutation who will be monitored by both flow and molecular methodology and any discrepancy between the two results will be reviewed centrally by the clinical coordinators.

In extramedullary disease/myeloid sarcoma, residual lesions persisting after the second cycle of treatment should be fully reassessed and should be imaged and biopsied to document disease activity. Patients who fail to clear disease at extramedullary sites after 2 courses of treatment are considered at high risk of relapse and will be discussed with the clinical coordinators.

7.2.1 Standard risk:

The standard risk group includes the following patients (Table 3):

Table 3: Standard risk patients at risk group assignment 2 (post course 2)

Cytogenetics/molecular genetics	MRD post course 1		MRD post course 2	
	Flow	Molecular♦	Flow	Molecular♦
Good	N/A	N/A	<0.1%	>3-log reduction
Intermediate	<0.1%	>3-log reduction	<0.1%	>3-log reduction

Standard risk patients will be subject to ongoing MRD monitoring throughout consolidation, however this will not affect their risk group assignment. Standard risk patients will be eligible for R3.

Isolated myeloid sarcoma patients with confirmed (imaging and biopsy) complete resoloution after course 2 will be considered as standard risk

7.2.2 Intermediate risk:

The intermediate risk group consists of the following patients (Table 4):

Table 4: Intermediate risk patients at risk group assignment 2 (post course 2)

Cytogenetics/molecular genetics	MRD post course 1		MRD post course 2	
	Flow	Molecular♦	Flow	Molecular♦
Good	N/A	N/A	>0.1%*	≤3-log reduction*
Intermediate	>0.1%	≤3-log reduction	<0.1%$	>3-log reduction$

All intermediate risk patients will have their treatment intensified with FLA-Ida as course 3.

*Patients with good risk cytogenetics/molecular genetics and a MRD level >0.1% (or ≤3-log reduction in leukaemic transcripts) post course 2 will have a further risk assessment after course 3: risk group assignment 3 (see section 7.3), which will determine subsequent treatment.

$Patients with intermediate cytogenetics/molecular genetics and a MRD level of <0.1% (or >3-log reduction in leukaemic transcripts) post course 2 will receive high dose cytarabine as course 4.

7.2.3 High risk:

The high risk group consists of the following patients (Table 5):

Table 5: High risk patients at risk group assignment 2 (post course 2)

Cytogenetics/molecular genetics	MRD post course 1		MRD post course 2	
	Flow	Molecular♦	Flow	Molecular♦
Intermediate	>0.1%	≤3-log reduction	>0.1%	≤3-log reduction

♦The trend in the level of leukaemic transcripts will also be considered

High risk patients will have their treatment intensified with FLA-Ida, proceed to HSCT and may be eligible for R4. These patients will be subject to ongoing MRD monitoring, but this will not affect their assignment to the high risk group.

7.3 Risk Group Assignment 3 (Post Course 3)

Risk group assignment 3 is only required for the intermediate risk patients with good risk cytogenetics defined in section 7.2.2 (Table 6).

Table 6: Patients who are subject to risk group assignment 3 (post course 3)

Cytogenetics/molecular genetics	MRD post course 1		MRD post course 2	
	Flow	Molecular*	Flow	Molecular*
Good	N/A	N/A	>0.1%	≤3-log reduction

♦The trend in the level of leukaemic transcripts will also be considered

This assessment will result in patients either remaining as intermediate risk or being reclassified as high risk. Risk group assignment 3 will be based on the MRD result post course 3.

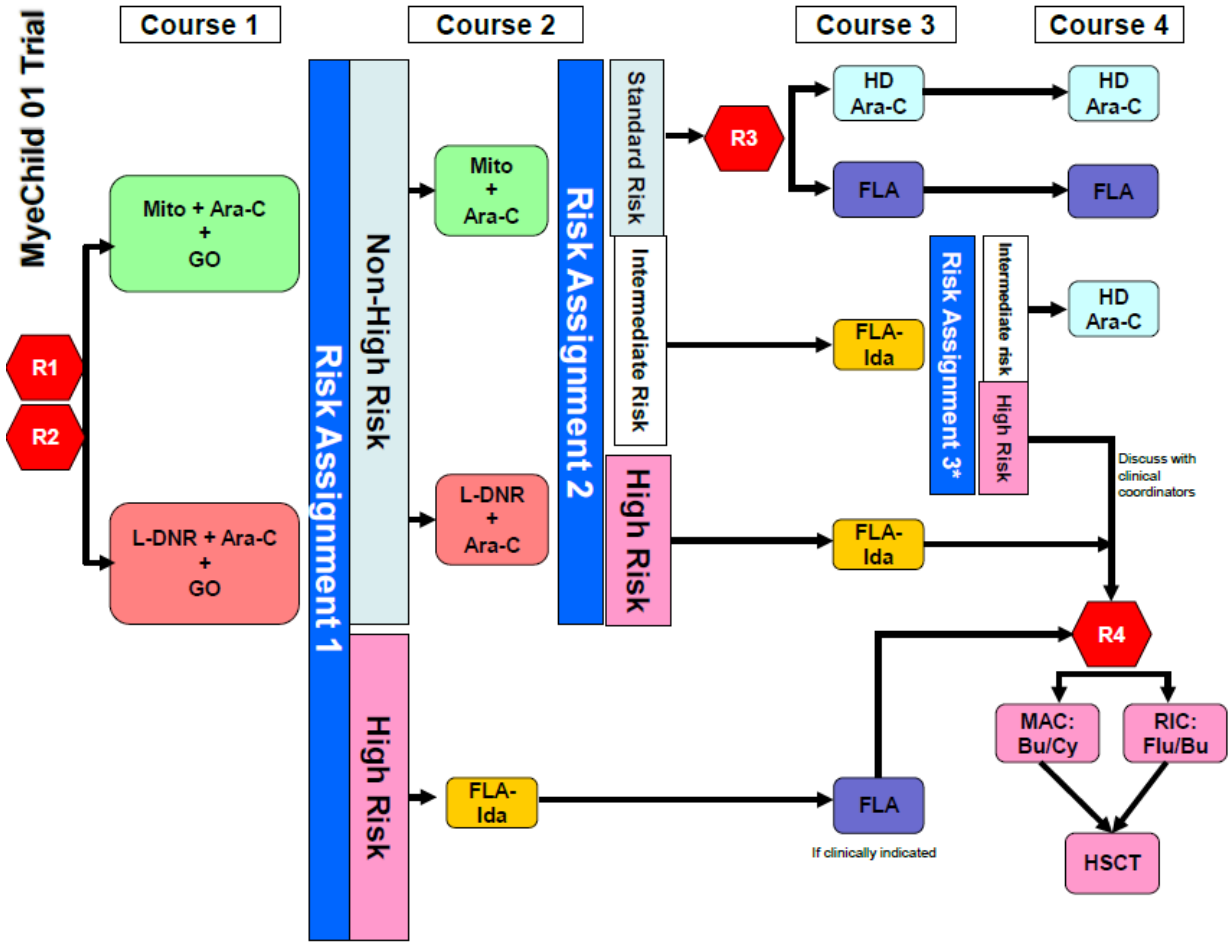
7.3.1 Intermediate risk:

Patients with good risk cytogenetics/molecular genetics who have a MRD level <0.1% (or a reduction in molecular leukaemic transcript levels > 3-logs) after course 3 continue to be classified as intermediate risk, and receive high dose cytarabine as course 4.

7.3.2 High risk:

Patients with good risk cytogenetics/molecular genetics who have a MRD level >0.1% (or a reduction in molecular transcript levels ≤ 3-logs) after course 3 will be re-classified as high risk and their treatment should be discussed with the Clinical Co-ordinators. They may be eligible for R4 if HSCT is indicated. Only patients with good risk cytogenetics and a MRD level of >0.1% , or a decrease in molecular MRD of <3 logs or rising transcript levels after course 3 despite treatment intensification (FLA-Ida) should be considered for HSCT.

Figure 7: Schema showing treatment allocation according to risk group assignment



* Risk assignment 3 is only for patients who are intermediate risk at risk assignment 2 with good risk cytogenetics/molecular genetics.

8. TRIAL TREATMENT

8.1 Investigational Medicinal Products (IMPs)

The following are regarded as IMPs for the purposes of this trial:

<u>During trial induction treatment</u>

- Intravenous mitoxantrone
- Intravenous liposomal daunorubicin
- Intravenous gemtuzumab ozogamicin

<u>During R3 consolidation treatment</u>

- Intravenous cytarabine
- Intravenous fludarabine

<u>During R4 HSCT conditioning treatment</u>

- Intravenous busulfan
- Intravenous cyclophosphamide
- Intravenous fludarabine

Gemtuzumab ozogamicin (Mylotarg ®) will be supplied free of charge by Pfizer Inc. for use in this trial. All other IMPs are licensed and should be obtained from routine hospital stock at sites.

Full details of IMPs, including preparation, labelling and accountability are contained in the national Pharmacy Manual. Country specific requirements for the safe handling of medicines must be adhered to.

8.2 Non Investigational Medicinal Products (NIMPs)

The following are regarded as NIMPs for the purposes of this trial:

During induction therapy
- Intravenous cytarabine
- Intrathecal therapy

During non-randomised consolidation therapy
- Intravenous fludarabine
- Intravenous cytarabine
- Intravenous idarubicin

Any drugs given during non-randomised HSCT conditioning treatment.

All mandatory supportive care.

9. INDUCTION TREATMENT

In the presence of clinical urgency patients may receive CNS directed therapy and up to two doses of cytarabine prior to trial entry. Alternatively, patients may receive hydroxycarbamide to reduce the WCC if that is the local preference. In exceptional circumstances, hydroxycarbamide may be given in addition to two doses of cytarabine.
If there is a clinical concern about performing a lumbar puncture (high WCC or haemorrhagic manifestations) then this may be delayed until that risk has abated. A diagnostic lumbar puncture without the administration of intrathecal chemotherapy should be avoided.

9.1 CNS Directed Treatment

The presence of CNS disease is defined as (all in an atraumatic tap):

CNS1 $<5 \times 10^6$/L WCC in Cerebral spinal fluid (CSF) with no blasts
CNS2 $<5 \times 10^6$/L WCC in CSF with blasts
CNS3 $\geq 5 \times 10^6$/L WCC in CSF with blasts

Traumatic tap: If the patient has circulating blasts in the peripheral blood and the lumbar puncture is traumatic (>10 RBC/ml) and contains >5 WBC/ml, Steinherz/Bleyer algorithm should be used to distinguish between CNS2 and CNS3:

CSF WBC/CSF RBC >2 x blood WBC/RBC

If CSF ratio is greater than two times the blood ratio, this is considered positive and the patient is defined as having CNS3; if less than two times the ratio the patient is classified as having CNS2.

Clinically significant neurological deficits (such as cranial nerve lesions) and/or radiological evidence of an intracranial or intradural mass consistent with MS should be considered to represent CNS positivity. Patients with extradural CNS chloromas should be treated as de novo AML as per the trial protocol.

Methotrexate is avoided in the first course due to concerns of increasing gemtuzumab ozogamicin associated hepatotoxicity and lack of superior efficacy over cytarabine monotherapy in clinical trials.The first lumbar puncture with intrathecal cytarabine may be performed along with other procedures prior to trial treatment as per local practice. If there are significant concerns about haemorrhagic risk or the patient is unfit for general anaesthetic, the intrathecal chemotherapy can be deferred until that risk has abated.

Patients with clinical CNS involvement such as cranial nerve lesions or parenchymal brain lesions on imaging should be treated as CNS 3.

CNS1

If there is no evidence of CNS disease at diagnosis, patients should receive a total of two injections of intrathecal cytarabine: one at the start of course 1 and one at the start of course 2 of chemotherapy.

CNS2 (or traumatic tap)

Due to concerns about the clear definition/characterisation of this group and evidence of some increase in CNS relapse rate (RR), these patients should receive two injections of intrathecal cytarabine per week from the start of course 1 until CNS is clear plus a further two injections of intrathecal cytarabine during course 1 (i.e. minimum of three injections of intrathecal cytarabine in course 1). CNS 2 patients should also receive one injection of intrathecal cytarabine at the start of course 2 of chemotherapy.

CNS3

Patients with CNS3 should receive two injections of intrathecal cytarabine per week from the start of course 1 until the CNS is clear plus a further two injections of intrathecal cytarabine during course 1. A minimum of six intrathecal injections of cytarabine should be given in a period of three weeks from diagnosis. Patients with cranial nerve lesions or parenchymal lesions on imaging should receive six intrathecal injections and be reassessed. Presence of persisting abnormalities should be discussed with the clinical co-ordinators.

This intensive phase during course 1 should be followed by triple intrathecal chemotherapy (as below) with each cycle of systemic chemotherapy.

Cranial irradiation should only be considered for CNS disease which is refractory to intrathecal chemotherapy and should be discussed with the clinical coordinators.

Scheduling of intrathecal chemotherapy

At start of course 1 (all patients)

At start of course 2 (CNS 1 or 2)

CNS 2 receive twice weekly IT cytarabine in first cycle until clear +2 (minimum 3 injections)

CNS 3 receive twice weekly IT cytarabine in first cycle until clear +2 (minimum 6 injections)

Age (years)	Cytarabine
<1	15 mg
1	20 mg
2	25 mg
>3 or over	30 mg

CNS3 receive triple IT at start of course 2 & with each subsequent course

Age	Methotrexate	Cytarabine	Hydrocortisone
<1	5 mg	15 mg	5 mg
1	7.5 mg	20 mg	7.5 mg
2	10 mg	25 mg	10 mg
>3 or over	12.5 mg	30 mg	12.5 mg

Intrathecal chemotherapy at the start of each course of treatment can be timed to coincide with other procedures requiring a general anaesthetic.

9.2 Recommendations for Pre Treatment Supportive Care

Management of tumour lysis risk and hyperleukocytosis can be according to local practice but the following guidance is strongly recommended.

9.2.1 Tumour lysis

All patients should be adequately hydrated with 2.5-3 l/m^2/day of hydration fluid at diagnosis. Potassium should not be added to hydration fluids during lysis. Allopurinol should be started prior to induction therapy and continued for at least 5 days. In patients considered to be high risk for tumour lysis syndrome (e.g. WCC >100 x 10^9/L, or renal impairment) rasburicase should be considered in place of allopurinol.

9.2.2 Management of hyperleukocytosis

Patients with high WCC leukaemia (hyperleukocytosis) are at risk of death or serious complications due to leukostasis/hyperviscosity syndrome, coagulopathy or tumour lysis syndrome. A high WCC leukaemia is generally defined as a WCC >100 x 10^9/L except in monocytic AML (FAB type M5) when a WCC of >50 x 10^9/L may be problematic because the cells are large, tend to aggregate, and cause coagulopathy more readily.

- Packed red cells, because of their high haematocrit (~70%), may exacerbate leukostasis. Generally red cell transfusion should be avoided or used with caution until the WCC has been reduced to safe levels as per standard clinical practice.
- Start rasburicase (if indicated check Glucose 6Phosphate Deficiency (G6PD) before starting), hyperhydrate and monitor biochemistry as per local supportive care protocols.
- Maintain the platelet count above 50 x 10^9/l. In the presence of active bleeding or coagulopathy, maintain platelets above 100 x 10^9/l.
- Correct any coagulopathy and keep the fibrinogen >1 g/l.
    - Commence chemotherapy urgently. Two doses of cytarabine as per induction chemotherapy or hydroxycarbamide to reduce WCC may be given before trial entry. In exceptional circumstances hydroxycarbamide may be given in addition to two doses of cytarabine.

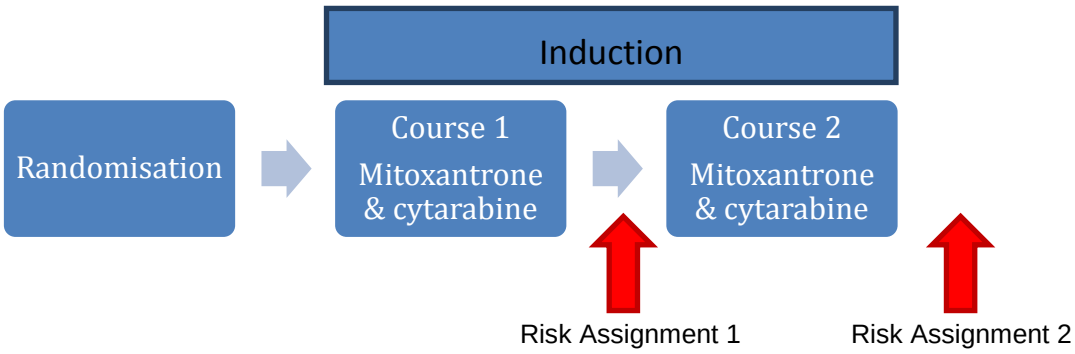
- The use of leukopheresis/exchange transfusion will be at the discretion of the treating physician.

9.3 R1: Induction Treatment

Induction treatment should start as soon as possible after trial entry at the point of R1.

9.3.1 R1 Arm A: mitoxantrone & cytarabine

Figure 8: R1 Arm A



Course 1:

See section 9.4 for details of gemtuzumab ozogamicin treatment during course 1 of induction.

Table 7: R1 Arm A course 1 treatment schedule

	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9	Day 10
Mitoxantrone 12 mg/m^2	●	●	●	●						
Cytarabine 100 mg/m^2/dose	● ●	● ●	● ●	● ●	● ●	● ●	● ●	● ●	● ●	● ●

- **Mitoxantrone**: 12 mg/m^2 daily by IV infusion over 1 hour on days 1, 2, 3 and 4 (total 4 doses)
- **Cytarabine:**100 mg/m^2 12 hourly by IV bolus on days 1-10 inclusive (total 20 doses)

Infants

Infants less than 12 months, weighing ≤10 kg or those with a BSA <0.5 m^2 should have all drug doses calculated as mg/kg:

- Mitoxantrone: 0.4 mg/kg/dose
- Cytarabine: 3.3 mg/kg/dose

Patients without CNS disease should receive age appropriate intrathecal cytarabine at the start of the course of chemotherapy. Patients with CNS disease should follow the instructions given in section 9.1.

Patients not in the dose finding study can start the next course of chemotherapy when the count has recovered to neutrophils 0.75 x 10^9/L and platelets to 75 x 10^9/L, when the risk group has been assigned and when the patient is clinically well.

For patients participating in the dose finding study, it is important to know whether they are experiencing a haematological DLT. Therefore, these patients should not start course 2 prior to day 45 in the absence of count recovery defined as neutrophil count 1.0 x 10^9/L and platelet count 80 x 10^9/L, unless absence of count recovery is due to residual disease. Refer to section 9.4.3.5 for definitions of DLTs.

Investigators will be informed of the risk group and the allocated treatment. Patients who fail to achieve CR after course 1 may start course 2 at the investigators discretion.

Course 2 (for non-high risk patients):

Table 8: R1 Arm A course 2 treatment schedule

	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9	Day 10
Mitoxantrone 12 mg/m^2	●	●	●							
Cytarabine 100 mg/m^2/dose	● ●	● ●	● ●	● ●	● ●	● ●	● ●	● ●		

- **Mitoxantrone:** 12 mg/m^2 daily by IV infusion over 1 hour on days 1, 2 and 3 (total 3 doses).
- **Cytarabine:** 100 mg/m^2 12 hourly by IV bolus on days 1-8 inclusive (total 16 doses).

Infants

Infants less than 12 months or weighing ≤10 kg or less or with a BSA <0.5m^2 should have all drug doses calculated as mg/kg:

- Mitoxantrone: 0.4 mg/kg/dose
- Cytarabine: 3.3 mg/kg/dose

Patients without CNS disease should receive age appropriate intrathecal cytarabine at the start of the course of chemotherapy. Patients with CNS disease should follow the instructions given in section 9.1.

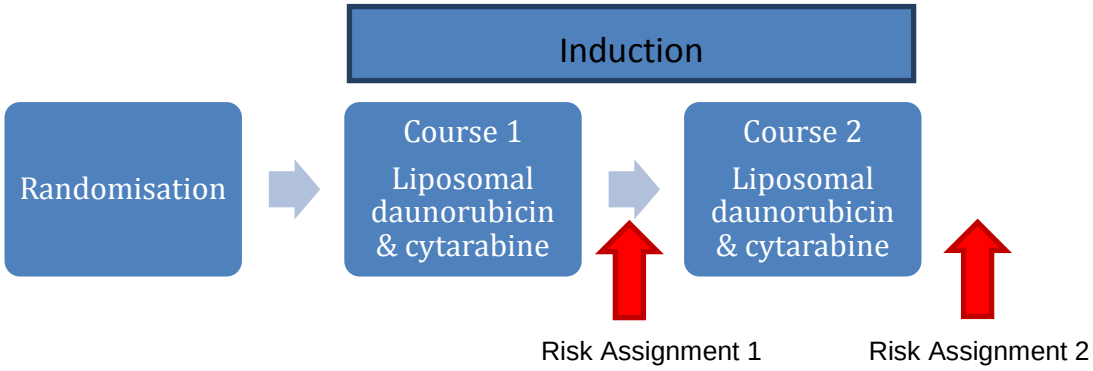
Patients can start the next course of chemotherapy when the count has recovered to neutrophils 0.75 x 10^9/L and platelets to 75 x 10^9/, when the risk group has been assigned and when the patient is clinically well.

For patients participating in the dose finding study, it is important to know whether they are experiencing a haematological DLT. Therefore, these patients should not start course 3 prior to day 45 in the absence of count recovery defined as neutrophil count 1.0 x 10^9/L and platelet count 80 x 10^9/L, unless absence of count recovery is due to residual disease. Refer to section 9.4.3.5 for definitions of DLTs.

Investigators will be informed of the risk group and the allocated treatment.

9.3.2 R1 Arm B: liposomal daunorubicin & cytarabine

Figure 9: R1 Arm B induction



Course 1:

See section 9.4 for details of gemtuzumab ozogamicin treatment during course 1 of induction for patients also participating in the dose finding study.

Table 9: R1 Arm B course 1 treatment schedule

	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9	Day 10
Liposomal daunorubicin 80 mg/m^2	●		●		●					
Cytarabine 100 mg/m^2/dose	● ●	● ●	● ●	● ●	● ●	● ●	● ●	● ●	● ●	● ●

- **Liposomal daunorubicin:** 80 mg/m^2 daily by 1 hour IV infusion on days 1, 3 and 5 (total 3 doses)
- **Cytarabine:** 100 mg/m^2 12 hourly by IV bolus on days 1-10 inclusive (total 20 doses)

Infants

Infants less than 12 months, weighing ≤10 kg or those with a BSA <0.5 m^2 should have all drug doses calculated as mg/kg:

- Liposomal daunorubicin: 2.6 mg/kg/dose
- Cytarabine: 3.3 mg/kg/dose

Patients without CNS disease should receive age appropriate intrathecal cytarabine at the start of the course of chemotherapy. Patients with CNS disease should follow the instructions given in section 9.1.

Patients can start the next course of chemotherapy when the count has recovered to neutrophils 0.75 x 10^9/L and platelets to 75 x 10^9/L, when the risk group has been assigned and when the patient is clinically well.

For patients participating in the dose finding study, it is important to know whether they are experiencing a haematological DLT. Therefore, these patients should not start course 2 prior to day 45 in the absence of count recovery defined as neutrophil count 1.0 x 10^9/L and platelet count 80 x 10^9/L, unless absence of count recovery is due to residual disease. Refer to section 9.4.3.5 for definitions of DLTs.

Investigators will be informed of the risk group and the allocated treatment.

Course 2 (non-high risk patients):

Table 10: R1 Arm B course 2 treatment schedule

	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9	Day 10
Liposomal daunorubicin 60 mg/m^2	●		●		●					
Cytarabine 100 mg/m^2/dose	● ●	● ●	● ●	● ●	● ●	● ●	● ●	● ●		

- **Liposomal daunorubicin:** 60 mg/m^2 daily by 1 hour IV infusion on days 1, 3 and 5 (total 3 doses)
  NB this dose differs from Course 1
- **Cytarabine:** 100 mg/m^2 12 hourly by IV bolus on days 1-8 inclusive (total 16 doses)

Infants
Infants less than 12 months, weighing ≤10 kg or those with a BSA <0.5 m^2 should have all drug doses calculated as mg/kg:
- Liposomal daunorubicin: 2.0 mg/kg/dose
- Cytarabine: 3.3 mg/kg/dose

Patients without CNS disease should receive age appropriate intrathecal cytarabine at the start of the course of chemotherapy. Patients with CNS disease should follow the instructions given in section 9.1.

Patients can start the next course of chemotherapy when the count has recovered to neutrophils 0.75 x 10^9/L and platelets to 75 x 10^9/L, when the risk group has been assigned and when the patient is clinically well. Investigators will be informed of the risk group and the allocated treatment.

For patients participating in the dose finding study, it is important to know whether they are experiencing a haematological DLT. Therefore, these patients should not start course 3 prior to day 45 in the absence of count recovery defined as neutrophil count 1.0 x 10^9/L and platelet count 80 x 10^9/L, unless absence of count recovery is due to residual disease. Refer to section 9.4.3.5 for definitions of DLTs.

9.3.3 Course 2 for high risk patients

High risk patients can receive FLA-Ida when the count has recovered to neutrophils 0.75 x 10^9/L and platelets to 75 x 10^9/L after course 1. Treatment may be started earlier in patients who have not achieved CR (≥ 5% blasts confirmed by flow).

For patients participating in the dose finding study, it is important to know whether they are experiencing a haematological DLT. Therefore, these patients should not start course 2 prior to day 45 in the absence of count recovery defined as neutrophil count 1.0 x 10^9/L and platelet count 80 x 10^9/L, unless absence of count recovery is due to residual disease. Refer to section 9.4.3.5 for definitions of DLTs.

If tissue-typing has not already been performed, all high risk patients and their siblings should be tissue-typed as soon as possible and if necessary an immediate search of donor registries initiated.

Fludarabine warnings for use:

All patients receiving fludarabine should receive irradiated blood products thereafter to prevent transfusion related graft-versus-host disease (GvHD).

Table 11: FLA-Ida high risk course 2 treatment schedule

	Day 1	Day 2	Day 3	Day 4	Day 5
Fludarabine 30 mg/m^2	●	●	●	●	●
Cytarabine 2g/m^2	●	●	●	●	●
Idarubicin 8 mg/m^2			●	●	●

- **Fludarabine:** 30 mg/m^2 every day by IV infusion over 30 minutes on days 1-5 inclusive (total 5 doses)
- **Cytarabine:** 2 g/m^2 every day by IV infusion over 4 hours on days 1-5 inclusive (total 5 doses). The cytarabine infusion should be started 4 hours from the start of the fludarabine infusion
- **Idarubicin:** 8 mg/m^2 every day by IV infusion over 1-6 hours on days 3, 4 and 5 (total 3 doses)

Infants

Infants less than 12 months or weighing 10 kg or less or with a BSA <0.5 m^2 should have all drug doses calculated as mg/kg:

- Fludarabine: 1.0 mg/kg/dose
- Cytarabine: 67 mg/kg/dose
- Idarubicin: 0.27 mg/kg/dose

NB: Patients should receive Prednisolone 0.5% eye drops (or local equivalent) 2 hourly (one drop per eye) during FLA-Ida and for 5 days after the last dose of cytarabine. Preservative free preparations are preferable.

Patients without CNS disease should receive age appropriate intrathecal cytarabine at the start of the block of chemotherapy. Patients with CNS disease should follow the instructions given in section 9.1.

Patients can start the next course of chemotherapy when the count has recovered to neutrophils 0.75 x 10^9/L and platelets to 75 x 10^9/L, and once the patient is clinically well.

For patients participating in the dose finding study, it is important to know whether they are experiencing a haematological DLT. Therefore, these patients should not start course 3 prior to day 45 in the absence of count recovery defined as neutrophil count 1.0 x 10^9/L and platelet count 80 x 10^9/L, unless absence of count recovery is due to residual disease. Refer to section 9.4.3.5 for definitions of DLTs.

Patients can proceed directly to HSCT (section 17) or have a third course of chemotherapy (FLA) if required to bridge to HSCT (section 10.3).

Any patients who fail to achieve CR or CRi at the end of course 2 will be deemed to have failed trial therapy and will not be eligible for further protocol treatment. Follow-up data should continue to be collected on these patients. Please see section 20 for further details on treatment discontinuation and patient withdrawal.

9.4 Treatment with Gemtuzumab Ozogamicin

Gemtuzumab ozogamicin will be given with course 1 of induction therapy only, either as part of the dose finding study, as one dose for patients treated in centres not taking part in the dose finding study (if R2 not open) or in R2 once open.

Note: The day of administration and the interval between doses should not be adjusted in the dose finding study.

To ensure that children are treated effectively without overdosing, the Body Mass Index (BMI) should be checked at diagnosis, prior to treatment with gemtuzumab ozogamicin. Please see Appendix 2 - Gemtuzumab Ozogamicin Dose Modification for Obesity for further details on dosing.

9.4.1 Gemtuzumab ozogamicin: warnings for use:

- Azoles should be withheld for 5 days before and 5 days after administration of gemtuzumab ozogamicin.
- Administration of gemtuzumab ozogamicin can result in severe hypersensitivity reactions (including anaphylaxis) and other infusion-related reactions which may include severe pulmonary events which infrequently have been fatal. In most cases, infusion-related symptoms occur during the infusion or within 24 hours of administration of gemtuzumab ozogamicin and resolve.
    - Vital signs should be monitored during the infusion and for the first four hours following infusion. A gemtuzumab ozogamicin infusion should be interrupted in patients experiencing dyspnoea or clinically significant hypotension. Patients should be monitored until signs and symptoms completely resolve (see section 11, Table 24).
    - Patients who experience anaphylaxis, pulmonary oedema or acute respiratory distress syndrome (ARDS) should not receive further doses of gemtuzumab ozogamicin. Similarly patients who develop VOD should not receive further gemtuzumab ozogamicin.
- Infants less than 12 months, weighing ≤10 kg or those with a BSA <0.5 m^2 should have doses of gemtuzumab ozogamicin calculated as mg/kg:
    - Gemtuzumab ozogamicin 0.1mg/kg/dose
- Dose capping:
    - Doses of gemtuzumab ozogamicin should be capped at the 98th percentile BMI for obese children. See Appendix 2 - Gemtuzumab Ozogamicin Dose Modification for Obesity
    - Doses of gemtuzumab ozogamicin should be capped at a maximum of 5 mg/dose.
- Gemtuzumab ozogamicin is given on day 4* of treatment to allow the WCC to fall to <30 x 10^9/l and avoid the risk of tumour lysis. If the WCC has not fallen to <30 x 10^9/l by day 4, gemtuzumab ozogamicin should still be administered, as tumour lysis is unlikely to be precipitated by gemtuzumab ozogamicin in this setting.

*Where production of gemtuzumab ozogamicin dose is impossible (e.g. on a Sunday), day 4 gemtuzumab ozogamicin can be given on day 3 providing the WCC is <30 x 10^9/l. The interval between further doses (where applicable) should be 72 hours. All efforts should be made to adhere to the stated schedule.

Any other alterations to timing of gemtuzumab ozogamicin doses must be referred to the Clinical Coordinators.

9.4.2 Gemtuzumab ozogamicin: pre-medication

All patients MUST receive pre-medication to prevent infusion related AEs with:

- Chlorphenamine and paracetamol (or local equivalent) given within 1 hour (maximum 2 hours) prior to the infusion and every 4 hours as required. Patients should be dosed as per British National Formulary for Children (BNFc) (below) or with equivalent antihistamine/paracetamol doses according to national/local practice.
- Further doses of paracetamol will reduce the risk of late reactions during the following 24 hours.
- Patients who suffer an infusion related reaction should receive methylprednisolone according to local practice 30 minutes prior to any subsequent infusion, in addition to anti-histamine and paracetamol.

Table 12: Paracetamol and antihistamine suggesting dosing

Age	Paracetamol (max 4 doses in 24 hours)	Parenteral chlorphenamine (max 4 doses in 24 hours)	Oral chlorphenamine (max 6 doses in 24 hours)
3–6 months	60 mg	250 microgram/kg (max 2.5mg) IV	1 mg (max 12 hourly)
6 months–2 years	120 mg		
2–4 years	180 mg	2.5mg IV	1 mg
4–6 years	240 mg		
6–8 years	240–250 mg	5mg IV	2 mg
8–10 years	360–375 mg		
10–12 years	480–500 mg		
12–16 years	480–750 mg	10mg IV	4 mg
16–18 years	500 mg–1 g		

9.4.3 Embedded Dose Finding Study for gemtuzumab ozogamicin

9.4.3.1 Major dose finding study: dose cohort 1

Gemtuzumab ozogamicin 3 mg/m^2 will be given on day 4 (total 1 dose) of course 1 induction chemotherapy by IV infusion over 2 hours after the chemotherapy scheduled for that day. Doses of liposomal daunorubicin/mitoxantrone and cytarabine are given in sections 9.3.1 and 9.3.2.

Table 13: Dose cohort 1 treatment schedule: R1 Arm A

Course 1	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9	Day 10
Gemtuzumab ozogamicin 3 mg/m^2				●						
Mitoxantrone	●	●	●	●						
Cytarabine	● ●	● ●	● ●	● ●	● ●	● ●	● ●	● ●	● ●	● ●

Children weighing ≤10 kg or with a BSA of <0.5 m^2 should have doses of gemtuzumab ozogamicin calculated as mg/kg:

- Gemtuzumab ozogamicin 0.1 mg/kg/dose

Table 14: Dose cohort 1 treatment schedule: R1 Arm B

Course 1	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9	Day 10
Gemtuzumab ozogamicin 3 mg/m^2				●						
Liposomal daunorubicin	●		●		●					
Cytarabine	● ●	● ●	● ●	● ●	● ●	● ●	● ●	● ●	● ●	● ●

Children weighing ≤10 kg or with a BSA of <0.5 m^2 should have doses of gemtuzumab ozogamicin calculated as mg/kg:

- Gemtuzumab ozogamicin 0.1 mg/kg/dose

9.4.3.2 Major dose finding study: dose cohort 2

Gemtuzumab ozogamicin 3 mg/m^2 will be given on days 4 and 7 (total 2 doses) of course 1 of randomised induction chemotherapy by IV infusion over 2 hours after the chemotherapy scheduled for that day. Doses of liposomal daunorubicin/mitoxantrone and cytarabine are given in sections 9.3.1 and 9.3.2.

Table 15: Dose cohort 2 treatment schedule: R1 Arm A

Course 1	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9	Day 10
Gemtuzumab ozogamicin 3 mg/m^2/dose				●			●			
Mitoxantrone	●	●	●	●						
Cytarabine	● ●	● ●	● ●	● ●	● ●	● ●	● ●	● ●	● ●	● ●

Children weighing ≤10 kg or with a BSA of <0.5 m^2 should have doses of gemtuzumab ozogamicin calculated as mg/kg:

- Gemtuzumab ozogamicin 0.1 mg/kg/dose

Table 16: Dose cohort 2 treatment schedule: R1 Arm B

Course 1	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9	Day 10
Gemtuzumab ozogamicin 3 mg/m^2/dose				●			●			
Liposomal daunorubicin	●		●		●					
Cytarabine	● ●	● ●	● ●	● ●	● ●	● ●	● ●	● ●	● ●	● ●

Children weighing ≤10 kg or with a BSA of <0.5 m^2 should have doses of gemtuzumab ozogamicin calculated as mg/kg:

- Gemtuzumab ozogamicin 0.1 mg/kg/dose

9.4.3.3 Major dose finding study: dose cohort 3

Gemtuzumab ozogamicin 3 mg/m^2 will be given on days 4, 7 and 10 (total 3 doses) of course 1 of the randomised induction chemotherapy by IV infusion over 2 hours after the scheduled chemotherapy for that day. Doses of liposomal daunorubicin/mitoxantrone and cytarabine are given in sections 9.3.1 and 9.3.2.

Table 17: Dose cohort 3 treatment schedule: R1 Arm A

Course 1	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9	Day 10
Gemtuzumab ozogamicin 3 mg/m^2/dose				●			●			●
Mitoxantrone	●	●	●	●						
Cytarabine	● ●	● ●	● ●	● ●	● ●	● ●	● ●	● ●	● ●	● ●

Children weighing ≤10 kg or with a BSA of <0.5 m^2 should have doses of gemtuzumab ozogamicin calculated as mg/kg:

- Gemtuzumab ozogamicin 0.1 mg/kg/dose

Table 18: Dose cohort 3 treatment schedule: R1 Arm B

Course 1	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9	Day 10
Gemtuzumab ozogamicin 3 mg/m^2/dose				●			●			●
Liposomal daunorubicin	●		●		●					
Cytarabine	● ●	● ●	● ●	● ●	● ●	● ●	● ●	● ●	● ●	● ●

Children weighing ≤10 kg or with a BSA of <0.5 m^2 should have doses of gemtuzumab ozogamicin calculated as mg/kg:

- Gemtuzumab ozogamicin 0.1 mg/kg/dose

9.4.3.4 Minor dose finding study: infant dose cohorts

Infants aged ≥12 weeks and <12 months will be recruited to the minor gemtuzumab ozogamicin dose finding study in separate infant dose cohorts. Infants less <12 weeks will not be eligible for the gemtuzumab ozogamicin dose finding study. The pre-medication and dosing schedules for each of the infant cohorts will be the same as the non-infant cohorts described in sections 9.4.3, 9.4.3.2 and 9.4.3.3.

Infants should have doses of gemtuzumab ozogamicin calculated as mg/kg:

- Gemtuzumab ozogamicin 0.1 mg/kg/dose

9.4.3.5 Definition of DLT

DLTs will be evaluable from the date of trial entry to count recovery to neutrophil count >1.0 x 10^9/L and platelet count >80 x 10^9/L after course 2 of treatment (up to day 45).

DLTs will be defined as any of the following events that are assessed as being possibly, probably or definitely related to any of the induction chemotherapy Investigational Medicinal Products (IMPs) as assessed by the treating Investigator:

- **Haematological DLT:**
  - Failure to recover neutrophil count to 1.0 x 10^9/L by day 45 post course 1 or 2 of treatment
  - Failure to recover non-transfusion dependent platelet count to 80 x 10^9/L by day 45 due to documented bone marrow aplasia/hypoplasia.

If failure to recover peripheral count by day 45 after the start of course 1 is due to leukaemic infiltration, this will render the patient non-evaluable for haematological DLT. If the bone marrow sample is considered morphologically unevaluable, this would also render the patient non-evaluable for haematological DLT. These patients will however be evaluable for non-haematological DLT.

- **Non-haematological DLTs**:
  - **Death** from any cause other than AML
  - **VOD**
  - **Cardiac Disorders:** Any grade ≥3 reduction of left ventricular systolic function, confirmed by local cardiology review
  - **Any grade 3 or higher non-haematological toxicity** persisting for >48 hours without resolution to grade ≤2, with the exception of:
    - Alopecia
    - Anorexia
    - Nausea
    - Grade 3 or 4 mucositis that resolves to grade ≤2 within 14 days
    - Grade 3 or 4 vomiting that resolves to grade ≤2 within 7 days
    - Grade 3 or 4 diarrhoea that resolves to grade ≤2 within 7 days
    - Grade 3 or 4 elevation in amylase, lipase, or direct or total bilirubin that is asymptomatic and that returns to grade ≤2 elevation within 14 days
    - Grade 4 elevation in hepatic transaminases (aspartate transaminase (AST), alanine transaminase (ALT) or gamma-glutamyl transferase (GGT)) and alkaline phosphatase that returns to grade ≤3 elevation within 14 days
    - Grade 3 or 4 fever with neutropenia, with or without infection
    - Grade 3 or 4 infection
    - Grade 3 or 4 electrolyte abnormalities that are not associated with clinical sequelae
    - Grade 3 or 4 hypotension that can be explained by sepsis

Haematological DLT post course 1 and 2 should be assessed by a bone marrow aspirate at count recovery but not later than 35 days from the start of the course (1 or 2) in the absence of count recovery. If the cause of slow count recovery can be reliably determined to be due to either myelosuppression or resistant disease, the bone marrow aspirate does not need to be repeated. However, if the sample obtained is inadequate for reliable assessment or the cause of delayed count recovery cannot be morphologically determined and confirmed by flow, the bone marrow aspirate should be repeated at weekly intervals until day 45 to determine the cause of delayed count recovery.

Delayed count recovery will be defined as neutropenia and/or thrombocytopenia more than 45 days from the start of a course of chemotherapy. The duration of myelosuppression will be collected.

The cardinal features of VOD are:

- Hyperbilirubinaemia
- Ascites
- Weight gain secondary to fluid retention
- Hepatosplenomegaly
- Platelet refractoriness

Definitive diagnosis can be challenging and ultrasound findings are not always conclusive, but patients with two or more of these features should be referred for ultrasound when slow or reversed hepatic blood flow is confirmatory. VOD cases will be discussed centrally with the Clinical

Coordinators to determine if the diagnostic criteria are met. A post mortem liver biopsy will be requested, but not mandated, on all VOD related deaths.

Suspected DLTs must be reported to the UK Trials Office on the Suspected DLT Form immediately upon awareness of the event.

Please fax to +44 (0)121 414 9520

9.4.4 Gemtuzumab Ozogamicin for Centres not Participating in the Dose Finding Study

Until the safety data from the first dose cohort of the major dose finding study has been reviewed and the treatment confirmed to be safe, only patients who are participating in the gemtuzumab ozogamicin dose finding study will receive gemtuzumab ozogamicin.

After the safety of a single dose of gemtuzumab ozogamicin has been confirmed by the DMC for use in patients aged ≥12 months patients who are not being treated in a centre participating in the major dose finding study, are ≥12 months of age and are eligible to receive gemtuzumab ozogamicin can receive a single dose of gemtuzumab ozogamicin on day 4 of course 1 of induction chemotherapy (see sections 9.4.1, 9.4.2 and 9.4.3 for details on pre-medication and dosing schedules). All centres which have not taken part in the embedded dose finding study will be formally activated by the NCC to enable them to give gemtuzumab ozogamicin on trial.

After one dose has been approved for use in infants aged between ≥12 weeks and <12 months by the DMC, all infants who are being treated in centres not participating in the minor dose finding study, are ≥28 days and <12 months and are eligible to receive gemtuzumab ozogamicin can receive a single dose of gemtuzumab ozogamicin on day 4 of course 1 of induction chemotherapy (see sections 9.4.1, 9.4.2 and 9.4.3 for details on pre-medication and dosing schedules). Infants less than 12 months, weighing ≤10 kg or those with a BSA <0.5 m^2 should have doses of gemtuzumab ozogamicin calculated as mg/kg:

- Gemtuzumab ozogamicin 0.1mg/kg/dose

Infants aged <28 days are not eligible to receive gemtuzumab ozogamicin in the trial.

9.4.5 R2: Gemtuzumab Ozogamicin Randomisation

If after the available data from cohort 2 of the dose finding study has been reviewed by the DMC and the DMC confirms that two doses of gemtuzumab ozogamicin can safely be delivered with the induction regimens, R2 will open in all centres not taking part in the embedded dose finding study. On completion of the dose finding study, R2 will be available in all centres.

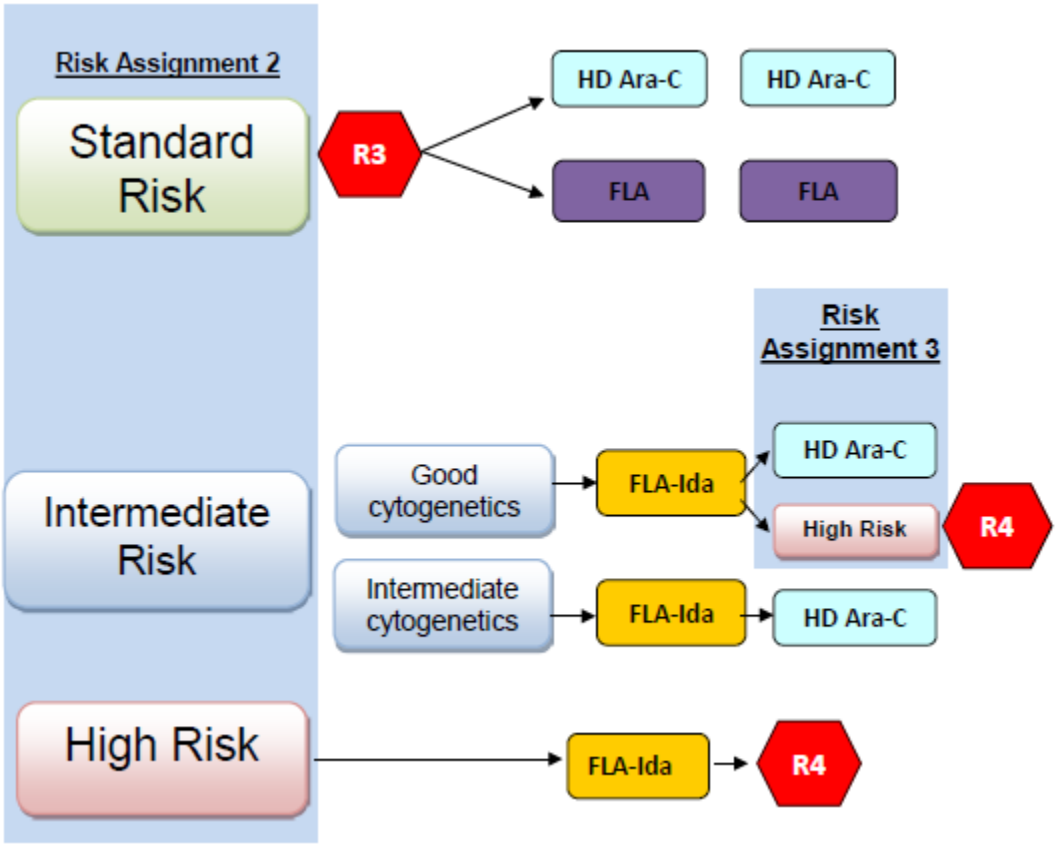
R2 will randomise 1 dose vs 2 doses of 3 mg/m^2 gemtuzumab ozogamicin. If 3 doses are confirmed to be safe to deliver with the induction regimens, R2 will be amended to randomise 1 dose vs 3 doses of 3 mg/m^2 gemtuzumab ozogamicin. R2 will open first for patients aged ≥12 months, and then expand to include patients aged ≥12 weeks and <12 months when the DMC recommends this action after reviewing data from the minor dose finding study.

See sections 9.4.1, 9.4.2 and 9.4.3 for details on warnings for use and pre-medication. Treatment will be given according to the schedules in section9.4.3.

10.CONSOLIDATION

Figure 10 shows consolidation treatment for patients classified as non-high risk post course 1 (risk group assignment 1). These patients are stratified into three clinical risk groups: standard, intermediate and high, after course 2 (risk assignment 2).

Figure 10: Consolidation



10.1R3 for Standard Risk Patients

The following patients (standard risk at risk assignment 2) will be eligible for R3 (please see eligibility criteria, section 4.1.5):

Cytogenetics/ Molecular	MRD post course 1		MRD post course 2	
	Flow	Molecular*	Flow	Molecular*
Good Risk	N/A	N/A	<0.1% MRD	> 3-log reduction
Intermediate Risk	<0.1% MRD	> 3-log reduction	<0.1% MRD	> 3-log reduction

See section 10.2 for patients classified as intermediate risk at risk assignment 2 and for patients classified as high risk at risk assignment 2.

Patients can start course 3 (consolidation) when the count has recovered to neutrophils 0.75 x 10^9/L and platelets to 75 x 10^9/L, when the risk group has been assigned and when the patient is clinically well.

For patients participating in the dose finding study, it is important to know whether they are experiencing a haematological DLT. Therefore, these patients should not start course 3 prior to day 45 in the absence of count recovery defined as neutrophil count 1.0 x 10^9/L and platelet count 80 x 10^9/L, unless absence of count recovery is due to residual disease. Refer to section 9.4.3.5 for definitions of DLTs.

Investigators will be informed of the risk group and the allocated treatment.

10.1.1 Arm C: high dose cytarabine (HD Ara-C)

Courses 3 and 4

Table 19: Arm C treatment schedule

	Day 1	Day 2	Day 3	Day 4	Day 5
HD cytarabine 3g/m^2/dose	● ●		● ●		● ●

- **Cytarabine:** 3 g/m^2 12 hourly by IV infusion over 4 hours on days 1, 3 and 5 (total 6 doses)

Infants
Infants less than 12 months or weighing ≤10 kg or with a BSA <0.5m^2 should have all drug doses calculated as mg/kg:

- Cytarabine: 100 mg/kg/dose

NB: Patients should receive prednisolone 0.5% eye drops (or local equivalent) 2 hourly (one drop per eye) during HD cytarabine and for 5 days after the last dose of cytarabine. Preservative free preparations are preferable.

Course 4 consolidation can start on when the count has recovered to neutrophils 0.75 x 10^9/L and platelets to 75 x 10^9/L from course 3 and when the patient is clinically well.

10.1.2 Arm D: fludarabine & cytarabine (FLA)

Fludarabine warnings for use:
All patients receiving fludarabine should receive irradiated blood products thereafter to prevent transfusion related GvHD.

Table 20: R3 Arm D treatment schedule

	Day 1	Day 2	Day 3	Day 4	Day 5
Fludarabine 30 mg/m^2	●	●	●	●	●
Cytarabine 2g/m^2	●	●	●	●	●

Courses 3 and 4

- **Fludarabine:** 30 mg/m^2 daily by IV infusion over 30 minutes on days 1-5 inclusive (total 5 doses).

- **Cytarabine:** 2 g/m^2 daily by IV infusion over 4 hours on days 1-5 inclusive (total 5 doses).The cytarabine infusion should be started 4 hours after the start of the fludarabine infusion.

Infants

Infants less than 12 months or weighing ≤10 kg or those with a BSA <0.5 m^2 should have all drug doses calculated as mg/kg:

- Fludarabine: 1 mg/kg/dose
- Cytarabine: 67 mg/kg/dose

NB: Patients should receive prednisolone 0.5% eye drops (or local equivalent) 2 hourly (one drop per eye) during FLA and for 5 days after the last dose of cytarabine. Preservative free preparations are preferable.

Course 4 consolidation can start when the count has recovered to neutrophils 0.75 x 10^9/L and platelets to 75 x 10^9/L from course 3 and when the patient is clinically well.

10.2 Intensified Consolidation for Intermediate and High Risk Patients

10.2.1 Course 3: FLA-Ida

All of the drugs given as part of non-randomised consolidation are NIMPs.

Patients classified as Intermediate risk at risk assignment 2 (as defined in section 7.2.2) are not eligible for R3 and should receive a course of fludarabine, cytarabine and idarubicin (FLA-Ida) as the 3rd course of treatment (1st course of consolidation therapy). This includes the following patients:

Cytogenetics/molecular genetics	MRD post course 1		MRD post course 2	
	Flow	Molecular*	Flow	Molecular*
Good Risk	N/A	N/A	>0.1% MRD	≤ 3-log reduction
Intermediate Risk	>0.1% MRD	≤ 3-log reduction	N/A	N/A

♦The trend in the level of leukaemic transcripts will also be considered

Patients classified as high risk at risk assignment 2 (as defined in section 7.2.3) are not eligible for R3 and should receive a course of fludarabine, cytarabine and idarubicin (FLA-Ida) as the 3rd course of treatment prior to moving to HSCT post course 3.

Patients can start FLA-Ida (course 3: consolidation) when the count has recovered to neutrophils 0.75 x 10^9/L and platelets to 75 x 10^9/L, when the risk group has been assigned and when the patient is clinically well.

For patients participating in the dose finding study, it is important to know whether they are experiencing a haematological DLT. Therefore, these patients should not start course 3 prior to day 45 in the absence of count recovery defined as neutrophil count 1.0 x 10^9/L and platelet count 80 x 10^9/L, unless absence of count recovery is due to residual disease. Refer to section 9.4.3.5 for definitions of DLTs.

Investigators will be informed of the risk group and the allocated treatment.

Fludarabine warnings for use:

All patients receiving fludarabine should receive irradiated blood products thereafter to prevent transfusion related GvHD.

Table 21: FLA-Ida course 3 treatment schedule

	Day 1	Day 2	Day 3	Day 4	Day 5
Fludarabine 30 mg/m^2	●	●	●	●	●
Cytarabine 2g/m^2	●	●	●	●	●
Idarubicin 8 mg/m^2			●	●	●

- **Fludarabine:** 30 mg/m^2 daily by IV infusion over 30 minutes on days 1-5 inclusive (total 5 doses)
- **Cytarabine:** 2 g/m^{2}daily by IV infusion over 4 hours on days 1-5 inclusive (total 5 doses). The cytarabine infusion should be started 4 hours from the start of the fludarabine infusion.
- **Idarubicin** 8 mg/m^{2}daily by IV infusion over 1-6 hours on days 3, 4 and 5 (total 3 doses)

Infants
Infants less than 12 months or weighing ≤10 kg or those with a BSA <0.5 m^2 should have all drug doses calculated as mg/kg:

- Fludarabine: 1.0 mg/kg/dose
- Cytarabine: 67 mg/kg/dose
- Idarubicin: 0.27 mg/kg/dose

NB: Patients should receive prednisolone 0.5% eye drops (or local equivalent) 2 hourly (one drop per eye) during FLA-Ida and for 5 days following the last dose of cytarabine. Preservative free preparations are preferable.

Following this course of FLA-Ida, treatment will be further stratified:

- Patients with good risk cytogenetics will be reassessed after course 3: risk assignment 3 (section 7.3), and will be classified as either:
  - Intermediate risk (MRD <0.1%) and will receive high dose cytarabine as course 4 (section 10.2.2)
    or
  - Reclassified as high risk (MRD>0.1%). All patients who are reclassified as high risk post course 3 should be discussed with the Clinical Coordinators, and may be eligible for R4.
- Patients with intermediate risk cytogenetics (and a MRD level <0.1% after course 2) will receive high dose cytarabine as course 4 (section 10.2.2)

Patients can start course 4 when the count has recovered to neutrophils 0.75 x 10^9/L and platelets to 75 x 10^9/L and when the patient is clinically well.

10.2.2 Course 4: High dose cytarabine (HD Ara-C) for intermediate risk patients

Table 22: HD Ara-C course 4 treatment schedule

	Day 1	Day 2	Day 3	Day 4	Day 5
HD cytarabine 3g/m^2/dose	● ●		● ●		● ●

- **Cytarabine:** 3 g/m^2 12 hourly by IV infusion over 4 hours on days 1, 3 and 5 (total 6 doses)

Infants
Infants less than 12 months or weighing 10 kg or less or those with a BSA <0.5 m^2 should have all drug doses calculated as mg/kg:

- Cytarabine: 100 mg/kg/dose

NB: Patients should receive prednisolone 0.5% eye drops (or local equivalent) 2 hourly (one drop per eye) during HD Ara-C and for 5 days after the last dose of cytarabine. Preservative free preparations are preferable.

10.3 Consolidation for High Risk Patients Post Course 1

All of the drugs given as part of non-randomised consolidation are NIMPs.

Course 3: FLA for patients classified as high risk after course 1 (risk assignment 1)

Patients identified as high risk after course 1 may receive a course of FLA as course 3 if a third course of chemotherapy is necessary to bridge to HSCT.

FLA can start when the count has recovered to neutrophils 0.75 x 10^9/L and platelets to 75 x 10^9/L after course 2 and when the patients is clinically well.

For patients participating in the dose finding study, it is important to know whether they are experiencing a haematological DLT. Therefore, these patients should not start course 3 prior to day 45 in the absence of count recovery defined as neutrophil count 1.0 x 10^9/L and platelet count 80 x 10^9/L, unless absence of count recovery is due to residual disease. Refer to section 9.4.3.5 for definitions of DLTs.

Fludarabine warnings for use:

All patients receiving fludarabine should receive irradiated blood products thereafter to prevent transfusion related GvHD.

Table 23: FLA course 3 treatment schedule

	Day 1	Day 2	Day 3	Day 4	Day 5
Fludarabine 30 mg/m^2	●	●	●	●	●
Cytarabine 2g/m^2	●	●	●	●	●

- **Fludarabine:** 30 mg/m^2 every day by IV infusion over 30 minutes on days 1-5 inclusive (total 5 doses)
- **Cytarabine:** 2 g/m^2 every day by IV infusion over 4 hours on days 1-5 inclusive (total 5 doses). The cytarabine infusion should be started 4 hours from the start of the fludarabine infusion.

Infants
Infants less than 12 months or weighing ≤10 kg or those with a BSA <0.5 m^2 should have all drug doses calculated as mg/kg:

- Fludarabine: 1.0 mg/kg/dose
- Cytarabine: 67 mg/kg/dose

NB: Patients should receive prednisolone 0.5% eye drops (or local equivalent) 2 hourly (one drop per eye) during FLA and for 5 days after the last dose of cytarabine. Preservative free preparations are preferable.

11.DOSE MODIFICATIONS FOR TOXICITY

Dose modifications for obesity are not recommended due to the risk of underdosing except for gemtuzumab ozogamicin. Doses of gemtuzumab ozogamicin should be capped for obese children at the 98th percentile BMI. See Appendix 2 - Gemtuzumab Ozogamicin Dose Modification for Obesity. Doses of gemtuzumab ozogamicin should be capped at a maximum of 5 mg/dose.

The exception is dose reductions for toxicity as detailed below for all patients. Cardiotoxicity monitoring guidelines should be rigidly adhered to in obese patients.

Course 1:

Abnormal hepatic or renal function at diagnosis may be secondary to leukaemic infiltration or an unrelated disorder such as Gilbert's disease. Therefore patients with abnormal hepatic or renal function at diagnosis require thorough investigation to identify the cause before consideration is given to reducing doses of anthracyclines in course 1. Please discuss with Clinical Coordinators before modifying the anthracycline dose in this course.

Table 24: Dose modifications for gemtuzumab ozogamicin course 1 of induction

Toxicity	Gemtuzumab ozogamicin
Prior to infusion:	
Bilirubin >2.5 x ULN	Omit further dose(s) until bilirubin falls to ≤2.5 x ULN
Prior to or during infusion:	
Symptomatic VOD	Omit further doses of gemtuzumab ozogamicin
Dyspnoea, significant hypotension and/or fever during infusion	Stop gemtuzumab ozogamicin infusion immediately. If symptoms resolve resume infusion at 50% and increment carefully as tolerated. If tolerated, any subsequent infusions should be preceded by methylprednisolone 30 minutes prior to the gemtuzumab ozogamicin infusion.
Anaphylaxis, pulmonary oedema or ARDS	Stop gemtuzumab ozogamicin. Omit further doses

All other courses:

Table 25: Dose modifications in all other courses

Toxicity	Mitoxantrone	Liposomal daunorubicin	Idarubicin	Fludarabine	High dose Cytarabine (≥2g/m^2/course)
Absolute Left Ventricular Fractional Shortening (LVFS) <28% or Ejection Fraction <55%	Discuss with Clinical Coordinators	Discuss with Clinical Coordinators	Discuss with Clinical Coordinators		
Bilirubin ≥2.5 x ULN	Treat with caution or 50% dose	Treat with caution or 50% dose	Treat with caution or 50% dose		
Occular irritation					Increase frequency of steroid eye

					drops or as local practice.
Cerebellar toxicity					Discuss with Clinical Coordinators/ Omit
Calculated creatinine clearance*:					
30-70ml/min/1.73m^2				75% dose	
<30ml/min/1.73m^2		50% dose	50% dose	Discuss with CI/Omit	Discuss with CI/Omit

*Calculate creatinine clearance from serum creatinine (SCr) according to the BNFc formula, or that in use nationally.

- Child > 1 year:
  Calculated creatinine clearance (ml/min/1.73m^2)= 40 x $\frac{\text{height (cm)}}{\text{SCr (µmol/L)}}$
- Child <1 year:
  Calculated creatinine clearance (ml/min/1.73m^2)= 30 x $\frac{\text{height (cm)}}{\text{SCr (µmol/L)}}$

Please discuss any other dose modifications or concerning toxicity not covered in this section with the Clinical Coordinators.

12. PATIENT ASSESSMENTS

12.1 Patient Assessments During Treatment

Please refer to Table 1 and Table 26 for the schedule of events. Please note that any HSCT specific assessments are detailed in section 17.2.6.

The following assessments should be performed before each course of treatment and as per local practice:

- Medical history and physical examination
- Karnofsky/Lansky performance status
- Weight and BSA
- Blood count
- Biochemistry
- Assessment of adverse reactions
- Bone marrow aspirate

The following assessment should be performed before courses 1 and 2:

- Lumbar puncture (local processing)

The following assessment should be performed prior to courses containing liposomal daunorubicin, mitoxantrone or idarubicin:

- Echocardiogram

Patients being treated with gemtuzumab ozogamicin should be monitored during the infusion and for 4 hours afterwards. Monitoring should include temperature, respiratory rate, heart rate and blood pressure.

After each course of treatment, a bone marrow aspirate should be performed on count recovery (neutrophil count >1.0 x 10^9/l and platelet count >80 x 10^9/l).

After courses 1 and 2, in the absence of count recovery, the bone marrow aspirate should be performed no later than 35 days after the start of the course of treatment. If the cause of slow count recovery can be reliably determined to be due to either myelosuppression or resistant disease, the bone marrow aspirate does not need to be repeated. However, if the sample obtained is inadequate for reliable assessment, a bone marrow aspirate should be repeated at weekly intervals until count recovery or until the cause for non-recovery is determined.

Patients with extramedullary disease should have a bone marrow aspirate after each course of chemotherapy to exclude bone marrow involvement which may occur despite the absence at presentation.

Bone marrow morphology should be assessed locally. Please see section 21 for details on central investigations and MRD monitoring. MRD monitoring is important to treatment assignment and therefore critical that samples are taken and sent centrally in a timely manner. To ensure that MRD samples are evaluable it is important that assessment marrows are scheduled to avoid sample transit over a weekend.

Please refer to section 18 for details of assessments to be performed at the end of treatment.

12.2 Patients With Non CNS Extramedullary AML

12.2.1 Isolated MS

Patients with MS will be assessed in a similar manner to all other patients. Additionally, appropriate cross sectional imaging should be performed according to local practice (not mandatory for the protocol). Significant residual disease post cycle 2 should be biopsied.

Patients with confirmed disease progression at the end of course 2 will not be eligible for further protocol treatment, but will be followed up within the trial. Patients with suspected disease progression, or confirmed/suspected residual disease should be discussed with the Clinical Coordinators.

12.2.2 Leukaemia cutis (LC)

Patients with LC will be assessed in a similar manner to other patients. Additionally, any leukaemia cutis post-course 2 should be biopsied where possible. Where biopsy has been possible, material obtained should be locally processed for cytogenetics, FISH and molecular genetics. Patients with confirmed disease progression at the end of course 2 will not be eligible for further protocol treatment, but will be followed up within the trial. Patients with suspected disease progression, or confirmed /suspected residual disease should be discussed with the Clinical Coordinators.

12.2.3 Extramedullary disease with marrow involvement

These patients will be assessed in a similar manner to all other patients. Additionally, any clinically residual extramedullary disease after course 2 should have the following assessments:

- appropriate cross sectional imaging according to local practice
- biopsy (where possible)

Material obtained where biopsy has been possible should be locally processed for cytogenetics, FISH and molecular genetics. Significant residual disease post cycle 2 should be biopsied. Patients with confirmed disease progression at the end of course 2 will not be eligible for further protocol treatment, but will be followed up within the trial. Patients with suspected disease progression, or confirmed /suspected residual disease should be discussed with the Clinical Coordinators.

13. ASSESSMENT OF RESPONSE

Response must be assessed in all patients after each course of treatment, regardless of the treatment pathway assigned as outlined in section 7.

Remission status will be assessed according to Creutzig et al [55, 56]. Bone marrow blast count should be assessed morphologically as well as by flow MRD. If the flow MRD result and morphological result are discrepant, the flow MRD result will be used to determine response.

The following definitions will be used:

Complete remission (CR):

All ofthe following must be achieved:

- Bone marrow blasts <5%. This must be confirmed by flow/molecular/FISH. For methodology see Figure 5.
- Absence of extramedullary disease
- Absolute neutrophil count (ANC) ≥1.0 x 10^9/l
- Platelet count ≥80 x 10^9/l

Morphological CR with incomplete count recovery (CRi):

- All CR criteria except for residual neutropenia (<1.0 x 10^9/l) or thrombocytopenia (<80 x 10^9/l)

Treatment failure due to resistant disease:

- Failure to achieve CR or CRi
- Patient survives ≥7 days after completion of initial treatment, with evidence of persistent leukaemia by blood and/or bone marrow examination

Relapse (after patient achieves CR):

- Bone marrow blasts ≥ 5%. This must be confirmed by flow cytometry
- Evidence of extramedullary disease

Any patients who fail to achieve CR or CRi at the end of course 2 will be deemed to have failed trial therapy and will not be eligible for further protocol treatment. Follow-up data should continue to be collected on these patients. Please see section 20 for further details on treatment discontinuation and patient withdrawal.

14. TREATMENT COMPLIANCE

Compliance with IMP treatment will be monitored by the relevant NCC and as specified in the national MyeChild 01 Pharmacy Manual and by the data on the Case Report Form (CRF).

15. SUPPORTIVE TREATMENT

AML therapy is intensive and associated with significant morbidity. Centres should consult their local supportive care protocols for further guidance. It is advisable to keep children in hospital during the induction period, and any block of treatment which includes anthracycline therapy, when severely neutropenic.

Specific pre-treatment medication and mandatory supportive care for each phase of treatment is provided within the relevant section.

15.1 GvHD Prophylaxis in Patients Receiving Fludarabine:

All patients receiving fludarabine should receive irradiated blood products thereafter to prevent transfusion related GvHD.

15.2 Tumour Lysis

All patients should be adequately hydrated with 2.5-3 l/m^2/day of hydration fluid at diagnosis. Potassium should not be added to hydration fluid during induction. Allopurinol should be started prior to induction therapy and continued for at least 5 days. In patients considered to be high risk for tumour lysis syndrome (e.g. WCC >100 x 10^9/l or renal impairment), rasburicase should be considered in place of allopurinol.

15.3 Pneumocystis Jirovecii Pneumonitis (PCP) Prophylaxis

PCP prophylaxis is recommended following fludarabine containing regimens and post HSCT. Co-trimoxazole is considered the first line agent. Dosing regimens should be according to local practice.

15.4 Antimicrobials

Antimicrobial prophylaxis, where considered appropriate, will be at the discretion of the treating physician. Antibiotic treatment of febrile neutropenia should be based on local supportive care guidance.

15.5 Antifungal Prophylaxis

Patients may either be nursed in high-efficiency particulate arrestance (HEPA) filtration or receive anti-fungal prophylaxis according to local practice. Azoles should be avoided in patients until 5 days after the final dose of gemtuzumab ozogamicin is administered.

15.6 Veno-Occlusive Disease (VOD)

VOD is a hepatic disorder that can occur as a result of the conditioning regimens post HSCT or due to gemtuzumab ozogamicin administration. (Please consult British Clinical Standards in Haematology (BCSH) Guidelines on diagnosis and management of VOD post stem cell transplantation [57]).

15.6.1 Diagnosis

VOD is largely a clinical disease requiring a high index of clinical suspicion. The cardinal features are:

- Hyperbilirubinaemia
- Ascites
- Weight gain secondary to fluid retention
- Hepatosplenomegaly
- Platelet refractoriness

Patients with two or more of these features should be referred for ultrasound. The diagnosis of VOD is confirmed by slow or reversed hepatic blood flow. However, definitive diagnosis can be challenging and ultrasound findings are not always conclusive. VOD cases will be discussed with the clinical coordinators to determine if they meet the criteria. A post mortem liver biopsy will be requested, but not mandated, on all VOD related deaths.

15.6.2 Prevention

Azole antifungal drugs should be avoided at diagnosis and for 5 days before and after the administration of gemtuzumab ozogamicin. The concomitant use of hepatotoxic drugs with gemtuzumab ozogamicin should be discouraged. Gemtuzumab ozogamicin frequently causes a transient elevation of liver function 8-10 days post infusion. This usually settles within 2-3 days and is not indicative of VOD. Defibrotide will not be used as prophylaxis of VOD.

15.6.3 Treatment

Defibrotide should be instigated promptly if VOD is suspected as treatment for VOD at a dose of 6.25 mg/kg given intravenously over 2 hours four times per day. The optimal duration of defibrotide in the treatment of VOD is unknown. Defibrotide should be continued for a minimum of 7 days and until the liver function is clearly improving.

15.7 Detection of Cardiotoxicity

Echocardiograms will be performed at the following time-points:

1. Baseline: prior to course 1 (Time point 1)
2. Prior to each course which includes anthracycline (Time point 2)
3. At end of treatment (time point 3)
4. At 12 months, 3 years, 5 years and 10 years after trial entry (Time point 4)

Subclinical cardiotoxicity will be defined as any of the following in the absence of clinical signs/symptoms;

- An ejection fraction <55%
- Fractional shortening <28%

Additional supportive therapy will be provided according to local practice. Consideration should be given to:

- Anti-bacterial, anti-fungal and anti-viral prophylaxis
- Management of febrile neutropenia with or without G-CSF support
- Interactions between hepatotoxic agents e.g. avoidance of azole therapy within 5 days of gemtuzumab ozogamicin

16. CONCOMITANT MEDICATION

Concomitant medication may be given as medically indicated.

Concurrent therapy that has the potential to interact with protocol medication should be avoided, if clinically possible. Please note that this is not a comprehensive list of drug interactions. Investigators should check for drug interactions as per local practice.

Stockley Drug Interactions lists potential interactions and recommends care in the following circumstances:

- Anti-convulsant therapy with anthracyclines
- High dose cytarabine with flucytosine and oral aciclovir therapy
- Hepatotoxic drugs should be avoided within 5 days of gemtuzumab ozogamicin therapy; however the PK parameters for patients pre-treated with paracetamol and anti-histamine are similar to those for patients who are not pre-treated with these drugs (as documented in the Investigator Brochure). Therefore, paracetamol and anti-histamines may be given.
- Heparin based anticoagulation is preferred to coumarin therapy should anticoagulation be necessary due to potential interactions.

17. R4: STEM CELL TRANSPLANT FOR HIGH RISK PATIENTS

THIS TREATMENT MUST BE GIVEN IN A CENTRE WHICH IS AN ACTIVE MEMBER OF A HSCT NATIONAL NETWORK.

Randomisation 4 (R4) will compare two different HSCT conditioning regimens. Patients will be randomised to receive either:

- Arm E: MAC regimen of busulfan and cyclophosphamide (Bu/Cy)
- Arm F: RIC regimen of fludarabine and busulfan (Flu/Bu)

Patients who decline randomisation to R4 may be transplanted at the discretion of the treating physician.

Table 26: Schedule of additional events: R4

			Months post-transplant								
	R4	**Pre-transplant**	**1**	**2**	**3**	**4**	**6**	**9**	**12**	**24**	**36**
Informed consent[1]	X										
Physical examination		X	X	X	X		X		X	X	X
Weight and BSA		X									
Blood count and biochemistry		X	X	X	X		X		X	X	X
Pregnancy test[2]		X									
Oxygen saturation		X	X	X	X						
Monitoring and recording of AEs					<<Continuous>>						
Assessment of Graft-versus-host disease (GvHD)			X	X	X	X	X	X	X	X	X
Virology/syphilis screening		X			<<Continuous as per local practice>>						
Peripheral blood sample for lineage specific chimerism		X	X	X	X	X	X	X	X		
Peripheral blood sample[3]		X	X		X		X		X		X
Clinical assessment of gonadal function including Tanner stage[4]									X		X
Blood samples for measurement of luteinizing hormone (LH)/follicle-stimulating hormone (FSH), Anti-mullerian hormone (AMH) (females), oestradiol (females) or inhibin B (males),		X							X		X

testosterone (males)[4, 5]											
Semen analysis (male age >16) or ovarian ultrasound (female age >16)											X
Bone marrow aspirate[3, 6]		X[7]	X		X		X	X	X		
Assessment for early regimen related toxicity			X		X						

1. Prior to any trial specific assessments
2. In female patients of child-bearing potential
3. Bone marrow and peripheral blood for MRD monitoring should be forwarded directly to the MRD laboratories, see the national MyeChild 01 laboratory manual
4. In patients ≥12 years
5. Where available
6. For consenting patients, bone marrow sample should be sent for LSC monitoring study
7. Bone marrow aspirate to be performed within 6 weeks prior to transplant

17.1 Donor Selection Hierarchy and Stem Cell Source

Patients and their siblings should be tissue-typed at diagnosis as part of routine standard of care.

For patients with intermediate and poor risk cytogenetics who have no HLA MFD, an unrelated and cord blood donor search should be initiated as soon as possible during induction course 1. Donors will be selected by the transplant centres using the following selection hierarchy approved by the UK Paediatric Bone Marrow Transplant Group or national equivalent. Medium/high resolution typing is required for adult unrelated donors (HLA A, B, C, DR and DQ) and unrelated cords (HLA A, B, C and DR loci).

MFD: Matched family donor

MUD: Matched unrelated donor

MMUD: Mismatched unrelated donor

MMFD: Mismatched family donor

MUCB: Matched unrelated cord blood

MMUCB: Mismatched unrelated cord blood

Table 27: Donor selection hierarchy

Choice	Family Donor	Unrelated Donor	Unrelated Cord Blood (CB)
1st	**MFD (bone marrow (BM), peripheral blood stem cells (PBSC) or CB)**		
2nd		**10/10 MUD** **9/10 1DQ MMUD**	**8/8 MUCB (Total Nucleated Cell (TNC) > 3 x 10^7/kg)**
3rd	**9/10 MMFD**	**9/10 (other) MMUD**	**5-7/8 MMUCB (TNC > 3 x 10^7/kg)**

NOTE: Patients in whom the best available donor is haploidentical, an 8/10 MMUD or a 4/8 MMUCB are excluded from the randomisation and should be transplanted as per local practice at their transplant centre.

For unrelated cord blood, a single cord is used if the cryopreserved TNC dose is > 3 x 10^7/kg. If TNC < 3 x 10^7/kg, a double cord transplant is preferred.

For family/unrelated donors, bone marrow is the preferred stem cell source in both arms but the use of peripheral blood stem cells is permissible. The use of peripheral blood stem cells from mismatched donors should be avoided wherever possible.

17.1.1 Serotherapy

Serotherapy is given only to patients transplanted from unrelated donors, 9/10 mismatched family donors or 5/8 matched unrelated cord blood units but not to patients receiving grafts from matched family donors or 6-8/8 unrelated cord blood units.

Appropriate cover for serotherapy should be given according to the local practice, and is recommended at least 12 hours pre the first dose and until 24 hours after the last dose, with steroid, antihistamine(s) and paracetamol.

Table 28: Serotherapy

Matched family donor	Unrelated donor	Unrelated cord blood
Full match - no serotherapy 9/10 match - serotherapy	9-10/10 match - serotherapy	6-8/8 match - no serotherapy 5/8 match – early serotherapy

17.2 Conditioning Regimen

17.2.1 Arm E: myeloablative busulfan/cyclophosphamide (MAC)

Table 29: R4 Arm E MAC conditioning treatment schedule

Please note that two schedules for anti-thymocyte globulin (ATG) are included on this table. ATG schedule should be selected based on donor type.

		D-11	D-10	D-9	D-8	D -7	D -6	D -5	D -4	D -3	D -2	D -1	D 0
Busulfan IV infusion over 3 hr AUC 70-100 mg/L x hr See below for starting doses	0 h		**X**	**X**	**X**[1]	**X**[1]							
	12 h		**X**	**X**	**X**[1]	**X**[1]							
Cyclophosphamide IV infusion, 1 hour 50 mg/kg /day with Mesna + hydration								**X**	**X**	**X**	**X**		
Unrelated or 9/10 mismatched family donor: **ATG**[2] IV infusion over 6-12 hours 2.5 mg/kg/day										**X**	**X**	**X**	

5/8 matched unrelated cord blood: **ATG**[2] IV infusion over 6-12 hours 2.5 mg/kg/day				X	X	X							
Clonazepam prophylaxis		X	X	X	X	X	X	X					
Ciclosporin (CSA) IV infusion 5 mg/kg/day in 2 divided doses										X	X	X	X Continuing
Stem cell infusion													X

1) Therapeutic drug monitoring will be performed. The busulfan dose will be adjusted on all doses marked X[1*] to achieve a cumulative AUC of 70-100 mg/L x hr. see section 17.2.3

2) Serotherapy with rabbit ATG (Genzyme) 2.5 mg/kg IV over 6-12 hours on day -3 to -1 pre-transplant for recipients of unrelated donor or 9/10 matched family donor transplants, or on day -9 to -7 for recipients of 5/8 matched unrelated cord blood units. The total dose of ATG is 7.5 mg/kg.

Busulfan is given twice daily IV as a 3 hour infusion for 4 days starting on D-10. The first dose of busulfan will be weight based as Table 30

Please note that the infusion (priming and flush) should be completed in 3 hours and the infusion rate should be calculated accordingly.

Table 30: Weight based busulfan dosing

WEIGHT	< 9 kg	9 to <16 kg	16 to 23 kg	> 23 to 34 kg	> 34 kg
DOSE	2 mg/kg	2.4 mg/kg	2.2 mg/kg	1.9 mg/kg	1.6 mg/kg

NB: No CNS directed radiotherapy will be given to patients with CNS disease at presentation.

Please note that cyclophosphamide, ATG and busulfan are dosed as mg/kg not mg/m^2

There should be no change to the dose or route of administration of busulfan and cyclophosphamide or to the timing, dose and route of administration of serotherapy with ATG. Any changes to the schedule must be agreed with the Chief Investigator or Clinical Coordinator prior to commencing the transplant schedule. The only exception to this is when necessary for logistical weekday administration/preparation.

17.2.1.1 MAC mandatory supportive care

Clonazepam or equivalent anti-epileptic prophylaxis should be given. Clonazepam prophylaxis is given on day -11 to day -5 according to institutional guidelines. Adequate hydration should be maintained throughout conditioning, with hyperhydration during cyclophosphamide administration according to institutional guidelines. Hydration and Mesna are mandatory from 4 hours prior to starting cyclophosphamide until 24 hours after completing the infusion according to local guidelines. Suggested fluids and rates are detailed in the pharmacy manual. Patients receiving ATG must have cover to prevent infusion related reactions according to local guidelines.

17.2.2 Arm F: Reduced intensity fludarabine/busulfan (RIC)

Table 31: R4 Arm F RIC conditioning treatment schedule

Please note that two schedules for anti-thymocyte globulin (ATG) are included on this table. ATG schedule should be selected based on donor type.

		D-9	D -8	D -7	D -6	D -5	D -4	D -3	D -2	D -1	D 0
Busulfan IV infusion over 3 hr AUC of 60-65 mg/L x hr See below for starting doses	0 h					**X**	**X**	**X**[1]	**X**[1]		
	12 h					**X**	**X**	**X**[1]	**X**[1]		
Fludarabine IV infusion, 30 mins 30 mg/m^2 /day [2]			**X**	**X**	**X**	**X**	**X**	**X**			
unrelated or 9/10 mismatched family donor: **ATG**[3] IV infusion over 6-12 hours 2.5 mg/kg/day								**X**	**X**	**X**	
5/8 matched unrelated cord blood: **ATG**[3] IV infusion over 6-12 hours 2.5 mg/kg/day		**X**	**X**	**X**							
Clonazepam prophylaxis					**X**	**X**	**X**	**X**	**X**	**X**	**X**
CSA IV infusion 5 mg/kg/day in 2 divided doses								**X**	**X**	**X**	**X** **Continuing**
Stem cell infusion											**X**

1. Therapeutic drug monitoring will be performed. The number of busulfan doses marked X[1] will be adjusted to achieve a cumulative AUC of 60-65 mg/L x hr. See section 17.2.3
2. Please note patients weighing <9kg should be treated with 1.2 mg/kg/dose
3. **Serotherapy** with rabbit ATG Genzyme 2.5 mg/kg/day IV over 6-12 hours on day -3 to -1 pre-transplant for recipients of unrelated donor or 9/10 matched family donor transplants or on day -9 to -7 for recipients of 5/8 matched unrelated cord blood units. The total dose of ATG is 7.5 mg/kg.

Busulfan is given twice daily IV as a 3 hour infusion for 4 days starting on D-5. The first dose of busulfan will be weight based as Table 32:

Please note that the infusion (priming and flush) should be completed in 3 hours and the infusion rate should be calculated accordingly.

Table 32: Weight based busulfan dosing

WEIGHT	< 9 kg	9 to <16 kg	16 to 23 kg	> 23 to 34 kg	> 34 kg
DOSE	2 mg/kg	2.4 mg/kg	2.2 mg/kg	1.9 mg/kg	1.6 mg/kg

Infants

Infants less than 12 months, weighing <9 kg should have fludarabine doses calculated as mg/kg:

- Fludarabine: 1.2 mg/kg/dose

NB: No CNS directed radiotherapy will be given to patients with CNS disease at presentation.

Please note that ATG and busulfan are dosed as mg/kg but fludarabine is dosed as mg/m^2 except in infants.

There should be no change in the dose or route of administration of fludarabine and busulfan or to the timing, dose and route of administration of serotherapy with ATG. Any changes to the schedule must be agreed with the Chief Investigator or Clinical Coordinator prior to commencing the transplant schedule. The only exception to this is when necessary for logistical weekday administration/preparation.

17.2.2.1 RIC mandatory supportive care

Adequate hydration is necessary throughout conditioning according to local guidelines. Suggested fluids and rates are detailed in the pharmacy manual. Patients receiving ATG must have cover to prevent infusion related reactions according to local guidelines. Clonazepam prophylaxis is given on day -6 to day 0 according to institutional guidelines.

17.2.3 Busulfan PKs

Therapeutic drug monitoring for busulfan should be performed according to local practice and the results used to adjust dosing to achieve the cumulative AUCs noted above. Blood samples (1 ml in EDTA) for PKs should be taken from the central line, from a different lumen to that down which the busulfan is administered, at the following time points: pre-busulfan and at 5, 15, 30 minutes and 1, 2 and 4 hours after completion of the first dose of busulfan and line flush. Samples should be centrifuged at the transplant centre and the plasma frozen within 30 minutes of collection.

Busulfan doses should be adjusted to achieve a cumulative AUC of 70-100 mg/L x hr for patients on the MAC arm and 60-65 mg/L x hr for the RIC arm. If the predicted AUC falls outside this range the dose is adjusted as outlined in Appendix 3 - Therapeutic Drug Monitoring for Busulfan.

17.2.4 GVHD prophylaxis

All patients will receive immunosuppression with CSA which should be commenced at a dose of 2.5 mg/kg IV twice daily 3 days prior to the stem cell infusion. Levels should be adjusted to achieve a trough of 100-150 µg/L. Once patients can tolerate oral medications, CSA may be converted to an oral preparation. Patients receiving grafts from mismatched donors or those in whom the stem cell source is PBSC or unrelated cord blood should receive additional prophylaxis with Mycophenolate mofetil (MMF) 15 mg/kg IV three times daily starting on the day of transplant. In the absence of GvHD, MMF will be stopped at day 28 post-transplant and CSA tailed over 4-6 weeks from day 60 or earlier if mixed chimerism is detected in the whole blood. Patients may transfer to oral GvHD prophylaxis once oral absorption is adequate.

Substitution of CSA due to AEs should be according to local guidelines.

17.2.5 HSCT supportive care

Local institutional guidelines on supportive care during transplant conditioning should aim to minimise hepatotoxicity while providing appropriate anti-emetic and anti-infective cover. VOD prophylaxis is at the discretion of the responsible clinician. Anti-epileptic prophylaxis, hydration and cover for ATG (if applicable) are mandatory (sections 17.2.1.1 and 17.2.2.1.).

Supportive care post-transplant, including blood product support, analgesia, anti-emetics, nutritional support, anti-infective prophylaxis and monitoring should be given as per local institutional guidelines, to monitor and minimise the risks of transplant complications. Defibrotide will not routinely be given as prophylaxis but early institution of therapy is recommended in the presence of clinical evidence of VOD. Clinicians should be particularly vigilant for VOD in patients who have received gemtuzumab ozogamicin pre HSCT. G-CSF will only be given in the case of delayed or incomplete neutrophil recovery.

Pneumocystis jirovecii prophylaxis should be given to patients who have been transplanted until at least 6 months post-transplant , omitting the period of neutropenia post stem cell infusion, unless temporarily stopped for clinical reasons.

17.2.5.1 Donor lymphocyte infusions (DLI)

Whilst immunotherapy post-transplant is not one of the objectives of MyeChild 01 trial, it is recognised this is an issue for clinicians with patients developing mixed chimerism/MRD and therefore the following guidance is suggested. Initially the use of DLI will be guided by mixed chimerism in the peripheral blood [58] but data on bone marrow MRD post-bone marrow transplant will be collected and once this is available, the indication for DLI may be revised. Data on DLI usage will be collected on the CRF.

Lineage specific chimerism will be assessed in the whole blood, T-cell and myeloid lineages in the peripheral blood at 1, 2, 3, 4, 6, 9 and 12 months post-transplant. If a patient shows mixed chimerism (MC), defined as >1% of autologous cells in the whole blood, this should initially be confirmed within a period of one week. Patients with confirmed mixed chimerism post-transplant without active acute GvHD >Grade I or chronic GvHD in the first year post-transplant may be offered immunotherapy. In the first instance, if patients are still receiving immunosuppression, this should be discontinued and chimerism reassessed a month later. In patients already off immunosuppression, chimerism should be reassessed a month after cessation of immunosuppression. If the percentage of recipient chimerism has increased by >2% in the whole blood at this time point, DLI may be given to recipients of MFD or MUD. DLI is not recommended in the context of 9/10 mismatched donor HSCT. The DLI cell dose administered is dependent on the donor source. A starting dose of 1 x 10^7 CD3+T-cell/kg is suggested for MFD and 1 x 10^6 CD3/kg in cases of 10/10 MUD. Chimerism should be reassessed 6 weekly until full donor chimerism is restored. Patients with persisting mixed chimerism and no GvHD >Grade I six weeks after the initial dose of DLI may be treated with a 2nd dose of DLI (3 x 10^7 CD3/kg in MSD transplants, 5 x 10^6/kg in MUD transplants). Likewise, patients with persisting mixed chimerism and no GvHD >Grade I six weeks after this 2nd dose of DLI may be treated where possible with a 3rd dose of DLI (1 x 10^8 CD3/kg in MSD transplants, 2x 10^7/kg in MUD transplants).

17.2.6 Patient assessments

Pre-transplant and post-transplant assessments will be performed as per local institutional policy but should as a minimum include those listed in Table 26: Schedule of additional events: R4.

17.2.6.1 Assessment of early regimen related toxicity

Patients will be assessed clinically and with blood tests/radiology as indicated for Early Regimen Related Toxicity at 1 month and approximately 100 days post-transplant.

17.2.6.2 Assessment of GvHD

Acute GvHD will be graded according to the modified Seattle criteria [59] and chronic GvHD using the National Institutes of Health (NIH) consensus criteria [60] at 1, 2, 3, 4, 6 and 9 months and 1, 2 and 3 years post-HSCT. See Appendix 4 – Assessment of GvHD for details.

17.2.6.3 Engraftment and Lineage specific chimerism

Lineage specific chimerism will be assessed using standardised PCR for short tandem repeats in the whole peripheral blood, T-cells (CD3 selected) and granulocytes (CD15 selected) pre-transplant and at 1, 2, 3, 4, 6, 9 and 12 months post-HSCT.

Engraftment will be assessed by haematological recovery from transplant up to 3 months post-transplant. Haematological engraftment will be defined as neutrophil recovery to >0.5 x 10^9 /L on the first of 3 consecutive days without GCSF; and platelet recovery to >20 x 10^9 /L on the first of three days without platelet transfusion.

17.2.6.4 Bone marrow assessments

Bone marrow samples will be obtained at 1, 3, 6 and 9 months and 1 year post-SCT. Morphological assessment, chimerism and cytogenetic characterisation, where a cytogenetic abnormality was present, should be performed by the transplant centre. Additionally 2-5 ml bone marrow samples should be taken for flow and molecular MRD analysis (for patients with an informative marker) and 2 ml bone marrow sample for LSC monitoring (for patients who have consented). Please refer to the national MyeChild 01 laboratory manual for further details.

17.2.6.5 Assessment of gonadal function

Gonadal function will be evaluated clinically and with blood tests pre-HSCT and at 1 and 3 years post-HSCT in patients 12 years or older at these time points. Clinical evaluation will consist of:

i) Pubertal development
   - Age and nature (spontaneous, delayed or arrested) of puberty, details of menstrual periods (regularity, frequency)
   - Tanner stage
   - Treatment required to induce or progress puberty
   - Sex hormone replacement treatment

ii) Fertility
   - Semen analysis (male age >16) or ovarian ultrasound (female age >16)
   - Pregnancies or paternity

In addition at the same time points patients should undergo measurement of Luteinizing Hormone (LH), Follicle-Stimulating Hormone (FSH), testosterone and inhibin B (male patients age 12 or older) or LH, FSH, oestradiol and Anti-Mullerian Hormone (AMH) (female patients age 12 or older) where available. Testicular volume should be measured using a Prader orchidometer.
The trial will establish a cohort of patients to enable subsequent prospective investigation of gonadotoxicity in both males and females, including a comparison of the different HSCT conditioning regimens. This will be undertaken in a future and separately funded study.

17.2.7 Long term follow-up

Careful long-term follow-up is strongly recommended following national or international (PanCareSurFup http://www.pancaresurfup.eu/) guidelines.

18. PATIENT ASSESSMENTS AT THE END OF TREATMENT

At the end of treatment, the following assessments should be performed on all patients:

- Medical history
- Physical examination including weight
- Assessment of toxicity
- Blood count
- Biochemistry
- Liver function tests
- Bone marrow aspirate for local morphology and central MRD assessment (see section 21)

- Echocardiogram

19. PATIENT FOLLOW-UP

Following completion of treatment, the frequency of follow-up assessments should be guided by local practice. Echocardiograms should be performed during years 1, 3, 5 and 10 years of follow-up.

Patients will be followed up for 5 years for disease relapse and late toxicity with particular attention to cardiac and hepatic toxicity. Data on relapse, death, cardiotoxicity and hepatic function will be collected on the CRF:

- 3 monthly for year 1
- 6 monthly for year 2
- Annually thereafter

After the first 5 years follow-up, basic data on relapse and survival will be captured annually, and echocardiogram results will be collected at 10 years.

The first analysis will be performed when all patients have had a minimum of 1 year follow up.

Please see section 17.2.7 for further follow-up investigations required post HSCT.

20. TREATMENT DISCONTINUATION AND PATIENT WITHDRAWAL

20.1 Discontinuation from MyeChild 01 Trial Treatment

If a patient stops MyeChild 01 protocol treatment prematurely, the reason should be recorded in the patient's medical records and should be reported on the CRF. Reasons for stopping protocol treatment may include, but are not limited to:

- The patient or parent/legal guardian withdraws consent to further data collection (section 20.2)
- Unacceptable toxicity
- Disease progression whilst on therapy
- If the patient becomes pregnant (section 22.1.2.2)
- Failure to achieve CR after course 2

MyeChild 01 will be analysed on an intention-to-treat (ITT) basis and any patients who stop trial treatment prematurely will remain in the trial for follow-up unless the patient and/or parent/legal guardian explicitly withdraws consent for data collection.

20.2 Withdrawal of Consent

The patient and/or parent/Legal guardian may withdraw consent at any time during the study. For the purposes of this trial, two types of withdrawal are defined:

- The patient or parent/legal guardian would like to withdraw from trial medication, but is willing to be followed up according to the schedule of assessments (i.e. the patient has agreed that data can be collected and used in the trial analysis).
- The patient or parent/legal guardian would like to withdraw from trial medication and is not willing to be followed up for the purposes of the trial at any further visits (i.e. only data collected prior to the withdrawal of consent can be used in the trial analysis).
  The details of withdrawal (date, reason and type of withdrawal) should be clearly documented in the patient's medical records. A Withdrawal of Consent Form should be completed.
  A patient's wishes with respect to their data must be respected.

20.3 Loss to Follow-up

If a patient is lost to follow-up, every effort should be made to contact the patient's Medical Practitioner/Primary Physician (if consented) to obtain information on the patient's status. Similarly, if a patient's care is transferred to another clinician, the patient should be followed up by that site and the relevant NCC should be informed.

21.SAMPLE COLLECTION

Bone marrow samples should all be taken on count recovery post courses of chemotherapy. Where possible, all samples should be taken at the same time as routine investigations. For further sample processing and shipment information, please refer to the national MyeChild 01 Laboratory Manual.

Table 33: Summary of sample collection

		Diagnosis	During course 1	Post course 1	Post course 2	Post course 3	Pre-transplant (if applicable)	End of treatment	1 month post-transplant (R4 only)	Day 100 (3 months) Post-transplant (R4 only)	6 months post-transplant R4 only)	9 months post-transplant (R4 only)	12 months post-transplant (R4 only)	Relapse
Mandatory samples	Bone marrow for flow MRD	X		X	X	X	X	X	X	X	X	X	X	X
	Peripheral blood for molecular MRD	X[1]		X	X	X	X	X	X	X	X	X	X	X
	Bone marrow for molecular MRD	X[1]		X	X	X	X	X	X	X	X	X	X	X
	Bone marrow for cytogenetic analysis	X												X
Optional Studies	Bone marrow for LSC monitoring	X		X	X	X	X	X	X	X	X	X	X	X
	Bone marrow for transcriptome sequencing	X												
	Buccal swab for transcriptome sequencing	To be taken at any time during treatment												
	Peripheral blood for liposomal daunorubicin and mitoxantrone PK study		X[2]											
	Saliva or buccal swab for pharmacogenomic study			X										
GO Dose finding study only	Blood samples for gemtuzumab ozogamicin PK studies and ADA analysis		X[2]											

1. At diagnosis it is preferable to send paired bone marrow and peripheral blood samples for molecular screening. If bone marrow cannot be obtained, peripheral blood should be sent.
2. Multiple sample time points, please see sections 21.6 and 21.5

21.1 Sample Collection for Flow MRD Monitoring

Diagnostic and Follow- up samples:

2ml bone marrow should be taken, ideally from the first pull. Post-HSCT samples should be 5ml.

If there is a dry tap, please send 3ml peripheral blood sample instead.

- The treating investigator will be provided with the results of the flow MRD analysis by report as part of the risk group assignment for all patients with a flow marker of sufficient sensitivity for MRD monitoring. If the sample is not adequate for analysis, the treating investigator will be notified and a second sample will be requested. This should be obtained, where possible, and is particularly important after course 1 of treatment.

For further sample processing and shipment information, refer to the national MyeChild 01 Laboratory Manual.

21.2 Sample Collection for Molecular MRD Monitoring

Diagnostic Sample for molecular screening:

Paired samples of 2-5ml bone marrow **and** 5-10ml peripheral blood should be taken. If bone marrow cannot be obtained, peripheral blood should be sent. These samples will be analysed for a leukaemia-specific molecular marker. The treating investigator will be provided with the result of this analysis via a report. If the sample was not adequate for analysis, further peripheral blood samples may be requested. Any further samples should be taken at the same time as routine investigations where possible.

Molecular MRD monitoring samples:

Paired samples 2-5ml bone marrow **and** 5-10ml peripheral blood should be taken. Where molecular monitoring is being used to guide risk group allocation, the randomising consultant will be provided with the result of this analysis via a report detailing the risk group assignment.

For further details on sample processing and shipment, refer to the national Myechild 01 Laboratory Manual.

21.3 Genetic and Functional Leukaemic Stem Cell (LSC) Monitoring

Consent for participation in the LSC monitoring is optional and will be collected on the main trial consent form. Please send 2 ml bone marrow from patients who have consented for this study. For further information on the study please refer to Appendix 5 - Genetic and Functional Leukaemic Stem Cell (LSC) Studies in Paediatric AML.

For further details on sample processing and shipment, refer to the national MyeChild 01 Laboratory Manual.

21.4 Transcriptome Sequencing

Consent for participation in the transcriptome sequencing study is optional and will be collected on the main trial consent form. Please send 2ml bone marrow and a buccal swab from patients who have consented for this study. For further information on the study please refer to Appendix 6 - Transcriptome Sequencing in Childhood Acute Myeloid Leukaemia: MyeChild 01 Study.

For further details on sample processing and shipment, refer to the national MyeChild 01 Laboratory Manual.

21.5 Gemtuzumab Ozogamicin Pharmacokinetic Analysis

There are a number of blood samples required for patients participating in the gemtuzumab ozogamicin dose finding study for PK and ADA analysis. For further information on the study please refer to Appendix 7 - Gemtuzumab Ozogamicin Pharmacokinetic Analysis.

Take 2 ml whole blood at the following time points, as detailed in the lab manual:

Table 34: Gemtuzumab ozogamicin PK sample time points

		Cohort 1			Cohort 2			Cohort 3		
Day of Dose	**Hour**	**Day of Draw**	**Gemtuzumab PK Plasma**	**ADA Plasma**	**Day of Draw**	**Gemtuzumab PK Plasma**	**ADA Plasma**	**Day of Draw**	**Gemtuzumab PK Plasma**	**ADA Plasma**
4	**Predose (0H)[1]**	**4**	X	X	**4**	X	X	**4**	X	X
	2		X			X			X	
	3		X							
	6		X			X			X	
	72	**7**	X							
	144	**10**	X							
7	**Predose (0H)[1]**				**7**	X		**7**	X	
	2					X			X	
	6					X				
	72				**10**	X				
	144				**13**	X				
10	**Predose (0H)[1]**							**10**	X	
	2								X	
	8								X	
	72							**13**	X	
Month 1				X			X			X

1. Pre-dose samples must be collected before the next infusion

X = Sampling time point

Collection windows

Plasma samples for determination of gemtuzumab ozogamicin drug concentrations should be collected within 1 hour prior to initiation of gemtuzumab infusion (Predose/0H). 2H, 3H, 6H, and 8H samples should be collected within ±1 hour of the specified times. 72H and 144H samples should be collected within ±12 hours of the specified time, if possible. Post infusion time points are based on time from the start of infusion.

As shown in the table, two blood samples are required for Anti-Drug Antibodies (ADA) analysis at the following time points:

- Prior to the first gemtuzumab ozogamicin dose
- 1 month after the first gemtuzumab ozogamicin dose (30 days ± 5 days)

In the event that the patients recruited to the pharmacokinetic study during the gemtuzumab ozogamicin dose finding study do not adequately represent the full paediatric age range, recruitment to the pharmacokinetic study may be extended to the gemtuzumab ozogamicin randomised study.

For further sample processing and shipment information, please refer to the national MyeChild 01 Laboratory Manual.

21.6 Liposomal Daunorubicin and Mitoxantrone Pharmacokinetic Sub-study (UK Only)

Participation in the liposomal daunorubicin and mitoxantrone PK sub-study is optional and consent will be collected using the main trial consent form. For further information on the study please refer to Appendix 8 – Liposomal Daunorubicin and Mitoxantrone Pharmacokinetic Sub-study.

The following blood samples should ideally be taken during the first course of induction chemotherapy:

Figure 11: Drug scheduling and PK sampling times for mitoxantrone

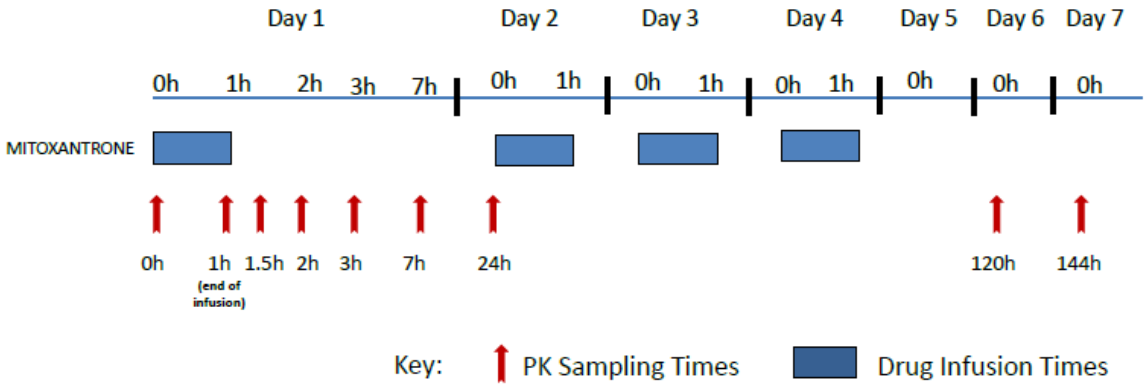
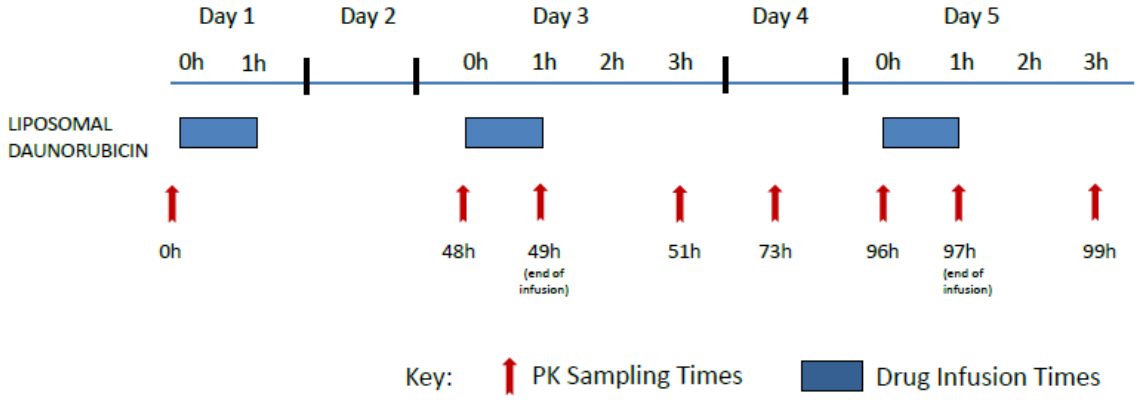


Figure 12: Drug scheduling and PK sampling times for liposomal daunorubicin



For further details on sample processing and shipment, please refer to the national MyeChild 01 Laboratory Manual.

21.7 Pharmacogenomic Sub-study

Participation in the Pharmacogenomic Sub-study is optional and consent will be collected using the main trial consent form. For further information on the study please refer to Appendix 9 – Pharmacogenomic Sub-study.

Either saliva (patients aged >3 years) or a buccal swab (patients aged ≤3 years) should be taken post course 1.

For further details on sample processing and shipment, please refer to the national MyeChild 01 Laboratory Manual.

22. ADVERSE EVENT REPORTING

The collection and reporting of AEs will be in accordance with the EU Directive for Clinical Trials 2001/20/EC and the Detailed Guidance on the Collection, Verification and Presentation of Adverse Events/Reaction Reports Arising From Clinical Trials of Medicinal Products For Human Use ('CT-3'). Definitions of different types of AE are listed in Appendix 10 - Definition of Adverse Events.

The Investigator should assess the seriousness and causality (relatedness) of all AEs experienced by the patient with reference to the Summary of Product Characteristics or Investigator Brochure. This should be documented in the patient's medical records.

22.1 Reporting Requirements

22.1.1 Adverse events

22.1.1.1 Adverse events for the gemtuzumab ozogamicin dose finding study:

All medical occurrences which meet the definition of an AE (see Appendix 10 - Definition of Adverse Events for definition) and are grade 3 or above should be reported. Please note this includes abnormal laboratory findings which meet the definition of the Common Terminology Criteria for Adverse Events (CTCAE) criteria. In addition, all grades of cardiac toxicity should be reported.

Similarly all VOD toxicity should be reported on a specific VOD AE form. AEs for the gemtuzumab ozogamicin dose finding study will be reported from the date of randomisation until count recovery after course 2 of treatment or day 45 post course 2.

22.1.1.2 Adverse events for all other parts of the protocol treatment:

AEs (see Appendix 10 - Definition of Adverse Events for definition) are commonly encountered in patients receiving chemotherapy. As the safety profiles of the IMPs used in this trial are well characterised, only selected Adverse Reactions (ARs) experienced during treatment will be reported. Please note that all AEs that meet the definition of a SAE should be reported (see section 22.1.2).

22.1.2 Serious adverse events (SAEs)

Investigators should report AEs that meet the definition of a SAE (see Appendix 10 - Definition of Adverse Events for definition) and are not excluded from the reporting process as described below. SAE reporting must be compliant with national and international regulations.

22.1.2.1 Events that do not require reporting on a Serious Adverse Event Form

The following events should not be reported on an SAE Form:

Hospitalisations for:

Protocol defined treatment

- Pre-planned elective procedures unless the condition worsens
- Treatment for progression of the patient's leukaemia
- Progression or death as a result of the patient's cancer, as this information is captured elsewhere on the CRF

The following events should be reported on an Expected Serious Adverse Reaction (SAR) Form rather than an SAE Form:

- Admissions to control symptoms of vomiting unless the condition is life threatening or proves fatal
- Prolongation of admissions for supportive treatment during an episode of myelosuppression unless this proves fatal or requires admission to a high dependency or intensive care facility

Expected SAR Forms should be completed and returned in the post as soon as possible.

22.1.2.2 Monitoring pregnancies for potential serious adverse events

The outcome of all pregnancies involving patients treated within this study must be collected to provide SAE data on congenital anomalies or birth defects.

In the event that a patient or their partner becomes pregnant during the SAE reporting period please notify the UK Coordinating Centre as soon as possible using a Pregnancy Notification Form. If it is the patient's partner who is pregnant, she should be asked to complete a Release of Medical Information Form. If the patient's partner is happy to provide information on the outcome of the pregnancy, she should sign the Release of Medical Information Form. Once consent has been obtained the Pregnancy Notification Form should be completed. If appropriate also complete an SAE Form.

22.1.3 Reporting period

For the gemtuzumab ozogamicin dose finding study:

Details of all AEs, AR's and SAEs, (except those listed in 22.1.2.1 above) will be documented and reported from the date of commencement of protocol defined treatment until count recovery or day 45 after the start of course 2.

For all other protocol treatment:

Details of all AEs, ARs and SAEs, (except those listed in 22.1.2.1 above) will be documented and reported from the date of commencement of protocol defined treatment until 30 days after the administration of the last protocol defined treatment.

For all parts of the trial, sites should continue to report SAEs which the Investigator feels meets the definition of a Suspected Unexpected Serious Adverse Reaction (SUSAR) using the procedure described below after this date.

22.2 Reporting Procedure

22.2.1 Site

22.2.1.1 Adverse events

For the gemtuzumab ozogamicin dose finding study:

AEs should be reported on an AE Form (and where applicable on an SAE Form). The AE Form should be updated throughout the reporting period i.e. until 45 days post course 2.

AEs will be reviewed using the CTCAE, version 4.0 (Appendix 11 - Common Toxicity Criteria Gradings). Any AEs experienced by the patient but not included in the CTCAE should be graded by an Investigator and recorded on the AE Form using a scale of (1) mild, (2) moderate or (3) severe. If the grade of the AE changes but the AE is still continuing, record this as a new event.

Suspected DLTs must be reported to the UK Trials Office on the Suspected DLT Form immediately upon awareness of the event.

Suspected DLT forms should be faxed to +44 (0)121 414 9520

For all other protocol treatment:

Selected ARs experienced during treatment should be recorded in the toxicity section of the Treatment Form.

AEs will be reviewed using the CTCAE, version 4.0 (Appendix 11 - Common Toxicity Criteria Gradings). For each sign/symptom, the highest grade observed since the last visit should be recorded.

22.2.1.2 Serious adverse events

For more detailed instructions on SAE reporting refer to the SAE Form Completion Guidelines contained in the ISF.

AEs defined as serious and which require reporting as an SAE (excluding events listed in Section 22.1.2.1 above) should be reported on an SAE Form. When completing the form, the Investigator will be asked to define the causality and the severity of the AE which should be documented using the CTCAE version 4. The Investigator (or delegate) must complete, date and sign an SAE Form. The SAE Form should be completed in English. The completed form should be faxed together with a SAE Fax Cover Sheet to the UK Coordinating Centre using one of the numbers listed below as soon as possible and no later than 24 hours after the Site Research Team first becoming aware of the event:

SAE forms should be faxed to +44(0)121 414 9520 or +44(0) 121 414 3700

On receipt the UK Coordinating Centre will allocate each SAE a unique reference number. This number will be transcribed onto the SAE Fax Cover Sheet which will then be faxed back to the site as proof of receipt. If confirmation of receipt is not received within 1 working day please contact the UK Coordinating Centre. The SAE reference number should be quoted on all correspondence and follow-

up reports regarding the SAE. The SAE Fax Cover Sheet completed by the UK Coordinating Centre should be filed with the SAE Form in the ISF.

For SAE Forms completed by someone other than the Investigator, the Investigator will be required to countersign the original SAE Form to confirm agreement with the causality and severity assessments. This must be done as soon as possible but can be done after the form has been faxed to the UK Coordinating Centre so as not to delay initial reporting. The form should then be returned to the UK Coordinating Centre in the post and a copy kept in the ISF.

Investigators should also report SAEs to the relevant bodies in accordance with local/national guidance.

For more detailed instructions on SAE reporting refer to the SAE Form Completion Guidelines contained in ISF.

22.2.1.3 Provision of follow-up information

Patients should be followed up until resolution or stabilisation of the event. Follow-up information should be provided on a new SAE Form (refer to the SAE Form Completion Guidelines for further information).

22.2.2 UK coordinating centre

On receipt of an SAE Form, seriousness and causality will be determined independently by a Clinical Coordinator. An SAE judged by the Investigator or Clinical Coordinator to have a reasonable causal relationship with the trial medication will be regarded as a SAR. The Clinical Coordinator will also assess all SARs for expectedness. If the event meets the definition of a SAR that is unexpected (i.e. is not defined in the Reference Safety Information) it will be classified as a Suspected Unexpected Serious Adverse Reaction (SUSAR).

22.2.3 Reporting to the competent authority and main research ethics committee (REC)

22.2.3.1 Suspected Unexpected Serious Adverse Reactions

The UK Coordinating Centre will report a minimal data set of all individual events categorised as a fatal or life threatening SUSAR to each NCC. Each NCC will be required to report SUSARs to the relevant Competent Authority and Ethics Committee in accordance with current regulations i.e. within 7 days. The UK Coordinating Centre will report to the Medicines and Healthcare products Regulatory Agency (MHRA) and UK REC within this timeframe. Detailed follow-up information will be provided within an additional 8 days.

All other events categorised as SUSARs will be reported by the mechanism above within 15 days.

22.2.3.2 Serious adverse reactions

The UK Coordinating Centre will include all SAEs, SARs (including SUSARs) in a Development Safety Update Report (DSUR) (or annual safety report) produced annually from receipt of the first Clinical Trials Authorisation for the trial to the End of Study Declaration. The NCCs will be responsible for forwarding this report to the relevant Competent Authority and Ethics Committee. The UK Coordinating Centre will report to the MHRA and UK REC.

22.2.3.3 Adverse events

Details of all AEs will be reported to the Competent Authorities on request.

22.2.3.4 Other safety issues identified during the course of the trial

The Competent Authorities and Ethics Committees will be notified immediately if a significant safety issue is identified during the course of the trial.

22.2.4 Investigators

Details of all SUSARs and any other safety issue which arises during the course of the trial will be reported to Principal Investigators of all participating sites by their respective NCC. A copy of any such correspondence should be filed in the ISF.

22.2.5 Data monitoring committee (DMC)

The independent DMC will review all SAEs.

22.2.6 Manufacturer of investigational medicinal product

All SAEs involving patients who have been allocated or randomised to receive gemtuzumab ozogamicin will be reported to Pfizer (the manufacturer of gemtuzumab ozogamicin), within 24 hours by fax by the UK Coordinating Centre (CRCTU). SAEs that are life threatening or result in death will be reported to Pfizer immediately.

23. DATA HANDLING AND RECORD KEEPING

23.1 Data Collection

This trial will use an eRDC system for completion of the CRF. Access to the eRDC system will be given to individuals via the UK Coordinating Centre. The MyeChild 01 eRDC system can be accessed from:

https://www.cancertrials.bham.ac.uk/MyeChild01Live

If the eRDC system is unavailable for an extended period of time a paper based CRF should be completed and forms returned to the relevant NCC for data entry.

Please Note: SAE reporting will be paper-based (refer to section 22.1)

The CRF will comprise the following forms:

Form	Summary of data recorded	Schedule for submission
Eligibility Checklist	Confirmation of eligibility and satisfactory staging investigations where necessary	Posted at point of randomisation
Randomisation/Registration Forms	Patient details; details of stratification variables; participation in optional sub-studies	As soon as possible after randomisation/registration
Baseline Form	Patient baseline details including blood counts and biochemistry	Within 1 month of randomisation
Treatment Forms (treatment arm/course specific)	Treatment details and details of adverse events	Within 1 month of completion of course of treatment
Disease Response Form	Details of assessment of response	Within 1 month of completion of course of treatment and count recovery
Gemtuzumab Ozogamicin Pfizer Sample Collection Form (cohort specific)	Details of Pfizer dose finding study PK and ADA samples (for patients on the gemtuzumab ozogamicin dose finding study only)	Within 1 month of the final sample taken

Transplant Assessment Forms (R4 only)	Transplant details and transplant follow up	Pre-transplant, and at post-transplant visits for 3 years post-transplant
End of Treatment Form	Details of end of treatment	Within 1 month of end of protocol treatment
Inpatient Admission Form	Details of all inpatient admissions	Ad hoc and until end of protocol treatment
Serious Adverse Event Form	Details of SAE	Immediately upon awareness of event
Suspected DLT Form	Details of suspected DLT (for patients on the gemtuzumab ozogamicin dose finding study only)	Immediately upon awareness of event
Suspected VOD Form	Details of VOD	Ad hoc – upon local diagnosis of VOD
Graft Versus Host Disease Form	Details of Graft Versus Host Disease	Ad hoc – upon loca diagnosis of GvHD
Pregnancy Notification Form	Details of Pregnancy	Ad hoc – As soon as possible upon awareness of pregnancy
Follow Up Form	Date of visit, patient status, details of any other treatment, remission status, late toxicities	In accordance with follow up schedule
Relapse Form	Date and site of relapse	Immediately upon patient relapse
Death Form	Date and cause of death	Immediately upon notification of patient's death
Deviation Form	Completed in the event of a deviation from the protocol	Immediately upon discovering deviation
Withdrawal Of Consent Form	Used to notify the Trials Office of patient withdrawal from the trial	Immediately upon patient withdrawal

The CRF must be completed by an Investigator or an authorised member of the site research team (as delegated on the Site Signature and Delegation Log, or country specific equivalent) within the timeframe specified above.

Data reported on each form should be consistent with the patient's medical records (source data) or the discrepancies should be explained. If information is not available, this must be clearly stated on the form. All missing and ambiguous data will be queried. All items on the form must to be completed.

It is the responsibility of the Investigator to ensure that the CRF has been completed correctly and that the data are accurate.

Trial forms may be amended by the UK Coordinating Centre, as appropriate, throughout the duration of the trial. Whilst this will not constitute a protocol amendment, sites will be notified of new versions when they become available on the eRDC. New versions of the SAE form must be implemented by participating sites immediately on receipt. Acknowledgment of receipt should be sent to the relevant NCC.

23.2 Archiving

It is the responsibility of the Principal Investigator to ensure that all essential trial documentation and source records (e.g. signed ICFs, ISFs, Pharmacy Files, patients' hospital notes, copies of CRFs, etc.)

at their site are securely retained for at least 25 years after the end of the trial. Do not destroy any documents without prior approval from the CRCTU Document Storage Manager.

24. QUALITY MANAGEMENT

24.1 Site Set-up and Initiation

Sites will be set up and initiated by the NCC. All sites will be required to sign a Clinical Study Site Agreement (or country specific equivalent) prior to participation. In addition, all participating Investigators will be asked to sign the necessary agreements and supply a current CV. All members of the site research team will also be required to sign the Site Signature and Delegation Log (or country specific equivalent), which should be returned to the relevant NCC.

Prior to commencing recruitment, all sites will undergo a process of initiation. Key members of the site research team will be required to attend either a meeting or a teleconference which will cover aspects of the trial design, protocol procedures, AE reporting, collection and reporting of data and record keeping.

Sites will be provided with an ISF and Pharmacy File containing essential documentation and instructions required for the conduct of the trial by the NCC. The relevant National Coordinating Centre must be informed immediately of any change in the site research team.

24.2 On-site Monitoring

Monitoring will be carried out as required following a risk assessment and as documented in the MyeChild 01 quality and trial management plan. Investigators will allow the MyeChild 01 trial staff access to source documents as requested.

24.3 Central Monitoring

If allowed by country specific legislation/guidance (as specified in the country specific quality and trial management plan) and if the patient and/or parent/legal guardian has given explicit consent, sites are requested to send in copies of signed ICFs to the relevant NCC for in-house review.

Trial staff will be in regular contact with the site research team to monitor progress and address any queries that they may have. Trial staff will check incoming data for compliance with the protocol, data consistency, missing data and timing. Sites will be sent Data Clarification Forms requesting missing data or clarification of inconsistencies or discrepancies.

Sites may be suspended from further recruitment in the event of serious and persistent non-compliance with the protocol and/or Good Clinical Practice (GCP), and/or poor recruitment. Any major problems identified during monitoring may be reported to the TMG, Trial Steering Committee (TSC) and the relevant regulatory bodies. This includes reporting serious breaches of GCP and/or the trial protocol.

24.4 Audit and Inspection

The Investigator will permit trial-related monitoring, audits, ethical review, and regulatory inspection(s) at their site, providing direct access to source data/documents.

Sites are also requested to notify the relevant NCC of any inspections by the relevant Competent Authority.

NCCs will notify the UK Coordinating Centre of any significant audit findings.

24.5 Notification of Serious Breaches

Country specific legislation may require the NCC to notify the Competent Authority and Ethics Committee in writing, within 7 days of becoming aware, of any serious breach of:

- The conditions and principles of GCP in connection with the trial
- The protocol relating to the trial

A “serious breach” is a breach which is likely to affect to a significant degree:

- The safety or physical or mental integrity of the patient in the trial; or
- The scientific value of the trial

Sites are therefore requested to notify the relevant NCC of a suspected trial-related serious breach of GCP and/or the trial protocol. Where the relevant NCC is investigating whether or not a serious breach has occurred, sites are requested to cooperate with the relevant NCC by providing sufficient information to report the breach to the relevant Competent Authority where required , and in undertaking any corrective and/or preventive action.

Please note: Persistent failure by sites to provide prompt and accurate information, particularly with regard to the reporting of SAEs, can be considered a serious breach.

The NCC will notify the UK Coordinating Centre of any serious breaches.

25. END OF TRIAL DEFINITION

The end of trial will be 12 months after the last data capture. This will allow sufficient time for the completion of protocol procedures, data collection input and cleaning of data.

The relevant NCC will notify the relevant Competent Authority and Ethics Committee that the trial has ended at the appropriate time and will provide them with a summary of the clinical trial report within 6 months of the declaration of the end of trial.

26. STATISTICAL CONSIDERATIONS

26.1 Definition of Outcome Measures

26.1.1 Primary outcome measures

Gemtuzumab ozogamicin dose finding study

- Incidence of DLTs within 45 days from date of randomisation in course 1 to count recovery after course 2 of treatment (up to day 45). DLT defined in section 9.4.3.5

Randomisation 1: Induction Randomisation (R1)

- EFS defined as the time from randomisation 1 (R1) to the first event.
  An event is defined as failure to achieve CR (which will be recorded as an event on day 1), relapse, secondary malignancy, or death from any cause. Patient who do not experience an event during the course of the trial will be censored at date last seen.
  CR is defined by Creutzig et al as CR or CRi [56]

Randomisation 2: Gemtuzumab Ozogamicin Randomisation (R2)

- EFS defined as time from randomisation 2 (R2) to the first of failure to achieve CR (recorded as an event on day 1), relapse, secondary malignancy or death from any cause. For patients who do not experience an event during the course of the trial, EFS time will be censored at the date last seen.

Randomisation 3: Consolidation Randomisation (R3)

- RFS defined as time from randomisation 3 (R3) to the first of relapse or death from any cause. For patients who have not experienced an event during the course of the trial, RFS time will be censored at date last seen.

Randomisation 4: HSCT Conditioning Randomisation (R4)

- Early treatment related adverse reactions defined as the incidence by day 100 post-transplant of grade 3-5 toxicity for the following systems using the National Cancer Institute (NCI) Common Terminology Criteria v4:
    - Cardiac (pericardial effusion/Left ventricular systolic dysfunction)
    - Respiratory, thoracic and mediastinal (hypoxia/ pneumonitis)
    - Gastrointestinal (GI) (diarrhoea/typhilitis/upper and lower GI haemorrhage)
    - Investigations (bilirubin)
    - Renal and Urinary (acute kidney injury/haematuria)
    - Nervous system (seizure)

- RFS defined as time from randomisation 4 (R4) to the first of relapse or death from any cause. For patients who have not experienced an event during the course of the trial, RFS time will be censored at date last seen.

26.1.2 Secondary outcome measures

Gemtuzumab Ozogamicin Dose Finding Study

- The nature, incidence and severity of AEs evaluated until day 45 post course 1 and course 2.
- Responses measured by bone marrow assessment using morphology and MRD assessment post course 1 and 2.

All randomisations

- CR defined by Creutzig et al [56]as CR or CRi and evaluated post course 1 and 2 of treatment. (R1 and R2 only)
- Reasons for failure to achieve CR evaluated post course 1 and 2 of treatment and classified as resistant disease, induction death or not evaluable. (R1 and R2 only)
- CIR defined as time from randomisation to the relevant question to relapse with death in remission being treated as a competing risk. Patients who do not relapse or die within the duration of the trial will be censored at date last seen.
- DCR defined as time from randomisation to relevant question to date of death from any cause in patients who have achieved CR with relapse being treated as a competing risk and patients who do not relapse or die prior to relapse during the trial being censored at date last seen.
- EFS defined as time from randomisation to the relevant question to the first of failure to achieve CR (recorded as an event on day 1), relapse, secondary malignancy or death from any cause. Patients who do not experience an event during the course of the trial will be censored at date last seen.
- OS defined as time from randomisation to the relevant question to death from any cause or date last seen for patients who are alive at the end of the trial.
- MRD negativity post course 1, course 2 and at end of treatment. (R1 and R2 only)
- Selected toxicities experienced within 30 days of end of trial treatment and defined as grade 3 or 4 using the NCI Common Toxicity Criteria v4 (R1, R2 and R3 only).
- Incidence of cardiotoxicity experienced within 10 years of randomisation and defined as a fall in fractional shortening to <28% or ejection fraction <55% (R1, R2 and R4 only)
- Incidence of Bilirubin of grade 3 or higher experienced within 30 days of end of trial treatment and defined by CTCAE 4 (R2 and R4 only)

- Incidence of confirmed VOD experienced within 30 days of end of trial treatment. (R2 and R4 only)
- Haematological recovery defined as neutrophil recovery to 1.0 x 10^9/L and platelet recovery to 80 x10^9/L by day 45 for DFS patients, and neutrophil recovery to 0.75 x 10^9/L and platelet recovery to 75 x 10^9/L for all other patients. (R1 and R2)
- Days in hospital per course of treatment
- Incidence of mixed chimerism at day 100 post-transplant. (R4 only)
- TRM defined as the time in days between randomisation to R4 and death which is unrelated to the underlying disease and considered related to the transplant procedure. Non transplant related deaths will be treated as a competing risk and patients who are still alive at the end of the trial will be censored at date last seen. (R4 only)
- Gonadal function at 1 year post-transplant and end of study follow up assessed by Tanner Stage, gonadotrophins and serum AMH (females)/inhibin B (males). (R4 only)

26.2 Sample Size

Randomisation 1 is between liposomal daunorubicin and mitoxantrone during induction. With 700 patients, 280 events are anticipated (based on EFS of 54% in AML12). If the observed HR was 0.89 or better in favour of a particular treatment, we could be >80% sure that this was indeed the more effective treatment. If the true HR was 0.8 in favour of a particular treatment, then we would have an 81% chance of observing this HR or better.

R2 is a comparison between 1 dose of gemtuzumab ozogamicin and the optimum tolerated number of doses of gemtuzumab ozogamicin and will be analysed using a classical hypothesis testing approach. As this randomisation will not open initially (i.e., once the optimum tolerated number of doses has been identified), only 550 patients will be eligible for this randomisation. If the higher dose of gemtuzumab ozogamicin leads to an increase in EFS from 60% to 70%, then 550 patients will provide >84% power to detect this difference on a 2-sided alpha of 0.15. Based on this it is expected that around 188 events will occur. The size of the study is based on a large treatment effect (HR 0.7) and a relaxed alpha, and we will use results from paediatric patients in other ongoing studies (e.g. AML17) to provide extra power to reliably detect smaller effects via a Meta-analysis.

R3 is a comparison between two consolidation therapies with known activity. An assumption is that only 60% of patients enrolled to the study qualify for this randomisation. Given this assumption and a historical event rate of 33%, 140 events are expected. This would mean that an observed HR of 0.86 or less would be required to be 81% sure that a particular treatment was better. If the true HR is 0.8 in favour of a particular treatment, then we have a 66% chance of achieving this level of certainty, but a 90% chance that the better treatment will be observed to perform best (HR<1).

R4 is a comparison between the current standard MAC and RIC regimens for SCT. The primary outcomes will be early regimen-related toxicity and DFS. We expect 150 patients to enter R4. We expect the severe toxicity rate (proportion of grade 3/4/5 toxicity) in the MAC arm to be 40%. In order for RIC to be considered worthwhile in this patient population, it would have to have a reduced toxicity and therefore a standard hypothesis testing approach will be used. If the true toxicity rate in the RIC arm is 20%, then this study will have 85% power to demonstrate the difference with a 2-sided alpha of 0.15. A relaxed alpha is necessary in this study as a more conventional alpha (0.05) would require an unfeasibly large number of patients. For RFS, a posterior probability plot of the underlying treatment effect will be produced as before. This analysis will not have great reliability. If uncertainty remains, this randomisation will be continued in the next trial.

26.3 Analysis of Outcome Measures

All analysis will be carried out on an ITT basis, with all patients analysed in the groups to which they were allocated at randomisation. The trial will analyse each randomisation in its own right and where appropriate will stratify by the treatment that the patient has received in previous randomisations. For example all patients randomised to liposomal daunorubicin and cytarabine will be compared to all

patients randomised to mitoxantrone and cytarabine with stratification by allocation of gemtuzumab ozogamicin. This approach is considered appropriate as there is no reason to anticipate any interaction between treatments in different randomisations. Interaction will however be assessed although there will be limited power to detect this. Stratification factors will have the following levels:

- Stratification by R1 treatment
  - Liposomal daunorubicin and cytarabine
  - Mitoxantrone and cytarabine
  - Mitoxantrone and cytarabine off trial (patient not randomised in R1)
- Stratification by dose of gemtuzumab ozogamicin allocated
  - Dose finding allocation, 1 dose, Dose finding allocation, 2 doses, Dose finding, 3 doses.
  - Randomised to1 dose via R2, Randomised to 2 doses via R2, Randomised to 3 doses via R2. (This will only apply if 3 doses is taken forward to R2).
  - No gemtuzumab ozogamicin allocated (patient not involved in R2/dose finding or registered in a centre no participating in the dose finding study prior to safety of 1 dose being established).

26.3.1 Primary outcomes

Gemtuzumab ozogamicin dose escalation study

- DLTs will be presented by dose of gemtuzumab ozogamicin.

Randomisation 1: Induction Randomisation (R1)

- EFS will be calculated using the method of Kaplan and Meier. A log rank test will be used to compare the treatment effect of liposomal daunorubicin and cytarabine v. mitoxantrone and cytarabine.
- As a Bayesian approach has been applied to this randomisation posterior probability plots of the treatment effect together with the probability that the true effect is less than 1 will be produced.
- Exploratory multivariable cox regressions will be used to compare EFS between the two arms adjusting for the dose of gemtuzumab ozogamicin allocated in either the dose finding study or throughout randomisation 2.

- **Randomisation 2: Gemtuzumab Ozogamicin Randomisation (R2)**
- EFS will be calculated using the method of Kaplan and Meier and a log rank test will be used to compare the treatment effect between 1 dose of gemtuzumab ozogamicin and the optimum tolerated number of doses. As a hypothesis testing approach has been applied HRs along with 95% CIs at 6 years will be produced
- Exploratory multivariable cox regressions will be used to compare EFS between the two arms adjusting for the assigned treatment in R1.

Randomisation 3: Consolidation Randomisation (R3)

- RFS will be calculated using the method of Kaplan and Meier and a log rank test will be used to compare the treatment effect of FLA with HD Ara-C.
- As a Bayesian approach has been applied to this randomisation posterior probability plots of the treatment effect together with the probability that the true effect is less than 1 will be produced.
- Exploratory multivariable cox regressions will be used to compare RFS between the two arms adjusting for both the assigned treatment in R1 and the dose of gemtuzumab ozogamicin received.

Randomisation 4: HSCT Conditioning Randomisation (R4)

- Toxicity will be assessed using a chi-squared test to compare the incidence of toxicities between Bu/Cy and Bu/Flu.

- Exploratory multivariable logistic regression will be used to compare the incidence of toxicities between the treatment arms while adjusting for both the assigned treatment in R1 and the dose of gemtuzumab ozogamicin received.
- RFS will be calculated using the method of Kaplan and Meier and a log rank test will be used to compare the treatment effect of Bu/Cy vs. Bu/Flu.
- As a Bayesian approach has been applied to this randomisation posterior probability plots of the treatment effect together with the probability that the true effect is less than 1 will be produced.
- Exploratory multivariable cox regressions will be used to compare RFS between the two arms adjusting for both the assigned treatment in R1 and the dose of gemtuzumab ozogamicin received.

26.3.2 Secondary outcomes:

All time-to-event outcomes (e.g. survival) will be calculated using Kaplan Meier methods and exploratory log rank tests will be used to compare treatment effect between arms. HRs and 95% CIs will be produced. Time-to-event outcomes with a competing risk (e.g. CIR) will be analysed using cumulative incidence curves and exploratory comparisons between arms will be made using Grey's test. Rate ratios and 95% CIs will be presented. Chi squared tests will be used to assess the treatment effect between arms for all categorical variables and t-tests or Mann Whitney tests will be applied for continuous measures as appropriate.

26.4 Planned Sub-group Analysis

Subgroup analysis will be carried out for WCC, age, type of disease and cytogenetics/molecular genetic risk group for all randomisations. Further sub group analysis will be carried out for donor type in R4 patients. Heterogeneity tests will also be performed.

26.5 Planned Interim Analysis

26.5.1 Major dose finding study

The major dose finding study's will take a similar form to the rolling design described by Skolnik et al[61], This means that interim analysis of cohorts will be presented in confidence to an independent DMC when 50 and then 100 percent of each cohort have been evaluated for DLTs. The DMC will therefore have the opportunity to make an initial decision to 'roll' on to the next cohort based on the available data at the timepoint, reducing the need to pause recruitment between cohorts. When each cohort has completed full recruitment the data from the complete cohort will be presented to the DMC (alongside any accumulating data from the current cohort). If a safety signal from the complete cohort is observed at this timepoint the DMC will have the opportunity to halt recruitment in the currently recruiting cohort and expand the previous cohort. If a safety signal is observed each cohort could be expanded as follows:

	Planned cohort size	Expansion size
Cohort 1	10	5
Cohort 2	20	10
Cohort 3	20	10

There will be no hard safety rules which will inform the decision to advance to the next gemtuzumab ozogamicin dose level, but the DMC will scrutinize the data and decide on causality/expected frequency of recognized events including:

- VOD
- SUSARs

- Death
- Prolonged neutropenia
- Prolonged thrombocytopenia

26.5.2 Minor dose finding study

Data from each complete cohort of patients recruited into the minor dose finding study will be presented at the next planned DMC for the major dose finding study. If the timing of available data from completion of a cohort of the minor dose finding study does not fit with the timing of the next planned DMC a short report of the data from the minor dose finding study will be sent to DMC for review.

26.5.3 Main phase III trial

Following opening of R2 accumulating data will be analysed by treatment arm and presented to the DMC on a 6 monthly basis in confidence. Additionally, safety monitoring of both arms of the gemtuzumab ozogamicin randomisation will be presented to the DMC.

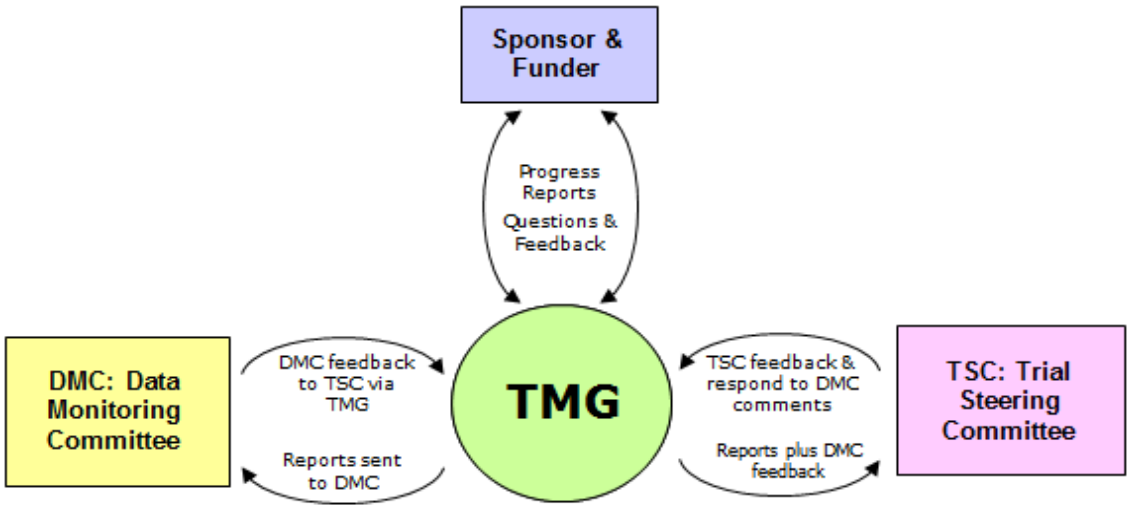
26.6 Planned Main Analyses

Following identification of the optimum tolerated number of doses of gemtuzumab ozogamicin from both the major and minor dose finding studies, the primary analysis of the data in the embedded dose finding study will be carried out.

The first analysis of data from randomisations 1, 2, 3 and 4 will take place after all patients have been followed up for a minimum of 1 year.

27. TRIAL ORGANISATIONAL STRUCTURE

Figure 13: Trial organisational structure



27.1 Co-ordinating Sponsor

The University of Birmingham is the Coordinating Sponsor. In addition, the University of Birmingham (UK Coordinating Centre) will undertake the responsibilities of NCC in the UK.

NCCs are responsible for the conduct of the trial within their own country in accordance with the delegated duties and in compliance with the applicable regulations.

27.2 National Co-Ordinating Centres

The Coordinating Sponsor has delegated the set-up, management and analysis of the trial to the UK Coordinating Centre. The role of the UK Coordinating Centre is assumed by the CRCTU, University of Birmingham, UK. The trial will be set-up, managed and analysed in the UK in accordance with CRCTU standard policy and procedures.

Each NCC will manage the trial in accordance with the trial protocol, and their standard policy and procedures.

27.3 Trial Management Group

The TMG is composed of the Chief Investigator, co-investigators, representatives from each NCC and the trial team at the CRCTU. The TMG is responsible for the day-to-day running and management of the trial and will meet by teleconference or in person at least every 3 months.

27.4 Trial Steering Committee

The TSC will provide overall supervision for the trial and provide advice though its independent chair. Membership includes independent clinicians, the Chief Investigator, a patient/parent representative and members of the TMG as appropriate. The TSC will assume responsibility for the oversight of the trial on behalf of the Coordinating Sponsor. The TSC will meet or hold teleconferences at least once a year, or more often as required. While the gemtuzumab ozogamicin dose finding study for gemtuzumab ozogamicin is open, the TSC will meet following each DMC.

27.5 Data Monitoring Committee (DMC)

Analyses will be supplied in confidence to an independent DMC, which will be asked to give advice on whether the accumulated data from the trial, together with the results from other relevant research, justifies the continuing recruitment of further patients. The DMC will operate in accordance with a trial specific charter based upon the template created by the Damocles Group.

During recruitment to the dose finding study for gemtuzumab ozogamicin, the DMC will meet mid-way through each dose cohort, and at the end of each dose cohort The DMC will also meet at the end of each dose cohort, once all of the patients recruited are evaluable for DLTs. Mid-way through each dose cohort, when half of the patients are evaluable for DLTs, the DMC will review the available safety data and recommend either rolling recruitment straight to the next dose cohort, or pausing recruitment until all the patients are evaluable for the DLTs. Once the gemtuzumab ozogamicin dose finding study has completed, the DMC will meet bi-annually during the recruitment period of the main trial. Additional meetings may be called if recruitment is much faster than anticipated and the DMC may, at their discretion, request to meet more frequently. An emergency meeting may also be convened if a safety issue is identified. The DMC will report to the TSC via the TMG who will convey the findings of the DMC to the Coordinating Sponsor and funders, where applicable. The DMC may consider recommending the discontinuation of the trial if the recruitment rate or data quality are unacceptable or if any issues are identified which may compromise patient safety. A randomisation may also stop early if the interim analyses showed differences between treatments that were deemed to be convincing to the clinical community

There will be no formal stopping rule for the phase III study. The decision will rest with the DMC but the difference in the two arms is usually at least 3 standard errors in the primary outcome to justify stopping early.

27.6 Finance

This is an investigator-initiated and led trial funded by Cancer Research UK (CRUK) in the UK and the National Institute for Cancer (INCa) in France. The embedded dose finding study for gemtuzumab ozogamicin is funded in the UK, France and Ireland by Pfizer. Pfizer are also providing gemtuzumab

ozogamicin free of charge for the duration of the trial. Galen Limited are providing an unrestricted grant for the management trial.

No individual per patient payment will be made to Investigators or patients. Sites will be compensated for their research activities carried out in relation to the trial as defined in the Clinical Study Site Agreement.

This study has been adopted into the NIHR CRN Portfolio in the UK.

In the event that another country wishes to join the trial, funding will have to be sought by the NCC to adequately support the running of the trial within that country. Approval from Pfizer will be sought to ensure appropriate funding for participation in the gemtuzumab ozogamicin dose finding study and drug supply is possible.

28. ETHICAL CONSIDERATIONS

The accepted basis for the conduct of clinical trials in humans is founded on the protection of human rights and the dignity of human beings with regard to the application of biology and medicine, and requires compliance with the principles of GCP and detailed guidelines in line with those principles (Directive 2001/20/EC (2) and Directive 2005/28/EC (1)).

GCP is a set of internationally recognised ethical and scientific quality requirements which must be observed for designing, conducting, recording and reporting clinical trials that involve the participation of human subjects. Compliance with GCP provides assurance that the rights, safety and well-being of trial subjects are protected, and that the results of the clinical trials are credible (Article 1 (2) of Directive 2001/20/EC).

The NCCs and Investigators shall consider all relevant guidance with respect to commencing and conducting the study in accordance with the GCP Directive (2005/28/EC)

The conduct of the trial shall be based on the following international ethical and statutory sources:

- The **WMA Declaration of Helsinki - Ethical Principles for Medical Research Involving Human Subjects** (adopted by the 18th World Medical Association General Assembly, Helsinki, Finland, June 1964 and amended by the 48th World Medical Association General Assembly, Somerset West, South Africa, October 1996**).** See Appendix 12 - WMA Declaration of Helsinki.

- If the region has adopted the Convention for the Protection of Human Rights and Dignity of the Human Being with regard to the Application of Biology and Medicine: **Convention on Human Rights and Biomedicine** (CETS No.: 164)(Council of Europe – Ratification signed in the following countries: Bosnia and Herzegovina, Bulgaria, Croatia, Cyprus, Czech Republic, Denmark, Estonia, Finland, France, Georgia, Greece, Hungary, Iceland, Italy, Latvia, Lithuania, Luxembourg, Moldova, Montenegro, Netherlands, Norway, Portugal, Romania, San Marino, Serbia, Slovakia, Slovenia, Spain, Sweden, Switzerland, The former Yugoslav Republic of Macedonia, Turkey, Ukraine).

- **Directive 2001/20/EC** of the European Parliament and of the Council of 4 April 2001 on the approximation of the laws, regulations and administrative provisions of the Member States relating to the implementation of good clinical practice in the conduct of clinical trials on medicinal products for human use (Official Journal L21, 01/05/2001 P. 0034 – 0044) and detailed guidance.

- **Directive 2005/28/EC** of 8 April 2005 laying down principles and detailed guidelines for good clinical practice as regards investigational medicinal products for human use, as well as the requirements for authorisation of the manufacturing or importation of such products (Official Journal L 91, 09/04/2005 P. 0013 – 0019).

- **Directive 95/46/EC** of the European Parliament and of the Council of 24 October 1995 on the protection of individuals with regard to the processing of personal data and on the free movement of such data **(**Official Journal L 281, 23/11/1995 P. 0031 – 0050).

- Scientific guidelines relating to the quality, safety and efficacy of medicinal products for human use, as agreed upon by the CHMP and published by the Agency, as well as the other Community guidelines

published by the Commission in the different volumes of the rules governing medicinal products in the European Community (Directive 2005/28/EC (9)).

This trial will be conducted under Clinical Trial Authorisation in each participating country. Appropriate country specific Ethics Committee approval must also be obtained prior to recruitment of patients within that country.

Before any patients are enrolled into the trial, the Principal Investigator at each site is required to obtain any necessary local approvals required within the country for the conduct of the trial at their site (see the country specific Trial and Quality Management Plan). It is the responsibility of the Principal Investigator to ensure that all subsequent amendments also gain the necessary local site specific approval prior to implementation. This does not affect the individual clinicians' responsibility to take immediate action if thought necessary to protect the health and interest of individual patients.

29.CONFIDENTIALITY AND DATA PROTECTION

Personal data recorded on all documents will be regarded as strictly confidential and will be handled and stored in accordance with the relevant data protection legislation in the member state.

Personal data recorded on all documents will be regarded as strictly confidential and will be handled and stored in accordance with the relevant data protection legislation in the member state. With the patient's consent (and where national legislation/guidance permits) their full name, date of birth, hospital number, medical practitioner details and national registry numbers (e.g. National Health Service (NHS) Number in the UK) will be collected at trial entry to allow long-term follow-up via other health care professionals (e.g. patient's medical practitioner) and national cancer registries.

Patients will be identified using only their unique trial number and, if national legislation permits, their initials and date of birth on the header section of the CRF/eRDC screens and in correspondence between the relevant NCC and participating sites. Any laboratory samples will be labelled with the patient's unique trial number, initials and date of birth to ensure that samples can be correctly identified. However, if local regulation/guidance permits patients are asked to give permission for the relevant NCC to be sent a copy of their signed ICF which will not be anonymised. This will be used to perform in-house monitoring of the consent process.

The Investigator must maintain documents not for submission to the relevant NCC (e.g. patient identification logs) in strict confidence. In the case of specific issues and/or queries from the regulatory authorities, it will be necessary to have access to the complete trial records, provided that patient confidentiality is protected.

The NCCs will maintain the confidentiality of all patient's data and will not disclose information by which patients may be identified to any third party other than those directly involved in the treatment of the patient and organisations for which the patient has given explicit consent for data transfer. Representatives of the MyeChild 01 trial team may be required to have access to patient's notes for quality assurance purposes but patients should be reassured that their confidentiality will be respected at all times.

30.INSURANCE AND INDEMNITY

The NCCs are responsible for obtaining insurance to set up and run the MyeChild 01 trial in their respective countries and for ensuring that sites in their country are adequately covered.

University of Birmingham employees are indemnified by the University insurers for negligent harm caused by the design or co-ordination of the clinical trials they undertake whilst in the University's employment.

The University of Birmingham cannot offer indemnity for non-negligent harm. The University of Birmingham is independent of any pharmaceutical company and, as such, it is not covered by the Association of the British Pharmaceutical Industry (ABPI) guidelines for patient compensation.

31.PUBLICATION POLICY

Results of this trial will be submitted for publication in peer reviewed journals. Manuscripts will be prepared by the TMG and authorship will be determined by mutual agreement.

Any publications and presentations prepared by Investigators must be reviewed and approved by the TMG. Manuscripts must be submitted to the TMG in a timely fashion and in advance of being submitted for publication to allow time for review and resolution of any outstanding issues. Authors must acknowledge that the trial was performed with the support of the University of Birmingham and where applicable other NCCs and other funding bodies. Intellectual property rights will be addressed in the agreements between the NCCs and the Clinical Study Site Agreement (or country specific equivalent) between the NCCs and sites.

APPENDIX 1 – RISK GROUP STRATIFICATION

Cytogenetic and molecular abnormalities

Good Risk cytogenetic and molecular abnormalities

- t(8;21)(q22;q22)/*RUNX1-RUNX1T1*
- inv(16)(p13q22)/t(16;16)(p13;q22)/*CBFB-MYH11*
- Mutation of *NPM1* without *FLT3*-ITD
- Double mutation of *CEPBA* without *FLT3*-ITD

Intermediate Risk Cytogenetic abnormalities

- t(9;11)(p21;q23)/*MLL-MLLT3*
- t(11;19)(q23;p13.3)/*MLL-MLLT1*
- All other *MLL* rearrangements not classified as high risk
- All other abnormalities which are neither good or poor risk

Poor risk cytogenetic and molecular abnormalities

- inv(3)(q21q26)/t(3;3)(q21;q26)/abn(3q26)
- -5/del(5q)
- -7
- t(6,9)(p23;q34)/*DEK-NUP214*
- t(9;22)(q34;q11)/*BCR-ABL1*
- 12p abnormalities
- t(6,11)(q27;q23)/*MLL-MLLT4*
- t(4;11)(q21;q23)/*MLL-AFF1*
- t(10;11)(p11~14;q23)/*MLL-MLLT10*
- t(5;11)(q35;p15.5)/*NUP98-NSD1*
- t(7;12)(q36;p13)/*MNX1-ETV6*
- inv(16)(p13.3q24.3)/*CBFA2T3-GLIS2*
- *FLT3*-ITD without NPM1 or CBF

Other poor risk categories

- Secondary leukaemia without good risk cytogenetics
- Induction failure after course 1: morphological failure confirmed by flow MRD in Good Risk /Standard Risk patients

Cytogenetics and FISH analysis will be carried out by local cytogenetic laboratories according to local practice. Molecular diagnostics will be centralised. Patients with no cytogenetic or molecular result will be classified as intermediate risk.

This risk stratification is significantly different from the previous MRC risk stratification and results in more children being classified as standard and high risk, and fewer as intermediate risk.

MRC stratification: Good Risk 20%, Intermediate Risk 68%, Poor Risk 11%

MyeChild stratification: Standard Risk 30%, Intermediate Risk 40%, High Risk30%

MRD monitoring

Patients will initially be stratified into high risk and non-high risk based on cytogenetic and molecular characteristics and confirmed morphological response to the first course of induction chemotherapy. Patients will subsequently be stratified into standard risk, intermediate risk and high risk by MRD assessment using multiparameter flow cytometry to LAIP or RT-qPCR for patients with an informative leukaemia-specific molecular marker, but no informative flow marker. This will guide treatment intensification and allocate HSCT. It is expected that over 90% of patients will have an informative LAIP marker and about 60% a molecular marker suitable for tracking by RT-qPCR.

Molecular markers which will be followed are:

- t(8;21)(q22;q22)*/RUNX1-RUNX1T1*
- inv(16)(p13q22)*/CBFB-MYH11*
- 11q23/*MLL* fusions
- t(6,9)(p23;q34)*/DEK-NUP214*
- t(5;11)(q35;p15.5)*/NUP98-NSD1*
- inv(16)(p13.3q24.3)*/CBFA2T3-GLIS2*
- other rare fusions
- *NPM1*mutations

APPENDIX 2 - GEMTUZUMAB OZOGAMICIN DOSE MODIFICATION FOR OBESITY

To ensure that children are treated effectively, without overdosing, the Body Mass Index (BMI) should be checked at diagnosis, prior to treatment with gemtuzumab ozogamicin.

In children > 2 years calculate using the following formula:

BMI= weight (kg) / height ^{2} (mxm)

The BMI can then be compared to the standard Child Growth foundation BMI charts for the appropriate sex. In children aged 6 months to 2 years use weight percentiles directly from the Child Growth Foundation charts.

For children > 2 years with a BMI that falls under the 98th percentile, dose by **actual weight** using the BSA charts to determine the surface area (SA) for dose calculation. For infants aged 6 months to 2 years with a weight that falls under the 98th percentile dose by **actual weight.**

For children who have a BMI >98th percentile read off the BMI at 98th percentile for their age. Child UK- WHO Growth Foundation charts can be downloaded from the Royal College of Paediatrics and Child Health at www.rcpch.ac.uk . The BMI charts are in the “school age chart” section and the infant weight charts in the “Early years charts- Growth Charts 0-4 years”

Calculate the **dosing weight** using the formula:

Dosing weight (kg) = BMI at 98th percentile x Ht^{2} (mxm)

For children aged 6 months to 2 years who have a weight >98th percentile read the weight at the 98th percentile for age as the **dosing weight.**

For children aged <6 months doses will be calculated on actual weight.

The BSA can be calculated using the **BSA charts in children to determine the surface area for dose calculation.** These can be found at the back of the BNF for Children.

For children ≤10kg, <1 year or BSA < 0.5m^{2} calculate the gemtuzumab ozogamicin dose as 0.1mg/kg.

For all patients if the calculated dose at 3mg/m^{2} exceeds 5mg then cap the dose at 5mg.

APPENDIX 3 - THERAPEUTIC DRUG MONITORING FOR BUSULFAN

Myeloablative Conditioning (Arm E):

Busulfan levels are taken after the first dose on day-10 pre-transplant as outlined in section 17.2.3 The results of busulfan levels and the Area Under the Curve (AUC expressed in mg*h/L) after a single dose should be available by the afternoon (4-5 PM) of the second day of treatment. Once the busulfan AUC is reported, the HSCT consultant/pharmacist at site should calculate the predicted cumulative AUC, by multiplying the actual AUC result by 8. For example, if the AUC after the first dose of Bu is 9 mg/L x hr, the cumulative AUC is: 9 mg/L x hr x 8= 72 mg/L x hr

A) If the cumulative AUC is within the range of 70 -100 mg/L x hr, there is no need for dose adjustment. Pharmacy should be informed to continue with the prescribed dose, for a total of 8 doses.
B) If the cumulative AUC is outside the range of 70 -100 mg/L x hr, the Bu dose needs to be adjusted to target a Bu AUC of 80 mg/L x hr. The remaining Bu doses to be given from the time of receipt of the result are adjusted as follows:

- Calculate the cumulative AUC given= **reported AUC x number of doses already given**

$$\text{Adjusted daily AUC} = \frac{80\text{mg/L- cumulative AUC given}}{\text{number of doses remaining}}$$

Then to calculate adjusted dose:

$$\text{Adjusted Bu dose (mg)} = \text{actual Bu dose given} \times \frac{\text{adjusted daily AUC}}{\text{reported AUC}}$$

The maximum permissible increase in an individual busulfan dose is 50%.

Reduced Intensity Conditioning (Arm F):

Busulfan levels are taken after the first dose on day-5 pre-transplant as outlined in section 17.2.3 The results of busulfan levels and the AUC expressed in mg*h/L after a single dose should be available by the afternoon (4-5 PM) of the second day of treatment. Once the busulfan AUC is reported, the HSCT consultant/pharmacist at site should calculate the number of doses of busulfan to administer, in order to target a cumulative AUC of 60-65 mg*h/L.

If the predicted cumulative AUC is less than 60 mg/L x hr even with 8 doses, the Bu dose needs to be adjusted to target a Bu AUC of 60 mg/L x hr. The remaining Bu doses to be given from the time of receipt of the result are adjusted as follows:

1. Calculate the cumulative AUC given = **actual AUC x number of doses already given**

$$\text{Adjusted daily AUC} = \frac{60\text{mg/L- cumulative AUC given}}{\text{number of doses remaining}}$$

Then to calculate adjusted dose:

$$\text{Adjusted Bu dose (mg)} = \text{actual Bu dose given} \times \frac{\text{adjusted daily AUC}}{\text{reported AUC}}$$

The maximum permissible increase in an individual busulfan dose is 50%.

APPENDIX 4 – ASSESSMENT OF GVHD

GvHD arises due to reactivity of donor T-cells against recipient cells, through both HLA and minor histo-incompatibility between the donor and the recipient. GvHD is divided into two forms, each of which produces distinct clinical syndromes as outlined below. Note acute GVHD may occur beyond 100 days post-transplant (late acute GvHD).

Grading of Acute Graft-versus-Host Disease (GvHD):

Acute GvHD is staged clinically according to the severity of involvement of each organ using the modified Glucksberg criteria below:

Stage	Skin rash (% body area)	Liver (bilirubin level μM)	GI tract (diarrhoea volume ml/kg/day)
1	Maculopapular< 25%	25-40 μM	10-15 ml/kg/d
2	25-50%	40-74 μM	16-20ml/kg/d
3	Generalised rash	75-200 μM	21-25 ml/kg/d
4	Vesicles and exfoliation	> 200 μM	Severe abdominal pain and ileus

The clinical grade of GvHD is then calculated as shown below, dependent on the most severely affected organ system.

Grade	Skin Stage	Liver Stage	Gut Stage	Clinical Performance
I	1-2	0	0	Normal
II	1-3	1	1	Mild decrease
III	2-3	2-3	2-3	Marked
IV	2-4	2-4	2-4	Incapacitated

Grading of Chronic GvHD (NIH):

Clinical Features	Score 0	Score 1	Score 2	Score 3
Skin ***Diagnostic[1]:*** Lichen planus-like features Poikiloderma Sclerotic features Morphea-like features Lichen sclerosus-like features ***Distinctive[2]:*** Depigmentation	No Symptoms	<18% BSA with disease signs but **NO** sclerotic features	9-50% BSA **OR** involvement with superficial sclerotic features "not hidebound" (able to pinch)	>50% BSA **OR** deep sclerotic features "hidebound" (unable to pinch) **OR** impaired mobility, ulceration or severe pruritus
Mouth ***Diagnostic:*** Lichen-type features Hyperkeratotic plaques Restriction of mouth opening from sclerosis ***Distinctive:*** Xerostomia Mucocele Mucosal atrophy Pseudomembranes* Ulcers*	No Symptoms	Mild symptoms with disease signs but not limiting oral intake significantly	Moderate symptoms with disease signs **with** partial limitation of oral intake	Severe symptoms with disease signs on examination **with** major limitation of oral intake
Eyes ***Distinctive:*** New onset dry, gritty, or painful eyes† Cicatricial conjunctivitis Keratoconjunctivitissica† Confluent areas of punctate keratopathy	No Symptoms	Mild dry eye symptoms not affecting ADL (requiring eyedrops ≤ 3 x per day) OR asymptomatic signs of keratoconjunctivitis sicca	Moderate dry eye symptoms partially affecting ADL (requiring drops > 3 x per day or punctal plugs), **WITHOUT** vision impairment	Severe dry eye symptoms significantly affecting ADL (special eyeware to relieve pain) OR unable to work because of ocular symptoms **OR** loss of vision caused by keratoconjunctivitissica
GI Tract ***Diagnostic:*** Esophageal web Strictures or stenosis in the upper to mid third of the	No Symptoms	Symptoms such as dysphagia, anorexia, nausea, vomiting, abdominal pain or diarrhoea without significant	Symptoms associated with mild to moderate weight loss (5- 15%)	Symptoms associated with significant weight loss >15%, requires nutritional supplement for most

esophagus*		weight loss (<5%)		calorie needs **OR** esophageal dilation

Clinical Features	Score 0	Score 1	Score 2	Score 3
Liver	Normal LFT	Elevated Bilirubin, ALP‡, AST or ALT <2 x ULN	Bilirubin >3 mg/dl or Bilirubin, enzymes 2-5 x ULN	Bilirubin or enzymes > 5 x ULN
Lungs ***Diagnostic:*** Bronchiolitis obliterans diagnosed with lung biopsy ***Distinctive:*** Bronchiolitis obliterans diagnosed with PFTs and radiology†	No Symptoms FEV1 > 80% **OR** LFS=2	Mild symptoms (shortness of breath after climbing one flight of steps) FEV1 60-79% **OR** LFS 3-5	Moderate symptoms (shortness of breath after walking on flat ground) FEV1 40-59% **OR** LFS 6-9	Severe symptoms (shortness of breath at rest; requiring 0_2) FEV1 <39% **OR** LFS 10-12
Joints and Fascia ***Diagnostic:*** Fasciitis Joint stiffness or contractures secondary to sclerosis ***Distinctive:*** Myositis or polymyositis†	No Symptoms	Mild tightness of arms or legs, normal or mild decreased range of motion (ROM) **AND** not affecting ADL	Tightness of arms or legs OR joint contractures, erythema thought due to fasciitis, moderate decrease ROM **AND** mild to moderate limitation of ADL	Contractures **WITH** significant decrease of ROM **AND** significant limitation of ADL (unable to tie shoes, button shirts, dress self etc.)
Genital Tract ***Diagnostic:*** Lichen planus-like features Vaginal scarring or stenosis ***Distinctive:*** Erosions* Fissures* Ulcers*	No Symptoms	Symptomatic with mild signs on exam **AND** no effect on coitus and minimal discomfort with gynaecologic exam	Symptomatic with moderate signs on exam **AND** with mild dyspareunia or discomfort with gynaecologic exam	Symptomatic **WITH** advanced signs (stricture, labial agglutination or severe ulceration) **AND** severe pain with coitus or inability to insert vaginal speculum

Notes:

1 Diagnostic: Sufficient to establish the diagnosis of chronic GvHD.

2 Distinctive: Seen in chronic GvHD but insufficient alone to establish a diagnosis of chronic GvHD.

* In all cases, infection, drug effects, malignancy, or other causes must be excluded.

† Diagnosis of chronic GvHD requires biopsy or radiology confirmation (or Schirmer test for eyes).

‡ ALP may be elevated in growing children, and not reflective of liver dysfunction.

Pulmonary scoring should be performed using both the symptom and pulmonary function testing (PFT) scale whenever possible. When discrepancy exists between pulmonary symptom or PFT scores the higher value should be used for final scoring.

Scoring using the Lung Function Score (LFS) is preferred, but if Diffusing Capacity of the Lung for Carbon Monoxide (DLCO) is not available, grading using Forced Expiratory Volume 1 (FEV1) should be used. The LFS is a global assessment of lung function after the diagnosis of bronchiolitis obliterans has already been established. The percent predicted FEV1 and DLCO (adjusted for hematocrit but not alveolar volume) should be converted to a numeric score as follows:

>80% = 1; 70-79% = 2; 60-69% = 3; 50-59% = 4; 40-49% = 5; <40% = 6.

The LFS = FEV1 score + DLCO score, with a possible range of 2-12. BSA - body surface area; ADL - activities of daily living; LFTs - liver function tests; ALP - alkaline phosphatase; ALT - alanine aminotransferase; AST - aspartate aminotransferase; ULN - upper limit of normal.

The Global Scoring of chronic GvHD is then calculated as shown below, dependent on the individual score of each affected organ:

Global chronic GvHD Score	**Individual score on affected organs**
None	No affected organs
Mild	Only 1 or 2 organs or sites (except Lung). No clinically significant impairment with maximum score of 1 in all affected organs
Moderate	(A) At least 1 organ or site with clinically significant but no major disability. Maximum score of 2 in any affected organ or site. *Or* (B) 3 or more organs or sites with no clinically significant functional impairment with maximum score of 1 in all affected organs or sites. A Lung score of 1 will be considered as Moderate
Severe	Major disability with a score of 3 in any organ or site. A lung score of 2 or greater will be considered as Severe

APPENDIX 5 - GENETIC AND FUNCTIONAL LEUKAEMIC STEM CELL (LSC) STUDIES IN PAEDIATRIC AML

AML is a genetically heterogeneous disease. At least 23 genes demonstrate a higher than expected mutation prevalence in AML; though like most cancers there is a much larger number of genes (at least 1893) where likely pathogenetic mutations (also known as Tier 1 mutations) exist at low frequency[62]. Any one AML sample has an average of 13 Tier 1 mutations but only 5 recurrent mutations (range 2-15), organised in at least 1-5 different clones (average 2). Although this raises the possibility of an almost infinite number of different clonal structures across AML as a whole, as AML driver mutations are often co-selected there are likely to be a more restricted number of common clonal genotypes across the majority of patients [62, 63]. Recent data suggest that acquired genetic mutations occur in a step-wise manner [64, 65]. Initiating mutation usually originates in a haemopoietic stem cell [66-68] to give rise to preleukemic stem cell populations that expand through clonal advantage. Further mutation acquisition results in clonal evolution and the imputed [64, 65] or proven clonal structures [66-70]are often branching.

Most current published comprehensive whole genome and exome sequencing data have been on adult AML samples. Many studies of karyotypic change and individual gene mutations have been performed in paediatric AML. These show differences in the spectrum and frequency of karyotypic abnormalities [28, 71] and specific genetic changes differ in childhood compared to adult AML. For example, chromosome 11q23 rearrangements are 5-fold commoner in childhood AML. Conversely, *NPM1* and *IDH1* and *IDH2* gene mutations occur in ~16-25% adult AML [72, 73], but are less common in childhood AML [74, 75]. *FLT3* mutations show an age-related increase in mutation prevalence [74, 76, 77].

MyeChild 01 provides an opportunity for comprehensive genetic analysis from a large uniformly treated paediatric cohort. We plan to perform whole genome analysis on diagnostic and relapse samples and more targeted gene mutation analysis in patients lacking known leukaemia-specific markers i.e. fusion gene/NPM1 mutation (i.e. assessment of MRD) on follow-up samples after each course of therapy and after cessation of therapy. This will allow comprehensive analysis of genetic variants that correlate with clinical outcomes at all time-points. Moreover, both bulk cell population and single cell genotyping will be performed to determine clonal structures of paediatric AML at diagnosis and assess how clonal structures change through treatment and into relapse. This will provide information on which clones are chemosensitive and which are chemoresistant and responsible for relapse. Ultimately, this will hopefully provide a rational basis for future combined therapy regimes.

In addition, to genetic heterogeneity, within any one patient there is functional heterogeneity at the cellular level – i.e. it is highly likely that not all leukaemic cells can propagate the disease. Functional heterogeneity has been principally investigated by immunophenotyping and purifying populations with stem cell function i.e. the ability to propagate leukaemia in immunodeficient mice through serial transplantation (thus demonstrating extensive self-renewal). These studies have identified multiple LSC populations [78-85].The clinical importance of experimentally identified LSC populations is still under investigation. LSC populations maybe more chemoresistant than bulk leukaemic cell populations [79] and may act as the cellular reservoir from which relapse originates [86-88]. As LSC populations can be detected by flow cytometry they provide an alternative flow-based method for tracking MRD.

Again most of the studies have been performed in adult AML with more limited studies in paediatric AML. We will use diagnostic, post-treatment, off treatment and relapse samples taken as part of Myechild 01 to:

- track LSC populations functionally through xenograft assays and quantitate LSC populations by flow cytometry
- We will compare the relative sensitivity of flow-cytometry leukaemia-aberrant phenotype MRD with flow cytometric LSC-detection based MRD and molecular MRD to determine the relative sensitivities of the three techniques
- This will provide correlative evidence if LSC populations are more chemoresistant and valuable to monitor clinically.

- We will also study clonal structures in LSC populations from diagnosis, through treatment, after therapy is complete and in relapse samples to understand which LSC clones are chemoresistant.

APPENDIX 6 - TRANSCRIPTOME SEQUENCING IN CHILDHOOD ACUTE MYELOID LEUKAEMIA: MYECHILD 01 STUDY

Evidence is accumulating on the role of gene mutations in AML from the point of view of understanding the biology of the disease and their association with outcome. For example, it is now well known that FLT3-ITD are associated with a poor outcome, particularly in those cases with an otherwise normal karyotype, and to the contrary mutations of NPM1 are linked to a favourable outcome. In addition, novel genetic rearrangements of prognostic significance have also recently been described. For example, the three rare rearrangements to be used in stratification to poor risk: t(5;11)(q35;p15.5)/*NUP98-NSD1*, t(7;12)(q36;p13)/*MNX1-ETV6* and inv(16)(p13.3q24.3)/*CBFA2T3-GLIS2,* were recently found to be cryptic (not visible at the cytogenetic level) and childhood AML specific. These transcripts also provide good molecular markers for disease monitoring. In approximately 42% of childhood AML cases, no suitable markers are available either for risk stratification or disease monitoring, screening of patients in MyeChild01 by transcriptome sequencing (RNA-Seq) provides an ideal opportunity for the discovery of novel disease specific biomarkers and to determine the relationships between them and known gene mutations/rearrangements in childhood AML on a large cohort of uniformly treated patients.

The aims and objectives of this study are:

1. To determine the types and incidences of known and novel genetic rearrangements and mutations within childhood AML through their study within a complete clinical trial.
2. Transcriptome sequencing of the whole genome will identify known and novel mutations from which their involvement in specific signalling pathways and their functional roles can be elucidated.
3. To examine the relationship of novel genetic abnormalities to other genetic changes of known prognostic relevance, which will be well annotated within this trial cohort.
4. To determine whether they have an impact on the outcome that is predicted from other features of risk stratification.
5. To determine whether specific combinations of abnormalities will impact on outcome within the different genetic subgroups.
6. To investigate the identified molecular markers as targets for MRD detection

The outcome of this study will demonstrate genetic differences and similarities between childhood and adult AML and highlight biological and related outcome differences between these age groups. Novel genetic abnormalities and the pathways which they activate may provide potential molecular targets for therapy for which targeted sequencing approaches can be designed.

APPENDIX 7 - GEMTUZUMAB OZOGAMICIN PHARMACOKINETIC ANALYSIS

Pharmacokinetic samples will be collected to evaluate the population pharmacokinetics (PK) of gemtuzumab ozogamicin, represented by total hp67.6 antibody, conjugated calicheamicin, and unconjugated calicheamicin, in children with AML. Samples will be analysed using validated analytical methods in compliance with Pfizer standard operating procedures at a Pfizer designated bioanalytical lab. Validated ADA (anti-drug antibody) assays will be used to measure ADA against gemtuzumab ozogamicin.

The concentration-time data will be summarized by descriptive statistics (mean, standard deviation, median, minimum, and maximum).

Population PK assessment will be conducted using the nonlinear mixed effect modelling approach in accordance with regulatory guidance. All patients from the Dose Finding Study who are treated with gemtuzumab ozogamicin and provide at least one post-dose drug concentration measurement will be included in the population PK analysis. A structural PK model based on prior information from a Wyeth-sponsor paediatric AML study may be used as a basis for the model. The population PK analysis will estimate typical value and variability for parameters including clearance (CL) and volume of distribution (Vd) of total hP67.6 antibody. Also, the influence of selected potential covariates on the PK parameters will be explored; the potential covariates to be explored may include selected demographics (e.g., body weight, sex), and ADA status.

APPENDIX 8 – LIPOSOMAL DAUNORUBICIN AND MITOXANTRONE PHARMACOKINETIC SUB-STUDY

Aims

(i) To investigate inter-individual variability in the PKs of liposomal daunorubicin and mitoxantrone in infants and young children with AML receiving reduced dosing regimens.

(ii) To compare drug exposures and degree of PK variability in infants and young children receiving reduced dosing regimens with data obtained from older children receiving standard doses.

(iii) To relate inter-individual variability in PKs and drug exposure to clinical toxicity and response.

(iv) To use PK data in conjunction with clinical information obtained following treatment to investigate the suitability of current dosing regimens for liposomal daunorubicin and mitoxantrone in infants and young children.

Background

The anthracycline liposomal daunorubicin and the anthracycline synthetic analogue mitoxantrone are now well established chemotherapeutics for use in induction therapy for children with AML. Current dosage regimens are based on clinical efficacy and toxicity data obtained from previous trials, with dose reductions implemented for infants and children <12kg in common with many other drugs and clinical trial protocols. However, the impact of these dose reductions on the PKs of the drugs involved is unknown. Indeed, in the case of mitoxantrone, almost no PK data have previously been published for children of any age.

Defining the most appropriate dosing regimens for anticancer drugs used in the treatment of infants and very young children represents a major challenge for paediatric oncologists and haematologists. Whereas dose reductions are commonplace for the vast majority of chemotherapeutics utilised in this patient group, for many drugs there are inconsistencies between tumour types and clinical protocols as to the magnitude of the dose reductions employed[89, 90]. Similarly, the cut-off point at which the reduced dosing regimen is implemented, commonly defined as below a specific age or body weight, is often variable. Although good reasons may exist for the implementation of variable cut-off points and dose reductions for different anticancer agents and tumour types, in many cases the scientific rationale behind the decisions is limited and dose reductions are largely historical, having been utilised in previous clinical studies. Although important differences in physiological characteristics in infants have the potential to impact on the PKs of anticancer drugs, only a handful of studies have actually been carried out to generate meaningful data in this area[89].

A number of studies have been published describing the PKs of liposomal daunorubicin in children. Liposomal daunorubicin PKs following administration of liposomal daunorubicin are characterised by increased drug plasma levels and an increased exposure (AUC), associated with a decreased volume of distribution following administration of the liposomal formulation. The liposomal drug exhibits one-compartment elimination PKs with an elimination half-life of 4-7 h and total plasma clearance of 0.2-1.0 l/h/m^2[91, 92]. Studies investigating liposomal daunorubicin (unchanged liposomal daunorubicin), free daunorubicin and daunorubicinol following a 1 hour IV infusion of liposomal daunorubicin, showed that liposomal daunorubicin accounted for approximately 96% of the drug recovered in the plasma of children aged 4-17 years[91]. Comparable levels of free daunorubicin and daunorubicinol were observed, with a daunorubicinol/daunorubicin AUC ratio of 0.8 reported. These data indicate a lower conversion of daunorubicin to daunorubicinol following administration of liposomal daunorubicin, as compared to treatment of patients with standard daunorubicin. This may be important as daunorubicinol is thought to contribute to the cardiac toxicity of the drug. As liposomal daunorubicin displays a different pharmacological profile from the standard formulation of daunorubicin, it is important to understand the PKs of total daunorubicin when administered as liposomal daunorubicin in infants and younger children receiving reduced drug doses, as compared to older children receiving standard dosing regimens. The studies published to date have reported on liposomal daunorubicin PKs in children aged 2-23 years of age.

Mitoxantrone is commonly administered by short IV infusion and its PKs reported in a number of published studies in adults. Mitoxantrone plasma concentrations have most frequently been measured

by high-performance liquid chromatography (HPLC) assay, with both two- and three-compartment models used to describe its PKs. Marked inter-patient variability in PK parameters has been reported in all studies. Mitoxantrone exhibits a short absorption half-life of 4-13 minutes, with wide variations in reported elimination half-lives of 9 hours to 4 days and reported clearance values ranging from 182 – 980 ml/min/m^2 [93-96].The explanation for these large discrepancies between studies is unclear but seems most likely to relate to differences in numbers of sampling time points at 24-96 hours post administration. Mitoxantrone is mainly eliminated from the body in the bile and several metabolites have been identified, including the major mono- and dicarboxylic acid derivatives. Few PK/pharmacodynamic relationships have been reported for mitoxantrone, although a correlation between nadir plasma levels and clinical response in patients with acute non lymphocytic leukaemia (ANLL) has been suggested.[95]

The current study will investigate inter-individual variability in the PKs of liposomal daunorubicin and mitoxantrone in infants and young children with AML receiving reduced dosing regimens, as compared to older children receiving standard drug doses. The observed inter-individual variability in PKs and drug exposure determined during induction therapy will be correlated with clinical toxicity and response in the patient groups being studied (reduced versus standard dosing). The results obtained will be used to investigate the suitability of current dosing regimens for liposomal daunorubicin and mitoxantrone in infants and young children.

Patients and treatment

Participation in this sub-study is optional, and separate informed consent must be obtained from the patient or parent/guardian prior to any samples being taken. Blood samples for analysis of liposomal daunorubicin and mitoxantrone PKs will be obtained from a total of 80 patients, male and female, receiving induction therapy in the defined treatment groups below:

Group 1:	Liposomal daunorubicin standard dosing (80 mg/m^2)	(n=20)
Group 2:	Liposomal daunorubicin reduced dosing for <10kg and/or <1yr	(n=20)
Group 3:	Mitoxantrone standard dosing (12 mg/m^2)	(n=20)
Group 4:	Mitoxantrone reduced dosing for <10kg and/or <1yr	(n=20)

PK sampling will be carried out on a single course of treatment as described below. The actual dose administered to the patient and time of administration should be clearly recorded on the sampling sheet (please refer to the MyeChild 01 Laboratory Manual) and it should be noted if this deviates in any way from the dose defined in the study protocol.

Samples

All patients must have a central venous catheter (single or multi-lumen catheter or portocath) or peripheral cannula in place in order for samples to be taken for PK analysis. Wherever possible, PK samples should be taken when clinical blood samples are obtained.

For liposomal daunorubucin, blood samples (1ml) will be obtained pre-treatment, immediately before the second infusion on Day 3, after the end of drug infusion on Day 3, 2 hours post-end infusion on Day 3, 24h post-end infusion Day 3, immediately before the third infusion on Day 5, after the end of drug infusion on Day 5 and 2 hours post-end infusion on Day 5, with exact sampling times clearly recorded on the sampling sheet (please refer to the MyeChild 01 Laboratory Manual).

For mitoxantrone, blood samples (2ml) will be obtained pre-treatment, immediately after the end of drug infusion on Day 1 and at 0.5, 1, 2 and 6 hours post-end of infusion on Day 1, immediately before drug infusion on Day 2 and, 48 hours and 72hours post-end infusion on the final day of mitoxantrone treatment, with exact sampling times clearly recorded on the sampling sheet (please refer to the MyeChild 01 Laboratory Manual).

Blood samples should be transferred to EDTA tubes immediately following collection, centrifuged for 5 min at 2,000 rpm and 4°C and the plasma obtained transferred to a clean labelled tube and stored at -20°C prior to transport to the Northern Institute for Cancer Research (NICR), Newcastle University.

APPENDIX 9 – PHARMACOGENOMIC SUB-STUDY

Introduction

Pharmacogenomics is a science that examines the inherited variations in genes that dictate drug response and explores the ways these variations can be used to predict whether a patient will have a favorable response, bad response, or no response at all to the drug. It is therefore important to investigate the genetic profile of cancer patients to determine the presence of an association between genotypes (genetic variants) and phenotypes (e.g. pharmacokinetics (PK) and pharmacodynamics (PD)). With an established association, it will be possible to personalise medicine to reduce toxicity and improve efficacy in turn reducing relapse by selecting the correct treatment for the correct patient at the correct dose and time. In oncology, it has been shown that 20% of patients do not respond to standard therapy. The therapeutic agents used in cancer chemotherapy are ideally suited to pharmacogenomic investigation as they are often administered at doses that produce severe toxicity with a wide interindividual response, but need to be given at optimal doses for the best effect. Short- and long-term toxicity affects more than 40% of cancer patients and can be life threatening or permanently disabling. Pharmacogenomics in this field has the potential to improve the safety and efficacy of drugs. For some treatments pharmacogenetics is already a reality. Examples in paediatrics include: *TPMT* SNPs & Mercaptopurine/Azathioprine. An increasing number of pharmacogenomic studies are being published, most include only adults. Only a few studies have shown the impact of pharmacogenomics in pediatrics, but those that have highlighted a key difference between children and adults, which is the contribution of developmental changes to therapeutic responses across different age groups, demonstrating the importance of separate analysis. Additional large knowledge banks of matched genomic-clinical data will be needed to support clinical decision-making in pediatrics.

Background of Project

In brief, gemtuzumab ozogamicin was withdrawn from the US market in 2010 owing to high treatment related toxicity (including hepatic, pulmonary and cardiac problems). Identification of risk patients according to their genetic make-up would benefit by adjusting the dose in the future. The top candidate gene for gemtuzumab ozogamicin is CD33. Since CD33 is the target of gemtuzumab ozogamicin, any genetic variation in CD33 that can influence its expression, surface localization or physiological role can have an impact on patient's response to gemtuzumab ozogamicin. The proposed pharmacogenomics study would be useful in identifying germline risk factors which could be combined with disease risk factors to stratify patients who would benefit from this treatment. Pharmacogenetics of cytarabine (Ara-C) was mostly reported in relation to PK. *CDA* and *DCK* genes seem to be important loci that should be further investigated regarding the outcome of Ara-C-based chemotherapy in leukemia patients. Mitoxantrone is a known substrate for ABC efflux transporters such as those encoded by the *ABCB1* and *ABCG2* genes. To date, there are no studies in the leukemic setting that would test whether genetic polymorphisms in these genes could affect mitoxantrone disposition and hence modulate the response and side effects to the drug. Fludarabine (Flu) is phosphorylated to the active form mainly with DCK enzyme. SNPs in *DCK* gene and the genes encoding some transporters have been shown to have the potential to influence PK/PD of Flu. For anthracycline antileukemic compounds, daunorubicin and its analog, idarubicin, there already exist published guidelines for identifying high-risk patients, particularly for cardiotoxicity and other anthracycline-related organ toxicities. Due to low number of these studies these genes should be investigated additionally in children diagnosed with AML. In the setting of Hematopoietic Stem Cell Transplantation (HSCT), our group has identified genetic markers for predicting the PK/PD of busulfan (BU) and clinical outcomes of HSCT following a busulfan/cyclophosphamide conditioning regimen, which we wish to validate in a homogenous cohort such as Myechild 01.

The aims and objectives of this study are:

1. To retrospectively and prospectively validate known risk variants that alter the efficacy and/or exposure of the treatment regimens used in children diagnosed with AML in the Myechild 01 study.

2. To identify new genetic markers of drug response (all drugs used in MyeChild 01) by performing an exploratory study using targeted sequencing and germline transcriptome.

Methodology:

Sample needed:

Saliva or buccal swab samples (2mL per patient) will be collected using Oragene DNA kit (OG-500, DNAgenotek) and stored/transported at room temperature.

Where sample collection is missed in error, DNA samples may be retrieved from remaining MRD samples from the central MRD labs.

Sample collection and kit distribution in UK centres will be coordinated by Dr Gareth Veal. All samples collected in UK centres will be gathered at Northern Institute for Cancer Research, Medical School, Newcastle University (UK) then the samples will be shipped in Geneva, CANSEARCH research laboratory, at Geneva Medical School, Switzerland for analysis and banking.

Sample collection and kit distribution in France will be coordinated by Prof Marc Ansari's laboratory, CANSEARCH research laboratory at Geneva Medical School, Switzerland.

All samples for this study will be gathered at Faculty of Medicine, University of Geneva by shipping in batches from Newcastle and as single samples from centres in France directly to the address in Geneva: Faculté de Médecine, Batiment Tulipe, 5th floor, Av De La Roseraie 64, 1205 GENEVE, Switzerland (Contact information: phone (+41 79 55 36 100) and e-mail: research@cansearch.ch).

OG-500 kits will be provided to either UK coordinator or France centres by the Oragene company, coordinated by the Faculty of Medicine, CANSEARCH research laboratory, University of Geneva (Switzerland).

DNA will be extracted using QIAsymphony automatic DNA extraction station (QIAgene) at the CANSEARCH research laboratory, Faculty of Medicine, University of Geneva (Switzerland)

Clinical data needed:

Clinical outcomes data related to the efficacy and toxicity of all investigational drugs in every randomisation arm will be used for statistical analysis with the genotypic data. Busulfan pharmacokinetic data from randomisation 4 would be used for validation of the association previously found with the functional GST variants.

The outcome of this study will be to confirm and identify novel biomarkers of drug response within AML in children for personalizing treatment for the future utility of these particular drugs.

Table 35: Summarizes all of the known candidate genes identified that influence the efficacy and toxicity of all the investigatory drugs which are going to be utilised in Myechild 01

Drug	List of Genes (symbols provided) Pharmacokinetics	List of Genes (symbols provided) Pharmacodynamics

Gemtuzumab Ozogamicin [97-99]	*ADH1A; SLCO1B1; SLC22A12; SULT2B1; GSTP1; GSTA1,2; GSTT1; GSTM1,2,3; NRF-2; NQO1; CTH; CBS; CYP3A4; CYP3A5; CYP2E1; NAT1; NAT2; ABCB1; ABCC2; ABCC1*	*CD33; SOCS3; XRCC5; LIG4; BCL2; BCL2L1; ATM; BRCA1, 2; Rad50; MRE11; NBS1; RAD9; RAD1; Other DNA Repair Pathway Genes*
Cytarabine [99-102]	*CDA; ENT1; ABCB1; SLC29A1; SLC2A3, 8; ABCB1; ALDH1A2; ME1; GK; PDK4; HK3; ACSL1; CYP2E1; SLC22A12; SLC14A1; SLC25A37; SLCO1B1; SULT2B1; NT5C2*	*DCK; RRM2; RRM2B; NOS3 ; NT5C2; NT5C3A; NDUFA13; ATP5L; HDAC4 ; PPARγ; KLF4; CREB5; CEBPβ; RARA; E2F4; MNDA; MTA3; RRM2; GATA3; DOK5; BMP7; MCC; GIT1; RAD51AP1*
Mitoxantrone [99]	*ABCB1; ABCG2; SLCO1B1*	*GALNT14; MECP2; DCK*
Fludarabine [103]	*SLC22A12; SLC28A3; CDA* *hCNT2; hCNT3; hENT1; hENT2; ABCG2; ABCC4*	*DCK; CXCL12*
Daunorubicin [99, 104-106]	*SLC28A1,3; SLC15A1; SLC22A2; SLCO1B1; SLC10A2; SLCO6A1 UGT1A6; ABCB1; ABCB11; ABCC1, 4, 5, 9; SULT2B1; GSTs (as mentioned above); FMO3; CYP4F2; CBR1,3; ADH7; XPO5*	*RARG; NOS3; HNMT; MTHFR; XDH; SOD2; SZRD1; Other DNA Repair Pathway Genes; NRP2; BMP7; DOK5; GATA3; 4*
Idarubicin [97]	*NT5C3A; ABCB1, 2; ABCC1; CYP2E1; SLC22A12; SLCO1B1; SULT2B1; ABCG2; PGP*	*DCK; NOD2*
Busulfan [99, 107-109]	*GSTA1,2; GSTM1,M2; GSTT1; GSTP1; NRF-2; SOD2; CTH; CYP2C9; FMO3; SLC22A4; SLC7A8; CYP2C19*	*APEX1; EXO1; ATM; CTH; MGMT; Other DNA Repair Pathway Genes*
Cyclophosphamide [99, 110, 111]	*CYP2A6; CYP2C8; CYP1B1; CYP2C9; CYP2B6; CYP2C19; CYP3A5; CYP3A4; CYP2E1; ABCB1; ABCC1; ABCC3; ABCC4; ABCG2; ALDH1A1; ALDH3A1; GSTs as mentioned above; NQO2; SLCO1B1; MTHFR; SOD2; GSTM3; NOS3; TPMT; SLC22A16; NAT2*	*TP53; RAD52; ERCC1, 2; KLC3; IKZF3; MGMT; LINC00251; GATA3; CXCL12; MUTYH; CTH; EPHX1; MTR; CTNNB1; VEGFA; FGFR4; NQO2*

*The list provided above includes examples of genes from some of the pathways. This study is not limited to the examples listed; based on arising evidence, reports and functional studies this list will be updated to include other potential candidate genes.

Supportive care drugs (cyclosporine, MMF etc.) would also be evaluated with this pharmacogenomics study.

APPENDIX 10 - DEFINITION OF ADVERSE EVENTS

Adverse Event

Any untoward medical occurrence in a patient or clinical trial subject administered a medicinal product and which does not necessarily have a causal relationship with this treatment.

Comment:

An AE can therefore be any unfavourable and unintended sign (including abnormal laboratory findings), symptom or disease temporally associated with the use of an investigational medicinal product (IMP), whether or not related to the IMP.

Adverse Reaction

All untoward and unintended responses to an IMP related to any dose administered.

Comment:

An AE judged by either the reporting Investigator or Sponsor as having causal relationship to the IMP qualifies as an AR. The expression reasonable causal relationship means to convey in general that there is evidence or argument to suggest a causal relationship.

Serious Adverse Event

Any untoward medical occurrence or effect that at any dose:

Results in death Is life-threatening*

Requires hospitalisation** or prolongation of existing inpatients' hospitalisation

Results in persistent or significant disability or incapacity

Is a congenital anomaly/birth defect

Or is otherwise considered medically significant by the Investigator***

Comments:

The term severe is often used to describe the intensity (severity) of a specific event. This is not the same as serious, which is based on patients/event outcome or action criteria.

* Life threatening in the definition of an SAE refers to an event in which the patient was at risk of death at the time of the event; it does not refer to an event that hypothetically might have caused death if it were more severe.

**Hospitalisation is defined as an unplanned, formal inpatient admission, even if the hospitalisation is a precautionary measure for continued observation. Thus hospitalisation for protocol treatment (e.g. line insertion), elective procedures (unless brought forward because of worsening symptoms) or for social reasons (e.g. respite care) are not regarded as an SAE.

*** Medical judgment should be exercised in deciding whether an AE is serious in other situations. Important AEs that are not immediately life threatening or do not result in death or hospitalisation but may jeopardise the subject or may require intervention to prevent one of the other outcomes listed in the definition above, should be considered serious.

Serious Adverse Reaction

An Adverse Reaction which also meets the definition of a SAE.

Suspected Unexpected Serious Adverse Reaction

A SAR that is unexpected i.e. the nature, or severity of the event is not consistent with the applicable product information.

A SUSAR should meet the definition of an AR, UAR and SAR.

Unexpected Adverse Reaction

An AR, the nature or severity of which is not consistent with the applicable product information (e.g. Investigator Brochure for an unapproved IMP or (compendium of) Summary of Product Characteristics (SPC) for a licensed product).

When the outcome of an AR is not consistent with the applicable product information the AR should be considered unexpected.

APPENDIX 11 - COMMON TOXICITY CRITERIA GRADINGS

Toxicities will be recorded according to the Common Terminology Criteria for Adverse Events (CTCAE), version 4.0. 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:

http://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm

APPENDIX 12 - WMA DECLARATION OF HELSINKI

WORLD MEDICAL ASSOCIATION DECLARATION OF HELSINKI

Recommendations guiding physicians

in biomedical research involving human subjects

Adopted by the 18th World Medical Assembly

Helsinki, Finland, June 1964

and amended by the

29th World Medical Assembly, Tokyo, Japan, October 1975

35th World Medical Assembly, Venice, Italy, October 1983

41st World Medical Assembly, Hong Kong, September 1989

and the

48th General Assembly, Somerset West, Republic of South Africa, October 1996

INTRODUCTION

It is the mission of the physician to safeguard the health of the people. His or her knowledge and conscience are dedicated to the fulfillment of this mission.

The Declaration of Geneva of the World Medical Association binds the physician with the words, "The Health of my patient will be my first consideration," and the International Code of Medical Ethics declares that, "A physician shall act only in the patient's interest when providing medical care which might have the effect of weakening the physical and mental condition of the patient."

The purpose of biomedical research involving human subjects must be to improve diagnostic, therapeutic and prophylactic procedures and the understanding of the aetiology and pathogenesis of disease.

In current medical practice most diagnostic, therapeutic or prophylactic procedures involve hazards. This applies especially to biomedical research.

Medical progress is based on research which ultimately must rest in part on experimentation involving human subjects.

In the field of biomedical research a fundamental distinction must be recognized between medical research in which the aim is essentially diagnostic or therapeutic for a patient, and medical research, the essential object of which is purely scientific and without implying direct diagnostic or therapeutic value to the person subjected to the research.

Special caution must be exercised in the conduct of research which may affect the environment, and the welfare of animals used for research must be respected.

Because it is essential that the results of laboratory experiments be applied to human beings to further scientific knowledge and to help suffering humanity, the World Medical Association has prepared the following recommendations as a guide to every physician in biomedical research involving human subjects. They should be kept under review in the future. It must be stressed that the standards as drafted are only a guide to physicians all over the world. Physicians are not relieved from criminal, civil and ethical responsibilities under the laws of their own countries.

I. BASIC PRINCIPLES

1. Biomedical research involving human subjects must conform to generally accepted scientific principles and should be based on adequately performed laboratory and animal experimentation and on a thorough knowledge of the scientific literature.

2. The design and performance of each experimental procedure involving human subjects should be clearly formulated in an experimental protocol which should be transmitted for consideration, comment and guidance to a specially appointed committee independent of the investigator and the

sponsor provided that this independent committee is in conformity with the laws and regulations of the country in which the research experiment is performed.

3. Biomedical research involving human subjects should be conducted only by scientifically qualified persons and under the supervision of a clinically competent medical person. The responsibility for the human subject must always rest with a medically qualified person and never rest on the subject of the research, even though the subject has given his or her consent.

4. 4. Biomedical research involving human subjects cannot legitimately be carried out unless the importance of the objective is in proportion to the inherent risk to the subject.

5. Every biomedical research project involving human subjects should be preceded by careful assessment of predictable risks in comparison with foreseable benefits to the subject or to others. Concern for the interests of the subject must always prevail over the interests of science and society.

6. The right of the research subject to safeguard his or her integrity must always be respected. Every precaution should be taken to respect the privacy of the subject and to minimize the impact of the study on the subject's physical and mental integrity and on the personality of the subject.

7. Physicians should abstain from engaging in research projects involving human subjects unless they are satisfied that the hazards involved are believed to be predictable. Physicians should cease any investigation if the hazards are found to outweigh the potential benefits.

8. In publication of the results of his or her research, the physician is obliged to preserve the accuracy of the results. Reports of experimentation not in accordance with the principles laid down in this Declaration should not be accepted for publication.

9. In any research on human beings, each potential subject must be adequately informed of the aims, methods, anticipated benefits and potential hazards of the study and the discomfort it may entail. He or she should be informed that he or she is at liberty to abstain from participation in the study and that he or she is free to withdraw his or her consent to participation at any time. The physician should then obtain the subject's freely-given informed consent, preferably in writing.

10. When obtaining informed consent for the research project the physician should be particularly cautious if the subject is in a dependent relationship to him or her or may consent under duress. In that case the informed consent should be obtained by a physician who is not engaged in the investigation and who is completely independent of this official relationship.

11. In case of legal incompetence, informed consent should be obtained from the legal guardian in accordance with national legislation. Where physical or mental incapacity makes it impossible to obtain informed consent, or when the subject is a minor, permission from the responsible relative replaces that of the subject in accordance with national legislation. Whenever the minor child is in fact able to give a consent, the minor's consent must be obtained in addition to the consent of the minor's legal guardian.

12. The research protocol should always contain a statement of the ethical considerations involved and should indicate that the principles enunciated in the present Declaration are complied with.

II. MEDICAL RESEARCH COMBINED WITH PROFESSIONAL CARE (Clinical Research)

1. In the treatment of the sick person, the physician must be free to use a new diagnostic and therapeutic measure, if in his or her judgement it offers hope of saving life, re-establishing health or alleviating suffering.

2. The potential benefits, hazards and discomfort of a new method should be weighed against the advantages of the best current diagnostic and therapeutic methods.

3. In any medical study, every patient - including those of a control group, if any - should be assured of the best proven diagnostic and therapeutic method. This does not exclude the use of inert placebo in studies where no proven diagnostic or therapeutic method exists.

4. The refusal of the patient to participate in a study must never interfere with the physician-patient relationship.

5. If the physician considers it essential not to obtain informed consent, the specific reasons for this proposal should be stated in the experimental protocol for transmission to the independent committee (I, 2).

6. The physician can combine medical research with professional care, the objective being the acquisition of new medical knowledge, only to the extent that medical research is justified by its potential diagnostic or therapeutic value for the patient.

III. NON-THERAPEUTIC BIOMEDICAL RESEARCH INVOLVING HUMAN SUBJECTS (Non-Clinical Biomedical Research)

1. In the purely scientific application of medical research carried out on a human being, it is the duty of the physician to remain the protector of the life and health of that person on whom biomedical research is being carried out.

2. The subject should be volunteers - either healthy persons or patients for whom the experimental design is not related to the patient's illness.

3. The investigator or the investigating team should discontinue the research if in his/her or their judgement it may, if continued, be harmful to the individual.

4. In research on man, the interest of science and society should never take precedence over considerations related to the wellbeing of the subject.

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