TITLE	A PHASE II TRIAL OF AN INTENSIVE PEDIATRIC PROTOCOL INCORPORATING POST-INDUCTION STRATIFICATION BASED ON MINIMAL RESIDUAL DISEASE LEVELS FOR THE TREATMENT OF ADOLESCENTS AGED 15 YEARS AND ABOVE, AND YOUNG ADULTS AGED UP TO 40 YEARS, WITH NEWLY DIAGNOSED ACUTE LYMPHOBLASTIC LEUKAEMIA (ALL)
SHORT TITLE	AYA ALL
PROTOCOL NUMBER	ALL06
UTN (Universal Trial Number)	U1111-1122-4935
ANZCTR Number	
PRINCIPAL INVESTIGATORS	Matthew Greenwood Royal North Shore Hospital St Leonards NSW 2065 Luciano Dalla Pozza Children's Hospital at Westmead Sydney, NSW, Australia 2145
VERSION NUMBER	Version 5.0
DATE OF PROTOCOL	09 June 2017

FOREWORD

Information in this protocol should not be disclosed other than to those involved in the execution or ethical review of the study without written authorisation from the Australasian Leukaemia and Lymphoma Group (ALLG).

This protocol complies with the Guidelines for Good Clinical Practice in clinical research.

This document is intended to describe an ALLG trial and to provide information about trial procedures. It is not intended that the Protocol be used as a guide for the treatment of patients who are not enrolled on this trial. ALLG will not accept any data for analysis unless the Human Research Ethics Committee (HREC) of each trial site has approved this trial for patient enrolment and participation.

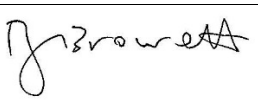
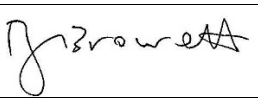
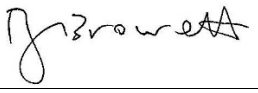
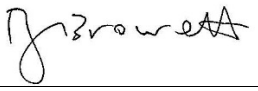
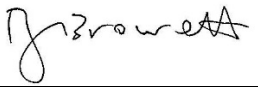
Amendments to the document will be circulated to participating trial sites. If in doubt about which is the correct version of the protocol please contact the Trial Centre Data Manager.

The Protocol and all other trial related documentation including the Patient Informed Consent Form (PICF) and Case Report Form (CRF) must be written in English and under no circumstances be translated into another language without prior written approval from ALLG.

Protocol History

Version	Date	Author	Reason
A	July 2010	Ken Bradstock	SDMC review
B	September 2010	Ken Bradstock	Incorporate changes recommended by SDMC
C	November 2010	Ken Bradstock	Incorporate changes recommended by SDMC
D	February 2011	Ken Bradstock	Incorporate changes recommended by SDMC
E	April 2011	Ken Bradstock	Incorporate changes recommended by SDMC
F	June 2011	Ken Bradstock	Approved , no changes
G	June 2014	Ken Bradstock	Incorporate changes recommended by SDMC

Protocol Approval

Version No	Date	Name and position of person approving	Approval signature
Version 1	28 June 2011	Peter Browett-SDMC chair	
Version 2	16 June 2014	Peter Browett-SDMC chair	
Version 3.0	25 July 2016	Peter Browett-SDMC chair	
Version 4.0	7 October 2016	Peter Browett-SDMC chair	
Version 5.0	09 June 2017	Peter Browett-SDMC chair	

SPONSOR SIGNATURE

I have read and approve this protocol. My signature, in conjunction with the signature of the investigator, confirms the agreement of both parties that the clinical study will be conducted in accordance with the protocol and all applicable local laws and regulations including, but not limited to, the International Conference on Harmonisation Guideline for Good Clinical Practice (ICH GCP), the ethical principles that have their origin in the Declaration of Helsinki and applicable privacy laws.

09 June 2017

SIGNATORY ON BEHALF OF SPONSOR | DATE

Prof Mark Hertzberg

NAME OF SIGNATORY (print)

PRINCIPAL INVESTIGATOR SIGNATURE

I have read and approve this protocol. My signature, in conjunction with the signature of the sponsor, confirms the agreement of both parties that the clinical study will be conducted in accordance with the protocol and all applicable local laws and regulations including, but not limited to, the ICH GCP, the ethical principles that have their origin in the Declaration of Helsinki and applicable privacy laws.

Nothing in this document limits the authority of a physician to provide emergency medical care under applicable regulations.

09 June 2017

PRINCIPAL INVESTIGATOR SIGNATURE | DATE

Dr Matthew Greenwood

PRINCIPAL INVESTIGATOR NAME (print)

Table of Contents

1. Contacts

Co-Chief Investigators

Dr Matthew Greenwood
Haematology Department
Royal North Shore Hospital, NSW, 2145
Ph: +61 2 9926 4393
Fax: +61 2 9926 4070
Mob: +61 (0) 434 568 082
Email: Matthew.Greenwood@health.nsw.gov.au

Dr Luciano Dalla Pozza
Oncology Department
Childrens Hospital Westmead, NSW, 2145
Ph: +61 2 9845 2134
Fax: +61 2 9845 2171
Mob: +61 (0) 418 464 606
Email: luciano.dallapozza@health.nsw.gov.au

Statistician

Dr Chris Frampton
Statistecol Consultants
45 Beveridge St
City Central, Christchurch, NZ, 8013
Ph: +64 27 2729378
Email: statistecol@xtra.co.nz

Sponsor

Australasian Leukaemia and Lymphoma Group
Ground Floor, 35 Elizabeth St
Richmond, VIC, Australia, 3121
Ph: +61 3 8373 9714
Fax: +61 3 9429 8277

Trial Coordinating Centre

Australasian Leukaemia and Lymphoma Group
Ground Floor, 35 Elizabeth St
Richmond, VIC, Australia, 3121
Ph: +61 3 8373 9714
Fax: +61 3 9429 8277

SAE and pregnancy notification for all sites within 24 hours

Fax: +61 3 9429 8277

Molecular MRD Analysis

Dr Rosemary Sutton
Childrens Cancer Institute Australia ustraliaio
C25 Lowy Cancer Research Centre
Lower Ground Floor
Gate 11, Botany Street, UNSW
Sydney, NSW 2052
Ph +61 2 9382 1829

Flow Cytometry MRD Analysis

Mrs Mary Sartor
Institute of Clinical Pathology and Medical Research
Level 2
Flow Cytometry Unit
Westmead Hospital
Darcy Rd
Westmead NSW 2145
Ph: +61 2 9845 6275

Trial Management Committee

Dr Matthew Greenwood
Royal North Shore Hospital
Reserve Rd
St Leonards, NSW, 2065
Ph: +61 2 9926 4393
Email: Matthew.Greenwood@health.nsw.gov.au

Dr Toby Trahair
Sydney Children's Hospital
High St
Randwick, NSW, 2031
Ph: +61 2 9382 2970
Email: Toby.Trahair@SESIAHS.HEALTH.NSW.GOV.AU

Prof Kenneth Bradstock
Mob: +61 (0) 430 526 925
Email: ken.bradstock@sydney.edu.au

Dr Luciano Dalla Pozza
Oncology Department
Childrens Hospital Westmead,
NSW, 2145
Ph: +61 2 9845 0000
Email: luciano.dallapozza@health.nsw.gov.au

Dr Andrew Wei
Alfred Hospital
Commercial Road
Prahran, Victoria, Australia, 3181
Ph: +61 3 9076 3451
Email: andrew.wei@monash.edu

Dr Michael Osborn
Royal Adelaide Hospital
North Terrace, Adelaide, SA, 5000
Ph: +61 8 8222 4000
Email: Michael.Osborn@sa.gov.au

HRQOL

Prof. Madeleine King
Cancer Australia Chair in Cancer Quality of Life Office
Psycho-oncology Co-operative Research Group School of Psychology
Brennan MacCallum Building (A18) University of Sydney NSW 2006
Ph: +61 2 9036 6114 Email: madeleine.king@sydney.edu.au

2. Abbreviations

AC	Additional concerns
ACD	Citrate anticoagulant
AE	Adverse event
ALL	Acute lymphoblastic leukaemia
ALLG	Australasian Leukaemia and Lymphoma Group
alloHCT	Allogeneic Haemopoietic Cell Transplantation
ALT	Alanine aminotransferase
ANC	Absolute neutrophil count
ANZCHOG	The Australian and New Zealand Children's Haematology/Oncology Group
Ara-C	Cytarabine
ASP	Asparaginase
AYA	Adolescent and young adult
BFM-95	Berlin-Frankfurt-Munster- 95 protocol
BSA	Body surface area
CI	Confidence interval
CNS	Central nervous system
CPA	cyclophosphamide
CR	Complete Remission
CRF	Case report form
CSF	Cerebospinal fluid
CT	Computed Tomography (=CAT scan)
CTN	Clinical trial notification
DEXA	Dexamethasone
DFS	Disease-free survival
DNR	Daunorubicin
DOX	doxorubicin
ECOG	Eastern Co-operative Oncology Group
E.coli	Escherichia Coli
eCRF	Electronic case report form
EDTA	Ethylenediaminetetraacetic acid
EORTC-QLQ-C30	European Organisation for Research and Treatment of Cancer – Quality of Life Questionnaire – core 30
EWB	Emotional wellbeing
FACIT	Functional Assessment of Chronic Illness Therapy
FeV1	Forced expiratory volume in 1 second
FSH	Follicle stimulating hormone
FWB	Functional wellbeing
g	gram
GCP	Good Clinical Practice
G-CSF	Granulocyte colony stimulating factor
h	hour
HBV	Hepatitis B virus
HCT	Hemopoietic cell transplant
HCV	Hepatitis C virus
HD-MTX	High dose methotrexate
HIV	Human immunodeficiency virus
HR	High Risk
HREC	Human Research Ethics Committee
HRQOL	Health-Related Quality of Life
ICH GCP	International Conference on Harmonisation, Good Clinical Practice
ICSH	International Committee for Standardization in Haematology
ID	Identification
IFO	Ifosfamide

IM	intramuscular
IP	Investigational product
IT	intrathecal
IV	intravenous
kg	kilogram
L	litre
LCV	Leucovorin
LDH	Lactate dehydrogenase
LH	Luteinsing hormone
m	metre
mg	milligram
MGG	Molecular and general genetics
mL	millilitre
MRD	Minimal residual disease
MP	6-Mercaptopurine
MTX	methotrexate
NaCl	Sodium chloride
NCI CTC	National Cancer Institute Common Toxicity Criteria
NZ	New Zealand
PICF	Patient Information and Consent Form
PB	Peripheral blood
PCR	Polymerase chain reaction
PD	Progressive disease
PI	Principal Investigator
PoCoG	Psycho Oncology Group
PR	Partial Response
PWB	Physical wellbeing
QOL	Quality of Life
SAE	Serious adverse event
SAP	Statistical Analysis Plan
SC	subcutaneous
SD	Stable disease
SDMC	Safety and data monitoring committee
SWB	Social/family welbeing
TCC	Trial Coordinating Centre
TGA	Therapeutic Goods Administration
TMC	Trial Management Committee
U	Units
ULN	Upper limit of normal
USA	United States of America
VCR	Vincristine
VDS	Vindesine
WBC	White blood count
WCC	White cell count
WHO	World health organisation

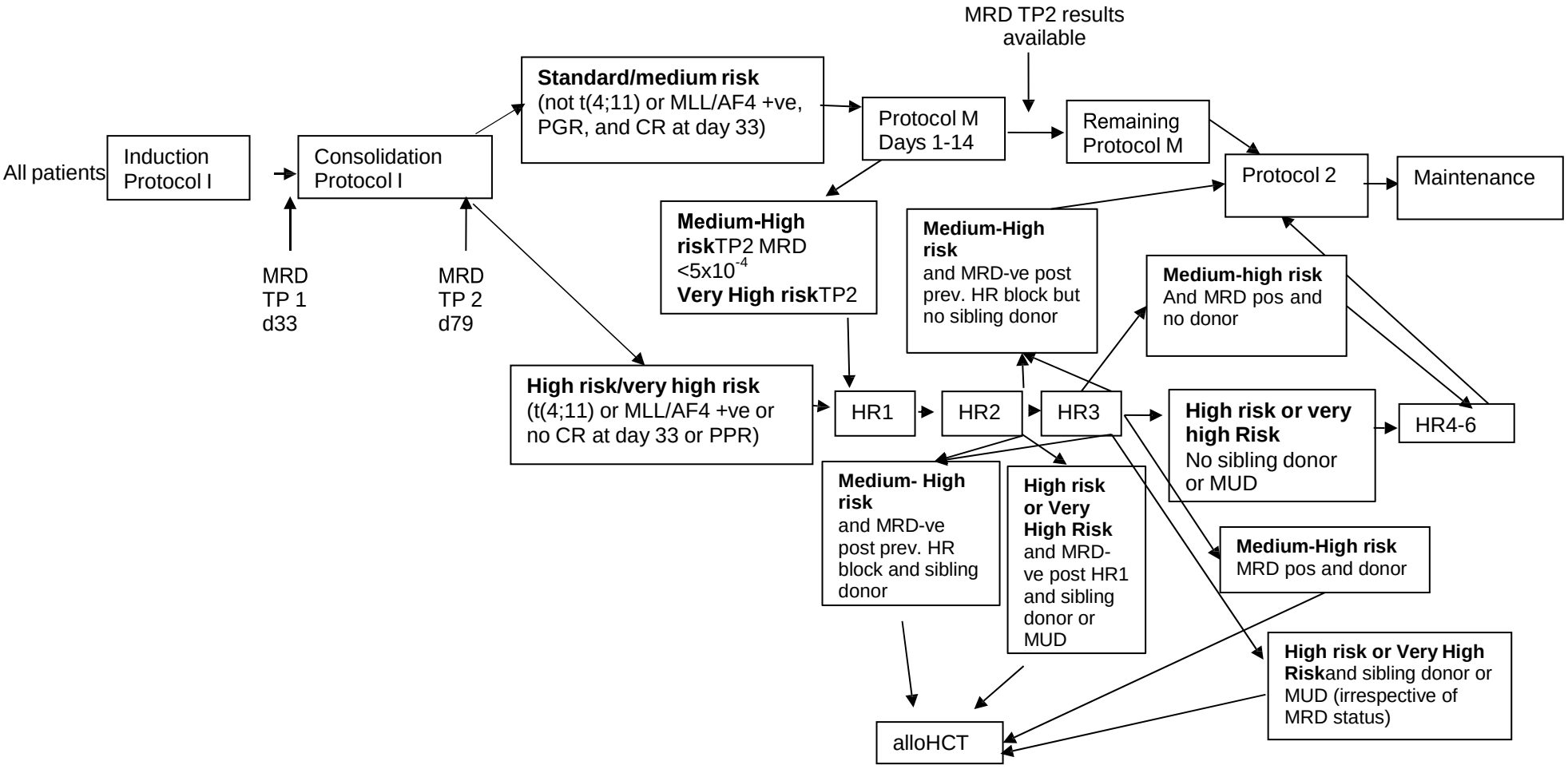
3. Protocol synopsis

Note: This is a synopsis. The body of the protocol must be referred to for the complete study information.

Title	A PHASE II TRIAL OF AN INTENSIVE PEDIATRIC PROTOCOL INCORPORATING POST-INDUCTION STRATIFICATION BASED ON MRD LEVELS FOR THE TREATMENT OF ADOLESCENTS AGED 15 YEARS AND ABOVE, AND YOUNG ADULTS AGED UP TO 40 YEARS, WITH NEWLY DIAGNOSED ACUTE LYMPHOBLASTIC LEUKAEMIA (ALL)
ALLG Study number	ALL06
Short title	AYA ALL
Sponsor	ALLG
Indication	Untreated ALL aged 15 to 40 years
Rationale	Determine deliverability of treatment in comparison to paediatric patients
Objectives	Primary objective To determine whether a modified form of the BFM-2000 protocol can be administered to patients with newly diagnosed and untreated ALL aged between 15 and 40 years in a comparable timeframe to patients under 15 years of age to be measured by the proportion of patients starting Protocol M by day 94 after commencing therapy. Secondary objectives To evaluate the complete remission (CR) rates, disease-free survival (DFS) and overall survival (OS) of patients treated on this protocol and to compare with results reported in a similar age group using protocols designed for use in adult patients. To evaluate toxicity, both hematologic and non-hematologic. To compare the proportions of AYA patients with standard, medium, and high risk disease to pediatric ALL, and to evaluate outcomes within these risk groups. To assess the level of residual disease after induction chemotherapy and compare this with levels reported in a similar age group using adult protocols. To conduct correlative laboratory scientific studies on blood and bone marrow samples taken from patients enrolled on this study To assess the impact of treatment on physical, functional, emotional and social wellbeing via HRQOL assessment at the beginning and end of each phase of treatment. To provide an indication of fertility status after treatment
Trial design	All patients will commence treatment with Protocol 1 which is an intensive chemotherapy induction protocol. Various prognostic factors such as cytogenetic abnormalities, initial response to prednisolone, achievement of remission, and MRD levels after induction and consolidation, will determine whether patients continue treatment outlined in Protocol M, or are to be treated on the High Risk protocol, or undergo allogeneic HCT. Patients removed for allogeneic HCT will be withdrawn from the study treatment, but will continue to be followed, with relapse, disease-free and overall survival data being recorded.
Number of participants	100
Duration of subject participation	2 years with a minimum of 2 years follow up
Recruiting period	3-3.5 years at a rate of 30 subjects/year
Study duration	7.5 years
Investigational products	All drugs used in this protocol and registered for the prescribed indication.
Main inclusion criteria	· A morphological diagnosis of ALL by WHO criteria, confirmed by immunophenotyping and cytogenetics. All clinico-pathological subtypes will be eligible, except for mature B or Burkitt ALL (L3). · Bone marrow blast count ≥20% · Adequate renal and hepatic function at Screening as defined by: a. Total bilirubin <2.5 x ULN unless medically correctable b. Serum creatinine ≤200 micromol/L unless medically correctable · Normal left ventricular ejection fraction, according to institutional criteria. If

	the clinical circumstances require that treatment must be given urgently before this can be ascertained, the absence of clinical cardiac impairment is acceptable, provided that a normal left ventricular ejection fraction is confirmed prior to the first consolidation cycle. · An ECOG performance status score of 0-3 Screening
Main exclusion criteria	· Patients known to have Philadelphia chromosome-positive disease · Subjects aged less than 15 or more than 40 years at Screening · Presence of serious cardiac, pulmonary, hepatic or renal disease · Previous treatment for ALL or history of cancer (other than basal cell skin cancer or carcinoma of the cervix in situ, or other localised cancer treated by surgical excision only more than 5 years earlier without evidence of recurrence in the intervening period). · Positive for HIV, or evidence of uncontrolled Hepatitis B or C infection · Severe active infection · Women who are pregnant at the time of diagnosis will not be excluded from the trial per se. A management plan will be devised between patient, obstetrician and haematologist.
Treatment	Based on the ANZCHOG ALL Study 8 protocol, the treatment is a modified form of the BFM-2000 protocol
Safety assessments	AEs; SAEs
Other assessments	Analysis of MRD that dictates treatment course will be assessed centrally at CCIA. QoL at multiple timepoints
Statistical considerations	A two-sided 95% confidence interval (CI) will be calculated for the primary endpoint. The regimen will be considered satisfactory for ongoing study if the two-sided 95% CI is entirely above 35%. With 100 subjects recruited, the probability that the observed 95% confidence interval is entirely above 35% is greater than 80% when the true proportion commencing Protocol M on or before day 94 is greater than or equal to 50% (equivalent to the previously observed pediatric results). The sample size of 100 patients, means that based on an estimated incidence of 30 cases in Australia annually, there will be an accrual period of approximately 3 years.
Interim analysis	Nil
Laboratory studies	• Laboratory studies procedures for MRD analysis on peripheral blood and bone marrow samples (appendix 8) • Comparison of MRD analysis on blood and bone marrow samples (appendix 9) • Xenografting and Biological Studies as a component of the ALL06 trial (appendix 10 & 11) • Investigating the prevalence of druggable novel gene fusions detectable by phospho-flow analysis (appendix 12) • Monitoring asparaginase therapy with serum asparagine levels (appendix 13) • Any leftover samples of those described in appendix 5 may be used for future HREC approved research projects

4. Schema


MRD TP2 results available
All patients
Induction Protocol I
Consolidation Protocol I
Standard/medium risk (not t(4;11) or MLL/AF4 +ve, PGR, and CR at day 33)
Protocol M Days 1-14
Remaining Protocol M
Protocol 2
Maintenance
MRD TP 1 d33
MRD TP 2 d79
Medium-High riskTP2 MRD <5x10-4
Very High riskTP2
Medium-High risk and MRD-ve post prev. HR block but no sibling donor
Medium-high risk And MRD pos and no donor
High risk/very high risk (t(4;11) or MLL/AF4 +ve or no CR at day 33 or PPR)
HR1
HR2
HR3
High risk or very high Risk No sibling donor or MUD
HR4-6
Medium- High risk and MRD-ve post prev. HR block and sibling donor
High risk or Very High Risk and MRD-ve post HR1 and sibling donor or MUD
Medium-High risk MRD pos and donor
High risk or Very High Riskand sibling donor or MUD (irrespective of MRD status)
alloHCT

5. Schedule of Assessments

Table 1 Schedule of Assessments

Every attempt should be made to schedule the assessments in the table below on the specified date. If due to scheduling difficulties this cannot be done, contact the chief investigator to discuss.

Protocol	Screening	Protocol I												Between Protocols	Protocol M [Z]			HR Blocks [Z]					Protocol II					Maintenance			Allo HCT	Relapse	FU
		Induction							Consolidation									Block 1		Block 2-6 [Z]		End final Block	Phase I			Phase II								
Days from start of specific protocol	-14 to 1	1	8	15	22	27	33	35	36	43	50	57	64	65-78	1	14	56	1	7	1	7		1	8	15	36	50	1	During	End			
Informed consent	X [A, B]	X [B]																															
Registration	X [C]																																
Confirm treatment patient should receive next														X [T]		X [U]		X [AG]		X [AG]		X											
Medical History [D]	X																																
Phys. Exam. [E]	X							X					X				X					X					X			X			X
BSA calculation		X													X			X		X			X					X					
Pulse oximetry																		X		X													
Chest X-ray	X							X [S]																									
ECG	X																							X [AC]									
LVEF [R]	X [F]																							X [AC]									
FBE [G]	X	X [H]	X [H]	X [H]	X [H]	X [H]	X [H]	X [H]	X [H]	X [H]	X [H]	X [H]	X [H]		X [H]	X [H]	X [H]	X [H]	X [H]	X [H]	X [H]	X [H]	X [H]	X [H]	X [H]	X [H]	X [H]	X	X [W]	X			X
Biochemistry [I]	X	X [J]	X [J]	X [J]	X [J]	X [J]	X [J]	X [J]	X [J]	X [J]	X [J]	X [J]	X [J]		X [J]	X [J]	X [J]	X [J]	X [J]	X [J]	X [J]	X [J]	X [J]	X [J]	X [J]	X [J]	X [J]	X	X [X]	X			
Coagulation profile [K]	X	X [L]	X [L]	X [L]	X [L]	X [L]	X [L]	X [L]										X [L]	X [L]				X [L]	X [L]	X [L]								
Pregnancy Test [Y]	X																																
Fertility assessments																																	X
Serology- HIV, Hep B, Hep C	X																																

Protocol	Screening	Protocol I												Between Protocols	Protocol M [Z]			HR Blocks [Z]					Protocol II					Maintenance			Allo HCT	Relapse	FU
		Induction							Consolidation									Block 1		Block 2-6 [Z]		End final Block	Phase I			Phase II								
Days from start of specific protocol	-14 to 1	1	8	15	22	27	33	35	36	43	50	57	64	65-78	1	14	56	1	7	1	7		1	8	15	36	50	1	During	End			
BM aspirate and trephine [M]	X			X			X[N]								X			X[AD]		X[AG]		X[AG]	X					X[AD]			X[AF]	X	
Immunophenotyping blood or BM cells	X																																
Molecular Studies [O]	X																	X[AD]		X[AG]		X[AG]											
Cytogenetic analysis	X																																
CSF examination	X					X		X[P]																									
Central Lab Samples [Q]	X		X	X	X	X	X[N]								X[N]			X[N]	X	X[AG]	X	X[AG]	X		X			X[AD]			X[AF]	X	
QOL [AD]	X								X				X					X		X			X					X	X[AA]	X	X[AB]		
Adverse Event review								X					X				X					X					X		X	X			X[AE]

A = The Screening Participant Information and Consent Form must be signed prior to any screening assessments
B = The Main Participant Information and Consent Form must be signed prior to any assessments that are not standard of care at the treating site. (see section 9 for further details)
C = Registration should occur no more than 5 days prior to day 1 and will not be allowed after day 8 systemic cytotoxics have been given (see section 9 for more details)
D = full medical history includes demographics
E = Complete physical exam includes oral exam, ECOG, vitals –height (at screening only), weight, temperature, respiratory rate, blood pressure, heart rate
F = Must be performed prior to registration. Results for eligibility must be submitted at the time of registration.
G = Full blood count with differential and blood film
H = If the patient is an inpatient recommended to be collected daily, if the patient is an outpatient recommended to be collected at a minimum of three times per week or more regularly as deemed clinically appropriate by the investigator.
I = including urea, creatinine, bilirubin, albumin, uric acid, liver enzymes including LDH, lipase, amylase
J = if the patient is an inpatient recommended daily, if the patient is an outpatient recommended minimum of three times per week or more regularly as deemed appropriate by the investigator
K = APTT, INR Fibrinogen, ATIII
L = minimum twice per week during specified protocol, or more frequently as deemed clinically appropriate by the investigator.
M = for morphology
N = it is recommended that the day 33 (time point 1) bone marrow aspirate be performed as close as possible to day 33 with count recovery sufficient to ensure adequate DNA yield for MRD and assessment of morphological CR. If DNA yield is insufficient, or bone marrow is hypocellular it is recommended that the bone marrow aspirate be performed again 4 days later (or sooner if count recovery permits) to assess morphological CR and MRD. For day 79 (time point 2) the bone marrow aspirate should be performed as soon as count criteria are met for the next protocol ie to commence Day 1 protocol M or day 1 HR block 1 (see section 11.7 or 11.8.2)
O = BCR-ABL, MLL/AF4, TEL/AML1, E2A/PBX1 transcripts
P = for patients with CNS involvement at commencement of protocol I induction

Q = per appendix 5 (including further explanation in appendices 8, 9, 10, 11, 12, 13)
R = a gated heart pool scan or echo can be used to determine LVEF
S = for patients with mediatinal tumor involvement at commencement of protocol I induction
T = confirm patient's risk stratification per section 11.5 & 11.6 to confirm which treatment protocol the patient should be receiving – HR blocks or protocol M
U = confirm patient's risk stratification per section 10.2 to confirm which treatment protocol the patient should be receiving – HR blocks or protocol M. If reclassified as very high risk per timepoint 2 MRD results cease protocol M treatment post the first high dose methotrexate block and commence HR block 1
V = for patients identified early HR block should commence immediately post protocol I. For standard/medium risk patients who are re-classified as very high risk patients after timepoint 2 MRD results, they will cease protocol M treatment post the first high dose methotrexate block and commence HR block 1 ~day 94
W = every 4 weeks
X = every 12 weeks
Y = serum or urine
Z = not all patients will undertake this protocol
AA = at months 3, 6, 12 and end of maintenance
AB = should be completed prior to commencing conditioning, on discharge from hospital (~4-6 weeks after alloHCT) and 3 and 6 months after discharge from hospital for alloHCT
AC = ECG and left ventricular function assessment before 1st doxorubicin dose (day8)
AD = prior to treatment commencing
AE = late emerging treatment related toxicities
AF = should be completed prior to commencing conditioning, on discharge from hospital (~4-6 weeks after alloHCT) and 3 months after discharge from hospital for alloHCT
AG = after count recovery 4-5 weeks after day 1 prior to the next HR block day 1 treatment

6. Introduction

Acute lymphoblastic leukaemia (ALL) is a neoplasm of B or T cell lymphoid precursors originating in the bone marrow or thymus respectively. Two-thirds of cases occur in children or adolescents. In children and young adolescents, treatment programs, originally developed in the 1960s and extensively revised over the past 3 decades, now produce excellent results, with cure rates of approximately 80%, and indications that, with further protocol refinements, an overall cure rate that may approach 90% [1]. In contrast, the results of treatment for adults are substantially poorer, with most large series reporting cure rates less than 40%[1]. The reasons for this are complex and incompletely understood.

The therapy of pediatric ALL is increasingly individualized. Clinical and biological features present at diagnosis together with markers of treatment response can be used to stratify patients into groups at low, intermediate or high risk of relapse. Children at low risk of relapse have an excellent outcome with conventional chemotherapy, with survival approaching 90%. Intensified treatment of high risk patients, defined by unfavourable biological features and/or a poor response to treatment, can improve survival from ≤30% to around 60-70%. Markers of treatment response known to have prognostic significance include response to prednisolone as a single agent in the first week of treatment, achieving a morphological bone marrow remission at the end of induction chemotherapy, and using sensitive measures of minimal residual disease (MRD) detection after achieving a remission. Quantitative polymerase chain reaction (PCR) or flow cytometric techniques can detect small numbers of residual leukemic cells surviving in the bone marrow after initial treatment[2,3].

All major pediatric oncology groups use risk and response adapted treatment protocols for children with ALL, in particular the BFM protocol, developed by the Berlin-Frankfurt-Munster group in Germany[4]. In contrast, there is no standard approach to the treatment of ALL in older adolescents and in young adults. A variety of treatment protocols have been reported, but the outcome for treatment of ALL in adults appears to be substantially inferior to that in children. The reasons for this may include different disease biology in older patients, poorer tolerance of therapy, inferior treatment protocols, less physician experience, poorer treatment compliance, or combinations of these factors. There is good evidence that, in general terms, ALL in adults is biologically different to that seen in children. There is a much higher incidence of poor-risk cytogenetic abnormalities in adults compared to children, particularly the t(9;22) abnormality (Philadelphia chromosome)[5]. There is a correspondingly lower incidence of good prognosis cytogenetic changes in adults, such as the t(12;21) translocation, and hyperdiploidy[6,7]. However, it has been unclear as to whether these inherent differences in the leukemic cells of adults with ALL are sufficient to account for the markedly different outcomes of therapy for adults compared to children. There is also little information about the biological differences in ALL in late adolescence compared with pediatric or adult cases. Thus, it remains unclear whether the inferior outcomes seen in adult ALL reflect fundamental biological differences in disease between age groups or are the results of inferior treatment intensity or delivery in olde r age groups. Variables that could potentially affect the latter include protocol design (the type of treatment) and adherence and compliance with protocols by physicians and patients (delivery of treatment).

In recent years, seven different retrospective studies have documented inferior results for late adolescents and young adults with ALL treated by adult physicians compared to pediatricians[8-14]. These studies are summarized in Table 1:

TABLE 2

STUDY	PATIENTS	% CR	% EVENT-FREE SURVIVAL AT 5 YEARS
USA	Age 16-21		
CCG 1882	196	96	64
CALGB 8811	103	93	38

FRANCE	Age 15-20		
FRALLE-93	77	94	67
LALA-94	100	83	41
HOLLAND	Age 15-20		
DCOG-ALL	47	98	69
HOVON	44	91	34
ITALY	Age 14-18		
AIEOP	153	94	83
GIMEMA	95	95	55
SWEDEN	Age 15-18/20		
NOPHO-92	36	NR	74
SAALL 94-2000	23	NR	39
DENMARK	Age 10-14/15-19		
Nordic ALL 1992	61	NR	60
Adult	38	NR	38
UK	Age 15-17		
UK MRC ALL97	61	NR	65
UKALL XII	67	NR	49

These results indicate that the type of treatment and its delivery may be very important in determining outcomes for patients in this age group. Pediatric protocols are more dose intensive than adult protocols, particularly with regard to dosing of vinca alkaloids, corticosteroids, L-asparaginase and methotrexate. While the above studies would suggest that such protocols can be effectively administered to older adolescents by experienced Pediatric Oncologists, it remains unclear whether similar results could be achieved by adult physicians using similar protocols in an older age group. In Australia, ALL patients older than 14-15 years must be treated in adult hospitals, usually by clinical hematologists. Treatment delivery in adult centres could be impaired by less strict adhesion to ALL treatment protocols, greater concerns about toxicity in the older patient, and less supportive hospital environments, potentially contributing to the poorer outcomes seen in late adolescent and younger adult (AYA) patients with ALL. Compliance issues, unique to this patient cohort, may also be a significant factor in impairing dose delivery in AYA ALL. Each of these factors, either alone or in combination, could reduce the effectiveness of chemotherapy by impairing dose delivery. Whether differences in dose delivery have a substantial impact in AYA ALL could be assessed by using an accepted pediatric ALL protocol, with known outcomes, in older patients and comparing differences in dose timing and intensity between age groups.

These issues have been examined in 2 prospective clinical trials conducted in Spain and France. In the PETHEMA ALL-96 trial, 81 ALL patients aged 15 to 30 years were treated with an intensive pediatric protocol, with a high remission rate (98%) and event-free survival at 6 years of 61%.[15] Toxicities observed were mainly hematologic. 68% patients developed grade 3 to 4 neutropenia and 44% developed grade 3 to 4 thrombocytopenia during induction. 25% received a delayed first consolidation course due to induction toxicity. Grade 3-4 hepatoxicity occurred in 8% of induction cycles. No severe mucositis or neurotoxicity was noted. Second consolidation courses were delayed or modified in 16% of cases due to toxicity in the previous consolidation course. Delays or modifications in consolidation courses were more common in young adults (aged 18-30 years) than adolescents (aged 15-18 years) as was the rate of grade 1 infection (13 vs 1, p=0.007). Dose reduction or modifications of asparaginase or vincristine during reinduction cycles occurred in 19% of cycles and were more common in young adults than adolescents (33% vs 15%, p=0.03), though the median interval from onset of CR to beginning of maintenance was no different between young adults (136 days) or adolescents (143 days). Toxic deaths were uncommon with 1 patient dying in induction from sepsis, 1 poorly responding patient dying during salvage therapy and one patient dying in remission during consolidation.

The French GRAALL-2003 trial enrolled 225 patients between 15 and 60 years over 3 years onto "pediatric-style" protocols. The remission rate was 93.5%, and event-free survival 55%.[16] Although relapse rates were not different, higher rates of toxicity and treatment-related deaths were seen in older patients, resulting in poorer event-free survival in those aged over45 years. Induction mortality (13% vs

4%, p=0.02) and cumulative incidence of death in CR1 were higher in the older age group (15% vs 2%, p=0.0001) with causes of death in first CR recorded as transplant related mortality (9 pts), sepsis (5 pts), secondary AML (2 pts), CNS hemorrhage (1), thromboembolic event (1) and sudden death unrelated to ALL treatment (1). Patients over45 years did not tolerate planned L-asparaginase dosing as well as younger patients leading to lower cumulative dosing in this group (29,000 U/m2 vs 81,000 U/m2). As a result, times for commencement of the first and fourth consolidation courses were significantly prolonged for patients over45 years when compared to younger patients (44 vs 39 days, p=0.03 and 95 vs 89 days, p=0.03). The most frequent grade 3-4 toxicity was hepatic, with thromboembolism, intolerance to L-asparaginase and peripheral neuropathy also observed in a small number of patients, with no difference seen between the rates of serious toxicity between older and younger patients.
The PETHEMA group trial made no direct comparison of treatment delivery or outcomes to the pediatric cohort receiving the same protocol, and in the GRAALL-2003 trial, treatment was sufficiently modified to make direct comparison to a pediatric cohort difficult. Hence no trial has yet reported direct comparisons of treatment delivery between paediatric and AYA ALL patients using the same treatment protocol. We hypothesize that the use of a pediatric protocol for ALL should be deliverable in a comparable time frame for AYA patients as for young children, and propose to formally test this in a cohort of AYA ALL patients. If this hypothesis is shown to be correct, then the differences in outcome between pediatric and AYA ALL must have other explanations. If incorrect, the factors leading to impaired dose delivery could be explored.

Both of the above studies confirmed that older adolescents and young adults can be treated with pediatric or pediatric –based protocols by adult physicians, with results that were superior in both trials to those previously obtained using adult ALL protocols in patients <45 years. The inferior results previously reported in adult ALL must be at least partly due to the use of treatment regimens with inferior efficacy compared to those used in the treatment of pediatric patients with ALL. However, in both studies, event-free survival rates were still worse than those reported in the 0-15 age group, with a relapse rate of 25% in the 15-45 year group observed in the GRAALL-2003 study.[16] This, together with the lower remission rate seen in this trial, suggest that there is a higher proportion of cases with inherent drug resistance in the AYA ALL population, and indicates a need for more intensive treatment in these high risk cases.

As discussed above, detection of residual disease by molecular or flow cytometry techniques provides a surrogate for inherent or early acquired drug resistance in ALL, and can identify patients at higher risk of relapse[2,3]. Several studies in adults with ALL have confirmed the prognostic significance of MRD detection demonstrated in pediatric patients, but also showed higher proportions of patients with detectable disease than in pediatric studies. In the GRAALL-2003 trial, a subset of 127 patients underwent MRD testing by PCR at the end of induction therapy. Using a very high cut-off of a < 2 log reduction in leukem ia cells, 15 patients were MRD-positive; however, the outcome of these patients was not described[16]. It is not known what proportion of patients in this study would have been classified as having hig h risk MRD using more accepted levels of $>10^{-4}$. Bruggemann and colleagues studied 196 adults with standard risk ALL enrolled on the GMALL 06/99 trial for MRD using molecular techniques.[17] MRD was detected in 63% of patients at day 24 at the end of induction therapy, and in 39% at week 11 at the end of consolidation treatment; detection of MRD was the only significant variable that affected outcome in this otherwise “standard” risk cohort. However, it is not clear whether a lower rate of MRD positivity would result from the use of a more intensive pediatric protocol in adult ALL patients. In a small Australian cohort of 38 patients aged 15 to 30 years treated off-study with the ANZCHOG Study 8 protocol, or closely related BFM protocols, the rates of MRD positivity by PCR at days 33 and 78 were 60% and 30% respectively, possibly comparable to those observed in the GMALL trial (R. Sutton, unpublished data).However, prospective data on a larger number of uniformly treated patients is needed to clarify this issue, and we propose to gather further information about MRD levels in the AYA ALL population using the ANZCHOG Study 8 protocol during this trial, and compare the data with that previously obtained on pediatric patients.

In the ANZCHOG Study 8, 20% of children were classified as standard risk (based on lack on high risk pre-treatment factors and MRD negativity at both post-treatment time points), 60% as medium risk (lack of high risk factors, with MRD positivity at the day 35 timepoint and negative at day 78), 5% high risk, and 15% very high risk. In the AYA cohort monitored by the Sutton laboratory, using the same criteria, 4 were standard risk, 7 were “standard-medium” (negative by one informative marker only), 24 were medium risk and 2 high risk.

In the GMALL trial, patients with MRD positivity $>10^{-4}$ at the end of induction course 1 on day 24 had a DFS of only 38%, significantly worse than the 69% DFS in those with lower or undetectable MRD levels. However, those who subsequently were MRD negative, or who had levels $< 10^{-4}$, at a week 16 timepoint, had an intermediate prognosis, with 53% DFS. It is unclear from this study whether the outcome of the latter group could be further divided on the basis of MRD negativity versus positivity below the threshold level at the later timepoint.

While retrospective results from the MRC UKALL XII trial suggest that residual MRD positivity following initial therapy for ALL does not adversely affect outcome for patients undergoing allogeneic HCT in first CR[18], overall, it is unclear whether outcomes for patients treated using "paediatric-style" protocols and at high risk of relapse, based on MRD positivity, can be improved by early intensification of treatment with "salvage" chemotherapy and/or allogeneic HCT.Adult ALL protocols have not utilised intensified "high-risk" blocks of chemotherapy to eradicate MRD, and until recently allogeneic HCT has been used only in adult patients with high risk ALL in CR1 or on relapse, with only moderate results. However, results from the UK ALL XII trial suggest an overall survival benefit for "standard risk" ALL patients transplanted using a matched sibling donor in CR1, though overall outcomes of this trial would be considered poor, with a 5 yr OS of only 43%, suggesting that chemotherapy may have been "inadequate". In addition, reports from UKALL XII and other studies have suggested that the poor prognosis of MRD positive patients treated using adult ALL protocols may be overcome by alloHCT[18-20]. In contrast, Uzunel et al reported 0% vs 50% relapse post allogeneic HCT for adult ALL patients who were MRD negative versus MRD positive pre-transplant, though small numbers limited the significance of these findings ($p=0.05$)[21]. Similarly, reports from paediatric groups consistently highlight the importance of MRD negativity in predicting outcome following allogeneic HCT. Knechtli et al reported the outcomes of 56 pts aged <18 yrs transplanted in CR1 or subsequent CR. MRD status pre-transplant was highly predictive of EFS, with positive versus negative MRD having 2 yr EFS of 17% versus 73% ($p<0.001$) respectively, with only MRD status significantly predicting outcome in this cohort[22]. Bader et al reported similar results in an update of this cohort with high level MRD ($>10^{-3}$), low level MRD and negative MRD associated with 5 yr EFS of 23%, 48% and 78% respectively[23]. Similar outcomes have been reported by other groups and highlight the prognostic significance of achieving MRD negativity in paediatric patients at high risk of relapse prior to allogeneic transplantation, in contrast to the reported outcomes for older MRD positive patients transplanted following treatment with adult ALL protocols in CR1[24,25]. We therefore consider that it may be very important for AYA ALL patients treated on a pediatric protocol and requiring allogeneic HCT, to be MRD negative prior to HCT.

Cyclical high risk based intensification therapy appears to be associated with efficient and progressive clearance of MRD for patients treated on ANZCHOG Study 8 protocol (R Sutton, personal communication). Hence we propose that medium-high risk patients by MRD criteria be treated with a 2-3 HR blocks, with MRD assessment after the completion of each block (11.7). Patients who become MRD negative after this intensification therapy will then proceed to allogeneic BMT if a matched sibling donor exists. For other patients lacking a suitable sibling donor, MRD negativity following HR intensification may still be associated with a satisfactory outcome, with GMALL 06/99 data suggesting 3 yr DFS 53.2% and OS 69.8% in those achieving MRD negativity at a "late" (ie: week 16) time point when treated with chemotherapy alone. Hence we would propose that such patients lacking a suitable sibling donor should resume the ANZCHOG Study 8 protocol, with re-induction with Protocol 2 followed by maintenance therapy (Section 11.10 and Section 11.11.)

Persistence of detectable MRD at late time points is associated with a poor outcome in pediatric and adult ALL. While the role of "salvage" allogeneic transplant for AYA patients in CR1 transplanted for residual MRD remains unclear, 3-5 yr EFS of 23-48% for paediatric patients transplanted with detectable MRD in CR1 and subsequent CR would suggest a superior outcome for allogeneic HCT over chemotherapy for such patients [21,24,25,26]. Therefore, given the poor prognosis associated with late MRD positivity, we suggest that medium-high risk patients who remain MRD positive at any level following 2-3 HR intensification blocks should be offered an allogeneic transplant with matched sibling or unrelated donor, or completion of intensified therapy with a total of 6 HR blocks if no donor is available, with the goal of reducing residual MRD to as low a level as possible. Such a strategy will allow us to assess the effectiveness of allogeneic HCT in AYA ALL, but limited only to those deemed at high risk of relapse based on residual

MRD positivity, as well as to assess the utility of an intensified chemotherapy-only approach in those patients considered to have poor prognosis disease based on MRD status, but who lack a suitable allogeneic donor. Since the quality of life impacts of this protocol are likely to be significant, and since they have not been documented previously, quality of life will be assessed as a secondary outcome.

The **objective of this study** is therefore to establish whether a BFM-based pediatric ALL protocol can be given effectively to patients aged 15-40 years and to examine factors that might impair dose delivery. The protocol chosen is based on BFM 2000, as used in the ANZCHOG Study 8, which will allow a direct comparison of dose delivery and MRD positivity with an equivalent pediatric cohort. Treatment stratification based on identical risk factors, including MRD assessment, will be used to allocate high risk patients to intensive chemotherapy or allogeneic HCT. The upper age limit has been arbitrarily chosen as 40 years, as toxicity and treatment delays were much more frequent in patients over 45 in the GRAALL-2003 study. Since the majority of ALL patients in both ANZCHOG Study 8 and the unpublished AYA ALL cohort fell into the medium risk group, we propose that MRD negativity at timepoint 2 may be an indicator of better prognosis, and will use this as a criterion for sub-dividing this risk group. Thus, the following risk classification and treatment allocation system will be used in this trial:

Risk Group	Clinical Features	MRD TP1	MRD TP2	Treatment
Standard Risk	Standard	neg	neg	Standard chemotherapy
Medium Risk	Standard	pos	neg	Standard chemotherapy
Medium -High Risk	Standard	pos	Pos. $< 5 \times 10^{-4}$	2-3X HR blocks, then if MRD neg, MSD alloHCT, or Protocol II chemotherapy if no donor, or if MRD+ alt donor HCT or 6XHR.[i]
High Risk	High	pos	Pos. $< 5 \times 10^{-4}$	2-3 X HR blocks if MRD neg and donor then alloHCT, if still MRD pos after HR3 and donor then alloHCT, otherwise 4-6 HR blocks.[i]
Very High Risk	High	pos	Pos. $\geq 5 \times 10^{-4}$	2-3 X HR blocks if MRD neg and donor then alloHCT, if still MRD pos after HR3 and donor then alloHCT, otherwise 4-6 HR blocks.[i]

Published information on long-term side effects of BFM protocols in the AYA ALL population is lacking and the impact of high-risk treatment on ovarian and testicular function in this age group is unknown. Fertility assessments conducted on trial participants at the end of treatment may assist in providing data on this issue.

[i]Some patients who are ultimately classified as very High risk may need to switch from standard chemotherapy to HR Block after the result from TP2 is obtained.

7. Study objectives and hypotheses

7.1 Hypothesis

That intensive pediatric treatment protocols, such as those based on BFM-95, will be tolerated by late adolescent and young adult (AYA) ALL patients, and will be more effective in curing these patients than the existing adult-based protocols used by Hematologists.

Objectives:

7.2 Primary objective

The primary objective of this study is to determine whether a modified form of the BFM-2000 protocol can be administered to patients with newly diagnosed and untreated ALL aged between 15 and 40 years in a comparable timeframe to patients under 15 years of age.

7.3 Secondary objectives

- To evaluate the complete remission (CR) rates, disease-free survival (DFS) and overall survival (OS) of patients treated on this protocol and to compare with results reported in a similar age group using protocols designed for use in adult patients.
- To evaluate toxicity, both hematologic and non-hematologic.
- To compare the proportions of AYA patients with standard, medium, and high risk disease to pediatric ALL, and to evaluate outcomes within these risk groups.
- To assess the level of residual disease after induction chemotherapy and compare this with levels reported in a similar age group using adult protocols.
- To conduct correlative laboratory scientific studies on blood and bone marrow samples taken from patients enrolled on this study
- To assess the impact of treatment on physical, functional, emotional and social wellbeing via HRQOL assessment at the beginning and end of each phase of treatment.
- To provide an indication of fertility status after treatment

8. Study population

8.1 Inclusion criteria

All of the following criteria must be satisfied for registration on the study.

1. A morphological diagnosis of ALL by WHO criteria, confirmed by immunophenotyping and cytogenetics. All clinico-pathological subtypes will be eligible, except for mature B or Burkitt ALL (L3).
2. Has provided written, informed consent
3. Available for follow up for at least 3 years
4. Males capable of parenting a child and women of childbearing potential must be using a medically acceptable and adequate method of contraception while undergoing protocol treatment and for 28 days following the last dose of protocol treatment. Note: due to a potentially increased risk of thrombosis in asparaginase containing regimens, cessation of the combined oral contraceptive in female patients should be considered and an alternative medically appropriate form of contraception be instituted.
5. Bone marrow blast count ≥20%
6. Adequate renal and hepatic function at Screening as defined by:
    a. Total bilirubin <2.5 x ULN unless medically correctable
    b. Serum creatinine ≤200 micromol/L unless medically correctable
7. Normal left ventricular ejection fraction, according to institutional criteria. If the clinical circumstances require that treatment must be given urgently before this can be ascertained, the absence of clinical cardiac impairment is acceptable, provided that a normal left ventricular ejection fraction is confirmed prior to the first consolidation cycle.
8. An ECOG performance status score of 0-3 at Screening - see Appendix 1

8.2 Exclusion criteria

Presence of any of the following criteria will exclude the subject from registration on the study.

1. Subjects aged less than 15 or more than 40 years at Screening
2. Patients known to have Philadelphia chromosome-positive disease
3. Presence of serious cardiac, pulmonary, hepatic or renal disease.
4. Previous treatment for ALL or history of cancer (other than basal cell skin cancer or carcinoma of the cervix in situ, or other localised cancer treated by surgical excision only more than 5 years earlier without evidence of recurrence in the intervening period).
5. Contraindication to the use of the study drugs.
6. Positive for HIV, or evidence of uncontrolled Hepatitis B or C infection
7. Severe active infection
8. Has any other clinically important abnormalities as determined by the investigator that may interfere with his or her participation in or compliance with the study
9. Presence of any psychological, familial, sociological or geographical condition potentially hampering compliance with the study protocol and follow-up schedule. This condition must be discussed with the patient prior to signing consent and registration in the trial.

Pregnancy-Women who are pregnant at the time of diagnosis will not be excluded from the trial per se. The specific circumstances will require discussion between the patient, the hematologist responsible for her

care, and the attending obstetrician. The management plan should then be discussed with one of the study

PI's. Options may include early termination, induced delivery, or commencement of antileukemic chemotherapy.

9. Registration

9.1 General guidelines

The ALL06 screening participant information and consent form allows for the collection of the diagnostic samples for central analysis (per appendix 5) and completion of study specific assessments (baseline QOL) prior to the patient's diagnosis being known. This will prevent the need to re- biopsy the patient to allow them on trial. Once the patient's diagnosis has been confirmed, samples will be shipped to the central laboratory for analysis. If the patient is not eligible per inclusion criteria 1 the samples will not be sent. Written informed consent must be obtained from each patient prior to performing screening procedures that are not part of standard of care.

After diagnosis is confirmed, consent must be obtained from all subjects using the main PICF. The nature of the study and the potential risks will be explained to all candidates.

9.2 Prior to commencing Treatment

Before the patient can commence day 1 treatment of prednisolone and IT MTX, the investigator should ensure that all of the following requirements are met:

- The patient has signed and dated the screening PICF
- All Screening assessments and investigations have been performed.
    - the only exception being that extenuating circumstances prevent the gated heart pool scan or echo pre-treatment to assess cardiac function– in this instance the test must be completed and results known prior to registration to confirm eligibility
- Blood and marrow samples (per appendix 5) are mandatory for study entry and must be collected before any treatment is commenced.
- All of the required samples have been sent or are in the process of being shipped to the central laboratories for MRD testing and laboratory studies.

If the investigator feels that the patient must commence treatment immediately, (prior to receiving the final screening test results), treatment with prednisolone & IT MTX can commence prior to registration if:

- the treatment specified in this protocol for day 1 to day 7 is standard of care at the treating hospital.
- the patients' eligibility is confirmed and the patient is registered prior to day 8 of treatment.

The day the prednisolone is commenced is regarded as day 1 of the protocol. Registrations will not be accepted once day 8 systemic chemotherapy has commenced.

9.3 Registration process to be followed by Trial site

Prior to patient registration, the investigator should ensure that all of the following requirements are met in addition to those specified above for commencing treatment:

- The patient has signed and dated the main PICF

- The patient has met all inclusion criteria and none of the exclusion criteria applied. The eligibility checklist should have been completed, signed and dated by the site investigator.
- The patient must be registered prior to day 8 systemic chemotherapy being commenced to be included on the ALL06 study.

An adequately qualified and authorised member of the research support team at the Trial Site must complete the relevant CRFs.

To register a patient, fax the Request for Registration to the Trial Coordinating Centre, on +61 3 9429 8277. The required CRFs and source data must be submitted to the TCC at the time of registration. The patient's eligibility will be rechecked and any queries will be dealt with by phone or email. Once enrolment of a patient to the trial has been approved, the TCC will notify the site of the patient registration number.

Patients registered prior to receiving treatment should commence day 1 within 3-5 days. Issues that may cause treatment delays should be initially discussed with the TCC and, if necessary, with the chief investigator. If a patient does not receive protocol therapy following registration, the TCC must be notified as soon as possible.

For any queries contact the Trial Coordinating Centre (TCC).

10.Investigational plan

10.1 Study design

The study Schema is outlined in Section 4

All patients will commence treatment with Protocol 1 which is an intensive chemotherapy induction protocol. Various prognostic factors such as cytogenetic abnormalities, initial response to prednisolone, achievement of remission, and MRD levels after induction and consolidation, will determine whether patients continue treatment outlined in Protocol M, or are to be treated on the High Risk protocol, or undergo allogeneic HCT. Patients removed for allogeneic HCT will be withdrawn from the study treatment, but will continue to be followed, with relapse, disease-free and overall survival data being recorded.

10.2 Definition of Risk Groups

Patients who are precursor B lineage, have a poor prednisolone response on day 8 (peripheral blood blast count $\geq 1.0 \times 10^9$/L) and are MRD negative at time point 2 (~day 79) will not meet the criteria for any of the below specified risk groups. In this instance the study doctor should contact the chief investigator to discuss the patient's risk classification and further treatment.

10.2.1Standard Risk

Must meet all of the following criteria:

- Prednisolone Good Response on Day 8 (blast count less than 1.0×10^9/L in peripheral blood after 7 days of prednisolone and one intrathecal injection of Methotrexate on day 1).
- Negative for t(4;11) and MLL/AF4
- CR at time point 1 (day 33) marrow biopsy per protocol section 14.0
- Either precursor-B or T lineage

  MRD: must be negative on time point 1 (day 33) and at time point 2 (day 79), measured with a sensitivity of $\leq 10^{-4}$.

Please note: Normal marrow cellularity is not required for assessment of time point 1 (day 33) CR.

10.2.2Medium Risk

For this trial, the Medium Risk group will be further divided on the basis of MRD result at Timepoint 2, with patients with negative results at this timepoint remaining as medium risk, while those with MRD positivity, but $<5x\ 10^{-4}$, being classified as Medium-High risk, and those with MRD positivity $\geq 5x10^{-4}$ being classified as very high risk.

Therefore in order to fulfil requirements for the medium risk group, the following criteria must be met:

- Prednisolone Good response on Day 8 (blast count less than 1.0 $x10^9$/L in peripheral blood after 7 days of prednisolone and one intrathecal injection of Methotrexate on day 1)
- Negative for t(4;11) and MLL/AF4
- CR at time point 1 (day 33) bone marrow biopsy
- Either precursor-B, including pro-B, or T lineage
- MRD criteria not fulfilled for standard risk, high risk, or very high risk, or not tested/no result. MRD will be positive at time point 1 (day 33) but must be negative at time point 2 (day 79) to remain in the medium risk group.
- Note: extramedullary disease at diagnosis does not exclude patients from this group.

10.2.3Medium-High Risk

In order to fulfil requirements for the medium-high risk group, the following criteria must be met:

- Prednisolone Good response on Day 8 (blast count less than 1.0 $x10^9$/L in peripheral blood after 7 days of prednisolone and one intrathecal injection of Methotrexate on day 1)
- Negative for t(4;11) and MLL/AF4
- CR at time point 1 (day 33) bone marrow biopsy
- Either precursor-B or T lineage
- MRD criteria not fulfilled for standard risk, high risk, or very high risk, or not tested/no result. MRD positive at time point 1 (day 33) and time point 2 (day 79) but must be $<5x\ 10^{-4}$.
- Note: extramedullary disease at diagnosis does not exclude patients from this group.

10.2.4High Risk

Must meet all of the following criteria:

- Prednisolone Poor Response (peripheral blood blast count $\geq$ 1.0 $x10^9$/L on day 8).
- Patient must be Pre-B phenotype (CD10+ CD19+) and have WCC $<100x\ 10^9$/L, not be T lineage or pro-B cell (CD10- CD19+) phenotype, negative for t(4;11) and MLL/AF4.
- Time point 1 (day 33) bone marrow biopsy in CR
- MRD positive but $<5x10^{-4}$ at timepoint 2 (day 79) or unknown.

10.2.5Very High Risk

Any one or more of the following criteria:

- MRD: $\geq 5x10^{-4}$ at timepoint 2.
- Prednisolone Poor Response and either T cell, Pro-B, or WCC $\geq 100\ x10^{9}/L$
- Positive for t(4;11) or MLL/AF4
- Time point 1 (day 33) bone marrow biopsy not in CR (see section 14 for definition ie ≥5% leukaemic blasts).

10.3 Expected duration

Accrual is expected to take approximately 3 years, at a rate of 30 new accruals per year. Participants are expected to be 'on study' for 24 months. All trial participants will be followed until 2 years after the last patient is registered onto the study.

11.Study Treatment

The study schema is in Section 4

11.1 Doses and side effects of Chemotherapeutic Agents

The intravenous and oral dosages are determined on the basis of body surface area calculations of the patient. This must be recalculated before each phase of treatment and doses adjusted accordingly (Protocol I; Protocol M; Protocol II, before each High Risk Block and before start of Maintenance phase).

Common side effects of drugs used in this protocol can be found in Appendix 3.

11.2 Asparaginase therapy guidelines for all protocols

Asparaginase is administered during protocol I induction, HR blocks and protocol II phase I, intramuscularly or intravenously without premedication. Pre-medications may mask development of systemic allergy associated with the development of asparaginase antibodies that render the drug ineffective.

Asparaginase must be the Pegylated preparation known as Oncaspar, available under SAS Category A from Link Pharmaceutical, at a dose of 1,000U/m2.

11.2.1 Guidelines for supportive care during Asparaginase therapy

Pegylated asparaginase therapy may be associated with a risk of thrombosis and bleeding in this population. Given this, a suggested approach for patients enrolled on ALL06 is detailed below. (Additional information and other supportive care guidelines for asparaginase therapy are detailed in Appendix 4.)

Thromboprophylaxis

Unless contraindicated, prophylactic **enoxaparin** (40mg SC daily) is strongly recommended for all patients during protocol I induction, and to continue till commencement protocol I consolidation to prevent VTE complications. It is also recommended during all inpatient admissions. For female patients, cessation of the combined oral contraceptive at diagnosis should be considered due to an increased risk of thrombosis in asparaginase containing regimens and alternative medically appropriate contraceptive prophylaxis be instituted.

Please refer to local departmental guidelines regarding prophylactic anticoagulation but the following approach is recommended

1. Prophylactic enoxaparin may be commenced 6 hours after the diagnostic lumbar puncture on day 1 during protocol I induction following commencement of prednisolone assuming it is clinically safe to do so and prior to the insertion of any central venous catheter
2. Withold prophylactic enoxaparin if platelets < 20 x 10^9/L (transfuse platelets and recommence enoxaparin ASAP)
3. Withhold prophylactic enoxaparin for bleeding complications
4. Withold prophylactic enoxaparin the evening prior (and for at least 12hours prior) to lumbar puncture
5. Continue enoxaparin till end of protocol I induction

Please refer to local guidelines regarding anticoagulation management but a suggested approach in the event of VTE is that 2 doses (and at least 24 hours) of therapeutic enoxaparin be withheld prior to lumbar puncture (ie the everning before and morning of LP) with post lumbar puncture enozaparin dose to be given no sooner than 6 hours post procedure

Prophylactic Management of Coagnulopathy, AT3 Deficiency

Assess PT, APTT, fibrinogen, AT3 twice weekly (more frequently if required) in protocol I induction and continue till commencement protocol I consolidation (and in all asparaginase based phases of treatment). Doses of asparaginase should not be withheld for laboratory abnormalities without clinical symptoms.

- avoid FFP for asymptomatic coagulopathy
- conservative cryoprecipitate infusions to maintain fibrinogen > 1g/L
- AT3 (Thrombotrol) infusions to maintain AT3 levels >60% (each infusion dosed to achieve $\geq$ 100% AT3 activity)

If a thrombohaemorrhagic complication develops in association with asparaginase therapy a suggested approach to management is outlined in Appendix 4.

For dose modifications and management of other toxicities for Asparaginase see appendix 4

11.3 Protocol I

All Risk Groups will receive protocol I. Figure 1 shows the plan for protocol I induction therapy.

Timing of treatment during this phase is important so that it should only be modified in exceptional cases. The absolute neutrophil count should not fall below 0.1 x 10^9/L, if possible. G-CSF may be given at the discretion of the treating physician during Phase 1.

Enoxaparin 40mg SC daily is strongly recommended for thromboprophylaxis during protocol I induction and to continue till commencement protocol I consolidation.

For dose modification and management of toxicities during protocol I refer to Appendix 4.

11.3.1Therapy: Protocol I – Induction, Days 1 - 35:

In the event that there is an excessive increase in the peripheral blast count during the administration of day 1-7 prednisolone, or due to clinical imperative, the administration of systemic cytotoxics prior to day 8 may be permitted after consultation with the Chief Investigator. Patients who require systemic cytotoxic chemotherapy prior to day 8 will be regarded as Prednisolone Poor Responders (PPR's) for the purposes of risk stratification. An increase in the WBC during initial prednisolone therapy is not in itself an indication for commencement of systemic cytotoxics unless the patient is clinically compromised.

i. IT MTX : 12mg intrathecal Methotrexate on days 1, 15, 33.

A lumbar puncture should be collected during screening or on day 1 prior to commencing treatment with prednisolone to determine CNS involvement. This is to minimize the risk of prednisolone eliminating any CSF blasts falsely excluding CNS disease. If the investigator wishes to treat the patient with prednisolone prior to the LP & IT-MTX administration they must discuss with the chief investigator. In these cases, IT MTX should be given as soon as possible after commencement of protocol I and by day 5 to maximize the impact of IT MTX on day 8 prednisolone response.

For initial CNS involvement or if unequivocal blasts are found in CSF, but cell count < 5/µl, additional IT MTX is administered on Days 18 and 27.

Traumatic lumbar puncture: For traumatic lumbar punctures at diagnosis which contain blasts on microscopy/cytocentrifuge, administer the additional intrathecal Methotrexate on Days 18 and 27.
To minimize traumatic lumbar puncture at diagnosis, consider ensuring that the platelet count is ≥ 50 x 10^9/L.

ii. PRED (Prednisolone): 60mg/m2 /day PO in 2 divided doses per day.

Days 1-7: It is recommended that therapy begins at full dose. In some situations (patients with high tumour burden or existing evidence of tumour lysis or pre-existing renal impairment), some consultants may wish to begin prednisolone therapy at a lower dose but this should be rapidly escalated to full dose. The use of Rasburicase should be considered in high risk patients (WBC >100 x 10^9/L and/or LDH >2 x ULN). The following is a guideline for rapid escalation of prednisolone to full dose.

Begin with about 25% of the calculated dose and increase rapidly, depending on the clinical response to therapy (reduction in cell count/organ size), laboratory results (urea, creatinine, uric acid, electrolytes, phosphate) and urine output, to the full dosage of 60mg/m^2/day (eg. daily increases to 50-75-100% of the full dose). This should be reached by Day 5 of the therapy at the latest. The total prednisolone dose in the first 7 days should be greater than 210mg/m^2.

Days 8 – 28: Prednisolone 60mg/m^2/day PO in 3 divided doses.

Days 29 – 38: Prednisolone taper in 3 stages every 3 days. Reduce dosage by half at each stage.

iii. VCR (Vincristine): 1.5mg/m2/day I.V. bolus (maximum single dose 2mg) Days 8, 16, 22 and 29.

TO PREVENT accidental administration of intrathecal vincristine, either or both of the following precautions must be taken: It must either NOT be administered:

- on the same day as intrathecal therapy or
- in the same room as concurrent intrathecal therapy.

iv. DNR (Daunorubicin): Daunorubicin 30mg/m^2 IV over 1 hr Days 8, 16, 22, 29.

v. PEGYLATED Asparaginase (LINK): 1,000U/m^2 IM or IV on Days 8 and 22.

All patients should continue treatment with the next phase of protocol I.

11.3.2Therapy: Protocol I – Consolidation, Days 36 - 64:

Guidelines for starting protocol I consolidation.:

- for those with initial CNS involvement, CR in the CNS.
- for those with initial mediastinal tumour, reduction of mass to <30% of original size.
- good general condition with no serious infections
- creatinine within upper normal limits according to age.

These are guidelines only and if the patient does not meet these criteria the investigator should discuss this with the CI before proceeding.

Ideally the bone marrow collected at time point 1 should be performed when there is adequate DNA yield and as close as possible to day 33.

If the bone marrow biopsy shows CR at time point 1 (day 33), as defined by section 14, the following hematological criteria should be met, unsupported by G-CSF for at least 48 hours, prior to the commencement of protocol I consolidation treatment.

- WCC $\geq 2.0 \times 10^9$/L
- neutrophils $\geq 0.5 \times 10^9$/L
- platelets $\geq 50 \times 10^9$/L

If the patient is not in morphological CR at time point 1 (day 33), as defined in section 14 ie ≥5% leukaemic blasts in the bone marrow biopsy, proceed with therapy without waiting for hematological recovery.

i. ARA-C (Cytarabine): 75mg/m^2/day IV or SC in 4 x 4 day blocks.

Days 36, 37, 38, 39; Days 43, 44, 45, 46; Days 50, 51, 52, 53; Days 57, 58, 59, 60.

Guidelines for beginning Cytarabine blocks 2-4 (unless indicated otherwise):

- white cell count $\geq 0.5 \times 10^9$/L

- platelets $\geq 30 \times 10^9$/L.

The Cytarabine blocks should not be interrupted, if possible. It is recommended that if the above WCC and platelet levels are not achieved on time after cytarabine blocks 1-3, that the next block be delayed by 1 week, and that if the delay is due to neutropenia, that G-CSF be started (filgrastim 5 ug/kg subcut daily rounded up to either 300ug or 480ug or lenograstim 263 ug daily). If after a 1 week delay, there is persistent pancytopenia, a clinical decision will need to be made as to whether to start the next cytarabine course with continued G-CSF therapy, and possibly platelet transfusion support, or to delay for a further week. Discussion with the Study chief investigators should be considered. If Ara-C is delayed the other drugs within this treatment phase should also be delayed.

For information relating to dose modifications and management of Ara-C syndrome refer to appendix 4.

ii. CPA (Cyclophosphamide): 1,000mg/m2/day IV over 1 hour, Days 36 and 64.

The first Cyclophosphamide dose commences on the same day as the first Cytarabine block for this phase: ie, Day 36.

Fluid and MESNA (Uromitexan®) support for cyclophosphamide administration should be as per institutional guidelines.

Frusemide 0.5mg/kg IV 6 hours after CPA may be needed. Check fluid balance.

Guidelines for the second cyclophosphamide dose for this phase on day 64:

- WCC > 1.0×10^9/L
- neutrophils $\geq 0.3 \times 10^9$/L
- platelets $\geq 50 \times 10^9$/L

Please note that the trend in counts is often more informative than the absolute levels (so that rising counts are more likely to reflect adequate bone marrow recovery rather than absolute counts).

For information relating to dose modifications and management of haematuria or renal dysfunction refer to appendix 4.

iii. MP (6-Mercaptopurine): 60mg/m^2/day PO, Days 36-63, 28 days total.

Dose must be given at night without milk products (at least one hour after meals)

If a Cytarabine block must be postponed or interrupted then the Mercaptopurine should also be stopped.

Omitted Mercaptopurine doses should be made up until the planned cumulative total dose of 1680 mg/m^2 (28 x 60 mg/m^2) is reached for this phase.

iv. IT MTX (Methotrexate): 12mg intrathecal methotrexate given on Days 43 and 57 with the second and fourth Cytarabine blocks.

If cytarabine is delayed, MTX should also be delayed.

For information relating to dose modifications and management of IT MTX toxicities refer to appendix 4.

11.4 Recommendations for supportive care during induction therapy

Transfusional support with red cell and platelet concentrates should be given according to the guidelines of each institution to correct anemia and thrombocytopenia.

It is recommended that mould active, non-triazole anti-fungal prophylaxis be given to all neutropenic patients throughout induction therapy as per institutional guidelines. Due to the potential for interactions between triazoles and certain anti-leukemic chemotherapy drugs (in particular vincristine and cyclophosphamide) the use of this class of anti-fungal drugs should be avoided during induction therapy.

It is also recommended that prophylaxis against Pneumocystis jiroveci pneumonia be given using trimethoprim-sulphamethoxazole 4 tablets weekly. For patients with documented sulphonamide allergies, the following alternatives may be considered: 1. Dapsone 50 mg bd or 100 mg daily; 2. Dapsone 50 mg daily with pyremethamine 50 mg weekly; 3. Pentamidine 300 mg by nebulizer every 4 weeks; 4. Atovaquone 1500 mg daily.

See section 11.2 for supportive care during asparaginase therapy.

11.5 Subsequent treatment for standard/medium risk patients

Patients who meet all of the following criteria:

- Are negative for t(4;11) and MLL/AF4
- Had a good response to prednisolone at day 8 (blast count $<1.0 \times 10^9$/L in peripheral blood after 7 days of prednisolone and one intrathecal injection of Methotrexate on day 1)
- Had CR at time point 1 (day 33) bone marrow biopsy

should continue treatment with Protocol M (see Section 11.7). Note that some patients in this group may later be classified as medium-high or very high risk based on the outcome of MRD testing at TP2 (d79).

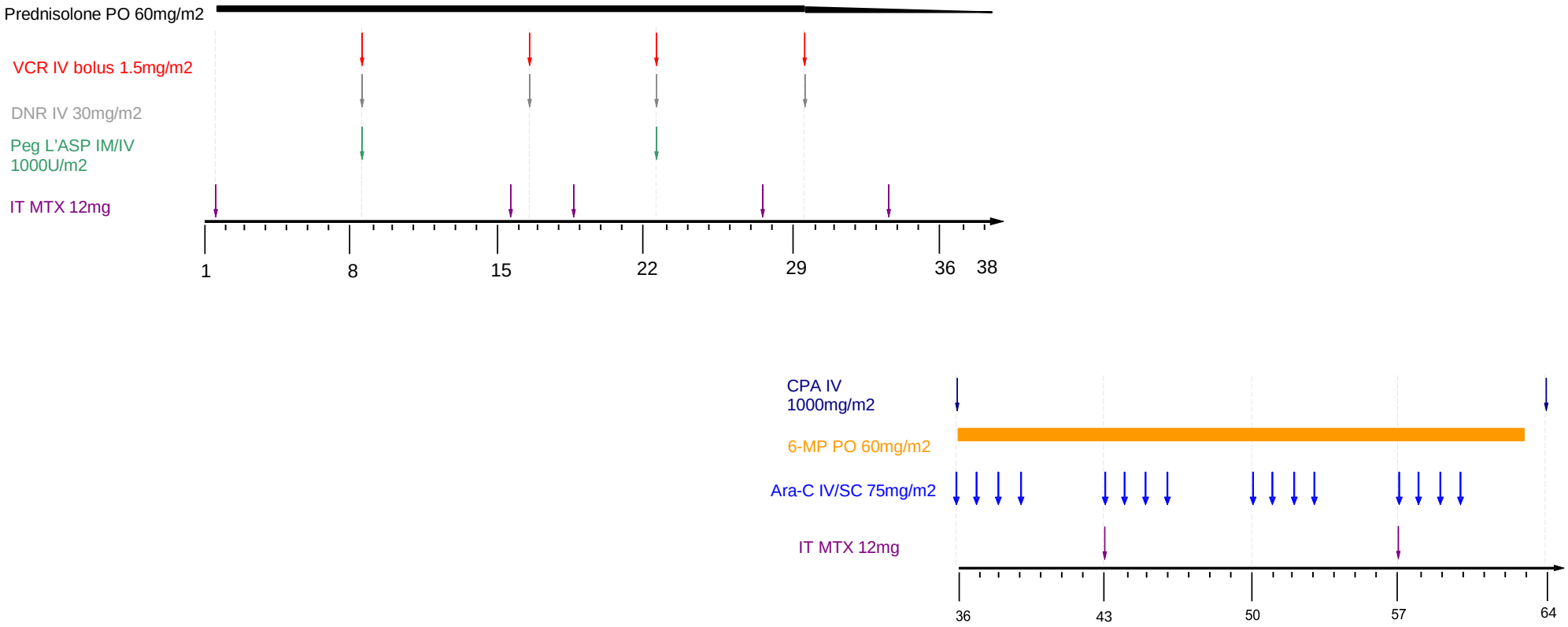
11.6 Subsequent treatment for high risk/very high risk patients

Patients who fulfil <u>at least one</u> of the following criteria:

- Are positive for t(4;11) or MLL/AF4
- Had a poor response to prednisolone at day 8 (peripheral blood blast count $\geq 1.0 \times 10^9$/L on day 8)
- No CR at time point 1 (day 33) bone marrow biopsy (per section 14 ie ≥5% leukaemic blasts)

should begin treatment with the High Risk protocol (see Section 11.8).

Figure 1 Protocol I: Induction/Consolidation Phase



* For patients with CNS disease at diagnosis

11.7 Protocol M

Following Protocol I, only Standard and Medium Risk patients will proceed through protocol M.

NOTE: Some patients proceeding to protocol M therapy will be subsequently identified on the basis of time point 2 MRD (ie day 1 of Protocol M, day 79 overall) to have medium high risk (MRD positive but $<5 \times 10^{-4}$) or very high risk (MRD positive $\geq 5 \times 10^{-4}$) disease. Most of these patients will be identified prior to Protocol M day 8 high dose methotrexate and should cease oral mercaptopurine and proceed to HR block 1 as soon as possible, once criteria for proceeding to HR block therapy are met. If MRD results are delayed, standard and medium risk patients may proceed to the first high dose methotrexate block of protocol M before MRD results are known. In these patients if time point 2 MRD suggests medium- high (MRD positive but $<5 \times 10^{-4}$) or very high risk (MRD $\geq 5 \times 10^{-4}$) disease, they should proceed to HR block 1 as soon as recovery permits, which will usually be between days 15 and 22 from the start of Protocol M assuming criteria for commencing HR block therapy are met.

This phase is scheduled to commence on Day 79 after the start of Protocol I, dependent upon adequate count recovery as detailed below. Protocol M is therefore scheduled to begin 2 weeks after the second dose of cyclophosphamide in Protocol I. This phase begins with daily oral mercaptopurine. The first of four high dose methotrexate infusions is administered on Day 8 of Protocol M.

The outline of therapy is provided in Figure 2.

A bone marrow aspirate is required on Day 1 (time point 2) of this Protocol M - for MRD assessmentby molecular and flow techniques (see Section 13 & Appendix 5)

Note: To enable sample processing; schedule the Timepoint 2 collection on Monday to Thursday.

Count Requirements for taking Timepoint 2 sample, unsupported by G-CSF for at least 48hours:

- WCC $\geq 1.5 \times 10^9$/L
- neutrophils $\geq 0.5 \times 10^9$/L
- platelets $\geq 50 \times 10^9$/L

Ideally timepoint 2 bone marrow should be performed when DNA yield is possible and as close as possible to day 79.

11.7.1Protocol M Therapy

Requirements for beginning Protocol M:

- Good general condition with no severe infections.
- Creatinine or creatinine-clearance within the normal limits
- Alanine aminotransferase (ALT)/ AST <5 x normal
- Bilirubin <3 x normal.
- Hematological criteria, unsupported by G-CSF for at least 48hours:
  - WCC $\geq 1.5 \times 10^9$/L
  - neutrophils $\geq 0.5 \times 10^9$/L
  - platelets $\geq 50 \times 10^9$/L

i. MP (6-Mercaptopurine): 25mg/m^2/day PO over 8 weeks. Days 1-56.

Give at night, 1 hour after meals, without milk products.

ii. HD-MTX (High Dose Methotrexate): 5g/m^2 as a continuous infusion over 24 hours, on Days 8, 22, 36, 50.

10% of the total Methotrexate dose is administered as a loading dose infused over 30 mins.
90% of the total dose is administered as continuous infusion over 23½ hours.

A suggested administration guideline for high dose methotrexate is given in Appendix 2. However, administration of intravenous fluids and urinary alkalization for high dose methotrexate blocks, may be as per institutional protocol for the administration of high dose methotrexate.

If the MTX infusion is started at 2pm, then the IT MTX administration and the sampling and measurement of MTX-levels at 24, 42, 48 hours fall within the regular workday. An alternative starting time is 6AM (requires admission for prehydration on the preceding day).

LCV: Leucovorin (Folinic Acid) Rescue:

For patients with normal Methotrexate excretion (ie. serum Methotrexate levels that fall within the guidelines), only three doses of Leucovorin (Folinic Acid) will be administered at hours 42, 48 and 54 after the START of the high dose Methotrexate infusion.

Table 3 Dose of LCV after MTX infusion

Time after start of MTX infusion (hours)	Dose of LCV (mg/m^2 IV)
42	15
48	15
54	15

iii. IT MTX (Intrathecal Methotrexate): 12mg intrathecal methotrexate. Administer the dose at 2 hours after the start of the MTX- infusion on Days 8, 22, 36, 50.

If not possible to administer dose at above time, the IT MTX should be administered before the end of the MTX infusion.

Determining the MTX Serum Level

Serum MTX level should be measured 24, 36, 42 and 48 hours after beginning the MTX-infusion.

Table 4 Expected MTX levels following MTX infusion

Time	Expected MTX level		
at 24 hours	MTX	≤	150,000 nM/L
at 36 hours	MTX	≤	3,000 nM/L
at 42 hours	MTX	≤	1,000 nM/L
at 48 hours	MTX	≤	400 nM/L

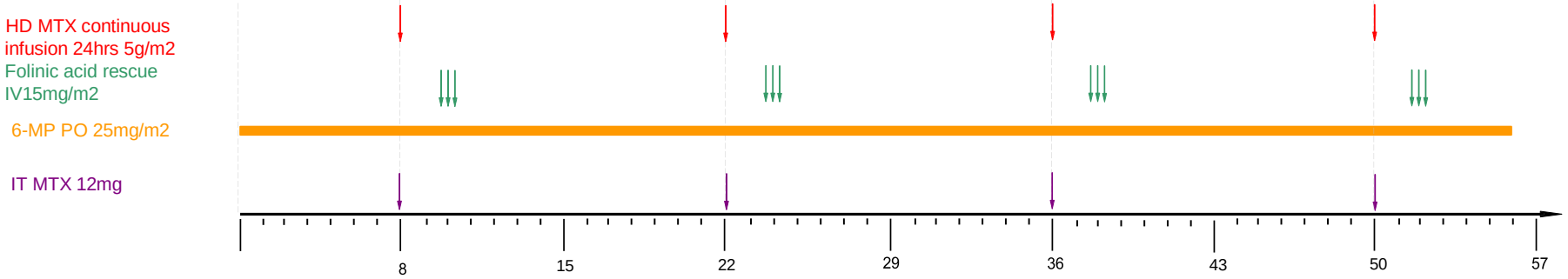
The level of MTX at 24, 42 and 48 hours must be determined promptly in the laboratory. The MTX level at 36 hours must be taken at the correct time. The 36 hour assay can be run at the same time as the 42 hour

sample. If the 24 hour MTX level is > 150,000nM/L (equivalent to 150µM/L) and/or there are concerns about elimination of Methotrexate (eg. a significant increase in the creatinine value, reduction of diuresis in spite of Frusemide), then the 36 hour level must be measured immediately. If the level is greater than expected according to the guidelines in this protocol then the folinic acid rescue should be started immediately.

Detailed instructions on measuring the MTX-level, alteration to the folinic acid rescue guidelines and further measures to be taken in situations of abnormal MTX-excretion and/or MTX-levels are found in Appendix 2.

In the event of acute renal failure secondary to Methotrexate (rare at this dose) consideration should be given to the use of carboxypeptidase (stocks available at Peter MacCallum and Childrens Hospital Westmead).

Figure 2 Protocol M: For SR and MR patients



11.8 Protocol For Medium-High, High, and Very-High Risk Patients

11.8.1General Guidelines

This protocol is to be used for all patients identified as being at medium-high, high or very high risk, even if a matched allogeneic stem cell donor is identified and allogeneic HCT is planned. The purpose of the protocol is to deliver intensified chemotherapy to reduce MRD levels, preferably to undetectable levels, prior to alloHCT, or re-commencing Protocol 2 if an appropriate donor is not available. The first block of treatment should be started after the completion of Protocol 1 for those patients identified early.

NOTE: Some patients proceeding to protocol M therapy will be subsequently identified on the basis of time point 2 MRD (ie day 1 of Protocol M, day 79 overall) to have medium high risk (MRD positive but $<5 \times 10^{-4}$) or very high risk (MRD positive $\geq 5 \times 10^{-4}$) disease. Most of these patients will be identified prior to Protocol M day 8 high dose methotrexate and should cease oral mercaptopurine and proceed to HR block 1 as soon as possible, once criteria for proceeding to HR block therapy are met. If MRD results are delayed, standard and medium risk patients may proceed to the first high dose methotrexate block of protocol M before MRD results are known. In these patients if time point 2 MRD suggests medium- high (MRD positive but $<5 \times 10^{-4}$) or very high risk (MRD $\geq 5 \times 10^{-4}$) disease, they should proceed to HR block 1 as soon as recovery permits, which will usually be between days 15 and 22 from the start of Protocol M assuming criteria for commencing HR block therapy are met.

In general, delays should be kept to a minimum in the first 3 HR blocks, and interruptions to therapy once a block of treatment is started should be avoided. Dosages should not be reduced; if necessary, postponement or complete omission of one drug is preferable.

Each HR block is started once there is evidence of bone marrow recovery, and if the patient's general condition is adequate. There must be absence of severe mucositis or infection, as well as no significant organ dysfunction.

A bone marrow aspirate for "Timepoint 2" MRD assessment must be performed prior to proceeding to HR Block-1 therapy (see Section 13)

Note: A bone marrow aspirate is collected in ACD after recovery from each block of HR therapy AND before and after allogeneic HCT for MRD analysis.

11.8.2Specific Guidelines

- Renal/hepatic function before each HR block
  - creatinine clearance within normal range
  - ALT <5x upper limit of normal
  - bilirubin< 2x upper limit of normal.
- Oxygen saturation >94% on pulse oximetry before each HR block
- Blood count Day 1 of each HR block (without GCSF support for at least 48hrs)
  - neutrophil count $> 0.2 \times 10^9$/L
  - platelet count $>50 \times 10^9$/L
- Prophylactic therapy should be given for opportunistic infection, using Bactrim DS (or equivalent) 1 tablet twice daily on Mondays and Fridays, and appropriate mould active anti-fungal prophylaxis be instituted as per institutional guidelines in neutropenic patients. Due to the potential for significant interactions, concomitant administration of a triazole and certain chemotherapeutic agents such as

vinca alkaloids or cyclophosphamide should be avoided.

- G-CSF is recommended following each high risk block until neutrophil recovery. G-CSF should be withheld at least 48hours prior to the initiation of the next HR block treatment to ensure that count criteria and adequate bone marrow recovery achieved before proceeding with the next HR block therapy.

11.8.3High Risk Protocol Therapy

Each HR block should be given at 4-5 week intervals, not less.

11.8.4Block HR-1

This first block of HR-1 normally begins directly after completion of Protocol I.

i. **DEXA (Dexamethasone): 20mg/m^2/d, PO/IV in 2 divided doses on days 1-5**

ii. **(VCR (Vincristine): 1.5mg/m^2/daily (maximum single dose 2mg) IV on days 1 and 6**

iii. **HD-MTX (High dose Methotrexate): 5g/m^2, infused IV over 24h on day 1.**

1/10 of the total dose as loading dose over 30 min.
9/10 of the total dose as continuous infusion IV over 23½ hours. Please use the infusion plan in Protocol M on page 38.
It is recommended that the MTX infusion be started at 2pm. The IT injection, (Triple drug), and the sampling and measurement of MTX-levels at 24, 42, 48 hours then fall within the regular workday.

LCV: Leucovorin Rescue

Please use the plan outlined in Protocol M on page 38.

iv. **CPA (Cyclophosphamide): 200mg/m^2/twice daily IV over 1h, days 2-4.**

5 doses: q12hr
Fluid and Mesna administration as per institutional guidelines.

v. **HD-ARA-C (Cytarabine): 2g/m^2/twice daily, IV over 3h on day 5.**

2 doses 12h apart.
Cerebellar checks required before each dose; discontinue high dose cytarabine if evidence of cerebellar damage.

vi. **ASP (Pegylated Asparaginase) 1,000U/m^2 IM or IV on day 6.**

If possible, use the same preparation as in Protocol I.
In the case of hypersensitivity, use alternative preparations.
Other side-effects: see Section 11.3.

vii. **MTX/ARA-C/HYDROCORTISONE IT**

Table 5 Intrathecal Methotrexate, Cytarabine, Hydrocortisone dosages on day 1 of HR-1

Drug	MTX IT(mg)	ARA-C IT (mg)	Hydrocortisone (mg)
Dose	12	30	50

The injection is given 2 hours after the start of the HD-MTX infusion (injections one after the other). If this is not possible, the IT injection should be given no later than at the end of the MTX-infusion.
Lowered-head position for at least 2 hours after intrathecal injection.

11.8.5Block HR-2

The doses and scheduling are the same as in block HR-1 for:

i. **DEXA** (Dexamethasone): 20mg/m2/d, PO/IV in 2 divided doses on days 1-5,

ii. **HD-MTX** (High dose Methotrexate): 5g/m2, infused IV over 24h on day 1,

iii. **LCV-Rescue** (use the plan outlined in Protocol M on page 38),

iv. **ASP** (Pegylated Asparaginase) 1,000U/m2 IM or IV on day 6,

v. **MTX (12mg IT)/ ARA-C (30mg IT) /HYDROCORTISONE (50mg IT) IT** 2 hours after the start of the HD-MTX infusion on day 1

In case of initial CNS involvement, triple IT is given twice: on days 1 + 5.

Additional Therapy in HR2:

vi. **VDS (Vindesine): 3mg/m2/daily (maximum single dose 5mg) IV on days 1 and 6.**

Caution: delayed clearance is possible when Asparaginase is given simultaneously.
Vincristine (same doses as HR1) can be substituted if the drug is not readily available.

vii. **IFO (Ifosfamide) 800mg/m2/ twice daily, IV over 1h, days 2 – 4.**

5 doses: every 12h.
Fluid and Mesna administration as per institutional guidelines.

viii. **DNR (Daunorubicin) 30mg/m2/daily, IV over 1 hr, day 5.**

11.8.6Treatment for Medium High Risk patients at the end of HR2

Medium High risk patients should proceed to alloHCT after completing the second of the high risk blocks, allowing at least 4 weeks for recovery from toxicity, provided all the following criteria are met.

- MRD-ve at end of HR1
- Sibling donor identified (i.e HLA-identical sibling)

Medium High Risk patients with no sibling donor but who are MRD-ve at end of HR1 should proceed after HR2 to Protocol II outlined in Section 11.10.

All other medium high risk patients should proceed to HR-3.

11.8.7Treatment for High Risk and Very High Risk patients at the end of HR2

High risk or Very High Risk patients should proceed to alloHCT after completing the second of the high risk blocks, allowing at least 4 weeks for recovery from toxicity, provided the following criteria are met.

- Sibling donor (i.e HLA-identical sibling) or
- matched unrelated donor (7 or 8 antigen matches at high resolution typing for HLA-A, B, C, and DRB1 alleles) identified. In some high risk/very high risk patients lacking such a donor, a suitable 1 antigen-mismatched family member, unrelated cord blood transplant or haploidentical transplant may also be considered
- MRD negative after HR1

All other High Risk or Very High Risk patients should proceed to HR-3.

11.8.8Block HR-3

The doses and scheduling are the same as in block HR-1 for:

i. **DEXA (Dexamethasone): 20mg/m2/d, PO/IV in 2 divided doses on days 1-5,**
ii. **ASP.** (Pegylated Asparaginase) 1,000U/m2 IM or IV on day 6,

Additional Therapy in HR3

iii. **HD-ARA-C (Cytarabine): 2g/m^2/ twice daily, IV over 3h, days 1 – 2.**

4 doses: q12h.
Cerebellar checks as for HR1

iv. **VP-16 (Etoposide): 100mg/m^2/ twice daily, IV over 1h, q 12 hr, days 3 – 5.**

5 doses: q 12 hr

Administration of etoposide should be as per institutional guidelines

v. **MTX/ARA-C/HYDROCORTISONE IT: Intrathecal Methotrexate, Cytarabine, Hydrocortisone on day 5.**

The intrathecal administration of this medication begins on day 5 in order to reduce the risk of neurotoxicity from simultaneous administration of HD-ARA-C. It is the same doses as in block HR-1.
Lowered-head position for at least 2 hours after intrathecal injection.

11.8.9Treatment of Medium-High Risk patients after HR3

Medium-High risk patients completing HR3 should proceed to alloHCT if:

1. A matched sibling donor is available
2. MRD negative after HR2

If MRD positive after HR2, patients should have a further MRD sample collected after HR3 and receive HR1therapy again (HR4), while awaiting MRD result from after HR3. If this is negative, they should proceed to Protocol II. If MRD positive after HR3, Medium-High risk patients with either a matched sibling donor, an alternative family donor or a matched unrelated donor should proceed to alloHCT. For patients MRD positive after HR3 and lacking any matched donor, it is recommended that HR blocks 4-6 be given, then Protocol II.

11.8.10Treatment for High Risk and Very High Risk patients at the end of HR3

High risk or Very High Risk patients should proceed to alloHCT after completing the third of the high risk blocks, irrespective of MRD status, allowing at least 4 weeks for recovery from toxicity, provided that a suitable matched donor is identified, either:

- Sibling donor (i.e HLA-identical sibling) or
- A matched unrelated donor (7 or 8 antigen matches at high resolution typing for HLA-A, B, C, and DRB1 alleles) may also be considered, depending on the policy of the treating centre and the transplant centre. In some high risk/very high risk patients lacking such a donor, a suitable 1

antigen-mismatched family member, unrelated cord blood transplant or haploidentical transplant may also be considered

All other High Risk or Very High Risk patients lacking a matched donor should proceed to HR4-6 (i.e repeat HR blocks 1-3).

When HR blocks 4-6 have been completed, these High Risk or Very High Risk patients should proceed to Protocol II.

11.9 Cranial Irradiation

Indications:

Cranial irradiation is only performed on patients with initial CNS-involvement and as preventive therapy for all HR/VHR-patients not undergoing alloHCT. Cranial radiation may also be considered for T-ALL patients with initial WCC >100, based on pediatric data [27]

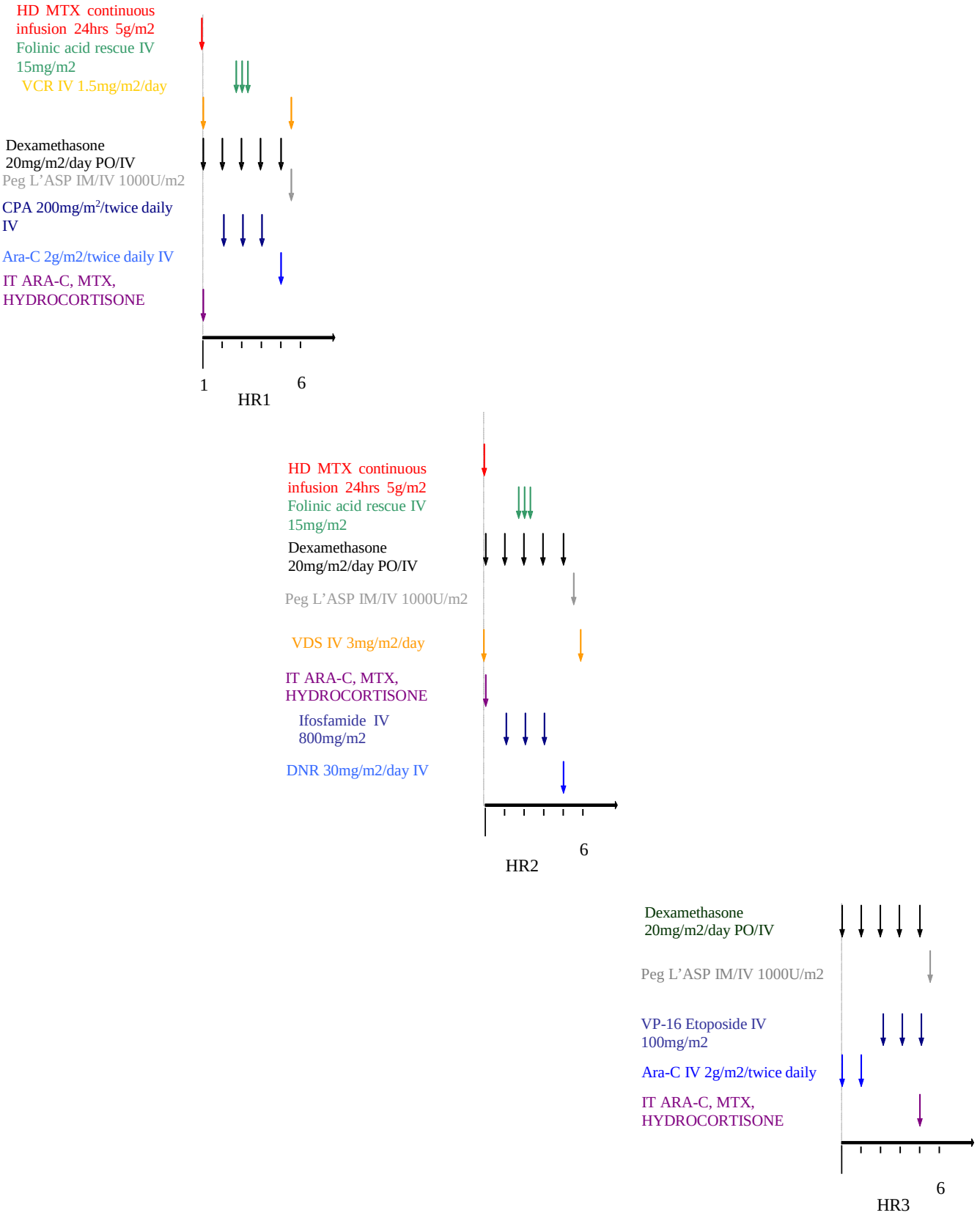
Irradiation Technique:

Cranial irradiation is performed under high-voltage conditions with a telecobalt-60-machine or linear accelerator. The daily settings must be exactly reproduced, for example with mask-technique. The CNS irradiation must include the whole neurocranium, with both upper vertebrae (C2), the retrobulbar space and the complete cranial base with its middle cranial groove. This assumes the use of individual filters. During irradiation a homogenous dose-delivery should be watched for, idealistically, every field should be irradiated in every session. The daily single dose is 1.5Gy. This is administered in 5 sessions per week until the total dose of 18 Gy has been applied. If irradiation-induced headaches occur, the administration of Dexamethasone (15mg/m2/d) is often helpful.

Dosages:

When the patient's clinical condition is good the cranial irradiation can usually begin on day 38 of Protocol II. Before cranial irradiation is begun there must be no signs of a central nervous system disorder.

Figure 3 High Risk protocol



11.10 Protocol II

All Risk Groups

Protocol II begins 2 weeks after the end of Protocol M for SR and MR patients. The duration of Protocol M is 56 days. Protocol II begins on day 70 after the start of Protocol M. For M-HR patients (and HR or VHR not proceeding to alloHCT), this phase begins 3 weeks after the end of the last High Risk Block (ie. Protocol II begins on Day 57 after the start of the last HR Block).

Requirements for beginning Protocol II:

- CR
- Good general condition with no severe infections.
- Hematological guidelines:

- WCC $\geq 2.5 \times 10^9$/L
- neutrophils $\geq 1.0 \times 10^9$/L
- platelets $\geq 100 \times 10^9$/L

11.10.1Therapy Guidelines: Protocol II Days 1 – 35 (Phase 1)

Therapy Management in Phase 1

In the case of severe neuropathy Vincristine can be omitted.
In the case of myelosuppression (WCC $< 0.5 \times 10^9$/L or neutrophil $< 0.2 \times 10^9$/L), the Doxorubicin/ Vincristine doses may be postponed/ omitted.

i. **DEXA (Dexamethasone): 10mg/m^2/day, PO in 2-3 divided doses, Days 1-21.**

From Day 22 on, reduce the dosage every 3 days by half and stop on Day 29.

ii. **VCR (Vincristine): 1.5mg/m^2/day, IV bolus (maximum single dose 2mg), Days 8, 15, 22, 29.**

iii. **DOX (Doxorubicin): 30mg/m^2/day, IV over 1 hour, Days 8, 15, 22, 29.**

Before the first Doxorubicin doses an echocardiogram or gated heart pool scan is necessary.
In case of a significant decrease in the ejection fraction, no further Doxorubicin should be given.

iv. PEGYLATED L'Asparaginase: 1000U/m^2 IM or IV on Day 1.

When possible, use the same asparaginase preparation as in Induction.
In Protocol II, there is a higher risk of allergic reactions (up to 35% of patients). This indicates the presence of antibodies (inactivating) to asparaginase, and requires a change in asparaginase preparation. All Asparaginase doses must be given in a setting which can cope with management of anaphylaxis.

Test doses before the full infusion are NOT recommended.
In the case of hypersensitivity, use alternative preparations:

1.Erwinia Asparaginase (SPEYWOOD): 10,000U/m^2 IM 3 days per week for 6 doses. Days 1, 3, 5, 8, 10 and 12
2.E Coli - Asparaginase 10,000U/m^2 IV over 1-2 hours. Days 1, 3, 5, 8, 10, 12, 15..

See section 11.2 for further details

v. IT MTX (Intrathecal Methotrexate): 12mg intrathecal methotrexate <u>Day 1, 18 ONLY IF CNS disease at diagnosis</u>.

11.10.2Therapy Guidelines: Protocol II Days 36 – 50 (Phase 2):

Requirements for beginning Protocol II Phase 2:

- Good general condition with no acute infections.
- Hematological guidelines:
  - WCC $\geq$ 2.0 x10^9/L
  - neutrophils $\geq$ 0.5 x 10^9/L
  - platelets $\geq$ 50 x 10^9/L

i. ARA-C (Cytarabine): 75mg/m^2/day, IV or SC in 2 x 4-day blocks. Days 36, 37, 38, 39 and Days 43, 44, 45, 46.

Cut-off values for beginning a Cytarabine block are:

- WCC $\geq$ 0.5 x 10^9/L
- platelets $\geq$ 30 x 10^9/L

The Cytarabine blocks should not be interrupted, if possible.

ii. CPA (Cyclophosphamide): 1,000mg/m^2, IV over 1 hour, Day 36.

Frusemide 0.5mg/kg IV 6 hours after CPA may be needed. Check fluid balance.
Fluid and Mesna (Uromitexan®) administration should be as per institutional guidelines for administration of cyclophosphamide. The first Cyclophosphamide dose is with Day 36 start of 1st Cytarabine block.

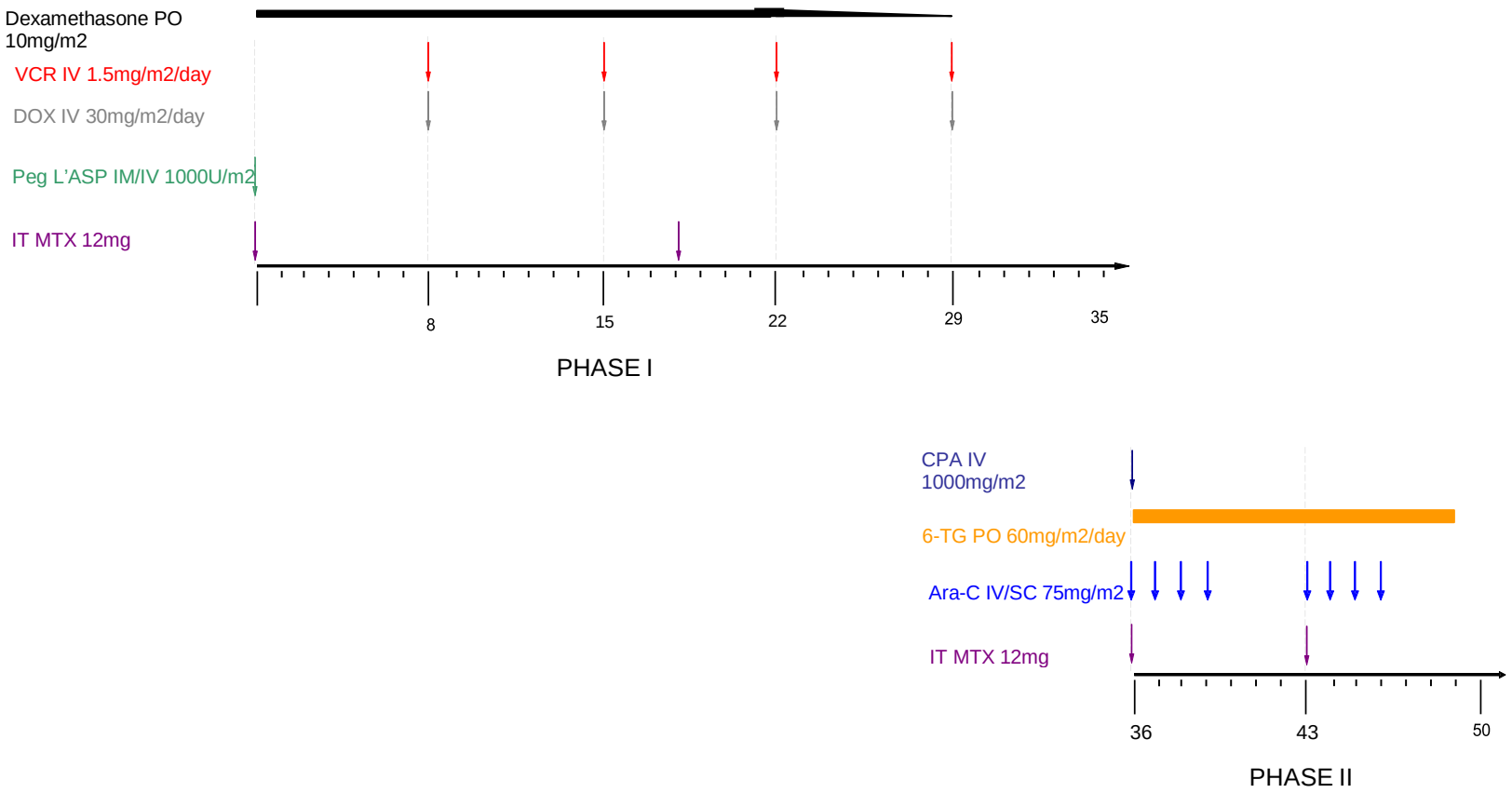
iii. 6-TG (6-Thioguanine): 60mg/m^2/day, orally Days 36-49, a total of 14 days.

Give at night, at least 1 hour after meals, without milk products.

If a Cytarabine block is interrupted, then Thioguanine should also be interrupted. Missed Thioguanine doses should be administered until the planned cumulative dose of 840mg/m^2 is reached.

iv. IT MTX (IT Methotrexate): 12mg intrathecal methotrexate. At the same time as the first dose of Cytarabine in Block 1 (Day 36) and Block 2 (Day 43).

Figure 4 Protocol II: For all risk groups



* For patients with CNS disease at diagnosis

11.11 Maintenance Therapy

Details:

Maintenance therapy begins, depending on bone marrow recovery and the patient's clinical state, two weeks after the end of Protocol II. Total therapy, calculated from the start of Protocol I, is 24 months for all patients.

Requirements for the start of Maintenance

1. Good general condition
2. No serious infection
3. Recovering (ieincreasing) blood counts with
   minimum a. WBC ≥ 1.0 X 10^9/L
   b. Neutrophils ≥ 0.5 x 10^9/L
   c. Platelets ≥ 50 X 10^9/L
4. Stable liver function
   a. AST/ALT < 10 X ULN
   b. Bilirubin within normal range.

Dose guidelines

Therapy management according to:	Count reading	% of dose MP/ MTX
WCC	< 1.0 x 10^9/L 1.0- 2.0 x 10^9/L >2.0- 3.0 x 10^9/L >3.0 x 10^9/L	0 50 100 to 150

Frequency (minimum) of blood tests in maintenance
Full blood count - every 4 weeks
Liver function tests - every 12 weeks

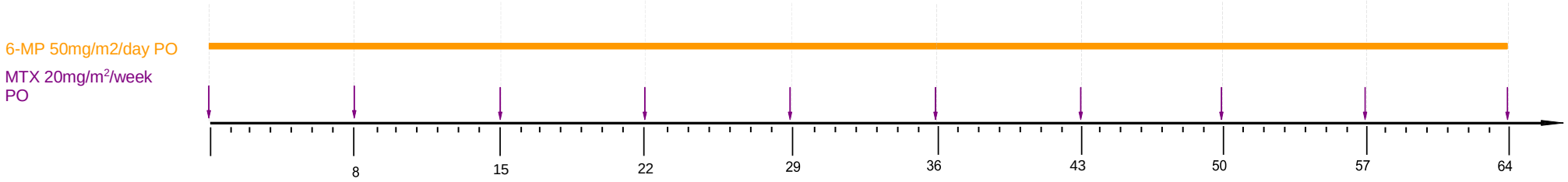
Figure 5 shows the standard maintenance therapy for all patients. For SR and MR patients this ten week cycle should be repeated a total of 6-7 times (ie. 7-8 courses - each 10 weeks in duration). For High Risk patients, the maintenance cycle is repeated 5 times (a total of 6 cycles – each 10 weeks in duration). For all patients the duration of all ALL06 therapy should not to be longer than 104 weeks, regardless of treatment delays during each protocol.

i. MP (6-Mercapoturine): 50mg/m^2/day, PO.

Daily in one dose in the late evening (at least 1 hour after meals).
Dose should not be taken with milk products.

ii. MTX: (Methotrexate): 20mg/m^2/week, PO once a week (at night).

Figure 5 Maintenance therapy- for all risk groups



11.12 Duration of therapy

Treatment duration on this protocol is for a total of 24 months (104 weeks), regardless of treatment delays, or until one of the following criteria applies:

- Disease progression,
- Intercurrent illness that prevents further administration of treatment,
- Unacceptable AE(s),
- Patient decides to withdraw from the study (i.e withdrawal of consent), or
- General or specific changes in the patient's condition render the patient unacceptable for further treatment in the opinion of the investigator.
- Allogeneic HCT

Details of patients who commenced protocol treatment but completed therapy prior to 24 months should be documented in the CRF, with a reason and date of exclusion.

12.Pharmaceutical information

All drugs used in this study are registered for the prescribed use and commercially available. Expected side effects of drugs are listed in Appendix 3. The package insert should also be consulted for more complete information.

12.1 Asparaginase

All forms of asparaginase deaminate asparagine, and therefore are lethal for cells which cannot synthesize asparagines. *E.coli* –derived Asparaginase powder for injection should be stored at room temperature. When asparaginase is reconstituted or further diluted for IV solutions, it is stable for 14 days when stored at 2-8°C.

Erwinia asparaginase is an enzyme from the plant pathogen Erwinia carotovora (Erwinia chrysanthemi). For injection: 10,000 U vial. Supplied as a lyophilized powder with glucose BP (dextrose monohydrate), 5mg and sodium chloride, 0.5 mg in 3 ml flint glass vials. Refrigerate the intact vials (2- 8°C). The intact vials are stable for at least 3 years both refrigerated (2-8°C) and at room temperature (22-25°C). Reconstitution as recommended results in a solution which is stable for *up* to 20 days both at room temperature or under refrigeration**. However, the manufacturer recommends that the product be used immediately after reconstitution**. Available from Speywood Pharmaceuticals Ltd., through its Australian distributor, Australasian Medical and Scientific.

Peg-asparginase is an L-asparaginase combination (L-asparaginase amidohydrolase), covalently attached to strands of monomethoxypolyethylene glycol of 5,000 daltons (PEG) by means of a coupling moiety. The source of the L-asparaginase is *Escherichia coli*. Pegaspargase is available in 3750 units per 5ml vial (750IU/ml). Keep under refrigeration at 2-8º C (36-40o). **DO NOT FREEZE.** If stored at room temperature, stable no greater than 48 hours. Do not use cloudy solutions or if particulate matter is seen. Do not shake the vials - the asparaginase portion of the compound may be separated from the PEG portion.

12.2 Cyclophosphamide

An alkylating agent, related to nitrogen mustard. Injectable form is available as white crystals with sodium chloride added, in vials containing l00mg, 200mg, 500mg, 1g. All preparations are stable at room temperature (not to exceed 30°C). Reconstitute with sterile water to a concentration of 20 mg/ml. Discard solution after 24 hours at room temperature; stable up to 28 days if refrigerated (2°-8°C).

12.3 Cytarabine

Deoxycytidine analogue which is metabolized to ARA-CTP, a substance which inhibits DNA polymerase and affects DNA synthesis. Cytarabine is available as 20mg/ml solution in 100mg vials, and 100mg/ml solution in 500mg, 1,000mg and 2,000mg vials. Further diluted solutions are stable for up to 7 days.

12.4 Dexamethasone

Dexamethasone is a synthetic fluorinated glucocorticoid devoid of mineralocorticoid effects. Available in 4mg tablets. It is also available as a 1mg/ml elixir, and as 4mg/ml solution for injection. Follow manufacturer's instructions for mixing.

12.5 Daunorubicin

An anthracycline compound derived from Streptomyces coeruleorubidus, which intercalates with DNA, interfering with DNA synthesis. Commercially available as a 2mg/ml solution in 20mg vials, stored at 2-8ºC. Protect from light. Diluted solutions are stable for up to 43 days at 2-8ºC.

12.6 Doxorubicin

An anthracycline antibiotic isolated from cultures of Streptomyces peucetius. Binds to DNA and inhibits nucleic acid synthesis. Available as 2mg/ml solution in 20mg, 50mg, 100mg vials. Store at 2-8ºC protect from light. Diluted solutions are stable for up to 43 days at 2-8ºC.

12.7 Etoposide

A semi synthetic derivative of podophyllotoxin that forms a complex with topoisomerase II and DNA which results in a single and double strand DNA breaks. Refer to package insert for appearance and storage conditions for individual formulations.

12.8 Hydrocortisone

Synthetic steroid akin to the natural adrenal hormone, cortisol. It binds with steroid receptors on nuclear membrane, impairs cellular mitosis and inhibits protein synthesis. Available as 100mg vials containing Hydrocortisone sodium succinate, a white, odourless, hygroscopic, amorphous solid powder which is very soluble in water, equivalent to hydrocortisone 100mg, sodium phosphate monobasic (monohydrate) 0.8mg, sodium phosphate dibasic (anhydrous) 8.73mg. Unreconstituted product should be stored below 30ºC and not frozen. Once reconstituted aseptically with 2mL of preservative-free Sodium chloride 0.9% for injection, the resulting solution is stable for 48 hours. When hydrocortisone is prepared in combination with cytarabine and methotrexate it is stable (Fridge 2 – 8ºC) for 24 hours. Available from Pharmacia Australia Pty Ltd.

12.9 Ifosfamide

Ifosfamide is a nitrogen mustard alkylating agent. Refer to package insert for appearance and storage conditions for individual formulations.

12.10 Leucovorin

Synthetic d,1-5 CHO tetrahydrofolate, which is used to bypass the inhibition of dihydrofolate reductase by Methotrexate (MTX). It competes with MTX for transport into the cells and "rescues" cells from the adverse effects of MTX. Available in 3mg/ml and 15mg/2ml ampoules and 50mg/5ml vials and in tablet form, l5mg.

12.11 6-Mercaptopurine

An analogue of the nucleic acid constituent adenine and the physiological purine base hypoxanthine. It must be metabolized to 6MPdR before it can be active. It is S phase specific and interferes with purine synthesis. Commercially available as a 50mg tablet that may be stored at room temperature, protected from light.

12.12 Methotrexate

A folate analogue that inhibits the enzyme dihydrofolate reductase, halting DNA, RNA, and protein synthesis. IV methotrexate: available in solutions of 2.5mg/mL (5mg vials), 25mg/mL (50mg vials) and 100mg/mL (500 and 1000mg vials). The diluted infusion solution is stable for up to 30 days. Solutions for intrathecal use should contain no preservatives.

12.13 Prednisolone

A glucocorticoid and synthetic congener of hydrocortisone, the natural adrenal hormone. It binds with steroid receptors on nuclear membrane, blocks mitosis, and inhibits protein synthesis. Commercially available in 1mg, 5mg and 25mg tablets; oral liquid, 5mg/ml.

12.14 Vincristine

Vincristine is an alkaloid isolated from Vinca rosea (periwinkle). It binds to tubulin, disrupting microtubules and inducing metaphase arrest. Available in solutions of 1mg/1ml in 2 or 5ml vials and in vials containing 1mg of powder. Refrigerate and protect from light.

12.15 Vindesine

Vindesine is an alkaloid. Refer to package insert for appearance and storage conditions for individual formulations.

12.16 6-Thioguanine

A purine analogue which may exert its effect during the S phase of the cell cycle by the incorporation of false bases into DNA and RNA. This results in single strand breaks and unilateral chromatid damage. It is converted to the active nucleotide, thioguanylic acid, via the enzyme hypoxanthine-guanine phosphoribosyl transferase (HGPRT) with phosphoribosyl pyrophosphate (PRPP) as a co-factor. Available in 40mg tablets that can be stored at room temperature for 2 years.

13.Schedule of evaluations

The following assessments will occur during the trial. Table 1 outlines the schedule.

13.1 Screening Assessments

13.1.1Pre- Treatment Assessments

A signed and dated Screening PICF must be obtained from the patient prior to any trial-specific evaluation not completed as part of standard of care (e.g. collection of central laboratory studies at diagnosis).

Pre-treatment assessments must be completed within 2 weeks prior to day 1. These assessments can be performed on day 1 if completed prior to treatment commencing. If the assessments have already been completed as part of standard of care prior to gaining written screening patient informed consent, they do not have to be repeated as long as they are within the above defined window from commencing treatment.

- Full Medical History (includes demographics)
- Complete Physical Examination including (but not limited to):
  - oral examination,
  - temperature,
  - blood pressure, heart rate, respiratory rate
  - height and weight
  - assessment of ECOG performance status (see Appendix 1)
- Blood samples will be taken for the following diagnostic tests:
  - Full blood count with differential and blood film
  - Biochemical analysis, including urea, creatinine, bilirubin, albumin, uric acid, liver enzymes including LDH, lipase, amylase.
  - Coagulation profile including APTT, INR, Fibrinogen, ATIII
  - Serology- HIV, Hep B, Hep C
  - Pregnancy Test (can be serum or urine test)
  - Either immunophenotyping on blood or bone marrow cells
  - Central laboratory tests as specified in Appendix 5
- CSF Examination (to confirm CNS involvement pre-treatment). Should be completed prior to commencing steroids. If this is not possible contact the CI to discuss.
- Bone marrow aspirate and trephine biopsy for the following diagnostic test:
  - Morphology
  - Either immunophenotyping on blood or bone marrow cells.
  - Cytogenetic analysis of leukemic cells.
  - Molecular studies for bcr-abl, MLL/AF4, TEL/AML1, E2A/PBX1 transcripts.
  - Central laboratory tests as specified in Appendix 5
- Chest X ray
- Electrocardiograph (ECG).
- A gated heart pool scan or echo to assess LVEF must be performed prior to day 8 of treatment. Results must be available for registration.
- HRQOL (see Section 18)

To be eligible for registration, the patient must meet all selection criteria as specified in Section 8. In addition, the patient must be thoroughly informed about all aspects of the trial, including the trial visit

schedule and the required evaluations, and all regulatory requirements for Informed Consent. Once the patient's diagnosis is confirmed and the patient has decided to participate in the trial, a signed and dated Main PICF must be obtained from the patient prior to registration. (see Section 9 for more details)

13.2 Treatment Assessments

For patients who are removed from the study and will not receive further treatment, a HRQOL should be collected at the end of treatment in the last protocol the patient received.

During treatment, the following assessments will be performed:

13.2.1Protocol I Induction d1-35

- BSA calculation on day 1

- Blood samples for the following tests
  - FBE with differential
    - If the patient is an inpatient recommended to be collected daily
    - If the patient is an outpatient recommended to be collected at a minimum of three times per week or more regularly as deemed clinically appropriate by the investigator.
    - Must be collected on days 1, 8, 15 & 33.
  - Biochemistry three times per week
    - including urea, creatinine, bilirubin, albumin, uric acid, liver enzymes including LDH, lipase, amylase
  - Coagulation profile minimum twice per week
    - including APTT, INR, Fibrinogen, ATIII
  - Central laboratory tests as specified in Appendix 5

- Bone marrow aspirate and trephine biopsy on days 15 & 33 for
  - morphology. If sample collected on day 33 is too hypocellular, the aspirate should be repeated at day 37.
  - central laboratory tests as specified in Appendix 5

- Complete Physical Examination on day 35 including (but not limited to):
  - oral examination,
  - temperature,
  - blood pressure, heart rate, respiratory rate
  - weight
  - assessment of ECOG performance status (see Appendix 1)

  Adverse events should be collected throughout the whole protocol. An adverse event review should occur on day 35

- For patients with mediastinal tumor involvement at commencement of protocol I induction, a chest xray on day 35 is required to assess response. If there is persistent CNS disease at day 36 contact the chief investigator.

13.2.2 Protocol I Consolidation d36-64

- HRQOL prior to treatment on day 36 and on day 64

- Blood samples for the following tests
  - FBE with differential.
    - If the patient is an inpatient recommended to be collected daily
    - If the patient is an outpatient recommended to be collected at a minimum of three times per week or more regularly as deemed clinically appropriate by the investigator.
    - Must be collected days 36, 43, 50, 57 & 64 prior to treatment to ensure guidelines detailed in section 11.3.2 are met.
  - Biochemistry three times per week
    - including urea, creatinine, bilirubin, albumin, uric acid, liver enzymes including LDH, lipase, amylase

- Complete Physical Examination on day 64 including (but not limited to):
  - oral examination,
  - temperature,
  - blood pressure, heart rate, respiratory rate
  - weight
  - assessment of ECOG performance status (see Appendix 1)

- Adverse events should be collected throughout the whole protocol. An adverse event review should occur on day 64

- At the end of the protocol confirm patient's risk stratification per section 11.5 & 11.6 to confirm which treatment protocol the patient should be receiving – HR blocks or protocol M

13.2.3Protocol M (for standard /medium risk patients only, commences d79 following protocol I) d1-56

- BSA calculation on day 1
- Blood samples for the following tests
  - FBE with differential three times per week
    - This must include day 1 prior to treatment to ensure guidelines detailed in section 11.7.1 are met.
  - Biochemistry three times per week
    - including urea, creatinine, bilirubin, albumin, uric acid, liver enzymes including LDH, lipase, amylase
  - Central laboratory tests as specified in Appendix 5 on days 1.
- Bone marrow aspirate and trephine biopsy on day 1 for
  - morphology.
  - central laboratory tests as specified in Appendix 5 on days 1
- Complete Physical Examination on day 56 including (but not limited to):
  - oral examination,
  - temperature,
  - blood pressure, heart rate, respiratory rate
  - weight
  - assessment of ECOG performance status (see Appendix 1)
- Adverse events should be collected throughout the whole protocol. An adverse event review should occur on day 56

13.2.4High Risk protocol (for medium high / high / very high risk patients, commences d79 following protocol I) d 1 – 6 given at 4-5 week intervals

- HRQOL
  - prior to treatment on day 1 of each HR block

- BSA calculation on day 1 of each HR block

- Blood samples for the following tests
  - FBE with differential
    - If the patient is an inpatient recommended to be collected daily
    - If the patient is an outpatient recommended to be collected at a minimum of three times per week or more regularly as deemed clinically appropriate by the investigator.
    - Must be collected on day 1 prior to treatment to ensure guidelines detailed in section 11.8.2 are met.
  - Biochemistry three times per week
    - including urea, creatinine, bilirubin, albumin, uric acid, liver enzymes including LDH, lipase, amylase
    - This must include day 1 prior to treatment to ensure guidelines detailed in section 11.8.2 are met.
  - Coagulation profile minimum of twice per week during HR block 1 only
    - including APTT, INR, Fibrinogen, ATIII
  - Central laboratory tests as specified in Appendix 5 (on day 79 overall, day 7 of each HR block and at the end of each HR block).

- Bone marrow aspirate and trephine biopsy at time point 2 (day 79) & at the end of each HR block (after count recovery 4-5 weeks after day 1 prior to the next HR block day 1 treatment) for
  - morphology.
  - Molecular studies as relevant (as determined by diagnostic BM to confirm CR)
  - central laboratory tests as specified in Appendix 5 (on day 79 overall and at the end of each HR block).

- Complete Physical Examination at the end of all HR blocks including (but not limited to):
  - oral examination,
  - temperature,
  - blood pressure, heart rate, respiratory rate
  - weight
  - assessment of ECOG performance status (see Appendix 1)

- Pulse oximetry on day 1 of each HR block
    - This must include day 1 prior to treatment to ensure guidelines detailed in section 11.8.2 are met.

- Adverse events should be collected throughout the whole protocol. An adverse event review should occur at the end of all HR blocks

13.2.5Protocol II Phase I d1-35 and Phase II d36-50

- BSA calculation on day 1

- HRQOL on day 1 prior to treatment

- Bone marrow aspirate and trephine biopsy on day 1 for
  - morphology.
  - central laboratory tests as specified in Appendix 5 (on day 1)

- Blood samples for the following tests
  - FBE with differential
    - If the patient is an inpatient recommended to be collected daily
    - If the patient is an outpatient recommended to be collected at a minimum of three times per week or more regularly as deemed clinically appropriate by the investigator.
    - Must be collected on days 1 and 36 prior to treatment to ensure guidelines detailed in section 11.10.1 & 11.10.2 are met.
  - Biochemistry three times per week
    - including urea, creatinine, bilirubin, albumin, uric acid, liver enzymes including LDH, lipase, amylase. This must include days 1 and 36.
  - Coagulation profile minimum of twice per week during phase I only
    - including APTT, INR, Fibrinogen, ATIII
  - Central laboratory tests as specified in Appendix 5 (on day 1 and day 15).

- Complete Physical Examination at the end of protocol (d50) including (but not limited to):
  - oral examination,
  - temperature,
  - blood pressure, heart rate, respiratory rate
  - weight
  - assessment of ECOG performance status (see Appendix 1)

- Adverse events should be collected throughout the whole protocol. An adverse event review should occur at the end of the protocol (d50)

- ECG and left ventricular function assessment before 1st doxorubicin dose (day8)

13.2.6Maintenance

- BSA calculation on day 1
- HRQOL on day 1 prior to treatment, then at 3, 6,12 months and at the end of maintenance treatment
- Bone marrow aspirate and trephine biopsy on day 1 for
  - morphology.
  - central laboratory tests as specified in Appendix 5 (day 1)
- Blood samples for the following tests
  - FBE with differential minimum every 4 weeks
  - Biochemistry minimum every 12 weeks
    - including urea, creatinine, bilirubin, albumin, uric acid, liver enzymes including LDH, lipase, amylase
  - Central laboratory tests as specified in Appendix 5 (day 1).
- Complete Physical Examination at the end of maintenance including (but not limited to):
  - oral examination,
  - temperature,
  - blood pressure, heart rate, respiratory rate
  - weight
  - assessment of ECOG performance status (see Appendix 1)
- Adverse events should be collected throughout the whole protocol. An adverse event review should occur at the end of each cycle

13.2.7Allogeneic HCT

- HRQOL prior to conditioning and upon discharge from hospital after allogeneic HCT, and at 3 and 6 months after discharge from allogeneic HCT admission
- Bone marrow aspirate and trephine biopsy immediately prior to conditioning for AlloHCT and 4 to 6 weeks after AlloHCT (prior to discharge from hospital) and at 3 months after discharge from hospital for alloHCT
  - morphology.
  - central laboratory tests as specified in Appendix 5
- Blood samples for the following tests immediately prior to conditioning for Allogeneic HCT and at 4 to 6 weeks after AlloHCT (prior to discharge from hospital) and at 3 months after discharge from hospital for allogeneic HCT
  - Central laboratory tests as specified in Appendix 5.

13.2.8Relapse

- Bone marrow aspirate and trephine biopsy
  - morphology.
  - central laboratory tests as specified in Appendix 5
- Blood samples for the following tests
  - Central laboratory tests as specified in Appendix 5.

13.3 Follow-up Assessments

The following assessments will occur monthly for 12 months, then 2 monthly for another 12 months after the end of treatment. Please note end of treatment is when the patient comes of study treatment for whatever reason. If the patient does not complete the protocol treatment in full, follow up is still required, unless the patient has withdrawn consent from any further follow up.

- Clinical assessment including measurements to assess objectives of study which include:
  - Complete Physical Examination at the end of maintenance including (but not limited to):
    - oral examination,
    - temperature,
    - blood pressure, heart rate, respiratory rate
    - weight
    - assessment of ECOG performance status (see Appendix 1)
  - Late emerging toxicities – adverse events related to ALL06 protocol treatment should be collected throughout the follow up period.
- Full blood count

In addition the following fertility assessments will occur during follow up:

For women:
Follicle Stimulating Hormone (FSH), Luteinising hormone (LH), estradiol 3 months after the end of therapy, then annually for 5 years

For men:
Semen analysis at the end of therapy, then annually until normal.

13.4 Long Term Data Sweep

A data sweep for all patients will be conducted at two time points, dates to be confirmed by the ALLG Trial Centre:

- when the last patient recruited completes day 94 and
- when all patients have completed 2 years post treatment follow up.

The data sweep will include an update on patient's survival status, disease status, whether the patient proceeded to transplant and if they have been diagnosed with a second primary malignancy.

14.Measurement of Response

14.1 Definition of Complete Remission (complete response)

- no morphological evidence of leukemic cells in the peripheral blood, and <5% blasts in the bone marrow aspirate
- no evidence of extramedullary disease.

14.2 Definition of Relapse

Leukemic relapse is defined as the presence of identifiable leukemic cells in the blood, more than 5% blasts in the bone marrow aspirate, or evidence of extramedullary leukaemia.

15.Statistical considerations

15.1 Endpoints

15.1.1Primary endpoints

The primary end-point will be the proportion of patients starting protocol M by day 94 after commencing therapy, or in the case of patients stratified to receive high risk treatment, the start of HR block 1.

15.1.2Secondary endpoints

1. CR rate at timepoint 1 (~day 33) and timepoint 2 (~day 79)
2. OS and DFS at minimum of 2 years post-ceasing therapy.
3. Incidence of grade 3 & 4 toxicities
4. Proportion of patients in all risk categories.
5. MRD levels measured by flow and molecular cytometry techniques.
6. Impact of treatment on physical, functional, emotional and social wellbeing via HRQOL assessment at the beginning and end of each phase of treatment.
7. Fertility assessments after treatment

15.2 Sample size calculation

The primary endpoint is the proportion of patients commencing protocol M, or HR1, of the regimen by day 94, after completion of the first 2 blocks of initial chemotherapy.

In the Australian and New Zealand Children's Cancer Study Group ALL Study 8, using the same treatment protocol in 210 children under the age of 12 years with ALL, the mean and median time taken to start Protocol M was 94 days, with a range of 70-129 days. It is anticipated that it will take a similar time to reach this protocol landmark in adolescents and young adults with ALL, therefore, it is expected that 50% of patients will start protocol M by day 94.

A two-sided 95% confidence interval (CI) will be calculated for the primary endpoint. The regimen will be considered satisfactory for ongoing study if the two-sided 95% CI is entirely above 35%.

With 100 subjects recruited, the probability that the observed 95% confidence interval is entirely above 35% is greater than 80% when the true proportion commencing Protocol M on or before day 94 is greater than or equal to 50% (equivalent to the previously observed paediatric results).

The sample size of 100 patients, means that based on an estimated incidence of 30 cases in Australia annually, there will be an accrual period of approximately 3 years.

15.3 Statistical Analysis Plan

As well as the calculation of a two-sided 95% confidence interval for the commencement rate for protocol M or HR1 by day 94, the distribution of the time taken to complete protocol M, measured from the date of day 1 of ALL06 protocol treatment to the date of last drug administered on protocol M, will also be estimated using the (Kalbfleisch and Prentice) non-parametric estimator of the cumulative incidence function. Both these endpoints will be summarised by risk stratification groups as appropriate and compared with existing data from paediatric ANZCHOG study 8.

DFS and OS will be estimated using the Kaplan-Meier product-limit method. DFS will be measured from the date on which remission (defined in section 14.1) is first documented until the date of relapse (defined in section 14.2) or the date of death, for those patients who die in remission. OS will be measured from the date of day 1 of ALL06 protocol treatment. Both survival analyses will use a close-out (study-censor) date defined at the time of analysis as the latest of the dates of last follow-up for the patients not known to have died and not deemed lost to follow-up. Kaplan-Meier product-limit estimates will also be made for the risk stratification

groups independently. DFS and OS will be estimated on two occasions. When all patients have commenced day 1 of Protocol M (or if commencing high risk therapy, day 1 of HR-1) and 2 years from all patients ceasing treatment.

The proportion of patients reaching morphological CR by time point 1 (~day 33) and time point 2 (~day 79) will be summarised with exact 95% confidence intervals and will be defined within risk groups as appropriate. The proportions of patients with standard, medium and high-risk disease will be compared for AYA patients registered on this study with pediatric patients registered on the ANZCCSG Study VIII using exact methods for two-way contingency tables. Exact methods for the multinomial distribution will also be used to calculate 95% confidence intervals for the proportions of AYA patients in each risk group.

The level of residual disease after induction chemotherapy (at ~day 79) will be summarised by reporting the mean, standard deviation, observed quartiles (lower quartile, median and upper quartile) and the minimum and maximum.

The percentage of the protocol defined dose received by each patient will also be summarized overall and by risk stratification group. This will be summarized as the median, range and IQR and additionally, as the percentage of patients within percentage band categories of the protocol defined dose.

Laboratory derived measures from blood and bone marrow samples will be correlated with clinical features at specific times using Spearman's and Pearson's correlation coefficients. Clinical measures include factors used to determine risk stratification, age, gender, treatment received (protocol M vs HR), ECOG at screening, CNS involvement, extramedullary involvement. These measures may also be related to CR, DFS and OS outcomes using logistic regression and Cox proportional hazards models.

The physical, functional, emotional and social wellbeing as assessed by the HRQOL per section 18 of this protocol, will be summarised descriptively using means, medians, standard deviations, and ranges. The changes during each treatment phase will be statistically assessed overall and within risk groups using paired t-tests or Wilcoxon signed-rank tests as appropriate.

Baseline demographic and clinical features (including factors used to determine risk stratification, age, gender, treatment received (protocol M vs HR), ECOG at screening, CNS involvement, extramedullary involvement) will be summarised for all subjects using standard descriptive statistics including frequencies, percentages, means, medians, standard deviations, ranges and quartiles. These summaries may also be presented separately for each risk group.

The incidences and percentages of subjects with AEs will be tabled by preferred terms, severity and relatedness classes as appropriate and further by risk stratification groups.
Fertility data will be listed in tables and may be presented separately for each risk group.

15.3.1Analysis populations

The analyses of efficacy and safety will be undertaken on the intent to treat (ITT) population which will include all subjects recruited into the study, irrespective of how compliant they are with the treatment protocols, but will not include any patients who subsequent to recruitment are found to violate any inclusion or exclusion criterion. The actual sample sizes may vary across the endpoint analyses depending on the availability of each endpoint.

15.3.2Missing data

Missing data will not be imputed or estimated, so that the sample size for some analyses (e.g. those involving risk stratification groups or residual disease levels) may not involve 100 subjects. Only verified follow-up status will be used for determining patient status (as defined above) in the Kaplan-Meier survival estimates.

15.3.3Main analysis

Accrual of the 100 patients is likely to take between 3 and 3.5 years. Accrual is expected to commence on 1 September 2011and accordingly the last patient to commence Protocol M is likely to do so in or before July

2016

Analysis of the primary endpoint (the percentage of patients who commence Protocol M or HR1 on or before day 94) will commence when the last patient in commences day of protocol M or for those receiving high risk therapy, day of 1 of HR-1 and will be presented, together with information on the morphological CR rates and the proportions of AYA patients in each risk group, the level of residual disease after induction and the factors associated with treatment compliance, at an international forum. Analyses of DFS and OS will be included in the main analysis with data up until the LPI commences day 1 of protocol M or day 1 of HR-1 if commencing high risk therapy.Sub group analyses of the primary endpoint, morphological CR, DFS and OS will be undertaken comparing outcomes between risk stratification groups, transplanted and not transplanted patients and also by protocol-defined dose compliance groups.

A final follow up analysis will be completed on DFS and OS which will include data up until all patients have completed 2 years follow up post treatment.

15.4 Premature termination of the study

The Trial Committee will meet by teleconference every 3 months to monitor the progress of the study. Data will be reviewed at each meeting on accrual, serious adverse events and deaths.

Specifically, the TMC will review data, and consider early termination of the trial, according to the following guidelines:

- Inadequate recruitment: less than 20 patients per year once a consistent accrual rate has been established;
- if the induction death rate appears unacceptably high, exceeding 4 of the first 20 patients or exceeding 12 of the first 50, ensuring with 95% confidence that the true underlying rate is not greater than 34% .
- if the death rate in HR blocks of treatment appears unacceptably high, exceeding 3 deaths in the first 10 patients to commence HR treatment, or exceeding 4 of the first 25, excluding deaths from subsequent Bone Marrow Transplant ensuring with 95% confidence that if the study does not stop at either of these reviews then the true underlying mortality is <33%.

16. Adverse events

16.1 Adverse Event Definitions

16.1.1 Adverse Event

An AE is defined in ICH GCP guidelines as an untoward medical occurrence in a trial participant. AEs are signs (including an abnormal laboratory finding), symptoms or diseases that are 'temporally associated' (occur within a related time period) to a medical treatment or procedure. There may or may not also be a causal relationship. AEs can be significant enough to lead to important changes in a trial. The Investigator and site staff are responsible for detection, recording and reporting of events that meet the criteria and definition of:

- a grade 3 or grade 4 or grade 5 AE or
- an SAE or
- grade 2 + avascular necrosis (G2-5), or
- grade 2 + peripheral neuropathy (G2-5) or
- grade 2 + thrombosis events (G2-5)

from time of signed consent until 30 days after the administration of the last study treatment (including alloHCT). If possible, a diagnosis should be reported (e.g. pneumonia, rather than cough, chest pain and fever).

Pre-existing events, which increase in frequency or severity or change in nature during or as a consequence of use of a drug in human clinical trials, will also be considered as adverse events, with the exception of the disease under study.

An AE does not include:

- medical or surgical procedures (e.g. surgery, endoscopy, tooth extraction, transfusion); the condition that leads to the procedure is an AE
- pre-existing diseases or conditions present or detected prior to start of study product administration, that do not worsen
- situations where an untoward medical occurrence has not occurred (e.g. hospitalisation for elective surgery, social and/or convenience admissions)
- overdose of either study product or concomitant medication without any signs or symptoms unless the subject is hospitalised for observation.

Laboratory abnormalities are only considered AEs if they fulfil one of the following criteria:

- Accompanied by clinical symptoms;
- Leading to a change in study medication (e.g. dose modification, interruption or permanent discontinuation);
- Requires a change in concomitant therapy (e.g. addition or change in a concomitant medication, therapy or treatment).
- Unexpected toxic side effect of treatment

Any medical condition or clinically significant laboratory abnormality with an onset date before the first date of study product administration is considered to be pre-existing, and should be documented in the CRF as medical history.

16.1.2 Serious Adverse Event

AEs and adverse drug reactions are considered ‘serious’ if they threaten life or function. Due to the significant information they provide, SAEs (including Serious ADRs) require expedited reporting. SAEs are defined as any AE or adverse drug reaction which:

- Results in death

- Is life-threatening (i.e. grade 4 CTC AE)
- Requires in-patient hospitalisation or prolongation of existing hospitalisation
- Results in persistent or significant disability/incapacity, or
- Is a congenital anomaly/birth defect
- Other medically relevant condition judged as serious

The term 'life-threatening' in the definition of 'serious' refers to an event in which the participant was immediately at risk of death at the time of event. It does not refer to an event which hypothetically might have caused death if it were more severe. However, important medical events may be considered as a SAE if they require medical or surgical intervention to prevent one of the listed definitions, e.g. an 'allergic bronchospasm' which required intensive treatment in an emergency room or at home.

An event that results in hospitalisation or prolongs an existing hospitalisation will not be considered a SAE if the only reason for the hospitalisation or prolongation was:

- administration of chemotherapy
- administration of trial procedures
- placement of a permanent intravenous catheter
- hospice placement for terminal care
- pre-trial scheduled elective surgery
- out-patient hospitalisation for procedures such as:
- elective day surgery
- convenience purposes (eg. transportation difficulties)
- admission for insertion of PEG tube or naso-gastric tube for commencement of enteral feeding

Complications that occur during hospitalisations are AEs. If a complication prolongs hospitalisation, it is a SAE.
"Other medically relevant condition judged as serious" is where medical and scientific judgment should be exercised in deciding whether expedited reporting is appropriate in other situations, such as important medical events that may not be immediately life-threatening or result in death or hospitalisation but may jeopardise the patient or may require intervention to prevent one of the other outcomes listed in the definition above. These should also usually be considered serious. Examples of such events are intensive treatment in an emergency room or at home for allergic bronchospasm; blood dyscrasias or convulsions that do not result in hospitalisation; or development of drug dependency or drug abuse. A diagnosis of a new cancer during the course of the treatment should be considered as medically important.

Progression of a patient's underlying condition leading to one of the above should always be reported as a serious (but expected) adverse event, which is either unrelated to protocol treatment, or caused by failure of the anticipated therapeutic effect of the study drugs.

16.2 Adverse Event Grading and Causality

AEs will be identified during each visit using criteria established by the National Cancer Institute Common Terminology Criteria for Adverse Events (CTCAE version 4. The current CTCAE version can also be downloaded from the CTEP website (http://ctep.cancer.gov). Where parameters are not addressed within the criteria, severity of AEs should be graded as:

Mild	Aware of sign or symptom, but easily tolerated
Moderate	Discomfort enough to cause interference with usual activities

Severe	Incapacitating with inability to work or perform usual activities
Life-threatening	Participant is at immediate risk of death
Fatal	Death

16.2.1Expected and Unexpected Adverse Events

An 'expected AE' is a known possible toxicity consistent with current available information (eg. drug product information or Investigator's Brochure). An 'unexpected AE' is the occurrence of an unknown toxicity or an event of greater severity or specificity than indicated in the available information (including the protocol, Investigator's Brochure or Informed Consent Form)

16.2.2Relationship to Study Treatment

The site investigator will initially assess each AE for the relationship between study intervention and the event. A summary of the grading is as follows:

Not suspected:	The temporal relationship of the AE to study drug administration makes a causal relationship unlikely or remote, or other medications, therapeutic interventions, or underlying conditions provide a sufficient explanation for the observed event
Suspected:	The temporal relationship of the AE to study drug administration makes a causal relationship possible, and other medications, therapeutic interventions, or underlying conditions do not provide a sufficient explanation for the observed event.

16.3 Adverse Event Reporting

16.3.1Adverse Event Reporting

AEs must be reported on the relevant trial CRFs. Documentation of an AE requires specific information regarding the sign, symptom or disease.

There are circumstances where AEs will not be reported. For this trial they are as follows:

- signs or symptoms associated with the disease or disorder under study, unless they are more severe than expected
- admission to hospital due to respite, family or social issues, or for pre-planned treatment

Any medications necessary for treatment of the AE must be recorded in the concomitant medication section of the subject's CRF.

There are some AEs that may necessitate rapid communication to regulatory authorities, such as information that may influence the benefit-risk assessment of a medicinal product, or information that would be sufficient to consider changes in medicinal product administration or in the overall conduct of a clinical investigation. Examples include lack of efficacy of a medicinal product used in treating life threatening disease, an increased rate of occurrence of an expected serious ADR, or a major safety finding from a recently completed safety study. Investigators should apply appropriate medical and scientific judgement in these circumstances, and notify ALLG or designee, if they believe the event requires expedited reporting.

In Australia, site Investigators are responsible for fulfilling any reporting requirements designated by the HREC.

In New Zealand (NZ) all recorded adverse events should be listed, evaluated and discussed by the Investigator and Sponsor, or designee, and included in the final report to the ethics committee and the NZ regulatory authority (Medsafe).

16.3.2Serious Adverse Event Reporting

All SAEs that occur whilst the trial participant is receiving trial treatment are required to be reported whether or not considered related to the treatment under investigation. An SAE must be reported for all events occurring from the time a participant is registered on the Trial until 30 days following the final treatment.

All SAE forms must include the sponsor trial number and the participant's trial registration number.

The PI (or Co Investigator) at the trial site must sign all reports. Should the Investigator not be available to sign the SAE form within the 24 hour period, a comment to this effect must be written on the form and the form faxed without signature. The investigator must sign the form as soon as possible and the SAE form must be re-faxed. The investigator may also be asked to provide follow-up information.

SAEs must be reported by completing the SAE form and FAXING it to the following:

Fax To:	Fax Number:
Trial Coordinating Centre	+61 3 9429 8277

SAE forms are required by the above mentioned parties at the following time points:

Initial report	Within one working day/24 hours of discovery or notification of the event. If the reporting of an SAE is delayed by more than 24 hours, an explanation must be provided in the comments section of the SAE form.
Incomplete initial reports	If all details are not available at the time the initial report a completed report must be sent within the next 10 days.
Updated report	If the event is not resolved (or 'on-going') at the time of the initial report, a new SAE Form must be submitted every 30 days until the event is resolved, death has occurred or the condition has stabilised. If a change occurs in a stable condition (i.e. either worsens or improves), then a new SAE Form should be faxed

The Sponsor, or designee, will review all SAEs for relationships to study treatment and the nature of the event (expected or unexpected). Events deemed to be both serious and unexpected and related to the study intervention must be reported by the TCC to the TGA using a "blue form" ADRAC card.

16.3.3Follow up of Adverse Events

Follow up of SAEs and AEs will continue through the last day on study (including the follow-up, off study medication period of the study), until the investigator and/or ALLG determine that the subject's condition is stable, or up to 30 days after the last dose of Study Product, whichever is longer. ALLG may request that certain AEs be followed until resolution.

16.4 SAE Responsibilities

16.4.1Trial Site

The Trial Site is responsible for:

1. Complying with the SAE reporting guidelines stated by the Protocol

2. Ensuring that any SAEs are reported within 24 hours of notification

3. Informing the responsible HREC of all AEs (including serious ADRs) that occur during the trial in accordance with local requirements.

16.4.2Trial Coordinating Centre

The TCC is responsible for:

1. Implementing and maintaining a suitable record to record information from all SAEs received from Trial Sites.

2. Ensuring that the trial PI is notified of each SAE to enable the SAE to be assessed by the trial PI and any other appropriate reviewers for nature (expected/unexpected), causality and whether the TGA needs to be notified of the SAE.

3. Under the direction of the trial PI, notifying the TGA (Australia) of any ADRs that are fatal and unexpected or life threatening and unexpected as soon as possible but no later than 7 days after the site gained first knowledge of the event. Incomplete reports must be completed and forwarded as soon as possible within 8 additional calendar days. All other serious, unexpected ADRs should be reported to the TGA within 15 days after the site gained first knowledge of the event.[k]

4. Considering information provided by (non-serious) AE data.

5. Under the direction of the trial PI, informing each trial site, and, if appropriate, the TGA, of new information arising from AEs, SAEs and adverse drug reactions that may affect the conduct of the Trial, or the rights, interests, safety or wellbeing of Trial Participants.

6. Under the direction of the trial PI, notifying the TGA of any significant issue that has arisen from analysis of overseas reports or action that has been taken by another country's regulatory authority within 72 hours of first knowledge.

16.5 Procedures to be Followed to Minimise Risks associated with Pregnancy

Foetal abnormalities have been reported following therapy with the study treatments described in this protocol. Females of childbearing potential must take contraceptive measures during study treatment and at least 4 weeks after cessation of study treatment.

---

[k] NZ sites are responsible for fulfilling any reporting requirements specific to NZ.

16.6 Procedures to be Followed in the Event of Pregnancy

The subject must be instructed to inform the investigator IMMEDIATELY if she becomes pregnant during the study, and seek advice regarding discontinuation of study medication. The female should be referred to an obstetrician/gynaecologist experienced in reproductive toxicity for further evaluation and counselling.

17.Data management and quality assurance

17.1 Case Report Forms

CRFs will be supplied by the TCC. Staff at participating Institutions should record data on CRFs as soon after each time point as possible. Originals of completed forms should be returned to the TCC at times requested (refer to CRFs) and a copy of each CRF should be kept at the Trial Site.

A copy of the case report form must be sent at the conclusion of each therapy phase to the TCC (Fax: +61 (0)3 9429 8277).

Trial participants are to be identified by unique identifier codes. Remission status, administration of therapy, reporting of severe therapy-associated side-effects and all other requested information must be entered on the CRFs. Information must be provided in the units indicated. All 'paper' CRFs should be completed in black ink. A correction should be made by striking through the incorrect entry with a single line and by entering the correct information adjacent to it. The correction must be initialled and dated by an adequately qualified and authorised member of the research support team at the Trial Site. If an item is not available or is not applicable, this fact should be indicated; do not leave a space blank.

For eCRFs, trial data must be recorded on the eCRFs provided. All required data entry fields must be completed. Data corrections will be done according to the instructions provided. The investigator will be asked to confirm the accuracy of completed eCRFs by signing key eCRFs as indicated.

The CRFs are confidential and remain the property of the ALLG.

17.2 Essential documents

Essential trial documents to be maintained at the trial site include, but are not limited to:

- HREC- approved study protocol and amended versions
- all source documents and laboratory records
- Sample CRF and completed CRF copies
- HREC-approved PICF and amended versions
- HREC membership list
- Any communication with the HREC
- Current version of the IB for the study drug and current PI for drugs that are already approved
- Laboratory reference ranges and accreditation
- Drug accountability logs
- Protocol deviation logs
- Staff curriculum vitae and training logs
- Signature sheet and delegation of responsibilities log
- Copies of PICF for each subject

Source documents pertaining to the trial must be maintained by investigational sites. Source documents may include, but are not limited to, a subject's medical records, hospital charts, clinic charts, the investigator's subject study files, treatment prescriptions, treatment administration sheets, X-rays, CT scans and laboratory tests.

Archiving requirements for study-related documentation are described in Section 17.6.

17.3 Confidentiality

The study will be conducted in accordance with applicable Privacy Acts and Regulations. All data generated in this study will remain confidential. All information will be stored securely and will only be available to staff directly involved with the study. Personal data identifying trial subjects will be held securely for the purpose of follow up after the conclusion of the protocol-specified period.

Trial participants will be allocated a unique identification (ID) number. The master list linking identifying participant information and ID number will be maintained in a secure location separate from the participant database. Analysis of trial-related data and all ongoing queries will be via trial participant ID number only. Each institution will maintain a list of its own trial participants. Data will be analysed by ID number and initials. Samples for laboratory scientific studies will also only be analysed by ID number and initials.

The central laboratories involved with sample processing may record the initials, trial ID and birth date of each participant contributing samples for ALL06 study research. This information is to enable retrieval of samples after study completion, should the patient later decide to withdraw samples. Access to trial-related information in the analysis of subsequent research studies utilising ALL06 samples will be subject to approval by the ALLG and the study PI(s), and will not enable identification of the trial participant.

Copies of any patient reports that are to be provided with CRFs MUST be de-identified and then clearly labelled with the trial patient identifier.

17.4 Database management and quality control

Clinical trial databases will be maintained by the TCC and will not record any information that may enable the subject to be identified. The CCT will conduct all data queries with individual trial sites. Once all data has been entered and verified, the database will be locked and the final analysis performed.

17.5 Site visits, monitoring and audits

17.5.1Site visits and monitoring

For all ALLG studies, the ALLG Chairperson and Bio-statistician must be satisfied of the integrity of the processes in place for data collection, checking, analysis and archiving.

All sites used in ALLG trials undergo an accreditation process encompassing assessment of clinical facilities, availability of qualified staff and evidence of access to an HREC and at least one ALLG member who has a significant clinical presence at the institution. Before a site is activated for a specific clinical trial, the site investigator, or someone able to sign on behalf of the institute, must undertake a commitment to assure access is available to resources for study participation, and that the study will be conducted in accordance with the protocol and GCP.

If required, ALLG or its designee must be able to directly inspect source documents, CRFs and other study-related documents at each trial site to verify that the trial is being run according to local ethical and regulatory guidelines and to verify that data recorded in the CRF is correct. ALLG, its designee or a representative of the supporting pharmaceutical company must be able to view drug accountability records, and drug storage and dispensing facilities.

Ongoing annual review of all active ALLG clinical trials is conducted by the ALLG SDMC (See section 20.7).

17.5.2Audits

This study may be subject to audit or inspection by representatives of the ALLG or designee, or representatives of regulatory bodies (e.g. TGA).

17.6 Document retention

Records from the study (both hard copy and electronic) will be retained for a minimum of 15 years after study completion in secure archiving facilities both at the trial site and at the Trial Centre. The Investigator or Trial Centre must notify the ALLG prior to destroying any clinical study records. Should either the Investigator or Trial Centre wish to assign the study records to another party or move them to another location, ALLG must be notified in advance.

If the investigator cannot guarantee this archiving requirement at the study site for any or all of the documents, special arrangements must be made between the investigator and ALLG to store these in sealed containers off site so that they can be returned sealed to the investigator in case of a regulatory audit.

18.Health-Related Quality of Life (HRQOL)

18.1 Measures

This trial is an ideal opportunity to promote the collaborations between the ALLG, Psycho Oncology Group (PoCoG), and the Paediatric Oncology Groups (ANZCHOG). Knowledge derived from researching Paediatric intensity regimens in the young adult population, via the selection of modules from the FACT suites of HRQOL questionnaires, will inform the basis for future AYA trials.

To research the specific impacts on physical, social and emotional wellbeing from the intense treatment regimen that the patients of this study will be exposed to, an exclusive number of questions have been assembled from the Functional Assessment of Chronic Illness Therapy (FACIT) suite. The precise questions and validated response scales have been selected from the following:

- the 27 items from the generic core module, FACT-G (Version 4), which cover the four core HRQOL domains: Physical Wellbeing (7 items), Social/Family Wellbeing (7), Emotional Wellbeing (6), and Functional Wellbeing (7)
- the 17 Additional Concerns items from the leukemia module, FACT-Leu (Version 4), which cover: fevers, chills, and night-sweats (3 items); infections (1); lumps and swelling (1); pain (1), bleeding and bruising (2); weight loss and appetite (2); fatigue (3); worries about future health (2); feelings of isolation (1); emotional ups and downs (1);
- the 11 Additional Concerns items from the neurotoxicity module, FACT-GOG-NTX (Version 4), which cover: peripheral neuropathy (7 items); tinnitus (2); joint pain/ muscle cramps (1); fatigue (1).
- A further 12 FACIT items are included to cover the remaining important issues in this clinical context: gastro-intestinal symptoms (7 items: swelling/cramps in stomach, control of bowels, diarrhea, digestion, eat solid foods, pain in mouth/throat/neck, mouth sores,); worries about low blood counts (3); worries about infection and delayed treatment (2).

This composite HRQOL measure is included in Appendix 6. All of these items have to the time frame of the past seven days, and five point response scale (not at all, a little bit, somewhat, quite a bit, very much). Completion time is estimated at approximately 10 minutes.

The FACT-G has previously been validated[26], and found to be reliable (Cronbach's alpha for total score = 0.89; for four domain subscores range 0.65-0.82). FACT-Leu has been shown to be valid and reliable[28], with Cronbach's alpha values for the eight possible summary scores in the range 0.77-0.94). FACT-GOG-NTX has been shown to be valid and reliable, with Cronbach's alpha values for the seven of the eight possible summary scores in the range 0.78-0.90, with only the social well-being score having a relatively low reliability coefficient (0.51) in this particular study in ovarian cancer).

The remaining 12 items have been selected from four modules and shown to be valid within those modules: FACT-N (Neutropenia), FACT-C (Colon)[29], FACT-H&N (Head and neck)[30], FACT-B (Breast)[31]. These items have not been combined previously; this aspect of the HRQOL assessment is therefore exploratory. We are currently corresponding with the FACIT administration centre about the use of data collected in this trial to validate this selection of items in this context.

18.2 Administration

- The HRQOL questionnaires should be administered according to the FACIT Measurement System manual. That is, the research nurse should instruct the patients in the following way:

- Patients should be instructed to complete the questionnaire by themselves, without the input of accompanying relatives, friends or clinic staff.
- Patients should be instructed to read the brief directions at the top of the page. After the patient's correct understanding has been confirmed, s/he should be encouraged to complete every item in order without skipping any. Some patients may feel that a given question is not applicable to them and will therefore skip the item. **Patients should be encouraged to circle the response that is most applicable.** If, for example, the patient is not currently receiving any treatment, the patient should circle "not at all" to the question "I am bothered by side-effects of treatment".
- HRQOL questionnaires can be completed in the clinic while patients wait to see their managing clinician, or while patients waiting for treatment, or while receiving chemotherapy if patients find this feasible. If a patient has difficulty reading or writing, the research nurse may provide assistance reading the questions or recording the patient's responses, but s/he must take care not to influence the patient's responses.
- The importance of good compliance to HRQOL questionnaire assessment cannot be over-emphasized. Missing HRQOL data cause difficulties during analysis and may compromise the interpretation of the results.
- HRQOL questionnaires should be thoroughly checked for completeness by the research nurse whilst patient is present; any missed items should be checked with the patient before s/he leaves the clinic, and an response recorded whenever possible.
- If patients do not wish to complete a questionnaire at any of these times, or if a patient does not complete a HRQOL questionnaire because s/he did not attend clinic, the research nurse should record the reason for non-completion on the HRQOL Checklist Form that can be found in 0.
- A contact-person who will be responsible for the correct administration of the HRQOL questionnaires, and for completion and accuracy of HRQOL Checklist forms should be identified at each centre.
- The above guidelines should be provided, in hardcopy form, to all sites where HRQOL questionnaires will be for administered.

18.3 The timing of HRQOL assessments

HRQOL assessments will occur at the beginning and end of each treatment block, that is:

- Beginning of induction Protocol I;
- Beginning of consolidation Protocol I;
- End of consolidation Protocol I (~day 64)
- Beginning of each high risk block;
- Beginning of Protocol II;
- Beginning of Maintenance
- Beginning and end of alloHCT.

In addition to these, QOL will be assessed

- at 3 and 6 months after discharge from hospital for alloHCT;
- during maintenance at 3, 6 and 12 months, then at end of maintenance.

18.4 Statistical considerations - Quality of Life

18.4.1Scoring QoL scales

The FACIT modules will be scored according to the FACIT Measurement System manual. Specifically:

- the 27 items of the FACT-G (Version 4) summarise as four subscales (Physical Wellbeing (7 items), Social/Family Wellbeing (7 items), Emotional Wellbeing (6 items), and Functional Wellbeing (7 items)) and a total HQL score;
- the 17 Additional Concerns items from the leukemia module, FACT-Leu (Version 4) summarise as a total score, and are added to the Physical and Functional Wellbeing items of the FACT-G to make the Trial Outcome Index Leukemia (TOI-Leu);
- the 11 Additional Concerns items from the neurotoxicity module, FACT-GOG-NTX (Version 4), summarise as a total score, and are added to the Physical and Functional Wellbeing items of the FACT-G to make the Trial Outcome Index Leukemia (TOI-Ntx).

Further to this standard scoring, as an exploratory analysis:

the additional 12 LL-specific Additional Concerns items will be summarised as a total score;

all 40 Additional Concerns items will be summarised as a total score, and added to the Physical and Functional Wellbeing items of the FACT-G to make the Trial Outcome Index Lymphoblastic Leukemia (TOI-LL);

For all scales, a higher score indicates higher levels of wellbeing, lower levels of symptoms, and better quality of life.

18.4.2Imputation of missed items

Missing items will be pro-rated as per the FACIT Measurement System manual. That is, as long as more than 50% of the items in a scale were answered, the missed items as imputed as the average of the answered items. If less than 50% of the items in a scale were answered, the scale will be recorded as missing.

18.4.3HRQOL hypotheses:

The HRQOL analysis is descriptive in nature. The following patterns are expected for Physical Wellbeing (PWB), Emotional Wellbeing (EWB), and Functional Wellbeing (FWB) and the sum of the Additional Concerns items (AC). These expectations are expressed as levels of wellbeing: H = high; M = moderate; L = low; VL = very low.

	FACT Domain Scale			
Treatment Phase	PWB	EWB	FWB	AC
Diagnosis	M	L	L	L
End of induction of Protocol I	L	L	L	L
End of consolidation of Protocol I	VL	VL	VL	VL
End of Protocol M;	M	M	M	M
End of Protocol II	VL	VL	VL	VL
End of each high risk block	VL	VL	VL	VL
End of alloHCT	VL	VL	VL	VL
During maintenance period				
3 monthly to 12 months	H	M	H	H
At end of maintenance	H	M	H	H

Since the other summary scores are simple summations of these domains scores, expectations for those are not tabulated, but can be implied.

No hypotheses are proposed for the Social/Family Wellbeing (SWB) scale, as this is expected to be highly variable among patients, with no systematic change expected.

18.4.4Analysis plan

Missing data

The rates of missed HRQOL assessments will be calculated for each phase of treatment, and reasons for missing data will be summarised. Within each treatment phase, the demographic and clinical characteristics of patients who do not complete HRQOL questionnaires will be compared with those that do. Similarly, for patients who complete HRQOL questionnaires at the beginning but not the end of a treatment phase, their demographic and clinical characteristics will be compared with those of patients that complete both HRQOL assessments, and their baseline HRQOL scores will be compared with those of patients who complete both HRQOL assessments. For continuous variables, if the comparison includes a subgroup less than 10 patients and/or the distribution of the variable is non-normal, Wilcoxon rank sum test will be used; otherwise a two-sample t-test will be used. For categorical variables, a chi-square test will be used, or Fishers exact test in cases where there are less than five patients in a cell. For each analysis, a type 1 error rate of 5% will be accepted.

Summary HRQOL scores by treatment phase

The mean and standard deviation of HRQOL scores of patients who complete HRQOL questionnaires will be calculated for each assessment time point, and tabulated and graphed by treatment phase. The HRQOL scores of patients who complete HRQOL questionnaires at the beginning but not the end of a treatment phase will be analysed separately from those of patients who complete both HRQOL assessments, and interpreted in light of the results of the missing data analysis. Confidence intervals (95%) around mean mean HRQOL scores will be calculated and plotted on graphs. The sample size per treatment phase and time point will be tabulated under graphs.

Power

Power calculations have not been attempted for the following reasons:

1. The analyses are descriptive.
2. Given the complex treatment protocol, and its novel application in this patient group, we cannot anticipate the number of patients who will enter and complete various phases of treatment.
3. Given that HRQOL assessment has not been reported for this patient group, we cannot anticipate missing data rates; this adds further uncertainty to the sample sizes in each treatment phase.

19.Ethical considerations

19.1 Ethical Principles

This Protocol has been designed to comply with the Declaration of Helsinki and any subsequent amendments, the ICH Guidelines for Good Clinical Practice (CPMP/ICH/153/95) annotated with TGA comments (July 2000), the NHMRC National Statement on Ethical Conduct in Research involving Humans (2007), the policies and procedures of ALLG and any applicable local guidelines.

At each trial site the trial will be conducted in compliance with the Protocol, ICH GCP Guidelines in Australia, the Interim Good Clinical Research Practice Guidelines in New Zealand issued by Medsafe and applicable regulatory requirements.

19.2 Regulatory Requirements

If applicable, a CTN form must be submitted to the responsible HREC and returned to the TCC. It is the responsibility of the investigator to not enter patients onto the trial before CTN acknowledgment is received from the TGA and all other documentation is completed as instructed by the TCC.

In NZ, all clinical trial applications that use a drug that is not yet registered at that particular dose, or for that particular indication, require review by SCOTT and Regional Ethics approval. Each clinical trial site must also ensure that any additional requirements of their own HREC have also been fulfilled prior to recruiting any patients to that site.

19.3 Informed Consent

A generic PICF, written in non-technical language, will be provided by the TCC to all sites, and will contain all the information that ALLG is legally obliged to supply to all patients interested in participating in the trial. This PICF can be modified to suit individual sites but any changes must be approved by the TCC prior to submission to the responsible HREC for approval. Consent for correlative studies is implicit in consent for trial participation and does not require a separate consent form.

Prior to the commencement of any study-related procedure (e.g Screening tests to fulfil eligibility criteria), the investigator must obtain written informed consent from each participant. The investigator must explain to each subject (or legally authorized representative) the nature of the study, its purpose, the procedures involved, the expected duration, the potential risks and benefits involved and any discomfort it may entail. Each subject must be informed that participation in the study is voluntary, that he/she may withdraw from the study at any time and that withdrawal of consent will not affect his/her subsequent medical treatment or relationship with the treating physician. In addition, the patient should be informed that participation in the trial includes consent to appropriate regulatory authorities and representatives of the Sponsor or sponsoring pharmaceutical company to inspect patient medical records in order to verify trial-related data. The subject should read and consider the PICF before signing and dating it, and should be given a copy of the signed document. No patient can enter the study before his/her informed consent has been obtained.

19.4 Human Research Ethics Committee

The PI at the Trial Site must submit this protocol, and other appropriate documentation to the responsible HREC. A copy of the letter detailing HREC approval of, or advice regarding, the protocol, must be forwarded to the TCC as soon as possible after it has been received by the Trial Site. The HREC approval/advice letter must include:

- a signature from the Chairperson of the HREC

- the date of HREC review
- the trial title
- the protocol number, date and version
- the name, date and version of all other trial related documents such as the PICF
- the length of Protocol approval, if applicable
- the requirements for trial progress report submissions (eg. annual).

19.5 Confidentiality and privacy

All information regarding trial participants must be treated in strict confidence. Data that identify any trial participant must not be revealed to anyone not directly involved in the trial or the clinical care of that participant. An exception is where the trial participant has provided written consent for his/her records to be included in source document verification. In this instance, the records may be inspected by (a) a representative of ALLG for the purposes of source document verification or quality audit as stipulated in the ICH GCP Guidelines, or (b) a representative of a government regulatory authority for the purposes of official inspection. Records must be made available for inspection on the understanding that all information relating to trial participants will be treated in strict professional confidence.

The participants will be allocated a unique ID number. The master list linking identifying participant information and ID number will be maintained in a secure location separate from the participant database. Analysis of trial-related data and all ongoing queries will be via patient ID number only. Each institution will maintain a list of its own trial participants. Data will be analysed by ID number and initials. Samples for correlative studies will also be analysed only by ID number.

19.6 Adherence to Protocol

Except for an emergency situation in which proper care for the protection, safety and well being of the trial participant requires that an alternative treatment be used, the trial shall be conducted exactly as described in the approved protocol. It is the responsibility of the investigator to document any protocol deviations in the appropriate log and the subject's CRF, accompanied by a suitable explanation and to satisfy any reporting requirements of their local HREC.

19.6.1Protocol amendments

Any change or addition to this protocol requires a written protocol amendment that must be prepared by the PI(s) in consultation with the ALLG, or designee. All protocol amendments will be reviewed by the ALLG SDMC and the ALLG Scientific Advisory Committee prior to submission to HREC.

All protocol amendments must be submitted to the HREC of all trial sites in accordance with local requirements. Significant changes affecting the safety of subjects, the scope of the investigation or the scientific quality of the study cannot be implemented until approval is obtained. A copy of the written approval by the HREC must be sent to the TCC.

Administrative changes of the protocol are defined as minor corrections and/or clarifications that have no effect on the way the study is to be conducted, or on the safety of the subjects. These administrative changes will be agreed upon by ALLG, or designee, and the PI(s), and will be documented in a memorandum and

disseminated to all trial sites. The investigator at each site will then notify the HREC of such administrative changes.

19.7 Safety and Data Monitoring Committee

The ALLG SDMC is responsible for:

- conducting a clinical review of all SAEs
- in the event of a significant incidence of SAEs, giving consideration to amending the trial.
- presenting summary reports, inclusive of SDMC comments and recommendation.
- Annual review of all ongoing trials, with particular reference to stopping rules. This study is to be reviewed six monthly by the ALLG SDMC
- Review of all protocol amendments

20.Publication and presentation policy

20.1 Reporting of results

Access to data during the trial will be limited to the ALLG SDMC and appropriate regulatory bodies. The primary analysis of trial results for publication, and any interim analyses, will be performed by a qualified statistician approved by the ALLG. The primary trial results will be published by the Principal Investigator after completion of the final report.

Acknowledgment of ALLG is required in all publications, abstracts and presentations. Publications must be provided to the ALLG for review and approved prior to submission.

Any formal presentation or publication of data from this trial will be considered as a joint publication by the investigators, in conjunction with the ALLG. For multicentre studies, it is mandatory that the first publication is based on data from all centres. Investigators participating in multicentre studies agree not to present data gathered from one centre or a small group of centres before the full publication, unless formally agreed to by all PIs and the ALLG.

Trial registration -
ALLG, or designee, is responsible for registering all trials with an appropriate clinical trials registry prior to the accrual of the first patient. All ALLG trials are registered at the Australian and New Zealand Clinical Trials Registry (ANZCTR) www.anzctr.org.au.

21.Reference List

1. Pui, C-H, Evans WD. Treatment of acute lymphoblastic leukaemia. New Engl. J. Med., 354: 166-178, 2006.
2. Nachman, JB, Sather HN, Sensel NG, et al. Augmented post-induction therapy for children with high-risk acute lymphoblastic leukaemia and a slow response to initial therapy. New Engl. J. Med., 338: 1663-71, 1998.
3. Borowitz, M, Devidas M, Hunger S, et al. Clinical significance of minimal residual disease in childhood acute lymphoblastic leukaemia and its relationship to other prognostic factors: a Childrens Oncology Group study. Blood, 111: 5477-85, 2008.
4. Burger B, Zimmermann M, Mann G, et al. Diagnostic cerebrospinal fluid examination in children with acute lymphoblastic leukaemia: significance of low leucocyte counts with blasts or traumatic lumbar puncture. J. Clin. Oncol., 21; 184-8, 2001.
5. Secker-Walker LM, Craig JM, Hawkins JM, Hoffbrand AV. Philadelphia-positive acute lymphoblastic leukaemia in adults; age distribution, BCR breakpoint and prognostic significance. Leukaemia, 5: 196-9, 1991.
6. Aguiar RC, Sohal J, van Rhee F, et al. TEL-AML1 fusion in acute lymphoblastic leukaemia in adults: MRC Adult Leukaemia Working Party. Br J Haematol., 95: 673-7, 1996.
7. Moorman AV, Chilton L, Wilkinson J et al. A population-based cytogenetic study of adults with acute lymphoblastic leukemia.Blood 115: 206-214, 2010
8. Boissel N, Auclere M-F, Lheritier V, et al. Should adolescents with acute lymphoblastic leukaemia be treated as old children or young adults? Comparison of the FRALLE-93 and LALA-94 trials. J. Clin. Oncol., 21: 774-80, 2001.
9. de Bont JM, Holt B, Dekker AW, van der Does-van den Berg A, Sonneveld P, Pieters R. Significant difference in outcome for adolescents with acute lymphoblastic leukaemia treated on paediatric vs adult protocols in the Netherlands. Leukaemia, 18: 2032-5, 2004.
10. Stock W, Sather H, Dodge RK, Bloomfield CD, Larson RA, Nachman J. Outcome of adolescents and young adults with ALL: a comparison of Children's Cancer Group (CCG) and Cancer and Leukaemia Group B (CALGB) regimens. Blood, 96: 467a, 2000.
11. Testi AM, Valsecchi MG, Conter V, et al. Difference in outcome of adolescents with acute lymphoblastic leukaemia enrolled in paediatric (AIEOP) and adult (GIMEMA) protocols. Blood, 104: 1954a, 2004.
12. Ramanujacher R, Richards S, Hann I, et al. Adolescents with acute lymphoblastic leukemia: outcome on UK national pediatric (ALL97) and adult (UKALL XII/E2993) trials. Pediatr.Blood Cancer, 48: 254-261, 2007.
13. Schroeder H, Kjeldahl M, Boesen AM, et al. Acute lymphoblastic leukemia in adolescents between 10 and 19 years of age in Denmark. Dan. Med. Bull. 53:76-79, 2006.
14. Hallbook H, Gustafsson G, Smedmyr B et al. Treatment outcome in young adults and children greater than 10 years of age with acute lymphoblastic leukemia in Sweden: a comparison between a pediatric protocol and an adult protocol. Cancer 107: 1551-1561, 2006
15, Ribera J-M, Oriol L, Sanz J-M, et al. Comparison of the results of treatment of adolescents and young adults with standard-risk acute lymphoblastic leukemia with the Programa Espanol de Tratiemento en Hematologia Pediatric-Based Protocol ALL-96. J Clin Oncol. 26: 1843- 1849, 2008.
16. Huguet F, Leguay T, Raffoux T, et al. Pediatric-inspired therapy in adults with Philadelphia chromosome-negative acute lymphoblastic leukemia: the GRAALL-2003 Study. J Clin Oncol. 27:911-918, 2009.

17. Bruggemann M, Raff T, Flohr T, et al. Clinical significance of minimal residual disease quantification in adult patients with standard risk acute lymphoblastic leukemia. Blood, 107: 1116-1123, 2006.

18. Patel B, Rai L, Buck G, et al. Minimal residual disease is a significant predictor of treatment failure in non T-lineage adult acute lymphoblastic leukaemia: final results of the international trial UKALL XII/ECOG2993 Brit J Haem 148: 80-89, 2010

19. Mortuza FY, Papaioannou M, Moreira IM et al. Minimal residual disease tests provide an independent predictor of clinical outcome in adult acute lymphoblastic leukemia. J Clin Oncol. 20: 1094-1104, 2002.

20. Spinelli O, Peruta B, Tosi M et al. Clearance of minimal residual disease alter allogeneic stem cell transplantation and the prediction of the clinical outcome of adult patients with high-risk acute lymphoblastic leukemia. Haematologica. 92: 612-618, 2007.

21. Uzunel M, Mattsson J, Jaksch M et al. The significance of graft-versus-host disease and pretransplantation minimal residual disease status to outcome after allogeneic stem cell transplantation in patients with acute lymphoblastic leukemia. Blood 98: 1982-1984, 2001

22. Knechtli CJ, Goulden NJ, Hancock JP et al. Minimal residual disease status before allogeneic bone marrow transplantation is an important determinant of successful outcome for children and adolescents with acute lymphoblastic leukemia. Blood 92: 4072-4079, 1998

23. Bader P, Hancock J, Kreyenberg H et al. Minimal residual disease (MRD) status prior to allogeneic stem cell transplantation is a powerful predictor for post transplant outcome in children with ALL. Leukemia 16: 1668-1672, 2002

24. Bader P, Kreyenberg H, Gunter HR et al. Prognostic value of minimal residual disease quantification before allogeneic stem cell transplantation in relapsed childhood acute lymphoblastic leukemia: the ALL-REZ BFM Study Group. J Clin Oncol. 27: 377-384, 2009

25. Smarkova L, Muzikova K, Fronkova E et al. Detectable minimal residual disease before allogeneic hematopoietic cell transplantation predicts extremely poor prognosis in children with acute lymphoblastic leukemia. Pediatr Blood Cancer. 48: 93-100, 2007

26. Cella DF, Tulsky DS, Gray G, et al. The Functional Assessment of Cancer Therapy (FACT) scale: Development and validation of the general measure. Journal of Clinical Oncology, 11: 570-579, 1993.

27. Conter et al. Role of cranial radiotherapy for childhood T cell acute lymphoblastic leukemia with high white cell count and good response to prednisone. J. Clin. Oncol. 15: 2786, 1997

28. Webster K, Chivington K, Shonk C, et al. Measuring quality of life (QOL) among patients with leukemia: The Functional Assessment of Cancer-Therapy-Leukemia (FACT-Leu). Quality of Life Research, 11: 678, 2002.

29. Ward WL, Hahn EA, Mo F, Hernandez L, Tulsky DS, & Cella D. Reliability and validity of the Functional Assessment of Cancer Therapy-Colorectal (FACT-C) quality of life instrument. Quality of Life Research, 8: 181-195, 1999.

30. List MA, D'Antonio LL, Cella, DF, Siston A, Mumby P, Haraf D, & Vokes E. The Performance Status Scale for head and neck cancer patients and the Functional Assessment of Cancer Therapy-Head and Neck (FACT-H&N) scale: A study of utility and validity. Cancer, 77: 2294-2301, 1996.

31. Brady MJ., Cella DF, Mo F. Reliability and validity of the Functional Assessment of Cancer Therapy-Breast (FACT-B) quality of life instrument. Journal of Clinical Oncology, 15: 974-986, 1997.

32. Stock W, Douer D, DeAngelo DJ et al. Prevention and management of asparaginase/pegasparaginase associated toxicities in adults and older adolescents: recommendations of an expert panel. Leukemia and Lymphoma, 12: 2237-2253, 2011.

Appendix 1 ECOG Performance status criteria

As published in Am J Clin Oncol: Oken et al, Toxicity and response criteria of the ECOG. Am J Clin Oncol (1982) 5:649-655

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

Appendix 2 Guidelines for high-dose Methotrexate and folinic acid rescue

Pre-hydration
Patients should be admitted the day before the methotrexate infusion, and given hydration fluids overnight, with 3 L/m^2 /24 hrs of dextrose-saline containing 20 mMol KcL and 40 mMol sodium bicarbonate per litre. Additional bicarbonate may be required in subsequent fluids to maintain urine pH >= 7.0.
Fluid and urinary alkalization protocols for administration of high dose methotrexate can also be given as per institutional guidelines.

High-dose methotrexate infusion
Methotrexate should preferably start at 6 am, to allow plasma levels to be determined during working hours. An alternative start time is 2 pm.
The MTX dose is 5 g/m^2, with:

10% given in the first 30 minutes (loading dose), in 50-100 mls normal saline;
90% given over next 23 hours and 30 minutes, in 250-300 mls saline.

The infusion must be completed in 24 hours; any drug remaining at this time should be discarded. Intravenous hydration fluids at 3 L/m^2 /24 hrs should be continued during this time.

Intrathecal therapy

1. For standard and medium risk patients, methotrexate should be given intrathecally within 2 hours of starting the intravenous infusion, or before the end of the infusion, depending on the time of day started.
2. For high risk patients, intrathecal therapy should consist of 3 drugs: methotrexate, cytarabine, and hydrocortisone, given at similar times as in (1).

Hydration fluids
After the methotrexate infusion is completed, continue IV fluids for at least 30 hours. Urine ph should be maintained at 7.5 or greater during this time. Combined oral and IV intake should remain above 3 L/m^2 /24 hrs until methotrexate levels are below 250 nM/L.

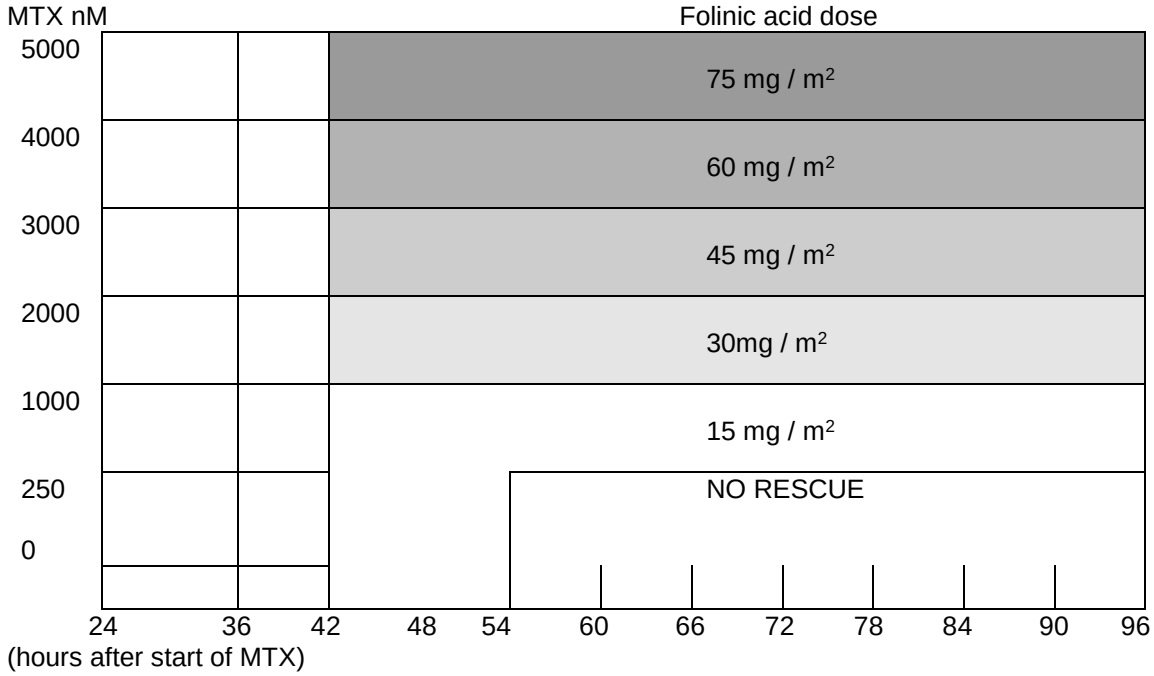
Monitoring of plasma methotrexate levels
Blood for plasma methotrexate levels should be taken at 24, 36, 42, 48, 54 and 72 hrs from the start of the MTX infusion. If necessary, samples should then be collected every 24 hrs until MTX levels are below 250 nM/L.

If the hour 24 level is less than 150,000 nM/L (usual case), then the 36 hr sample can be batched and run with the 42 hr sample.
If the 24 hr level is above 150,000 nM/L, the 36 hr level must be measured, so that a decision can be made about the dose of folinic acid to start rescue at 42 hrs. If the 36 hr level is is <3000 nM/L the 42 and 48 hr levels can be run in the usual manner. If the 36 hr level is >3000 nM/L, folinic acid rescue must be started immediately, at the dose planned for 42 hr.

Folinic acid rescue

1. For patients with a normal excretion pattern (the majority), folinic acid rescue must start at 42 hrs from the start of the MTX infusion. The dose is 15 mg/m^2 given IV at 42, 48, and 54 hrs after the start of the MTX infusion. Continue giving folinic acid at a dose determined by the plasma level until the level is < 250nM/L. If excretion is slow, the folinic acid dose will need to be increased according to the MTX level, as below:

Diagram for calculating Folinic Acid Dose, based on MTX-Level



2. For patients with delayed excretion, if the 42 hr level is >5000 nM/L, folinic acid should be continued every 6 hrs until the level falls below 5000 nM/L, according to the formula: dose in mg = NTX level (nM/L) x weight (kg)/1000, where the MTX level is the one taken 6 hrs before (ie: folinic acid dose at 48 hrs is based on MTX level at 42 hrs). Once the level is below 5000 nM/L, the above diagram is used to calculate doses.
3. If the patients is well and not needing additional folinic acid rescue, it may not be necessary to run the 36 and 42 hr levels, but samples should be taken anyway. If the 48 hr level is > 1000 nM/L, the 54 hr level must be run, together with the 42 hr level, to determine the rate of MTX excretion.
4. If the 54 hr level is less than 250 nM/L, the patient can be discharged without folinic acid. If the 54 hr level is >250 nM/L, the patient can be discharged on oral folinic acid, with a further plasma level to be collected and run the next day.

Appendix 3 DRUG SIDE EFFECTS

DRUG AND HOW GIVEN	SIDE EFFECTS
PEG-L′asparaginase (IM or IV)	Possible allergic reaction such as hives (red, itchy, swollen spots), high or low blood pressure, a type of diabetes (high blood sugar), pancreatitis (swelling of the pancreas with pain in the mid-stomach through to the back that could be very strong, causing vomiting, and could lead to shock with cold, clammy skin, very low blood pressure and kidney failure if not treated), easy bruising of the skin and bleeding from the gums, from other body organs such as the bladder, the digestive tract, and/or the brain. Bone marrow suppression, and liver damage, and pain at the site of injection may occur. In rare cases, it may affect the brain causing confusion, slow Response, and the sensation of seeing or feeling things that are not real.
Cyclophosphamide (IV)	Nausea and vomiting, low blood counts, hair loss, blood in the urine or irritated urinary bladder, lowered sperm count or sterility (rare at doses used in this study). Rarely, children may develop a second form of cancer known as acute myeloid leukemia as a result of treatment with cyclophosphamide. Experience so far suggests that the chance of this happening is very small. However, not enough information has been gathered in children to be able to give an accurate picture. Data are now being collected on all studies using this drug in order to find out the relationship between the drug and second cancers. Your doctor will provide you with new information as it becomes available.
Cytarabine (IV)	Nausea (stomach sickness) and vomiting, mouth sores, pain in the arms and legs, bone marrow suppression, rash, fever.
Intrathecal Cytarabine (IT)	Possible irritation around the brain or spinal cord, rarely causing temporary paralysis (inability to move arms or legs) and brain damage that may result in sluggishness, slow reactions and long-term problems with learning, convulsions, bone marrow suppression, mouth sores, rarely liver damage, headache, backache, and fever.
Daunorubicin or Doxorubicin (IV)	Bone marrow suppression (as described), hair loss, stomach sickness and vomiting, red urine may occur for up to 48 hours after infusion, mouth sores, return of side effects from radiation therapy (skin redness at site), severe skin sores with blistering and bleeding if drug leaks from vein, blotchy skin, watery eyes. May cause heart damage, although the doses to be used in this study are well below the range usually associated with such problems. Heart function will be carefully monitored during treatment.
Dexamethasone (PO)	Same as Prednisolone (below)
Etoposide (IV)	Nausea and vomiting, temporary hair loss, low blood counts, mouth ulcers, skin pigmentation and allergic reactions. Rarely, children may develop a second form of cancer known as acute myeloid leukemia as a result of treatment with etoposide.
Hydrocortisone	Refer to package insert for detailed side effects
ldarubicin (IV)	Side effects are the same as Cyclophosphamide
Ifosfamide (IV)	Refer to package insert for detailed side effects
Leucovorin (IV)	Allergic reactions (rash, itching)
Mercaptopurine (MP) (PO)	Bone marrow depression, decreased appetite, nausea or vomiting, rarely liver damage, fever, and rash.

DRUG AND HOW GIVEN	SIDE EFFECTS
Methotrexate (IV)	Nausea (stomach sickness) and vomiting, mouth sores, redness and swelling of the lining of the digestive tract (from the mouth to the stomach through the intestines), bone marrow suppression, rarely kidney damage.
Intrathecal Methotrexate (IT)	Possible irritation around the brain or spinal cord, rarely causing temporary paralysis (inability to move arms or legs) and brain damage that may result in sluggishness, slow reactions, and long-term problems with learning, convulsions, bone marrow suppression, mouth sores, rarely liver damage, headache, backache, and fever.
Prednisolone (PO)	Weight gain, water retention, increased thirst and urination, irritability, high blood pressure, diabetes, immune suppression (decrease in ability to fight infections).
Thioguanine (PO)	Bone marrow depression, nausea and vomiting, rarely liver damage, fever, and rash.
Vincristine (IV)	Pain and blistering of the skin if the drug leaks out of the vein when it is being given, jaw pain, leg pain, constipation, mild bone marrow suppression (explained earlier), convulsions and hair loss.
Vindesine (IV)	AS ABOVE

Appendix 4 Dose Modifications and management of toxicities in ALL06 Protocol

Dose modifications and management of toxicities for Asparaginase

Allergy

Of the 3 preparations of asparaginase, PEG asparaginase is the least immunogenic with treatment limiting reactions occurring in 9% of all patients. E.Coliasparaginase has the highest rate of allergic reaction at 15-35%. Note that E.coli preparations have been shown to be more effective than Erwiniapreparation .

If allergic reactions occur, then administration is stopped, and appropriate measures taken (Hydrocortisone, Promethazine, Adrenaline).

Local Allergic Reactions following IM injection (inflammation at injection site, swelling)

Grade 1 allergic reaction (transient flushing or rash, drug fever <38C and intervention not indicated) –continue with PEG Asparaginase administration. If administered IM, recurrent local allergic reactions should prompt substitution with Erwinia if available. (See dosage guidelines below.)

Systemic Allergic Reactions
Discontinuation should be considered for grade ≥ 2 systemic reactions (requiring intervention or interruption of infusion). Consider substitution with Erwinia asparaginase if available. (See dosage guidelines below.)

Anaphylaxis
PEG Asparaginase should be discontinued for grade ≥ 3 systemic reactions (bronchospasm, urticaria, oedema, hypotension, requiring parenteral therapy). Consider substitution with Erwinia asparaginase. If systemic allergy develops to an alternative preparation then asparaginase therapy should be stopped.

If discontinuing PEG asparaginase or E. Coliasparaginase because of allergy/anaphylaxis the following schedule for Erwinia administration should be adopted:

Protocol I
PEG Asparaginase 1000 iU/m2 IV/IM day 8 + 22. In the event of serious reactions, each dose of PEG asparaginase should be replaced with:

- Erwinia preparation from Speywood used at dose of 10,000 iU/m^2 IM 3 days/week (Mon, Wed, Fri) X 6 doses or
- colaspase, "Leunase" at a dose of 5,000iU/m^2 given by 1-2 hour intravenous infusion 3 days/week (Mon, Wed, Fri) X 6doses.

High Risk Blocks 1, 2, 3
PEG asparaginase 1000U/m2 IM or IV on day 6. When possible use the same asparaginase preparation as used in protocol I. If required PEG asparaginase should be replaced with:

- Erwinia preparation from Speywood used at dose of 10,000 iU/m2 IM 3 days/week (Mon, Wed, Fri) x 6 doses OR
- colaspase "Leunase" at a dose of 5000 iU/m2 3 days/week (Mon, Wed, Fri) x 6 doses.

Protocol II
PEG asparaginase 1000U/m2 IM or IV on day 1. When possible use the same asparaginase preparation as used in protocol I. Anaphylaxis to asparaginase is more common in this block. If required PEG Asparaginase should be replaced with:

- Erwinia preparation from Speywood used at dose of 10,000 iU/m^2 IM 3 days/week (Mon, Wed, Fri) X 6 doses OR
- colaspase "Leunase" at a dose of 5000 iU/m2 3 days /week (Mon, Wed, Fri) X 6 doses

Coagulopathy

Please also refer to "guidelines for Supportive Care During Asparaginase Therapy" in section 11.2.1.

Most patients will develop an asymptomatic coagulopathy and should have coagulation studies (including fibrinogen and AT3 levels) twice weekly. Doses of asparaginase should not be withheld for laboratory abnormalities without clinical symptoms.

A suggested approach to asymptomatic coagulopathy is as follows:

Assess PT, APTT, fibrinogen, AT3 twice weekly (more frequently if required) in protocol I phase I and continue till commencement protocol I phase II (and in all asparaginase based phases of treatment)

1. avoid FFP for asymptomatic coagulopathy
2. conservative cryoprecipitate infusions to maintain fibrinogen > 1g/L
3. AT3 (Thrombotrol) infusions to maintain AT3 levels >60% (each infusion dosed to achieve ≥ 100% AT3 activity)

Prevention of Thrombosis

Unless contraindicated, prophylactic enoxaparin (40mg SC daily) is strongly recommended for all patients during protocol I phase I induction, and to continue till commencement protocol I phase II consolidation to prevent VTE complications. It is also recommended during all inpatient admissions. For female patients, it is recommended that the combined oral contraceptive should be discontinued at diagnosis due to a potential increased risk of thrombosis and alternative medically appropriate contraceptive prophylaxis be considered.

CNS Events (haemorrhage, thrombosis)

No prospective data regarding the management of asaparaginase thrombosis in adults is available. A suggested approach is outlined below:

Hold asparaginase.
AT3 (Thrombotrol) and cryoprecipiate infusions should be considered to treat thrombohaemorrhagic events due to ATIII deficiency or hypofibrinogemia respectively.
In the event that AT3 concentrates are unavailable, in the acute setting 20ml/kg FFP may raise AT3 levels by ~20% but may reverse the antileukaemic effect of asparaginase.
For CNS thrombosis, patients should be therapeutically anticoagulated with enoxaparin with or without AT3 infusions. AT3 infusions may be required in maintaining therapeutic anti-Xa levels. If at risk of haemorrhage consider running low range therapeutic anti-Xa.

For patients who experience significant CNS events (thrombosis or hemorrhage), rechallenge with asparaginase is generally not recommended.

Non – CNS Thrombosis

For all clinically significant thrombotic events hold asparaginase.
Patients should be therapeutically anticoagulated with enoxaparin with or without AT3 infusions to correct AT3 deficiency or maintain therapeutic anti-Xa once enoxaparin initiated.
Rechallenge with asparaginase may be considered when acute toxicity and clinical signs resolve and anticoagulation stable or completed. Administer missed doses if possible. For VTE associated with pulmonary embolism asparaginase therapy should be discontinued.

Non-CNS Bleeding

Hold asparaginase
Administer cryoprecipitate, FFP, VIIa as appropriate
Avoid FFP for nonurgent bleeding events
Asparaginase can be recommenced once bleeding resolves (grade ≤ 1) and factor replacement is stable or complete.
Administer missed asparaginase doses if possible.

Pancreatitis (Grade3/4)

Evidence of clinical pancreatitis (radiological and/or clinical findings (abdominal pain > 72 hrs) and/or lipase/amylase > 2 X ULN) or haemorrhagic pancreatitis is an absolute **contraindication** to further asparaginase therapy. Asparaginase should be discontinued and not restarted.

Note that pancreatitis may develop in the absence of hyperamylasemia. Mild amylase or lipase elevation in the absence of symptoms does not warrant discontinuation of asparaginase.

Hyperbilirubinaemia

Asparaginase therapy may need to be withheld in patients with an elevated bilirubin. No specific guidelines are available but consideration should be made to withhold asparaginase if bilirubin exceeds > 2.5 X ULN.

Hyperglycaemia

Do not withhold dose. Treat as medically indicated – insulin therapy may be required for blood glucose ≥ 15 mmol/L

Ketoacidosis

Withhold asparaginase until blood sugar can be stabilised with insulin. Recommence asparaginase therapy once stable.

Hyperlipidaemia

Do not withhold dose. In the case of markedly elevated triglycerides, close monitoring of pancreatic enzymes is recommended due to the increased risk of pancreatitis in this group.

Hyperammonemia

Elevated serum ammonia levels have been reported in children receiving either conventional or pegylated forms of L-asparaginase during treatment for ALL. This may be asymptomatic, but has been associated with neurological symptoms, including unusually prolonged nausea and vomiting, headaches and drowsiness, and frank encephalopathy. One recent report suggests a high incidence in children receiving intensive pegylated asparaginase. Serum ammonia levels should be measured weekly during protocol I induction, and, if elevated, asparaginase therapy be withheld if the patient is symptomatic until symptoms have resolved. Intavenous butyric acid and glucose has been recommended for treatment of severe hyerammonemic encephalopathy.

Dose modifications and management of toxicities for Cyclophosphamide

Haematuria

Omit in the presence of macroscopic haematuria. Cyclophosphamide should be administered as per ALL06 protocol with hydration and Mesna support as per institutional guidelines.

Renal Dysfunction

If CrClis < 10 mL/min/1.73 m^2, reduce dose ofcyclophosphamide by 50%. Prior to dose adjustment of cyclophosphamide, the creatinine clearance should berepeated with good hydration.

Dose modifications and management of toxicities for Cytarabine (Ara-C)

Ara-C Syndrome

Do not withhold cytarabine for fever if thought to be related to cytarabine. Blood cultures should be obtained. Forrash or conjunctivitis, cytarabine can be withheld for grade 3-4 toxicity until resolved. Missed doses **SHOULD** be made up and consideration given to concurrent hydrocortisone or dexamethasone therapy. During high risk blocks of therapy containing high dose cytarabine, dexamethasone eye drops should be administered to prevent conjunctivitis.

Once Protocol I consolidation or Protocol II have commenced do not interrupt cytarabine blocks for uncomplicated myelosuppression. In Protocol I consolidation, each 4 day block can recommence once ANC ≥

0.5 x 10^9/L and platelets≥ 30 X 10^9/L. G-CSF should not be routinely administered except according to physician discretion during periods of severe infection related to neutropenia. Cytarabine blocks can be withheld if severe infection develops. Missed cytarabine blocks **SHOULD** be made up.

Dose modifications and management of toxicities for Daunorubicin and Doxorubicin (Anthracyclines)

Cardiac Toxicity: Discontinue for clinical or echocardiographic/GHPS evidence of cardiomyopathy (SF <27% or EF< 50%) or Grade 3-4 left ventricular systolic dysfunction as per CTCAE version 4.0.

Anthracyclines are contraindicated in heart failure and cardiac dysfunction. Resuming anthracycline therapy will depend upon the cause of cardiac dysfunction and the results of further cardiac evaluation. Contact PI if patient cannot have further anthracycline therapy.

Myelosuppression: do not hold for myelosuppression during induction (ie days 8, 16, 22, 29) or in High Risk Block 2 (day 5).

Severe infection or mucositis in protocol II (days 8, 15, 22, 29)If patient has severe infection or mucositis (grade 3-4) and are neutropenic (ANC < 0.5 X 10^9/L) therapy should be delayed until signs of infection have resolved. Subsequent doses should be given at full dose.

Hyperbilirubinemia: a suggested approach to anthracycline dose reduction is outlined below

Total Bilirubin	Dose Reduction
≤ 26μmol/L	Full dose
26 – 51μmol/L	25% dose reduction
52 – 85 μmol/L	50% dose reduction
> 85 μmol/L	Withhold dose and administer next scheduled dose if toxicity has resolved. Do not make up missed doses.

An alternative approach could be to assess direct (conjugated) bilirubin and dose according to Childrens Oncology Group (COG) guidelines

Direct Bilirubin	Dose Reduction
<20 μmol/L	Full dose
21-51μmol/L	50% dose reduction
52-85 μmol/L	75% dose reduction
>85 μmol/L	Withold dose and administer next scheduled dose if toxicity has resolved. Do not make up missed doses.

Extravasation:
In the event of an extravasation, discontinue the IV administration of the drug and institute appropriate measures to prevent further extravasation and damage according to institutional guidelines. Suggestions below may be helpful but none are considered definitive:

1. Stop infusion, aspirate drug and blood if possible, remove needle.

2. Apply DMSO (concentrations in various guidelines go from 50% to 99%) topical solution to area twice that affected by extravasation. Allow DMSO to air dry. Do not cover. Repeat QID for 7-14 days.

3. Elevate limb if possible.

4. Apply ice pack for one hour, can repeat up to QID for 24 hours.

5. Injecting steroids is recommended by some, not all as is the use of Vitamin E topically together with the methods mentioned above.

Dose modifications and management of toxicities for Etoposide

Hypotension, Allergic reactions: Etoposide allergic reactions may be managed with pre-medications (eg anti-histamines and/or hydrocortisone) and by slowing the rate of the infusion. For those reactions which are

unable to be controlled in this way, etoposide phosphate may be substituted in the same dose and at the same rate. Pre-medication for etoposide phosphate is recommended.

Dose modifications and management of toxicities for Ifosfamide

Haematuria
Omit ifosfamide in the presence of macroscopic haematuria. If there is a history of previous significant haematuria, hydrate before ifosfamide until specific gravity is <1.015 and hydrate at a rate of 1000ml IV every 4-6 hours for 24 hours after a dose. Administer MESNAas per institutional protocol.

Encephalopathy
Confusion and comahave been associated with the use of ifosfamide. Administration of methylene blue is recommended in an attempt to reverse any ifosfamide-associated encephalopathy. Subsequent ifosfamide doses can be substituted with cyclophosphamide which should be administered at 25% of the ifosfamide dose.

Dose modifications and management of toxicities for Intrathecal Methotrexate (IT MTX)

Do not withhold on day 1 of induction protocol. IT MTX should be administered regardless of circulating blasts. Consider platelet transfusion if platelets < 50 X 10^9/L and radiologic guidance if concerns regarding traumatic puncture for day 1 LP.

Systemic toxicity:
Dosage of IT MTX should not be reduced for systemic toxicity. If there are concerns of worsening myelosuppression, mucositisetc, then Leucovorin may be used, beginning 48 hours after the IT therapy has been delivered at adose of 5 mg/m2/dose every 12hours x 2 doses PO/IV, beginning 48 hours after the IT therapy has been delivered. Do not administer leucovorin solely to prevent myelosuppression.

For patients with Down syndrome, leucovorin should be administered after every dose of IT MTX during ALL phases of therapy EXCEPT maintenance.

Dose modifications following an episode of subacute neurotoxicity/encephalopathy
Neurotoxicity has been described with IT and systemic high dose MTX and can range from transient events, seizures or episodes of acute hemiparesis, to severe necrotizing encephalopathies. Generally such symptoms will resolve in hours to days. The exclusive use of IT cytarabine as CNS prophylaxis in ALL has not been studied.

The following guidelines are offered for consideration following an acute neurotoxic event, but it must be recognized that there is little data to support these approaches or any others. Bearing in mind that CNS prophylaxis is a mandatory component of therapy for ALL,the treating physician must evaluate the patient and make an individualised assessment with respect to the relative risks and benefits of continued therapy.

Following an acute neurotoxic event,**imaging studies of the CNS are mandatory and should include MR angiogram or CT if MR not immediately available – to exclude the differential diagnoses of haemorrhage, thrombosis or infection.** Subacute MTX neurotoxicity may present with MRI based white or grey matter changes that may resemble recent infarcts. Seizures and other transient events may be linked to fever, infection, encephalitis, meningitis, hypertension, electrolyte disturbance, hypoglycemia, trauma, intracranial hemorrhage or thrombosis, narcotic withdrawal, illicit drug use, or other causes in addition to the direct side effects of chemotherapy. Appropriate laboratory studies may include blood cultures, a FBC, electrolytes, including glucose, calcium, magnesium and phosphorus, renal and liver function studies and/or an examination of the CSF. Posterior reversible encephalopathy may be present on MRI with extensive diffusion abnormalities, but these do not appear to correlate with subsequent demyelination or gliosis.

Many acute events are temporally related to the administration of IT therapy, commonly 9 to 11 days after IT administration. Following an acute event, with recovery, there are few data to support or guide therapeutic interventions. The risk of a second neurological event does not seem to be significantly increased in such

patients, though it may be prudent to withhold further IT or HD MTX in the acute period following a recent neurological event until imaging abnormalities and symptoms have resolved. It may also be reasonable to substitute IT Ara-C for one dose of IT MTX. Leucovorin rescue beginning 48 hours after subsequent IT MTX may also be considered in patients with a previous IT MTX related neurological event. If the event does not recur, resumption of standard therapy should be considered, following one modified or omitted IT dose. In the face of multiply recurrent events, or evidence of progressive encephalopathy, another evaluation is warranted and the treating physician may consider a more prolonged or definitive change in therapy. Decisions regarding ongoing therapy in this context must balance the risk of relapse versus quality of life impacts arising from progressive toxicity.

Since the greatest impact of CNS prophylaxis occurs early in therapy, the timing of these events may also influence clinical decisions. Cranial radiation has been suggested as an alternative to continued IT therapy though much of the literature on long-term neurocognitive dysfunction supports a more deleterious effect from CRT than IT therapy. The use of dextromethorphan (DM) has been suggested as a neuroprotectant. However, in the absence of a clinical trial there are few data to support the addition of DM.

Hydrocephalus, microcephaly or known abnormality of CSF flow precluding intrathecal chemotherapy via lumbar puncture

Intraventricular chemotherapy via Ommaya catheter may be used in place of intrathecal therapy delivered by LP. Intraventricular chemotherapy should be given according to the same schedule, but at 50% of the corresponding dosethat would be given by LP.Obstruction to CSF flow may be a contraindication to intrathecal and/or intraventricular therapy.

Viral, bacterial, or fungal meningitis

Omit IT methotrexate until meningitis has resolved.

Dose modifications and management of toxicities for Intravenous High Dose Methotrexate (HD-MTX)

High Dose Methotrexate (HD-MTX) Guidelines (see also Appendix 2)

- Avoid the administration of cotrimoxazole, nonsteroidal anti-inflammatory medications, and penicillins simultaneously to HD-MTX and until the MTX level is <0.25 µmol/l.
- Avoid sun exposure (also solarium) during HD-MTX-containing treatment elements.

This protocol is based on BFM dosing schedules. There is minimal folinic acid rescue. Please adhere strictly to folinic acid rescue guidelines

Each HD-Methotrexate block should only be commenced -

1. after cessation of cotrimoxazole (Bactrim, Septra, Resprim) if being administered
2. when creatinine is normal and Cr Cl> 100 ml/min
3. bilirubin is ≤ 3 x normal and ALT/AST≤ 5 X ULN
4. an alkaline diuresis is established. A suggested protocol is 3000ml/m2/day with dextrose-saline containing solution with 20mmol KCl, and 40mmol $NaHCO_3$ per litre to maintain urinary pH > 7.0
5. in the absence of third space fluid accumulation

The IV high dose methotrexate dose is 5000 mg/m^2 with:

- 10% (ie 500 mg/m^2) given in the first 30 minutes (loading dose)
- 90% (ie 4500 mg/m^2) given over next 23.5 hours

Check that the MTX is running to time, as the entire dose should be administered in 24hrs. If any methotrexate is left over at the 24 hour point, the infusion should be stopped and the remainder discarded.

Continue hydration (3000ml/m^2/24h) and alkalinization (urine pH 7.0-8.0) throughout the HD-MTX infusion, and until the plasma MTX concentration is below 0.25μM/L (usually ~ 48 hours after HD-MTX infusion has completed).

The IT methotrexate can be administered at any time during the 24 hour high dose IV methotrexate infusion.

Monitoring of plasma MTX levels: Take plasma MTX levels at 24, 36, 42, 48, 54, 72, and 96 hours from start of MTX infusion. Then measure MTX levels every 24 hours until MTX level is <0.25μM/L.

Leucovorin (folinic acid) rescue:Theleucovorin must start at 42 hours from the start of the IV HD-MTX infusion. Continue giving leucovorin, at doses depending on MTX levels, until level is < 0.25μM/L. For slow excretors, the dose of folinic acid will have to be increased according to the MTX level, as outlined in Appendix 2.

If serum creatinine rises significantly, at any time point, assure appropriate urine pH and urine output. If urine output fails to continue at 80% of the fluid intake, consider giving frusemide. Administration of carboxypeptidase G2 (glucarpidase) should also be considered in patients who develop significant renal dysfunction.

Even with a normal pattern of methotrexate excretion, some patients develop significant mucositis after discharge. Such patients may require re-admission for IV fluids and pain relief.

Acute Neurotoxicity/MTX Encephalopathy

Acute neurotoxicity has been described with both oral and parenteral MTX in various doses. Symptoms may manifest as headache, lethargy, confusion, agitation, aphasia, paresis and seizures and are generally associated with delayed MTX excretion. Aminophylline may result in prompt and complete resolution of symptoms in a majority of patients.

Aminophylline dosing from literature (though experience in adults is limited)

- 2.5mg/kg IV over 45-60mins
- 0.5mg/kg as continuous infusion over 12 hours

Generally acute MTX neurotoxicity is fully reversible and should not be a contraindication to further MTX therapy.

For cases of delayed or subacute MTX toxicity please see above guidelines

Liver Dysfunction

LFT's must be checked immediately prior to each course of intravenous HD-MTX. LFT's should not be drawn immediately following the MTX infusion as 100% of patients are expected to have significant elevations at that time. Dependent upon AST/ALT values the following is recommended:

- AST/ALT ≤ 5 x normal: proceed with HD-MTX
- AST/ALT > 5 X normal: assess other liver parameters and delay HD-MTX till transaminases fall ≤ 5 X normal. Withhold cotrimoxazole if necessary
- If ALT/AST persistently > 5 x normal: assess US, HBV, HCV, HAV, CMV, EBV, adenovirus serology and PCR. Discuss with study centre if abnormalities persist

Mucositis

For Grade 3-4 mucositis, withhold IV MTX until resolved. Consideration may be given to reducing the next dose of HD-MTX to 2g/m2. If no recurrence of grade 3-4 mucositis then subsequent HD-MTX dosing should be at full dose. If mucositis persists or recurs, consider culturing lesions for herpes simplex.

Nephrotoxicity

HD-MTX should be postponed until CrCimproves. HD-MTX should not be given to patients if CrCl<

60mls/min. HD-MTX dosing may be prorated to CrCl between 100 and 60 mls/min. Please discuss with PI.

Weekly PO Methotrexate and Daily 6-Mercaptopurine (MP)
Guidelines for the administration of oral methotrexate and oral 6-MP, including dosage modifications for myelosuppression are outlined in 11.11 of ALL06 protocol.

Dose modifications and management of toxicities for Therapy during Maintenance

Doses of 6-MP and MTX should be reduced by 50% if WBC < 2.0 X 10^9/L and withheld if WBC < 1.0 X 10^9/L. A suggested approach to dose modification is to alter, the doses of the two drugs 6-MP and MTX in the same ratio. If WBC > 3.0 X 10^9/L then 6-MP and MTX are commenced at 150% of standard dose. Alternatively, methotrexate can be ceased and the dose of mercaptopurine modified, with methotrexate being later re-introduced at a lower dose.

When commencing maintenance, FBC should be performed weekly or at a minimum second weekly with dosing adjustments made as per protocol. If blood counts are steady and the patient is clinically stable, intervals can be lengthened to 2 weeks and at a minimum every 4 weeks. It is recommended to administer weekly MTX dose at the evening of the day of regular blood count measurement in order to adjust the dose to the current blood count.

Routine measurements of liver parameters during maintenance should be performed at least every 12 weeks, more frequently in symptomatic patients, in those who have received high risk blocks of therapy or those receiving > 100% standard dosing. Dose reductions should be based on a rise in bilirubin > 3 X ULN or ALT/AST > 10 X ULN and rising. In such cases, other causes of liver dysfunction such as Gilberts syndrome or viral hepatitis should also be considered.
.
Long treatment breaks should be avoided if possible. A dose reduction or resumption of chemotherapy at reduced dose is generally favored over longer intervals without therapy. An uncomplicated (viral) infection without fever and with stable blood counts, for example, is not in every case an indication for treatment interruption.

Infection
If blood counts are stable and the patient is clinically improved then chemotherapy could be reintroduced (possibly at a reduced dose) once fever has resolved for 24 hours.

Mucositis
Grade 3-4: MTX should be reduced to 50% if Grade 3 toxicity develops; withhold in the presence of Grade 4 toxicity until there is a resolution, then resume at 50% of original dose with gradual dose escalation. If mucositis persists or recurs, consider culturing for herpes simplex. 6-MP can usually be continued at the same dose.

Liver Toxicity
Dose reductions or temporary discontinuation should be based on abilirubin > 3 X ULN or ALT/AST > 10 X ULN and rising. Stable mild increases in ALT/AST with normal or mildly elevated bilirubin are generally well tolerated and should not prompt a dose adjustment. More regular (every 4 weeks) monitoring of LFT's are recommended in these circumstances.

MTX Pneumonitis
MTX must be discontinued if there is a suspicion of MTX pneumonitis. Systemic steroid therapy may be worthwhile in severe cases.

Dose modifications and management of toxicities for Steroid

Hypertension
Dose should not be reduced. Sodium restriction and anti-hypertensives should be employedin an effort to control hypertension.

Hyperglycemia
Dose should not be reduced for hyperglycemia. Rather, insulin therapy should be employedto control the blood glucose level.

Pancreatitis
Do not modify dose for asymptomatic elevations of amylase and/or lipase. Discontinue steroids,except for stress doses, in the presence of hemorrhagic pancreatitis or severe pancreatitis (abdominal pain>72 hours and > Grade 3 amylase elevation > 2.0x ULN).

Osteonecrosis/Avascular Necrosis
Do not modify corticosteroid therapy for osteonecrosis.

Severe Psychosis
Steroid dose may be reduced by 50%.

Dose modifications and management of toxicities for Vincristine

Vincristine can result in central, peripheral, and autonomic neuropathy. The majority of vincristine-associated neuropathies are ultimately reversible. Severe peripheral neuropathies, with or without a positive family history, might suggest the need for a molecular diagnostic evaluation to rule out conditions such as Charcot Marie Tooth Disease (CMT). Vincristine dose modifications during Induction therapy should be avoided. Post-induction, if significant vincristine-associated neurotoxicity persists or recurs, vincristine can be administered at a reduced dosage or can be substituted with vindesine (3mg/m2, max dose 5mg)

Severe neuropathic pain (≥ grade 3)
Hold dose/s. When symptoms subside, resume at 50% previous dose, then escalate to full dose as tolerated.

Vocal cord paralysis
Hold dose/s. Organise ENT review. When symptoms subside, resume at 50% previous dose, then escalate to full dose as tolerated.

Foot drop
Evidence of foot drop should prompt discontinuation of vincristine therapy.

Constipation or ileus (≥ grade 3) or typhlitis
Hold dose/s. Institute aggressive regimen to treat constipation if present. When symptoms abate resume at 50% dose and escalate to full dose as tolerated.

Jaw pain
Treat with analgesics. Do not modify vincristine dose.

Hyperbilirubinemia: a suggested approach to vinca alkaloid dose reduction is outlined below

Total Bili	*% Dose Reduction*
≤25μmol/L	Full dose

26-50 µmol/L	50% dose reduction
>50µmol/L	75% dose reduction

An alternative approach could be to assess direct (conjugated) bilirubin and dose according to COG guidelines

Direct Bilirubin	Dose reduction
<53 µmol/L	Full dose
53-85 µmol/L	50% dose reduction
86-102 µmol/L	75% dose reduction
>102 µmol/L	withhold dose and administer next dose once toxicity has resolved. Do not make up missed doses.

Dose modifications and management of toxicities for Vindesine

Guidelines are as per Vincristine above.

Dose modifications and management of toxicities for Supportive Care Guidelines

Antibacterial Prophylaxis

As per institutional guidelines Antifungal Prophylaxis

It is recommended that neutropenic patients be given prophylaxis against invasive fungal and mold infections using an appropriate mold active agent such as itraconazole, posaconazole or voriconazole and appropriate monitoring of drug levels be performed to ensure adequate absorption.

Note that due to significant interactions, particularly with vinca alkaloids, that the concomitant use of a triazole is not recommended during treatment phases involving vincristine or vindesine. If required, alternative non-azole mould prophylaxis (as per institutional guidelines) should be used during treatment with a vinca alkaloid to avoid concomitant administration with a triazole and subsequent severe neurotoxicity in neutropenic patients.

Pneumocystis jiroveci Prophylaxis

All patients should receive pneumocystis prophylaxis throughout the entire duration of the treatment protocol until 4 weeks following the completion of maintenance therapy.

Prophylaxis should be administered as per institutional guidelines. **Note that if TMP/SMX is to be used it should not be administered within 48 hours of the commencement of HD-MTX and should only be reinstituted once MTX levels are <0.25uM/L.**

VZV and HSV Prophylaxis

It is recommended that VZV and HSV IgG positive individuals receive at least valaciclovir or acyclovir prophylaxis throughout therapy until maintenance.

G-CSF

The routine use of G-CSF outside of HR therapy is not routinely recommended. All patients receiving HR blocks of therapy will be administered either daily G-CSF or pegylated G-CSF following completion of each HR block of chemotherapy until neutrophil recovery. G-CSF should be ceased at least 2 days prior to the next round of chemotherapy.

In the case of severe neutropenic infection, G-CSF should be offered to all patients regardless of risk status or until neutrophil recovery or resolution of infection.

Special Considerations

Downs Syndrome

Downs Syndrome patients are known to be at high risk of severe treatment related toxicity but are also prone to higher rates of relapse if offered less intensive treatment.

Treatment tolerance is variable in this patient group and general dose reductions are not recommended, **except for HD-MTX (see below)**, unless based on actual toxicity.

Because of the increased risk of severe methotrexate toxicity in some Down syndrome patients, an *a priori* dose reduction of methotrexate is recommended in Protocol M. The first HD-MTX (Protocol M or HR-1) should be administered at a dose of **500mg/m^2/24 h** in all patients. If not associated with significant toxicity, a dose increase to 2000 mg/m^2/24 h should be used. If well tolerated, a dose of 5000 mg/m^2/24 h should then be used for subsequent courses of HD-MTX. In the case of relevant systemic toxicity (myelosuppression or mucositis) due to intrathecal methotrexate, intrathecal dosing should not be reduced, but leucovorin rescue would be recommended.

Appendix 5 Blood and bone marrow collections

Timepoint	Patient type	Sample type and volume	Reason	Storage and Transport conditions	Destination
Diagnosis (pre-treatment)	all	2mL BM in ACD	Flow MRD (appendix 8) & Research studies (appendix 10 & 11)	RT on day of collection[2]	ICPMR
	all	4ml BM in ACD	Molecular MRD (appendix 8)	RT on day of collection[1]	CCIA
	all	30 ml PB in LiHep	Research (appendix 12)	Courier on gel packs overnight on day of collection [3]	SAHMRI on day of collection
	all	3mL BM in EDTA	Research (appendix 12) & PB vs BM MRD analysis (appendix 9)	Courier on gel packs overnight on day of collection [3]	SAHMRI on day of collection
	all	5mL PB in EDTA	PB vs BM MRD analysis (appendix 9)	Courier on gel packs overnight on day of collection [3]	SAHMRI on day of collection
	all	2ml PB in SST	Asparaginase activity (appendix 13)	Centrifuge, aliquot serum & store at -20ºC	Freeze on site & batch to UniMelb
Day 8	all	5mL PB in ACD	Flow cytometry (appendix 8)	RT on day of collection[2]	ICPMR
Day 15	all	5mL PB in EDTA	PB vs BM MRD analysis (appendix 9)	RT on day of collection [2]	ICPMR
	all	2mL BM in ACD	Flow MRD (appendix 8) & Research Studies (appendix 11)	RT on day of collection [2]	ICPMR
	all	5ml BM in ACD	Molecular MRD (appendix 8)	RT on day of collection[1]	CCIA
	all	2ml PB in SST	Asparaginase activity (appendix 13)	Centrifuge, aliquot serum & store at -20ºC	Freeze on site & batch to UniMelb
Day 18	all	2ml PB in SST	Asparaginase activity (appendix 13)	Centrifuge, aliquot serum & store at -20ºC	Freeze on site & batch to UniMelb
Day 22	all	2ml PB in SST	Asparaginase activity (appendix 13)	Centrifuge, aliquot serum & store at -20ºC	Freeze on site & batch to UniMelb
Day 27	all	2ml PB in SST	Asparaginase activity (appendix 13)	Centrifuge, aliquot serum & store at -20ºC	Freeze on site & batch to UniMelb
Day 33	all	2ml PB in SST	Asparaginase activity (appendix 13)	Centrifuge, aliquot serum & store at -20ºC	Freeze on site & batch to UniMelb
	all	5mL PB in EDTA	PB vs BM MRD analysis (appendix 9)	RT on day of collection [2]	ICPMR
	all	2mL BM in ACD	Flow MRD (appendix 8) & Research Studies (appendix 11)	RT on day of collection[2]	ICPMR
	all	5mL BM in ACD	Molecular MRD (appendix 8)	RT on day of collection[1]	CCIA
Day 79	all	5mL PB in EDTA	PB vs BM MRD analysis (appendix 9)	RT on day of collection[2]	ICPMR
	all	2mL BM in ACD	Flow MRD (appendix 8)	RT on day of collection[2]	ICPMR
	all	5mL BM in ACD	Molecular MRD (appendix 8)	RT on day of collection[1]	CCIA

1 If collected on a Friday, Saturday or day prior to a public holiday, contact Rosemary Sutton (see contacts list for contact details) for directions on how to process and ship. If collected Sunday to Thursday send at room temperature via overnight delivery

2 If collected on Friday, Saturday or day prior to a public holiday, add 1ml of tissue culture medium (eg RPMI) and send to ICPMR on next business day

3 See study coordinators manual for courier details.

Timepoint	Patient type	Sample type and	Reason	Storage and Transport conditions	Destination
Day 7 of each HR block	MHR, HR, VHR patients	2ml PB in SST	Asparaginase activity (appendix 13)	Centrifuge, aliquot serum & store at -20ºC	Freeze on site & batch to Uni Melb
At end of each HR block	MHR, HR, VHR patients	2mL BM in ACD	Flow MRD (appendix 8)	RT on day of collection[2]	ICPMR
		5mL PB in EDTA	PB vs BM MRD analysis (appendix 9)	RT on day of collection	ICPMR
		5ml BM in ACD	Molecular MRD (appendix 8)	RT on day of collection[1]	CCIA
Immed. before alloHCT (prior to conditioning)	MHR, HR, VHR patients	5mL PB in EDTA	PB vs BM MRD analysis (appendix 9)	RT on day of collection	ICPMR
		2mL BM in ACD	Flow MRD (appendix 8)	RT on day of collection[2]	ICPMR
		5mL BM in ACD	Molecular MRD (appendix 8)	RT on day of collection[1]	CCIA
4 to 6 weeks post alloHCT (prior to discharge)	MHR, HR, VHR patients	5mL PB in EDTA	PB vs BM MRD analysis (appendix 9)	RT on day of collection	ICPMR
		2mL BM in ACD	Flow MRD (appendix 8)	RT on day of collection[2]	ICPMR
		5mL BM in ACD	Molecular MRD (appendix 8)	RT on day of collection[1]	CCIA
3 months post discharge from hospital for alloHCT	MHR, HR, VHR patients	5mL PB in EDTA	PB vs BM MRD analysis (appendix 9)	RT on day of collection	ICPMR
		2mL BM in ACD	Flow MRD (appendix 8)	RT on day of collection[2]	ICPMR
		5ml BM in ACD	Molecular MRD (appendix 8)	RT on day of collection[1]	CCIA
Day1 of Protocol II	all	5mL PB in EDTA	PB vs BM MRD analysis (appendix 9)	RT on day of collection	ICPMR
		2ml BM in ACD	Flow MRD (appendix 8)	RT on day of collection[2]	ICPMR
		5mL BM in ACD	Molecular MRD (appendix 8)	RT on day of collection[1]	CCIA
Day15 of Protocol II	all	2ml PB in SST	Asparaginase activity (appendix 13)	Centrifuge, aliquot serum & store at -20ºC	Freeze on site & batch to Uni Melb
Prior to commencement of maintenance	all	5mL PB in EDTA	PB vs BM MRD analysis (appendix 9)	RT on day of collection	ICPMR
	all	2ml BM in ACD	Flow MRD (appendix 8)	RT on day of collection[2]	ICPMR
	all	5mL BM in ACD	Molecular MRD (appendix 8)	RT on day of collection[1]	CCIA
	all	30 ml PB in LiHep	Research (appendix 12)	Courier on gel packs overnight on day of collection[3]	SAHMRI
Upon relapse	all	5mL PB in EDTA	PB vs BM MRD analysis (appendix 9)	RT on day of collection	ICPMR
	all	2mL BM in ACD	Flow MRD (appendix 8) & Research Studies (appendix 11)	RT on day of collection [2]	ICPMR
	all	5mL PB in ACD	Flow cytometry (appendix 8)	RT on day of collection [2]	ICPMR
	all	4mL BM in ACD	Molecular MRD (appendix 8)	RT on day of collection[1]	CCIA
	all	2mL BM in EDTA	Research (appendix 12)	Courier on gel packs overnight on day of collection[3]	SAHMRI
	all	30 mls PB in LiHep	Research (appendix 12)	Courier on gel packs overnight on day of collection[3]	SAHMRI

1 If collected on a Friday, Saturday or day prior to a public holiday, contact Rosemary Sutton (see contacts list for contact details) for directions on how to process and ship. If collected Sunday to Thursday send at room temperature via overnight delivery

2 If collected on Friday, Saturday or day prior to a public holiday, add 1ml of tissue culture medium (eg RPMI) and send to ICPMR on next business day

3 See study coordinators manual for courier details.

Appendix 6 Composite HRQOL measure

FACT-G (Version 4)

Below is a list of statements that other people with your illness have said are important. **By circling one (1) number per line, please indicate how true each statement has been for you during the past 7 days.**

	PHYSICAL WELL-BEING	Not at all	A little bit	Some-what	Quite a bit	Very much
GP1	I have a lack of energy	0	1	2	3	4
GP2	I have nausea	0	1	2	3	4
GP3	Because of my physical condition, I have trouble meeting the needs of my family	0	1	2	3	4
GP4	I have pain	0	1	2	3	4
GP5	I am bothered by side effects of treatment	0	1	2	3	4
GP6	I feel ill	0	1	2	3	4
GP7	I am forced to spend time in bed	0	1	2	3	4

	SOCIAL/FAMILY WELL-BEING	Not at all	A little bit	Some-what	Quite a bit	Very much
GS1	I feel close to my friends	0	1	2	3	4
GS2	I get emotional support from my family	0	1	2	3	4
GS3	I get support from my friends	0	1	2	3	4
GS4	My family has accepted my illness	0	1	2	3	4
GS5	I am satisfied with family communication about my illness	0	1	2	3	4
GS6	I feel close to my partner (or the person who is my main support)	0	1	2	3	4
Q1	*Regardless of your current level of sexual activity, please answer the following question. If you prefer not to answer it, please check this box* ☐ *and go to the next section.*					
GS7	I am satisfied with my sex life	0	1	2	3	4

FACT-G (Version 4)

By circling one (1) number per line, please indicate how true each statement has been for you during the past 7 days.

	EMOTIONAL WELL-BEING	Not at all	A little bit	Some-what	Quite a bit	Very much
GE1	I feel sad	0	1	2	3	4
GE2	I am satisfied with how I am coping with my illness	0	1	2	3	4
GE3	I am losing hope in the fight against my illness	0	1	2	3	4
GE4	I feel nervous	0	1	2	3	4
GE5	I worry about dying	0	1	2	3	4
GE6	I worry that my condition will get worse	0	1	2	3	4

	FUNCTIONAL WELL-BEING	Not at all	A little bit	Some-what	Quite a bit	Very much
GF1	I am able to work (include work at home)	0	1	2	3	4
GF2	My work (include work at home) is fulfilling	0	1	2	3	4
GF3	I am able to enjoy life	0	1	2	3	4
GF4	I have accepted my illness	0	1	2	3	4
GF5	I am sleeping well	0	1	2	3	4
GF6	I am enjoying the things I usually do for fun	0	1	2	3	4
GF7	I am content with the quality of my life right now	0	1	2	3	4

FACT-Leu (Version 4)

By circling one (1) number per line, please indicate how true each statement has been for you during the past 7 days.

	ADDITIONAL CONCERNS	Not at all	A little bit	Some-what	Quite a bit	Very much
BRM 3	I am bothered by fevers	0	1	2	3	4
P2	I have certain parts of my body where I experience significant pain	0	1	2	3	4
BRM 2	I am bothered by the chills	0	1	2	3	4
ES3	I have night sweats	0	1	2	3	4
LEU 1	I am bothered by lumps or swelling in certain parts of my body (e.g., neck, armpits, or groin)	0	1	2	3	4
TH1	I bleed easily	0	1	2	3	4
TH2	I bruise easily	0	1	2	3	4
HI 12	I feel weak all over	0	1	2	3	4
BMT 6	I get tired easily	0	1	2	3	4
C2	I am losing weight	0	1	2	3	4
C6	I have a good appetite	0	1	2	3	4
An7	I am able to do my usual activities	0	1	2	3	4
N3	I worry about getting infections	0	1	2	3	4
LEU 5	I feel uncertain about my future health	0	1	2	3	4
LEU 6	I worry that I might get new symptoms of my illness	0	1	2	3	4
BRM 9	I have emotional ups and downs	0	1	2	3	4
LEU 7	I feel isolated from others because of my illness or treatment	0	1	2	3	4

English

2/8/2005
Page 3 of 4

FACT/GOG-Ntx (Version 4)

By circling one (1) number per line, please indicate how true each statement has been for you during the past 7 days.

	ADDITIONAL CONCERNS	Not at all	A little bit	Some-what	Quite a bit	Very much
NTX 1	I have numbness or tingling in my hands	0	1	2	3	4
NTX 2	I have numbness or tingling in my feet	0	1	2	3	4
NTX 3	I feel discomfort in my hands	0	1	2	3	4
NTX 4	I feel discomfort in my feet	0	1	2	3	4
NTX 5	I have joint pain or muscle cramps	0	1	2	3	4
HI 12	I feel weak all over	0	1	2	3	4
NTX 6	I have trouble hearing	0	1	2	3	4
NTX 7	I get a ringing or buzzing in my ears	0	1	2	3	4
NTX 8	I have trouble buttoning buttons	0	1	2	3	4
NTX 9	I have trouble feeling the shape of small objects when they are in my hand	0	1	2	3	4
An6	I have trouble walking	0	1	2	3	4

ALL (Version 1)

(A composite of items from the FACT-N, FACT-C, FACT-H&N and FACT-B)

By circling one (1) number per line, please indicate how true each statement has been for you during the past 7 days.

	ADDITIONAL CONCERNS	Not at all	A little bit	Some-what	Quite a bit	Very much
C1	I have swelling or cramps in my stomach area...	0	1	2	3	4
H&N 11	I can eat solid foods.....	0	1	2	3	4
C3	I have control of my bowels.....	0	1	2	3	4
C4	I can digest my food well.....	0	1	2	3	4
H&N 12	I have pain in my mouth, throat or neck.....	0	1	2	3	4
C5	I have diarrhea.....	0	1	2	3	4

		None of the time	A little of the time	Some of the time	Most of the time	All of the time
N1	I worry about getting sick due to low blood counts.....	0	1	2	3	4
N2	I avoid public places for fear of getting an infection.....	0	1	2	3	4
N4	I worry my condition will not improve if my treatment is delayed.....	0	1	2	3	4
N6	I have mouth sores.....	0	1	2	3	4
N7	My partner worries about me when my blood counts are low.....	0	1	2	3	4
N8	My low blood counts interfere with my intimate relationships.....	0	1	2	3	4

Page 3 of 3

Appendix 7HRQOL Checklist Form

DataFax #035 **Plate #015**

Month Number 0 0

Assessment Number 0 1

IBCSG Patient ID 3 5 (*Study No.*) ____ (*Patient ID No.*)

Patient Initials ____ (*f m fl sl — first, middle, first last, second last*)

Patient's Date of Birth __ (*day*) __ (*month*) ____ (*year*)

Date Form Completed __ (*day*) __ (*month*) ____ (*year*)

Center Code ____

Participating Center Name/Affiliate _____ **Version No.** 1 **Language No.** 0 1

QL ASSESSMENT CHECKLIST (Form 35-AC)

Please submit this checklist for every patient participating in the Quality of Life substudy at each scheduled date for quality of life assessment. For each assessment, the patient should complete the QL Form and PRS Form. If any forms are missing, please indicate the reason for missing data.

1. Were the following forms completed at this scheduled assessment?

Please complete each box below with one of the following codes: 1 = No, 2 = Yes

☐ QL Questionnaire (Form QL)

☐ Patient Reported Symptoms Form (Form PRS)

If both boxes = 2 (Yes), sign and date form and submit to DataFax.
If any box = 1 (No) continue on to Q2.

2. Reason the patient did not complete the QL Form and/or the PRS Form

Please select one of the following:

☐ Patient felt too ill to complete the assessment

☐ Forms not presented to patient. Specify reason: _____

☐ Patient refused to participate in this assessment.

☐ Patient did not come to clinic. Select one reason patient missed this scheduled appointment:

- ☐ Patient on holiday
- ☐ Patient in hospital or nursing home
- ☐ Not known why patient missed this scheduled appointment
- ☐ Other _____

☐ Patient wishes to withdraw from further participation in the Quality of Life Study.

☐ Other, specify _____

_____ Investigator/*Designee* Signature

Date __ (*day*) __ (*month*) ____ (*year*)

20Apr07

Appendix 8 Laboratory Studies Procedures for MRD analysis on peripheral blood and bone marrow samples.

Aims:

1. To use MRD testing on post-treatment bone marrow (BM) aspirates at time points 1 and 2 (day 33 and day 79) to determine risk group stratification.
2. To use MRD testing in patients receiving the high risk treatment strategy, to determine the effectiveness of this treatment.
3. To collect data to determine the value of MRD testing on bone marrow at day 15, and on blood at day 8.
4. To examine the predictive value of MRD immediately before and after each block of high risk therapy, and before alloHCT.
5. To compare the results of molecular testing by PCR to those obtained by flow cytometry.

Methods:

- Molecular testing for antigen receptor gene rearrangements. PCR and DNA sequencing will be used to identify specific antigen receptor gene rearrangements in the leukemic cells at diagnosis. Real-time quantitative (RQ)-PCR assays with patient-specific primers will then be used to detect and quantify these markers of the patient's leukaemia in remission marrow samples, with sensitivity levels of 10^{-4} or greater.
- Flow cytometry analysis. Leukemic cells will be immuno-phenotyped at diagnosis using appropriate monoclonal antibodies, and specific abnormal phenotypes identified for each case. MRD will then be assessed in remission bone marrow aspirate samples using the most informative antibody combination, with a sensitivity of 10^{-4} or greater.

Collection and transport of bone marrow samples for MRD analysis

Samples should be sent fresh on day of collection whenever possible. Please do NOT delay sending samples because the diagnosis is not clear.

DIAGNOSIS SAMPLES

- Diagnosis samples. Bone marrow aspirate samples taken at diagnosis will need to include material for both immunophenotyping and molecular testing. This should be done via 1 major draw, mixing and splitting the aspirate into separate tubes for local analysis and three tubes for ALL06.
  - 1 x 2ml ACD tube will be used for Flow cytometry and
  - 1 x 4ml ACD tube will be used for Molecular MRD.
  - 1 x 1ml EDTA tube will be used for Appendix 9 laboratory studies

  The diagnosis samples should be collected in an ACD tubes of marrow aspirate. For molecular MRD, 4ml is required with a minimum of $1x\ 10^7$ mononuclear cells after ficoll purification, in order to yield at least 40 ug DNA both for PCR to identify markers and to provide enough DNA for the standard curves used in the subsequent real-time PCR MRD assays. For flow MRD at 2ml is required to provide 10^6 leukemic cells for analysis.

  In a case with a dry tap, a bone marrow trephine sample should be collected and placed in ACD collection tube. In addition to the trephine the site should also send a 20ml peripheral blood sample in EDTA tube. These samples should be sent directly to Rosemary at CCIA (who will share the sample as required with Mary Suttor at ICPMR). In some patients with high blast cell counts, a peripheral blood sample may be a suitable substitute for bone marrow at diagnosis. If peripheral blood blasts are >15% sites can send a 20ml PB samples

  Note that patients with insufficient diagnosis material cannot be MRD stratified and cannot participate in the ALL06 study.

SUBSEQUENT SAMPLES

- Subsequent samples for MRD analysis
  - Flow MRD 1 x 2ml collected in ACD tube
  - Molecular MRD 1 x 5ml collected in ACD tube

  must not be frozen and not chilled. Each sample should sent at ambient temperature on the day of collection, using an overnight courier for centres outside of NSW.

- **Relapse** samples for MRD analysis
  - Flow MRD 1 x 2ml collected in ACD tube
  - Molecular MRD 1 x 4ml collected in ACD tube
  - Additional sample collected for appendix 12 research (refer to appendix 12)

  must not be frozen and not chilled. Each sample should sent at ambient temperature on the day of collection, using an overnight courier for centres outside of NSW.

-
- The post-treatment bone marrow samples required for all patients are day 15, time point 1 (day 33), and time point 2 (day 79). In addition, 5mls of blood collected in ACD on day 8 and at relapse should be sent for flow cytometry. For high risk patients, bone marrow samples are required before each of the HR blocks (HR1, HR2, HR3), immediately before and 3 months after alloHCT. Relapse samples should be sent in all cases for both molecular and flow studies. (see appendix 5 for details)

- Marrow aspirates taken in remission should be on the first "suck" aspirate, with collection of at least 4ml for molecular MRD testing in ACD tube to provide a minimum of 5 $x10^6$ cells after ficoll purification and yield at least 10 ug DNA to enable molecular MRD assays to be done, and at least 2 ml for flow MRD in ACD tube to provide a minimum of 10^6 cells. Peripheral blood cannot be substituted for bone marrow remission samples. If the time point 1 bone marrow sample is too hypocellular, the aspirate should be repeated at day 37.

- Samples for MRD analysis after starting therapy should wherever possible be collected on a Monday to Thursday, and sent promptly, in order to allow receipt and processing of the sample before the end of the working week. If samples are collected on Fridays, and are unable to be received by the processing laboratory before close of business on Friday, they should be processed as per the below requirements.

PROCESSING SAMPLES

- **Flow MRD Samples**
  - If collected Sunday to Thursday send at room temperature via overnight delivery
  - If collected on Friday, Saturday or day prior to a public holiday, add 1ml of tissue culture medium (eg RPMI) and send to ICPMR on next business day
  - All Flow MRD samples should be sent to Mary Sartor, Flow Cytometry Unit, Level 2, Institute of Clinical Pathology and Medical Research (ICPMR), Westmead Hospital, Darcy Rd, Westmead, NSW 2145, phone 02-9845-6257, after hours 02-9845-5555 pager 27762, Mary.Sartor@swahs.health.nsw.gov.au.
  - Email Mary to notify her of the dispatch of samples to ICPMR

- **Molecular MRD Samples**

- If collected Sunday to Thursday send at room temperature via overnight delivery

- If collected on a Friday, Saturday or day prior to a public holiday, contact Rosemary Sutton (see contacts list for contact details) for directions on how to process and ship.

- MRD request forms: Each sample should be labelled with a hospital patient sticker and accompanied by a request form preferably with the same sticker. Either use the specific ALLG form or a haematology request form. This request needs to clearly specify MRD for ALL06 patient, bone marrow sample and the specific therapy timepoint. If the diagnosis is not clear at the time of sending the sample, please write ?ALL and ? MRD for ALL06 patient on the form. This is especially important if MRD is NOT required to avoid the significant waste of money and effort in finding markers. Within a week of diagnosis please provide any information missing from the request form and confirm the ALL diagnosis; ALL type (T-ALL or precursor B-ALL); and name and email addresses for those to receive the MRD report (consultant and study coordinator).

- All Molecular MRD samples should be sent to Rosemary Sutton, Childrens Cancer Institute Australia, C25 Lowy Cancer Research Centre, Lower Gound Floor, Gate 11, Botany Street, UNSW, Sydney, NSW, 2052, phone 02 9382 1829, RSutton@ccia.org.au or mrd@ccia.unsw.edu.au

- Contact for queries – either Dr Rosemary Sutton as above or Nicola Venn phone 02 9385 1604, NVenn@ccia.org.au

- Email Rosemary to notify her of the dispatch of samples to CCIA

Reports for patient Molecular MRD results (by PCR):

1. The results of MRD determined by PCR for Tp1 and Tp2 for each patient will be sent as a pdf attachment by email to the address(es) provided for the consultant and study coordinator in 6-10 working days after receipt of the Tp2 sample. A copy of the report should be de-identified by the site and sent to the TCC.

2. The MRD report will provide the necessary information on the sensitivity of the MRD assays and specify MRD levels at Tp1 and Tp2 to enable stratification of the patient into risk groups.

3. Results of MRD testing at other research time points will provide information for the next trial. Only samples used to determine the patient's treatment pathway will be reported to the consultants, eg at the end of each HR block. Other MRD results that are not used to determine therapy prospectively will be batched and analyzed separately, and will not be reported to consultants.

4. Results of the MRD testing in transplant patients (pre and post-transplant MRD levels) will be provided 2 weeks after the post-transplant sample if requested. If required please request the results via email (mrd@ccia.unsw.edu.au).

5. Results of MRD testing in any patients who relapse will be available 2 weeks after the relapse sample is received if requested

Appendix 9 Laboratory Studies- comparison of MRD analysis of peripheral blood and bone marrow

Rationale

Quantification of minimal residual disease (MRD) in B-lineage acute lymphoblastic leukaemia (ALL) by the detection of immunoglobulin heavy chain (IgH) gene rearrangements is widely used, and provides powerful prognostic information. The level of MRD measured in the peripheral blood in ALL is lower than in parallel BM specimens, and for this reason BM is preferred. However, if the Q-PCR method is highly sensitive, the lower level of MRD in the PB might still be detectable. In addition, the level of MRD at different sites in the BM may be heterogeneous, whereas the level in the PB is likely to represent an average of the haematopoietic output. This may reduce the risk of falsely high or low readings based on sampling error. Samples of PB can be obtained safely and with less discomfort than BM aspiration. This sub-study therefore aims to answer the following two questions:

1. Is the prognostic information provided by PB MRD analysis equivalent to that provided by BM MRD analysis?
2. Can PB MRD surveillance identify impending relapse?

Sample collection and processing (per Appendix 5)

- Peripheral blood 5 mL in EDTA to be collected at the following time points:
  - At diagnosis
  - Day 15
  - Time point 1 (~Day 33)
  - Time point 2 (~Day 79)
  - At end of each HR block
  - Immediately before alloHCT
  - 3 months post alloHCT
  - Day 1 of protocol II
  - Prior to commencement of maintenance
  - Upon relapse

- Bone Marrow Aspirate at diagnosis only: 1mL in EDTA (T lineage ALL. Approximately 20% of T-ALL patients will have an informative IgH gene rearrangement. Please send samples for all patients.) In case of dry tap at diagnosis, contact Sue Latham for further instructions

Ship samples per protocol appendix 5. If samples cannot be sent on day of collection, freeze peripheral blood and bone marrow aspirate samples below -70 ^{0}C on day of collection. No additional processing is required. ALLG should be notified if you are storing these samples at site.

Sample transport

Samples should be shipped per protocol appendix 5. If samples cannot be sent on the day of collection they should be frozen on site and held until they can be sent to the SAHMRI in Adelaide batched (on dry ice) with other ALLG study samples. The samples' labels should include at minimum the study number (ALL06), the patient's ALL06 registration number, the time point (protocol and day), the date of collection. If your site is not sending any frozen samples to the SAHMRI please contact us to make alternative arrangements at the end of the trial.

Shipping details

- SAHMRI Leukaemia Research Laboratory SAHMRI, Cancer Theme, Level 5, North Terrace, Adelaide SA 5000

Contact details for clinical queries - Dr David Ross or Prof Alec Morley

- Email: david.ross@health.sa.gov.au Phone: 08 8204 5231, Fax: 08 8204 5114. After hours via FMC switchboard 08 8204 5511
- Email: alec.morley@flinders.edu.au Phone: 0433101555

Contact details for sample collection/transport queries – Sue Latham

- Email: sue.latham@flinders.edu.au Phone 08 82044019

Appendix 10 Laboratory Studies: Xenografting and Biological Studies as a Component of the ALL06 Trial

Overview

It is recognised that ALL xenografts established in severely immune-deficient mouse strains [NOD/SCID and more recently NOD/SCID/γc^{null} (NOG or NSG)] represent a highly clinically relevant experimental model of the disease [1-3]. Direct inoculation of pediatric ALL biopsy specimens in these mouse strains via the tail vein results in the development of a systemic disease, with leukemia cells infiltrating the bone barrow, spleen, liver and disseminating into the peripheral blood, therefore providing an orthotopic model of the disease [1,4]. Therefore, the efficient engraftment of diverse pediatric ALL subtypes and clinical outcomes, as well as the ability to establish "continuous" xenografts by the re-inoculation of leukemia cells from the bone marrows and spleens of engrafted mice into secondary and tertiary recipients, have added a new dimension to the scope of preclinical investigations in the pediatric ALL field [2,4]. Moreover, the availability of almost limitless numbers of spleen-derived human leukemia xenograft cells, which for the most part accurately reflect the morphology, immunophenotype, and molecular characteristics of the original disease [1,2,5], has the potential to significantly and rapidly advance our understanding of the biology and treatment of pediatric ALL. Therefore, establishing xenografts of AYA-ALL represents a unique opportunity to improve treatment outcomes in this high-risk patient population.

Goals of the Project

1. Establish continuous xenografts from AYA-ALL samples by inoculation of leukemic cells from bone marrow or peripheral blood into NSG mice
2. Utilise continuous xenografts for cell and molecular biology analysis, and for preclinical drug testing

Experimental Plan

1. Xenograft AYA-ALL samples by inoculation of mononuclear cells from bone marrow or peripheral blood into NSG mice

We have well-established methodology for the routine xenografting of primary ALL cells into immune-deficient mice [2,4], which will be used for this AYA ALL study. In those cases where sufficient numbers of leukemic blasts are available (>10 million), 2.5-5 million cells will be inoculated into the tail veins of groups of 3-4 sub-lethally irradiated (250 cGy) NSG mice. At weekly intervals thereafter engraftment will be monitored by flow cytometric enumeration of the proportion of human $CD45^+$ cells in the murine peripheral blood. Upon exceeding 50% human $CD45^+$ cells, animals will be culled, leukemia cells harvested from bone marrows and spleens, and cryopreserved. The extent of leukemia infiltration of the bone marrow and spleen, as well as other major organs, will also be assessed by flow cytometry.

In our experience inoculation of as few as 1 million cells into a NOD/SCID mouse can yield up to 2 billion cells from the spleen upon harvest. Therefore, the availability of these samples that have been subsequently expanded in NSG mice will represent a valuable resource.

Continuous xenografts will be subsequently be established by inoculating spleen-derived leukemia cells from primary engrafted mice into secondary and tertiary recipient mice [2]. This will result in an almost limitless supply of xenograft cells.

2. Utilise continuous xenografts for cell and molecular biology analysis, and for preclinical drug testing

Establishing continuous xenografts from AYA ALL patients will facilitate studies aimed at improving outcome for this high-risk subgroup. Such investigations will include cell and molecular analysis for identification of pathways to target with novel drugs, as well as preclinical evaluation of novel drugs for prioritisation in future clinical trials.

Projects investigating the biology of the disease will focus on the interaction of ALL cells with micro-environmental factors and ALL cell trafficking. Examination of these interactions has previously identified the PI-3K/AKT/mTOR pathway as a potential therapeutic target leading to the initiation of

a clinical trial of the mTOR inhibitor RAD001. Current and planned studies will examine mechanisms of in vivo acquired resistance to mTOR inhibition and the potential of blockade of additional pathways to overcome such resistance. Projects examining how micro-environmental niches provide protection from chemotherapeutic agents, including the mechanisms involved in the retention of ALL cells within these niches and the impact of these niches on ALL cell biology, will also be undertaken.

Sample collection and transport requirements

To facilitate these studies, 10 million ALL cells collected from the diagnostic bone marrow biopsy (typically obtainable from 2 mls if hypercellular aspirate) collected in ACD will be required. This amount of marrow will need to be collected in addition to the requirements for diagnostic morphology, immunophenotyping, cytogenetics, and MRD studies required at diagnosis. Alternatively, in cases where marrow aspirate yields are limited, leukemic cells may be obtained from blood where white cell counts are sufficiently elevated. Samples must be sent to Mary Sartor, Flow Cytometry Laboratory, ICPMR, Westmead Hospital, NSW 2145. Leukemic cells will be purified by Ficoll Isopaque centrifugation and cryopreserved and transported to A/Prof Lock's laboratory at CCIA Randwick for engraftment into immuno-deficient mice. Alternatively samples may be engrafted in Dr Bendall's laboratory at Westmead Millennium Institute. A/Prof Lock at CCIA and Dr Bendall at WMI will use the cells recovered from the spleens of these animals for the projects outlined above.

Sample collection should occur per appendix 5.

References

1. Borgmann A, Baldy C, von Stackelberg A, et al. Childhood ALL blasts retain phenotypic and genotypic characteristics upon long-term serial passage in NOD/SCID mice. Pediatr Hematol Oncol 2000:17(8):635-650.
2. Liem NL, Papa RA, Milross CG, et al. Characterization of childhood acute lymphoblastic leukemia xenograft models for the preclinical evaluation of new therapies. Blood 2004:103(10):3905-3914.
3. Nijmeijer BA, Mollevanger P, van Zelderen-Bhola SL, et al. Monitoring of engraftment and progression of acute lymphoblastic leukemia in individual NOD/SCID mice. Exp Hematol 2001:29:322-329.
4. Lock RB, Liem N, Farnsworth ML, et al. The nonobese diabetic/severe combined immunodeficient (NOD/SCID) mouse model of childhood acute lymphoblastic leukemia reveals intrinsic differences in biologic characteristics at diagnosis and relapse. Blood 2002:99(11):4100-4108.
5. Neale G, Su X, Morton CL, et al. Molecular characterization of the pediatric preclinical testing panel. Clin Cancer Res 2008:14(14):4572-4583.

Appendix 11 Laboratory Studies: Xenografting and Biological Studies as a Component of the ALL06 Trial

Project Description

Acute lymphoblastic leukaemia (ALL) is the most common childhood cancer. Approximately 15% of children and the majority of adults diagnosed with ALL relapse following treatment and once this occurs the outlook for the patient is poor.

Relapse results from a small number of leukaemia cells that survived chemotherapy. Currently these cells are measured during and after treatment to estimate the chance that a patient will relapse. Although this monitoring is very helpful, it is not perfect. Not all patient destined to relapse are identified and some patients that are predicted to relapse remaining disease free. This shortcoming of the current method of testing was highlighted in a recent clinical trial where patients receiving the new protocol did much better than those on the old protocol but the monitoring of remaining leukaemia cells during treatment did not predict this outcome.

Recently it has been suggested that leukaemia is dependent on a tiny population of leukemic stem cells. These cells can be identified by the proteins they express on their surface in some types of leukaemia, but this is not the case in ALL. The ability to expel a particular dye called Hoechst 33342, has also been used to identify leukemic stem cells. In normal blood forming cells, cells with the ability to expel this dye are referred to as side population (SP) cells. The SP cells are highly enriched for blood forming stem cells. The reason why SP cells can expel the dye is because they have a protein that can pump out this dye. This protein pump can also pump out chemotherapy drugs. As a result SP cells tend to be more resistant to chemotherapy.

We have identified SP cells in the bone marrow of patients with ALL and shown that these cells are more resistant to chemotherapy. We propose that it is the SP cells that are responsible for relapse. If true, detecting these cells would be a better predictor of relapse than current methods that detect all remaining leukemic cells. To examine this hypothesis we propose the following studies: Enumerate SP cells in patients on current clinical trials, and correlate the findings with patient outcomes; and Test the response of SP cells to chemotherapy using human ALL cells in a special mouse model. Performing studies in patients enrolled on clinical trials where clinical outcomes are rigorously assessed will allow us to translate positive results into the clinic.

Quiescent ALL cells Study Contact Person: Linda Bendall Westmead Millennium Institute Darcy Rd Westmead. NSW. 2145 Please notify: Linda Bendall via email of sample shipment at linda.bendall@sydney.edu.au Phone: 02 8627 3770 or Mobile: 0407453716.	2ml sample (ACD tube) collected at the following time points will be used for both flow MRD (per appendix 8) and for identifying quiescent ALL cells in the marrow (appendix 11). (see appendix 5 for collection details) At Diagnosis Day 15 Day 33 At Relapse

Appendix 12. Investigating the Prevalence of Druggable Novel Gene Fusions, Detectable by Phospho-Flow Analysis.

Chief Investigator: **Professor Deborah White**
Co-Investigator(s): **Professor Timothy Hughes and A/Professor Charles Mullighan Project**

Description: To define the proportion of ALL cases who harbour the novel gene fusions using phospho-flow analysis of blood and/or marrow at the time of diagnosis.

Background B-lineage ALL is characterized by recurring chromosomal abnormalities including aneuploidy, chromosomal rearrangements (e.g*ETV6-RUNX1*, *BCR-ABL1*, *TCF3-PBX1*), and rearrangements of *MLL* and *CRLF2*[1-5]. However, many patients who relapse lack known chromosomal alterations, and identifying the full repertoire of genetic alterations in high-risk ALL is essential to design novel targeted therapies. Ph-like ALL represents up to 15% of childhood B-progenitor ALL, is three times more common than Ph-positive ALL, and is associated with an inferior outcome compared to non-Ph-like ALL. Using complementary genomic approaches, it has been shown that a majority of Ph-like ALL patients harbor novel rearrangements and sequence mutations activating tyrosine kinase signaling and importantly, that these patients may benefit from treatment with tyrosine kinase inhibitors.
As alterations driving activated kinase signaling are a hallmark of Ph-like ALL, it is now important to identify these patients at the time of diagnosis. To date the frequency of these novel fusions has not been identified in the adult ALL setting.
In view of the diverse range of genetic lesions underlying this phenotype, it is unlikely that detection of each alteration will be feasible. **Importantly phosphoflow cytometric analysis, may identify Ph-like patients that are candidates for treatment with targeted agents.**

Experimental Detail:
Phospho-flow analysis for p-Crkl and total p-Tyr will be used to determine the prevalence of these fusions in ALL patients at the time of diagnosis. Sensitivity to tyrosine kinase inhibition (using imatinib {Novartis} and dasatinib {BMS}) and Jak2 inhibition using the BMS compound and ruxolitinib {Incyte} will be simultaneously tested.

Result Reporting
As this is an assay under development results will not be made immediately available.

Sample Collection per appendix 5:
***Diagnosis*:** (Kit provided) 30ml blood collected in LiHep no gel

Prior to commencement of maintenance (Kit provided) 30ml blood collected in LiHep no gel

Relapse (Kit provided) 30ml blood collected in LiHep no gel & 2mls BM in EDTA

Any queries please contact: Prof. Deb White on 08 8128 4302 or deborah.white@sahmri.com

Transport.
Please send EDTA blood & Relapse BM to:
Prof Deb White
Leukaemia Research Laboratory
SAHMRI, Cancer Theme, Level 5
North Terrace
Adelaide SA 5000

Contact World Courier on 1800 023 560 and Quote Acct # 3514.
Any queries please contact: Stephanie Arbon on 08 8128 4304 or Stephanie.arbon@sahmri.com

References:

1. Pui CH, Robison LL, Look AT. Acute lymphoblastic leukaemia. Lancet 2008;371:1030-43.
2. Mullighan CG, Collins-Underwood JR, Phillips LA, et al. Rearrangement of CRLF2 in B-progenitor- and Down syndrome-associated acute lymphoblastic leukemia. Nat Genet 2009;41:1243-6.
3. Harvey RC, Mullighan CG, Chen IM, et al. Rearrangement of CRLF2 is associated with mutation of JAK kinases, alteration of IKZF1, Hispanic/Latino ethnicity, and a poor outcome in pediatric B-progenitor acute lymphoblastic leukemia. Blood 2010;115:5312-21.
4. Russell LJ, Capasso M, Vater I, et al. Deregulated expression of cytokine receptor gene, CRLF2, is involved in lymphoid transformation in B-cell precursor acute lymphoblastic leukemia. Blood 2009;114:2688-98.
5. Yoda A, Yoda Y, Chiaretti S, et al. Functional screening identifies CRLF2 in precursor B-cell acute lymphoblastic leukemia. Proc Natl Acad Sci U S A 2010;107:252-7.

Appendix 13 Monitoring asparaginase therapy with serum asparagine levels

Background:

The enzyme asparaginase has narrow substrate specificity and hydrolyses asparagine (and glutamine) to aspartic acid and ammonia. Leukaemic blasts in ALL can not resynthesise asparagine, leading to cytotoxicity. Despite the in vitro studies demonstrating up regulation of asparagine synthase by ALL blasts, this doesn't result in resistance to asparaginase in vivo[1]. In vitro data demonstrates that leukaemia cell cytotoxicity occurs when asparagine is completely depleted for a minimum of four days[2]. The purpose of monitoring is primarily for the early detection of loss of efficacy thus ensuring the ongoing delivary of effective therapy.

Complete asparagine depletion is achieved more readily and the time to serum asparagine recovery is longer (11 days vs 4 days, respectively) with the E.coli compared to Erwinia preparation. Consistent with this, a prospective randomised trial comparing E.Coli and Erwinia asparaginase for the management of childhood ALL demonstrated a superior overall (84% vs 75%) and event free survival (73 vs 60%) in favour of E.Coli preparation[3]. Another prospective randomised study using peg-asparaginase comparing weekly or twice weekly schedule for treating children with relapsed ALL demonstrated higher complete remission rates in those with higher asparaginase levels[4].

The CALGB Study 9511 used peg-asparaginase in 102 adult patients with newly diagnosed ALL (figure 1)[5]. This study demonstrated that older patients failed to achieve complete asparagine depletion; the median age of those failing to achieve complete asparagine depletion was 48 years compared to 32 years in whome asparagine was completely depleted. Furthermore, even after adjusting for other poor risk characteristics (age, performance status, white cell count and karyotype), both the DFS and OS were worse in those failing to achieve complete asparagine depletion (HR 1.8). This was further supported by the Children's Oncology Group Study which in a randomised trial comparing native E. Coli asparaginase with peg-asparaginase demonstrated that failure of asparagine depletion is predictive of relapse in children with standard risk ALL06. Fifty five percent of patients with inadequate asparagine depletion relapsed compared to 9% of those who achieved complete depletion.

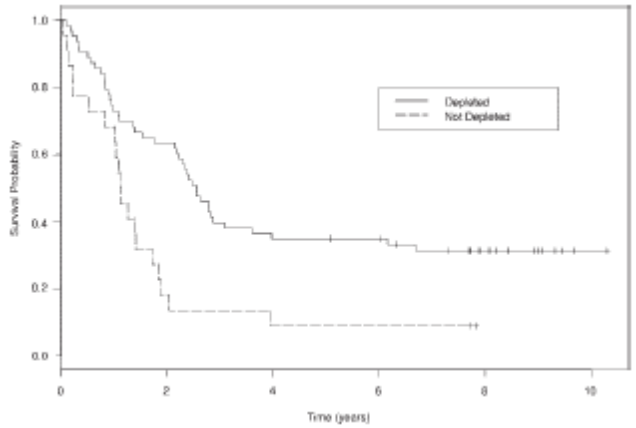


Figure 1

The question whether the dose of asparaginase can be adjusted according to serum asparagine levels was addressed by Ahlke and colleagues[7]. Using gradually decreasing E. Coli asparaginase doses from 10,000 u/m^2 to 2,500 u/m^2, they demonstrated that the lower dose (2,500 u/m^2) is sufficient to deplete asparagine completely in some patients; thus avoiding un-necessary higher doses. Higher doses were associated with higher vascular complications. Importantly not all patients on lower dose achieved adequately suppress serum asparagine, thus highlighting the importance of monitoring asparagine levels.

From above it is apparent that: a) adequate asparagine depletion is a necessary prerequisite for improved disease free survival and reduction in toxicity and that b) adequate asparagine depletion is not achieved predictably after each asparaginase dose even in asparaginase naive patients. The situation is complicated further by development of neutralising anti-asparaginase antibodies during the course of treatment with asparaginase.

As asparaginase is a foreign protein, allergic reactions and antibody formation is a clinically significant problem. The reported frequency of antibody formation after i.v. or intramuscular administration of E. Coli asparaginase is up to 70% in children and 80% in adults[8]. It was also noted that adults are more likely to develop allergic reactions, antibody formation and reduction in the enzyme activity. In addition, 'silent inactivation' of asparaginase in the absence of clinical hypersensitivity further compounds the problem. A similar phenomenon is described with the use of Erwinia asparaginase as well[9].

Antibody formation is not an 'innocent' and bystander event. Antibodies are neutralising in 94% of cases and are associated with decline in asparaginase levels along with increasing asparagine levels. This has been correlated with worse clinical outcome[10]. In the CCG-1961 study, 1001 paediatric patients were treated with E. Coli asparaginase. Elevated antibody titres were found in 61% of patients. Those having salient inactivation (30% of patients) and continuing to receive asparaginase had a far worse outcome (HR 3.2) than those with clinical allergy and antibody formation. This is because the latter group received Erwinia asparaginase after allergic reaction to E. Coli asparaginase. It has been demonstrated previously that formation of antibodies to the native E. Coli preparation predicts development of antibodies to peg-asparaginase after subsequent use but not to the Erwinia asparaginase[11] (a more expensive preparation). Thus, antibodies formed to E. Coli asparaginase are not cross reactive to the Erwinia preparation. Finally, in a cohort of 47 children with newly diagnosed ALL Zalewska-Szewczyk and colleagues demonstrated that development of antibodies to asparaginase resulted in an inferior DFS and OS (figure 2)[12]. The need to make a safe and effective switch between asparaginase preparations reinforces the need to monitor adequacy of asparagine depletion in patients receiving asparaginase.

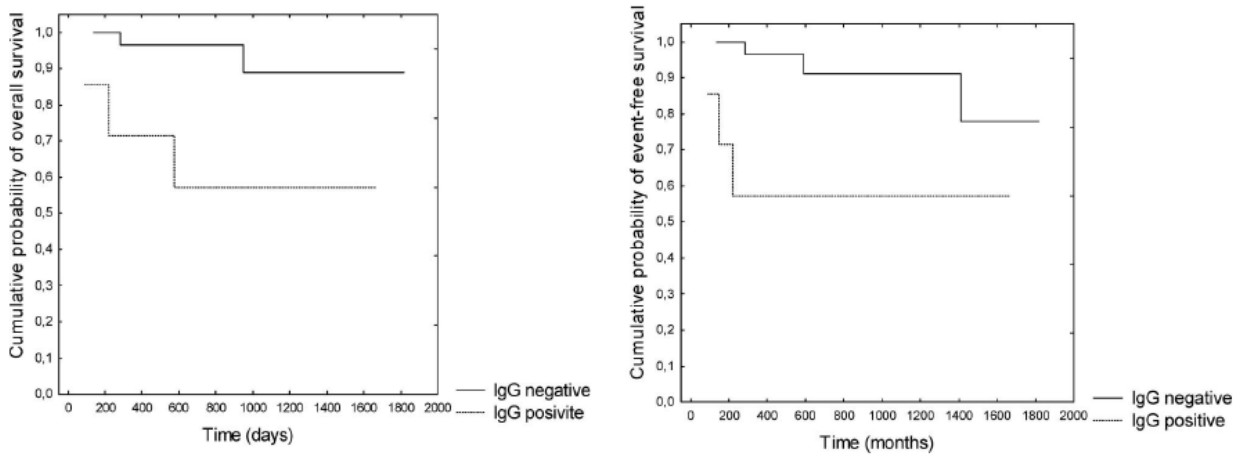


Figure 2

Therefore, it is apparent that the efficacy of asparaginase therapy be monitored, either by monitoring the enzyme levels, by monitoring antibody titres or by measuring asparagine levels. Of these, asparagine level monitoring is perhaps most ideal as it represents the end effect of all variables including dietary supplementation of the amino acid.

We propose monitoring of asparagine levels during ALLG ALL06 study (A Phase II trial of an intensive pediatric protocol incorporating post - induction stratification based on minimal residual disease levels for the treatment of adolescents aged 15 years and above, and young adults aged up to 40 years, with newly diagnosed acute lymphoblastic leukaemia (ALL) and assess the impact of depth

of asparagine depletion on leukaemia free survival (LFS).

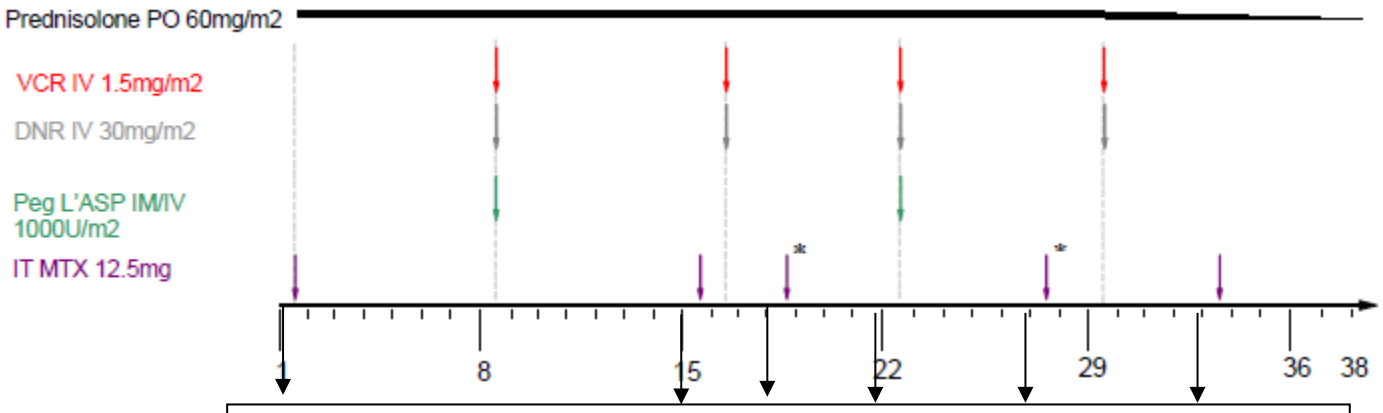
Primary objective: To collect the preliminary data exploring a correlation between the serum asparaginase activity and leukaemia free survival in adolescents and adults upto 40 years treated with pegylated asparaginase

Secondary objectives:

1) To study impact of serum pegylated asparaginase activity on neurological relapse
2) To study the impact of serum asparaginase activity on MRD status at time point 1 (day 33) and 2 (day 79)
3) To characterize the correlation between serum asparaginase activity and non haematological toxicity

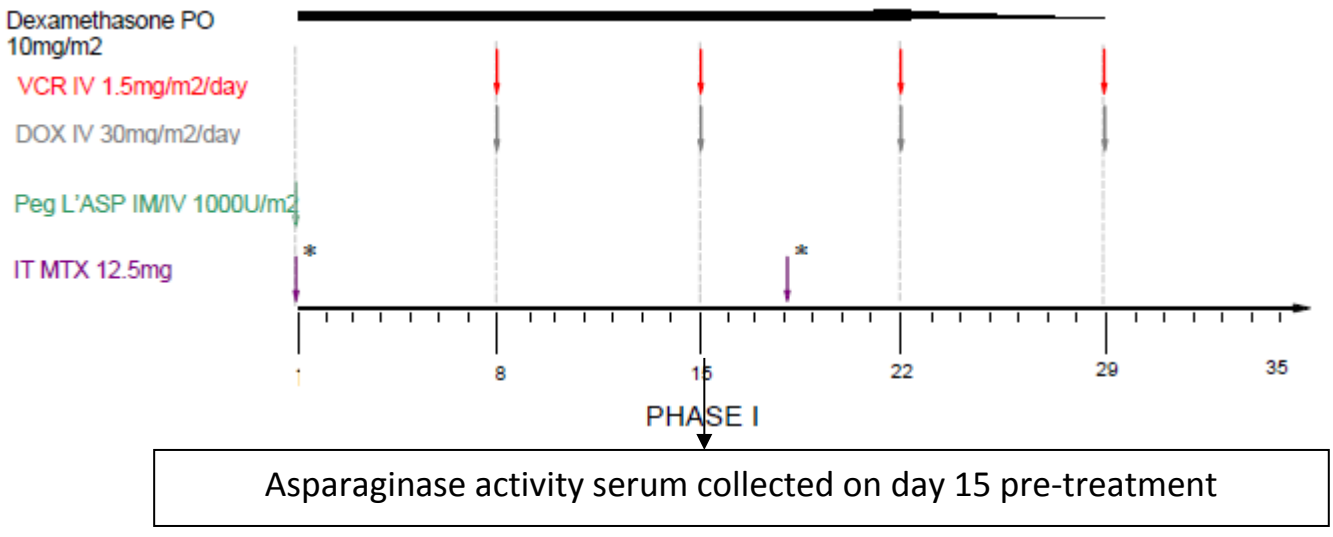
Proposed time points for collection of asparaginase activity (plasma and CSF)

1) Asparaginase levels during Protocol I: Induction/Consolidation Phase:



2) Asparaginase levels during HR blocks
Serum at day 7 following each asparaginase dose

3) Asparaginase levels during protocol II



Sample collection and handling

1. At each of the specified time points (see appendix 5), collect a minimum of 2ml peripheral blood in an SST tube
2. Invert tube not less than 5 times
3. Centrifuge within 2hours at 800 rpm x g for 10mins
4. Use pipette to transfer all of the serum into a cryovial
5. Freeze immediately at -20^{o}C or -80^{o}C

The samples labels should include at minimum the study number (ALL06), the patient's ALL06 registration number, the time point (protocol and day), the date of collection.

Samples will be stored frozen at site and batched to Dr Patil at the end of the trial. The ALLG TC will contact the site with further details regarding shipping at that time.

References:

1) Appel IM et al. Upregulation of asparagine synthase expression is not linked to the clinical response to L-asparaginase in pediatric acute lymphoblastic leukemia. Blood 2006; 107: 4244-9.

2) Asselin BL et al. In vitro and in vivo killing of acute lymphoblastic leukaemia cells by L-asparaginase. Cancer Res 1989; 49: 4363 - 4368.

3) Duval M et al. Comparison of Escherischia Coli asparaginase with Erwinia asparaginase in the treatment of childhood lymphoid malignancies: results of randomised European Organisation for Research and Treatment of Cancer – Children's Leukemia Group phase 3 trial. Blood 2002; 99: 2734 – 2739.

4) Abshire T et al. Weekly polyethylene glycol conjugated L-asparaginase compared to biweekly dosing produces superior induction remission rates in childhood relapsed acute lymphoblastic leukaemia: a pediatric oncology group study. Blood 200; 96: 1709 – 1715.

5) Wetzler M et al. Effective asparagine depletion with pegylated asparaginase results in improved outcomes in adult acute lymphoblastic leukemia: Cancer and Leukemia Group B study 9511. Blood 2007; 109: 4164 – 4169.

6) Avramis VI, Holcenberg JS, Ettinger AG, Stork LC, La M, Gaynon PS. Failure of asparagine (ASN) depletion, not inadequate asparaginase (ASNase) activity, predicts relapse in standard risk (SR) childhood acute lymphoblastic leukemia (ALL): A Children's Oncology Group (COG) Report. Blood 2007; 110: [Abstract 588].

7) Ahlke E, Nowak-Gottl U, Schulze-Westhoff P et al. Dose reduction of asparaginase under pharmacokinetic and pharmacodynamic control during induction therapy in children with acute lymphoblastic leukaemia. Br J Hematol 1997; 96: 675 – 681.

8) Killander D, Dohlwitz A, Engstedt L et al. Hypersensitivity reactions and antibody formation during L-asparaginase treatment of children and adults with acute leukemia. Cancer 1976; 37: 220 – 228.

9) Albertsen B, Schroder H, Jakobsen P et al. Antibody formation during intravenous and intramuscular therapy with Erwinia asparaginase. Med Pediatr Oncol 2002; 38: 310 – 316.

10) Panosyan E, Seibel N, Martin-Aragon S et al. Asparaginase antibody and asparaginase activity in children with high-risk acute lymphoblastic leukemia. J Pediatr Hematol Oncol 2004; 26: 217 – 226.

11) Wang B, Relling M, Storm M et al. Evaluation of immunological cross reaction of

antiasparaginase antibodies in acute lymphoblastic leukemia (ALL) and lymphoma patients. Leukemia 2003; 17: 1583 – 1588.

12) Zalewska-Szewczyk B, Anderzejewski W, Mlynarski W, Jedrychowska-Danska K, Witas H, Bodalski J. The anti-asparaginase antibodies correlate with L-asparaginase activity and may affect clinical outcome of childhood acute lymphoblastic leukemia. Leuk & Lymph 2007; 48(5): 931 – 936.