UKALL14

A randomized trial for adults with newly diagnosed acute lymphoblastic leukaemia

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Protocol Version 4.0 (date 24.04.2012) authorised by:

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Senior Lecturer in Haematology

Sign & Date 24/4/12

For the Sponsor: Professor Jonathan Ledermann
Director Cancer Research UK & UCL Cancer Trials Centre

Sign & Date 14/5/12

Introduction:

Cancer Research UK is supporting central coordination through the Cancer Research UK & UCL Cancer Trials Centre (UCL CTC) – the coordinating centre for the trial. Problems relating to this trial should be referred, in the first instance, to the UCL CTC.

This trial will adhere to the principles of ICH Harmonised Tripartite Guideline for Good Clinical Practice (CPMP/ICH/135/95) as set out in Schedule 1 of the Medicines for Human Use (Clinical Trials) Regulations 2004 and the GCP Directive 2005/28/EC, and any amendments thereto. It will be conducted in compliance with the protocol, the Data Protection Act 1998, the Medicines for Human Use (clinical trials) regulations 2004, as amended from time to time, and other regulatory requirements as appropriate.

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Table of Contents

1.0 Protocol Summary

1.1 Summary of Trial Design

Title:	A randomized trial for adults with newly diagnosed acute lymphoblastic leukaemia
Short Title:	UKALL14
EuDRACT No.:	2009-012717-22
MREC No:	09/H0711/90
Clinicaltrials.gov No.:	NCT01085617
UK Sponsor & No:	University College London Cancer Research UK & UCL Cancer Trials Centre 90 Tottenham Court Road, London, W1T 4 TJ, UK tel – +44207 679 9869 email – bnli@ctc.ucl.ac.uk UCL/08/0167
UK Funder & No:	Cancer Research UK 61 Lincoln's Inn Fields, London, WC2A 3PX, UK C27995/A9609
Design:	Multisite, randomized controlled trial
Aims:	Aim 1B. (precursor-B lineage) To determine if the addition of Rituximab to standard induction chemotherapy results in improved EFS in patients with precursor B-cell lineage ALL. Aim 1T (T lineage) To determine if the addition of nelarabine following standard induction therapy (arms T 1 and T2) improves outcome for patients with T cell ALL. Aim 2 To determine the tolerability of pegylated asparaginase in induction (for all patients) and to compare anti-asparaginase antibody levels between patients in the 2 randomisation groups from aim 1B. Aim 3 To determine whether risk-adapted introduction of unrelated donor HSCT (myeloablative conditioning in patients aged up to and including 40 years at time of study entry and non-myeloablative conditioning in patients aged greater than 40 years, ie having reached their 41st birthday at time of study entry) result in greater EFS for patients at highest risk of relapse. Aim 4 To compare 2 schedules of administration (standard P1 vs., 'collapsed' P2) of keratinocyte growth factor (palifermin) for efficacy in preventing the severe mucosal toxicity of etoposide/TBI HSCT conditioning regimen.
Primary endpoint:	Event free survival (applies to all interventions) Toxicity related to pegylated asparginase (applies to asparaginase evaluation only) Mucositis score by Oral Daily Mucositis Score (ODMQ) (applies to palifermin randomisation only)
Secondary endpoints:	Anti-asparaginase antibodies (induction randomisation only) Overall Survival Complete remission rate Minimal residual disease quantification after 1st phase of induction (applies to all patients) and MRD quantification post transplant (applies to the reduced intensity conditioned transplant randomisation only) Grade 3/4 graft versus host diseases (applies to transplant patients only) Death in complete remission

Patients:	The target overall recruitment is 720 patients with newly diagnosed, untreated acute lymphoblastic leaukaemia with or without evidence of Philadelphia chromosome. 20% of patients are expected to be T lineage, so out of a total of 720 patients 144 will be T-lineage and 576 precursor -B-lineage.
Planned number of sites:	100
Target countries:	United Kingdom and Eire
Treatment Summary: **(see also trial schema)**	**PHASE 1 & 2** Treatment for all patients will consist of a steroid pre-phase followed by 2 phases of induction therapy during which novel agents will be tested (precursor-B-lineage: 2 randomisation arms termed B1 and B2 and T lineage ALL 2 randomisation arms termed T1 and T2), as indicated in study aims, above. **After PHASE 2** **A risk assessment will be carried out for each patient based on presenting characteristics, cytogenetics and minimal residual disease analysis** Patients achieving CR & aged up to and including 40 years at time of study entry with an HLA-matched sibling identified move directly to allogeneic SCT* For all other patients further therapy depends on risk: Standard Risk: Continuing chemotherapy based on a modified UKALL12/ECOG2993 schedule (4 cycles of combination chemotherapy and then standard maintenance therapy). High Risk and aged up to and including 40 years at time of study entry will be allocated to allogeneic SCT with a matched unrelated donor and receive a myeloablative conditioning regimen*(Etoposide-TBI is preferred but Cyclophosphamide can be used.) High risk and aged greater than 40 years (ie having reached 41st birthday at time of study entry) will be allocated to allogeniec SCT with a matched unrelated donor and receive a reduced intensity conditioning regimen (fludarabine-melphalan +/- alemtuzumab). Intensification with high-dose methotrexate and PEG-ASP similar to the UKALL12/ECOG2993 trial will be given in the reduced intensity conditioned setting. *All Patients receiving the myeloablative regimen will be randomized between 2 dose schedules of palifermin as detailed in study aims, above.
Anticipated duration of recruitment:	Six years
Duration of patient follow up:	Until death
Definition of end of trial:	Recruitment: 6 years Active treatment: 2.5 years when maintenance chemotherapy is given Follow-up: 2 years after the last patient has completed maintenance therapy The end of the follow-up phase signifies the end of the trial.

Correlative Science:	1. To determine the relationship between CD20 expression on ALL blasts and response to monoclonal antibody therapy 2. To determine whether the administration of an anti-B cell monoclonal antibody as part of induction therapy for ALL limits the extent anti-asparaginase antibodies formation and promotes asparagine depletion. 3. To perform genomic profiling in order to discover and characterise novel prognostic markers and to identify known sub-microscopic copy number alterations (CNA) beyond the resolution of standard diagnostic testing (i.e. cytogenetics and FISH). 4. To determine whether the speed at which full donor chimerism is achieved in the T –cell compartment correlates with the level of molecularly determined minimal residual disease. 5. To formally assess the late effects of ALL therapy for all patients on the trial, whether they have received chemotherapy alone or an allograft.
Drug Supply:	1. Rituximab – Supplied free of charge from Roche until Rituximab comes off patent at the end of Nov 2013 after which it will be provided from hospital commercial stock. 2. Nelarabine – Supplied free of charge from Glaxo SmithKline 3. Palifermin – Supplied free of charge from Biovitrum 4. Pegylated Asparaginase – Supplied from MEDAC UK (not free of charge) All other drugs specified in the protocol must be provided from pharmacy stock at participating sites, see UKALL14 drug supply guidelines for more information.

UKALL14 TRIAL SCHEMA

1.2 Trial schema:

***Phase 1 Induction (4 weeks)*: precursor B cell & T cell patients (Philadelphia –ve patients only) to receive Pegylated-Asparaginase plus standard phase 1 induction therapy**

Pre B-cell ALL

- B1: Standard Phase 1 Therapy Alone
- B2: Standard Phase 1 Therapy + CD20 (Rituximab)

T-cell ALL

- Standard Phase 1 Therapy

***Phase 2 Induction (4 weeks)*: precursor B cell & T cell patients to receive standard phase 2 induction therapy**

Pre B-cell ALL

- Standard Phase 2 Therapy

T-cell ALL

- T1: Standard Phase 2 Therapy
- T2: Standard Phase 2 Therapy + Nelarabine

PATIENTS IN COMPLETE REMISSION (CR) – Risk Assessment performed on all patients at this time point

SIBLING DONOR

Over 40 years old*

Intensification with HD-Methotrexate + PEG-ASP

Conditioning regimen with fludarabine-melphalan and Alemtuzumab

To receive allo SCT (sibling)

40 years and under*

Myeloablative: Conditioning with Etopside-TBI

To receive allo SCT (sibling)

- **P1: Standard**
- **P2: Collapsed**

NO SIBLING DONOR

High Risk

Over 40 years old*

Intensification with HD-Methotrexate + PEG-ASP

Conditioning regimen with fludarabine-melphalan and Alemtuzumab

To receive allo SCT (MUD)

40 years and under*

Myeloablative: Conditioning with Etopside-TBI

To receive allo SCT (MUD)

- **P1: Standard**
- **P2: Collapsed**

Standard Risk

Continue methotrexate intensification, consolidation and maintenance

Randomisation to Palifermin Dose

*Age at study entry

2.0 Background

2.1 Introduction

The treatment of children with acute lymphoblastic leukaemia (ALL) is a shining example of the success of combination chemotherapy in curing malignancy. Results of recent trials suggest that up to 90% of children may be cured of their disease . Adult patients with ALL now have a 90% chance of entering first complete remission (CR) with modern chemotherapy. However, most patients still relapse, and leukaemia-free survival with three to seven years of follow-up is only 30-40%. The poor outcome of chemotherapy in adults with ALL as compared to children relates to multiple factors, some of which are known (e.g. adults have a higher incidence of poor prognostic subtypes such as Ph+ /t(9;22)/BCR-ABL ALL and a lower incidence of favourable subtypes such as t(12;21)(p13;q22)/ETV6-RUNX1 and high hyperdipliody) but others, are unknown.

2.2 Induction therapy for ALL

The primary goal of induction therapy is a complete eradication of ALL cells from blood, bone marrow and CNS or other extramedullary sites (when initially involved). This should be achieved in as many patients and as early as possible, and with as few toxic side effects as possible, in order to start rapidly the postremission consolidation. For Ph- ALL, induction therapy involve three sequential, connected steps, a pre-phase, induction I and induction II with the latter applied regardless of CR after induction I. Many examples of highly effective induction protocols have been reported with CR rates of 90% or more. Because of the complexity of existing induction regimens and the fact that it is already possible to obtain a CR in 90% or more of unselected patients, the evaluation of any new treatment element will also have to evaluate alternative endpoints as early surrogate marker of long-term response. The induction regimen used in this protocol is based on that used in UKALLXII/ECOG2993[3] with some modifications some of which are rooted in changes in practice since the design of UKALLXII and some of which comprise the questions to be asked.

2.3 Steroid for induction of ALL

Corticosteroids are one of the most important drugs in the treatment of ALL, and recent trials in paediatric ALL patients have suggested that the use of dexamethasone, as opposed to prednisolone may improve outcome. This is based on data suggesting that dexamethasone has greater in vitro anti-leukaemia activity than prednisolone, better penetration of the CNS and causes fewer thromboembolic events.[63] Randomized trials have demonstrated improved survival in children receiving dexamethasone as opposed to prednisolone [4], although this has not been shown in every study.[64] Based on these data this trial will substitute a discontinuous schedule of dexamethasone for prednisolone during induction.

2.4 Monoclonal antibodies in induction of precursor B lineage ALL

2.4.1 Anti CD20 antibody: Rituximab

Rituximab, a chimeric IgG-1 anti-CD20 monoclonal antibody, has found ever expanding uses since its approval by the FDA in 1997. When combined with CHOP chemotherapy in the treatment of diffuse large B-cell lymphoma, the combination of chemotherapy with rituximab was shown to have an overall survival advantage.[66]
Rituximab has been combined with chemotherapy in a wide range of schedules of Lymphoma therapy without any evidence of increased toxicity.

CD20 is expressed on nearly 70% of pre-B ALL cells, although at lower intensity than in non-Hodgkin lymphoma or chronic lymphocytic leukaemia (E. Paietta, personal communication). The ability to combine rituximab with chemotherapy in the treatment of lymphoma and the expression of CD20 in ALL of B cell precursor-cell type has led to the use of rituximab in the treatment of B cell precursor ALL in case reports and smaller case series [5,6].

A recent report of 28 patients with adult-Burkitt-type lymphoma and ALL (B-ALL) suggested an advantage with a Hyper-CVAD plus rituximab compared to historical controls treated with hyper CVAD alone. Hyper-CVAD and rituximab have also been combined for treatment of B cell precursor-ALL patients. The addition of rituximab to Hyper-CVAD appeared to improve disease-free survival in CD20-positive patients compared to historical controls treated with Hyper-CVAD alone [7]. These data are encouraging enough to warrant a randomized trial comparing chemotherapy with or without rituximab in induction for newly diagnosed patients with bcr-abl negative B cell precursor-ALL. This data has recently been updated with longer follow up at the American Society of Haematology meeting December 2008.

The expression of CD20 and CD22 on ALL blasts varies widely (E. Paietta, personal communication) CD20 is less commonly or less highly expressed on B-ALL blasts than some other B cell antigens. However, there is accumulating evidence that it is of prognostic significance . No data in ALL - or other tumour types - are available to define the relationship between antigen expression and response and suggest a threshold level for response. Particularly intriguing in this regard is a recent demonstration that the relatively modest expression level of CD20 at diagnosis was often dramatically up-regulated, both in numbers of cells expressing the antigen and levels of expression per cell, following induction chemotherapy. This was shown in vitro to occur on exposure to glucocorticoids, and correlated well with in-vitro rituximab-induced killing[9].
We propose to compare the efficacy and toxicity of induction chemotherapy in ALL alone or in combination with Rituximab.

2.5 Nelarabine for induction of T-cell ALL

Guanine arabinoside (ara-G) was first synthesized in 1964 and subsequently shown to have pre-clinical activity against human T cell lymphoid malignancies. A pro-drug with increased solubility, known as compound 506U78, was developed . A phase I study was carried out in 93 patients with refractory haematologic malignancies utilizing a daily one-hour intravenous infusion schedule of administration for five days. Doses ranging from 5 to 75 mg/kg/day were given with dose-limiting neurotoxicity encountered at the dose of 75 mg/kg/day. One patient

each had grade 3 neurotoxicity at doses of 20 and 60 mg/kg/day. Maximum tolerated doses were defined as 40 mg/kg/day in adults and 60 mg/kg/day in children. To facilitate future phase II studies, dosing was converted to doses given per m^2 of body surface area during the study and it was projected that 1.2 grams/m^2/day (approximately 30 mg/kg/day in adults and 40 mg/kg/day in children) would be a reasonable phase II dose. Both central and peripheral neurotoxicity were the main toxicities seen and were largely reversible [10] A subsequent phase II study of nelarabine conducted by the Children's Oncology Group (COG) in patients with refractory T cell malignancies treated four groups of patients: first bone marrow (BM) relapse, second or greater BM relapse, central nervous system (CNS) relapse and extramedullary (EM) relapse. An initial dose of 1.2 grams/m^2/day for five days was chosen, but was de-escalated to 900 mg/m^2/day and then to 650 mg/m2/day because of severe neurotoxicity. The third and fourth groups with CNS and EM relapse were treated with 400 mg/m^2/day to obtain pilot response data with the anticipation that this dose might be utilized in combination with chemotherapy in future trials. Complete plus partial response rates in the four groups were 55% (first BM relapse), 27% (second BM relapse), 33% (CNS relapse) and 14% (EM relapse). Neurological events of grade 3 or greater were seen in 18% of patients [11] The COG has recently reported the safety and feasibility of combining nelarabine at a doses of both 650 and 400 mg/m^2/day for five days with an intensive modified BFM regimen in children with T cell precursor ALL. A cohort of patients on the study was treated without nelarabine. Neurological and non-neurological toxicities were similar between the two dose levels of nelarabine and those treated without nelarabine. A phase III trial of nelarabine in combination with augmented BFM chemotherapy in consolidation therapy in newly diagnosed intermediate and high-risk children and young adults with T-ALL has recently opened in COG (AALL0434). A study in adults showed approximately 30% response in relapsed and resistant ALL with neurotoxicity being minimal and haematological toxicity being the most common. In the present trial we propose to add a course of nelarabine to induction in a randomized fashion following phase II of induction therapy in patients with T-ALL.

2.6 Role of L-asparaginase

L-asparaginase is arguably one of the most valuable drugs in the treatment of ALL. However, it is associated with numerous toxicities including hepatic dysfunction, pancreatitis and thrombohaemorrhagic complications related to depletion of coagulation factors. An additional complicating feature of the use of L-asparaginase clinically is the development of antibodies to the enzyme that can either result in hypersensitivity reactions (IgE) or via neutralising antibodies (IgG) decrease in enzyme activity with loss of therapeutic efficacy. Neutralizing antibodies developing in the absence of a clinical reaction is known as silent inactivation. When toxicity occurs early in treatment, therapeutic delays are often generated which can compromise the aims of therapy. The appropriate dose, preparation and formulation of L-asp remain unresolved. In paediatric practice, pegylated L-asp (peg-asp) is less immunogenic and gives the most appropriate pharmacokinetic and pharmacodynamics but evidence that this agent could be properly used in adults was lacking until a recent CALGB phase II study used peg-asp as part of a multi-agent regimen. Effective asp depletion was achieved in some adults [15]although increasing age was associated with significantly decreased peg-asp doses and less asparagine depletion. Furthermore, there was still a significant number of patients who developed of anti-asparaginase antibodies; this correlated with a less successful asparagine depletion. Within the associated clinical study UKALL14, peg-asp will be administered to adults for the first time in a large phase 3 setting.

L-asparaginase is prepared from bacterial sources of either Escherichia coli or Erwinia chrysanthemi. E Coli asparagnase is available either as a native asparaginase or conjugated to polyethylene glycol (peg-asp). Pegylation extends the half-life and lessens the frequency of injections .The use of PEG-ASP has been compared to the Ecolab native asparaginase preparation in two randomized trials. In the DFCI 91-01 study, patients were randomized to receive PEG-ASP 2500 IU/m^2 every other week for 15 doses or E. coli asparaginase 25,000 IU/m^2 every week for 30 doses in intensification. Event-free survival and toxicities were similar between the two asparaginase arms[17] A CCG trial compared PEG-ASP 2500 IU/m^2 on day 3 of induction to nine doses of native E. coli asparaginase, 6000 IU/m^2 every 3 days in induction and found a lower rate of high titre antibody formation with PEG-ASP, but similar rates of adverse events and similar EFS [18]. Recent data from the CALGB has shown that effective asparagine depletion with PEG-ASP results in improved outcomes compared with patients not achieving effective asparagine depletion [15].

Based on these and other data, we propose to evaluate the treatment of patients in this study with PEG-ASP and ask specific questions about the toxicity and some scientific questions about asparagine depletion and anti-asparaginase antibody formation. Because of regulatory restrictions two different forms of PEG-ASP will be used in this study, the Medac product in the United Kingdom and the Enzon product in the United States. The precise relative efficacy of the two products is not known so doses will be chosen based on published reports of the use of each respective product.

In the case of hypersensitivity to peg-asp, Erwina asparaginase should be substituted at a dose of 20,000units/m^2 IM (x6 doses) as a replacement for each scheduled dose of PEG-asparaginase. Please see appendix 14 for further guidance.

2.7 Prophylaxis against Central Nervous System Disease in ALL

The presence of leukaemic blasts in the central nervous system (CNS) is a more frequent occurrence in patients with ALL than AML and the importance of prophylactic treatment to prevent the development of progression to the CNS has long been recognized [19]. The results of therapy in patients with CNS involvement at diagnosis in the current MRC UK ALL XII/ECOG E2993 trial have recently been published. Seventy-seven of 1508 (5%) patients had CNS involvement at diagnosis. The incidence is higher in patients with T cell ALL. Of these 77 patients, 69 (90%) achieved complete remission. This study has demonstrated that, while long-term disease-free survival is attainable in patients who present with CNS involvement, overall survival at five years was inferior at 29% compared to 38% for patients without CNS involvement ($p=0.03$)[20]).

The role of prophylactic cranial irradiation in the era of combined intrathecal and high-dose systemic therapy has been questioned in recent studies with the stated intent of reducing the risk of late sequelae . This has been of particular interest to paediatricians and trials in children have demonstrated that CNS irradiation can be eliminated without worsening overall outcome [22] . In adults several trials have reported CNS recurrence rates less than 10% with the combined use of high dose systemic and intrathecal chemotherapy without the use of cranial irradiation[24-26]. In the UKALLXII/E2993 the risk of CNS recurrence was 4%. We propose to discontinue the use of cranial irradiation in the trial proposed here, but will plan to perform interim analyses to detect any increase in CNS recurrence rate.

2.8 Role of Haematopoietic Stem Cell Transplantation (HSCT) in adult ALL

Numerous trials have been conducted incorporating autologous and allogeneic SCT into the treatment algorithm. These trials, while varying in design, have generally demonstrated that allogeneic transplant is more effective than autologous transplant or chemotherapy in either high-risk patients or in all patients treated.

One of the most important analyses was the comparison of the outcome of Ph- patients assigned to HLA-matched sibling SCT compared to patients randomized to autologous SCT or chemotherapy (so-called donor vs. no donor analysis) in UKALLXII/E 2993. In a comparison of 389 patients with a donor to 530 patients without a donor, the donor groups had superior EFS (50% versus 41%, p=0.009) and OS (53% versus 45%, p=0.02). A similar statistically significant benefit was seen when the no donor group was restricted to those who were randomized to the chemotherapy arm only. Of importance, this benefit was primarily seen in the standard risk patients (OS 63% for donor versus 51% for no donor patients, p=0.01), but not in high-risk patients (OS 40% versus 36%, p=0.6). The lack of difference in outcomes between donor and no donor patients in the high-risk group were related to a high non-relapse mortality of 39% at two years (20% at two years for the standard risk patients)[27] which in large part was seen in older patients. An additional justification for considering allogeneic SCT in first CR is the dismal outcome of patients who relapse from first CR in the current UKALLXII/E2993 trial with an overall survival at 5 years of 7% [28].

Hence allogeneic transplant is currently the treatment of choice for eligible adults in first complete remission (CR1). At present, a group of adults with ALL in whom the risk of relapse is less than the risk of sibling allogeneic SCT cannot be defined. Accordingly, this study continues to propose sibling allogeneic SCT for every eligible patient where a sibling donor is available.

2.8.1 Myeloablative conditioning regimen

The "Stanford" conditioning regimen of high dose etoposide and total body irradiation (TBI) was originally chosen for UKALLXII/E2993 as it offered the best published results in SCT for ALL . There are no randomized controlled studies comparing conditioning regimens. Best available evidence is a retrospective analysis of IBMTR data . This suggests no difference in relapse risk or OS between conditioning regimens containing cyclophosphamide and etoposide when patients are transplanted in CR1, while there was an advantage to etoposide and higher doses of TBI in patients transplanted in CR2. There is now considerable experience with the use of the etoposide/TBI regimen among centres carrying out allogeneic SCT for ALL, the appropriate course of action is to continue to use the etoposide-TBI conditioning regimen for this study in patients under the age of 40. Since the major acute toxicity of this regimen is severe mucositis, attempts to reduce the incidence of mucositis are warranted and this will be a study question – aim 4.

2.8.2 Prevention of oral mucositis – the role of Palifermin

The development of palifermin and demonstration of its efficacy in reducing the incidence and severity of oral mucositis following a TBI-containing conditioning regimen in the autologous setting has generated considerable interest in the BMT community[32]. However, the schedule of administration of palifermin is cumbersome in that it mandates administration of three doses prior to initiation of the conditioning regimen. Palifermin has been tested

in the setting of allogeneic BMT in a phase I/II study and shown to be safe . The etoposide-TBI regimen to be utilized in this study has considerable anti-leukaemic efficacy, but is associated with a 100% incidence of grade 3-4 mucositis. This toxicity has an important consequence for GVHD prophylaxis as most patients cannot receive all 4 planned methotrexate (MTX) doses required for the standard cyclosporin and short course MTX GVHD prophylaxis regimen. Two major studies confirm the importance of MTX dose delivery [34,35],.in preventing severe acute GVHD, which is an important cause of mortality that can affect transplant outcome. Preliminary data suggest that a 'collapsed dose' of Palifermin is as effective as the standard administration schedule. There are some phase 2 data and data from murine models, which also suggest the hypothesis that a collapsed schedule may be more effective. A randomized comparison of standard (6 doses) versus 'collapsed' (4 doses) will be incorporated into the current trial in combination with the etoposide-TBI-containing regimen in an attempt to reduce the morbidity and mortality of this intensive regimen.

2.8.3 Reduced intensity conditioning regimens in ALL

The current UKALLXII/E2993 trial confirmed the benefit of allogeneic SCT in inducing a potent GVL effect, but was associated with unacceptable toxicity in older patients. Reduced intensity conditioning SCT (RIC-SCT) has allowed the extension of the use of allogeneic SCT to older and infirm patients There is a paucity of data on the use of RIC-SCT in the treatment of patients with ALL [37,38].These small studies have, however, demonstrated the feasibility of this approach with preservation of a GVL effect.

Most RIC-SCT conditioning regimens combine a purine nucleoside analogue (most commonly fludarabine) to induce potent T cell immunosuppression with low dose TBI or an alkylating agent such as cyclophosphamide or melphalan with additional immunosuppression with antithymocyte globulin or alemtuzumab [39]. The fludarabine-melphalan regimen has been extensively used for RIC-SCT for both myeloid and lymphoid malignancies and shown antitumor activity with acceptable toxicity [40-42]Recent data from the City of Hope National Medical Centre reported on 21 patients primarily consisting of patients with high risk ALL in first or more advanced stages of remission who were not eligible for conventional BMT and received a conditioning regimen of fludarabine and melphalan combined with a matched related (33%) or unrelated (67%) donor allograft. With 17 months of follow-up, the one year cumulative probability of overall or disease-free survival and relapse were 77%, 71% and 8%, respectively. The incidence of acute GVHD, grades II-IV and III-IV, were 50% and 15%, respectively. The 100-day non-relapse mortality was 10%. In the United Kingdom, alemtuzumab has been combined with fludarabine and melphalan to help ensure engraftment of donor cells and reduce the risk of GVHD. These studies have shown low rates of 100 day non-relapse mortality (<15%) with low rates of acute GVHD, grades III-IV, of <10%), but a significant incidence of viral and fungal infections [40-42]. This trial will include a phase II sub-study of a fludarabine-melphalan-alemtuzumab regimen for RIC-SCT with either related or unrelated donors for patients over the age of 40 to determine if we can retain a GVL effect in older adults while reducing non-relapse mortality. The cut-off age of 40 years was based on a combination of data from UKALLXII/ECOG2993 and informed opinion from the transplant communities in the UK and the USA.

Please note that as patients receiving reduced intensity Conditioning regimens DO NOT receive TBI there is a need to maintain appropriate CNS directed therapy. These patients will therefore receive 8 x 3 monthly intrathecal methotexate injections at a dose of 12 mg (total dose) for 2 years post transplant starting at 3 months post transplant - see section 7.2.11.

2.8.4 The use of unrelated donors

Evidence has accumulated to suggest that risk of MUD allogeneic SCT for patients with ALL is now not much greater than risk of sibling allogeneic SCT [45], 38). The outcome for patients at high risk of relapse is sufficiently poor that an evaluation of MUD allogeneic SCT is proposed for those patients deemed to be at the highest risk of relapse. In the UKALLXII/E2993 trial, only those patients with Ph- ALL were deemed to be at high enough risk for relapse to warrant the risk of MUD allogeneic SCT. In the current study, other high-risk factors confirmed both by data from UKALLXII/E2993[46,47] and from other published studies have been introduced. Patients older than age 40, those with precursor B cell disease with WBC >30 X 10^9/L or T cell precursor disease with WBC>100 x 10^9/L) or the presence of high-risk cytogenetic abnormalities; Philidelphia chromosome/t(9;22)(q34;q11)/BCR-ABL1, t(4;11), low hypodiploidy/near triploidy and complex karyotype, will each confer "high-risk".

2.8.5 The source of stem cells

Peripheral blood stem cells have a number of advantages compared to bone marrow in the setting of related donor allogeneic SCT. They result in faster haematopoietic recovery and shorter length of hospitalization but may also result in a higher incidence of chronic graft versus host disease [48,49] Larger patients (>80 kg) should certainly receive peripheral blood stem cells as first preference. There is no evidence that for patients in first remission that peripheral blood stem cells result in superior survival to bone marrow and therefore no recommendation can be made with regard to stem cell source in the sibling donor setting. When peripheral blood stem cells are used a more prolonged period of immunosuppression for GVHD prophylaxis should be considered and careful monitoring for chronic graft versus host disease should occur.

Controversy exists over the preferred source of stem cells for matched unrelated donor (MUD) allogeneic SCT for ALL due to a retrospective analysis of European registry data, which suggested that survival after MUD allogeneic SCT for ALL may be reduced by the use of PBSC as compared to BM [50]. The survival advantage for BM in this study related to both procedure related mortality and relapse risk. The preferred source of stem cell for MUD allogeneic SCT may be BM, but in the absence of confirmatory prospective data, PBSC remain acceptable.

2.9 Measurement of Minimal Residual Disease (MRD)

Despite the identification of the risk factors described above, many patients with ALL with or without high-risk features still relapse. These relapses presumably occur as a result of minimal residual disease (MRD) not detected by conventional methods of assessing remission status. The introduction of more sensitive molecular techniques to detect MRD in ALL patients and predict their risk of relapse utilizing clonal immunoglobulin or T-cell receptor gene rearrangements have been very effective .In paediatric ALL, the presence of residual disease at the end of induction chemotherapy or at later time points was a powerful predictor of relapse independent of other risk factors [52] .In adult ALL, the use of these molecular markers has been introduced more recently and also shown to be of prognostic significance. The German Multi-Centre Study Group for adult ALL has recently published data indicating that patients who have a rapid decline in their minimal residual disease within the first month of therapy had a three-year relapse rate of 0%. Another subset of patients who had MRD detectable until week 16 of therapy had a three-year relapse rate of 94%. Patients in between these two groups had an intermediate risk of 47% Measurement of MRD in a subset of patients in the on-going MRC/ECOG UKALLXII/E2993 trial has also shown

evidence of prognostic significance, especially in the pre-B ALL group. The detection of MRD at the end of the second month of induction therapy appeared to be the most sensitive time point (Patel et al, submitted). Measurement of MRD will be incorporated into the trial presented here to help stratify patients with low risk clinical prognostic features into those who are MRD positive at the end of induction therapy and therefore, re-categorized as high risk and now eligible for allogeneic SCT, and those who are MRD negative who will be treated with consolidation and maintenance chemotherapy and not be allocated to allogeneic SCT. Measurement of MRD will be performed in all patients and correlated with outcome.

2.10 Summary of study aims

Primary

To determine if the addition of monoclonal antibody to standard induction chemotherapy results in improved EFS in patients with precursor B-cell ALL (aim 1B).

To determine if the addition of nelarabine improves outcome for patients with T cell ALL (aim 1T).

Secondary

To determine the tolerability of pegylated asparaginase in induction treatment of all patients (aim 2) and to compare anti-asparaginase antibody levels between patients in the 2 randomisation groups from aim 1B (patients with B lineage ALL only).

To determine whether risk-adapted introduction of unrelated donor HSCT (myeloablative conditioning in patients ≤40 years old and non-myeloablative conditioning in patients >40 years old) results in greater EFS for patients at highest risk of relapse (aim 3).

To compare 2 schedules of administration (standard vs., 'collapsed') of keratinocyte growth factor (palifermin) for efficacy in preventing the severe mucosal toxicity of etoposide/TBI HSCT conditioning regimen (aim 4).

To formally assess the late effects of ALL therapy for all patients on the trial, whether they have received chemotherapy alone or an allograft. To identify and describe some of the adverse physical and psychosocial consequences of the disease and its treatment.

3.0 Selection of Trial Sites/Site Investigators

3.1 Trial Site selection

"Site" the hospital or site participating in the conduct of the Trial;

Sites should be able to comply with:

- Trial treatments, sample collection procedures and follow up schedules
- Research Governance Framework and the Medicines for Human Use (clinical trials) Act (SI 2004/1031)
- The trial protocol
- Data collection requirements.

Each site should also have:

- Haematology Multidisciplinary Team (MDT), which must include a leukaemia specialist
- Transplant centre or a relationship with a referring transplant centre to whom they are prepared to refer every patient for a donor search at diagnosis.
- Transplant centres must be JACIE accredited or working towards JACIE accreditation

3.2 Selection of site investigators

Each site must have an appropriate Principal Investigator (PI) ie a health care professional authorised by the site, ethics committee and Competent Authority to lead and coordinate the work of the trial on behalf of the site.

The PI, other clinicians and all staff involved in the conduct of the trial at the site must be identified on the site delegation log, held at site and copied to UCL CTC prior to site activation. This must be updated to reflect all staff changes, signed by the PI and copied to UCL CTC.

3.3 Site set up

The following documentation must be in place prior to a site being opened to recruitment by the UCL CTC trial team:

- All relevant institution approvals (including Trust R & D approval)
- Clinical Trial Site Agreement (CTSA) between the Sponsor and the Trust
- UKALL14 Declaration of Participation & Delegation Log

Please note: Non-transplant sites will not be activated by UCL CTC until their referring transplant centre has approval.

3.3.1 Site initiation

All sites will be required to have a site initiation session before recruitment can begin. Site initiation will take the form of either a site visit or a teleconference involving the trial coordinator and site staff involved in the trial. The exact nature of the site initiation will be agreed between the CTC and site staff.

3.3.2 Site activation (UCL CTC responsibility)

Once the trial team at UCL CTC have confirmed that all documentation is in place a site activation letter will be issued to the PI, at which point the site may start to approach patients.

Please note: Non-transplant sites will not be activated by UCL CTC until their referring transplant centre has approval.

4.0 Informed consent

Sites are responsible for ensuring all patients are fully informed about the trial and have confirmed their willingness to take part in the trial by signing a consent form. Site investigators are required to provide a full explanation of the trial and all relevant treatment options to each patient prior to trial entry. During these discussions a detailed patient information sheet and consent form for the trial will be given to the patient. A minimum of twenty four hours must be allowed for the patient to consider and discuss participation in the trial. Written informed consent must be obtained before any trial-specific procedures are conducted.

Site staff are responsible for:

- checking information on consent forms is complete and legible
- checking that the patient has initialled all relevant sections and signed and dated the form
- countersigning and dating consent forms to confirm that they provided information to the patients
- recording the informed consent process in patient medical notes
- storing the original signed consent form at site and giving a copy to the patient

The right of the patient to refuse to participate in the trial without giving reasons must be respected. All patients are free to withdraw at any time from the protocol treatment without giving reasons and without prejudicing further treatment. Any patient's withdrawal of consent from the trial must be explicitly documented in the source documents and UCL CTC informed.

5.0 Selection of Patients

5.1 Pre-registration & pre-1st-randomisation evaluation (study entry)

All patients will undergo their first randomisation upon registration for the trial and the following assessments and procedures are required prior to registration (please note that there are further assessments & procedures required prior to transplant – see section 5.4)

- Full medical history & physical examination
  - Including cardiac history, any history of mental health issues and employment status pre-ALLdiagnosis (baseline measurements for the late effects assessment at 2 years follow up in section 8.3)
- Height, Weight & BSA
- Assessment of performance status (ECOG)
- Full blood counts & biochemistry
- Bone marrow aspirate & trephine
- Pregnancy test for all women of childbearing age
- Confirmation of disease diagnosis from bone marrow aspirate (or peripheral blood where there is high presenting WCC) using Immunophenotyping/Flow Cytometry
- Cytogenetic, FISH and molecular genetic analysis on a pre-treatment bone marrow is essential to
  - (a) confirm the presence or absence of the Philadephia (Ph) chromosome \ t(9;22)(q34;q11) \ *BCR-ABL1*. Where patient is Ph pos, imatinib will be administered with the therapy.
  - (b) identify the following high risk abnormalities (refer to Appendix 6 for cytogenetic definitions and detection strategy;
    - Philadephia chromosome \ t(9;22)(q34;q11) \ *BCR-ABL1*.
      - These patients will needs imatinib added to therapy
    - t(4;11)(q21;q23) / *MLL-AF4*
      - NB *AFF1* was previously known as *AF4* and *MLLT2*
    - low hypodiploidy / near-triploidy (Ho-Tr)
    - complex karyotype (five or more chromosomal abnormalities)
  - These tests are to be performed by the local or regional cytogenetic/genetic laboratory and a copy of the report of all diagnostic and relapse cytogenetic, FISH and genetic testing must be sent to the cytogenetic coordinating centre - the Leukaemia Research Cytogentics Group (LRCG). See Appendix 6 for contact details.
- The cytogenetic co-ordinating centre (LRCG) may request left-over fixed cell suspension, DNA, RNA or other material from the local genetics laboratory or the treating clinician to undertake further cytogenetic, molecular cytogenetic and genetic testing (including but not restricted to FISH, array CGH and RT-PCR) to refine the definition of known abnormalities and characterise novel subgroups. All these additional tests will be performed with the full knowledge of the CI and clinical coordinators.
- Lumbar puncture is not required at diagnosis except in the case of suspected central nervous system involvement. Otherwise, it should be avoided (in case of traumatic puncture and CNS seeding) until the first dose of intrathecal methotrexate is due, at which time blasts should have been cleared from the peripheral blood. The first lumbar puncture should always be given by the most experienced operator available, to reduce the incidence of traumatic taps.

Please note, as detailed in section 8.2.1 and Appendix 7:

	Specimens for local assessment	**Specimens for correlative studies to be sent to central laboratory (see app 7)***
At Diagnosis	Cytogenetics/molecular assessment of BCR-ABL and MLL on bone marrow. A copy of the results should be sent to the Leukaemia Research Cytogenetics Group (LRCG) at Newcastle University. (Please refer to Appendix 6 for details of the LRCG) Tissue typing of patient and any siblings to be carried out. Donor search to be initiated if no matched sibling donor available.	Bone marrow 3-5ml in EDTA (OR peripheral blood 30-50ml in EDTA if WCC > 30x 10^9/l). BCR-ABL status will also be checked.

5.2 Patient Eligibility for Study Entry

For eligibility for transplantation, see section 5.5

5.2.1 Patient Inclusion Criteria (study entry)

a) Subjects must be aged ≥ 25 and ≤ 65 years old with acute lymphoblastic leukaemia
b) Newly diagnosed, previously untreated ALL (a steroid pre-phase of 5-7 days is required and can be started prior to registration)
c) Written informed consent

5.2.2 Patient Exclusion Criteria (study entry)

a) Known HIV infection
b) Pregnant or lactating women
c) Blast transformation of CML
d) Mature B-cell leukemia i.e. Burkitt's lymphoma t(8;14)(q24;q32) and variant c-myc translocations e.g. t(2;8)(p12 ;q24), t(8;22)(q24;q11)

5.2.3 Management of patients with poor organ function at study entry

In order for the protocol to reflect 'real-life' management of ALL, patients with poor organ function at diagnosis are not excluded, but extra care should be taken with their management. If patients present with poor renal or hepatic function, relationship to the presenting condition should be sought; imaging should be performed and liver biopsy could be considered, to seek involvement by ALL. A steroid pre-phase is particularly necessary in such patients to gain clinical improvement and normalisation of renal and/or liver function prior to starting study chemotherapy. In the case of persistently abnormal renal or hepatic function at start of protocol therapy, dose adjustments to the non-IMP chemotherapy drugs should be made according to appendix 4 and in conjunction with local network guidelines. Dose modifications should be recorded on the CRF. Dose modification for IMPs, where necessary, are also indicated in appendix 4. If a patient presents with prior history of poor cardiac function, clinically indicated tests should be performed at site and UCL CTC should be contacted to discuss treatment options within the trial.

5.2.4 Pregnancy, Lactation & Birth Control

Women of childbearing potential should use effective contraceptive methods during treatment and for 12 months following the trial. Women should not breastfeed while on trial and for 12 months following.

All male patients should take adequate contraceptive precautions during and up to 12 months following the trial. Male patients may consider the option of sperm banking before treatment. Please advise patients according to local procedures.

5.3 UKALL14 Registration & Randomisation Procedures

Patients will be registered to the trial at study entry and there are 3 randomisations at different timepoints in the trial, **each patient will undergo at least one but no more than 2 randomisations.**

All randomisations will be performed at the appropriate timepoint at UCL CTC upon submission of the relevant form by sites.

Sites will be informed by UCL CTC of the randomisation group as follows:

Table 5.3 – UKALL14 Randomisations

Randomisation	Randomisation Timepoint (protocol section)	Patient Group	Randomisation group	Treatment involved	Treatment timepoint	Treatment Protocol section
B	Study entry (5.3.1)	Precursor B lineage	**B1**	Standard Phase I induction therapy alone	to be given alongside phase I induction therapy	7.2.3b
			B2	+ Rituximab		
T**	Study entry (5.3.1)	T lineage	**T1**	Standard Phase 2 induction therapy alone	to be given following Phase 2 induction therapy *(withhold randomised drug if current evidence of grade 2 or higher neurotoxicity)	7.2.4b
			T2	+ Nelarabine		
P**	Pre-transplant (5.6)	Patients aged ≤40 at study entry proceeding to transplant	**P1**	Standard dose Palifermin	to be given prior to & post infusion of donor cells according to schedules provided	7.2.10 (c & d)
			P2	Collapsed dose Palifermin		

*Neurological adverse events <u>with the use of NELARABINE</u>

Severe neurological events have been reported with the use of Nelarabine. These events have included altered mental states including severe somnolence, central nervous system effects including convulsions, and peripheral neuropathy ranging from numbness and paresthesias to motor weakness and paralysis. There have also been reports of events associated with demyelination, and ascending peripheral neuropathies similar in appearance to Guillain-Barr Syndrome.

Full recovery from these events has not always occurred with cessation of Nelarabine. Therefore, close monitoring for neurological events is stongly recommended, and Nelarabine must be discontinued at the first sign of neurological events of NCI common Toxicity Grade 2 or greater.

Sites will be informed by UCL CTC when the UKALL14 trial is opened to recruitment of patients.
All eligible patients may enter the trial at any time point.
However, please note that due to Trial Drug Supply Agreements, the trial may open initially with only the B cell (Rituximab) randomisation available.
The T cell (Nelarabine) and P (Palifermin) randomisations will open when the respective Trial Drug Supply Agreements are in place.
To reiterate - all eligible patients may enter the trial at any time point regardless of which randomisations are open:
Eligible T-lineage patients may enter the trial before the T cell (Nelarabine) randomisation is open, these patients will receive Standard Phase 2 induction therapy alone.
Patients who proceed to a myeloablative transplant before the P (Palifermin) randomisation is open will not receive either schedule of Palifermin.
All participating sites will be notified by UCL CTC when the T & P randomisations are open.

5.4 Registration & Randomisations (B & T) at study entry

a. Please see section 5.7 for the registration to transplant and for randomisation procedure for high risk transplant patients (Randomisation P).
b. Patient registration & randomisation will be performed centrally at the UCL CTC and must be performed prior to commencement of any trial treatment (a steroid pre-phase of 5-7 days is required and can be started prior to study entry).
c. Pre-treatment evaluations should be carried out at sites as detailed in section 5.1.
d. A registration form must be fully completed and faxed to UCL CTC and will be used to confirm patient eligibility at UCL CTC.
e. Initial randomisations (performed via minimisation) (B and T) will be stratified on gender, age (≤40, >40 years old) and WBC ≥30 x 10^9/L (precursor-B), WBC ≥100 x 10^9/L (T-lineage)
f. A trial number and treatment allocation will be assigned and details added to the form. UCL CTC will fax confirmation of the patient's inclusion in the trial, their trial number and treatment allocation (either B1 or , B2, T1 or T2, see table 5.3) to the main contact.
g. Patient specific Case Report Forms (CRFs) to cover phase I and 2 induction therapy will be emailed to the main contact at site.

Registration & Randomisation fax number:	+44 (0)20 7679 9861
Office hours:	09:00 to 17:00 (UK Time) Monday to Friday

Once a patient has been registered onto the trial they must be provided with the following:

- A copy of their signed consent form (if not given at time of consent).

- A patient contact card. Site on call contact details for out of hours medical care must be added to this card and patients informed to carry this with them at all times while on the trial.

5.5 Pre-transplant evaluation

Please contact the Transplant co-ordinator (see front of protocol) if you have any queries about the eligibility of a patient for transplant or any other transplant queries.

1. Medical history including allergies, previous chemotherapy, prior radiotherapy, hormonal or immunotherapy and response to treatment, end-organ toxicity and infections.
2. Physical examination.
3. ECOG performance status (Appendix 10).
4. Karnofsky performance status (Appendix 16).
5. Comorbidity Index (Appendix 9).
6. Full blood count with differential
7. Biochemistry. (to include LDH) Liver and renal function should be assessed as per local practice.
8. Urinalysis.
9. Clotting screen including PT/APTT.
10. Pregnancy test (urine) as clinically indicated.
11. Microbial titers (CMV, HIV I+2, HBsAg, HBcAb, HC, HTLV 1+2, EBV, VZV, TPHA, toxoplasma +/- HSv). Syphilis serology also required.
12. Bone marrow aspirate and trephine biopsy (The bone marrow sample taken at the end of Phase II will be acceptable here as long as there are no significant delays. Please discuss with the transplant co-ordinator for advice if you anticipate a delay.)
13. Cadiac function (to be assessed as per local practice).
14. Pulmonary function (to be assessed as per local practice).
15. DNA specimen from patient and donor should be screened for informative markers for subsequent lineage-specific chimerism studies. Residual DNA should be stored indefinitely.

Please note – as detailed in section 8.2.1 and Appendix 7:

	Specimens for local assessment	**Specimens for correlative studies to be sent to central laboratory (see app 8)***
Pre-transplant		20ml Blood in EDTA from donor and recipient for assessment of mini-satellite regions

5.6 Patient eligibility for transplant

5.6.1 Inclusion criteria (transplant)

a. Completion of Phase 1 and Phase 2 treatment within the trial
b. HLA-compatible sibling or unrelated donor (8/8 molecular match at A,B,C and DR. DQ mismatch is permitted.
c. Two subgroups of patients will proceed to transplant:
    i. Any patient with an HLA-compatible sibling donor.
    ii. High Risk patients with a molecularly matched donor at HLA-A,B,C and DR (see 5.5.2)

5.6.2 Eligibility for high-risk arm – unrelated donor stem cell transplantation:

Any one of the factors below makes the patient high-risk

a. Age over 40 years
b. WBC ≥30 x 10^9/L (precursor-B), ≥100 x 10^9/L (T-lineage)
c. Cytogenetics – any one or more of the abnormalities below
    i. t(4;11)(q21;q23)/*MLL-AF4*
    ii. Low hypodiploidy/near triploidy (30-39 chromosomes / 60-78 chromosomes
    iii. Complex karyotype (five or more chromosomal abnormalities)
    iv. Philadelphia chromosome t(9;22) (q34;q11)/BCR-ABL1 (detected by cytogenetic or molecular methods)
d. High Risk Minimal Residual Disease (MRD) post phase 2 of induction.

NB. Patients age >40 at time of study entry will be given non-myeloablative conditioning and patients age ≤40 at study entry will be given myeloablative conditioning.

5.6.3 Exclusion criteria (transplant)

a. Relapsed disease
b. Standard risk patients without a sibling donor (these patients will continue chemotherapy consolidation and maintenance)

5.7 Procedure for Registration to either maintenance/consolidation or transplant (and Palifermin Randomisation of transplant patients aged ≤ 40 years at study entry)

- Registration to either maintenance/consolidation or transplant (& Palifermin (P) randomisation for the patients aged 40 years and under at time of study entry) will be performed centrally at the UCL CTC and must be performed prior to commencement of any transplant activity.

- Pre-transplant evaluations should be carried out as detailed in section 5.5.

- A maintenance/consolidation registration form or transplant registration form must be fully completed and faxed to UCL CTC and will be used to confirm transplant eligibility at UCL CTC.

- UCL CTC will fax confirmation to the main contact at site of either:
    - Patient not eligible to proceed to transplant and will be treated using consolidation and maintenance as detailed in sections 7.2.8 and 7.2.9.
    - Patient may proceed to transplant (and for those patients receiving a myeloablative transplant, Palifermin treatment allocation: P1 or P2, see table 5.3) as detailed in section 7.2.10.

- Randomisation (performed using minimisation) of myeloablative transplant patients aged 40 years or under at study entry into either P1 or P2 will be stratified by gender, sibling/MUD donor and precursor B lineage/T-lineage.

- Patient specific Case Report Forms (CRFs) will be sent to the main contact at site.

Registration & Randomisation fax number:	+44 (0)20 7679 9861
Office hours:	09:00 to 17:00 (UK Time) Monday to Friday

6.0 Initial trial drug supply

For full instructions for drug ordering, delivery and labelling for the UKALL14 trial, please consult the UKALL14 Drug Supply Guidelines.

7.0 Trial Treatments

7.1 Summary of IMPs to be evaluated

For the purpose of this protocol, the IMPs are

1. Rituximab (supplied by Roche, free of charge, until the patent for Rituximab expires in November 2013, after which this will be supplied from hospital stock.)
2. Pegylated asparaginase (made available for the trial by MEDAC UK via UDG, at sites own cost)
3. Nelarabine (supplied by GlaxoSmithKline, free of charge)
4. Palifermin (supplied by Biovitrum, free of charge)

These drugs will be supplied free of charge except Pegylated asparaginase. All other drugs specified in the protocol are standard treatment for this disease and are not IMP's and must be provided from pharmacy stock at the participating sites.

Full details of all IMPs and supporting medications are supplied in Appendix 2.

For full instructions for drug ordering, delivery and labelling for the UKALL14 trial, please consult the UKALL14 Drug Supply Guidelines.

7.2 Treatment Schedule

7.2.1 Recommended supportive care:

Allopurinol should be started 24 hours prior to induction chemotherapy and should be continued for a minimum of 5 days. Rasburicase should be considered as an alternative to allopurinol if the white cell count is high i.e > 100 x 10^9/L or the patient has bulky disease eg. large mediastinal mass or elevated urate at diagnosis. All patients need prophylaxis against HSV and VZV reactivation. It is recommended that patients are given aciclovir 200mg bd throughout therapy although local policies may be followed.
All patients need prophylaxis against PCP from day zero of induction. The recommended PCP prophylaxis is Co-trimoxazole 960mg bd for 2 days each week, avoiding the day that methotrexate is given when the patient is on maintenance therapy. In the event of the patient being allergic to co-trimoxazole, local policies should be followed but alternative prophylactic agents include nebulised pentamidine or dapsone.
Antifungal prophylaxis is mandatory for all patients on ALL therapy from the time of induction. Azoles must be avoided when the patient is on vincristine. There is no clear evidence to suggest which anti-fungal prophylaxis regimen should be used in this situation but one option is to give AmBisome® 7mg/kg weekly. Local policies may be followed. Azoles can be used safely after phase 1 of induction.
Antifungal prophylaxis is not generally required when a patient is on maintenance therapy unless that patient is deemed to be high risk for fungal disease.

The use of granulocyte colony stimulating factor (G-CSF) is strongly recommended for all patients to hasten neutrophil recovery following induction phases 1 and 2. It can be given as per local policy.

Chugai Pharma UK will provide a 25% retrospective reimbursement in stock for rHuG-CSF (Lenograstim) used within the study period.
Please contact medicalaffairs@chugai-pharm.co.uk with regards to making a claim.

Granocyte is available at NHS contract prices through AAH Hospital Service Telephone 0845 607 6699.

Coagulation issues during asparaginase therapy:

- The management of coagulation issues during treatment with L-asparaginase varies widely world-wide. There is no clear evidence base. Although L-asparaginase is associated with very deranged coagulation parameters, clinically, bleeding episodes are rare and thrombosis is the most common clinical event.
- Thrombosis can be expected in approximately 10% of adults being treated with L-asparaginase.
- There is no evidence that infusion of fresh frozen plasma can correct the coagulation abnormalities or prevent thrombosis or bleeding events.
- However, there is some evidence that the use of antithrombin (AT) concentrate infusions, aiming to maintain AT levels above 60%, is associated with fewer thromboses related to L-asparaginase therapy and possibly a better clinical outcome. [54] It is therefore acceptable for investigators to adopt this practice if they wish, although there is no requirement within the protocol to give AT replacement routinely. Evidence suggests that a median of 31IU/kg per patient will be required to maintain AT levels above 60%.
- We recommend that coagulation parameters are not routinely checked during during L-asparaginase treatment unless the patient requires a clinical procedure such as an LP or there is clinical concern about bleeding.

Thrombosis and central venous catheters:

- The greatest risk of thrombosis (CNS and central line related) is in early induction. The presence of a central catheter considerably increases this risk and there is some evidence that survival is affected. The risk is reduced when dexamethasone is used compared to prednisolone.
- Centres should consider delaying indwelling central venous catheterization until the end of phase I induction.

Management of thrombosis

- LMWH is the treatment of choice for the management of central line related thrombosis. In this situation, asparaginse should be suspended for that course but can be given with heparin prophylaxis in subsequent courses.
- The use of LMWH in patients immediately following a diagnosis of a CNS thrombosis is more contentious. All heparinised patients must be monitored using anti Xa levels or the APTT depending on the type of heparin given. It is important to note that a proportion of patients will be resistant to heparin due to the depletion of AT caused by asparaginase. In this instance replacement of AT may be indicated. This should be done after seeking specialist advice from a thrombosis and haemostasis expert. Alternatively please contact the CI or one of the clinical coordinators for advice.

- All thrombotic events should be reported as SAEs

Methotrexate encephalopathy management:

- Methotrexate encephalopathy presents with fits, focal neurological deficit or impaired consciousness and occurs within one day to about 3 weeks of exposure to Intrathecal methotrexate. Full recovery is usual.
- Other causes of CNS events should be considered such as sagittal sinus thrombosis or central nervous system involvement with ALL.
- Methotrexate should be discontinued whilst the patient is also receiving cytarabine systemically.
- Re-challenge is possible without recurrence but if recurrence happens, the intrathecal regimen should be changed to cytarabine 50mg in association with 12.5mg hydrocortisone. Local policy may be followed for the hydrocortisone dose if necessary.

7.2.2 Steroid pre-phase

All patients should be treated with a steroid pre-phase of 5-7 days. Study entry is still permissible if the steroid pre-phase has started prior to study registration. The steroid pre-phase consists of Dexamethasone 6mg/m^2/d, PO for 5 to 7 days.

7.2.3 Phase 1 induction

Phase 1 induction, weeks 1-4

To be given to all patients regardless of phenotype. Patients with B precursor lineage disease will be randomised to receive either, Rituximab or no antibody (randomisation groups B1 and B2, see table 5.3).

Patients with Philadelphia positive disease should also receive continuous daily Imatinib, PO, starting at 400mg, aiming to escalate to 600mg within 2 weeks, if tolerated.

For schedules see Tables 7.2.3a & 7.2.3b overpage

Table 7.2.3a – Phase I induction

Drug	Dose	Route of administration	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9	Day 10	Day 11	Day 12	Day 13	Day 14	Day 15	Day 16	Day 17	Day 18	Day 19	Day 20	Day 21	Day 22	Day 23	Day 24	Day 25	Day 26	Day 27	Day 28
Daunorubicin	**30 mg/m^2**	IV	1							8							15							22						
Vincristine***	**1.4mg/m^2 Max 2mg**	IV	1							8							15							22						
Dexamethasone**	**10mg/m^2**	PO	1	2	3	4				8	9	10	11				15	16	17	18										
PEG- asparaginase **Philadelphia Positive patients** should NOT be given Pegylated Asparaginase	**1000IU/m^2**	iv				4*														18										
Methotrexate[#]	**12.5mg**	Intrathecal														14														
Imatinib - Patients with **Philadelphia positive disease** should also receive continuous daily Imatinib, PO, starting at 400mg, aiming to escalate to 600mg within 2 weeks, if tolerated. This should be continued until transplant wherever possible			1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28

Omit Day 4 Pegylated Asparaginase for Philadelphia Negative patients OVER 40. *These patients should only receive Day 18 Pegylated Asparaginase.*

***Dexamethasone should be capped at 20mg for larger patients*

****Do not give azoles as antifungal prophylaxis within 72 hours before or after vincristine. Please note: This rules out any azole from phase 1.*

Notes on lumbar puncture and treatment of established CNS disease:

 # Timing of Intrathecal therapy can be moved +/- 3 days to allow administration on specified lists as per local and national guidance.

In the case of traumatic lumbar puncture (> 10 red blood cells per microlitre), patients should be treated as having CNS disease IF they still have blasts within the peripheral blood at the time of occurrence or have blasts in the CSF. In this case and in the case where there is existing evidence of established

CNS disease, intrathecal therapy with methotrexate should be escalated to twice per week and given at this frequency until the cytospin is clear of blasts. Such patients should also receive cranial irradiation, prior to consolidation, if they are not going to receive myeloablative allogeneic transplant.

Table 7.2.3b – B lineage antibody randomisation during Phase I induction

ANTIBODIES SCHEDULED BELOW ONLY TO BE GIVEN TO PRECURSOR-B LINEAGE PATIENTS DEPENDING ON RANDOMISATION																															
Drug	Dose	Route of administration	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9	Day 10	Day 11	Day 12	Day 13	Day 14	Day 15	Day 16	Day 17	Day 18	Day 19	Day 20	Day 21	Day 22	Day 23	Day 24	Day 25	Day 26	Day 27	Day 28
Rituximab plus phase 1; Arm B2																															
Rituximab*	**375mg/m^2**	IV			3							10							17							24				

**Rituximab to be given to patients with precursor-B Lineage ALL according to randomisation after chemotherapy.*

Please note as detailed in section 8.2.1 and Appendix 7:

	Specimens for local assessment	**Specimens for correlative studies to be sent to central laboratory (see app 8)***
During Phase 1 therapy		5ml peripheral blood in a serum tube (for Asparaginase activity assay and anti-Asparaginase antibodies) taken on d3 or d4 and d18 (immediately prior to doses 1 and 2 of peg-asp)

7.2.4 End of phase 1 induction

Following recovery from phase 1 therapy (neutrophils 0.75 x 10^9/l and platelets to 75 x 10^9/l), remission should be confirmed by morphological bone marrow examination (please don't forget to send a specimen centrally for MRD examination - see section 8.2.1 and Appendix 7 for full details). This is the absolute minimum count recovery needed for adequate assessment of response and of MRD. Hence the bone marrow aspirate to assess MRD may be postponed for a few more days after reaching this level and can be carried out once the patient has been admitted to begin the next course of therapy, to make sure there is adequate cellularity for the end of phase assessment in order to confirm CR with count recovery. **The bone marrow aspirate must be done by day 35 at the latest.** Please indicate the peripheral blood counts on the form when you submit specimens for central analysis.

However, progression to phase 2 should not be delayed more than a few days once haematopoietic recovery has occurred.

If the patient is not in CR at the end of phase 1, swift progression to phase 2 treatment is indicated.

Please note as detailed in section 8.2.1 and Appendix 7:

	Specimens for local assessment	**Specimens for correlative studies to be sent to central laboratory (see app 8)***
At recovery post Phase 1	Bone marrow aspirate for remission assessment locally. Same day local FBC to determine peripheral count.	Bone marrow from biopsy: 3-5ml in EDTA for MRD assessment (IgH/TCR rearrangements for Ph-ALL, BCR-ABL for Ph+ALL)

7.2.5 Phase 2 induction

Phase 2 induction, weeks 5-8 (please see section 7.2.4 for recovery pre-phase 2)

To be given to all patients regardless of phenotype. Patients with T-lineage ALL will be randomised to receive either Nelarabine as an additional course, following phase 2 or no additional treatment.

Patients with Philadelphia positive ALL should also receive continuous daily Imatinib, PO, starting at 400mg, aiming to escalate to 600mg within 2 weeks, if tolerated.

Table 7.2.5a – phase 2 induction

Drug	Dose	Route of administration	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9	Day 10	Day 11	Day 12	Day 13	Day 14	Day 15	Day 16	Day 17	Day 18	Day 19	Day 20	Day 21	Day 22	Day 23	Day 24	Day 25	Day 26	Day 27	Day 28
Cyclophosphamide*	**1000 mg/m^2**	IV	1														15													
Cytarabine#	**75mg/ m^2**	IV		2	3	4	5				9	10	11	12				16	17	18	19				23	24	25	26		
Mercaptopurine	**60mg/ m^2**	PO	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28
Methotrexate#	**12.5mg**	Intrathecal	1							8							15							22						
Imatinib - Patients with **Philadelphia positive disease** should also receive continuous daily Imatinib, PO, starting at 400mg, aiming to escalate to 600mg within 2 weeks, if tolerated. This should be continued until transplant wherever possible			1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28

 # Timing of Intrathecal therapy can be moved +/- 3 days to allow administration on specified lists as per local and national guidance. Likewise, timing of the cytarabine blocks can be scheduled so that they can take place during the week as long as the full doses are given.

** Cyclophosphamide 1000mg/m2 IV over 20-30 minutes on days 1 and 15. Give 125 mls/m2/hour of Dextrose/ Saline for 30 minutes before cyclophosphamide and for 3.5 hours afterwards ie 4 hours in total. Do not add potassium. Mesna is not needed.'*

Table 7.2.5b – Nelarabine schedule for T lineage patients following phase 2 induction

- Only for patients with T lineage disease randomised to Nelarabine
- If patients have current grade 2 or greater neurotoxicity, nelarabine should not be given as randomised and patients should continue to whatever treatment is next scheduled

Drug	Dose	Route of administration	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9	Day 10	Day 11	Day 12	Day 13	Day 14	Day 15	Day 16	Day 17	Day 18	Day 19	Day 20	Day 21
Phase II plus Nelarabine: Arm T2																							
Nelarabine	**1.5 g/m^2**	IV	1		3		5																

Neurological adverse events

Severe neurological events have been reported with the use of Nelarabine. These events have included altered mental states including severe somnolence, central nervous system effects including convulsions, and peripheral neuropathy ranging from numbness and paresthesias to motor weakness and paralysis. There have also been reports of events associated with demyelination, and ascending peripheral neuropathies similar in appearance to Guillain-Barr Syndrome. Full recovery from these events has not always occurred with cessation of Nelarabine. Therefore, close monitoring for neurological events is stongly recommended, and Nelarabine must be discontinued at the first sign of neurological events of NCI common Toxicity Grade 2 or greater.

7.2.6 End of phase 2 induction

Following recovery from phase 2 therapy (neutrophils 0.75 x 10^{-9}/l and platelets to 75 x 10^{-9}/l), remission should be confirmed by morphological bone marrow examination (please don't forget to send a specimen centrally for MRD examination - see section 8.2.1 and Appendix 7 for full details). This is the absolute minimum count recovery needed for adequate assessment of response and of MRD. Hence the bone marrow aspirate to assess MRD may be postponed for a few more days after reaching this level and can be carried out once the patient has been admitted to begin the next course of therapy, to make sure there is adequate cellularity for the end of phase assessment in order to confirm CR with count recovery. Please indicate the peripheral blood counts on the form when you submit specimens for central analysis.

However, progression to intensification or bone marrow transplant should be as swift as possible.

If the patient is not in CR at the end of phase 2, protocol therapy ceases. Please refer to Section 13.0.

Advice on non-protocol therapy can be obtained from the CI or one of the clinical coordinators.

Please note as detailed in section 8.2.1 and Appendix 7:

	Specimens for local assessment	**Specimens for correlative studies to be sent to central laboratory (see app 8)***
At recovery post Phase 2	Bone marrow aspirate for remission assessment locally. Same day local FBC to determine peripheral count.	Bone marrow from biopsy: 3-5ml in EDTA for MRD assessment (IgH/TCR rearrangements for Ph-ALL, BCR-ABL for Ph+ALL)

7.2.7 Intensification/Central nervous system prophylaxis

N.B This may be omitted if a myeloablative transplant is to be carried out but if there are delays in transplant start (> 3 weeks following recovery from phase 2 induction), the patient should continue with per-protocol intensification. If there are still delays in donor procurement following intensification, the patient *should not be left without any anti-leukaemia therapy*. Depending on the projected duration of delay, either 2 monthly cycles of interim maintenance therapy should be given (as per maintenance phase of this protocol, with vincristine and steroid and an intrathecal MTX given each month) OR if anticipated delay is longer than 2 months, patients should instead receive the first cycle of consolidation therapy.

The initial Creatinine Clearance before starting methotrexate should ideally be > 100 mls/minute. Dose reductions must be made if the Cr Cl is < 80mls /min.

Treatment to begin upon recovery from induction phase 2 .

Patients with Philadelphia positive disease should also receive continuous daily Imatinib, PO, starting at 400mg, aiming to escalate to 600mg within 2 weeks, if tolerated.

For schedule see Tables 7.2.7 overpage

Table 7.2.7 – Intensification/CNS prophylaxis

Drug	Dose	Route of administration	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9	Day 10	Day 11	Day 12	Day 13	Day 14	Day 15	Day 16	Day 17	Day 18	Day 19	Day 20	Day 21	Day 22	Day 23	Day 24	Day 25	Day 26	Day 27	Day 28
Methotrexate*	**3 g/m^2**	IV	1														15													
PEG- asparaginase**	**1000IU/ m^2**	IV		2														16												
Imatinib - Patients with **Philadelphia positive disease** should also receive continuous daily Imatinib, PO, starting at 400mg, aiming to escalate to 600mg within 2 weeks, if tolerated. This should be continued until transplant wherever possible			1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28

*<u>Please consult **appendix 15** for full guidance on administration of High Dose MTX</u> – (please note: after treatment with methotrexate, folinic acid rescue must be given. Please see Appendix 15 for details).*

**Please note as detailed in section 8.2.1 and Appendix 7:

	Specimens for local assessment	**Specimens for correlative studies to be sent to central laboratory (see app 8)***
During intensification:		5ml peripheral blood in a serum tube (for Asparaginase activity assay and anti-Asparaginase antibodies) taken on d2 and d16 of intensification (immediately prior to doses 3 and 4 of peg-asp)

7.2.8 Consolidation therapy

To be given to patients not eligible for transplantation. The first cycle of consolidation therapy should begin after intensification, when neutrophils > 0.75 x 10^9/L and platelet > 75 x 10^9/L (Please see tables 7.2.8 a-d for details). For patients with central nervous system involvement, cranial irradition will be given before consolidation begins. Maintenance therapy with 6-Mercaptopurine should be given throughout the period of CNS irradiation. In the event of cytopenias, 6-Mercaptopurine therapy should be reduced or omitted rather than radiotherapy being delayed. The dose of thiopurine should not be increased as per the maintenance protocol but should be continued at 75mg/m^2 in the absence of cytopenias.

Table 7.2.8a - Cycle 1 Consolidation – to begin after intensification , when neutrophils > 0.75 x 10^9/L and platelet > 75 x 10^9/L.

Drug	**Dose**	Route of administration	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
Cytarabine	**75mg/m^2**	IV	1	2	3	4	5		
Etoposide	**100mg/m^2**	IV	1	2	3	4	5		
PEG- asparaginase	**1000 IU/m^2**	IV					5		
Methotrexate[#]	**12.5 mg**	Intrathecal	1						
Imatinib - Patients with **Philadelphia positive disease** should also receive continuous daily Imatinib, PO, starting at 400mg, aiming to escalate to 600mg within 2 weeks, if tolerated. . This should be continued until transplant wherever possible			1	2	3	4	5	6	7

 # Timing of Intrathecal therapy can be moved +/- 3 days to allow administration on specified lists as per local and national guidance. Likewise, timing of the cytarabine blocks can be scheduled so that they can take place during the week as long as the full doses are given.

Table 7.2.8b - Cycle 2 Consolidation – to commence 3 weeks from day one cycle 1 or when neturophils are >0.75 x 10^9/l and platelets >75 x 10^9/l

Drug	Dose	Route of administration	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
Cytarabine	**75mg/m^2**	IV	1	2	3	4	5		
Etoposide	**100mg/m^2**	IV	1	2	3	4	5		
Methotrexate	**12.5 mg**	Intrathecal	1						
Imatinib - Patients with **Philadelphia positive disease** should also receive continuous daily Imatinib, PO, starting at 400mg, aiming to escalate to 600mg within 2 weeks, if tolerated. . This should be continued until transplant wherever possible			1	2	3	4	5	6	7

Table 7.2.8ci – Cycle 3 (DAYS 1-28) - Consolidation/Delayed intensification - to commence 3 weeks from day one cycle 2 or when neutrophil count> 0.75 x 10^9/L and platelets >75 x 10^9/l

DAYS 1-28

Drug	Dose	Route of administration	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9	Day 10	Day 11	Day 12	Day 13	Day 14	Day 15	Day 16	Day 17	Day 18	Day 19	Day 20	Day 21	Day 22	Day 23	Day 24	Day 25	Day 26	Day 27	Day 28
Daunorubicin	**25mg/m^2**	IV	1							8							15							22						
Vincristine	**1.4mg/m^2** (Max 2mg)	IV	1							8							15							22						
Peg-asparaginase	**1,000 IU/m^2**	IV				4																								
Dexamethasone	**10mg/m^2**	PO	1	2	3	4				8	9	10	11				15	16	17	18				22	23	24	25			
Methotrexate	**12.5 mg**	Intrathecal		2															17											
Imatinib - Patients with **Philadelphia positive disease** should also receive continuous daily Imatinib, PO, starting at 400mg, aiming to escalate to 600mg within 2 weeks, if tolerated. . This should be continued until transplant wherever possible				2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28

This phase runs from day 1 to day 42 inclusive (i.e. 6 weeks). Patients should have ANC >0.75x10^9/L and platelets of >75x10^9/L to start and have recovered again to this level from before the 29th day of therapy is started. Once begun, therapy is not interrupted for myelosuppression alone. Any serious infection, such as Varicella, pneumocystis pneumonia, or neutropenia with fever, and presumed or proven infection, warrants chemotherapy interruption at any time during this block. Before the patient completes day 29-42 (table 7.2.8cii), i.e. before the d29 cyclophosphamide, the counts should be ANC >0.75x10^9/L and platelets of >75x10^9/L

Table 7.2.8cii – Cycle 3 (DAYS 29-42) – Consolidation/Delayed intensification - to commence 3 weeks and 29 days from day one cycle 2 or when neutrophil count> 0.75 x 10^9/L and platelets >75 x 10^9/l

DAYS 29-42

Drug	Dose	Route of administration	Day 29*	Day 30	Day 31	Day 32	Day 33	Day 34	Day 35	Day 36	Day 37	Day 38	Day 39	Day 40	Day 41	Day 42
Cyclophosphamide	**1000 mg/m^2**	IV	29													
Cytarabine	**75mg/m^2**	IV		30	31	32	33				37	38	39	40		
Mercaptopurine	**60mg/m^2**	PO	29	30	31	32	33	34	35	36	37	38	39	40	41	42
Imatinib - Patients with **Philadelphia positive disease** should also receive continuous daily Imatinib, PO, starting at 400mg, aiming to escalate to 600mg within 2 weeks, if tolerated. . This should be continued until transplant wherever possible			29	30	31	32	33	34	35	36	37	38	39	40	41	42

This phase runs from day 1 to day 42 inclusive (i.e. 6 weeks). Patients should have ANC >0.75x10^9/L and platelets of >75x10^9/L to start and have recovered again to this level from before the 29th day of therapy is started. Once begun, therapy is not interrupted for myelosuppression alone. Any serious infection, such as Varicella, pneumocystis pneumonia, or neutropenia with fever, and presumed or proven infection, warrants chemotherapy interruption at any time during this block. Before the patient completes day 29-42 (table 7.2.8cii), i.e. before the d29 cyclophosphamide, the counts should be ANC >0.75x10^9/L and platelets of >75x10^9/L.

Table 7.2.8d - Cycle 4 – Consolidation: Identical to Cycle 2, and will begin when neutrophils > 0.75 x 10^9/L and platelets >75 x 10^9/l

Drug	Dose	Route of administration	Day -1	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
Cytarabine	**75mg/m^2**	IV		1	2	3	4	5		
Etoposide	**100mg/m^2**	IV		1	2	3	4	5		
Methotrexate	**12.5mg**	Intrathecal		1						
Imatinib - Patients with **Philadelphia positive disease** should also receive continuous daily Imatinib, PO, starting at 400mg, aiming to escalate to 600mg within 2 weeks, if tolerated. . This should be continued until transplant wherever possible				1	2	3	4	5	6	7

7.2.9 Maintenance therapy (non-transplant patients only)

To start as soon as neutrophils are 0.75 x 10^9/l and platelets are 75 x 10^9/l following consolidation 4 and to continue for 2 full years.

Patients with Philidelphia positive disease should continue with daily Imatinib throughout maintenance.

Table 7.2.9 – Maintenance therapy

Drug	Dose	Route of administration	Frequency
Vincristine	1.4 mg/m^2 (max 2 mg/dose)	IV	every 3 months
Prednisolone	60 mg/m^2	PO	5 days every 3 months
Mercaptopurine*	75 mg/m^2	PO	Daily
Methotrexate*	20 mg/m^2	PO or IV	once per week (not to be given on the same day at the Co-trimoxazole)
Intrathecal therapy during maintenance (to be given once neutrophils reach 0.75 x 10^9/l and platelets reach 75 x 10^9/l)			
Methotrexate	12.5mg	Intrathecal	Every 3 months

Dosing of maintenance therapy should be adjusted to maintain the neutrophil count between 0.75 and 1.5 x 10^9/l and platelet count between 75 and 150 x 10^9/l.

*Dose of MP and MTX should be alterered in 25% increments or decrements to achieve the above counts. eg if neutrophils > 1.5 x 10^9/l, increase 6-MP dose by 25%. If neutrophils remain > 1.5 x 10^9/l after 4 weeks, increase MTX by 25% etc. There are no maximum doses of MP and MTX. If neutrophils fall below 0.75 x 10^9/l, reduce both drugs by 50%, if neutrophils fall below 0.5 x 10^9/l, stop maintenance and restart at 100% when neutrophils > 0.75 x 10^9/l. Similar adjustments need to be made for the platelet count to maintain above counts.

Maintenance should not be interrupted unnecessarily but if doses are omitted for cytopaenias or infectious complications, they do not need to be made up with additional doses later.

Co-trimoxazole (960mg bd, twice per week, not on the same day as the weekly oral MTX) and aciclovir (200mg bd) prophylaxis against PCP and HSV/VZV reactivation should be given throughout maintenance. Local practice may be followed regarding the aciclovir dose if necessary.

If cytopaenias occur and maintenance is halted, consideration should be given to stopping the co-trimoxazole if blood counts do not recover within 2-3 weeks. Doses of mercaptopurine and MTX should not be compromised in order to permit continuation of co-trimoxazole.

Alternative prophylaxis against PCP should be given, for example monthly nebulised pentamindine, or oral dapsone.

7.2.10 Transplant conditioning regimens

Please see appendix 8 for Donor Peripheral Blood Stem Cell Collection & Return.

Please note – as detailed in section 8.2.1 and Appendix 7:

	Specimens for local assessment	**Specimens for correlative studies to be sent to central laboratory (see app 8)***
Pre-transplant		20ml Blood in EDTA from donor and recipient for assessment of mini-satellite regions

7.2.10.1 Myeloablative conditioning regimen

- The following regimen is strongly recommended for patients aged ≤40 years at study entry who are eligible for myeloablative transplantation.
- At the discretion of the transplant centres, cyclophosphamide 60mg/kg on days -3 and -2 is acceptable.
- Local practice for the scheduling of the conditioning regimen may be followed.
- TBI dose should not be less than 13.2Gy.
- T cell depletion is not recommended for unrelated donor SCT. Where T cell depletion is deemed necessary by individual centres, '*in-vivo*' Alemtuzumab is recommended at 30mg IV days -2 and -1. The dose given should be documented on the relevant CRF.

Table 7.2.10a – Myeloablative conditioning regimen – preferred option: Etoposide

Drug	Dose	Route of administration	Day –7	Day –6	Day –5	Day-4	Day-3	Day-2	Day-1	Day 0
Fractionated TBI	**total dose 1320 cGY in 8 fractions**		-7	-6	-5	-4				
Etoposide	**60 mg/kg**	IV					-3			
Haematopoetic stem cell infusion										0

Table 7.2.10b – Myeloablative conditioning regimen – alternative option: Cyclophosphamide

Drug	Dose	Route of administration	Day –7	Day –6	Day –5	Day-4	Day-3	Day-2	Day-1	Day 0
Fractionated TBI	**total dose 1320 cGY in 8 fractions**		-7	-6	-5	-4				
Cyclophosphamide	**60 mg/kg**	IV					-3	-2		
Haematopoetic stem cell infusion										0

Patients eligible for a myeloablative stem cell transplant will have been randomised between standard dose Palifermin (60micrograms/kg x 6 doses) and collapsed dose Palifermin (180micrograms/kg x 1 dose plus 60micrograms/kg x 3 doses).

Table 7.2.10c – Palifermin schedule: P1- Standard dose (only for those patients randomised)

Drug	Dose		Route	Day -10	Day -9	Day -8	Day -7	Day -6	Day -5	Day -4	Day -3	Day -2	Day -1	Day 0	Day 1	Day 2	Day 3	Day 4
Palifermin	**P1 - Standard**	**60 micrograms /kg**	IV bolus injection	-10	-9	-8								0		2		4

Table 7.2.10d – Palifermin schedule: P2-Collapsed doses (only for those patients randomised)

Drug	Dose		Route	Day -10	Day -9	Day -8	Day -7	Day -6	Day -5	Day -4	Day -3	Day -2	Day -1	Day 0	Day 1	Day 2	Day 3	Day 4
Palifermin	**P1 - Collapsed**	**180 micrograms/ kg**	IV bolus injection		-9													
		60 micrograms/ kg	IV bolus injection											0		2		4

7.2.10.2 Non-myeloablative conditioning therapy

The following regimen should be used for patients aged ≥40 at study entry who are eligible for non-myeloablative transplantation:

Table 7.2.10e – Non-myeloablative conditioning regimen schedule

Drug	Dose	Route of administration	Day -7	Day -6	Day -5	Day-4	Day-3	Day-2	Day-1	Day 0
Fludarabine	**30 mg/m^2**	IV over 1 hour	-7	-6	-5	-4	-3			
Melphalan	**140 mg/ m^2**	IV							-1	
For recipients of unrelated donor allografts:								-2	-1	
Alemtuzumab*	**30 mg**	IV								
For recipients of sibling allografts:									-1	
Alemtuzumab*	**30 mg**	IV								

**Pre-medication with steroids prior to Alemtuzumab is strongly recommended, in addition to antihistamines and paracetamol*

Please note that as patients receiving Reduced Intensity Conditioning regimens DO NOT receive TBI there is a need to maintain appropriate CNS directed therapy. These patients will therefore receive 8 x 3 monthly intrathecal methotrexate injections at a dose of 12.5 mg, for 2 years post transplant starting at 3 months post transplant - see section 7.2.11.

Please note, as detailed in section 8.2.1 and appendix 7:

	Specimens for local assessment	**Specimens for correlative studies to be sent to central laboratory (see app 8)***
Post-transplant (non-myeloablative only):		20ml peripheral blood in EDTA for chimerism. To be repeated every 3 months. 3-5 ml Bone marrow in EDTA for MRD assessment. To be repeated every 3 months until 2 years post bone marrow transplant or until relapse.

7.2.10.3 Supportive care during and after transplant

Investigating transplant centres should use local protocols with regard to anti-emetic and anti-infective prophylaxis.

7.2.10.4 Graft versus host disease prophylaxis

Ciclosporin

Ciclosporin will be given as follows: total dose of 3 mg/kg/day I.V. from day -1 to +28. (Local practices for GVHD prophylaxis may be followed). The dose will be adjusted to maintain a therapeutic blood level and the patient will be switched to oral ciclosporin once clinically indicated. Ciclosporin will tapered from 2 months and discontinued at 3 months in the absence of GVHD.

Methotrexate

Short course methotrexate is given as per the original Seattle protocol to patients receiving a myeloablative transplant.

The dosing is as follows:

15 mg/m^2 day 1 and 10 mg/m^2 days 3, 6 and 11

We strongly advise that 4 doses of methotrexate should be given as lower doses of methotrexate may result in increased incidences of acute and chronic GVHD.

Moderate mucositis should not prevent full methotrexate dosing and the amount of methotrexate given is a secondary endpoint of the palifermin randomisation.

Dose reductions should occur in renal impairment:

CrCl 61-80 mL/minute: Reduce dose to 75% of usual dose

CrCl 51-60 mL/minute: Reduce dose to 50% of usual dose

CrCl < 50 mL/minute: no methotrexate

Investigators are permitted to give omitted doses of methotrexate when the renal impairment has resolved and can discuss this with the transplant coordinator of the trial.

Dosage adjustment in hepatic impairment:
Bilirubin 50-75 umol/l or AST >180 units: Administer 50% of usual dose
Bilirubin >75 umol/l : no methotrexate

Patients at particular risk of progressive renal or hepatic impairment may require greater reductions in dosage. Discussion with the transplant coordinator of the trial is advised.

Folinic acid rescue may be given as per local policy.

7.2.10.5 Prophylaxis against infectious diseases

All patients should receive prophylaxis against infection with HSV, VZV, fungal organisms and PCP according to local guidelines. CMV monitoring by PCR should be undertaken.

7.2.10.6 Donor lymphocyte infusions (DLI)

Disease and chimerism status will be assessed in all patients as per schedule in correlative studies section post non-myeloablative allogeneic transplant. If needed, DLI will be given in escalating doses at 3 monthly intervals.

Indications for DLI:

1. mixed chimerism

2. continued or progressive minimal residual disease

DLI at 6 months post transplant.

Doses:

6 months: 1 x 10^6 T cells/kg
9 months: 3 x 10^6 T cells/kg
12 months: 1 x 10^7 T cells/kg
15 months: 3 x 10^7 T cells/kg
18 months: 1 x 10^8 T cells/kg

The chimerism status will be assessed 2 months after each infusion as per chimerism monitoring section (7.2.10.7) and correlative science section (8.2.1) and no further infusions will be given if both remission of the immune phenomena and full donor chimerism is achieved. However, if the patient fails to achieve this after the final dose of donor lymphocytes (1 x 10^8 T-cells/kg), this is considered a treatment failure.

Patients **in clinical remission with full donor chimerism** at 6 months post transplant will **not** receive DLI. If, at future assessment, there is evidence of either disease relapse OR mixed chimerism, patients may commence DLI as per the above dose schedule.

7.2.10.7 Multi Lineage Chimerism monitoring:

Peripheral blood samples for determination of the donor or recipient origin of T cells and myeloid cells will be performed centrally, as per correlative science studies section. Results of chimerism analyses will be available within 1 week of the sample. All patients should be followed very carefully until they achieve a high level of donor chimerism (>95%). Subsequent falls in donor chimerism should be confirmed and these patients should be followed carefully, for evidence of relapse especially if peripheral blood counts have fallen or there are symptoms suggestive of leukaemia.

The usual specimen for peripheral blood chimerism is 20 mls of blood in an EDTA (green) tube. Regular monitoring of chimerism by XY FISH in sex-mismatched allografts locally is also encouraged as supporting evidence.

Patients with persistent mixed chimerism or a fall in chimerism, are candidates for immunotherapy at the protocolised doses. Investigators are encouraged to contact the transplant co-ordinator (Professor David Marks) to discuss these patients and immunotherapy decisions.

7.2.10.8 T cell depletion in the unrelated donor myeloablative HSCT setting an alternative protocol for centres committed to T cell depletion.

T cell depletion is not permitted for sibling allografting and T cell depletion is not recommended during full intensity unrelated donor allografting. For those centres committed to T cell depletion, in-vivo CAMPATH 1-H (60mg pretransplant as 2 x 30mg doses) is permissible.

7.2.11 Post-transplant Intrathecal therapy:

For those patients having reduced intensity conditioned transplant, it will be necessary to complete further intrathecal therapy post-transplant. The 8 doses intrathecal methotrexate (12.5mg) which would be given in maintenance should be given post RIC transplant, at 3 monthly intervals over 2 years.

7.3 Management after treatment withdrawal

If a patient withdraws consent or stops trial treatment for any reason e.g. toxicity or lack of efficacy then subsequent treatment will be at the discretion of the treating clinician.

In these cases patients remain within the trial for the purposes of follow-up and data analysis according to the treatment option to which they have been allocated.

7.4 Out-of-hours medical care

Medical care, including out-of-hours medical care is the responsibility of the site. Sites must ensure that all patients registered onto the trial receive a copy of the UKALL14 patient card and that the site on-call contact details have been added.

8.0 Assessments

8.1 Assessment time points

- Information is required for **all** patients at the following time points:
    - Initial diagnosis & pre treatment (pre-registration – section 5.1)
    - Post-induction phase 1 (at count recovery)
    - Post-induction phase 2 (at count recovery)
    - After intensification
    - Annual follow-up after study treatment completion until death
- For patients treated with Consolidation
    - After each cycle of Consolidation
- For patients treated with maintenance:
    - Every 3 months during maintenance
- For **Transplant** recipients:
    - Pre-transplant (section 5.5)
    - Day 100 post transplant
    - Every 3 months for 2 years following transplant
    - Any patients participating in the Palifermin/KGF randomization, Oral Mucositis Daily Questionnaire (OMDQ) to completed daily by all patients during in-patient therapy from day -12 until day 28 or date of discharge (whichever is sooner)

PLEASE SEE STUDY VISIT SCHEDULE (APPENDIX 11) FOR FURTHER DETAILS

8.2 Routine Clinical and Laboratory Assessments during treatment

a) Clinical examination will be carried out daily or more often, if the patient is acutely unwell, as part of routine clinical care.

b) Assessment of performance status ECOG will be carried out before each discrete block of therapy.

c) Height/Weight & BSA will be assessed as needed to prescribe each block of therapy.

d) Full blood count/ and other laboratory tests e.g. LFTs, U&Es will be carried out at the clinicians discretion as part of the routine management of acute leukaemia. These tests are usually carried out a minimum of three times weekly during inpatient stays.

8.2.1 Schedule of testing for MRD and correlative science testing

	Specimens for local assessment	Specimens for correlative studies to be sent to central laboratory (see app 8)*
At Diagnosis	Cytogenetics/molecular assessment of BCR-ABL and MLL on bone marrow. A copy of the results should be sent to the Leukaemia Research Cytogenetics Group (LRCG) at Newcastle University. (Please refer to Appendix 6 for details of the LRCG) Tissue typing of patient and any siblings to be carried out. Donor search to be initiated if no matched sibling donor available.	Bone marrow 3-5ml in EDTA (OR peripheral blood 30-50ml in EDTA if WCC > 30x 10^9/l). BCR-ABL status will also be checked.
During Phase 1 therapy		5ml peripheral blood in a serum tube (for Asparaginase activity assay and anti-Asparaginase antibodies) taken on d3 or d4 (patients under 40 only) and d18 (immediately prior to doses 1 and 2 of peg-asp) **Not applicable to Philadelphia positive patients**
At recovery post Phase 1**	Bone marrow aspirate for remission assessment locally. Same day local FBC to determine peripheral count.	Bone marrow from biopsy: 3-5ml in EDTA for MRD assessment (IgH/TCR rearrangements for Ph-ALL, BCR-ABL for Ph+ALL)
At recovery post Phase 2**	Bone marrow aspirate for remission assessment locally. Same day local FBC to determine peripheral count.	Bone marrow from biopsy: 3-5ml in EDTA for MRD assessment (IgH/TCR rearrangements for Ph-ALL, BCR-ABL for Ph+ALL)
During intensification:		5ml peripheral blood in a serum tube (for Asparaginase activity assay and anti-Asparaginase antibodies) taken on d2 and d16 of intensification (immediately prior to doses 3 and 4 of peg-asp)
Pre-transplant		20ml Blood in EDTA from donor and recipient for assessment of mini-satellite regions
Post-transplant (non-myeloablative only):		20ml peripheral blood in EDTA for chimerism. To be repeated every 3 months. 3-5 ml Bone marrow in EDTA for MRD assessment. To be repeated every 3 months until 2 years post bone marrow transplant or until relapse.
At Relapse		Bone marrow 3-5ml in EDTA (OR peripheral blood 30-50ml in EDTA if WCC > 30x 10^9/l).

***Please refer to Appendix 7 for guidelines for sending specimens to the central laboratory at the Royal Free Hospital, London**

** "Recovery" is defined as neutrophils >0.75 x 10^9/l, platelets >75 x 10^9/l.

8.3 Assessments during follow up

All patients will be followed up annually until death.

Follow-up begins when trial treatment is completed, i.e. after the last dose of maintenance therapy has been given. In the case of patients treated by transplantation, precise definition of when treatment becomes follow-up is difficult due to the wide variation in the clinical scenarios. Hence follow-up will be arbitrarily defined to commence 2 years after the date of stem cell infusion, to coincide with the length of maintenance therapy.

a) Clinical examination as necessary (often there are no expected abnormal findings)
b) Full blood count
c) Any other tests as dictated by the patient's clinical condition
d) Assessment of disease status
e) Record of any occurrence of AVN or serious cardiac problems
f) Employment history post treatment of ALL
g) GVHD

At the 2 year follow up appointment (2 years after stopping maintenance/4 years post stem cell infusion), as well as the above annual assessments, patients will also be assessed for late effects of treatment:

a. General Health Questionnaire (GHQ-12) Appendix 12
b. Symptoms of heart failure
c. Echocardiogram
d. Avascular necrosis
e. GVHD
f. Mental Health
g. Fertility
h. Any other cancer diagnosis since ALL diagnosis.
i. Employment history post treatment of ALL

If a patient fails to attend clinic for any visit then the site must make every effort to gain follow up information as requested. If a patient no longer attends clinic at the treating transplant centre (e.g. moves away or is discharged from clinic) it is the duty of the site to inform UCL CTC of where follow up information may be obtained (e.g. GP, alternate transplant centre). Patients will also be consented to follow up through the NHS Information Centre.

9.0 Correlative Science

PLEASE ALSO SEE SECTION 8.2.1 and APPENDICES 7, 8 and 12 FOR FURTHER DETAILS INCLUDING CONTACT NAMES AND ADDRESSES.

Four distinct but inter-related scientific correlative studies are planned, closely integrated with the clinical questions being asked. Minimal residual disease (MRD) testing will be carried out as a matter of course, as an integral part of the risk-stratified treatment allocation. The Adult ALL MRD laboratory at UCL Royal Free Campus, will provide specimen reception, processing and storage facilities and carry out the MRD testing, providing the infrastructure for the duration of the study.
Tests and analyses will be performed in 3 places (a) the central laboratory, UCL Royal Free Campus, London (b) Northern Institute of Cancer Research (NICR), Newcastle University & CRUK-PIMS (c) Medac, Germany. Data will be ultimately be collated with clinical outcome data held by UCL Cancer Trials Unit.

9.1 Aim 1. To determine the relationship between CD20 on ALL blasts and response to monoclonal antibody therapy

9.1.1 Background

The ability to combine rituximab (antiCD20) with chemotherapy (resulting in considerable improvement in outcome) in the treatment of lymphoma coupled with the expression of CD20 in ALL of B cell precursor-cell type has lead to the introduction of rituximab in the treatment of B cell precursor (BCP) ALL. The expression of CD20 on ALL blasts varies widely (E. Paietta, personal communication) CD20 is less commonly or less highly expressed on B-ALL blasts than some other B cell antigens. However, there is accumulating evidence that it is of prognostic significance.Upon instituting an investigation into the use of anti-B cell monoclonal antibodies in ALL, the investigators realised that there was no answer to the recurrent question asked in review 'why do you not limit anti-CD20 antibody administration only to those with CD20 positive tumors'. No data in ALL - or other tumour - were available to define the relationship between antigen expression and response and suggest a threshold level for response. Particularly intriguing in this regard is a recent demonstration that the relatively modest expression level of CD20 at diagnosis was often dramatically up-regulated, both in numbers of cells expressing the antigen and levels of expression per cell, following induction chemotherapy. This was shown in vitro to occur on exposure to glucocorticoids, and correlated well with in-vitro rituximab-induced killing[9].We think this kind of study offers an ideal opportunity to investigate this important issue further.

9.1.2 Plan of investigation

In order to investigate the hypothesis that response to monoclonal antibody therapy may have no clear relation to the density of relevant antigen expression at diagnosis, we plan to investigate the relationship between density of CD20 antigen expression at diagnosis and response to monoclonal antibody therapy in all patients with B-lineage ALL who are participating in the induction randomisation. Flow cytometry will be used to quantify CD20 antigen expression (percentage expression and mean fluorescence intensity MFI) at diagnosis. Correlation between percentage CD20 expression and response to initial therapy (CR rate and

quantitative MRD level after both phases of induction) will be determined for all 4 randomisation groups, the group receiving no monoclonal antibodies serving as a negative control group.

9.2 Aim 2. To determine whether the administration of an anti-B cell monoclonal antibody as part of induction therapy for ALL limits the extent of anti-asparaginase antibody formation and promotes asparagine depletion.

9.2.1 Background

L-asparaginase is arguably one of the most valuable drugs in the treatment of ALL. However, it is associated with numerous toxicities. When these occur early in treatment, therapeutic delays are often generated which can result in compromise of overall therapy. The appropriate dose, preparation and formulation of L-asp remain unresolved. In paediatric practice, pegylated L-asp (peg-asp) is less immunogenic and gives the most appropriate pharmacokinetic and pharmacodynamics but evidence that this agent could be properly used in adults was lacking until a recent CALGB phase II study used peg-asp as part of a multi-agent regimen. Effective asp depletion was achieved in some adults [15]although increasing age was associated with significantly decreased peg-asp doses and less asparagine depletion, furthermore, there was still a significant number of patients who developed of anti-asparaginase antibodies; this correlated with a less successful asparagine depletion. Within the associated clinical study UKALL14, peg-asp will be administered to adults for the first time in a large phase 3 setting.

9.2.2 Plan of Investigation

We hypothesise that the rate of anti-asparaginase antibody formation will be reduced by the co-administration of antiCD20 and that this can result in higher levels of asparaginase activity. We will test this hypothesis by quantifying anti-asparaginase antibody levels and asparaginase activity prior to peg-asp infusion and at time-points specified in section 8.2.2 post peg-asp infusion. The 2-way randomization provides the control groups (antiD20, or neither) necessary to adequately address this question.

9.3 Aim 3. To perform genomic profiling in order to discover and characterise novel prognostic markers and to identify known copy number alterations (CNA).

9.3.1 Background

ALL is a heterogeneous disease at the genetic level and numerous genetic abnormalities have been described which correlate with demographic, clinical and outcome parameters. Karyotype is one of the most important risk factors in adult ALL[46]. Genomic copy number arrays have now revolutionised our understanding of the genetic basis of childhood ALL and have identified novel subgroups which correlate with clinical parameters, genetic subtypes and outcome[55] [56]Currently, there is very limited genomic information available for adult ALL. In one study, SNP arrays revealed a series of cryptic genomic abnormalities but it was too small to establish any novel subgroups or correlate the findings with clinical parameters or outcome[57]. UKALL14 provides a valuable opportunity to collect diagnostic and remission samples to identify novel CNAs within a large cohort. We will focus on Philadelphia chromosome (Ph) negative BCP-ALL, since T-ALL is better understood at the genetic level and shares a greater homology with paediatric ALL[58].

9.3.2 Plan of Investigation

We plan to analyse DNA from 400 diagnostic samples using the Affymetrix SNP6.0 array (or later version). We will generate a normal cohort for comparison by analysing DNA from 50 MRD-negative post phase II remission samples. Data from this cohort will be used alongside the publicly available HapMap control dataset to remove copy number variants (CNV), using paired and unpaired normalisation. All labelling and hybridisation procedures will be outsourced to CRUK-PIMS. The raw data files will be returned to the LRCG where it will be processed, stored and analysed using a combination of in-house tools, freeware and commercially available software (e.g. Affymetrix Genotyping Console, dChip, Partek and Nexus). Where necessary, CNAs identified by the SNP arrays will be confirmed using home-grown and commercially available FISH probes. The LRCG has experience analysing large complex datasets and integrating data from different technologies e.g. genomic, gene expression and micro-RNA data [59-61]

Using cytogenetic and FISH data generated during the patients diagnostic work-up we will determine whether each novel CNA co-exists with known chromosomal abnormalities. This will allow us to assess whether these novel lesions are likely to represent primary genetic aberrations which might define new biological subgroups or secondary abnormalities which are likely to be cooperating mutations. Running high resolution SNP arrays will reveal novel CNAs and also identify known CNAs which are beyond the detection limits of standard diagnostic technologies (e.g. Ikaros deletions, which have shown to be associated with a poor outcome in childhood ALL and are frequently <85kb[56]. Running this array-based project in conjunction with a clinical trial will allow the clinical relevance of these novel and existing genetic lesions to be assessed in a reasonable timeframe. We will investigate the relationship of each CNA with the age, sex, white cell count, immunophenotype and other clinical parameters. Most importantly, we will be able assess their prognostic relevance in the context of a modern treatment protocol and be able to consider a variety of clinically relevant endpoints (e.g. CR status, MRD status, BM and CNS relapse and death). Assuming an overall event rate of 50%, the analysis of 400 patients will give us 80% power to detect a 20% difference in outcome for an abnormality found in 20% patients. This magnitude of effect was observed for cytogenetic subgroups in the previous trial [46]and novel CNAs of this frequency have been discovered in childhood ALL using the same platform[55]. Standard statistical tests will be used and all analyses will be conducted in collaboration with the trial statistician (Dr Sue Richards) as in previous studies.

This project will identify a number of genomic abnormalities whose functional consequence will need to be fully evaluated in order to elucidate their contribution to leukaemogenesis and assess their potential as therapeutic targets. Utilising total RNA extracted from the same diagnostic samples used for the SNP arrays we plan to further investigate different genetic lesions using gene expression arrays (Affymetrix Exon 1.0 ST array) and micro-RNA arrays (Agilent miRNA microarray Rel12.0). These additional scientific studies will require additional funding; however, the LRCG is an excellent position to attract such funding. We have ongoing collaborations within NICR with experts in functional analysis (Dr Olaf Heidenreich), molecular pharmacology (Dr Julie Irving) and molecular carcinogenesis (Dr James Allan). In addition, we are well positioned to take forward any identified therapeutic targets through the CRUK Drug Discovery programme in collaboration with Herbie Newell, Professor of Cancer Therapeutics, NICR and Roger Griffin, Professor of Medicinal Chemistry.

9.4 Aim 4. To determine whether the speed at which full donor chimerism is achieved in the T –cell compartment correlates with the level of molecularly determined minimal residual disease.

9.4.1 Background

Since the age threshold at which TRM exceeds reduction in relapse risk may be as low as 35 to 40 years old [27], it is very reasonable to examine non-myeloablative HSCT (previously reported only in retrospective studies) as a way to provide a graft versus leukaemia effect with reduced toxicity in adult ALL and this will be investigated in UKALL14. The success of this approach is likely to be disease burden dependent - absence of MRD at the time of transplant and the speed at which full donor chimerism[42] can be achieved may be of crucial importance. This has never previously been studied. Hence, the main scientific question to be addressed in this correlative study is whether the graft versus leukaemia effect is of sufficient magnitude and appropriate in timescale to deliver an effective anti-leukaemia therapy.

9.4.2 Planned Investigation

In order to investigate this question thoroughly, mixed-lineage chimerism analysis will be performed on peripheral blood - fractionated into myeloid, T cell and B cell populations using immunomagnetic beads - at 3, 6, 9, 12, 18 and 24 months post transplant. The degree of donor/recipient chimerism will be assessed with PCR analysis of informative mini-satellite regions. Peripheral blood (20 ml EDTA) must be collected and stored from both the donor and patient prior to the transplant.

For patients receiving DLI as defined in the protocol, the chimerism status will be assessed 2 months after each DLI infusion, until full donor chimerism is obtained.

Minimal residual disease will be assessed at the same time-points, using immunoglobulin gene or T cell receptor gene re-arrangements as identified at diagnosis.

9.5 Aim 5. Assessment of late effects

9.5.1 Aims

To formally assess the late effects of ALL therapy for all patients on the trial, whether they have received chemotherapy alone or an allograft

To identify and describe some of the adverse physical and psychosocial consequences of the disease and its treatment.

9.5.2 Background & Planned Investigations

A great deal of research has been carried out on the late effects caused by chemotherapy for acute lymphoblastic leukaemia in children. It is well documented that approximately 2 out of every 3 survivors from childhood cancers, including ALL, will suffer at least one late effect. One in four survivors will experience a late effect that is life threatening (1,2).

There are data on late effects of myeloablative transplantation in adults with ALL (EBMT refs), but less research has been done in adult ALL sufferers treated with chemotherapy alone and there are minimal data on the effects of RIC allografting for ALL to date. We presume that the late effect rate in adults will be at least equivalent to that seen in children, if not worse, but we do not know that this is the case. Part of our

role as clinicians is to inform patient choice on the best therapeutic choice available to them. Survival rates are obviously a key part of any such discussions, but increasingly we need to look at the quality of survival offered by available therapies when helping patients consider their options. The aim of this part of the trial is to collect some basic information regarding late effects of diagnosis and treatment of ALL in adults that may inform such discussions with our patients in the future.

Late effects that are known to occur as a consequence of chemotherapy include neurocognitive problems, premature menopause, cardiorespiratory dysfunction, sexual impairment, infertility, chronic fatigue and pain syndromes, and second malignancies. Research shows that many survivors also experience significant negative psychosocial outcomes, including fear of recurrence, poor self-esteem, anxiety and depression, employment and insurance discrimination, and relationship difficulties (5).
Historically, survivorship research has sought to identify and address the adverse physical and psychosocial consequences of the disease. An emerging body of evidence suggests that cancer survivors, similar to survivors of other traumatic life events, also experience positive life changes following a cancer diagnosis, and that these positive effects of cancer frequently coexist with the negative (6, 7). Studies in adult cancer survivors have shown that many survivors identify their illness experience as an event that has allowed them to make positive lifestyle changes resulting in higher quality of life scores than the general population (8, 9). We therefore want to use the general health questionnaire-12 (GHQ-12) as part of our screening for treatment related late effects. This is a reliable screening instrument for psychological distress in all clinical groups and can be used for assessing both positive and negative aspects of mental health.

Whilst some late effects may occur after many years eg second malignancies, other effects such as infertility can be assessed at much earlier time points. The aim of this part of the study is to initially assess each patient for late effects 2 years following the end of therapy. This will be 4 years from diagnosis for those having received chemotherapy only whereas it will be less than 3 years from diagnosis in some cases where allografting has been used. This part of the study is in addition to the routine data collected for all transplant patients.

9.6 Schedule of testing for correlative science studies

Please see section 8.2.1 - Schedule of testing for MRD and correlative science (please also see appendices 7, 8 and 12)

10.0 Data Collection & Management

All documents must be available for inspection by the appropriate authorities upon request.

10.1 Completing Forms

The original CRFs must be sent to the UCL CTC and a copy kept at site. All entries must be clear and legible. The use of abbreviations and acronyms must be avoided. The treating clinician is responsible for the accuracy of all data reported in the CRF. All CRFs must be signed off by staff who are listed on the site staff delegation log as performing this duty.

10.2 Corrections

Any corrections must be made by drawing a single line through the incorrect item whilst ensuring that the previous entry is not obscured. Each correction must be dated and initialled. Tippex must not be used.

10.3 Missing Data

To avoid the need for unnecessary data queries CRFs must be checked at site for blank fields before sending to the UCL CTC. When data is unavailable because a measure has not been taken or test not performed, enter "ND" for not done. If a measure was not required at the particular time the form relates to, enter "N/A" for not applicable. When data is unknown enter the value "NK" (only use if every effort has been made).

10.4 Queries

Data arriving at the UCL CTC will be checked for completeness, accuracy and consistency of data. Queries will be sent out sites. When completing a query, attach an amended copy of your case report form and please mail to the UCL CTC. All amendments must be initialled and dated.

10.5 Submission Timelines

UK sites must complete and return CRFs to the UCL CTC within one month of the patient being seen.
Sites outside the UK must complete and return CRFs to their country co-ordinating centre within one month of the patient being seen. Country co-ordinating centres must forward all CRFs to the UCL CTC within 5 business days.

10.6 Archiving of Trial Documentation

At the end of the trial, the UCL CTC will archive securely all trial related documentation for 5 years. Arrangements for confidential destruction will then be made. It is the responsibility of PIs to keep all essential documents relating to the trial for a minimum of 5 years after the end of the trial and in accordance with national legislation and for the maximum period of time permitted by the site. Essential documents are those which enable both the conduct of the trial and the quality of the data produced to be evaluated and show whether the site complied with the principles of Good Clinical Practice and all applicable regulatory requirements.

11.0 Pharmacy

Please see separate UKALL14 drug supply document for initial drug supply information, section 7.0 for treatment schedule and appendices 2-4 for full details of IMPs and supporting medications.

11.1 Pharmacy responsibilities

Please see separate UKALL14 drug supply document and Clinical Trial Site Agreement.

11.2 Drug accountability

Accountability for Rituximab, , Nelarabine, Palifermin and Pegylated Asparaginase at participating sites is the responsibility of the Principal Investigator, who may delegate this responsibility to the local pharmacist, or other appropriately qualified personnel. The responsible person will ensure that the Rituximab, Nelarabine, Palifermin and Pegylated Asparaginase are used only in accordance with this protocol and that appropriate drug accountability records are maintained.

The trial drugs must not be used outside the context of this protocol. Under no circumstances should the site investigator or other site personnel supply trial drug to other investigators, patients, or clinics, or allow supplies to be used other than directed by this protocol without prior authorisation from the Supplier and notification to the Sponsor.

The site pharmacy must maintain drug accountability records for the 4 drugs listed above including receipt dispensing, returned medication, storage conditions and destruction of returned/unused medication.
Sites are permitted to use their own drug accountability systems as long as the required information above is recorded and available to the Sponsor.

12.0 Pharmacovigilance

12.1 Definitions of Adverse Events

The following definitions have been adapted from Directive 2001/20/EC, ICH E2A "Clinical Safety Data Management: Definitions and Standards for Expedited Reporting" and ICH GCP E6:

Adverse Event (AE)

Any untoward medical occurrence or effect in a patient treated on a trial protocol, which does not necessarily have a causal relationship with a trial treatment. An AE can therefore be any unfavourable and unintended sign (including an abnormal laboratory finding), symptom or disease temporally associated with the use of a trial treatment, whether or not related to that trial treatment.

Adverse Reaction (AR)

All untoward and unintended responses to a trial treatment related to any dose administered. A causal relationship between a trial treatment and an adverse event is at least a reasonable possibility, i.e. the relationship cannot be ruled out.

Serious Adverse Event (SAE) or Serious Adverse Reaction (SAR)

An adverse event or adverse reaction that at any dose:

- Results in death
- Is life threatening (The term "life-threatening" refers to an event in which the subject was at risk of death at the time of the event. It does not refer to an event that hypothetically might have caused death if it were more severe.)
- Requires in-patient hospitalisation or prolongs existing hospitalisation
- Results in persistent or significant or disability/incapacity
- Is a congenital anomaly or birth defect
- Is otherwise medically significant (i.e. 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 above)

Suspected Unexpected Serious Adverse Reaction (SUSAR)

A serious adverse reaction, the nature or severity of which **is not consistent** with the applicable trial treatment information.

12.2 Reporting Procedures

12.2.1 All Adverse Events (AEs)

All adverse events must be recorded in the patient notes. The maximum severity grade of all adverse events that occur between informed consent and 30 days post consolidation (for non transplant patients) or 30 days post transplant (for transplant patients) must be recorded on the trial CRFs. Those meeting the

definition of a Serious Adverse Event (SAE) must also be reported to the UCL CTC using the trial specific SAE Report (see Serious Adverse Events section for details).
Pre-existing conditions do not qualify as adverse events unless they worsen.

12.2.1.1 Overdoses

All accidental or intentional overdoses, whether they result in an adverse event or not, must be recorded in the patient notes and CRFs. Overdoses resulting in an adverse event are classified as SAEs and must also be reported to UCL CTC according to SAE reporting procedures (see Serious Adverse Events section for details). Sites must inform UCL CTC immediately when an overdose has been identified.

12.2.1.2 Adverse Event Term

An adverse event term must be provided for each adverse event, preferably using the term listed in the Common Terminology Criteria for Adverse Events (CTCAE) v4.0 available online at:
http://evs.nci.nih.gov/ftp1/CTCAE/CTCAE_4.02_2009-09-15_QuickReference_8.5x11.pdf

12.2.1.3 Severity

Severity for each adverse event must be determined by using the Common Terminology Criteria for Adverse Events (CTCAE) v4.0 as a guideline, wherever possible. The criteria are available online at
http://evs.nci.nih.gov/ftp1/CTCAE/CTCAE_4.02_2009-09-15_QuickReference_8.5x11.pdf

In those cases where the CTCAE criteria do not apply, severity should be coded according to the following criteria:

1 = Mild
2 = Moderate
3 = Severe
4 = Life threatening
5 = Fatal

12.2.1.4 Causality

The PI, or other delegated site investigator must perform an evaluation of causality for each adverse event. Causal relationship to the trial treatment must be determined as follows:

- **None**

There is no evidence of any causal relationship.

- **Unlikely**

There is little evidence to suggest a causal relationship (e.g. because the event did not occur within a reasonable time after administration of the trial treatment). There is another reasonable explanation of the event (e.g. the patient's clinical condition, other concomitant medications).

- **Possible**

There is some evidence to suggest a causal relationship (e.g. because the event occurs within a reasonable time after administration of the trial treatment). However, the influence of other factors may have contributed to the event (e.g. the patient's clinical condition, other concomitant medications).

- **Probable**

There is evidence to suggest a causal relationship and the influence of other factors is unlikely.

- **Definitely**

There is clear evidence to suggest a causal relationship and other possible contributing factors can be ruled out.

12.2.2 Serious Adverse Events (SAEs)

All SAEs that occur between informed consent and 30 days post the last IMP administration, or 30 days post transplant for transplant patients (or after this date if the site investigator feels the event is related to one of the IMPs.) must be submitted to UCL CTC by fax within **1 business day** of observing or learning of the event, using the trial specific SAE Report. All sections on the SAE Report must be completed. If the event is not reported within 1 business day to UCL CTC, the circumstances that led to this in the SAE report must be detailed to avoid unnecessary queries.

12.2.2.1 Events which do not Require Immediate Reporting on an SAE Report

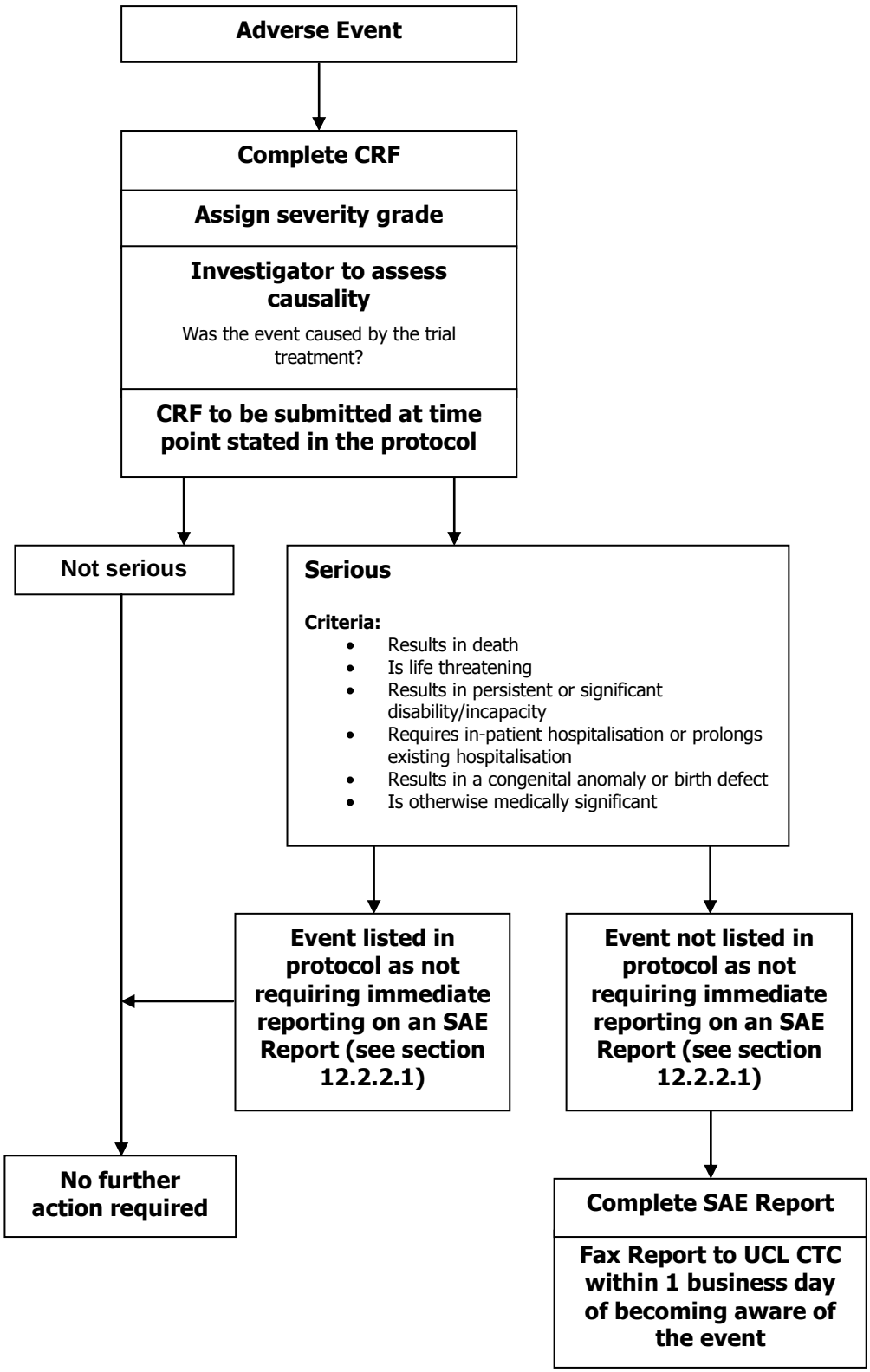
The following events do not require immediate reporting on an SAE Report for this trial, but must be recorded in the relevant section(s) of the CRF (please see section 12.2.1)

- Disease progression
- Disease related deaths
- Admissions for palliative care
- Admissions to intensive care that would be expected as part of the non-IMP treatment
- GvHD
- Graft Failure
- Secondary Malignancy

Please note that hospitalisation for elective treatment or palliative care does not qualify as an SAE.

All SAEs must be reported by faxing a completed SAE Report,
within 1 business day of becoming aware of the event, to UCL CTC
Fax: 020 7679 9861

12.2.2.3 Adverse Event Reporting Flowchart



12.2.2.4 SAE Follow-Up Reports

All SAEs must be followed-up until resolution and until there are no further queries. The PI, or other delegated site investigator must provide follow-up SAE Reports if the SAE had not resolved at the time the initial report was submitted.

12.2.2.5 SAE Processing at UCL CTC

On receipt of the SAE Report, UCL CTC will evaluate the event for seriousness and expectedness to determine whether or not the case qualifies for expedited reporting.

Expectedness will be evaluated using the list of expected adverse events in the current SmPCs for Rituximab, Oncaspar, Nelarabine and Palifermin.

The SAE Report will be submitted immediately to the CI or their delegate (e.g. a clinical member of the TMG) for review and for them to perform an evaluation of causality on behalf of UCL CTC. If UCL CTC has considered expectedness difficult to determine, the CI will be consulted for their opinion. The CI must respond to the trial team within 1 business day.

UCL CTC will fax required SAE Reports to pharmaceutical companies involved within 1 business day, as agreed in the Trial Drug Supply Agreements held with each company.

12.3 SUSARs

If the event is evaluated as a Suspected Unexpected Serious Adverse Reaction (SUSAR), UCL CTC will submit a report to all relevant regulatory authorities, MREC and country co-ordinating centres/country lead sites within 6 calendar days for fatal/life threatening events, with a follow-up report within a further 7 calendar days, and 14 calendar days for all other events. Country co-ordinating centres/country lead sites must forward all SUSAR reports to their ethics committee(s) as required within 1 business day. UCL CTC will ensure that consideration is given where the reporting deadline falls over the weekend to allow reporting within the required timeframes. In the case of conflicting evaluations of causal relationship by the site and UCL CTC/CI, both opinions will be reported.

UCL CTC will inform all Principal Investigators of any SUSARs which occur on the trial. Site investigators will receive a quarterly line listing which must be processed according to local requirements. Country co-ordinating centres/country lead sites must forward all SUSAR reports received from the UCL CTC to all Principal Investigators in that country within 1 business day.

UCL CTC will forward reports regarding SUSARs that have occurred on other trials using the same trial treatment to all country co-ordinating centres/country lead sites. Country co-ordinating centres /country lead sites must forward all reports to all Principal Investigators in that country within 1 business day. These must be processed by Principal Investigators according to local requirements and filed with the IB for the drug concerned.

12.4 Clinical Review

UCL CTC will provide safety information to the Trial Management Group and the Independent Data Monitoring Committee on a periodic basis for review. Should the outcome of the review result in upgrading/downgrading of SAEs to SUSARs and vice versa, UCL CTC will provide relevant reports to all regulatory authorities, MREC and country co-ordinating centres/country lead sites. Country co-ordinating centres/country lead sites must forward all reports to their ethics committee(s), as required, within 1 business day.

12.5 Additional Safety Monitoring at UCL CTC

UCL CTC will monitor safety data for any trial related events that are not considered related to the trial treatment. In the event that any trial procedures appear to be resulting in adverse events, the Trial Management Group will be contacted for their opinion. If it is declared necessary to review the conduct of the trial, UCL CTC will inform the MHRA, MREC and all country co-ordinating centres/country lead sites, as appropriate. Country co-ordinating centres/country lead sites must forward all notifications to the regulatory authority and ethics committee(s) in that country, as required, within 1 business day.

If UCL CTC detect a higher incidence in rare events than is stated in the IB(s)/SmPC(s) for the trial treatment, a report detailing the finding will be submitted to all regulatory authorities, MREC and country co-ordinating centres. Country co-ordinating centres must forward all reports to their ethics committee(s), as required, within 1 business day.

12.6 Pregnancy

If a female patient or a female partner of a male patient becomes pregnant at any point during the trial, a completed trial specific Pregnancy Report must be submitted to UCL CTC by fax within **1 business day** of learning of its occurrence.

All pregnancies must be reported by faxing a completed Pregnancy Report within 1 business day of becoming aware of the pregnancy to UCL CTC
Fax: 020 7679 9861

12.6.1 Pregnancy Follow-Up Reports

All pregnancies must be followed-up until an outcome is determined. Follow-up Pregnancy Reports must be submitted to UCL CTC by fax within **1 business day** of learning of the outcome. Reports must include an evaluation of the possible relationship of the trial treatment to the pregnancy outcome. Consent to report information regarding pregnancy outcomes must be obtained from the mother, if not a trial patient. The Pregnant Partner Information Sheet and Consent Form provided by UCL CTC must be used for this purpose.

12.6.2 SAEs During Pregnancy

Any SAE occurring in a pregnant patient must be reported using the trial specific SAE Report, according to SAE reporting procedures.

12.6.3 Pregnancy Report Processing at the UCL CTC

UCL CTC will fax all Pregnancy Reports to pharmaceutical companies involved within 1 business day, as required.

UCL CTC will submit areports to all relevant regulatory authorities, MREC and country co-ordinating centres/country lead sites should the pregnancy outcome meet the definition of a SUSAR. . See section 12.3 (SUSARs) for details.

12.7 Annual Safety Reports

The UCL CTC will submit Annual Safety Reports to the MHRA, MREC and all country co-ordinating centres/country lead sites. This will commence one year from the date of the first CTA approval obtained for the trial. Country co-ordinating centres/country lead sites must forward all reports to the regulatory authority and ethics committee(s) in that country, as required, within 1 business day.
The final Annual Safety Report will be submitted in the year following trial closure. See section 15.0 (Discontinuation/End of Trial) for details.

13.0 Incident Reporting and Serious Breaches

Organisations must notify UCL CTC of all deviations from the protocol or GCP immediately. UCL CTC may require a report on the incident(s) and a form will be provided if a Trust Incident Form is unavailable at the site. UCL CTC will assess all incidents to see if they meet the definition of a serious breach.

Systematic or persistent non-compliance by a site with GCP and/or the protocol, including failure to report SAEs occurring on trial within the specified timeframe, may be deemed a serious breach.

In cases where a serious breach has been identified, UCL CTC will inform the MHRA within 7 calendar days and country co-ordinating centres and/or country lead sites within 6 calendar days of becoming aware of the breach. Country co-ordinating centres and/or country lead sites must forward all reports to the regulatory authority in that country, as required, within 1 business day.

UK sites must have written procedures for notifying the sponsor of serious breaches (MHRA Guidance on the Notification of Serious Breaches, 2009).

UCL CTC will use an organisation's history of non-compliance to make choices on future collaborations.

14.0 Withdrawal of patients

In consenting to the trial, patients are consenting to trial treatment, trial follow-up and data collection.

14.1 Withdrawal from Trial Treatment

The site investigator may withdraw a patient from the trial treatment whenever continued participation is no longer in the patient's best interests, but the reasons for doing so must be recorded. Reasons for discontinuing treatment may include:

- Disease progression whilst on therapy
- Unacceptable toxicity
- Intercurrent illness which prevents further trial treatment
- The patient withdraws consent to further trial treatment
- Any alterations in the patient's condition which justifies the discontinuation of treatment in the site investigator's opinion

In these cases patients remain within the trial for the purposes of follow-up and data analysis according to the treatment option to which they have been allocated. If a patient wishes to withdraw from trial treatment, sites should explain the importance of remaining on trial follow-up, or failing this of allowing routine follow-up data to be used for trial purposes.

14.2 Withdrawal of Consent to Data Collection

If a patient explicitly states their wish not to contribute further data to the trial their decision must be respected and recorded on the relevant CRF.
Details should be recorded in the patient's hospital records and no further CRFs must be completed.

14.3 Moving

For patients moving from the area, every effort should be made for the patient to be followed up at another participating trial site and for this new centre to take over the responsibility for the patient, or for follow-up via GP.

14.4 Lost to follow-up

If a patient is lost to follow-up every effort should be made to contact the patient's GP (if consented) to obtain information on the patient's status.
Patients lost to follow up can be tracked via the NHS Information Centre

15.0 Discontinuation/End of the Trial

15.1 Trial closure

For regulatory purposes the end of the trial will be defined as the end of the follow up phase (2 years after the end of maintenance or 4 years after the date of stem cell infusion for transplant patients) at which point the 'declaration of end of trial' form will be submitted to participating regulatory authorities and ethical committees, as required. However, this will be followed by the non-interventional phase of long-term follow-up, which will continue indefinitely after the final patient entered onto the trial has completed trial follow up (2 years after the end of maintenance or 4 years after the date of stem cell infusion for transplant patients).

15.2 Archiving of Trial Documentation

At the end of the trial, the UCL CTC will archive securely all centrally held trial related documentation for 5 years. Arrangements for confidential destruction will then be made. It is the responsibility of the PI's to keep all data and essential documents relating to the trial held at site, for a minimum of 5 yeares after the end of the trial and in accordance with national legislation and for the maximum period of time permitted by the site.

If a patient withdraws consent for their data to be used, it will be confidentially destroyed.

All documents must still be available for inspection by the appropriate authorities upon request.

15.3 Early discontinuation of trial

The trial may be stopped before completion upon recommendation of the TSC or IDMC (see section 16 Sites will be informed by UCL CTC in writing of reasons for early closure and actions to be taken with regards to trial patients. Patients should continue to be followed up as per protocol.

15.4 Withdrawal from trial participation by sites

When closing the trial at a site Investigators must inform the UCL CTC in writing with reasons for withdrawal. Follow up as per protocol should continue for all patients recruited into the trial at that site.

If a patient withdraws consent for their data to be used, it will be confidentially destroyed immediately.

16.0 Trial Monitoring and oversight

16.1 Monitoring

Participating sites and Principal Investigators must agree to allow trial-related on-site monitoring, including Sponsor audits, ethics committee review and regulatory inspections by providing direct access to source data/documents as required. Patients are informed of this in the patient information sheet and are asked to consent to their medical notes being reviewed by UCL CTC on the consent form. .

16.1.1 Central monitoring

Data stored at the UCL CTC will be checked for missing or unusual values (range checks) and checked for consistency over time. If any problems are identified, a data query sheet will be issued to the site. Sites are required to resolve any queries and update the relevant CRF as required. All changes must be initialled and dated. The amended version must be sent to the UCL CTC and a copy retained at site. The UCL CTC will send reminders for any overdue data or query sheets.

Sites will also be requested to submit screening logs and staff delegation logs to UCL CTC on request, and these will be checked for consistency and completeness.

16.1.2 Self Assessment monitoring:

Participating sites will be requested to complete a self-assessment monitoring report periodically, at a frequency detailed in the trial monitoring plan. This report may include, but is not limited to, Investigator Site File and Pharmacy File document version checklists, recruitment status for consistency checks, and review of informed consent forms. Responses will be reviewed at UCL CTC to indentify areas of non-compliance/fraud and to indicate training needs. Findings may trigger an on-site monitoring visit.

16.1.3 Non-Compliance

On-site monitoring visits may be scheduled where there is evidence or suspicion of non-compliance by a site with important aspect(s) of the trial/GCP requirements. Sites will be sent a letter in advance outlining the reason(s) for the visit, a list of the documents that are to be reviewed, interviews that will be conducted, planned inspections of the facilities, who will be performing the visit and when the visit will likely occur.

16.1.4 Monitoring report

Following any monitoring visit, the monitor/TC will provide to the site a report, which will summarise the documents reviewed, along with a statement of findings, deviations, deficiencies, conclusions, actions taken or recommended and actions required. The Principal Investigator at the site will be responsible for ensuring that monitoring findings are addressed (this may be delegated to an appropriate member of staff).

16.2 Trial Management and Trial Committees

16.2.1 Trial Management Group (TMG)

The TMG will include the Chief Investigator, clinicians and experts from relevant specialities and UKALL14 trial staff from UCL CTC (see page 2).The TMG will be responsible for overseeing the trial. The day to day management of the trial will be coordinated through the UCL CTC. The group will meet regularly and will send updates to Principal Investigators (via newsletters or at Investigator meetings) and to report to the appropriate NCRI Clinical Studies Group.

16.2.2 Independent Trial Steering Committee (TSC)

The role of the ITSC is to provide overall supervision of the trial and ensure that it is conducted in accordance with GCP and the Protocol. The ITSC will review the recommendations of the Independent Data Monitoring Committee and, on consideration of this information, recommend any appropriate amendments/actions for the trial as necessary. The ITSC acts on behalf of the funder(s) and Sponsor.

16.2.3 Independent Data Monitoring Committee (IDMC)

The role of the IDMC is to provide independent advice on the data and safety aspects of the trial. Meetings of the Committee will be held periodically, or as necessary to address any issues. The IDMC is advisory to the Independent Trial Steering Committee (TSC) and can recommend premature closure of the trial to the TSC.

16.3 Trial Administration

16.3.1 Role of Trials Centre

The Trial Centre (UCL CTC) will be responsible for the day to day coordination and management of the trial and will act as custodian of the data generated on the trial (on behalf of UCL). UCL CTC will ensure that all SAEs and SUSARs are appropriately reported to the MHRA and Main REC and pharmaceutical companies. In addition all reports of SUSARs will be reported promptly to all Principal Investigators.

16.3.2 PI and Site Responsibilities

Individual site investigators should seek research ethics committee approval and once approved are responsible for recruiting patients, adhering to the most recent version of the protocol (taking into account any updated safety information and protocol amendments), safe conduct of the trial, collection of data on the CRFs and prompt notification of adverse events, as detailed in section 3. Site investigators should also refer SAEs as required by their Research Ethics Committee/R & D department. An agreement (CTSA) between the site and the UCL CTC will be signed at the start of the trial.

16.3.3 Protocol Amendments

The TMG will agree protocol amendments on behalf of the Principal Investigators prior to submission to the Main REC and MHRA. All Principal investigators will be kept informed of substantial amendments.

17.0 Statistics

17.1 Population for analysis

The population for this trial will be adult patients age 25-65 years, inclusive. The primary objective of this study is to compare the event-free survival (EFS) in a phase III trial of patients with B cell precursor ALL receiving either Rituximab in conjunction with chemotherapy to chemotherapy alone. The primary endpoint of the trial is event free survival (EFS) and all analysis for this endpoint will be on an intention to treat basis.

The incidence of cases of ALL within the age range of the trial (25-65 years) in England in the decade between 1997 and 2006 suggests that there will be 150 eligible patients per year in the UK. The aim is to recruit 80% of eligible patients, giving 120 per year. The target overall recruitment is thus 720 patients over 6 years or 840 patients over 7 years. This would give 576 or 672 patients expected to be B lineage-ALL and randomized equally between two treatment arms..
20% of patients are expected to be T lineage, so there will be 144 or 168 T-lineage.

The Trial Steering Committee will assess at the time whether it is feasible and necessary to continue recruitment for the seventh year, blinded to randomised treatment results.

17.2 Analysis of the primary objective(s)

The primary endpoint, EFS, is defined as time from randomization to relapse or to death from any cause. Curves for the comparison of EFS will be produced using the method of Kaplan-Meier and compared using the log-rank test.

Subgroup analyses will be performed in patient groups aged <40 and ≥40 years. If a relationship between CD20 expression and response is found, this will be used to define positive and negative groups for subgroup analysis of EFS by treatment.

Primary objective in patients with B-lineage disease: effect of antibody on EFS

For the evaluation of additional benefit of antibody to chemotherapy in terms of EFS in patients with B cell precursor ALL, the effect of the antibody will be tested at a two-sided significance level of 0.05, using the log rank test using all randomized patients (intention-to-treat analysis).

Primary objective in patients with T lineage disease: effect of nelarabine on EFS

Patients with T-lineage disease enrolled in this study will be randomized between chemotherapy and the combination of Nelarabine and chemotherapy as a phase II study to determine if Nelarabine improves the EFS of patients with T-cell ALL.

17.3 Analysis of secondary objectives

Tolerability of pegylated asparaginase in induction

Cases of hypersensitivity to peg-asparaginase will be recorded, plus changes to Erwinia or withdrawal of asparaginase treatment.

Toxicity of RIC BMT

An early loss of 16% of patients due to induction failure or relapse is expected before transplant can be reached. About 1/3 of patients will have a matched sibling donor and about ½ of those without such a donor might be expected to be high risk, giving 2/3 eligible for transplant, of whom about half can be expected to be over 40 years old. Thus 720 or 840 recruited patients would result in 201 or 235 who might receive a reduced intensity conditioned transplant. Based on UKALLXII/E2993 data, some of these will be excluded on the basis of liver function, or other, tests, so we would expect perhaps 150 or 175 to receive the transplant. Currently the treatment related mortality (TRM) of transplant in this age group is over 40%. Unfortunately it would be impractical to randomize between standard and RIC BMT, and thus a historical comparison is necessary and results should be treated with the appropriate caution. It is expected that the reduction in toxicity will be substantial, maybe as much as halved. As there may be an increase in relapses, EFS will be the primary outcome with the current rate of 40% at 3 years post transplant.

Palifermin randomization

In the palifermin study, approximately -150 or 175 patients treated with myeloablative HSCT will be randomized to evaluate the "standard dose" of palifermin vs. a single "collapsed" dose in reducing the incidence of Grades 3 or 4 mouth and throat soreness (MTS) scores. The randomization will be stratified by gender, sibling/MUD donor & precursor-B/Tlineage.

Outcome measures are:

1. OMDQ 2. Number of doses of MTX administered 3. Acute GVHD

17.3.1 Efficacy (secondary)

- Secondary outcomes will be
- Overall survival
- Complete remission rate
- Death in complete remission
- Relapse rate (actuarial incidence of relapse, excluding non-remitters and censoring at death in remission)
- Bone marrow relapse rate(actuarial incidence of relapse involving the bone marrow, excluding non-remitters and censoring at relapse at other sites and death in remission)
- Central nervous system (CNS) relapse rate (actuarial incidence of relapse involving the CNS, excluding non-remitters and censoring at relapse of other sites and death in remission)
- For the antibody randomisations only: anti-asparaginase levels and asparaginase activity prior to asparaginase infustions.

- Minimal Residual Disease at the end of Phase 1 Induction(antibody randomisation) or post transplant

17.3.2 Safety

All grade 3 or 4 toxicities will be reported.

17.4 Sample size calculations

Addition of monoclonal antibody to standard induction chemotherapy in patients with precursor B-ALL

The power calculations of the comparisons are made under the assumption that the 3-year EFS of the chemotherapy in this patient population (based on UKALLXII/E2993 data) is approximately 40%. With 576 (or 672 with 7 years accrual) patients randomised, there is 84% (or 89%) power to detect an improvement in 3-year EFS from 40% to 52%.

Addition of Nelarabine to standard induction chemotherapy in patients with precursor T-ALL

With 144 (or 168 with 7 years accrual) patients randomized, we would have over 86% power to detect an improvement in 3-year EFS from 50% to 75%, and about 68% (or 75% with 7 years accrual) power to detect an improvement to 70%, at a two-sided significance level of 0.05.

17.5 Power for analysis of critical secondary variables

Palifermin randomisation

Based on the references from Spielberger et al., and T. Shea et al., we assumed a range of incidence of Grades 3 or 4 MTS scores of 60%, 70% and 80% for the standard dose arm, and 30%, 40% and 50% for the collapsed dose arm, and a two-sided alpha level of 0.05, the table below provides the power for 75 patients per arm.

	30%	40%	50%	60%
60%	97%	69%	23%	NA
70%	99%	97%	71%	26%
80%	99%	99%	99%	84%

The null hypothesis is that the incidence is the same between the standard dose and the collapsed dose arms. The alternative hypothesis is that there is a difference between the two arms. With 75 patients per arm, the study would have at least 69% power to detect a treatment difference of at least 20%.

Since the patients are hospitalized, the MTS evaluation period is relatively short (until discharge or 28 days post-transplant) and the worst score for a patient will be used for the incidence calculation, the sample size loss due to non-compliance and loss to follow up will be small and the resulting power loss will be negligible.

17.6 Interim analysis

Interim analyses of the main endpoints will be supplied approximately annually, in strict confidence, to a data monitoring committee (DMC) starting in the fourth year of recruitment, or when 300 patients have been entered, whichever is the sooner. This will allow 4 or 5 planned interim analyses to be performed before the completion of the trial at 8 years, with the first occuring when about 25% of the expected events have happened. In the light of these interim analyses, the DMC will advise the MRC Leukaemia Steering Committee if, in their view, the randomized comparisons in the trial have provided proof beyond reasonable doubt ($2P<0.001$) that for all or for some types of patient one treatment is clearly indicated or clearly contraindicated.

The main analyses will be performed using standard log-rank methods based on the intention to treat, i.e. all patients believed to be eligible at the time of randomization will be included in the analysis, irrespective of protocol compliance, early relapse, etc. All analyses will assume that there may be some **quantitative** differences in the size of any treatment effects in different strata, but that there is unlikely to be any **qualitative** difference (i.e. harm in one group, benefit in another).

Final analyses will be performed when the last patient randomized has been followed up for 2 years, i.e. after all patients have finished their initial treatment.

18.0 Ethical and Regulatory

18.1 Regulatory Compliance

In conducting the Trial the Sponsor, UCL CTC and Sites shall also comply with all laws and statutes, as amended from time to time, applicable to the performance of clinical trials including, but not limited to: The principles of ICH Harmonised Tripartite Guideline for Good Clinical Practice (CPMP/ICH/135/95) as set out in Schedule 1 (Conditions and Principles of Good Clinical Practice and for the Protection of Clinical Trial Subjects) of the Medicines for Human Use (Clinical Trials) Regulations 2004 and the GCP Directive 2005/28/EC, as set out in SI 2006/1928.

18.2 Ethical Compliance

The trial will be conducted in accordance with the ethical principles founded in the Declaration of Helsinki.

The trial has received a favourable opinion from the Charing Cross Hospital Research Ethics Committee. The UCL UCL CTC will maintain contact with MREC and submit any protocol amendments to them. The UCL CTC will provide relevant documentation to participating sites.

The PI will provide the relevant ethics committee(s) with the final version of the protocol, patient information and consent forms, any other written information given to patients and any revisions to the protocol or any other trial documentation. It is the responsibility of the PI to obtain all necessary local approvals for the trial, and for any subsequent amendments where required. Evidence of local approval must be provided to the UCL CTC prior to site activation. The trial will only be conducted at sites where all necessary approvals have been acquired.

18.3 Patient Confidentiality & DPA

The Cancer Trials Centre will act to preserve patient confidentiality and will not disclose or reproduce any information by which patients could be identified. Data will be stored in a secure manner and UCL CTC trials are registered in accordance with the Data Protection Act 1998 with the Data Protection Officer at UCL.

19.0 Sponsorship and Indemnity

19.1 Sponsor Details:

Name:	**Senior Clinical Trials Manager or Divisional Manager**
Telephone:	020 7380 9995/6978 (unit admin)
Fax:	+44 (0) 20 7380 9937
Address:	Joint UCL and UCL Biomedical Research Unit Ground Floor, Rosenheim Wing 25 Grafton Way London WC1E 5DB

19.2 Indemnity:

University College London holds insurance to cover participants for injury caused by their participation in the clinical trial. Participants may be able to claim compensation if they can prove that UCL has been negligent. However, as this clinical trial is being carried out in a hospital, the hospital continues to have a duty of care to the participant of the clinical trial. University College London does not accept liability for any breach in the hospital's duty of care, or any negligence on the part of hospital employees. This applies whether the hospital is an NHS Trust or not. This does not affect the participant's right to seek compensation via the non-negligence route.

Participants may also be able to claim compensation for injury caused by participation in this clinical trial without the need to prove negligence on the part of University College London or another party. Participants who sustain injury and wish to make a claim for compensation should do so in writing in the first instance to the Chief Investigator, who will pass the claim to the Sponsor's Insurers, via the Sponsor's office.

Hospitals selected to participate in this clinical trial shall provide clinical negligence insurance cover for harm caused by their employees and a copy of the relevant insurance policy or summary shall be provided to University College London, upon request.

20.0 Publication Policy

All publications and presentations relating to the trial will be authorised by the TMG. The first publication of the trial results will be in the name of the TMG, if this does not conflict with the journal's policy. The TMG will form the basis of the writing committee and advise on the nature of publications. If there are named authors, these should include the Chief Investigator, Trial Coordinator, and Statistician involved in the trial. Contributing site investigators in this trial will also be acknowledged. Data from all sites will be analysed together and published as soon as possible. Participating sites may not publish trial results prior to the first publication by the TMG or without prior written consent from the TMG.

The trial data is owned by UCL CTC. Drug companies who have provided grants towards the trial will be permitted to see the draft manuscripts and make comments at least 30 days prior to submission for publication. (to be confirmed)

The Eudract number (2009-012717-22) or the clinicaltrials.gov number once allocated to this trial will be quoted in any publications resulting from this trial.

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Appendix 1: Abbreviations

AE	Adverse Event
ALL	Acute Lymphoblastic Leukemia
ALT	Alanine transaminase
ANC	Absolute Neutrophil Count
AR	Adverse Reaction
AST	Aspartate aminotransferase
AVN	Avascular Necrosis
BMT	Bone Marrow Transplant
CI	Chief Investigator
CMV	Cytomegalovirus
CNA	Copy Number Alteration
CNS	Central Nervous System
CR	Complete response
CRF	Case Report Form
CrCl	Creatinine Clearance
CT	Computerised Tomography
CTA	Clinical Trial Authorisation
CTAAC	Clincal Trials Advisory & Awards Committee
CTCAE	see NCI CTCAE
CTSA	Clincal Trial Site Agreement
DFS	Disease Free Survival
DLI	Donor Lymphocyte Infusion
DPA	Data Protection Act
ECG	Electrocardiogram
ECOG	Eastern Cooperative Oncology Group
EDTA	Ethylene Diamine Tetra Acetate
EFS	Event Free Survival
EudraCT	European Clinical Trials Database
FISH	Flourescent In Situ Hybridization
FBC	Full Blood Count
G-CSF	Granulocyte Colony Stimulating Factor
GVHD	Graft versus Host Disease
GFR	Glomerular Filtration Rate
GVL	Graft versus Lymphoma
HSCT	Hematopoietic Stem Cell Transplantation
Hb	Haemoglobin
HLA	Human Leukocyte Antigen
HSv	Herpes Simplex Virus

IBMTR	International Blood and Marrow Transplant Research
ICH GCP	International Conference of Harmonisation-Good Clinical Practice
IDMC	Independent Data Monitoring Committee
IMP	Investigational Medicinal Product
ISRCTN	International Standard Randomised Controlled Trial Number
IV	Intravenous
LDH	Lactic Dehydrogenase
LFT	Liver Function Tests
LMWH	Low Molecular Weight Heparin
MUD	Matched Unrelated Donor
MRC	Medical Research Council
MREC	Multicentre Research Ethics Committee
MRD	Minimal Residual Disease
MHRA	Medicines and Healthcare products Regulatory Agency
MP	Mercaptopurine
MTX	Methotrexate
NCI CTCAE	National Cancer Institute Common Terminology Criteria for Adverse Events
NCRI	National Cancer Research Institute
NCRN	National Cancer Research Network
NRES	National Research Ethics Service
OMDQ	Oral Mucositis Daily Questionnaire
OS	Overall Survival
PCP	Pneumocystis pneumonia
PD	Progressive Disease
PFS	Progression Free Survival
Ph	Philadelphia chromosome /t(9;22)(q34;q11) / BCR-ABL1
PI	Principal Investigator
PO	By mouth
PR	Partial Response
RIC-SCT	Reduced Intensity Conditioning Stem Cell Transplant
SAE	Serious Adverse Event
SCT	Stem Cell Transplant
SD	Stable Disease
SmPC	Summary of Product Characteristics
SUSAR	Suspected Unexpected Serious Adverse Reaction
TBI	Total Body Irradiation
TRM	Transplant Related Mortality
TMF	Trial Master File
TMG	Trial Management Group
TSC	Trial Steering Committee

UCL CTC	CR UK and UCL Cancer Trials Centre
U&E	Urea and Electrolyte
ULN	Upper Limit of Normal
VZV	Varicella Zoster Virus
WBC	White Blood Cells
WCC	White Cell Count

Appendix 2: IMP Information

It is recommended that sites follow local guidelines for dose adjustments for obese patients.

Rituximab (Mabthera)

Formulation and storage

Chemical Name:	Rituximab
Other Names:	Mabthera®
Physical Characteristics:	Concentrate for solution for infusion. Clear, colourless liquid

Description

Rituximab is a genetically engineered chimeric mouse/human monoclonal antibody representing a glycosylated immunoglobulin with human IgG1 constant regions and murine light-chain and heavy-chain variable region sequences. The antibody is produced by mammalian (Chinese hamster ovary) cell suspension culture and purified by affinity chromatography and ion exchange, including specific viral inactivation and removal procedures.

Rituximab is supplied as a clear, colourless liquid. It is a concentrate for solution for infusion. Rituximab comes in 500mg and 100mg vials containing concentrate for solution for infusion. Each solution contains 10mg/ml of rituximab.

Rituximab should be stored in a refrigerator (2 °C – 8 °C). Keep the container in the outer carton in order to protect from light.

The prepared infusion solution of Rituximab is physically and chemically stable for 24 hours at 2 °C - 8 °C and subsequently 12 hours at room temperature.

Please refer to the SmPC for more information.

Administration

Aseptically withdraw the necessary amount of MabThera, and dilute to a calculated concentration of 1 to 4 mg/ml rituximab into an infusion bag containing sterile, pyrogen-free sodium chloride 9 mg/ml (0.9 %) solution for injection or 5 % Glucose in water. For mixing the solution, gently invert the bag in order to avoid foaming. Care must be taken to ensure the sterility of prepared solutions. Since the medicinal product does not contain any anti-microbial preservative or bacteriostatic agents, aseptic technique must be observed. Parenteral medicinal products should be inspected visually for particulate matter and discoloration prior to administration. Any unused product or waste material should be disposed of in accordance with local requirements

The prepared Rituximab solution should be administered as an intravenous infusion through a dedicated line. It should not be administered as an intravenous push or bolus.

Rituximab infusions should be administered under the close supervision of an experienced physician, and in an environment where full resuscitation facilities are immediately available.
Premedication consisting of an anti-pyretic and an antihistaminic, e.g. paracetamol and diphenhydramine, should always be administered before each infusion of Rituximab. Premedication with glucocorticoids should also be considered.
The recommended initial rate for infusion is 50 mg/hr; after the first 30 minutes, it can be escalated in 50 mg/hr increments every 30 minutes, to a maximum of 400 mg/hr.
Subsequent doses of MabThera can be infused at an initial rate of 100 mg/hr, and increased by 100 mg/hr increments at 30 minutes intervals, to a maximum of 400 mg/hr.
For second and subsequent doses, it is acceptable to give Rituximab according to the escalated infusion protocol- to give 20% of the infusion over 30 minutes, with the remaining 80% given over an hour.
Patients should be closely monitored for the onset of cytokine release syndrome. Patients who develop evidence of severe reactions, especially severe dyspnoea, bronchospasm or hypoxia should have the infusion interrupted immediately. Patients should be evaluated for evidence of tumour lysis syndrome including appropriate laboratory tests and, for pulmonary infiltration, with a chest x-ray. In all patients, the infusion should not be restarted until complete resolution of all symptoms, and normalisation of laboratory values and chest x-ray findings. At this time, the infusion can be initially resumed at not more than one-half the previous rate. If the same severe adverse reactions occur for a second time, the decision to stop the treatment should be seriously considered on a case by case basis.

Mild or moderate infusion-related reactions usually respond to a reduction in the rate of infusion. The infusion rate may be increased upon improvement of symptoms.

A dose of 375mg/m^2 should be given by IV on days 3,10,17 & 24 of Phase 1 Induction Therapy.

Pegylated Asparaginase

Formulation and storage

Chemical Name:	Pegylated asparaginase
Other Names:	Pegaspargase, Oncaspar ®
Physical Characteristics:	Solution for injection

Description

Oncaspar® is a medicinal product (cytostatic agent) which lowers the L-asparaginase level in the tumour cells so that the protein synthesis in these cells is inhibited.

Administration

When administered intravenously Oncaspar® should be given over a period of 1-2 hours in 100ml sodium chloride 0.9% or glucose 5% into a free flowing infusion.

Oncaspar® is available in packs with:

1 vial (type I glass) containing 5 ml ready-to-use solution for injection [N1] (Geman labelling) or

1 vial (type I glass) containing 5 ml ready-to-use solution for injection [N1] (Geman-English labelling).

Not all pack sizes may be marketed.

1 vial contains 3750 I.U. pegaspargase (equivalent to 750 I.U./ml), in a clear colourless phosphate-buffered sodrium chloride solution, pH 7.3.

Oncaspar should be stored in a refrigerator (2°C – 8°C), and should not be frozen or shaken. Discard any drug that remains unused. Do not use if the solution is cloudy or a precipitate has formed..

Please refer to the SmPC for more information.

Nelarabine (Atriance)

Formulation and storage

Chemical Name:	(2*R*,3*S*,4*R*,5*R*)-2-(2-amino-6-methoxy-purin-9-yl)-5-(hydroxymethyl)oxolane-3,4-diol
Other Names:	Nelarabine, Atriance
Physical Characteristics:	Solution for injection, Clear, colourless liquid

Description

Nelarabine is a purine analogue and is provided as a clear, colourless 5mg/ml solution for infusion. Each ml contains 5mg of nelarabine and each vial contains 250mg of nelarabine.

This medicinal product does not require any special storage conditions. Nelarabine is stable for up to 8 hours at up to 30°C once the vial is opened.

Please refer to the SmPC for more information.

Administration

Nelarabine is for intravenous use only and must only be administered under the supervision of a physician experienced in the use of cytotoxic agents. Nelarabine is not diluted prior to administration. The appropriate dose of nelarabine is transferred into polyvinylchloride (PVC) or ethyl vinyl acetate (EVA) infusion bags or glass containers and administered as a two-hour infusion in adult patients.

A dose of 1.5grams/m^2 should be given by IV on days 1, 3 & 5 following Phase 2 induction treatment.

Neurological adverse events

Severe neurological events have been reported with the use of Nelarabine. These events have included altered mental states including severe somnolence, central nervous system effects including convulsions, and peripheral neuropathy ranging from numbness and paresthesias to motor weakness and paralysis. There have also been reports of events associated with demyelination, and ascending peripheral neuropathies similar in appearance to Guillain-Barr Syndrome.

Full recovery from these events has not always occurred with cessation of Nelarabine. Therefore, close monitoring for neurological events is stongly recommended, and Nelarabine must be discontinued at the first sign of neurological events of NCI common Toxicity Grade 2 or greater.

Palifermin (Kepivance)

Formulation and storage

Chemical Name:	Palifermin
Other Names:	Kepivance
Physical Characteristics:	Solution for injection.

Description

Palifermin is a human keratinocyte growth factor (KGF), produced by recombinant DNA technology in *Escherichia coli*. It is provided in a vial with 6.25mg of powder for solution for injection. Each vial contains 6.25 mg of palifermin. Reconstituted Kepivance contains 5 mg/ml of palifermin.

Palifermin should be stored in the original package to protect from light in a fridge (2°C - 8°C). Once reconstituted it can be stored in a fridge (2°C - 8°C), protected from light, for 24 hours.

Administration

The standard dosage of Kepivance is 60 micrograms/kg/day, administered as an intravenous bolus injection for three consecutive days before and three consecutive days after myeloablative therapy for a total of six doses (see below). Kepivance should not be administered subcutaneously due to poor local tolerability.
In the collapsed dose schedule the the 1st 3 doses at 60mcg/kg are replaced by one 180mcg/kg dose as an intravenous bolus (see below).
Reconstituted Kepivance should not be left at room temperature for more than one hour, and should be protected from light. Prior to administration, visually inspect the solution for discolouration and particulate matter before administration.

STANDARD DOSE SCHEDULE:

A dose of 60micrograms/kg should be given by IV bolus injection on days -10, -9 -8 and, 0, 2 & 4.

COLLAPSED DOSE SCHEDULE:

A dose of 180micrograms/kg should be given by IV bolus injection on day -9 and a dose of 60 micrograms/kg should be given on days 0, 2 & 4.

Appendix 3: Non-IMP General Drug information

This appendix is intended as a guide for the administration of non-IMPs in the UKALL14 trial.
Those centres with a firm local policy which differs in administration detail (but not dose) from the information provided below may follow their local guidelines.

Body surface area should be calculated according to the Dubois formula:
Body Surface Area (m) = 0.007184 x (patient height in cm)$^{0.725}$ x (patient weight in kg)$^{0.425}$
Body surface area should be recalculated after each cycle of chemotherapy and with any major weight change.

It is recommended that sites follow local guidelines for dose adjustments for obese patients.

Drugs used in Induction

Cyclophosphamide

Formulation	Powder for solution for injection. A white crystalline powder contained in clear glass injection vials.
Storage	Do not store above 25°C. Store in original container. After reconstitution (for either intravenous or oral administration), store at 2 - 8°C and protect from light.
Administration	A dose of 1000mg/m^2 to be given in 250ml sodium chloride 0.9% over 30mins. Give 125 mls/m2/hour of Dextrose/ Saline for 30 minutes before cyclophosphamide and for 3.5 hours afterwards ie 4 hours in total. Do not add potassium. Mesna is not needed.'

Cytarabine

Formulation	Clear, colourless solution for injection.
Storage	Do not store above 25°C. Keep container in the outer carton.
Administration	A dose of 75mg/m^2 to be given at a concentration of 20mg/ml in syringe by slow IV bolus.

Dexamethasone

Formulation	Each tablet contains 2mg Dexamethasone PhEur.
Storage	Store below 25°C protected from light.
Administration	10mg/m^2 to be given orally in one dose with breakfast.

Daunorubicin

Formulation	Vial containing a red lyophilised powder for intravenous administration following reconstitution in water for injections and dilution with saline. Each vial contains 21.4 mg Daunorubicin hydrochloride (equivalent to 20 mg as base).
Storage	Daunorubicin vials should be stored below 25°C, protected from light. After reconstitution Daunorubicin should be stored at 2 - 8°C, protected from light.
Administration	60mg/m^2 dose to be diluted in sodium chloride 0.9% to give final concentration of 1mg/ml and inject over 20 mins into side arm of freely running intravenous infusion of sodium chloride 0.9%. Alternatively the Daunorubicin may be added to a 100ml minibag of sodium chloride 0.9% and this solution infused over 20 minutes into the side arm of a freely running infusion of sodium chloride 0.9%. Take care to avoid extravasation.

Imatinib

Formulation	Glivec® 100 mg film-coated tablets & Glivec® 400 mg film-coated tablets
Storage	Do not store above 30°C. Store in the original package in order to protect from moisture.
Administration	The prescribed dose should be administered orally with a meal and a large glass of water to minimise the risk of gastrointestinal irritations. Doses of 400 mg or 600 mg should be administered once daily. For patients unable to swallow the film-coated tablets, the tablets may be dispersed in a glass of mineral water or apple juice. The required number of tablets should be placed in the appropriate volume of beverage (approximately 50 ml for a 100 mg tablet, and 200 ml for a 400 mg tablet) and stirred with a spoon. The suspension should be administered immediately after complete disintegration of the tablet(s).

Methotrexate

Formulation	Methotrexate 2.5mg/ml for intrathecal use
Storage	See relevant SPC
Administration	12.5mg to be given intrathecally,ensure national guidance on the safe administration of intrathecal chemotherapy is followed (Department of Health).

Vincristine

Formulation	A sterile, colourless solution for injection. Each 1 ml contains 1.0 mg of vincristine sulphate
Storage	Store at 2 - 8°C. Keep container in the outer carton.
Administration	Ensure NPSA guidance for using Vinca Alkaloid minibags is followed (reference NPSA/2008/RRR 004. When vinca alkaloids are prescribed, dispensed or administered in adult and adolescent units: • Doses in syringes should no longer be used. • The prescribed dose should be supplied from the hospital pharmacy ready to administer in a 50ml minibag of sodium chloride 0.9% (for some brands of vinorelbine glucose 5% solution for injection may be used instead of sodium chloride 0.9%). • The following warning should be prominently displayed on the label of ALL vinca alkaloid doses 'For Intravenous Use Only – Fatal If Administered by Other Routes'. • The vinca minibag should be infused intravenously over 5 - 10 minutes and the patient closely monitored for signs of extravasation. Incidents of extravasation should be reported and shared via the National Extravasation Information Service (www.extravasation.org.uk).

Mercaptopurine

Formulation	50mg tablets containing 50mg of Mercaptopurine
Storage	Store below 25ºC. Keep the bottle tightly closed.
Administration	60 mg/m^2 orally taken once a day before breakfast.

Drugs used in Intensification

Imatinib

Formulation	Glivec® 100 mg film-coated tablets & Glivec® 400 mg film-coated tablets
Storage	Do not store above 30°C. Store in the original package in order to protect from moisture.
Administration	The prescribed dose should be administered orally with a meal and a large glass of water to minimise the risk of gastrointestinal irritations. Doses of 400 mg or 600 mg should be administered once daily. For patients unable to swallow the film-coated tablets, the tablets may be dispersed in a glass of mineral water or apple juice. The required number of tablets should be placed in the appropriate volume of beverage (approximately 50 ml for a 100 mg tablet, and 200 ml for a 400 mg tablet) and stirred with a spoon. The suspension should be administered immediately after complete disintegration of the tablet(s).

Methotrexate - HIGH DOSE

Formulation	1g or 5g vials
Storage	As per relevant SPC
Administration	3g/m^2 to be given by IV infusion (see **Appendix 15** for high dose Methotrexate guidelines)

Drugs used in Consolidation

Cyclophosphamide

Formulation	Powder for solution for injection. A white crystalline powder contained in clear glass injection vials.
Storage	Do not store above 25°C. Store in original container. After reconstitution (for either intravenous or oral administration), store at 2 - 8°C and protect from light.
Administration	A dose of 650mg/m^2 given at a concentration of 20mg/ml in syringe by IV bolus

Cytarabine

Formulation	Clear, colourless solution for injection.
Storage	Do not store above 25°C. Keep container in the outer carton.
Administration	A dose of 75mg/m^2 to be given at a concentration of 20mg/ml in syringe by slow IV bolus or by IV depending on local practice.

Daunorubicin

Formulation	Vial containing a red lyophilised powder for intravenous administration following reconstitution in water for injections and dilution with saline. Each vial contains 21.4 mg Daunorubicin hydrochloride (equivalent to 20 mg as base).
Storage	Daunorubicin vials should be stored below 25°C, protected from light. After reconstitution Daunorubicin should be stored at 2 - 8°C, protected from light.
Administration	25mg/m^2 dose to be diluted in sodium chloride 0.9% to give final concentration of 1mg/ml and inject over 20 mins into side arm of freely running intravenous infusion of sodium chloride 0.9%. Alternatively the Daunorubicin may be added to a 100ml minibag of sodium chloride 0.9% and this solution infused over 20 minutes into the side arm of a freely running infusion of sodium chloride 0.9%. Take care to avoid extravasation.

Dexamethasone

Formulation	Each tablet contains 2.0mg Dexamethasone PhEur.
Storage	Store below 25°C protected from light.
Administration	10mg/m^2 to be given orally in one dose after breakfast.

Etoposide: HIGH DOSE

Formulation	Concentrate for solution for infusion (to dilute).
Storage	Store below 25° C, protected from light (keep vials in the outer carton). Do not freeze. Diluted solutions: Do not store the diluted product in a refrigerator (2 – 8 °C) as this might cause precipitation. Solutions showing any sign of precipitation should not be used.
Administration	Concentrate for solution for infusion 20 mg/ml must be diluted prior to use with either 5 % dextrose in water, or 0.9 % sodium chloride solution to give a final concentration of 0.2 to 0.4 mg/ml. (or as recommended by manufacturer). Give over at least 30 minutes.

Imatinib

Formulation	Glivec® 100 mg film-coated tablets & Glivec® 400 mg film-coated tablets
Storage	Do not store above 30°C. Store in the original package in order to protect from moisture.
Administration	The prescribed dose should be administered orally with a meal and a large glass of water to minimise the risk of gastrointestinal irritations. Doses of 400 mg or 600 mg should be administered once daily. For patients unable to swallow the film-coated tablets, the tablets may be dispersed in a glass of mineral water or apple juice. The required number of tablets should be placed in the appropriate volume of beverage (approximately 50 ml for a 100 mg tablet, and 200 ml for a 400 mg tablet) and stirred with a spoon. The suspension should be administered immediately after complete disintegration of the tablet(s).

Methotrexate

Formulation	Methotrexate 2.5mg/ml for intrathecal use
Storage	See relevant SmPc
Administration	12.5mg to be given intrathecally,ensure national guidance on the safe administration of intrathecal chemotherapy is followed (Department of Health).

Vincristine

Formulation	A sterile, colourless solution for injection. Each 1 ml contains 1.0 mg of vincristine sulphate
Storage	Store at 2 - 8°C. Keep container in the outer carton.
Administration	Ensure NPSA guidance for using Vinca Alkaloid minibags is followed (reference NPSA/2008/RRR 004. When vinca alkaloids are prescribed, dispensed or administered in adult and adolescent units: • Doses in syringes should no longer be used. • The prescribed dose should be supplied from the hospital pharmacy ready to administer in a 50ml minibag of sodium chloride 0.9% (for some brands of vinorelbine glucose 5% solution for injection may be used instead of sodium chloride 0.9%). • The following warning should be prominently displayed on the label of ALL vinca alkaloid doses 'For Intravenous Use Only – Fatal If Administered by Other Routes'. • The vinca minibag should be infused intravenously over 5 - 10 minutes and the patient closely monitored for signs of extravasation. Incidents of extravasation should be reported and shared via the National Extravasation Information Service (www.extravasation.org.uk).

Mercaptopurine

Formulation	50mg tablets containing 50mg of -Mercaptopurine
Storage	Store below 25ºC. Keep the bottle tightly closed.
Administration	60 mg/m^2 orally taken once a day on an empty stomach (usually in the evening), ensure patient avoids milk products for 2 hours either side of dose.

Drugs used in Maintenance

Methotrexate oral

Formulation	2.5mg tablets
Storage	As per relevant SPC
Administration	20mg/m^2 to be given orally once a week

Methotrexate IV

Formulation	Injection
Storage	As per relevant SPC
Administration	20mg/m^2 to be given intravenously once a week (as IV bolus or infusion)

Prednisolone

Formulation	Enteric coated Tablet
Storage	As per relevant SPC
Administration	60mg/m2 by mouth in the morning for 5 days every three months

Vincristine

Formulation	A sterile, colourless solution for injection. Each 1 ml contains 1.0 mg of vincristine sulphate
Storage	Store at 2 - 8°C. Keep container in the outer carton.
Administration	Vincristine Sulphate Injection may be injected into the tubing or side arm of a free-flowing intravenous infusion or directly into a vein over a one-minute period. For safety reasons when administering Vincristine Sulphate Injection into a side arm of a fast running infusion, please ensure that pressure is maintained on the syringe plunger during administration, to avoid back pressure from the infusion forcing the plunger out of the syringe barrel. Care should be taken to avoid extravasation as this may cause local ulceration.

Mercaptopurine

Formulation	50mg tablets containing 50mg of Mercaptopurine
Storage	Store below 25ºC. Keep the bottle tightly closed.
Administration	75 mg/m^2 orally taken once a day before breakfast.

Appendix 3 continued on next page

Drugs used in transplant conditioning regimens

Alemtuzumab

Formulation	MabCampath 30 mg/ml concentrate for solution for infusion
Storage	Store in a refrigerator (2°C-8°C). Do not freeze. Store in the original package in order to protect from light. Alemtuzumab should be used within 8 hours after dilution. Solutions may be stored at 15°C-30°C or refrigerated. This can only be accepted if preparation of the solution takes place under strictly aseptic conditions and the solution is protected from light
Administration	The required amount of the vial contents should be added to 100 ml of sodium chloride 9 mg/ml (0.9%) solution for infusion or glucose (5%) solution for infusion. The bag should be inverted gently to mix the solution. Care should be taken to ensure the sterility of the prepared solution particularly as it contains no antimicrobial preservatives. All doses should be administered by intravenous infusion over approximately 2 hours. Patients should be premedicated with oral or intravenous steroids, an appropriate antihistamine and analgesic 30-60 minutes prior to each alemtuzumab infusion

Cyclophosphamide: HIGH DOSE

Formulation	Powder for solution for injection. A white crystalline powder contained in clear glass injection vials.
Storage	Do not store above 25°C. Store in original container. After reconstitution (for either intravenous or oral administration), store at 2 - 8°C and protect from light.
Administration	A dose of 60mg/kg to be given, as 20mg/ml cyclophosphamide in sodium chloride 0.9% in empty ethyl vinyl acetate (EVA) bag over 2 hours

Etoposide: HIGH DOSE

Formulation	Concentrate for solution for infusion (100mg in 5ml vials)
Storage	Room temperature
Administration	60mg/kg To be given undiluted via central venous catheter over approx 4 hours. Use polyethylene or polyethylene lined line – new line for each syringe, primed with saline. Do not flush line, disconnect at source and use new line for each syringe. An extra 2ml Etoposide is supplied in each syringe to account for Etoposide lost in line. Ensure anti-emetics are prescribed. Ensure patient remains well hydrated. Patient must have a baseline ECG before starting the infusion and be coupled to a cardiac monitor throughout. Regular recordings of pulse and blood pressure should be made throughout the infusion, which can be slowed if necessary. Maintenance of blood pressure using fluid support or colloids may be necessary

Fludarabine

Formulation	Fludarara 50mg powder for solution for injection or infusion
Storage	See relevant SPC
Administration	Fludara should be prepared for parenteral use by aseptically adding sterile water for injection. When reconstituted with 2 ml of sterile water for injection, the powder should fully dissolve in 15 seconds or less. Each ml of the resulting solution will contain 25 mg of fludarabine phosphate, 25 mg of mannitol, and sodium hydroxide (to adjust the pH to 7.7). The pH range for the final product is 7.2 - 8.2. Dilution The required dose (calculated on the basis of the patient's body surface) is drawn up into a syringe. For intravenous bolus injection this dose is further diluted in 10 ml sodium chloride 9mg/ml (0.9%). Alternatively, for infusion, the required dose may be diluted in 100 ml sodium chloride 9mg/ml (0.9%) and infused over approximately 30 minutes.

Melphalan

Formulation	Freeze-dried powder for injection.
Storage	Store below 30° C, Protect from light & Do not refrigerate.
Administration	For intravenous administration, Melphalan Injection solution may be administered diluted in an infusion bag. Melphalan is not compatible with infusion solutions containing dextrose and it is recommended that only sodium chloride intravenous infusion 0.9% w/v is used. When further diluted in an infusion solution, Melphalan has reduced stability and the rate of degradation increases rapidly with rise in temperature. If Melphalan is infused at a room temperature of approximately 25°C, the total time from preparation of the injection solution to the completion of infusion should not exceed 1.5 hours. Should any visible turbidity or crystallisation appear in the reconstituted or diluted solutions, the preparation must be discarded. Hydration and forced diuresis is recommended but not mandatory- please follow your local practice.

Please check relevant Summary Product Characteristics for brand of drug used for storage , special precautions and contraindications, interactions, undesirable effects and stability information.

Appendix 4: Dose modifications for toxicity

Steroids

Hypertension: Steroid should not be reduced. Sodium restriction and anti-hypertensives should be employed in an effort to control hypertension.

Malignant Hypertension: Reduce dose 33%. Sodium restriction and anti-hypertensive drugs may also be utilised.

Hyperglycemia: Steroids should not be reduced if the patient develops clinical signs of diabetes. Rather, insulin therapy should be employed to control the blood glucose level such that symptoms and signs are minimal.

Pancreatitis: Do not modify dose.

Psychosis: Administer half dosage of steroid.

Suspected steroid-induced myopathy: Measure CPK with isoenzymes, consider EMG studies.

Avascular necrosis: Contact CI or clinical coordinators if AVN develops before Maintenance therapy has begun. Omit further steroids if AVN develops during maintenance.

Varicella Zoster: Steroids should be held during active infection except during Induction (Discuss with co-ordinators). They should not be held during incubation period following exposure to Varicella,.

Severe Dexamethasone intolerance – change to Prednisolone 40 mg/m^2.

Vincristine

Seizures: Hold 1 dose, then reinstitute.

Severe foot drop, paresis or ileus: Hold dose(s); institute aggressive regimen to treat constipation (except enemas if neutropenic), if present. When symptoms abate, resume at 1.0 mg/m^2; escalate to full dose as tolerated.

Jaw pain: Treat with analgesics; do not modify Vincristine dose.

Hyperbilirubinemia: Withhold if total bilirubin > 50. Administer 50% of dose if total bilirubin 25 – 50. Do not alter dose for abnormal transaminases.

Asparaginase

Anaphylaxis or anaphylactoid reactions: PEG-asparaginase should be discontinued if the patient develops a Grade 2 – 4 toxicity. Send blood samples to the Adult ALL MRD laboratory for asparaginase antibodies and change to Erwinase (appendix 14).

Symptomatic pancreatitis: Discontinue L-asparaginase in the presence of symptomatic pancreatitis documented by an elevated serum amylase or lipase level or ultrasonographic abnormalities. Do not give further doses if there is a prior history of asparaginase induced pancreatitis.

Hyperglycaemia: Do not modify dose. Administer Insulin as required.

Ketoacidosis: Hold L-Asparaginase until blood glucose can be regulated with insulin.

Coagulopathy: When significant symptomatic coagulopathy occurs, withhold L-asparaginase until resolved. Routine clotting screens are not recommended. Coagulopathy without bleeding is not an indication to withhold L-asparaginase.

Liver Dysfunction: Check LFTs only if patient jaundiced. Withhold if total bilirubin > 50.
Do not alter dose for abnormal transaminases.

Anthracyclines (Doxorubicin and Daunorubicin)

Hyperbilirubinemia: If total bilirubin > 120 omit dose; if > 90 but < 120 give 25% of dose. If > 50 but < 90 give 50% of dose, and if < 50 give full dose. Check LFTs only if patient jaundiced. Do not alter dose for abnormal transaminases.

Intrathecal Methotrexate

Any significant neurotoxicity not due to lumbar puncture syndrome (low opening pressure, slow CSF flow, orthostatic symptoms) should be reported.
Systemic toxicity: The dosage for Intrathecal Methotrexate will not be reduced for systemic toxicity (myelosuppression, mucositis, etc).
Viral, bacterial or fungal meningitis: Omit until resolved.
Encephalopathy attributed to intrathecal Methotrexate: see appendix.

Oral Methotrexate

Mucositis: For grade 2 mucositis of over 3 days duration, decrease MTX dose by 30%. For grade 3-4, mucositis, withhold MTX until resolved; resume at 50% of the previously attained dose and subsequently escalate to 75% to 100% dose at 10 day intervals provided grade 3-4 toxicity does not recur. Consider culturing lesions for herpes simplex if mucositis persists or recurs.
Liver: Check LFT's only if patient jaundiced. If bilirubin is >50 micromoles/L omit MTX until it is less than 20 micromoles/L, and then restart at half of the previously attained dose. Escalate from 50% to 75% to 100% dose at 10-day intervals provided hyperbilirubinaemia does not recur. Do not modify dosage for elevated aminotransferases.
Kidney (Grade 3-4): Omit MTX until grade 0 toxicity (ie completely resolved). Resume at 100% of the previously attained dose and continue at 10-day intervals.

Intravenous Methotrexate
PLEASE SEE APPENDIX 15

Cyclophosphamide

Prior history of gross haematuria or microscopic haematuria: Hydrate at 125 ml/m^2/hr for 24 hours after dose and use Mesna 360 mg/m^2 pre, and 4, 7, 11 hours post dose.
Acute Fluid retention: Treat with Frusemide and saline; do not modify dose.

Cytarabine

Hyperbilirubinaemia: if total bilirubin > 120 omit dose; if > 90 but < 120 give 25% of dose. If > 50 but < 90 give 50% of dose, and if < 50 give full dose. Check LFT's only if patient jaundiced. Do not alter dose for abnormal transaminases.

Mercaptopurine

Hyperbilirubinaemia: If bilirubin >50micromol/l omit mercaptopurine until it is less than 20micromol/l and then restart at helf the previously attained dose. Escalate from 50% to 75% to 100% dose at 10-day intervals provided hyperbilirubinaemia does not recur. Do not modify dosage for elevated aminotranseferases.

Nelarabine

Nelarabine must be discontinued at the first sign of neurological events of National Cancer Institute Common Terminology Criteria Adverse Event (NCI CTCAE) grade 2 or greater. Delaying subsequent dosing is an option for other toxicities, including haematological toxicity.

Renal Impairment

Nelarabine has not been studied in individuals with renal impairment. Nelarabine and 9-β-D-arabinofuranosylguanine (ara-G) are partially renally excreted (see section 5.2 — Renal impairment). There are insufficient data to support a dose adjustment recommendation for patients with a renal clearance of creatinin CrCl less than 50 ml/min. Patients with renal impairment must be closely monitored for toxicities when treated with nelarabine.

Hepatic Impairment

Nelarabine has not been studied in patients with hepatic impairment. These patients should be treated with caution.

Patients receiving nelarabine are recommended to receive intravenous hydration according to standard medical practice for the management of hyperuricemia in patients at risk of tumour lysis syndrome. For patients at risk of hyperuricemia, the use of allopurinol should be considered.

Appendix 5: Assessment of GVHD

Graft Versus Host Disease (GVHD) arises due to reactivity of cytotoxic T lymphocytes against recipient cells, through both HLA- and minor histo-incompatibility between the donor and the recipient. After the transplant the reaction usually requires 10 or more days for priming and proliferation of T cells to occur.

GVHD is divided into two forms, each of which tend to produce distinct clinical syndromes:

- Acute GVHD: <100 days post transplant (grades I to IV)
- Chronic GVHD: >100 days post transplant (limited or extensive)

Organ involvement for GVHD should be staged using the criteria outlined in the tables below. Biopsy of each organ site at diagnosis or major change in disease activity will be performed unless clinical circumstances make it impossible.

Acute GVHD

Acute graft versus host disease predominantly affects three organs, either singly or together:

- Skin - maculopapular rash, erythema, desquamation
- Gastrointestinal tract - nausea, vomiting, diarrhoea
- Liver - raised alkaline phosphatase, bilirubin, later hepatitis

Glucksberg criteria for assessment of Acute GVHD: **Table A**

Stage	Skin	Liver	Gut
1	rash <25% body	bilirubin 35 - 50 uM/l	Diarrhoea <1 l/day
2	rash 25-50% body	bilirubin 51-100 uM/l	Diarrhoea 1-1.5 l/day
3	rash >50% body	bilirubin 101-250 uM/l	Diarrhoea >1.5 l/day
4	desquamation or bullae	bilirubin >250 uM/l	Pain or ileus

Glucksberg criteria for assessment of Acute GVHD: **Table B**

Grade	Skin stage	Liver stage	Gut stage
I	1-2	0	0
II	1-3	1	1
III	2-3	2-3	2-3
IV	2-4	2-4	2-4

Chronic GVHD

Classification scheme for chronic GVHD (Shulman *et al*):

Limited Either or both: 1. Localised skin involvement 2. Hepatic dysfunction due to chronic GVHD
Extensive Either: 1. Generalised skin involvement; or 2. Localised skin involvement and/or hepatic dysfunction due to chronic GVHD, plus: a. Liver histology showing chronic aggressive hepatitis, bridging necrosis or cirrhosis; or b. Involvement of eye: Schirmer's test with less than 5mm wetting; or c. Involvement of minor salivary glands or oral mucosa demonstrated on labial biopsy; or d. Involvement of any other target organ

Appendix 6: Cytogenetic Definitions and Detection Strategy

Definition and detection of high risk abnormalities

It is essential that conventional G-banded cytogenetics analysis is performed on all trial patients. In addition, FISH or RT-PCR to detect high risk cytogenetic abnormalities will be necessary in all patients with a normal karyotype or failed cytogenetics.

The four cytogenetic subgroups which will be treated as high risk in UKALL14 are:

- Philadelphia chromosome / t(9;22)(q34;q11)/*BCR-ABL1*
- t(4;11)(q21;q23) / *MLL-AFF1*
    - NB *AFF1* was previously known as *AF4* and *MLLT2*
- low hypodiploidy / near-triploidy (Ho-Tr)
- complex karyotype (CK).

Philadelphia chromosome / t(9;22)(q34;q11)/*BCR-ABL1*

Patients with Philadelphia positive ALL will receive imatinib in induction in UKALL14 and therefore all patients must be identified as quickly as possible. All patients must be screened by FISH using a dual-colour dual-fusion translocation probe or RT-PCR using standard primers.

t(4;11)(q21;q23) / *MLL-AF4*:

This translocation is readily detectable by conventional cytogenetics but patients with a normal karyotype or failed cytogenetics must be screened using either RT-PCR using standard primers or the following FISH strategy:

- FISH with a *MLL* dual colour break apart probe.
- Patients with a *MLL* split signal pattern should be screened using an appropriate *AF4* dual colour break apart probe, unless a t(4;11) is visible cytogenetically. Currently, no *AF4* probe is commercially available. Thus we advise the use of a home-grown dual-colour probe comprising the tiling path clones RP11-397E7 and RP11-476C8 which cover the 5' and 3' portions of the gene respectively. For further advice please contact the LRCG (see below). If necessary the LRCG will undertake this specific FISH test centrally.

Low hypodiploidy (30-39 chromosomes) / near-triploidy (60-78 chromosomes) (Ho-Tr):

This cytogenetic subgroup comprises two related entities a low hypodiploid clone and its "doubled" near-triploid counterpart [Charrin et al (2004) Blood 104:2444]. Although both entities are usually detectable by conventional cytogenetics occasionally just one is visible. In cases where only the near-triploidy clone is visible there may be some confusion with high hyperdiploidy if the karyotype has 60-65 chromosomes. Distinction between the two subgroups can be made on the basis of chromosomes gained and the proportion of tetrasomic chromosomes. In particular, near-triploidy karyotypes in this subgroup are usually

tetrasomic for chromosomes 1, 6, 11 and 18, and are almost always disomic for chromosomes 3, 7 and 15. If there is any doubt in classifying patients please contact the LRCG (see contact details below).

Patients with a normal karyotype or failed cytogenetics should be screened for Ho-Tr using one of the following techniques:

- Flow cytometry to determine the DNA content of the blast cells – cases with Ho-Tr typically display two aneuploid peaks – low hypodiploid (0.7-0.9) and near-triploid (1.4-1.6).
- FISH using the Chromoprobe Multiprobe-I System (CytoCell, www.cytocell.com) or equivalent, which allows the simultaneous enumeration of all chromosomes on a single slide.
- FISH using selected centromeric probes. Defining precise chromosomes to test is difficult as the key feature of this subgroup is the modal chromosome number of the karyotype rather than the gain or loss of any individual chromosome or sets of chromosomes. However, characteristic chromosomes include 1, 6, 11 and 18 which are usually disomic and tetrasomic and 3, 7 and 15 which are usually monosomic and disomic in the low hypodiploid and near-triploid clones respectively.

Complex karyotype (CK):

The definition of a complex karyotype is five or more chromosomal abnormalities in the absence of an established translocation (e.g. t(1;19)(q23;p13), t(11;19)(q23;p13), t(12;21)(p13;q22)/*ETV6-RUNX1* etc.) or ploidy subgroup (e.g. low hypodiploidy / near –triploidy, high hyperdiploidy, tetraploidy). Detection is by conventional cytogenetics only. If there is any doubt in classifying patients please contact the LRCG (see contact details below).

Contact details for Leukaemia Research Cytogenetics Group (LRCG)

Dr Anthony V Moorman, 0191 282 1323, anthony.moorman@ncl.ac.uk
Professor Christine J Harrison, 0191 282 1320, christine.harrison@ncl.ac.uk
Ms Claire Schwab, 0191 282 1324, claire.schwab@ncl.ac.uk

Northern Institute for Cancer Research, Level 5, Sir James Spence Institute,
Royal Victoria Infirmary, Queen Victoria Road, Newcastle upon Tyne, NE1 4LP
tel: 0191 282 1324 | fax: 0191 282 1326

Appendix 7: Adult ALL MRD Laboratory and trial schedule

The laboratory will quantify MRD by immunoglobulin/T cell receptor gener re-arrangements for Ph- ALL and by BCR-ABl quantification for Ph+ ALL.

Request forms

These will be provided by our laboratory. They have been designed to provide sufficient information for each patient to be reliably identified and for MRD results to be correctly interpreted for reporting. it is important that you let us know the blast percentage in the marrow as we need this to interpret the result. It is important to let us know as much immunphenotyping information as you have available, since it assists us to set up the correct MRD marker panel.

MRD sample Collection and Transport

At diagnosis

2 - 5mls of bone marrow from all adults with suspected leukaemia should be placed into EDTA. In patients with peripheral white counts greater than 30 x109 /litre, 10 -20 mls of blood collected into EDTA is also acceptable at diagnosis. Please note that peripheral blood is of no value as an MRD sample at other time points.

Follow Up Samples

5mls of bone marrow should be placed into EDTA at the following timepoints. (Please note peripheral blood is NOT an acceptable alternative for follow-up specimens.)

Samples required: Absolute (taken as standard practice)
Bone Marrow sample following Phase 1 therapy (this is for the secondary end-point of the antibody randomisations and will not be reported)
Bone Marrow sample following Phase 2 induction therapy (this is for the risk stratification and will be reported within 10 working days of specimen receipt)

If the MRD result is not available (failed or specimen not sent) patient should be considered standard risk in absence of any other high risk features.

Bone Marrow sample following bone marrow transplant and then at 3 monthly intervals until 2 years post bone marrow transplant or until relapse.

At relapse: We hope that relapse will be infrequent, but if it does occur, we would be pleased to receive a specimen of bone marrow (3-5ml, in EDTA) so that we can identify whether or not the relapse has originated, or not, from the Ig-TCR confirmed clone which we have been using as an MRD marker.

Transport

All samples should be sent by courier or by 1st class post to arrive the same day or overnight to the following address:

Minimal Residual Disease Laboratory
(URGENT: FAO Lena Rai, Aditi Dey, Bella Patel or Adele Fielding UKALL14 STUDY SAMPLE)
Department of Haematology
Royal Free Campus
UCL School of Medicine
Rowland Hill St
London NW3 2PF

The package should be clearly marked on the outside as "URGENT, FEASIBILITY STUDY SAMPLE"

Sample processing

On receipt of bone marrow aspirates, the MRD laboratory will assign the patient and the sample a unique number according to the standard operating procedure. Cell counts will be recorded and DNA extracted within 48 hours of receipt of the sample. A minimum of 10 micrograms of DNA is required at diagnosis and 5 micrograms for follow- up samples.

UK MRD Network of Laboratories

MRD monitoring for this study will be undertaken at the Royal Free Hospital headed by Dr Adele Fielding. This laboratory has recently joined the UK network of MRD laboratories. This network was conceived for the purpose of MRD monitoring for the current UK childhood trial (UKALL 2003) as such it has demonstrated a robust framework for providing quality assured results.

The UK MRD network acts as a virtual single laboratory using a standard operating procedure and centrally distributed reagents to measure MRD by the Real time quantitative (RQ PCR) Allele Specific Oligonucleotide (ASO) PCR method. This technique is considered to be the most widely applicable and sensitive approach to MRD detection in ALL. The network is co-ordinated by clinical and scientific leads based at Bristol Children's Hospital (Dr J Hancock to co-ordinate) and headed by a Steering committee chaired by Professor Nicholas Cross. It participates in national and European External Quality Assurance schemes under Professor J Van Dongen's direction.

Funding

The Royal Free MRD Laboratory will be required to charge participating centres for MRD testing as it has been deemed by CR-UK as an 'excess NHS treatment cost'.

Appendix 8: Donor Peripheral Blood Stem Cell Collection & Return

The donor will be given G-CSF 10 micrograms/kg/day s.c. from day -4 to day 0. Mononuclear cells will be collected by leucapheresis on days 0 and +1. If sufficient cells are harvested on day 0 no further collection is required. The leucapheresis product will be assessed for nucleated cell count, CD34 content and NK and T cell subset content.
NB: It is mandatory to assess the leucapheresis product for nucleated cell cout and CD34. Evaluation of other cell subset content (NK cells, T cells) is desirable but it is at the centre's discretion.

A minimum dose of 2 $x10^6$ CD34+ cells/kg will be returned to the patient on Day 0 of the transplant. The target dose for returned cells is > 4 $x10^6$ CD34+ cells/kg.
G-CSF 5 micrograms/kg s.c. (or 300 micrograms Filgrastim) will be given to the patient from day +6 until neutrophils > 1 x 10^9/l on 2 consecutive days.

Chugai Pharma UK will provide a 25% retrospective reimbursement in stock for rHuG-CSF (Lenograstim) used within the study period.

Please contact **medicalaffairs@chugai-pharm.co.uk** with regards to making a claim.

Granocyte is available at NHS contract prices through AAH Hospital Service Telephone 0845 607 6699.

Please refer to the SpC for Lenograstim: www.medicines.org.uk

Appendix 9: Haematopoietic Cell Transplantation-Specific Comorbidity Index

Comorbidity index = sum of scores defined in table below

Comorbidities	Definitions	Score
Arrhythmia	Atrial fibrillation or flutter, sick sinus syndrome, or ventricular arrhythmias	1
Cardiac	Coronary artery disease*, congestive heart failure, myocardial infarction, or EF≤50%	1
Inflammatory bowel disease	Crohn's disease or ulcerative colitis	1
Diabetes	Requiring treatment with insulin or oral hypoglycaemic, but not controlled with diet alone	1
Cerebrovascular disease	Transient ischaemic attacks or cerebrovascular accident	1
Psychiatric disturbance	Depression/anxiety requiring psychiatric consult and/or treatment at the time of transplant	1
Hepatic (mild)	Chronic hepatitis, bilirubin>ULN to 1.5xULN, or AST/ALT>ULN to 2.5xULN	
Obesity	BMI>35 for adults or with BMI-for-age percentile of ≥95th percentile for children	1
Infection	Documented infection or fever of unknown aetiology requiring anti-microbial treatment before, during and after the start of conditioning regimen	1
Rheumatological	SLE, RA, polymyositis, mixed CTD and polymyalgia rheumatica	2
Peptic ulcer	Requiring treatment	2
Renal (moderate/severe)	Serum creatinine>2mg/dL†, on dialysis or prior to renal transplantation	2
Pulmonary (moderate)	DLCO and/or FEV_1 66-80% or dyspnoea on slight activity	2
Prior solid tumour	Treated at any point in the patients history, excluding non-melanoma skin cancer	3
Heart valve disease	Except asymptomatic mitral valve prolapse	3
Pulmonary (severe)	DLCO and/or FEV_1 ≤65% or dyspnoea at rest or requiring oxygen	3
Hepatic (moderate/severe)	Liver cirrhosis, bilirubin>1.5xULN, or AST/ALT> 2.5xULN	3

EF – ejection fraction; ULN – upper limit of normal; AST – aspartate aminotransferase; ALT – alanine aminotransferase; BMI – body mass index; SLE – systemic lupus erythematosus; RA – rheumatoid arthritis; CTD – connective tissue disease; DLCO – diffusion capacity of carbon monoxide; FEV_1 – forced expiratory volume in 1 second.

* one or more vessel coronary artery stenoses requiring medical treatment, stent or bypass graft.

† to convert creatinine from mg/dL to µmol/L, multiply by 88.4

Appendix 10: ECOG performance status

Score	Definition
0	Asymptomatic and fully active
1	Symptomatic; fully ambulatory; restricted physically strenuous activity
2	Symptomatic; ambulatory; capable of self-care; more than 50 percent of waking hours are spent out of bed
3	Symptomatic; capable of limited self care; spends more than 50 percent of time in bed but not bedridden.
4	Completely disabled; no self-care; bedridden

Appendix 11: Schedule of Assessments (including testing for MRD & Correlative Science)

Table 12.1a: Treatment & Follow up (for Non Transplant patients)

Timing / **Assessment**	Registration	During Phase 1 treatment (d3 or d4 and d18)	Upon recovery from Phase 1 induction	Upon recovery from Phase 2 induction	During intensification (d2 and d16)	After intensification	After Cycle 1 consolidation	After Cycle 2 consolidation	Before Cycle 3 consolidation d29	After Cycle 3 consolidation	After cycle 4 consolidation	During maintenance therapy (every 3months)	Annual Follow up (until patient death)	2 year follow up appointment	At Relapse
Informed consent	X														
Full medical history & physical examination	X												X	X	
Height, Weight, & BSA	X														
ECOG Status	X														
FBC	X		X	X		X	X	X	X	X	X	X	X	X	
Biochemistry	X														
Bone marrow aspirate & trephine (**SENT TO MRD LAB @ ROYAL FREE)**	X[1]		X[2]	X[2]											X[1]
Pregnancy test	X														
Confirmation of disease diagnosis	X														
Cytogenetics	X[3]														
Lumbar puncture	X4														
Peripheral blood sample- for asparaginase activity (**SENT TO MRD LAB @ ROYAL FREE**)		X[5]			X[5]										
Assessment of disease status													X	X	
Echocardiogram														X	
General Health Questionnaire (GHQ-12)														X	
Late Effects of Treatment Assessment														X	
Record of AVN or serious cardiac problems													X	X	

1= Bone marrow 3-5ml in EDTA (OR peripheral blood 30-50ml in EDTA if WCC > 30x 10^9/l). (**Please see protocol section 8.2.1 & Appendix 7)**
2= Bone marrow from biopsy: 3-5ml in EDTA for MRD assessment. (**Please see protocol section 8.2.1 & Appendix 7**)
3= Cytogenetics/molecular assessment of BCR-ABL and MLL on bone marrow. A copy of the results should be sent to the Leukemia Research Group. (**Please see Appendix 6**)
4= Lumbar puncture is not required at diagnosis except in the case of suspected central nervous system involvement. (**Please see protocol section 5.1**)
5= 5ml peripheral blood in a serum tube (for Asparaginase activity assay and anti-Asparaginase antibodies). (**Please see protocol section 8.2.1 & Appendix 7**)

Table 12.1b Transplant & Follow up (for transplant patients)

Transplant patients should follow the schedule of assessments on the previous page for Phase I & II treatment, and for intensification and at relapse, if applicable.

Timing **Assessment**	Pre-Transplant	100 days Assessment	3 monthly assessments (for 2 years following transplant)	Annual Follow up (until patient death)	2 year follow up appointment
Full medical history & physical examination	X				
ECOG Status & Karnofsky Performance Status	X	X	X	X	X
Co morbidity Index	X				
Full blood count with differential	X			X	X
Biochemistry (including LDH) & assessment of liver and renal function	X				
Pregnancy test, Microbial Titres, Urinalysis & clotting screen (including PT/APTT)	X				
Bone marrow aspirate & trephine (**SENT TO MRD LAB @ ROYAL FREE)**	X		X[1]		
Assessment of cardiac & pulmonary function	X				
Peripheral blood sample- mini satellite regions (**SENT TO MRD LAB @ ROYAL FREE)**	X[2]				
Chimerism studies (**SENT TO MRD LAB @ ROYAL FREE)**	X	X[3]	X[3]		
GVHD Assessment		X	X		
Donor Lymphocyte Infusion		X	X		
Clinical examination (as necessary)				X	X
Assessment of disease status		X	X	X	X
Oral Mucositis Daily Questionnaire (OMDQ)		X[4]			
Employment status			X	X	X
Record of AVN or serious cardiac problems				X	
Echocardiogram					X
General Health Questionnaire (GHQ-12)					X
Late Effects of Treatment Assessment					X

1= 3-5ml Bone Marrow in EDTA for MRD assessment. FOR NON MYELOABLATIVE TRANSPLANT PATIENTS ONLY.
2= 20ml blood in EDTA from donor and recipient for assessment of mini-satellite regions
3= 20ml peripheral blood in EDTA for chimerism. FOR NON MYELOABLATIVE TRANSPLANT PATIENTS ONLY.
4= For patients participating in the Palifermin randomisation, ODMQ to be completed daily by all patients during in-patient therapy

Appendix 12: General Health Questionnaire (GHQ-12)

General Health Questionnaire

Name.....

We want to know how your health has been in general over the last few weeks. Please read the questions below and each of the four possible answers. Circle the response that best applies to you. Thank you for answering all the questions.

Have you recently:

1. been able to concentrate on what you're doing?

better than usual (0) | same as usual (1) | less than usual (2) | much less than usual (3)

2. lost much sleep over worry?

Not at all | no more than usual | rather more than usual | much more than usual

3. felt that you are playing a useful part in things?

more so than usual | same as usual | less so than usual | much less than usual

4. felt capable of making decisions about things?

more so than usual | same as usual | less than usual | much less than usual

5. felt constantly under strain?

Not at all | no more than usual | rather more than usual | much more than usual

6. felt you couldn't overcome your difficulties?

Not at all | no more than usual | rather more than usual | much more than usual

7. been able to enjoy your normal day to day activities?

more so than usual same as usual less so than usual much less than usual

8. been able to face up to your problems?

more so than usual same as usual less than usual much less than usual

9. been feeling unhappy or depressed?

not at all no more than usual rather more than usual much more than usual

10. been losing confidence in yourself?

not at all no more than usual rather more than usual much more than usual

11. been thinking of yourself as a worthless person?

not at all no more than usual rather more than usual much more than usual

12. been feeling reasonably happy, all things considered?

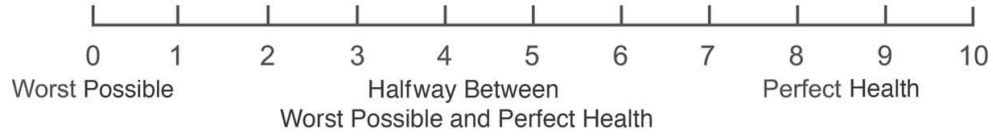
more so than usual same as usual less so than usual much less than usual

General Health Questionnaire Scoring

Scoring – Likert Scale 0, 1, 2, 3 from left to right.
12 items, 0 to 3 each item
Score range 0 to 36.
Scores vary by study population. Scores about 11-12 typical.
Score >15 evidence of distress
Score >20 suggests severe problems and psychological distress

Appendix 13: Oral Mucositis Daily Questionnaire

1. How would you rate your OVERALL HEALTH during the LAST 24 HOURS? (*circle one number*)



2. During the LAST 24 HOURS, how much MOUTH AND THROAT SORENESS did you have? (*circle one number*)

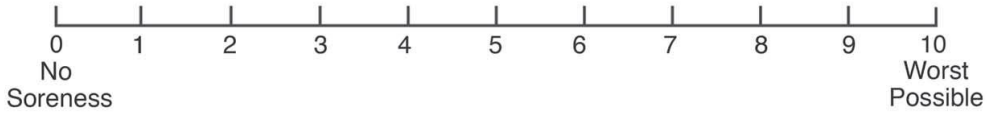
No soreness ---------- 0
A little soreness ---------- 1
Moderate soreness ---------- 2
Quite a lot of soreness ---------- 3
Extreme soreness ---------- 4

If you circled 0, please skip to question 5

3. During the LAST 24 HOURS, how much did MOUTH AND THROAT SORENESS limit you in each of the following activities? (*circle one number*)

	Not Limited	Limited A Little	Limited Some	Limited A Lot	Unable To Do
a. Swallowing	0	1	2	3	4
b. Drinking	0	1	2	3	4
c. Eating	0	1	2	3	4
d. Talking	0	1	2	3	4
e. Sleeping	0	1	2	3	4

4. On a scale of 1 to 10, how would you rate your OVERALL MOUTH AND THROAT SORENESS during the LAST 24 HOURS? (*circle one number*)

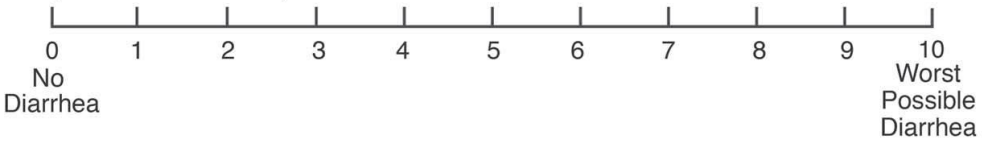


5. During the LAST 24 HOURS, how much DIARRHEA did you have? (*circle one number*)

No diarrhea ---------- 0
A little diarrhea ---------- 1
Moderate diarrhea ---------- 2
Quite a lot of diarrhea ---------- 3
Severe diarrhea ---------- 4

If you circled 0, STOP here

6. On a scale of 1 to 10, how would you rate your OVERALL DIARRHEA during the LAST 24 HOURS? (*circle one number*)



Appendix 14: Guidance on Use of Erwinia Asparaginase (Erwinase®) in patients with systemic reactions to Pegylated-Asparaginase.

1. A licensed preparation of Erwinia Asparaginase (Erwinase®) is now available, thus providing an effective alternative for patients with hypersensitivity to E.Coli Asparaginase.
2. Erwinase®.will be marketed and distributed by EUSA Pharma
3. Erwinase® should be used in place of Pegylated E. Coli Asparaginase in the following circumstances:
   - Systemic hypersensitivity reactions to n Pegylated E.Coli Asparaginase (Oncaspar). This includes patients with generalised rash with or without anaphylactic symptoms, but not those with only local pain or redness at the site of injection.
   - Patients with previously documented systemic reactions to Pegylated E.Coli Asparaginase should receive Erwinase® in any remaining Asparaginase containing courses.
4. Each dose of Pegylated Asparaginase (Oncaspar) should be replaced with 6 doses of 20,000 Units/m^2 Erwinase® given on Mondays, Wednesdays and Fridays. (*or this could be 6 doses q 48 hours if preferred)*
5. Erwinase® should be administered by intra-muscular injection. The individual dose may be split between two injection sites if injection volume more than 4ml.
6. Please notify the trials office of patients switching to Erwinase®.

Chemical name - *Erwinia* L-asparaginase

Other names - ERWINASE®, Crisantaspase (Asparaginase from *Erwinia chrysanthemi*; *Erwinia* L-asparaginase)

Formulation - 10,000 Units/vial, Lyophilisate for solution for injection, White lyophilised powder in a vial.

Special warnings and precautions for use

Warnings: Anaphylactic reactions have been observed after the use of Erwinase. Facilities should be made available for management of an anaphylactic reaction, should it occur, during administration.

Careful observation is required on re-exposure to L-asparaginase after any time interval, which may increase the risk of anaphylactic reactions occurring.

Careful monitoring before and during therapy is necessary:

• Serum amylase, lipase and/or insulin levels should be monitored to exclude hyperglycaemia and severe pancreatitis. Hyperglycaemia may be treated with insulin, if needed.

• Routine clotting screening may be performed before treatment initiation. If significant symptomatic coagulopathy occurs withhold L-asparaginase treatment until resolved then continue according to protocol.

• Hepatic function tests should be monitored regularly during therapy.

Storage *– Store at 2-8°C*

Administration – The contents of each vial should be reconstituted in 1 ml to 2 ml of sodium chloride (0.9%) solution for injection. Slowly add the reconstitution solution against the inner vial wall, do not squirt

directly onto or into the powder. Allow the contents to dissolve by gentle mixing or swirling maintaining the vial in an upright position. Avoid froth formation due to excessive or vigorous shaking.

The solution should be clear without any visible particles. Fine crystalline or thread-like wisps of protein aggregates may be visible if shaking is excessive. If there are any visible particles or protein aggregates present the reconstituted solution should be rejected.

The solution should be administered within 15 minutes of reconstitution. If a delay of more than 15 minutes between reconstitution and administration is unavoidable, the solution should be withdrawn into a glass or polypropylene syringe for the period of the delay. The solution should be used within 8 hours.

Give by intra-muscular injection, if the volume is over 4ml the individual dose may be split between two injection sites.

See also SPC at www.medicines.org.uk

Appendix 15: Guideline for the administration of Intravenous High-Dose Methotrexate

Regimen for administration of high-dose methotrexate

NOTE: The guidance for administration of high dose methotrexate is a detailed suggestion for trial participants to follow. It contains all the necessary information to give the drug safely. However, those centres who have a firm local policy in place which differs in administration detail (but not dose) from the suggestions put forward here can administer high dose methotrexate within UKALL14 according to their local policies.

One week before admission for the 1st methotrexate infusion the Creatine Clearance (measured according to local practice) should be determined. The initial Creatinine Clearance before starting methotrexate should ideally be > 100 mls/minute.

Patients with a clearance between 80-100 mls / minute before the first dose of High Dose Methotrexate MUST have a measured Creatinine clearance (24 urine collection) BEFORE the second dose and dose adjustments (as above) made if the result is less than 80 mls /minute.

Also repeat Creatinine Clearance before the 2nd infusion if there is delayed methotrexate excretion after the first course.

Based on a dose of 3g/m^2 of Methotrexate and renal function pre-treatment use Dose reductions as follows:

Pre Cycle 1

CrCl(ml/min)	Dose
> 80 mls/min	100%
50-80 mls/min	50 %
<50 mls/min	0 %

Pre Cycle 2

CrCl(ml/min)	Dose
> 50 mls/min	100%
<50 mls/min	0 %

Consult the TMG if in any doubt regarding the high dose methotrexate.

METICULOUS ATTENTION SHOULD BE PAID AT ALL TIMES TO CHANGES IN CREATININE CLEARANCE DURING THE HIGH DOSE METHOTREXATE PHASE. (BOTH WITHIN AND BETWEEN EACH COURSE OF METHOTREXATE).

On admission for each methotrexate infusion, measure:

- Serum creatinine
- Bilirubin and AST or ALT
- Plasma sodium and potassium
- FBC

Guidelines for dosing high dose methotrexate in Liver impairment

Bilirubin (micromol/L)	AST	Dose
<50	And < 180	100%
51-85	Or > 180	75%
> 85		Contraindicated

It is expected that patients receiving high dose methotrexate will develop hypertransaminasemia and occasionally hyperbilirubinemia.These elevations can last up to 2 weeks following the methotrexate infusion and are not considered toxicities requiring discontinuation of repeated administration of methotrexate. Persistant hyperbilirubinemia and/or grade 3-4 hypertransaminasemia for longer than 3 weeks should result in discontinuation of the drug. Dose reduce, particularly in patients with concomitantly impaired renal function. The drug is contraindicated in severe hepatic impairment.

Pre-hydration - For at least 6 hours prior to the commencement of the intravenous methotrexate.

Hydration fluid - 1 litre dextrose saline to which has been added 50 mmol sodium bicarbonate and 20 mmol potassium chloride.

Infusion rate - 125 ml/m^2/hour.

Check urine pH - Adjust the sodium bicarbonate concentration to maintain the urinary pH between 7 and 8 (i.e. alkaline). A urinary pH of 7.5 or greater must be achieved before starting the methotrexate infusion.
Alternating bags of sodium chloride 0.9% and glucose 5% is acceptable.

HIGH-DOSE METHOTREXATE INFUSION
Methotrexate dose
Methotrexate 3 g/m^2 with:
10% (i.e. 300 mg/m^2) given over 1 hour (loading dose) in 200 mls sodium chloride 0.9%
90% (i.e. 2700 mg/m^2) given over next 23 hours in 1 litre sodium chloride 0.9%

NOTE: The infusion of methotrexate must always stop at 24 hours even if not completed for any reason.

FOLINIC ACID RESCUE MUST START AT 36 HOURS FROM THE START OF METHOTREXATE.
The first dose of folinic acid (to be given at 36 hours after the start of methotrexate infusion) must be written up at the time of prescribing the methotrexate infusion.

Dosage of folinic acid:
At 36 hours: Give 15 mg/m^2 iv.
36-48 hours: Give 15 mg/m^2 iv every 3 hours.
From then on: Give doses as per table below until methotrexate level is less than 0.1micromol/litre.

Monitoring of plasma methotrexate levels following infusion.
Times given are from time 0 (time of starting intravenous methotrexate infusion).
The following plasma samples are **required for patient's safe rescue** with folinic acid:
48 hours, 72 hours, and then every 24 hours until methotrexate level is less than 0.1micromol/litre

Table for the calculation of folinic acid rescue on the basis of MTX plasma levels.

Time after starting MTX	**MTX plasma concentration (micromol/litre)**				
	<0.1	0.1-2	2-20	20-100	>100
48h	None[a]	15mg/m^2q6h[b]	15mg/m^2q6h	10mg/m^2q3h	100mg/m^2q3h

72h	None	15mg/m^2q6h	10mg/m^2q3h	100mg/m^2q3h	1g/m^2q3h
96h	None	15mg/m^2q6h	10mg/m^2q3h	100mg/m^2q3h	1g/m^2q3h
120h[c]	None	15mg/m^2q6h	10mg/m^2q3h	100mg/m^2q3h	1g/m^2q3h

Notes

a No extra folinic acid is required provided MTX levels are below 0.1 micromol/litre at 48h.
b Dose and schedule of folinic acid: q6h = every 6 hours.
c At time points after 120h folinic acid administration should be continued as recommended for 120h.

Hydration regimen during and after completion of intravenous methotrexate infusion

Continue to infuse at a rate of 125 ml/m^2/hour for a minimum of 48 hours after start of methotrexate with:
1L dextrose saline containing 50 mmol of sodium bicarbonate and 20 mmol potassium chloride.
Alternating bags of sodium chloride 0.9% and glucose 5% is acceptable.
Continue to ensure that urinary pH is above 7 by adjusting sodium bicarbonate dose.

After 48 hours from the start of the intravenous methotrexate, **ENSURE** a combined oral and/or intravenous intake greater than 3 litres/m^2/24 hours until plasma methotrexate levels<0.1 micromols/litre.

Check fluid balance at regular intervals (at least 4-hourly) through each day, taking early action if fluid overload occurs by giving furosemide if the urine output falls below 400 ml/m^2 in any given 4-hour period.

Other investigations during folinic acid rescue:
Daily Creatinine, sodium and potassium.
Alternate days Bilirubin, AST, ALT, albumin, full blood count.

These investigations should also be checked at least twice during the week following the first and second methotrexate infusion to detect any toxicity that might occur.

Conversion table for methotrexate levels expressed in different units

Molar (M)	**µmol/l**
1×10^{-3}	1013.0
2×10^{-4}	202.0
1×10^{-4}	101.0
2×10^{-5}	20.0
1×10^{-5}	10.1
2×10^{-6}	2.0
1×10^{-6}	1.01
2×10^{-7}	0.2
1×10^{-7}	0.10

Drug interactions

Drugs which compromise renal function eg. aminoglycosides and cisplatin can decrease clearance of methotrexate and lead to systemic toxicity. Avoid concurrent use of Non steroidal anti inflamatories **(NSAIDs) including salicylates and sulphonamides**.
Large doses of penicillin may interfere with the active renal tubular secretion of methotrexate.
It is recommended that prophylactic co-trimoxazole be stopped one week before high dose MTX therapy, until maintenance therapy starts.

Appendix 16- Karnofsky Performance Status

KARNOFSKY PERFORMANCE STATUS SCALE DEFINITIONS RATING (%) CRITERIA

Able to carry on normal activity and to work; no special care needed.	100	Normal no complaints; no evidence of disease.
	90	Able to carry on normal activity; minor signs or symptoms of disease.
	80	Normal activity with effort; some signs or symptoms of disease.
Unable to work; able to live at home and care for most personal needs; varying amount of assistance needed.	70	Cares for self; unable to carry on normal activity or to do active work.
	60	Requires occasional assistance, but is able to care for most of his personal needs.
	50	Requires considerable assistance and frequent medical care.
Unable to care for self; requires equivalent of institutional or hospital care; disease may be progressing rapidly.	40	Disabled; requires special care and assistance.
	30	Severely disabled; hospital admission is indicated although death not imminent.
	20	Very sick; hospital admission necessary; active supportive treatment necessary.
	10	Moribund; fatal processes progressing rapidly.
	0	Dead

Appendix 17: – Protocol Version History

Protocol:		**Amendments:**		
Version no.	**Date**	**Amendment no.**	**Section (no./title)**	**Summary of main changes from previous version.**
1.0	11nov09	n/a	n/a	n/a
2.0	17may10			
3.0	18aug10			