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A PHASE II STUDY OF THERAPY FOR PEDIATRIC RELAPSED OR REFRACTORY PRECURSOR B-CELL ACUTE LYMPHOBLASTIC LEUKEMIA AND LYMPHOMA

IDE 11,533 (CliniMACS™ System)

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St. Jude Children's Research Hospital
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August 3, 2018

To: IRB

From: Sima Jeha, MD

RE: ALLR18, A Phase II Study of Therapy for Pediatric Relapsed or Refractory Precursor B-Cell Acute Lymphoblastic Leukemia and Lymphoma.

Clarification Memo

Due to the unavailability of the drug Teniposide (VM-26), etoposide (VP-16) will be substituted for the remaining doses for patients currently on this study. **The protocol Section 5.1.3 allows this substitution**. Participants will be informed of the substitution verbally, and this will be documented in the medical record. The substitution of VP-16 will continue until 46 participants are enrolled to ensure that 40 evaluable patients are enrolled.

1. During Interim Continuation (Section 5.3), Week 3 teniposide will be substituted with Etoposide 300 mg/m^2 on Day 1

Week	Agent	Dosage and route	# Doses	Schedule
1	VP16 (etoposide)	300 mg/m^2 IV	1	Day 1
	CYCLO (cyclophosphamide)	300 mg/m^2 IV	1	Day 1
2	MTX (methotrexate)	40 mg/m^2 IV	1	Day 1
	6MP (mercaptopurine)	75 mg/m^2 PO	7	Days 1-7
3	VP16 (etoposide)	300 mg/m^2 IV	1	Day 1
	ARA-C (cytarabine)	300 mg/m^2 IV	1	Day 1
4	DEX (dexamethasone)	12 mg/m^2 PO	15	Days 1-5 TID
	VINBLAST (vinblastine)	6 mg/m^2 IV	1	Day 1

2. During Continuation Treatment (Sections 5.5.1 and 5.5.2), Weeks 3 and 7 teniposide will be substituted with etoposide 300 mg/m^2 on Day 1.

Week	Agents
1	MTX + 6MP
2	MTX + 6MP
3	VP16 + ARA-C
4	DEX + VCR
5	MTX + 6MP
6	MTX + 6MP
7	VP16 + ARA-C
8	DEX + VINBLAST

5.5.2 Drug dosages, schedules and routes – Continuation treatment

MTX (methotrexate)	40 mg/m^2	1 dose, IV* day 1 (weeks 1, 2, 5 & 6)
6MP (mercaptopurine)	75 mg/m^2	days 1-7, PO (weeks 1, 2, 5 & 6)
VP16 (etoposide)	300 mg/m^2	1 dose, IV day 1 (weeks 3 & 7)
VCR (vincristine)	2 mg/m^2	(max 2 mg), 1 dose, IV push day 1 (week 4)
ARA-C (cytarabine)	300 mg/m^2	1 dose, IV day 1 (weeks 3 & 7)
DEX (dexamethasone)	12 mg/m^2	15 doses, PO day 1-5 TID (weeks 4 & 8)
VINBLAST (vinblastine)	6 mg/m^2	1 dose, IV day 1 (week 8)

Protocol and Title: ALLR18, A PHASE II STUDY OF THERAPY FOR PEDIATRIC RELAPSED OR REFRACTORY PRECURSOR B-CELL ACUTE LYMPHOBLASTIC LEUKEMIA AND LYMPHOMA
Principal Investigator: Sima Jeha, M.D.
IDE holder: St. Jude Children's Research Hospital, IDE 11,533 (CliniMACS system)
Brief overview: This phase II trial is studying risk-directed therapy (standard and high risk participants will receive different therapy) for precursor B-cell acute lymphoblastic leukemia or lymphoma in first relapse.
Interventions: Biologicals: rituximab, Interleukin-2 Drugs: vincristine, dexamethasone, clofarabine, cyclophosphamide, etoposide, PEG-asparaginase, Erwinia asparaginase, methotrexate, mercaptopurine, leucovorin, cytarabine, mitoxantrone, teniposide, vinblastine, imatinib, dasatinib, nilotinib Device: CliniMACS™ System Procedure: Intrathecal chemotherapy (ITMHA), NK cell infusions, allogeneic hematopoietic stem cell transplant (HSCT) Other: CNS irradiation, testicular irradiation
Brief outline of treatment plan: The general treatment plan will consist of chemotherapy for standard-risk participants and chemotherapy followed by HSCT for high risk participants in first relapse of B-precursor ALL or lymphoblastic lymphoma. Remission induction for all participants consists of three blocks of therapy, wherein the first block is a novel immunotherapy regimen that includes cytotoxic chemotherapy, rituximab and infusion of haploidentical NK cells. Standard-risk patients will continue to receive chemotherapy for a total duration of approximately 2 years. High-risk patients will be candidates for HSCT and will proceed to transplant once a suitable donor is found and the patient achieves negative MRD.
Study design: Phase II multi-center, non-randomized trial. Primary outcome measure is 3-year survival rate.
Sample size: 40 evaluable participants over 6 years. **Data management:** Data management and statistical analysis will be provided locally by the Hematological Malignancies Program clinical research staff, Leukemia/Lymphoma Division and Biostatistics Department at St. Jude Children's Research Hospital.
Human subjects: The primary risk to participants in this research study is toxicity from the multi-modality therapies. For patients with relapsed ALL, there is no salvage regimen that offers a good cure rate. The current standard of care is intensive chemotherapy using different regimens, and allogeneic transplant for those who have a suitable donor. The primary objective of this study is to increase the 3-year survival rate. Rituximab and NK cell therapy has been shown to be safe and effective in similar pediatric malignancies, but both are investigational therapies for ALL in first relapse. As such, an additional risk to patients participating in this trial may be that rituximab and NK cell therapy increase incidence of side effects. Potential benefits for patients who participate in this study include a possible increase in progression free survival. Since the standard of care is intensive chemotherapy and allogeneic transplant, participants will be receiving the standard of care.

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1.0 OBJECTIVES

The overall objective of this protocol is to improve the cure rate of relapsed precursor B-cell acute lymphoblastic leukemia (ALL) and lymphoblastic lymphoma.

1.1 Primary Objective

To estimate the 3-year survival rate of participants with first relapse or primary refractory precursor B-cell ALL and lymphoblastic lymphoma treated with risk-directed therapy.

1.2 Secondary Objectives

1.2.1 To determine minimal residual disease (MRD) levels at the end of remission induction therapy for participants with relapsed precursor B-cell ALL and compare the results with those in ALLR17.

1.2.2 To estimate levels of CD20 expression at baseline, during treatment with dexamethasone-containing chemotherapy and following rituximab treatment in Block 1 of remission induction therapy for relapsed precursor B-cell ALL.

1.3 Exploratory Secondary Objectives

1.3.1 To assess donor natural killer (NK) cell immunoglobulin-like receptor (KIR) repertoire and explore the efficacy of NK cell infusions followed by rituximab in relapsed ALL.

1.3.2 To study whether pre-existing or emerging development of serum antibodies to asparaginase is related to hypersensitivity reactions or exposure to asparaginase or to anti-leukemic or adverse effects of therapy, in participants with relapsed ALL.

1.3.3 To describe the effect of prophylactic antibiotics on:

a. The evolution of antibiotic resistance in peri-rectal swab isolates of *Escherichia coli*, *Klebsiella pneumonia*, *Pseudomonas aeruginosa*, *Streptococcus mitis* and enterococci.
b. Resistance patterns of bacterial isolates from all sterile site cultures.

Completed with amendment 3.0

1.3.4 To characterize global gene expression and copy number changes in leukemic cells at relapse and to compare to initial diagnosis (when available) to improve knowledge of mechanisms of relapse.

1.3.5 To determine *in vitro* sensitivity of pretreatment relapsed leukemic cells to anti-leukemic agents and compare to in *vitro* sensitivity at diagnosis (when available).

1.3.6 To engraft freshly harvested relapsed ALL cells into murine models of ALL to gain insights into therapy resistance.

1.3.7 To study the development of resistant clones during therapy for relapsed ALL.

2.0 BACKGROUND AND RATIONALE

Cure rates for newly diagnosed ALL have improved dramatically over the past 50 years. In the recent St Jude Total XV study, the estimated 5-year event-free survival (EFS) and overall survival (OS) rates for all 498 patients were 85.6% and 93.5% respectively.[1] On the other hand, for children who relapse outcome is generally poor despite intensified therapy.[2] In particular, outcome for those who relapse while still on therapy is dismal across multiple co-operative group and single institution studies (5-year OS between 11% and 29%).[3,4] Preliminary analyses of the St Jude ALLR16 study, which utilized an upfront topotecan window treatment followed by intensive multi-agent chemotherapy, demonstrated a 5-year OS of 40% while in the subsequent ALLR17 study with an etoposide and teniposide based regimen 3-year OS estimate is 49% for all patients. Hence, there is a need for novel therapeutic approaches. The outcome for patients with primary induction failure is also poor.[5] The 10-year survival rate is estimated at 32%. Thus, these patients will be eligible for ALLR18. An exception is children < 6 years of age with induction failure and favorable cytogenetics (hyperdiploidy or ETV6-RUNX1). This group of patients has a higher survival rate of approximately 72% with chemotherapy alone and will be excluded from ALLR18.

2.1 Remission Induction

MRD is an excellent surrogate marker for relapse free survival.[4] Interim analyses of ALLR17 revealed that 71% of participants continue to be MRD positive at the end of remission induction therapy and the outcome of these participants is poor. Similar results have been reported by the Children's Oncology Group.[5] Thus, in ALLR18, we will attempt to decrease this rate of MRD positivity by utilizing a novel immunotherapy regimen of rituximab, cytotoxic chemotherapy and haploidentical NK cells. The hypotheses for the induction regimen in ALLR18 are:

1) Immunotherapy with the anti-CD20 monoclonal antibody rituximab can be given safely in children with relapsed precursor B-cell ALL in combination with cytotoxic chemotherapy.
2) Pre-treatment with dexamethasone will cause up-regulation of CD20 expression in relapsed ALL blasts leading to increased rituximab mediated cell kill.

3) NK cell infusions from a haploidentical donor in addition to direct anti-leukemia effects will enhance efficacy of rituximab.
4) Cytotoxic chemotherapy will be synergistic with rituximab and will also augment NK cell function by induction of neutropenia.
5) This strategy will lower MRD levels, improve complete remission (CR) rates and ultimately event free survival.

2.1.1 Rationale for rituximab

The CD20 antigen is a B-cell specific marker which is expressed on the surface of B-lymphocytes from the pre B-cell stage through the memory cell stage. Rituximab, a chimeric anti-CD20 monoclonal antibody is approved by the FDA for the treatment of patients with non-Hodgkin lymphoma and chronic lymphocytic leukemia. In the pediatric setting, rituximab is shown to be safe and efficacious in immune mediated disorders and non-Hodgkin lymphoma.[6-8] Though all its mechanisms of action are not completely understood, rituximab's anti-tumor effects are attributed to direct inhibition of signaling pathways, complement mediated cytotoxicity and antibody-dependent cellular cytotoxicity (ADCC).[9] Studies have also demonstrated synergy with cytotoxic chemotherapy.[9] Rituximab has been extensively used for the treatment of lymphomas but its use in ALL is limited. Nevertheless, a recent study in adult patients with ALL incorporated rituximab in a backbone of multi-agent chemotherapy (HyperCVAD) and demonstrated a survival benefit in patients less than 60 years (3-year overall survival rates of 75% versus 47% with standard HyperCVAD alone).[10] The potential therapeutic role of rituximab in childhood ALL is not defined but interest in this agent was ignited by a study showing that pretreatment of leukemic blasts with steroids either *in vitro* or *in vivo* caused up-regulation of CD20 and increased their susceptibility to rituximab *in vitro.*[11] At diagnosis, 52% of patients expressed CD20 on their peripheral blood blasts while on Day 8 of therapy, CD20 expression was noted in 75% of patients. These finding, which we have confirmed in preliminary results at St Jude, suggest that rituximab therapy could be incorporated in the treatment of ALL and can potentially augment leukemic cell kill.

2.1.2 Rationale for the choice of chemotherapeutic agents in the first induction block

Cytotoxic chemotherapy will include clofarabine, a highly active nucleoside analog approved by the FDA for children with relapsed/refractory ALL.[12,13] Clofarabine was rationally designed as a hybrid molecule to improve the activity and overcome the toxicities associated with fludarabine and cladribine. The cytotoxic effects of clofarabine are due to its inhibition of DNA synthesis and repair, induction of apoptosis and possibly other mechanisms.[14] In the pediatric phase 1 study, the maximum tolerated dose (MTD) was determined to be 52 mg/m^2/day for 5 days. Of the 17 ALL patients enrolled, 4 achieved CR and one PR, resulting in an overall response rate of 29%.[12] Dose limiting toxicities were reversible elevations in transaminases and skin rash. In the pediatric phase II study, 61 patients with ALL were enrolled and the overall response rate was

30%.[15] *In vitro* studies have demonstrated synergy between clofarabine and the alkylator cyclophosphamide.[16] Cells treated with clofarabine impede repair of cyclophosphamide induced DNA damage. In the multicenter pediatric phase I CLO-218 study, clofarabine was given in combination with cyclophosphamide and etoposide.[17] Dose escalation was performed for all three drugs. An MTD was not reached, but the recommended phase II doses for the three agents were 40, 440 and 100 mg/m^2/day respectively for 5 consecutive days. The overall response rate for the 20 ALL patients enrolled was 55% (9 CR and 2 CRp). Treatment related toxicities were mainly infectious with documented infections in 72% of patients. Elevation in transaminases was commonly noted. This combination of agents has been subsequently used for patients with relapsed and refractory ALL by multiple centers. Three reports are available: a study of 25 patients from Italy[18], a retrospective review of 18 patients from the UK[19] and the St Jude experience of 11 patients with relapsed or refractory ALL.[20] All three reports concluded that the regimen was efficacious with an acceptable safety profile even in patients who had received a prior transplant and in infants. Supportive care, especially prophylaxis against infectious diseases was imperative. The St Jude frontline ALL study Total XVI incorporates this regimen for high-risk patients and for infants. NKHEM is another ongoing St Jude study that utilizes the same combination followed by infusion of haploidentical natural killer cells. In ALLR18, we will add non- myelosuppressive agents such as vincristine, dexamethasone and PEG-asparaginase in induction block I while awaiting count recovery. Prophylaxis against infectious agents (bacterial and fungal) will be required. Due to anticipated risk of increased hepatotoxicity, myelosuppression and infections in patients with prior hematopoietic stem cell transplant, these patients will be evaluated in a separate stratum for toxicity.

2.1.3 Rationale for the addition of NK cells

NK cells are normal lymphocytes that can kill target cells without the need for prior sensitization or activation. The ability of NK cells to perceive alterations in target cells is mediated by a balance of signals through many surface receptors including KIRs and activating receptors such as CD16a. CD16a is a low-affinity transmembrane Fragment c gamma receptor IIIA (FcγRIIIA) found predominantly in NK cells and is essential for ADCC.[21] The FcγRIIIA binds clustered IgG molecules bound to antigens on the cell surface of target cells and does not bind circulating monomeric IgG. Therefore, ADCC is highly specific and occurs only when the target cells are coated with antibody. The CD16b (FcγRIIIB) GPI-linked form on neutrophils binds to IgG but is inefficient in inducing any signal or functional effect.[21] Because one of the primary mechanisms of action of rituximab is primarily through ADCC, strategies to optimize NK cell mediated ADCC will be crucial to improve the outcomes of patients treated with rituximab.

Four strategies may be used to optimize NK cell therapy. First is to adoptively transfer normal NK cells from a healthy donor to the patients. This is particularly important in this protocol, as the patient's NK cell number and function are

expected to be low after chemotherapy. Using the St. Jude NKCELL protocol, we performed 12 consecutive purifications of NK cells from 12 normal volunteers. The system uses a two-step procedure.[22] First, mononuclear cells obtained by leukapheresis are depleted of T cells by CD3+ cell depletion using the CliniMACS. Second, the CD3-depleted product is enriched for CD56+ cells using the CliniMACS. The final products contained a median of 1.6 x 10^8 mononuclear cells and 91% CD3-CD56+ cells. In addition, the final products had minimal contamination with T cells (at or below the lower limit of detection in 10 of the 12 products) or B cells (median 0.2%). Thus, recipients are expected to be at low risk for GVHD or EBV lymphoproliferative disease. We also found that the expression of KIRs, adhesion molecules, intracellular cytokines, perforin, and granzyme B in NK cells was not significantly different before and after cell purification. Extensive proliferative capacity and potent antitumor activity of the NK cells were demonstrated by using an immunodeficient mouse model.[23] In addition, GVHD developed in all mice transplanted with unpurified mononuclear cells, but in none of the 10 mice transplanted with purified NK cells.

Based on these preclinical data, four clinical protocols have been opened at St. Jude to transplant normal NK cells from parents into leukemia patients. The data from the first cohort of 10 patients from the NKAML protocol (FDA IDE 11533) were published recently.[24] This study demonstrated that haploidentical NK cell infusions were safe, feasible, and efficacious. The conditioning, IL-2, and NK cell transplantation were all well tolerated. The median NK cell dose was 27 x 10^6/kg (range, 5 to 80 x 10^6/kg). The average hospital stay was only 2 days. None of the patients have acute or chronic graft-versus-host disease. Correlative laboratory studies showed that all patients had transient donor NK cell engraftment for a median of 10 days. NK cell cytotoxicity against K562 cells normalized in all patients by Day 7 after NK cell transplantation. Importantly, there was a significant expansion of KIR mismatched cells in the blood, from a median of only 210/ml on Day 2 to a median of 5,800/ml on day 14.

The second strategy to optimize NK cell therapy in this protocol is the use of chemotherapy that is efficacious in ALL to induce cytopenia and to up-regulate stress ligand expression before rituximab and NK cell administration. We have shown that T cell activity may dominate NK cell activity; thus, T-cell lymphopenia may facilitate NK cell function.[25,26] Furthermore, we have recently shown that blood DCs may suppress NK cell through IL-6 and IL-10.[27] Taken together, these data suggest that giving chemotherapy before NK cell administration may augment NK cell function and expansion by induction of leukopenia. Furthermore, DNA damage is known to be able to trigger ATM and ATR pathways to up-regulate genotoxic stress ligands such as NKG2D ligand expression.[28] NKG2D is a potent NK cell activating receptor and signals through DAP10 for degranulation and IF-γ production.

The third approach to augment NK cell cytotoxicity towards ALL is to capitalize on the unique ability of St. Jude in selecting an optimal KIR mismatched donor.[29]

The laboratory of Dr. Leung has set up comprehensive KIR typing assays for the highly polymorphic KIR genes.[30] In humans, KIRs are encoded by a gene family on chromosome 19q13 and recognize specific HLA class I alleles in HLA-A, -B and -C. The receptors specific for MHC class I molecules on target cells inhibit NK effector functions such as cytotoxicity and cytokine production. Clinical data suggest that KIR2DL2 and KIR2DL3 that recognize an epitope shared by HLA-C group 1 allotypes, KIR2DL1 that recognizes an epitope shared by HLA-C group 2 allotypes, and KIR3DL1 that recognizes an epitope shared by HLA-Bw4 allotypes are important determinants of anti-tumor effect. In hematopoietic stem cell transplantation (SCT), the NK cells of the donor may exert potent anti-leukemia effects if the cognate MHC class I epitope is absent on the patient's leukemia cells for the donor's inhibitory KIRs.[31] This potency has been demonstrated in both mouse models and in clinical transplantation. In a study of 51 patients at the St. Jude Children's Research Hospital with direct measurement of the donor KIR repertoire, patients with a KIR matched donor had a 4-fold higher risk of relapse than those with a KIR mismatched donor.[29]

The fourth strategy to optimize NK cell therapy is the use of IL2. In the four clinical trials at St. Jude using haploidentical NK cell transplantation, all patients received low dose IL-2 (1 million units/m^2/dose SQ) every other day starting the night before NK cell transplantation. The purpose of the low dose IL-2 administration is to expand NK cells *in vivo* and to augment their cytotoxicity. Correlative laboratory studies in the first cohort of participants showed that all patients had expansion of donor NK cells with a median peak at Day 14 and normalization of NK cell cytotoxicity against K562 cells by Day 7 after NK cell transplantation.[24] Importantly, there was a significant expansion of KIR mismatched cells in the blood in the first 2 weeks after transplantation. The IL2 was well tolerated - there was no associated grade II-IV toxicity, no patients required hospitalization, and no patients required discontinuation of IL-2.

2.1.4 Induction Block II

Two doses of HDMTX 5 g/m^2 will be given a week apart together with 6MP. This regimen was used in Total XIV and will provide good CNS coverage. The second dose of methotrexate will be targeted to achieve a steady-state plasma concentration of 65 µM as has been done in our frontline studies since Total XV. Our goals are to continue to avoid unacceptably low concentrations (albeit in a small % of courses), minimize unacceptably high concentrations and avoid GI toxicity. Using this approach, we had only 5.1% of courses with grade 3-4 mucositis (compared to as high as 44% of courses with BFM's administration of 5 g/m^2 without targeting). In patients who have experienced renal dysfunction, require concurrent drugs that might affect MTX clearance, or who are otherwise unstable, we will employ intra-course targeting of HDMTX.

Rationale for change in administration of mercaptopurine (revision 2.1)

Prior studies assessing the pharmacological and therapeutic impact of giving oral mercaptopurine (MP) with food or with dairy products, or giving 6MP at different times of day have produced variable results. All of the early studies[32-34] enrolled small numbers of patients and revealed trends in differences in peak plasma concentrations of parent drug or plasma area-under-the-concentration-time curve (AUC), but these results were not statistically significant. Other studies[35,36] reported statistically significant differences in time of maximum drug concentration in plasma (tmax) and AUC in the fasting state of parent drug[35] or changes in ALL disease free survival[36] associated with time of day of oral 6MP administration (with better results reported for nighttime drug administration in that the risk of relapse was 4.6 times greater with the morning administration schedule).

However, these older studies did not assess these variables in the context of treatment protocols that utilize erythrocyte thioguanine nucleotides (TGN) levels to guide therapy adjustments, nor did they include thiopurine dosing as part of multivariate analyses for outcome predictors. A more recent study of 532 patients by Schmiegelow (2014) used a Cox multivariate model to show that the circadian schedule (morning vs. evening vs. mixed) of MTX/6MP was not of prognostic significance for the risk of relapse, and the 10-year cumulative relapse risk was below 20% in all groups.[37]

A separate recent study assessed the effects of "pill-taking" habits on treatment adherence, erythrocyte TGN levels & relapse risk in 441 children enrolled on the COG AALL03N1 study.[38] This study reported no association between relapse risk and whether 6MP was taken with food (p=0.5), with dairy (p=0.2), whether tablets were swallowed whole vs. crushing/chewing (p=0.7); IV), or time of day: evening/night vs. morning/mid-day (p=0.9), varying times vs. non-varying times (p=0.9). The 6MP taking habits were also not associated with DI- and age-adjusted TGN levels. The authors concluded that the commonly practiced restrictions concerning administration with food or at bedtime did not significantly influence outcome in their protocol and patient cohort.

Based on these larger and newer studies, that include more careful attention to 6MP dosing than was true in the earlier studies, and on the fact that 6MP dose is adjusted to desired blood counts, we propose to lift the restrictions concerning administering MP at bedtime and on an empty stomach to allow patients to take 6MP at a time of day which is most likely to yield full adherence to the prescribed daily dosing of 6MP. As for any daily drug, we recommend that parents administer and/or patients take 6MP at a consistent time every day. We also strongly recommend that erythrocyte TGN levels continue to be monitored to guide 6MP therapy (detect non-adherence and/or inappropriate dosing). Likewise, TPMT phenotype/genotype should continue to be assessed for each patient to guide the selection of optimal 6MP dosages for each patient. Finally, ALL continuation therapy should continue to be guided by measurement of WBC throughout therapy, with dosage adjusted made as stipulated in the primary ALL treatment protocol.[32-40]

2.1.5 Induction Block III

High dose cytarabine and mitoxantrone will be used. This combination is well tolerated in multiple AML regimens.[41,42] In the recent UK ALLR3 study for ALL in first relapse, mitoxantrone was shown to be beneficial.[43] The estimated 3-year overall survival was 69% in patients who received mitoxantrone in induction versus 45.2% in patients randomized to receive idarubicin. In addition, patients are not exposed to mitoxantrone in frontline studies for ALL and it is anticipated that the use of this agent will circumvent drug resistance. High dose cytarabine will provide good CNS coverage.

2.2 Re-Induction

A five day course of clofarabine, cyclophosphamide and etoposide along with non-myelosuppressive agents dexamethasone, vincristine and PEG-asparaginase, similar to Block I induction (without rituximab and NK cells) will be used to consolidate remission.

2.3 Continuation and Interim Continuation

These phases are similar to ALLR17 with the use of rapidly rotating drug pairs (modeled after Total XI and ALLR11). This continuation regimen is well tolerated in an outpatient setting. Four weeks of this regimen will be used as interim continuation between the induction Block III and re-induction phases to allow time for recovery from intensive chemotherapy. During continuation cyclophosphamide and etoposide will be omitted as patients would have received these agents during the induction and re-induction blocks. We will use vinblastine in interim continuation and alternating with vincristine in weeks 4 and 8 of each cycle of continuation. There are *in vitro* data showing sensitivity of ALL to vinblastine.[44] Considering the hematological toxicity of vinblastine, the dose will be adjusted according to the patient's ANC.

Additional interim continuation weeks of chemotherapy will also be given to the occasional patients who are deemed unable to tolerate intensive therapy as prescribed by the study. Criteria include but are not limited to systemic fungal infection, severe thrombosis or grade 3 or 4 hepatic or renal dysfunction.

2.4 MRD to Determine Indication and Timing of HSCT

Bone marrow MRD will be done at the end of each block of induction therapy. Provisional standard-risk patients (i.e. late relapse) with positive MRD (≥0.01%) at the completion of Block II will be re-assigned to the high-risk group. Multiple studies in first relapse have demonstrated the prognostic implication of positive MRD (≥ 0.01%) at the end of 4-6 weeks of therapy.[45] In the COG AALL01P2 study, the 12 month EFS was 80% versus 48% in patients with positive versus

negative MRD at the end of the first block of remission induction therapy.[5] In the UKALLR3 study, patients with MRD ≥ 0.01% at week 5 were candidates for stem cell transplant.[43]

MRD status immediately prior to HSCT is predictive of outcome in precursor B-cell ALL. In a report from the BFM group, the cumulative incidence of subsequent relapse was 57% in patients with $\geq 10^{-4}$ leukemic cells versus 13% in patients with $\leq 10^{-4}$ leukemic cells prior to HSCT.[46] Thus, patients with positive MRD (≥0.01%) at the end of induction Block II will receive Block III (+/- interim continuation) prior to HSCT. If MRD is still detectable or they are not candidates for HSCT, they may continue to re-induction therapy or be offered alternate studies. They will receive HSCT as soon as MRD is negative and a suitable donor is available.

2.5 Background and Rationale for Biologic and Ancillary Studies

2.5.1 Minimal residual disease (MRD)

MRD is an excellent surrogate marker for response and survival in childhood ALL at initial diagnosis.[4] At first relapse, studies at St Jude and other institutions have also demonstrated the prognostic implication of positive MRD (≥ 0.01%) at the end of 4-6 weeks of therapy. In St Jude ALLR11 and ALLR15 studies, the cumulative incidence of second relapse was 70.2% for MRD positive patients (≥ 0.01%) versus 28% for MRD negative patients (p=0.008).[45] In the COG AALL01P2 study, the 12 month EFS was 80% versus 48% in patients with positive versus negative MRD at the end of the first block of remission induction therapy.[5] The proportion of patients who are MRD positive at the end of re-induction therapy is relatively high: 68% in AALL01P2 and 71% in ALLR17. Thus, in ALLR18, we will attempt to decrease the rate of MRD positivity by utilizing a novel induction regimen. It remains unclear whether patients with insufficient reduction of MRD can be salvaged by more intensive treatment.

2.5.2 CD20 expression

The CD20 antigen is expressed on the surface of peripheral blood B-lymphoblasts in approximately 50% of patients with precursor B-cell ALL.[11] The administration of steroids increased this proportion to 75% by day 8 of therapy. Up-regulation of CD20 expression sensitizes cells to the cytotoxic effects of rituximab.[11] Post rituximab, the number of CD20-positive cells is expected to diminish rapidly. Thus, we will measure CD20 expression by flow cytometry frequently during the first three weeks of induction Block I to study the kinetics of CD20 expression in relation to the various components of therapy.

2.5.3 Donor NK cell KIR repertoire and NK cell infusions followed by rituximab

In humans, NK cells are regulated by KIRs that are encoded by a gene family on chromosome 19q13 and recognize specific HLA class I alleles.[47] KIRs includes receptors with activating as well as inhibitory potential, receptors that have no known ligand, and receptors with specificity for MHC class I such as HLA-A, -B and -C. The receptors specific for MHC class I molecules on target cells inhibit NK effector functions such as cytotoxicity and cytokine production. Each NK cell generally expresses at least one inhibitory receptor that recognizes a self HLA molecule, thus preventing autoimmunity. Fourteen members of the KIR family have been identified on human NK cells thus far.[48] The NK cells of the donor may exert potent anti-leukemia effects if the cognate MHC class I epitope is absent on the patient's leukemia cells for the donor's inhibitory KIRs. In a landmark article evaluating NK cell alloreactivity in 57 adult patients with AML, none of the 34 patients who received a KIR-ligand mismatched haploidentical HSCT had a disease relapse.[49] In an analysis of 130 patients with hematologic malignancies undergoing unrelated donor SCT, those with KIR-ligand incompatibility (including patients with ALL, CML, and MDS) had higher probabilities of overall survival and disease free survival and lower rates of transplant-related mortality and relapse.[50] In an exclusively pediatric study, with 51 research participants at St. Jude, the effect of KIR mismatch on myeloid leukemia was confirmed and a KIR mismatch effect was also noted in pediatric lymphoid leukemias.[31] In this study, with direct measurement of the donor KIR repertoire, participants with a KIR matched donor had a 4-fold higher risk of relapse than those with a KIR mismatched donor. In contrast to KIR mismatch, KIR-ligand mismatch was not a significant factor for the prediction of relapse. The absence of beneficial effects of KIR-ligand mismatch has also been observed in additional studies.[51] Taken together, these data underscore the importance of direct assessment of donor KIR repertoire rather than donor KIR-ligands. Peripheral blood will be obtained from the donor and the patient prior to Induction Block 1 and on Day 19 for immunophenotyping and genotyping for NK cell receptors as described previously.[31] We will assess NK cell receptors including KIRs, NCRs, NKG2D, DNAM-1, 2B4, and NTBA.

2.5.4 Asparaginase antibodies

Because patients have been treated previously with asparaginase, we expect there will be variability in both pre-existing asparaginase antibodies and in the development of antibodies on ALLR18. We will incorporate single serum samples to measure antibodies to asparaginase as well as to measure asparaginase activity in serum (which may be affected by changes in immune response). Antibodies against native *E. coli* (Elspar), PEGylated *E. coli* (Oncaspar), and Erwinia asparaginase are measured by an ELISA. Those with possible allergic reactions will also have measurement of anti-asparaginase antibodies and asparaginase at the time of the reaction and, if re-challenged, after the next dose. Asparaginase will be measured using a spectrophotometric assay based on conversion of asparagine to aspartic acid.[52] These measures will be available to use as covariates in analyses of objectives 1.1.1 and 1.2.1.

2.5.5 Surveillance for antibiotic resistance (completed with amendment 3.0)

A COG study evaluating a 3-block platform of intensive chemotherapy for children with first marrow relapse noted the rate of febrile neutropenia and clinically or microbiologically documented infections to be 59.7%, 39.6% and 79.4% per block of therapy.[5] Studies of the use of prophylactic antibiotics in neutropenic adult oncology patients conducted over the last 30 years have consistently shown efficacy in reducing the incidence of fever and microbiologically documented bacterial infections.[53] What is less well documented is the evolution of antibiotic resistance in colonizing and invasive bacterial isolates in recipients of such antibiotic prophylaxis. Showing that antibiotic exposure can increase the rate of resistance among colonizing organisms is not an absolute confirmation that colonization by a resistant organism can result in infection. There have, however, been a number of studies in patients with acute leukemia and those receiving HSCT that illustrate that invasive infection is often linked to previously noted colonization by the same organism.[54-56] Resistance not only to the antibiotic that a patient is being exposed to for antibiotic prophylaxis but cross-resistance to other antibiotic classes remains a major concern and needs to be systematically tracked. We will determine whether ciprofloxacin or cefepime prophylaxis will result in the selective emergence of colonization with Gram negative organisms resistant to fluoroquinolones, higher generation of cephalosporins or other unrelated antimicrobial agents such as carbapenems. Additionally, we will define whether fluoroquinolone or cefepime prophylaxis will result in the emergence of colonization or invasive infections with *S. mitis* that are resistant to fluoroquinolones, higher generation cephalosporins or penicillin. Finally, we will assess the impact of antibiotic prophylaxis on colonization with vancomycin resistant enterococci and incidence of *C. difficle* infections. Peri-rectal swabs will be collected pre and post antibiotic prophylaxis during inductions Blocks I, III and re-induction.

2.5.6 Global gene expression and copy number changes

Matched diagnosis and relapse leukemic samples from the same patient offer an excellent resource to study the mechanisms of relapse and to identify potential new therapeutic targets. Global changes in gene expression have been studied on Affymetrix microarrays and have demonstrated that pathways involved in cell cycle and DNA repair to be predominant in early relapse.[57] Survivin, an anti-apoptotic gene was up-regulated at relapse and these findings have led to the initiation of a phase I study of survivin antagonists in relapsed ALL. Studies of DNA changes (copy number variations, loss of heterozygosity) at St Jude have provided insights into the clonal evolution of relapse[58] and a recent re-sequencing effort identified novel mutations including those in the *CREBBP* gene at relapse.[59] Another study of matched samples from the Children's Oncology group identified deletions of the *MSH6* gene in a subset of patients specifically at relapse.[60] The studies mentioned above had a limited number of patient samples and thus it was

not possible to investigate changes specific to various ALL subtypes at relapse and correlate to multiple clinical features and response. In addition, recent discoveries of genomic aberrations (e.g. rearrangements of *CRLF2,* activating mutations of *JAK2*) in high risk ALL have not been studied in detail in relapsed ALL.[61-64] In ALLR18, we will collect bone marrow samples at the time of relapse and retrieve cells from initial diagnosis from the tissue bank (if available). Consent for TBANK is required for participation in these studies.

2.5.7 Drug sensitivity studies

In newly diagnosed ALL, *ex vivo* drug sensitivity predicts long-term relapse risk, is associated with drug-specific gene expression patterns, and is being coupled with preclinical studies in cell lines and murine models to identify determinants of drug sensitivity.[65-67] Not much data is available for *in vitro* drug resistance in relapsed ALL though it is well known clinically that relapsed blasts are relatively chemo-resistant. In 1995, Klumper *et al*[68] demonstrated that relapsed blasts (N=137) were highly resistant to glucocorticoids, L-asparaginase, anthracyclines and thiopurines, but not to other agents like vincristine, and epipodophyllotoxins when compared to samples from initial diagnosis (N=141). In this study, matched samples were only available for 16 patients which showed *de novo* as well as acquired drug resistance. At St Jude, *in vitro* drug resistance profiles of leukemic blasts at diagnosis to a panel of chemotherapeutic agents have been performed in Studies Total XIII-XVI and have led to the identification of genes correlating with sensitivity to single agents as well as combination chemotherapy.[66,67] Findings also point to previously unrecognized potential targets for therapeutic intervention. Profiles at relapse are available in a subset of patients too. In ALLR18, we will continue to study *ex vivo* sensitivity of primary ALL blasts to multiple anti leukemic agents (e.g., mercaptopurine, thioguanine, prednisolone, dexamethasone, vincristine, L-asparaginase, cytarabine, and daunorubicin) and/or chemo-sensitivity modulators (e.g., rolipram, a PDE4B inhibitor that improves sensitivity to glucocorticoids).

2.5.8 Xenograft models of ALL

Establishing xenografts of primary human ALL samples has proven to be a powerful tool to establish bio-repositories of human leukemic cells to examine the genetic and biologic basis of treatment failure, to examine clonal heterogeneity and to use as a platform for the testing of novel therapeutic agents *ex vivo* and *in vivo*. At St Jude, several investigators have performed xenotransplants of B-progenitor ALL, including *CRLF2*-rearranged ALL, infant leukemia and hypodiploid ALL, and have achieved up to 80% engraftment rates with remarkable reproducibility between the tempo of engraftment between replicate mice transplanted with the same tumor. Moreover, for *CRLF2* rearranged and hypodiploid ALL downstream genomic assays demonstrated that the genomic alterations in the relapsed tumor

recapitulate those present in the primary leukemia. The use of xenograft models has also proven valuable in determining the genetic basis of the aggressiveness of disease, and the basis of relapse.[62,69,70] At St Jude, these studies have been performed with cryopreserved viable cells stored by the St Jude Biorepository. Cells harvested from patients in ALLR18 will be used for engraftment into murine models and will contribute to the ongoing efforts at SJCRH to establish a panel of xenografts of high risk acute leukemia samples to serve as a resource for a range of studies of the biologic basis of relapse in acute leukemia, as well as establishing a platform for the preclinical testing of novel therapeutics.

2.5.9 Resistant clones during therapy for relapsed ALL

Recent genomic profiling studies from our group and others identified somatic mutation within the *NT5C2* gene directly related to drug resistance in relapsed ALL (~15-20% in T and B-lineage ALL at relapse).[71,72] Cells with *NT5C2* gain-of-function mutation are extremely resistant to thiopurine therapy compared to those with wildtype *NT5C2*, and we expect a substantial proportion of patients in this trial would be *NT5C2* mutant. Therefore, this protocol offers a unique opportunity to examine the dynamics of *NT5C2* mutation during therapy (e.g., the change of % mutant cells at a function of time), especially as patients progress through various blocks of therapy. Therefore, it is important to determine the feasibility of monitoring *NT5C2* mutation during ALL therapy. Our ultimate goal is to examine the clinical value of prospectively identifying *NT5C2* mutant patients and possibility of implementing *NT5C2*-guided treatment individualization. Bone marrow samples will be collected for this objective to coincide with the timing of MRD evaluations. Consent for TBANK is required for participation in these studies.

3.0 ELIGIBILITY CRITERIA AND STUDY ENROLLMENT

According to institutional and NIH policy, the study will accession research participants regardless of gender and ethnic background. Institutional experience confirms broad representation in this regard.

3.1 Inclusion Criteria for Participant

3.1.1 Must have relapsed or refractory **precursor B-cell acute lymphoblastic leukemia or acute lymphoblastic lymphoma.**

3.1.1.1 Participants with **leukemia** must meet one of the following:

1) In first hematologic relapse*, or

2) Refractory to one or two courses of frontline induction therapy (≥ 5% blasts in the bone marrow or peripheral blood confirmed by flow cytometric analysis).

Note: see Exclusion Criterion 3.2.1. Participants aged 1 to 5 years with induction failure and favorable cytogenetics (i.e., hyperdiploid or ETV6-RUNX1) will not be eligible for this protocol. Other patients younger than 6 years will be eligible.

3.1.1.2 Participant with **lymphoma** must meet one of the following:

1) In first relapse, or

2) Refractory to one or two courses of frontline induction therapy with measurable disease

*Relapse in ALL is defined as the reappearance (in a patient who has previously achieved remission) of leukemic blasts in the bone marrow or peripheral blood.

- Should flow cytometric analyses suggest relapse (by the reappearance of a similar immunophenotype to the original leukemia) in the presence of <5% blasts morphologically, a repeat bone marrow test is recommended to confirm relapse.

- Molecular or genetic relapse is characterized by the reappearance of a cytogenetic or molecular abnormality.

- Early relapse is defined as relapse on therapy or < 6 months after completion of frontline therapy. Late relapse is defined as relapse occurring ≥ 6 months after completion of frontline therapy.

3.1.2 Participants age is < 22 years at time of enrollment (e.g., participant is eligible until 22nd birthday).

3.1.3 Prior therapy

3.1.3.1 There is no waiting period for participants who relapse while receiving frontline therapy and are free from acute side effects attributable to such therapy.

3.1.3.2 Emergent radiation therapy, one dose of intrathecal chemotherapy, and up to 7 days of steroids for treatment of relapse are permitted before start of treatment in participants who relapse after completion of frontline therapy.

3.1.3.3 At least 90 days have elapsed since bone marrow transplant and participant is off immune suppression for ≥ 2 weeks, if applicable.

Participants with ALL or NHL who were transplanted in first remission are eligible for this study.

3.1.4 Organ function requirements

3.1.4.1 Hepatic: Total bilirubin ≤ ULN for age, or if total bilirubin is >ULN, direct bilirubin is ≤ 1.4 mg/dL.

3.1.4.2 Cardiac: Shortening fraction ≥ 28%.

3.1.4.3 Renal: Glomerular filtration rate >50cc/min/1.73 m^2, OR serum creatinine based on age as follows:

Age (years)	Maximum serum creatinine (mg/dL)	
	Male	Female
1 to 2 years	0.6	0.6
2 to 6 years	0.8	0.8
6 to 10 years	1	1
10 to <13 years	1.2	1.2
13 to 16 years	1.5	1.4
> 16 years	1.7	1.4

The threshold creatinine values in this Table were derived from the Schwartz formula for estimating GFR (Schwartz et al. J. Peds, 106:522, 1985) utilizing child length and stature data published by the CDC.

3.2 Exclusion Criteria for Participant

3.2.1 Leukemia participants aged 1 to 5 years with induction failure **and** favorable cytogenetics (i.e., *ETV6-RUNX1* or hyperdiploidy defined as DNA Index ≥ 1.16 or modal chromosome number ≥ 51).

3.2.2 Hepatitis B or HIV infection.

3.2.3 Pregnant or breast-feeding.

3.2.4 Inability or unwillingness or research participant or legal guardian/representative to give written informed consent.

3.3 Screening criteria for NK cell donors

3.3.1 Donor is at least 18 years of age.

3.3.2 Donor is a family member.

Potential donor(s) will first be screened for eligibility and suitability (e.g., HIV testing, pregnancy, and blood samples to Pathology & BMT&CT to select the optimal donor(s). This will be accomplished using the donor eligibility checklist and donor consent. The BMT&CT and Oncology teams will then select the best donor(s) based on established institutional practice, among the screened donor(s) who meet the eligibility criteria listed in Section 3.3. Only the selected donor(s) will proceed to the donor procedures. This will not require a re-consent or re-enrollment, although donors may withdraw consent at any time before the apheresis procedure is completed.

3.4 Inclusion criteria for Selected NK cell donor

3.4.1 Donor is not pregnant or breast-feeding.

3.4.2 Donor is HIV negative.

3.4.3 Donor does not have any other medical condition that, in the opinion of an independent physician, precludes performance of an apheresis procedure.

3.5 Research Participant Recruitment and Screening

Three institutions will collaborate in the proposed project: St. Jude Children's Research Hospital (SJCRH); Rady Children's Hospital in San Diego, CA and Cook Children's Medical Center in Fort Worth, TX. It is anticipated that additional sites will be added as new collaborations are established.

3.6 Enrollment on Study at St. Jude

A member of the study team will confirm potential participant eligibility as defined in Section 3.1-3.2, complete and sign the 'Participant Eligibility Checklist'. The study team will enter the eligibility checklist information into the St. Jude Clinical Trials Management System (CTMS). Eligibility will be reviewed, and a research participant-specific consent form and assent document (where applicable) will be generated. The complete signed consent / assent form(s) must be faxed or emailed to the CPDMO at [redacted] to complete the enrollment process.

The CPDMO is staffed 7:30 am-5:00 pm CST, Monday through Friday. A staff member from the St. Jude Cerner Millennium (MILLI) helpline is on call Saturday, Sunday, and holidays from 8:00 am to 6:00 pm. If you have a prospective research enrollment and need assistance releasing your consent, please call the MILLI helpline [redacted] on call number.

3.7 Enrollment on Study at Collaborating Sites

All participants must receive Block I therapy at St. Jude (includes NK cell infusions). Therefore, all participants will be consented and enrolled at St. Jude. Collaborating sites will assess preliminary eligibility on-site and will refer potentially eligible participants to St. Jude for final eligibility determination and work-up for NK cell infusions.

4.0 RISK ASSIGNMENT

Provisional risk assignment will be based on timing of relapse. Final risk assignment will incorporate MRD measurements after Block II of remission induction therapy.

4.1 Standard Risk

1) Late relapse ($\geq$ 6 months after completion of frontline therapy) AND
2) MRD < 0.01% at the end of Block II of remission induction therapy.

Provisional standard risk participants (i.e., late relapse) will be re-assigned to High risk if MRD $\geq$ 0.01% at the end of Block II.

Participants with lymphoma must be in complete remission at the end of Block III.

4.2 High Risk

1) Early relapse (on therapy or < 6 months after completion of frontline therapy), OR
2) Any relapse after hematopoietic stem cell transplant, OR
3) MRD $\geq$ 0.01% at the end of Block II of remission induction therapy, OR
4) Re-emergence of MRD at any time after attaining negative MRD on ALLR18

Biopsy of residual mass should be attempted (if feasible) for participants with lymphoma who are not in complete remission at the end of Block III.

5.0 TREATMENT PLAN

5.1 General Overview

Refer also to Appendix V

The general treatment plan will consist of chemotherapy for standard-risk patients and chemotherapy followed by HSCT for high risk patients. Remission induction for all patients comprises of three blocks of therapy wherein the first block is a novel immunotherapy regimen that includes cytotoxic chemotherapy, rituximab and infusion of haploidentical NK cells. Standard-risk patients will continue to

receive chemotherapy for a total duration of approximately 2 years. High-risk patients will be candidates for HSCT and will proceed to transplant once a suitable donor is found and the patient achieves negative MRD.

As the chemotherapy is intensive and participants with ALL will require supportive care measures and the majority of the participants will receive HSCT, placement of double-lumen Hickman line before starting the treatment is strongly recommended.

All participants with ALL receive remission induction. All high-risk participants will be offered HSCT, which will be performed after a suitable donor is identified and preferably after MRD becomes negative (see section 5.5). Standard-risk participants continue chemotherapy if MRD is negative after Block II of induction. If the assay is unsuccessful or the specimen is inadequate, the MRD will be assumed negative for purposes of treatment decisions and it will be handled as missing data for data analysis of MRD. Repeat MRD should be obtained as soon as possible.

Interim continuation treatment will be given to the occasional participants who are deemed unable to tolerate dose intensive chemotherapy. Specific criteria to use interim continuation therapy include disseminated fungal infection, recent development of cerebral thrombosis, or grade 3 or 4 renal or hepatic dysfunction. Interim treatment will consist of the plan for "interim continuation" as per section 5.3. Protocol specified therapy will be resumed when the participant's physical condition improves.

5.1.1 General assumptions regarding chemotherapy administration for all treatment phases:

- The timing and duration for administration for all commercially available agents are provided in the treatment phase sections as guidelines only. Variations in the timing and duration of chemotherapy infusions according to institutional practice or variations based on patient care needs are acceptable, as long as the treating investigator and/or PI determines that there was no impact on patient safety. These variations will not be considered protocol deviations, as long as the total dose is given within 10% of protocol specified dose.
- The term "day" does not refer to an absolute calendar day. It refers to a general 24-hour period as per St. Jude Nursing P&P.
- Medication dosing may be modified for research recipients based upon actual body weight or adjusted ideal body weight when clinically indicated. Criteria for medication calculations based on body weight/body surface area can be found in any version of the St. Jude Formulary. Medication doses may be rounded to the nearest integer or to the nearest appropriate quantity when clinically or pharmaceutically indicated as per the M.D. and Pharm.D.

5.1.2 NK cell therapy for collaborating site participants

NK cell therapy for participants from collaborating sites will be done at St. Jude. The patient and donor will need to travel to Memphis and stay for about 3 weeks. St. Jude will cover travel costs for the patient, donor and one parent, housing for up to 4 family members, and all medical costs during this time. St. Jude will also cover the travel costs for the NK cell donor.

5.1.3 Guidance during times of drug shortages and unavailability:

Treating investigators are urged to consult with the PI and to use their best clinical judgment in optimizing therapeutic intent and ensuring patient safety in managing the protocol-specified therapy. Although these decisions may constitute "Protocol Violations," they are unavoidable and made in consideration of the best interest of an individual patient. These will not be considered monitoring/audit findings if appropriately documented. All protocol deviations must be noted in the research database and the alterations in therapy due to the agent shortage will be captured. This should be accomplished by entering "dose modified" and details noted in the comments field. These deviations will also be noted in the Deviation Log with the notation "Drug substitution/reduction due to unavoidable drug shortage/unavailability".

5.2 Remission Induction

Required baseline evaluations:

- History and physical exam
- CBC with diff
- CMP, LDH, uric acid
- Coagulation screen
- Urine analysis
- Hepatitis and HIV screen
- EBV, CMV. HSV, varicella, histoplasma and toxoplasma titres
- Bone marrow aspirate for MRD, mutant cells, cytogenetics, biology and molecular studies
- Serum for asparaginase and anti-asparaginase antibodies
- CSF cell count, diff and cytology
- Chest X-ray
- CT scan of involved areas (lymphoma participants only)
- ECHO and EKG (can be performed within 1 week of initiation of therapy)
- QCT for bone density
- Peri-rectal swab for infectious disease research
- HLA typing
- KIR genotyping and NK cell phenotyping

- VNTR or variable nucleotide tandem repeat (chimerism studies)
- Pregnancy test of female adolescent patient

5.2.1 Block I - (approximately 5 weeks, until count recovery). **Block I therapy must be administered at St. Jude.**

Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
VCR DEX ITMHA	DEX	DEX	RITUX DEX	DEX (ITMHA)	CLO CYCLO VP16 DEX	CLO CYCLO VP16 DEX
Day 8	Day 9	Day 10	Day 11	Day 12	Day 13	Day 14
CLO CYCLO VP16 DEX ITMHA	CLO CYCLO VP16	CLO CYCLO VP16	IL-2 *(ITMHA)**	NK cell infusion	RITUX IL-2	
Day 15	Day 16	Day 17	Day 18	Day 19	Day 20	Day 21
IL-2		IL-2		IL-2	RITUX	PEG-ASP VCR DEX (ITMHA)
Day 22	Day 23	Day 24	Day 25	Day 26	Day 27	Day 28
DEX	DEX	DEX	DEX	DEX	RITUX DEX	VCR DEX ITMHA
Day 29	Day 30	Day 31	Day 32	Day 33	Day 34	Day 35
						PEG-ASP VCR**

RITUX (rituximab) - 375 mg/m^2/dose IV: Days 4, 13, 20, 27
CLO (clofarabine) - 40 mg/m^2/day IV: Days 6-10
CYCLO (cyclophosphamide) - 300 mg/m^2/day IV: Days 6-10
VP16 (etoposide) - 100 mg/m^2/day IV: Days 6-10
DEX (dexamethasone) - 8 mg/m^2/day divided TID PO or IV: Days 1-8 and Days 21-28
(Avoid dexamethasone for at least 3 days prior to NK cell infusion)
PEG-ASP (PEG-asparaginase) - 2500 units/m^2/dose IV: Day 21 and 35
VCR (vincristine) - 1.5 mg/m^2/dose (max. 2 mg) IV: Day 1, 21, 28, 35
IL-2 (interleukin-2) - 1 x 10^6 units/m^2 SQ QOD for 5 doses (Day 11-19)

*ITMHA will be repeated on Day 11 if Day 8 CSF is not clear. Additional ITMHA on Days 5 and 21 for participants with CNS2, CNS3 or traumatic taps with blasts. See Sections 5.2.1.2 and 5.10 for details of IT.
**Day 35 PEG-ASP and VCR may be skipped if counts recover sooner than Day 35.

See table 9 and appendix 5 for required research studies.

5.2.1.1 Enrollment of initial 5 participants

Enrollment of the initial participants will be done in a staggered fashion to ensure safety of the experimental treatment. For the first 5 enrollments, participants must be enrolled one at a time and each participant must complete therapy through Block I with recovery of counts prior to the next enrollment. If the safety profile is acceptable for the first 5 participants, enrollment will proceed as planned.

Recovery of counts is defined as: WBC $\geq 1500/mm^3$, ANC $\geq 300/mm^3$ and platelet count $\geq 50 \times 10^9/L$

5.2.1.2 Triple Intrathecal therapy (ITMHA) – Block I

- CNS1: Days 1, 8 and 28
- CNS2 and CNS3 and traumatic tap with blasts: Days 1, 5, 8, 21 and 28.
    - If CNS blasts are not cleared by Day 8, additional ITMHA will be given on day 11.

5.2.1.3 Bone marrow aspirate and MRD – Block I

Bone marrow aspirate/MRD will be performed after count recovery (WBC $\geq 1500/mm^3$, ANC $\geq 300/mm^3$ and platelet count $\geq 50 \times 10^9/L$

5.2.1.4 Participants who do not receive NK cell therapy for any reason (e.g., donor found to be ineligible after workup): IL-2 will NOT be given and chemotherapy Day 21 onwards may be moved up by a week to Day 14.

5.2.1.5 NK cell therapy – Block I

Note: When scheduling needs warrant, Day 11 of therapy onwards may be delayed for 1-3 days if necessary. Please contact PI or the transplant team for scheduling questions.

Donor selection and testing

1) HLA-typing in the HLA laboratory to confirm that the donor is greater than or equal to 3 of 6 HLA match to recipient.

2) KIR phenotyping and genotyping in Dr. Leung's laboratory (Mnemonic: NK TYPING). The results of these assays will not be used for donor selection (i.e., KIR matched or mismatched donors will be able to donate NK cells).

3) Donors are required to undergo screening, examination, and testing as described in 21 CFR 1271 and the Guidance "Eligibility Determination for Donors of Human Cells, Tissues, and Cellular and Tissue-Based Products (HCT/Ps)" to determine eligibility. This includes the following:

    a) Medically approved by a non-Department of Bone Marrow Transplantation and Cellular Therapy (St. Jude or non- St. Jude) to serve as an NK cell donor. Medical evaluation must be done within 60 days of NK cell collection (i.e. apheresis procedure).
    b) Medically cleared by the Blood Donor Center physician or designee to undergo apheresis within 7 days of apheresis procedure.
    c) Infectious disease testing within 7 days of apheresis procedure

Participants with ALL will receive NK cell transplantation from an adult family member who shares at least one HLA-haplotype with the recipient if available. Specific exclusion criteria include pregnancy and any other medical condition that, in the opinion of an independent physician, precludes performance of an apheresis procedure. If a suitable donor is not available, NK cell therapy as well as IL2 administration will be omitted. Subsequent chemotherapy can be initiated a week earlier (i.e., chemotherapy planned day 20 onwards can begin on day 14).

Histocompatibility testing is performed in a CLIA-certified, ASHI- and CAP-inspected laboratory of the Department of Pathology, SJCRH. Molecular techniques will be utilized to characterize donor and recipient HLA Class I gene content to the level sufficient to determine the ligand repertoire for KIR recognition.

NK cell collection and selection

On day 11 the donor will undergo apheresis once. The cells obtained will be purified for CD56+ cells by the two-step procedure described previously.[22] The CliniMACS selection column will be operated using the Standard Operating Procedures of the Human Applications Laboratory. For this protocol, our goal is to infuse immediately after processing on Day 12 all the NK cells collected to give $>2x10^6$ $CD56^+$ cells/kg of recipient body weight, but allowing for a $CD3^+CD56^-$ cell dose of no greater than $0.05x10^6$ /kg. The maximum dose of cells that would be infused is 400 x 10^6 CD56+ cells/kg. The minimum dose that would be infused is 0.1 x 10^6 cells/kg**.** The NK cell product will undergo quality control testing that includes assays for viability, sterility and purity before release for infusion following standard operating procedures of the Human Applications Laboratory.

Quality assurance for cellular products

The Department of Therapeutic Production and Quality has established an independent division of Quality Assurance (QA). This group is responsible for the management of Quality Control, Quality Assurance and Quality Improvement processes for the Human Applications Laboratory. Production and QA Systems that are in place include:

- Standard Operation Procedures (SOPs) for production and quality processes
- Documentation of Donor Eligibility
- Documentation of processes captured in Batch Records.
- In-process quality control testing including sterility
- Release Specifications established for all products.
- Out of Specification Reporting and Investigation Process
- Authorization by QA for the release of all products after review of records and release specification test results.
- Product labeling procedures with multiple person review.
- Variance Management Process
- Personnel Competence and Proficiency Program
- Inventory control and documentation of product history through patient infusion.

Test results that are out of specification for products that are needed on a clinically urgent basis will be evaluated by the laboratory medical director. The patient's physician or attending transplant physician will be informed of the test result prior to infusion of the product. Notification regarding positive sterility results before or after infusion will be given to the primary attending physician and patient and/or parent/guardian. Notification to the FDA and St. Jude IRB will be given and will include testing results or adverse events and any required intervention. An investigation following TPQ/HAL SOPs will be completed, reviewed by TPQ Quality Assurance and outcomes of the investigation reported.

5.2.2 Induction Block II (duration 3 weeks)

Note: patients with high-risk relapse who will require cranial irradiation prior to transplant will NOT receive this block of therapy in order to prevent neurological toxicity.

5.2.2.1 Drug dosages – Block II

Agent	Dosage and route	# Doses	Schedule
HDMTX (high dose methotrexate)	5 g/m^2 IV or targeted 65 µM	2	Days 1 and 8
6MP (mercaptopurine)	50 mg/m^2/day PO	21	Days 1 to 21

Block II will begin after count recovery from Block I (i.e., WBC ≥ 1500/mm^3, ANC ≥ 300/mm^3 and platelet count ≥ 50 x 10^9/L).

5.2.2.2 Intrathecal therapy – Block II

- ITMHA on day 1

5.2.2.3 Bone marrow aspirate/MRD – Block II

Bone marrow aspirate and MRD will be performed on Day 22, provided counts are satisfactory (WBC ≥ 1500/mm^3, ANC ≥ 300/mm^3 and platelet count ≥ 50 x 10^9/L.

High-risk participants will proceed to BMT once MRD is negative. They may continue to receive protocol directed therapy while awaiting transplant workup. All participants with positive MRD will proceed to receive Block III.

5.2.2.4 Mercaptopurine administration – Block II

Mercaptopurine should be given daily at a consistent time that maximizes adherence with the prescribed treatment. If a dose is inadvertently missed, then it should be given as soon as the omission is noted, as long as it is at least 6 hours prior to the next scheduled dose of MP.

In participants for whom high dose methotrexate treatment is delayed, mercaptopurine may be continued until 7 days after the last course of high dose methotrexate. Mercaptopurine may be held in the presence of ANC < 300/mm^3, WBC < 1,500/mm^3, platelet count < 50 x 10^9/L or grade 3 or 4 mucositis. Dosage of mercaptopurine may be reduced to 25 mg/m^2/day in participants who have developed neutropenia after the first high dose methotrexate and mercaptopurine treatment. See section 6.13 for modifications of mercaptopurine based on TPMT status.

5.2.2.5 HDMTX Administration – Block II

Patients will receive 5 gram/m^2 (or a dose targeted to achieve a steady-state plasma concentration of 65 µM) administered over 24 hours intravenously. The subsequent dose of high dose methotrexate and mercaptopurine will be delayed if ANC <300/mm^3, WBC < 1500/mm^3, platelet count < 50 x 10^9/L, SGPT >500 U/L, total bilirubin >2 mg/dl and direct bilirubin >1.4 mg/dl, or mucositis is present. For patients with Down syndrome, high dose methotrexate administration will be modified (see section 6.7.1)

HDMTX pre-hydration

At least two hours before high dose methotrexate, pre-hydration IV fluid (D5W + 40 mEq NaHCO3/L + 20 mEq KCl/L) will be administered at the rate of 200 ml/m^2/hr. At start of pre-hydration, one IV dose of NaHCO3 (25 mEq/m^2) diluted in 50 ml D5W will be given over 15 minutes. Pre-hydration fluid may also be given overnight at a rate of at least 125 ml/m^2/hr. High dose methotrexate treatment will follow, provided that urinary pH is ≥ 6.5; exceptions must be cleared with the pharmacokinetics service and the attending physician.

HDMTX infusion

Methotrexate loading dose will be given over 1 hour, followed immediately by maintenance infusion over 23 hours. During the methotrexate infusion, participants should receive hydration fluid with D5W + 40 mEq/L NaHCO3 + 20 mEq KCl/L at 125-150 ml/m^2/hr. Urine pH will be monitored with each void during infusion. An IV bolus of 12 mEq/m^2 NaHCO3 will be given if urine pH is 6.0; and 25 mEq/m^2 will be given if urine pH is <6.0. Acetazolamide 500 mg/m^2 orally every 6 to 8 hours may be used if systemic alkalosis limits the administration of bicarbonate for urinary alkalinization. Participants with evidence of renal dysfunction or delayed clearance during the methotrexate infusion may receive less than a 24 hour methotrexate infusion. Blood samples for methotrexate pharmacokinetics will be drawn as described below.

HDMTX leucovorin rescue

Leucovorin, 15 mg/m^2 (IV or PO) will be started at 42 hours after the start of methotrexate and repeated every 6 hours for a total of three to five doses, as determined by primary physician and Pharm D based on patient's prior treatment history or present condition. The dosage of leucovorin will be increased in participants with high plasma methotrexate concentrations (>1.0 µM at 42 hours) and continued until the methotrexate concentration is less than 0.10 µM. Additional measures, such as hydration, hemoperfusion, or carboxypeptidase will be considered in participants with 42-hour methotrexate levels > 10µM. Participants with a history of delayed Grade 3 or 4 gastrointestinal toxicity with

prior methotrexate or a history of typhlitis with any chemotherapy should have leucovorin continue for 5 doses; those with early toxicity should have leucovorin begin at 36 hours with subsequent methotrexate; if toxicity recurs, the baseline leucovorin dosage should also be increased.

See Appendix VIII, "HDMTX administration and monitoring guidelines, Induction Block II" for additional information.

5.2.3 Induction Block III (approximately 3 weeks until count recovery)

Block III will begin when WBC ≥ 1500/mm^3, ANC ≥ 300/mm^3 and platelet count ≥ 50 x 10^9/L.

5.2.3.1 High dose mitoxantrone and cytarabine (MA) – Block III

Agent	Dosage and route	# Doses	Schedule
ARA-C (cytarabine)	1 g/m^2 IV over 2 hours every 12 hours	8	Days 1-4
MITO (mitoxantrone)	12 mg/m^2 (0.4 mg/kg for participants < 10 kg) IV over 1 hour	3	Days 3-5

5.2.3.2 Drug dosages, schedules and routes – Block III

ARA-C (cytarabine): 1 g/m^2 IV over 2 hours every 12 hours on days 1-4 (8 doses)

MITO (mitoxantrone: 12 mg/m^2 (0.4 mg/kg for participants less than 10 kg) IV over 1 hour on days 3-5 (3 doses)

5.2.3.3 Intrathecal treatment – Block III

- ITMHA on Day 7

5.2.3.4 Conjunctivitis prophylaxis – Block III

Dexamethasone ophthalmic solution (0.1%), 2 drops to both eyes four times per day, or artificial tears (e.g., hydroxymethylcellulose, hypromellose, polyvinyl alcohol), 2 drops to both eyes every 2-6 hours, may be used during HDAC administration and for 24 hours after completion to prevent conjunctival irritation.

5.2.3.5 Bone marrow aspirate/MRD – Block III

Bone marrow aspirate and MRD will be performed after count recovery (WBC ≥ 1500/mm^3, ANC ≥ 300/mm^3 and platelet count ≥ 50 x 10^9/L.

5.3 Interim Continuation (4 weeks)

Interim continuation will begin when WBC ≥ 1500/mm^3, ANC ≥ 300/mm^3 and platelet count ≥ 50 x 10^9/L

Week	Agent	Dosage and route	# Doses	Schedule
1	VP16 (etoposide)	300 mg/m^2 IV	1	Day 1
	CYCLO (cyclophosphamide)	300 mg/m^2 IV	1	Day 1
2	MTX (methotrexate)	40 mg/m^2 IV	1	Day 1
	6MP (mercaptopurine)	75 mg/m^2 PO	7	Days 1-7
3	VM26 (teniposide)	200 mg/m^2 IV	1	Day 1
	ARA-C (cytarabine)	300 mg/m^2 IV	1	Day 1
4	DEX (dexamethasone)	12 mg/m^2 PO	15	Days 1-5 TID
	VINBLAST (vinblastine)	6 mg/m^2 IV	1	Day 1

5.3.1 Intrathecal therapy – Interim Continuation

ITMHA on Day 1 of week 1.

5.3.2 Mercaptopurine administration

Mercaptopurine should be given daily at a consistent time that maximizes adherence with the prescribed treatment. If a dose is inadvertently missed, then it should be given as soon as the omission is noted, as long as it is at least 6 hours prior to the next scheduled dose of 6MP

5.3.3 Dose modifications and delays – Interim Continuation

Chemotherapy except dexamethasone may be delayed or the dose will be reduced as follows:

- WBC ≥1,500/mm^3 and ANC ≥300/mm^3; give full dose
- WBC ≥ 1,000/mm^3 but <1,500/mm^3 and ANC >300/mm^3; reduce dose by 50%
- WBC < 1000/ mm^3 or ANC <300/ mm^3; hold chemotherapy
- Participants with a variant TPMT allele should receive no more than 60 mg/m^2 of 6MP

5.3.3 Bone marrow/aspirate/MRD – Interim Continuation

Bone marrow aspirate and MRD will be performed at the end of week 4 provided counts are satisfactory (WBC ≥ 1500/mm^3, ANC ≥ 300/mm^3 and platelet count ≥ 50 x 10^9/L).

5.4 Re-Induction Therapy

Approximately 3 weeks until count recovery.

5.4.1 Overview - Re-Induction treatment will begin when WBC ≥ 1500/mm^3, ANC ≥ 300/mm^3 and platelet count ≥ 50 X 10^9/L

Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
CLO CYCLO VP16 DEX ITMHA	CLO CYCLO VP16 DEX	CLO CYCLO VP16 DEX	CLO CYCLO VP16 DEX	CLO CYCLO VP16 DEX	PEG-ASP VCR DEX	
Day 8	Day 9	Day 10	Day 11	Day 12	Day 13	Day 14
					VCR	
Day 15	Day 16	Day 17	Day 18	Day 19	Day 20	Day 21
(ITMHA)					PEG-ASP VCR	

5.4.2 Drug dosages, schedules and routes – Re-Induction therapy

CLO (clofarabine)	40 mg/m^2/day: IV Days 1-5
CYCLO (cyclophosphamide)	300 mg/m^2/day: IV Days 1-5
VP16 (etoposide)	100 mg/m^2/day: IV Days 1-5
DEX (dexamethasone)	8 mg/m^2/day: PO Days 1-6
PEG-ASP (PEG-asparaginase)	2500 units/m^2/dose: IV Day 6 and 20
VCR (vincristine)	1.5 mg/m^2/dose (max. 2 mg): IV push Day 6, 13, 20

5.4.3 Intrathecal treatment – Re-Induction therapy

- CNS1: ITMHA on Day 1

- CNS 2, 3 and traumatic tap with blasts: ITMHA on Days 1,15

5.4.4 Bone marrow aspirate/MRD – Re-induction therapy

Bone marrow aspirate and MRD will be performed after count recovery (WBC ≥ 1500/mm^3, ANC ≥ 300/mm^3 and platelet count ≥ 50 x 10^9/L.

5.5 Continuation Treatment

5.5.1 Overview of Continuation treatment

Continuation treatment begins after completion of Re-Induction, provided that the ANC ≥ 300/mm^3, WBC ≥ 1500/mm^3 and platelet count ≥ 50 x 10^9/L as well as no evidence of grade 3 or 4 mucositis. Duration of continuation: 10 cycles of 8-week rotations for a total of 80 weeks. Entire duration of treatment is approximately 2 years.

Week	Agents
1	MTX + 6MP
2	MTX + 6MP
3	VM26 + ARA-C
4	DEX + VCR
5	MTX + 6MP
6	MTX + 6MP
7	VM26 + ARA-C
8	DEX + VINBLAST

5.5.2 Drug dosages, schedules and routes – Continuation treatment

MTX (methotrexate)	40 mg/m^2	1 dose, IV* day 1 (weeks 1, 2, 5 & 6)
6MP (mercaptopurine)	75 mg/m^2	days 1-7, PO (weeks 1, 2, 5 & 6)
VM26 (teniposide)	200 mg/m^2	1 dose, IV day 1 (weeks 3 & 7)
VCR (vincristine)	2 mg/m^2	(max 2 mg), 1 dose, IV push day 1 (week 4)
ARA-C (cytarabine)	300 mg/m^2	1 dose, IV day 1 (weeks 3 & 7)
DEX (dexamethasone)	12 mg/m^2	15 doses, PO day 1-5 TID (weeks 4 & 8)
VINBLAST (vinblastine)	6 mg/m^2	1 dose, IV day 1 (week 8)

**MTX should be given IM or as a 2 hour IV infusion if the participant has had previous cranial irradiation. Mercaptopurine should be given daily at a consistent time that maximizes adherence with the prescribed treatment. If a dose*

is inadvertently missed, then it should be given as soon as the omission is noted, as long as it is at least 6 hours prior to the next scheduled dose of MP

5.5.3 Intrathecal therapy – Continuation treatment

- CNS1: ITMHA on Day 1 of week 1 of all cycles
- CNS2, 3 and traumatic tap with blasts:
  - Cycles 1-5: ITMHA on Day 1 of week 1 and Day 1 of week 5
  - Cycles 6-10: ITMHA on Day 1 of week 1
  - Participants who are candidates for cranial irradiation will receive 4-5 ITMHAs during radiation therapy. These patients will not receive additional ITMHA following completion of radiation therapy. Lumbar puncture will be done every 16 weeks for surveillance only. See section 6.3.3.3 for details

5.5.4 Dose modifications and delays – Continuation treatment

Dexamethasone and vincristine can be given regardless of counts. For all other drugs, dose will be reduced as follows:

- WBC $\geq$1,500/mm^3 and ANC $\geq$300 /mm^3; give full dose
- WBC $\geq$ 1,000/mm^3 but <1,500/mm^3 and ANC $\geq$ 300 /mm^3; reduce the dose by 50%
- WBC < 1,000/mm^3 or ANC <300/mm^3; hold chemotherapy
- Participants with a variant TPMT allele should receive no more than 60 mg/m^2 of 6MP

5.5.5 Bone marrow aspirate/MRD – Continuation treatment

Bone marrow aspirate/MRD will be performed for all participants in week 1 of every 2 cycles (i.e. every 16 weeks) and at the end of therapy.

5.5.6 End of therapy evaluations

- CBC with diff
- CMP, LDH, uric acid
- Bone marrow aspirate and MRD
- CSF cell count, differential and cytology
- ECHO/EKG

5.6 Plan for HSCT

All high-risk participants are eligible for HSCT. HSCT will be performed as soon as MRD becomes negative (<0.01%). If MRD becomes negative and a donor has not been found, the patient will continue chemotherapy phases until a suitable donor is found. All attempts will be made to attain a negative MRD prior to HSCT. If MRD is persistently positive, the plan will be discussed with the PI and the transplant team. Donor will be selected according to institutional practices and

transplant regimens will be used according to institutional HSCT protocols and guidelines.

5.7 Prophylaxis and Treatment for CNS Disease

All participants will receive ITMHA. Irradiation will be given to some participants with CNS involvement at relapse, see section 6.3.

5.7.1 Criteria for CNS status

- CNS-1: No blasts in CSF
- CNS-2: < 5 WBC/μL of CSF with blasts
- CNS-3: ≥ 5 WBC/μL of CSF with blasts or cranial nerve palsy
- Traumatic tap with blasts: > 10 RBC/μL of CSF with blasts

5.7.2 Dosage of ITMHA

Age	< 1 year	1-2 years	2-3 years	> 3 years
methotrexate	6 mg	8 mg	10 mg	12 mg
hydrocortisone	12 mg	16 mg	20 mg	24 mg
cytarabine	18 mg	24 mg	30 mg	36 mg
total volume	6 ml	8 ml	10 ml	12 ml

Leucovorin 5 mg/m^2/dose, max 5 mg will be given PO or IV at 24 and 30 hours after each intrathecal treatment during induction Block I, Block III and Re-induction. Leucovorin may be given during other phases of therapy if the patient has an adverse reaction with previous intrathecal treatments or methotrexate treatment or is neutropenic.

5.8 Participation of St. Jude Collaborating Sites in the Treatment Plan

Block I Induction therapy will be administered at St. Jude. After completion of Block I Induction therapy, participants will return to collaborating site to complete the remainder of ALLR18 therapy.

5.9 Participation of St. Jude Affiliates the Treatment Plan

St. Jude participants may return to affiliate to receive some parts of ALLR18. Blocks I, II, III of induction and Re-induction therapies, and HSCT must be given at St. Jude. Participants may return to affiliates to receive interim continuation and continuation treatments.

5.10 Summary of Intrathecal Therapy on ALLR18

CNS Status	Induction-Block I		Induction – Block II		Induction – Block III		Interim continuation		Re-induction		Continuation		All phases
CNS-1	**Days**	**Total**	**Day**	**Total**	**Day**	**Total**	**Day**	**Total**	**Days**	**Total**	**Days**	**Total**	**Total –all phases**
	1, 8, 28	3	1	1	7	1	1	1	1	1	Cycles 1-10: Day 1 of Week 1	10	**17**
CNS-2, CNS-3, & Traumatic tap w/blasts	1, 5, 8, (11*), 21, 28	5 or 6*	1	1	7	1	1	1	1, 15	2	Cycles 1-5: Day 1, Week 1 & Day 1, week 5 Cycles 6-10: Day 1, week 1	15	**25** *(26 if given on Day 11 Block I)*

**If CNS blasts are not cleared by Day 8, additional ITMHA will be given on Day 11*

Those patients receiving CNS irradiation should receive 4-5 ITHMA during radiation. They will not receive subsequent ITMHA following radiation therapy. See section 6.3.3.3 - Concurrent chemotherapy with irradiation. Consult with PI and radiation oncologist.

6.0 TREATMENT MODIFICATIONS

6.1 Philadelphia Chromosome-Positive (Ph+) ALL

The use of tyrosine kinase inhibitors has improved the outcome of children with Ph-positive ALL[73]. Imatinib and dasatinib are the agents used most frequently in frontline therapy. Unfortunately, resistance develops commonly due to point mutations within the kinase binding domain of BCR-ABL. By using sensitive detection methods such as denaturing high-performance liquid chromatography, these mutations can be detected in a small sub-clone of leukemic cells in 40% of newly diagnosed patients, but in the dominant clone in 90% at relapse, thus implying selective pressure of the resistant clone after treatment with tyrosine kinase inhibitors.[74]

Ph-like ALL is a high-risk subtype of ALL with a gene expression profile similar to Ph-positive ALL, but lacking the Philadelphia chromosome.[75] Recent studies have demonstrated that a subset of these cases harbor a diverse range of genomic alterations that activate tyrosine kinases such as ABL1, ABL2, CSF1R and PDGFR.[76,77] In preclinical studies, Ph-like leukemic cells with targetable lesions are highly sensitive to tyrosine kinase inhibitors.[76,77] Various case reports also demonstrate that patients with Ph-like ALL respond well to TKIs despite resistance to standard chemotherapeutic agents.[78] In view of compelling preclinical data, TKIs will be added to frontline therapy for patients with Ph-like ALL in Total XVII as well as ongoing COG studies.

In addition to patients with Philadelphia chromosome (Ph)-positive ALL, patients with the Ph-like subtype of ALL and a targetable lesion will also receive a tyrosine kinase inhibitor. Testing for specific mutations will be done in a CLIA-approved laboratory and patients with fusions predicted to respond to ABL1 inhibitor will receive a TKI (ex. imatinib, dasatinib, nilotinib). Please discuss individual cases with the PI.

The choice of tyrosine kinase inhibitor in ALLR18 will be at the discretion of the treating physician and principal investigator dependent on prior exposure and resistance patterns. Examples include imatinib, dasatinib and nilotinib.

Tyrosine kinase inhibitors should be introduced after Day 20 of Induction Block I therapy to avoid drug interactions and interference with NK cell function. Consider holding during HDMTX in induction Block II to avoid cumulative toxicity. Dose should be adjusted to avoid myelosuppression to enable administration of therapy without interruption. Please discuss with the PI.

6.2 PEG-Asparaginase Allergies/Hypersensitivity

Participants with allergic reactions or intolerance to PEG-asparaginase will be given *Erwinia* L-asparaginase intramuscularly or intravenously over 30-60 minutes duration.[79] Each dose of PEG-asparaginase will be replaced by Erwinia at 25,000 units/m^2/dose thrice weekly (2 to 3 days apart, e.g. Monday, Wednesday and Friday).

Participants with possible allergic reactions should have measurements of anti-asparaginase antibodies and asparaginase at the time of the reaction and if re-challenged, after the next dose. Native *E. coli* L-asparaginase (L-asparaginase, Elspar®) may be given when clinically indicated, but this should be discussed and approved by the PI.

6.3 Irradiation for Participants with CNS Disease

6.3.1 Participants with CNS3 disease

Craniospinal irradiation will be given for participants whose first CR was less than 18 months and only cranial irradiation will be given for those whose CR was more than 18 months. The doses are 18 Gy cranial and 12 Gy spinal.

6.3.2 Participants with CNS2 disease

Not all participants with CNS2 disease will require irradiation. These cases will be discussed individually with the radiation oncologist and PI.

6.3.3 Timing of cranial/craniospinal irradiation

6.3.3.1 Participants who will not undergo HSCT (i.e. standard-risk participants): After completion of the 3rd course of continuation therapy (i.e. approximately one year from diagnosis of relapse). These participants will resume the protocol at cycle 4 of continuation after completion of irradiation but will not receive subsequent intrathecal chemotherapy. A lumbar puncture will be done every four months for surveillance only.

Methotrexate should be given IM or as a 2 hour IV infusion if the participant has had previous cranial irradiation.

6.3.3.2 Participants who will undergo HSCT (i.e. high-risk participants): Participants who will receive total body irradiation (TBI) for pre-transplant conditioning will receive a cranial irradiation boost (6 Gy) just prior to the TBI. Participants who will not receive TBI conditioning will receive cranial or craniospinal irradiation as per section 6.3.1 just prior to HSCT.

6.3.3.3 Concurrent chemotherapy with irradiation

Those participants receiving cranial irradiation should only receive 4 to 5 triple intrathecal therapy with leucovorin rescue during irradiation. Mercaptopurine and methotrexate will be withheld for at least one week prior to and during irradiation; systemic chemotherapy during irradiation will include dexamethasone and vincristine with or without PEG-asparaginase.

6.4 Treatment for Testicular Involvement

The plan for testicular irradiation for participants with testicular involvement will be discussed with the radiation oncologist and PI at the time of diagnosis of relapse and at the end of induction therapy. The need for testicular radiation will be determined for individual participants based on response to therapy, ultrasound findings and possibly testicular biopsy.

6.5 Obesity

Actual body weight will be used to calculate body surface area in all participants and used for dosage calculations (with the exception that vincristine dosage is capped at 2 mg).

6.6 Infants

With the exception of vincristine and mitoxantrone, all dosages given to infants (< 1 year) will be based on body surface area. For infants < 1 month of age, or for infants < 3 months of age born significantly prematurely (< 36 weeks gestation), a 50% reduction in dosages of asparaginase, etoposide, methotrexate, thiopurines, cyclophosphamide, clofarabine, and cytarabine should be made. The vincristine dosage for participants < 12 months of age or < 10 kg weight is 0.05 mg/kg/dose. The mitoxantrone dose for participants less than 10 kg is 0.4 mg/kg/dose

6.7 Down Syndrome Participants

Participants with Down syndrome should be closely monitored. Doses should be reduced (30% to 50%) as clinically indicated (especially dexamethasone and high-dose cytarabine, and for those who have higher than expected toxicity in earlier phases).

6.7.1 HD-MTX

Dosages of HD-MTX will be modified because participants with Down syndrome have altered MTX pharmacokinetics and enhanced tissue sensitivity to the effects of MTX. The hydration and alkalinization regimen should be the same as outlined in section 5.1.2. However, the dose of HD-MTX should be 500 mg/m^2 (50 mg/m^2 over 1 h and 450 mg/m^2 given over 23 h). Baseline leucovorin rescue will begin early (at hour 30 at 30 mg/m^2 IV q 6 h × 2 doses, followed by 10 mg/m^2 IV q6 h × 6 doses).

If MTX plasma levels are elevated, increased leucovorin rescue will be recommended by the Pharmacokinetics Service. Vigorous hydration should be ensured until the 42-hr MTX level is known.

6.7.2 Continuation low-dose MTX

The low-dose weekly MTX (40 mg/m^2) should be administered at full dosage if possible. If the patient has severe neutropenia or leucopenia (which delays subsequent therapy) or grade 4 mucositis (or mucositis which delays subsequent therapy) after being administered 40 mg/m^2 low-dose MTX, the dosage should be decreased to 30 mg/m^2, and further to 20 mg/m^2 and finally to 10 mg/m^2 if necessary. If 10 mg/m^2 is also not tolerated, then leucovorin should be added at 5 mg/m^2 every 6 h for 4 doses, starting 42 h from the MTX dosage, with titration to acceptable toxicity.

6.8 Participants with Renal Dysfunction

Subclinical renal impairment (normal serum creatinine but decreased GFR) may be present in participants receiving concurrent nephrotoxic drugs (e.g. IV acyclovir and vancomycin) which, if possible, should be held during and for 20 hours after HDMTX infusions or until adequate MTX clearance has been documented. Consideration to delaying MTX should be given if a patient's serum creatinine indicates renal impairment (e.g., glomerular filtration rate <50cc/min/1.73 m^2, however this will be a clinical decision, please contact PI, and/or PharmD).

In the event of toxicity secondary to high dose methotrexate, consider carboxypeptidase (glucarpidase).

6.9 Participants with Hepatic Dysfunction

6.9.1 Mitoxantrone, clofarabine, etoposide and vincristine

Dosages for mitoxantrone, clofarabine, etoposide and vincristine should be modified in participants with elevated direct bilirubin concentrations or other evidence of biliary obstruction.

- Direct bilirubin 2-4 mg/dl: 50% dosage decrease
- Direct bilirubin 4-6 mg/dl: 75% dosage decrease
- Direct bilirubin >6 mg/dl: withhold dose

6.9.2 PEG-Asparaginase

PEG-asparaginase may need to be withheld in participants with elevated direct bilirubin concentrations, especially if there is evidence of mucositis.

6.9.3 HDMTX – High dose methotrexate

HDMTX should be withheld if there is evidence of existing mucositis or if total bilirubin >2 mg/dl and direct bilirubin >1.4 mg/dl.

Subclinical hypertransaminasemia (SGPT >500 IU/L) is an indication to delay only high dose methotrexate but no other chemotherapy.

6.10 Cardiotoxicity

Hold mitoxantrone until fractional shortening is $\geq$ 28%. Consultation with cardiologist is suggested if clinically indicated.

6.11 Vinca Alkaloid Neurotoxicity

Mild vinca alkaloid toxicities are anticipated. These include jaw pain, constipation, decreased deep tendon reflexes. If there is persistent severe abdominal cramps, gait impairment, severe pain that requires narcotics or SIADH develops, the dose may be reduced to 50%. Motor paralysis or typhlitis will warrant temporary discontinuation of vinca alkaloids.

6.12 Pancreatitis

Acute hemorrhagic pancreatitis is a contraindication to continue PEG-asparaginase treatment. In the case of mild to moderate pancreatitis, PEG-asparaginase should be held until symptoms and signs subside, and amylase and lipase levels return to normal and then resumed. Any participants with abdominal pain suspected of pancreatitis should have serum amylase and lipase measured as well as an abdominal sonogram or CT scan done. In the case of severe pancreatitis (i.e. abdominal pain of 72 hours or more, amylase and lipase level three times or more of the upper limit of normal, and sonographic or CT scan evidence of pancreatitis), PEG-asparaginase may be discontinued permanently when the possibility of glucocorticoid- or mercaptopurine induced pancreatitis is excluded. In cases with mild to moderate pancreatitis (abdominal pain less than 72 hours and amylase and lipase level less than three times the upper limit of normal), PEG-asparaginase should be held and resumed once symptoms and signs subsided. Call the PI to discuss the management if the patient is asymptomatic (without abdominal pain) and has only elevated amylase or lipase levels. Consideration should be given to use native asparaginase (10,000 units/m^2 thrice weekly for 3 doses) for participants who are re-challenged. Consideration should also be given to dexamethasone- or mercaptopurine- related pancreatitis. Contact the PI to discuss the management if there is a possibility that the pancreatitis is due to either of these two drugs.

6.13 TPMT Status and Thiopurine Dosage

Participants who have at least one mutant TPMT allele often require dosage decreases of 6MP to avoid severe myelosuppression.[80,81] In a setting in which 6MP dosage was preferentially adjusted in about 1/3 of participants with TPMT defects, overall leukemia-free survival was outstanding among participants with TPMT defects.[82] Thus, we recommend that participants with phenotype or genotype consistent with at least one variant allele start mercaptopurine at 60 mg/m^2 and adjust the subsequent doses according to blood count and red blood cell 6 thioguanine nucleotide level (in consultation with Pharm D). For the participants who did not have TPMT testing in the frontline protocol, a blood sample (5-10 ml) will be drawn along with routine lab work at the start of induction Block B to allow for timely TPMT genotyping and/or phenotyping.[83] In participants in whom TPMT genotype and phenotype are discordant, or in whom suspected noncompliance, problems with toxicities or high blood counts are present, samples for red blood 6TGN and/or repeat TPMT activity may be measured. The dose of 6MP may be adjusted based on participants' TPMT status, 6TGN levels and WBC in consultation with Pharmaceutical Sciences.

7.0 SUPPORTIVE CARE GUIDELINES

These guidelines are provided to help physicians caring for participants treated on this protocol. They are guidelines and not protocol requirements. Nothing in these guidelines is intended to supplant the judgment of the treating physician regarding patient management. Current institutional practice may dictate other approaches to the management of the areas discussed in this section.

7.1 Management of Tumor Lysis Syndrome

It is suggested that baseline serum chemistries (uric acid, urea, creatinine, electrolytes, Ca, Mg, and PO_4) be obtained pre-treatment and frequently after therapy is started, and a central venous line (Double Lumen Hickman Catheter or DLHC) be inserted for fluid administration and monitoring.

Preventative measures

1. Give allopurinol 300 mg/m^2 daily divided TID. Alternatively, rasburicase may be given daily until the lysis period is over.
2. Intravenous fluids should be started at least 12 hours before chemotherapy, 3000 mL/m^2/day. **NO ADDED POTASSIUM.**
3. Phosphate binder for hyperphosphatemia as per institution guidelines

Suggested monitoring during Induction chemotherapy

1. Strict monitoring of fluid balance is essential.
2. Check blood pressure frequently.
3. Daily weights.

4. Frequent measurement of serum chemistries

7.2 Management of Hyperleukocytosis

In participants with WBC $\geq$ 400 x 10^9/L or symptoms of hyperviscosity, leukapheresis or exchange transfusion may be used according to local institutional guidelines.

7.3 Prophylaxis for Pneumocystis Jiroveci Pneumonia (REQUIRED)

All participants should receive TMP/SMZ (trimethoprim 150 mg/m^2/day in 2 divided doses on Monday, Tuesday, and Wednesday of each week). For those who cannot tolerate TMP/SMZ, monthly pentamidine may be substituted. Other options include atovoquone or dapsone. Please consult with clinical pharmacists and local institutional guidelines.

7.4 Prophylaxis for Fungal Infections (REQUIRED*)*

Participants with relapsed ALL are at especially increased risk for fungal infections, most commonly candidiasis and aspergillosis. Although there is no national standard for antifungal prophylaxis of these participants, effective regimens include voriconazole and posaconazole. Micafungin and caspofungin are acceptable alternatives, but fluconazole and itraconazole are not recommended because of lack of activity against Aspergillus. Antifungal prophylaxis should be initiated when ANC < 500 or <1000 and falling or predicted to fall in induction blocks I, III and re-induction and continue until count recovery (ANC >100 and rising).

All participants on ALLR18 must receive prophylactic antifungal therapy, although the agent(s) used may be based on local institutional guidelines. Acceptable antifungal prophylaxis options are listed below, in order of preference. Currently, we recommend voriconazole or posaconazole. Please consult with clinical pharmacists for therapeutic drug monitoring and refer to local institutional guidelines for voriconazole and posaconazole. Consult with the PI if other antifungal agents other than one listed below will be used.

Voriconazole and other azoles should be avoided during administration of vinca alkaloids and high dose methotrexate.

- Voriconazole
  - $\geq$ 1 – 11 years: 7 mg/kg/dose PO BID (rounded up to 50 mg or 100 mg dose increments)
  - $\geq$ 12 years, < 40 kg: 200 mg PO BID x 1 day then 100 mg PO BID
  - $\geq$12 years, $\geq$ 40 kg: 400 mg PO BID x 1 day then 200 mg PO BID
- Posaconazole
  - Oral suspension 40 mg/mL (recommend adequate oral intake or nutritional supplement with at least 14 g of fat and avoid H2 antagonist and proton pump inhibitors.

- Participants ≥ 13 years old: 200 mg PO 3 times a day with meals
- Participants < 13 years old: dose not established

Delayed-release tablets (do not cut or crush tablets and may administer without regards to meals)

- Participants ≥ 13 years old: 300 mg PO 2 times a day for 1 day, then 300 mg once daily
- Participants < 13 years old: dose not established

- Micafungin:
  - ≤ 40 kg: 1 mg/kg/day IV (max 50 mg/day) or per local institutional guidelines
  - 40 kg: 50 mg/day IV
- Liposomal amphotericin B IV: 3-5 mg/kg/day
- Caspofungin: 1 mg/kg/day IV (max 50 mg/day)

7.5 Prophylaxis for Bacterial Infections (REQUIRED)

Because patients with relapsed ALL are at high risk for bacterial sepsis, all participants on ALLR18 must receive prophylactic antibiotics. Prophylactic antibiotics should be started in induction blocks I, III and re-induction when the ANC < 500 or < 1000 and falling or predicted to fall, and continue until the ANC > 100 and rising. Antibiotics may be given by the parents or other caregivers at home, according to local institutional guidelines. Acceptable prophylactic regimens, in order of preference, include the following:

- Levofloxacin 10 mg/kg PO/IV every 12 hours if < 5 years of every 24 hours if ≥ 5 years (maximum 500 mg per dose)
- Vancomycin 400 mg/m^2/dose IV every 12 hours (maximum 1 gram per dose) plus ciprofloxacin 250-350 mg/m^2/dose PO every 12 hours (maximum 500 mg per dose)
- Cefepime 1500 mg/m^2/dose IV every 12 hours (maximum 2 gram per dose)
- Vancomycin 400 mg/m^2/dose IV every 12 hours (maximum 1 gram per dose) plus cefepime 1500 mg/m^2/dose IV every 12 hours (maximum 2 gram per dose). *Note that this regimen is not recommended by the PI, but is acceptable if preferred by the local institution or treating physician.*
- Please consult with the PI if you wish to use a prophylactic regimen other than one listed above. Because local infection rates, organisms, and susceptibilities vary, other prophylactic regimens will be allowed. However, regimens should be uniform at each collaborating

site and data on each regimen will be captured to explore the efficacy of each regimen.

7.6 Management of Febrile Neutropenia

Episodes of fever and neutropenia should be managed according to institutional guidelines. Participants with fever (defined as a single oral temperature ≥ 38.3° C (101°F) or temperature of ≥ 38.0° C (100.4° F) sustained over a one hour period and neutropenia (defined as ANC < 500 cells/μL) should be given IV antibiotics immediately. We recommend the following guidelines:

- Participants who develop fever while receiving prophylactic levofloxacin or vancomycin and ciprofloxacin:
  - Begin cefepime (1500 mg/m^2/dose IV every 8 hours)
  - Add or increase vancomycin to 400 mg/m^2/dose IV every 8 hours
  - Discontinue ciprofloxacin or levofloxacin

- Participants who develop fever while receiving prophylactic cefepime:
  - Begin meropenem (20 mg/kg/dose IV every 8 hours)
  - Add vancomycin (400 mg/m^2/dose IV every 8 hours) if recent history of receiving intensive chemotherapy that produces substantial mucosal damage
  - Discontinue cefepime

Participants who have suspected catheter-related infection, have evidence of sepsis (including shock, hypotension, rigors, septic emboli, unexplained respiratory distress or hypoxemia, or poor peripheral perfusion), or are known to be colonized by *Pseudomonas aeruginosa*, should also receive tobramycin, 60 mg/m^2/dose IV every 6 hours.

Participants who have severe abdominal pain or radiographic findings suggesting typhlitis, severe abdominal pain with evidence of sepsis, focal findings suggestive of intra-abdominal infection on physical examination, or known or suspected infection with *Bacillus cereus* should receive meropenem, 20 mg/kg/dose IV every 8 hours, instead of cefepime.

7.7 Growth Factors

Prophylactic use of hematopoietic growth factors (GM-CSF or G-CSF) is not recommended. However, GM-CSF (250 μg/m^2/day) or G-CSF (5-10 μg/kg/day) may be considered for participants who have life threatening fungal infections or bacterial sepsis after discussion with PI.

7.8 Management of Capillary Leak Syndrome Related to Clofarabine

In pediatric studies, during or shortly after clofarabine administration a few participants developed signs and symptoms consistent with capillary leak

syndrome. In these heavily pretreated participants, it has been difficult to separate potential drug-related cases of capillary leak syndrome from concurrent medical conditions such as infection/sepsis, progressive disease, or other underlying problems resulting from prior anti-leukemic therapies. For these reasons, during and after each dose of clofarabine investigators are to assess participants for the onset of the following signs or symptoms ≥ grade 2:

- Tachypnea or other evidence of respiratory distress;
- Unexplained hypotension; and/or
- Unexplained tachycardia.

If one or more of these signs or symptoms occurs during study drug infusion, clofarabine administration is to be interrupted or held as clinically indicated. It is recognized that the total infusion time for this clofarabine dose in this circumstance may exceed 1 hour. Thus, if the patient's condition stabilizes or improves, clofarabine administration may resume. Supportive care, strict monitoring of intake and output and investigation for other etiologies of the signs and symptoms, such as infection/sepsis, must be pursued.

7.9 Etoposide Reactions

7.9.1 Cardiovascular effects - Transient hypotension has occurred in about 1 to 2 % of participants following rapid IV administration of etoposide during clinical trials. However, hypotension has not been associated with cardiac toxicity or electrocardiogram changes. Blood pressure usually normalizes within a few hours after discontinuation of the infusion. To avoid this complication, etoposide should be infused over 30 – 60 minutes. If hypotension should occur, stop the infusion, and if necessary, give 10 mL/kg NS bolus over 15 minutes. Repeat as necessary. Once symptoms resolve, resume infusion at ½ the previous infusion rate until the full dose administered. If hypotension recurs, stop infusion and administer 10 mL/kg NS bolus as indicated. Once hypotension resolves, resume infusion at ½ previous infusion rate until complete. Consider infusing NS at 1 – 1.5 x maintenance during remainder of infusion. For all subsequent doses, further dilute and infuse over 2 hours.

7.9.2 Sensitivity reactions - Anaphylactoid reactions consisting principally of chills, rigors, diaphoresis, pruritis, loss of consciousness, nausea, vomiting, fever, bronchospasm, dyspnea, tachycardia, hypertension, and/or hypotension have occurred in 0.7 – 3% of patients receiving etoposide. Other manifestations include flushing, rash, substernal chest pain, lacrimations, sneezing, coryza, throat pain, back pain, abdominal cramps, and auditory impairment. Facial/lingual swelling, coughing, diaphoresis, cyanosis, tightness in the throat, and laryngospasm have also occurred. If an anaphylactoid reaction should occur:

1. Stop the infusion immediately and notify H/O Fellow or Attending MD
2. Administer the following as indicated:
    a) diphenhydramine 1mg/kg IV (max dose 50 mg)

b) hydrocortisone 50 – 100 mg/m^2 IV
c) epinephrine to be administered according to institutional policy
d) fluid bolus 10 mL/kg NS infused over 15 minutes

3. Once symptoms have resolved, resume infusion at ½ previous rate until infusion complete. Consider infusing NS at 1 – 1.5 x maintenance during remainder of infusion.
4. If anaphylaxis recurs, stop the infusion and re-treat as above. Do not administer remainder of dose. Consider substituting etoposide with etoposide phosphate (Etopophos®) for all subsequent doses.
5. If anaphylaxis does not recur, pre-medicate all subsequent doses with diphenhydramine 1mg/kg (max 50 mg) and hydrocortisone 50 – 100 mg/m^2. Consider slowing the loading dose to be administered over 1 hour.
6. Have at bedside all of the following for all subsequent infusions:
    a) Diphenhydramine 1mg/kg IV (max 50 mg)
    b) Hydrocortisone 50 – 100 mg/m^2 IV
    c) epinephrine to be administered according to institutional policy

Please note: anaphylactoid reactions are still possible with etoposide phosphate. If the patient cannot tolerate the substitution, drug is contraindicated and must be discontinued.

7.10 Drug Interactions

Because concurrent use of enzyme inducing anticonvulsants (e.g. phenytoin, phenobarbital, and carbamazepine) with anti-leukemic therapy has recently been associated with inferior EFS, every effort should be made to avoid these agents, as well as rifampin, which also induces many drug metabolizing enzymes. Gabapentin does not induce hepatic drug metabolizing enzymes and may be a suitable alternative anticonvulsant. Azole antifungals (fluconazole, itraconazole, voriconazole, and ketoconazole) and the macrolide group of antibiotics (e.g. erythromycin, rifampin, and zithromax) may have potent inhibitory effects on drug-metabolizing enzymes, and the doses of some antileukemic drugs (e.g. vincristine, anthracyclines, steroids, etoposide) may need to be reduced in some patients on chronic azole antifungals or antibiotics. Consult Pharmacokinetics if long-term use of these interacting drugs is unavoidable.

Penicillins interfere with tubular excretion of methotrexate, and it is recommended that an alternative non-penicillin antibiotic be used.

Ph+ participants receiving tyrosine kinase inhibitors (e.g. imatinib, dasatinib and nilotinib) should not receive drugs known prolong the QT interval and should avoid strong CYP3A4 inhibitors. Participants should also avoid food 2 hours before and 1 hour after taking dose.

7.11 Cytokine Release Syndrome Related to Administration of IL-2 and NK cells

Cytokine release syndrome is a disorder characterized by nausea, headache, tachycardia, hypotension, rash and shortness of breath. It is caused by the release of cytokines from the cells. IL-2 will be discontinued for cytokine release syndrome > grade 2. Supportive care with IV fluids, correction of hypoalbuminemia with albumin infusions and close monitoring should be performed. In severe cases, corticosteroids may be required. Corticosteroids should be avoided (if possible) during the 3 days prior to NK cell infusion or during the first 7 days after the infusion.

8.0 DRUG/DEVICE/BIOLOGIC AGENT INFORMATION

See Appendix III for information on the individual drugs to be used in this protocol.

9.0 REQUIRED EVALUATIONS, TESTS, AND OBSERVATIONS

	Baseline	Induction Block I	Induction Block II	Induction Block III	Interim continuation	Re-induction	Continuation	Off therapy
H&P	X	Q 3-7 days	Q 3-7 days	Q 3-7 days	Q week	Q 3-7 days	Q week	X
CBC with diff	X	Q 3-7 days	Q 3-7 days	Q 3-7 days	Q week	Q 3-7 days	Q week	X
CMP	X	Q 3-7 days	Q 3-7 days	Q 3-7 days	As indicated	Q 3-7 days	As indicated	X
Coagulation screen	X							
Urine analysis	X							
Hepatitis, HIV screen	X							
EBV, CMV, HSV, varicella, histoplasma and toxoplasma titres	X							
Bone marrow for MRD and mutant cells	X	End of block	End of block	End of block	End of block	End of block	Every 16 weeks	X
Bone marrow for biology studies	X							
#Peripheral blood for MRD		Day 6						
#Peripheral blood for CD20	X	Days 2, 4, 6, 11, 14 and 21						
Serum for asparaginase and anti-asparaginase antibodies	X	Days 21 & 35 (pre-PEG each day)			Day 1 of week 1	Days 6 & 20 (pre-PEG each day)		
*TPMT genotyping		Day 3						
Germline DNA		Day 3	Day 1					
CSF cell count, diff, cytology	with each IT	with each IT	with each IT	with each IT	with each IT	with each IT	with each IT	with each IT
Chest X-ray	X							
ECHO/EKG	X							X
QCT for bone density	X							5 years after completion of therapy
CT scan of involved areas (lymphoma pts. only)	X			X (end of Block III)				X

	Baseline	Induction Block I	Induction Block II	Induction Block III	Interim continuation	Re-induction	Continuation	Off therapy
HLA typing of patient, parents and full siblings	X							
KIR genotyping (recipient and donor)	X							
NK cell phenotype (recipient)	X	Day 19						
NK cell phenotype (donors)	X							
NK Chimerism (recipient)		Day 19						
Variable nucleotide tandem repeat (recipient and donor)	X							
Pregnancy test, if applicable								
Donor evaluations and services as per "NK Donor Pre-Evaluations and Scheduled Evaluations Prior to Cell Collection" standard order set	X							

Peripheral blood MRD and Peripheral blood CD20 evaluations will be discontinued for a patient following a negative MRD result (i.e., less than 0.01%)

*TPMT genotyping will be sent if previous results are not available from frontline therapy

Participants with possible allergic reactions to peg-asparaginase should have measurements of anti-asparaginase antibodies and asparaginase at the time of the reaction, and again if re-challenged (see Section 6.2)

See Appendix I for listing of standard of care versus research studies

9.1 Response Evaluations

Bone marrow aspiration for microscopic evaluation and MRD by flow cytometry will be performed at the following time points:

- End of block I of induction
- End of block II of induction
- End of Block III of induction
- End of interim continuation
- End of re-induction
- Every 16 weeks during continuation
- End of therapy
- As clinically indicated to plan for transplant or for suspicion of relapse

9.2 Suggested Long-Term Follow-up Evaluations

The following evaluations are recommended for participants who are off-treatment after completion of protocol specified therapy:

- It is recommended that participants will be followed every 4 months for 1 year, every 6 months for 1 year and then yearly until the patient is in remission for 10 years and is at least 18 years old. Thereafter, the patient will become alumni and will be followed according to the institution's policy.
- During the St. Jude visit, CBC with differential and other laboratory studies as clinically indicated will be obtained.
- It is recommended that QCT for bone density be performed at 5 years off-therapy.
- Participants will be considered off therapy 30 days after last treatment taken. Adverse events will not be reported while patient is off-therapy unless they are unanticipated problems (see Section 12.0) deemed related to therapy by the site PI.
- After attaining continuous, complete remission for 5 years or more, SJCRH participants will be invited to participate in the long-term follow-up umbrella protocol and may be referred to the After Completion of Therapy Clinic (ACT). Collaborating sites will follow participants as per local guidelines.
- When a SJCRH patient has been in remission for 10 years and is at least 18 years of age, the patient will become SJCRH alumni and will be followed according to the SJCRH institutional policy.

Note: Participants removed from therapy for other reasons (i.e., relapse, toxicity, etc.) before treatment is completed will be followed for survival only, and evaluations will be done as per treating physician's/institution's standard practice.

10.0 EVALUATION CRITERIA

10.1 Response Criteria for Leukemia

The response after each course of chemotherapy will be determined by the examination of the bone marrow. For the purposes of this protocol, MRD-negative is defined as < 0.01% blasts with a leukemia associated phenotype detected by flow cytometry. Because morphologic examination of the bone marrow during periods of hematopoietic recovery after intensive chemotherapy may be unreliable, response will be based on blast percentage by flow cytometry. Blast percentages determined by morphology will be used in cases that are not evaluable by flow cytometry. If the blast percentage is less than 5% in such cases, they will be classified as complete response, MRD not evaluable.

10.1.1 Complete response (CR), MRD-negative
< 0.01% blasts by flow cytometry

10.1.2 Complete response (CRM), MRD-positive 0.01% to < 5% blasts by flow cytometry

10.1.3 Partial response (PR) - A decrease of at least 50% in the percentage of blasts and 5% to 25% blasts by flow cytometry.

10.1.4 No response (NR) - No change in clinical or laboratory status.

10.1.5 Progressive disease (PD): Deterioration of initial disease status

10.1.6 Relapse

- Bone marrow relapse: Subsequent appearance, after achievement of CR, of ≥ 5% blasts in the bone marrow with confirmation by flow cytometry
- CNS relapse: ≥ 5 WBC/μL of CSF with definite blasts on cytospin
- Other extra-medullary relapse: Development of extra-medullary disease after achievement of CR.

10.2 Response Criteria for Lymphoblastic Lymphoma

10.2.1 Complete response (CR) - No clinical or radiographic evidence of disease with M1 marrow (<5% blasts) status and with normal CSF and hemogram.

10.2.2 Partial response (PR) - defined as >50% but <100% decrease in the sum of the product of the maximum perpendicular diameter of all lesions and decrease in bone marrow involvement by tumor cells by at least 50%

10.2.3 No response (NR) - No change in clinical or laboratory status, response not qualifying for PR or Progressive disease

10.2.4 Progressive Disease (PD) - Worsening of disease, evidenced by >25% increase in the size of any lesion, increased organomegaly or appearance of new disease.

10.3 Toxicity Evaluation Criteria

Common Terminology Criteria for Adverse Events v4 (CTCAE): This study will utilize the CTCAE of the National Cancer Institute (NCI) for toxicity and performance reporting. A copy of the current version of the CTCAE can be downloaded from the Cancer Therapy Evaluation Program (CTEP) home page (http://ctep.info.nih.gov). Additionally, toxicities are to be reported on the appropriate data collection screens/forms.

10.4 Acceptable Percentage of Missed Doses for Commercially Available Drugs

It is understood that deviations from the protocol should be avoided, except when necessary to eliminate an immediate hazard to a research participant. In such case, the deviation must be reported to the IRB. However, it is expected that participants will occasionally miss some doses or receive the wrong dose of oral chemotherapy. Compliance with oral medication will be captured in the CRIS database and appropriately documented in the participants' medical records. Appropriately documented doses of missed or wrong doses of chemotherapy will not constitute a deviation unless the amount in question is over *10%* of the expected total dose due in the respective protocol cycles (these are specified in the CRIS ALLR18 database). Missed doses do not include doses held or reduced for medical reasons (toxicity, illness) and will not be considered protocol deviations or violations.

11.0 OFF THERAPY AND OFF STUDY CRITERIA

11.1 Off-Study Criteria

- Death
- Withdrawal of consent
- Found to be ineligible (e.g., incorrect diagnosis)
- For St. Jude participants: completion of protocol-required evaluations and follow-up period (10 years), then survival outcomes will be followed per SJLTFU. For participants from collaborating sites, participants will be followed for survival status and relapse until one of the above criteria are met (e.g. death, withdrawal of consent, found to be ineligible).
- For donors: 8 days after apheresis, or sooner if he/she is deemed ineligible after donor workup.

11.2 Off-Treatment Criteria

Off-treatment participants will continue to be followed for survival status until an off-study criterion is met. The first relapse/progression will be documented and grade 3-4 adverse events will be followed until they have resolved to ≤ grade 2, or until the participant receives other anti-leukemia/lymphoma therapy.

- Research participants who fail to achieve complete remission after induction block III of treatment.
- Relapse as defined in section 10.1.6.
- Second malignancy
- Development of unacceptable toxicity during treatment (with concurrence of the PI)
- Participant/family decision to withdraw from protocol treatment at any time for any reason
- Discretion of the study PI, such as the following:
  - The researcher decides that continuing in the study would be harmful
  - A treatment is needed that is not allowed on this study
  - The participant misses so many appointments that the data cannot be used in the study
  - The participant's condition gets worse
  - New information is learned that a better treatment is available, or that the study is not in the participant's best interest
- 30 days after completion of all protocol prescribed treatment, or if participant proceeds to transplant, off treatment date will be defined as the first day of conditioning therapy.

12.0 SAFETY AND ADVERSE EVENT REPORTING REQUIREMENTS

12.1 Reporting Adverse Experiences (AEs) and Deaths on Treatment

Only "unanticipated problems involving risks to participants or others" referred to hereafter as "unanticipated problems" are required to be reported to the St. Jude IRB promptly, but in no event later than 10 working days after the investigator first learns of the unanticipated problem. Regardless of whether the event is internal or external (for example, an IND safety report by the sponsor pursuant to 21 CFR 312.32), only adverse events that constitute unanticipated problems are reportable to the St. Jude IRB. As further described in the definition of unanticipated problem, this includes any event that in the PI's opinion was:

- Unexpected (in terms of nature, severity, or frequency) given (1) the research procedures that are described in the protocol-related documents, such as the IRB-approved research protocol and informed consent document, as well as other relevant information available about the research; (2) the observed rate of occurrence (compared to a credible

baseline for comparison); and (3) the characteristics of the subject population being studied; and
- Related or possibly related to participation in the research; and
- Serious; or if not serious suggests that the research places subjects or others at a greater risk of harm (including physical, psychological, economic, or social harm) than was previously known or recognized.

Unrelated, expected deaths do not require reporting to the IRB. Though death is "serious", the event must meet the other two requirements of "related or possibly related" and "unexpected/unanticipated" to be considered reportable.

Deaths meeting reporting requirements are to be reported immediately to the St. Jude IRB, but in no event later than 48 hours after the investigator first learns of the death.

The following definitions apply with respect to reporting adverse experiences:

Serious Adverse Event: Any adverse event temporally associated with the subject's participation in research that meets any of the following criteria:

- results in death;
- is life-threatening (places the subject at immediate risk of death from the event as it occurred);
- requires inpatient hospitalization or prolongation of existing hospitalization;
- results in a persistent or significant disability/incapacity;
- results in a congenital anomaly/birth defect; or
- any other adverse event that, based upon appropriate medical judgment, may jeopardize the subject's health and may require medical or surgical intervention to prevent one of the other outcomes listed in this definition (examples of such events include: any substantial disruption of the ability to conduct normal life functions, allergic bronchospasm requiring intensive treatment in the emergency room or at home, blood dyscrasias or convulsions that do not result in inpatient hospitalization, or the development of drug dependency or drug abuse), a congenital anomaly/birth defect, secondary or concurrent cancer, medication overdose, or is any medical event which requires treatment to prevent any of the medical outcomes previously listed.

Unexpected Adverse Event:

- Any adverse event for which the specificity or severity is not consistent with the protocol-related documents, including the applicable investigator brochure, IRB approved consent form, Investigational New Drug (IND) or Investigational Device Exemption (IDE) application, or other relevant sources of information, such as product labeling and package inserts; or if it

does appear in such documents, an event in which the specificity, severity or duration is not consistent with the risk information included therein; or

- The observed rate of occurrence is a clinically significant increase in the expected rate (based on a credible baseline rate for comparison); or
- The occurrence is not consistent with the expected natural progression of any underlying disease, disorder, or condition of the subject(s) experiencing the adverse event and the subject's predisposing risk factor profile for the adverse event.

Internal Events: Events experienced by a research participant enrolled at a site under the jurisdiction of St. Jude IRB for either multicenter or single-center research projects.

External Events: Events experienced by participants enrolled at a site external to the jurisdiction of the St. Jude Institutional Review Board (IRB) or in a study for which St. Jude is not the coordinating center or the IRB of record.

Unanticipated Problem Involving Risks to Subjects or Others: An unanticipated problem involving risks to subjects or others is an event which was not expected to occur and which increases the degree of risk posed to research participants. Such events, in general, meet all of the following criteria:

- unexpected;
- related or possibly related to participation in the research; and
- suggests that the research places subjects or others at a greater risk of harm (including physical, psychological, economic, or social harm) than was previously known or recognized. An unanticipated problem involving risk to subjects or others may exist even when actual harm does not occur to any participant.

Consistent with FDA and OHRP guidance on reporting unanticipated problems and adverse events to IRBs, the St. Jude IRB does not require the submission of external events, for example IND safety reports, nor is a summary of such events/reports required; however, if an event giving rise to an IND safety or other external event report constitutes an "unanticipated problem involving risks to subjects or others" it must be reported in accordance with this policy. In general, to be reportable external events need to have implications for the conduct of the study (for example, requiring a significant and usually safety-related change in the protocol and/or informed consent form).

Although some adverse events will qualify as unanticipated problems involving risks to subjects or others, some will not; and there may be other unanticipated problems that go beyond the definitions of serious and/or unexpected adverse events. Examples of unanticipated problems involving risks to subjects or others include:

- Improperly staging a participant's tumor resulting in the participant being assigned to an incorrect arm of the research study;
- The theft of a research computer containing confidential subject information (breach of confidentiality); and
- The contamination of a study drug. Unanticipated problems generally will warrant consideration of substantive changes in the research protocol or informed consent process/document or other corrective actions in order to protect the safety, welfare, or rights of subjects or others

12.2 Recording AEs and SAEs

Adverse events (AEs) will be evaluated and documented by the clinical staff and investigators throughout inpatient hospitalizations and each outpatient visit. CRAs are responsible for reviewing documentation related to AEs and entering directly into CRIS protocol-specific database. The data to be recorded are 1) the event description, 2) the NCI CTCAE v4.0 code and grade, 3) the onset date, 4) the resolution date (or ongoing), 4) action taken for event, 5) patient outcome 6) relationship of AE to protocol treatment/interventions, 7) if AE was expected or unexpected, and 8) comments, if applicable. AEs that are classified as serious, unexpected, and at least possibly related will be notated as such in the database as "SAEs". These events will be reported expeditiously to the St. Jude IRB within the timeframes as described above.

Cumulative summary of Grades 1-5 events will be collected in Induction Block I; and Grade 3-5 events will be reported throughout rest of the protocol therapy as part of the progress reports to IRB at the time of continuing review. Hematological adverse events will not be collected in database. Specific data entry instructions for AEs and other protocol-related data will be documented in protocol-specific data entry guidelines, which will be developed and maintained by study team and clinical research informatics.

The study team will meet regularly to discuss AEs (and other study progress as required by institutional DSMP). The PI will review Adverse Event reports generated from the research database, and corrections will be made if applicable. Once the information is final the PI will sign and date reports, to acknowledge his/her review and approval of the AE as entered in the research database.

12.3 Reporting Requirements to the FDA

Any unexpected fatal or unexpected life-threatening event judged by the PI to possibly be due to the investigational agent, will be reported to the FDA by telephone or fax as soon as possible but no later than seven calendar days after notification of the event and followed by a written safety report as complete as possible within eight additional calendar days (i.e. full report 15 calendar days total after notification of event).

Unexpected, non-fatal and non-life-threatening SAEs, which occur in on-study participants during the time periods specified in Section 9.1 that are considered due to or possibly due to the investigational agent, will be reported to the FDA by written safety report as soon as possible but no later than 15 calendar days of the notification of the occurrence of the event. Expected SAEs, even unexpected fatal SAEs, considered by the PI to be not related to the study, will be reported to the FDA in the Annual Review Report along with non-serious AEs. All FDA correspondence and reporting will be conducted through the St. Jude Office of Regulatory Affairs.

12.4 Reporting to St. Jude Regulatory Affairs Office (RAO)

Copies of all correspondence to the St. Jude IRB, including SAE reports, are provided to the St. Jude Regulatory Affairs office by the St. Jude study team. FDA-related correspondence and reporting will be conducted through the Regulatory Affairs office.

12.5 Reporting AEs to and from St. Jude and Collaborating Sites/Affiliates

Adverse events from collaborating sites will also be reviewed by the PI and discussed in study team meetings as described above. SAE report from collaborating sites for AEs that are serious, unexpected, and at least possibly related to protocol treatment or interventions will be reported to site IRB and the St. Jude IRB within the reporting requirements described above. The PI will determine if this is an event that will need to be reported expeditiously to all participating sites, considering the following criteria:

- Is the AE serious, unexpected, and related or possibly related to participation in the research?
- Is the AE expected, but occurring at a significantly higher frequency or severity than expected?
- Is this an AE that is unexpected (regardless of severity that may alter the IRB's analysis of the risk versus potential benefit of the research *and*, as a result, warrant consideration of substantive changes in the research protocol or informed consent process/document?

With the submission of the "Reportable Event" in St. Jude TRACKS application, the PI will indicate if all sites should be notified to report to their IRBs, and if the protocol and/or consent should be amended (consent will be amended if event is information that should be communicated to currently enrolled subjects). Generally, only events that warrant an amendment to the protocol and/or consent will be reported expeditiously to all sites. However, any event may be reported expeditiously to all sites at the discretion of the PI.

A cumulative summary of Grades 1-5 adverse events that occur during Block I, Grade 3-5 AEs adverse events during remainder of protocol therapy, and expected/unrelated deaths that occur more than 30 days off last protocol treatment

will be reported to all sites with study progress report at the time of continuing review.
For collaborating sites: Serious AND unexpected events are to be reported to the St. Jude PI (Dr. Sima Jeha) within 48-72 hours via fax or email.

Unexpected deaths must be reported to the St. Jude PI via phone call or email within 24 hours of the event. A written report must follow.

All correspondence and reports must also be sent by email to **ALLR18 study team**.

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13.0 DATA COLLECTION, MONITORING AND CONFIDENTIALITY

13.1 Data Collection

Electronic case report forms (e-CRFs) will be completed by the SJCRH Leukemia/Lymphoma CRAs. Data will be entered from record directly into a secure CRIS database, developed and maintained by St. Jude Clinical Research Informatics.

Data Management will be supervised by the Director of Clinical Trials Management, and Manager of Clinical Research Operations for the Leukemia/Lymphoma Division, working with Dr. Jeha or her designee. All protocol-specific data and all grade 3-5 adverse events will be recorded by the clinical research associates into the CRIS database, ideally within 2-4 weeks of completion of study phase. All questions will be directed to the attending physician and/or PI and reviewed at regularly-scheduled working meetings. The attending physicians (or their designees) are responsible for keeping up-to-date roadmaps in the patient's primary SJCRH medical chart.

Regular (at least monthly) summaries of toxicity and protocol events will be generated for the PI and the department of Biostatistics to review

13.2 Data Collection Instructions for Collaborating Sites

Collaborating sites will collect abstract data from medical records and submit by using e-CRFs via remote electronic data entry. All protocol-specific data and all grade 3-5 adverse events will be recorded by the clinical research associates into the CRIS database, ideally within 2-4 weeks of completion of study phase.

13.3 Study Monitoring

This study is considered high risk (HR-3) for monitoring purposes. Protocol and regulatory compliance, including essential regulatory documentation, will be assessed as well as the accuracy and completeness of all data points relating to the primary and secondary objectives semi-annually. If the study design has strata, accrual will be tracked continuously. The first two enrollees will be monitored and 15% of the study enrollees thereafter, semi-annually.

The PI and study team are responsible for protocol and regulatory compliance, and for data accuracy and completeness. The study team will meet at appropriate intervals to review case histories or quality summaries on participants and retain copies of the minutes that are signed by the PI.

The Eligibility Coordinators in the Central Protocol and Data Monitoring Office (CPDMO) will verify informed consent documentation and eligibility status on 100% of St. Jude participants within 5 working days of enrollment completion.

The Clinical Research Monitor (CRM) will verify informed consent documentation and eligibility status of all non-St. Jude participants and perform a quality verification of select St. Jude participants during routine monitoring intervals (every 6 months). Overall study conduct, compliance with primary and secondary objectives, age of majority consenting, safety assessments and reporting, and the timeliness and accuracy of database entries are monitored routinely.

Study documents routinely monitored on selected participants include medical records, database entries, study worksheets, and case report forms. Study documents are monitored for participant status, demographics, staging, subgroup assignment, treatments, investigational drug accountability, evaluations, responses, participant protocol status, off-study and off-therapy criteria, and for all other specifics as detailed in a separate study-specific monitoring plan. The study-specific monitoring plan may be revised over time, to adapt monitoring frequency and/ or intensity to a changing environment when appropriate (for example: new safety signals; positive history of compliance; all participants are in long term follow-up; or the enrollment period has ended).

The recording and reporting of Adverse Events, Serious Adverse Events (SAEs), and Unanticipated Problems (UPs) to include type, grade, attribution, duration, timeliness and appropriateness will be reviewed by the Monitor/ CRM. The CRM will generate a formal report which is shared with the Principal Investigator (PI), study team and the Internal Monitoring Committee (IMC).

Continuing reviews by the Internal Review Board (IRB) and Clinical Trials-Scientific Review Committee (CT-SRC) will occur at least annually. In addition, unanticipated problems are reviewed in a timely manner by the IRB.

St. Jude affiliates and domestic collaborating study sites will be monitored on-site by a representative of St. Jude as needed.

13.4 Confidentiality

Study numbers will be used in place of an identifier such as a medical record number. No research participant names will be recorded on the data collection forms. The list containing the study number and the medical record number will be maintained in a locked file and will be destroyed after all data have been analyzed. The medical records of study participants may be reviewed by the St. Jude IRB, FDA, and St. Jude clinical research monitors.

14.0 STATISTICAL CONSIDERATIONS

14.1 Accrual

The institutional trial ALLR17 has been activated for 7 years and enrolled 41 participants thus far. The first 6 participants were considered not evaluable due to an amendment to ALLR17. The previous trial ALLR16 opened in January 1997 and closed to accrual in September 2003 with 40 evaluable participants enrolled. Thus historically there would be on average 5 to 6 participants enrolled per year in our institution. Thus, at the institution's historical rate, we expect 7 to 8 years accrual time for the desired sample size of 40. The ALLR18 trial will be a multi-institution study and it is predicted that the accrual rate will be higher, at approximately 10 evaluable participants per year. Assuming this rate, we expect to accrue 40 evaluable participants in approximately 4 years for the primary objective.

Update amendment 2.0: The first 5 patients were enrolled in a staggered manner (until completion of Block I) due to FDA stipulations to ensure safety of the experimental regimen. As a consequence, enrollment was slow for the first 2 years until the restricted enrollment period was completed on 7/28/14. Therefore, we will extend the accrual of the trial by 2 years (i.e., we expect to accrue 40 evaluable patients in approximately **6 years** for the primary objective).

14.2 Monitoring of Induction Toxicities and Short-Term Efficacy

The chemotherapy in the remission induction phase of this protocol is more intensive than the previous trials. We will closely monitor grade 4 or higher infections and toxic deaths during remission induction.

Grade 4 infection or higher: The rate of grade 4 or higher infection during Induction of ALLR16 and R17 is 15% and 17.6% respectively. The following group sequential rule will be in place to monitor if there is sufficient evidence that the true probability of grade 4 or higher infection is higher than 20%.

Number of participants completing Induction	Suspend trial if # Gr 4 Infection >= or toxic death during Block I
10	5
20	8
30	11

Based on the Binomial distribution, the overall significance level is less than 0.091 based on the inclusion-exclusion formula, and the statistical power to stop the trial at each look is 0.49, 0.74, and 0.86, respectively if the true probability is 45%.

Toxic deaths during Induction Block I. All toxic deaths (not related to progressive disease) during Induction will be monitored closely. In ALLR16 and ALLR17, all disease-unrelated deaths were toxic deaths: 2/40 on ALLR16 and 2/39 on ALLR17. Thus, on ALLR18, we will not tolerate more than 3 toxic deaths in the first block of induction therapy. If, at any time, the third toxic death occurs during the first block of induction, we will suspend the trial and re-evaluate the safety of the therapy.

One-year event-free survival: The 1-year EFS rate of ALLR16 and R17 is about 50%. For monitoring purpose failures we will exactly include death, failure to attain CR, relapse, second malignancy, excessive toxicity resulting in cessation of ALLR18 treatment, and lost to follow-up (but we fully expect to follow every patient for at least one year). The following binomial based group sequential rule will be in place to monitor if there is sufficient evidence that the true 1-year failure rate is higher than 55%.

Number of participants completing one-year Tx	Suspend trial if # failures >=
15	10
30	20

Based on the binomial distribution, only when the number of failures greater than or equal to 12 and 22 at each look respectively can the overall significance level be controlled below 0.075, but these numbers are too high to be clinically acceptable. We thus set the boundary at 10 and 20. Based on the binomial distribution, if the probability of failure at one year is 0.7 (i.e. 0.2 higher than the historical 0.5 and 0.15 higher than the monitoring target of 0.55), then the stopping boundary gives the probability to stop at the first look 0.73 and at the second look 0.72. [Note that based on the binomial distribution, only when the number of failures is greater than or equal to 12 and 22 at each look respectively can the overall type-I error (i.e., erroneously stop when the true 1-year failure rate is in fact 0.5) probability be controlled below 0.075, but these numbers are too high to be clinically acceptable. We thus set the boundary at 10 and 20.]

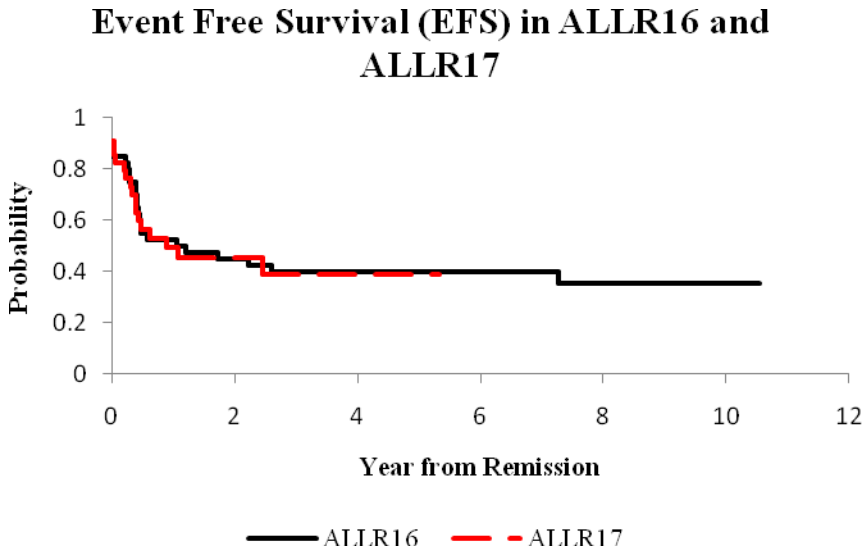
14.3 Primary Objective

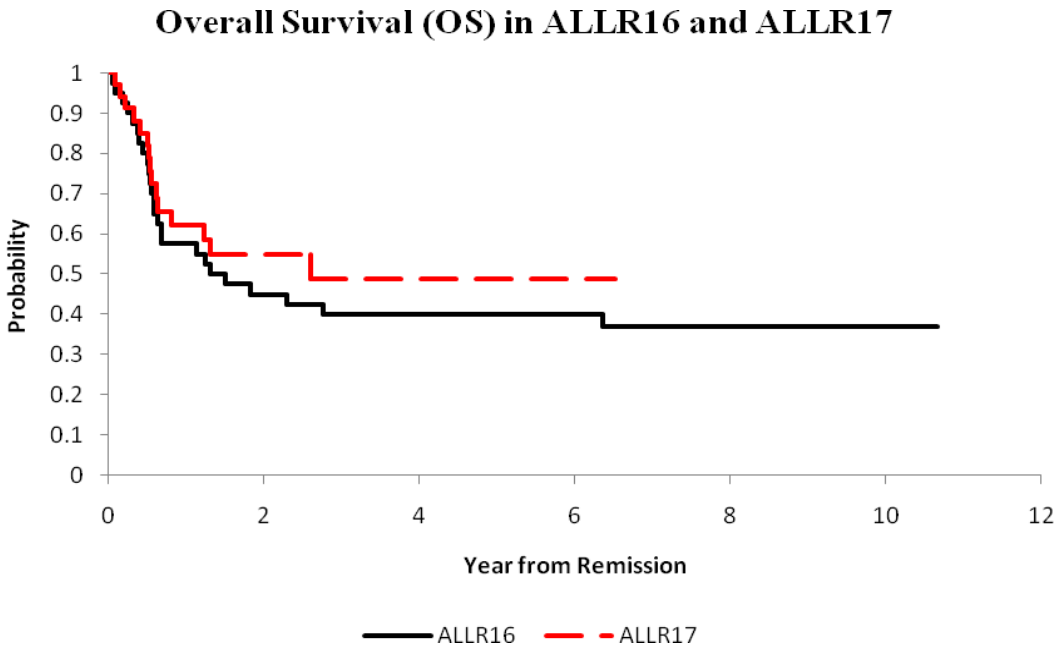
To estimate the 3-year survival rate of participants with 1st relapse or primary refractory precursor B-cell ALL treated with risk-directed therapy

Analysis and power: This is a phase-II clinical trial. The outcome will be analyzed in terms of overall survival (OS) since diagnosis of first relapse. Only death will be considered a failure for OS. Event-free survival (EFS) will also be estimated. For EFS, relapse and second malignancies will be considered as failures in addition to death in complete remission. The time to EFS will be set to 0 for participants who fail to achieve complete remission. Kaplan-Meier estimates of the OS and EFS functions will be computed, along with estimates of standard errors by the method of Peto. Three-year OS and EFS rates, as well as longer term survival rates (5 year and 10 year) will be estimated with 95% confidence intervals.

Additionally, EFS and OS will be estimated separately in two strata: participants with prior bone marrow transplant (BMT) and those never had any BMT because it is expected that participants with prior BMT may have worse survival. The follow-up time on each participant will be three years since enrollment.

Kaplan-Meier estimates of EFS and OS in R16 and R17 are shown below:





The objective here is estimation instead of comparison. Comparisons to historical controls are considered exploratory. With the sample size n=40, the statistical power will be limited. Analysis will begin after 3 years of follow-up since the on-study date of the last enrolled patient.

14.4 Secondary Objectives

1) To determine minimal residual disease (MRD) levels at the end of remission induction therapy and compare with ALLR17.

Probability (proportion) of positive MRD will be compared with that from the ALLR17 trial using Fisher's exact test. MRD will also be put into ordered categories such as negative (<0.01%), low (0.01% - 1%) and high (>=1%) and compared with those in ALLR17 using exact Chi-square test. We are aware that the power is limited given the small sample size, unless the true difference is unusually large. Analysis will begin no later than 1 year after the last enrollment.

2) To estimate levels of CD20 expression at baseline, during treatment with dexamethasone-containing chemotherapy and following rituximab treatment in week 1 of Block I of remission induction therapy for relapsed precursor B-cell ALL

Mean and median of CD20 expression levels will be computed at each time point along with 95% confidence intervals. Trajectory of CD20 levels as a function of time will be modeled and predicted using a longitudinal data model, with time (and possibly other clinical and demographic variables) and an intra-patient random effect as factors; this analysis is considered exploratory. Analysis will begin no later than 1 year after the last enrollment.

14.5 Exploratory Objectives

1) To assess donor NK cell killer immunoglobulin-like receptor (KIR) repertoire and explore the efficacy of NK cell infusions followed by rituximab in relapsed ALL.

The donor KIR repertoire will be described by summary statistics (counts and proportions). Three and five year EFS of participants who received NK cell infusion will be estimated by the KM estimator along with 95% confidence intervals. Analysis will begin no later than 1 year after the last enrollment.

2) To study whether pre-existing or emerging development of serum antibodies to asparaginase is related to hypersensitivity reactions or exposure to asparaginase or to anti-leukemic or adverse effects of therapy, *in participants with relapsed ALL.*

Due to heterogeneity among participants and prior therapy, we will use exploratory analytic tools to assess whether anti-asparaginase antibodies are related to clinical hypersensitivity reactions. Analysis will begin no later than 1 year after the last enrollment.

3) To describe the effect of prophylactic antibiotics on the evolution of antibiotic resistance in peri-rectal swab isolates of Escherichia coli, Klebsiella pneumonia, Pseudomonas aeruginosa, Streptococcus mitis and enterococci and resistance patterns of bacterial isolates from all sterile site cultures (completed with amendment 3.0)

Resistance of selected colonizing and invasive organisms to representative antibiotics from various classes (ex. Cephalosporins, carbapenems, fluoroquinolones) will be described pre and post antibiotic prophylaxis at baseline (pre-antibiotic prophylaxis) and post-antibiotic prophylaxis at any time point for the entire study population and separately based on type of antibiotic prophylaxis the patients received. All suspected or proven infections which occur while a patient is on antibiotic prophylaxis as well as *C. difficle* infections during and up to one month post antibiotic prophylaxis will be described.

Analysis will begin no later than 1 year after the last enrollment.

4) To characterize global gene expression and copy number changes in leukemic cells at relapse and to compare to initial diagnosis (when available) to improve knowledge of mechanisms of relapse

The analyses will be performed using established methods.[17-19] Analysis will begin no later than in 1 year after the last enrollment.

5) To determine in vitro sensitivity of pretreatment relapsed leukemic cells to anti-leukemic agents and compare to in vitro sensitivity at diagnosis (when available)

Descriptive statistics of sensitivity such as IC50 will be calculated. Analysis will begin no later than 1 year after the last enrollment.

6) To engraft freshly harvested relapsed ALL cells into murine models of ALL to gain insights into therapy resistance

After engraftment, various downstream analyses will be done on the leukemic cells including immunophenotype, histologic and genomic studies. Analysis will begin no later than 1 year after the last enrollment.

8) *To study the development of resistant clones during therapy for relapsed ALL.*

NT5C2 mutation detection will be performed by targeted re-sequencing using next generation platforms. We plan to sequence *NT5C2* coding regions on the Mi-seq platform, but are mindful of continuing evolvement of sequencing technologies to adopt the most cost-effective and efficient platforms for this study, with the help from Hartwell Center and PCGP. Analysis will begin no later than 1 year after the last enrollment.

15.0 OBTAINING INFORMED CONSENT

15.1 Consent/Assent at Enrollment

The process of informed consent for ALLR18 will follow institutional policy. The informed consent process is an ongoing one that begins at the time of diagnosis and ends after the completion of therapy. Informed consent should be obtained by the attending physician or his/her designee, in the presence of at least one non-physician witness. Initially, informed consent will be sought for the institutional banking protocol (research study), blood transfusion and other procedures as necessary. After the diagnosis of relapse ALL is established, we will invite the patient to participate in the ALLR18 protocol. Consent/assent for initial enrollments will be done at St. Jude for all participants (including NK cell donors).

Throughout the entire treatment period, participants and their parents receive constant education from health professionals at SJCRH and collaborating sites, and are encouraged to ask questions regarding alternatives and therapy. All families have ready access to chaplains, psychologists, social workers, and the St. Jude ombudsperson for support, in addition to that provided by the primary physician and other clinicians involved in their care.

We will also obtain verbal assent from children 7 to 14 years old and written assent for all participants $\geq$14 years of age. Participants who reach the age of majority while on study will be re-consented for continued participation on ALLR18, according to Cancer Center and institutional policy.

15.2 Consent at Age of Majority

The age of majority in the state of Tennessee is 18 years old. Research participants must be consented at the next clinic visit after their 18th birthday. If an affiliate or collaborating site is located in a country or state where a different age of majority applies, that location must consent the participants according to their local laws.

15.3 Consent When English is Not the Primary Language

When English is not the patient, parent, or legally authorized representative's primary language, the Social Work department will determine the need for an interpreter. This information documented in the participant's medical record. Either a certified interpreter or the telephone interpreter's service will be used to translate the consent information. The process for obtaining an interpreter and for the appropriate use of an interpreter is outlined on the Interpreter Services, OHSP, and CPDMO websites.

Collaborating Sites will follow institutional policy for consenting non-English speaking participants (and will provide institutional policy to St. Jude).

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APPENDIX I: TESTS PERFORMED FOR GOOD CLINICAL CARE AND FOR RESEARCH

The standard of care services in this study are as follows:

- History & physical exams
- CBC with diff
- Coagulation screen
- Urine analysis
- Hepatitis, HIV screen
- NK cell recipient - EBV, CMV, HSV, varicella, histoplasma, and toxoplasma titres
- Bone marrow aspirate and biopsy procedure and MRD
- Peripheral blood for MRD
- TPMT genotyping
- CSF cell count, diff
- Chest x-ray
- ECHO and EKG
- QCT for bone density
- HLA typing of patient and potential donors
- Pregnancy test for females of childbearing potential (patient)
- All agents used in this trial are commercially available. Rituximab and IL-2 use are experimental

The research services are as follows:

- CliniMACS system and NK cell infusions
- Bone marrow sample for biology studies (taken at same time bone marrow procedure is done for routine care to confirm relapse – baseline)
- Peripheral blood for CD20
- Bone marrow for mutant cells
- Serum for asparaginase and anti-asparaginase antibodies (at specified time points – baseline, Induction Block I - Days 21, 28, 35 (pre-PEG each day); Interim continuation - Day 1 of week 1; Re-Induction – Days 6, 13, 20 (pre-PEG); and at time of reaction (if applicable); again if re-challenged with asparaginase (section 6.2)
- Germline DNA sample Day 3 Induction Block I and Day 1 induction Block II
- NK PHENO (patient) – baseline and Day 19 of Induction Block I
- NK PHENO (donor) – baseline
- Variable nucleotide tandem repeat (VNTR) and KIR typing for recipient participant
- Donor testing – KIR typing, VNTR, serologic infectious disease testing, CBC, pregnancy test, complete metabolic panel; see NK Cell Donor Power Plan order set for full details

APPENDIX II: PEDIATRIC RITUXIMAB INFUSION GUIDELINES

Dose = 375 mg/m^2

- Dilute in NS to final concentration 1 mg/ml for ease of administration.
- Administer intravenously through a dedicated line

Pre-medicate with:

- Acetaminophen 15 mg/kg PO (max 650 mg)
- Benadryl 1 mg/kg IV or PO (max 50 mg)

FIRST INFUSION: Participants treated at St. Jude will be as per current St. Jude formulary from Intranet (collaborating sites will follow institutional formulary/policy)

SUBSEQUENT INFUSIONS: Participants treated at St. Jude will be as per current St. Jude formulary from Intranet (collaborating sites will follow institutional formulary/policy)

GENERAL GUIDELINES:

1. Monitor blood pressure, pulse, respiration and temperature every 15 minutes for the first hour or until the participant is stable, then hourly until the infusion is complete.
2. Have epinephrine and diphenhydramine available along with resuscitation equipment for the emergency management of anaphylactic reactions.
3. If infusion related events occur, slow the infusion or stop the infusion until resolution, treating the participant if necessary. If it is determined that the participant can be safely re-challenged, begin the infusion at 50% of the rate at which the infusion was running when stopped.
4. Verify that the participant has not taken antihypertensive medications within 12 hours of beginning the rituximab infusion. Hypotension may occur during the infusion.
5. Participants with a high number of circulating cancer cells (> 25,000/mm^3) may be at a higher risk for tumor lysis syndrome. These participants should receive allopurinol and intravenous hydration and should be monitored.
6. Participants who experience severe infusional symptoms may need to be hospitalized for observation. Participants and families should be counseled to be seen in the emergency room if infusion-related symptoms occur again after the infusion is complete (i.e. at home at a later time).
7. Monitor participants during and for a few weeks after receiving rituximab for the development of mucositis with a sore throat or mouth ulcers followed by a diffuse skin rash which can worsen rapidly and result in total body skin sloughing and can become life threatening.
8. Carriers of hepatitis B should be closely monitored for clinical and laboratory signs of active HBV infection and for signs of hepatitis throughout their study participation.

APPENDIX III: DRUG/DEVICE/BIOLOGIC AGENT INFORMATION

1. VINCRISTINE (Oncovin®)

Source and pharmacology: Vincristine is an alkaloid obtained from the periwinkle *(Vinca rosea)* plant. It reversibly binds to microtubule and spindle proteins to cause metaphase arrest. Vincristine has poor penetration into the CSF. It is extensively protein bound (~75%). Extensive metabolism occurs in the liver. Excretion is primarily in the bile. A dosage decrease is recommended in participants with elevated bilirubin (see section 7.3).

Formulation and stability: Vincristine is supplied in multiple-dose 1 mg/mL vials containing 1 mL, 2 mL and 5 mL. The intact vials should be stored under refrigeration and protected from light.

Supplier: The drug is commercially available.

Toxicity: Dose-limiting toxicity is neurotoxicity, which is characterized by constipation and/or paralytic ileus, ptosis, vocal cord paralysis, weakness, jaw pain, abdominal pain, peripheral neuropathies, loss of deep tendon reflexes, and "foot drop." Peripheral neuropathy is often the first sign of neurotoxicity and is initially reversible. Other toxicities reported include alopecia, mild nausea and vomiting, SIADH, myelosuppression, orthostatic hypotension, optic atrophy, transient cortical blindness, and auditory damage. Acute shortness of breath and severe bronchospasm have been reported after administration of vinca alkaloids. Myelosuppression is rare at usual doses. Vincristine is a vesicant and may cause severe tissue damage if extravasation occurs. Note that dose reduction may be necessary in participants <1 year of age or <10 kg in weight; dosing on a "per kg" (rather than per m^2) basis has been advocated for infants in order to decrease toxicity.

Guidelines for administration: intravenous, see Treatment section 5.2.1 (Remission induction Block I), section 5.4 (Re-Induction) and section 5.5 (Continuation).

2. DEXAMETHASONE (Decadron®)

Source and pharmacology: Dexamethasone is a synthetic congener of the natural adrenal hormone hydrocortisone. Dexamethasone is a white or yellowish crystalline powder. It binds with steroid receptors on nuclear membranes to impair cellular mitosis and inhibit protein synthesis. Dexamethasone also has potent anti-inflammatory effects and suppresses the immune system. Dexamethasone is absorbed well orally. It is metabolized in the liver, and the metabolites are excreted mainly in the urine.

Formulation and stability: Dexamethasone is available as tablets of various strengths and as an elixir. It is also available as a solution for parenteral use. All formulations of the drug can be stored at room temperature. The injectable form may be further diluted in 5% dextrose or 0.9% NaCl-containing solutions and is stable for at least 24 h at room temperature.

Supplier: The drug is commercially available.
Toxicity: The side effects of dexamethasone vary depending on the duration of its use. Side effects that can occur with short-term use include peptic ulcers with possible perforation and hemorrhage, increased susceptibility to infections, emotional instability, insomnia, increased appetite, weight gain, acne, and hyperglycemia. Side effects more commonly associated with prolonged use include cataracts, increased intraocular pressure and associated glaucoma, development of a "cushingoid" state, compression fractures, menstrual irregularities, suppression of growth in children, secondary adrenocortical and pituitary unresponsiveness particularly in times of stress as in trauma, surgery or illness, osteoporosis and muscle wasting.

Guidelines for administration: PO, see section 5.2.1 (Remission induction, Block I), section 5.3 (Interim continuation), section 5.4 (Re-Induction), section 5.5 (Continuation)

3. HYDROCORTISONE, Intrathecal (Cortef, Solu-Cortef)

Source and pharmacology: Hydrocortisone is a synthetic steroid akin to the natural adrenal hormone cortisol. Hydrocortisone has phase-specific cytotoxicity, killing lymphoblasts primarily during S phase. It has catabolic effect on proteins and alters the kinetics of peripheral blood leukocytes. It is excreted in the urine and catabolized in the liver.

Formulation and stability: Solu-Cortef sterile powder is supplied in the following package: 100 mg plain, and 100 mg, 250 mg, 500 mg, and 1000 mg ACT-O-VIAL (MIX-O-VIAL). Store unreconstituted product at controlled room temperature 15-30°C (59-86°F). Store reconstituted solution in the refrigerator and protect from light. Unused solution should be discarded after 3 days. Use Solu-Cortef (plain vial) for intrathecal use, and reconstitute with 0.9% sodium chloride, USP for injection.

Supplier: Commercially available

Toxicity: If given intrathecally, sterile arachnoiditis may occur. Headache, seizures, unusual feelings or sensations, loss of feeling or ability to move arms or legs, and difficulty with urination or bowel movements may also occur.

Guidelines for administration: Intrathecal, see section 5.2.1 (Remission induction Block I), section 5.2.2 (Remission induction Block II), 5.2.3 (Remission induction Block III), section 5.3 (interim continuation), section 5.4 (Re-induction), section 5.5 (Continuation), and 5.7 (prophylaxis and treatment for CNS disease), 5.10 (summary of IT therapy).

4. METHOTREXATE

Source and pharmacology: Methotrexate is a folate analogue that acts by inhibiting dihydrofolate reductase. Dihydrofolate reductase is an enzyme important in the conversion of folic acid to tetrahydrofolic acid, which is necessary in the synthesis of purine nucleotides and thymidylate. By inhibiting the production of tetrahydrofolic acid, methotrexate interferes with

DNA, RNA and protein synthesis. Methotrexate is poorly and variably absorbed orally, with an average of ≈ 40% for doses of ≤ 30 mg/m^2. At higher dosages, the extent of absorption decreases. Methotrexate is approximately 50% protein bound. It distributes widely into body tissues and fluids with sustained concentrations in the kidney and the liver. Methotrexate undergoes metabolism by cytosolic aldehyde oxidase to hydroxy methotrexate. It is excreted mainly in the urine as unchanged drug with small amounts being excreted in the bile and feces. The percent recovered as unchanged drug in the urine is higher with short infusions than with prolonged infusions. Methotrexate has a biphasic elimination with an initial half-life of ≈ 2-3 hours and a terminal half-life of 10-12 hours. Methotrexate may be "sequestered" in body fluid collections and eliminated slowly from these areas. Patients with effusions or GI obstruction should have plasma levels monitored closely for delayed excretion following high-dose methotrexate.

Formulation and stability: Methotrexate is supplied in single-dose vials containing 50mg, 100mg, 200mg, and 250 mg of methotrexate as a 25 mg/ml preservative-free solution and in vials containing 20mg, 50 mg, 100mg, 250 mg and 1000mg of lyophilized drug. It is also available in 2.5 mg tablets. Methotrexate preservative-free solution and lyophilized drug should be stored at room temperature and protected from light. Methotrexate tablets can also be stored at room temperature. The vials containing 20, 50, 100 and 250 mg of lyophilized product can be reconstituted by adding sterile water, 0.9% NaCl or D5W to a final concentration not exceeding 25 mg/ml. The 1000mg vials containing lyophilized product are reconstituted to a final concentration of 50 mg/ml.

Supplier: commercially available

Toxicity: The dose limiting toxicities of methotrexate are generally bone marrow suppression, ulcerative stomatitis, severe diarrhea or acute nephrotoxicity. Toxicities reported frequently include nausea and vomiting, diarrhea, anorexia, alopecia, hepatic toxicity and alopecia. Less common side effects include blurred vision, photosensitivity, anaphylaxis, headache, pneumonitis, skin depigmentation or hyperpigmentation, rash, vasculitis and encephalopathy. During high-dose methotrexate therapy, most patients experience a transient decrease in GFR, but renal failure can occur, particularly if the patient does not receive urinary alkalinization and aggressive hydration before, during and after receiving high dose methotrexate. Leucovorin rescue should be initiated within 48 hours of starting high-dose methotrexate and adjusted based on MTX levels to prevent bone marrow toxicity and mucositis. Leucovorin may also be necessary after IT administration, especially if IT methotrexate therapy is given to patients with renal dysfunction. Patients with Down syndrome have a tendency to have delayed methotrexate clearance and a greater risk of toxicity, despite increased leucovorin rescue.

Guidelines for administration: Intrathecal and intravenous, see section 5.2.1 (Remission induction Block I), section 5.2.2 (Remission induction Block II), 5.2.3 (Remission induction Block III), section 5.3 (interim continuation), section 5.4 (Re-induction), section 5.5 (Continuation), and 5.7 (prophylaxis and treatment for CNS disease), 5.10 (summary of IT therapy).

5. CYTARABINE (Ara-C) (Cytosar-U®)

Source and pharmacology: Cytarabine is a deoxycytidine analogue. It must be tri-phosphorylated to its active form, ARA-CTP, by deoxycytidine kinase and other nucleotide kinases. Ara-CTP inhibits DNA polymerase. In addition, ara-CTP is incorporated into DNA as a false base, causing inhibition of DNA synthesis. It is cell cycle, S phase specific. Cytarabine does penetrate the blood brain barrier. It is converted to its inactive form, uracil arabinoside, by pyrimidine nucleoside deaminase. Approximately 80% of the dose is recovered in the urine, mostly as uracil arabinoside (ara-U).

Formulation and stability: Cytarabine is available in multi-dose vials containing 100, 500, 1000 and 2000mg of lyophilized drug. Intact vials can be stored at room temperature. For IV use, either sterile water for injection or bacteriostatic water for injection can be used to reconstitute the lyophilized drug. For intrathecal use, only sterile water for injection should be used for reconstitution. The 100 and 500 mg vials are reconstituted with 2 and 10 ml respectively resulting in a final concentration of 50mg/ml. The 1000 and 2000mg vials are reconstituted with 20ml and 40 ml respectively resulting in a final concentration of 50mg/ml. After reconstitution, the drug is stable for 8 days at room temperature.

Supplier: commercially available

Toxicity: Myelosuppression is the dose limiting adverse effect, with leukopenia and thrombocytopenia being predominant. Other adverse effects reported commonly include nausea and vomiting (may be severe at high doses), diarrhea, mucositis, anorexia, alopecia, skin rash and liver dysfunction. A flu-like syndrome characterized by fever, muscle and bone aches is common. Less common side effects include allergic reactions and cellulitis at the injection site. High doses of cytarabine can cause conjunctivitis, hepatitis, and a group of CNS symptoms including somnolence, peripheral neuropathy, ataxia and personality changes. CNS symptoms are usually reversible and are more common in patients who have received previous cranial irradiation. In addition, a syndrome of sudden respiratory distress progressing to pulmonary edema has occurred.

Guidelines for administration: Intrathecal and intravenous; see section 5.2.1 (Remission induction Block I), section 5.2.2 (Remission induction Block II), 5.2.3 (Remission induction Block III), section 5.3 (interim continuation), section 5.4 (Re-induction), section 5.5 (Continuation), and 5.7 (prophylaxis and treatment for CNS disease), 5.10 (summary of IT therapy).

6. RITUXIMAB (Rituxan®)

Source and pharmacology: Rituximab is a murine /human chimeric monoclonal antibody. It is specific for the CD20 antigen located on B-cells. Rituximab has been shown to mediate complement-dependent tumor cell lysis and antibody-dependent cellular cytotoxicity. Direct binding to the CD20 antigen is thought to play a role in inhibition of cell growth. Rituximab is

administered intravenously. The mean serum half-life after a single IV dose of 375 mg/m^2 is 59.8 hours (range 11.1-104.6 hours).
Formulation and stability: Rituximab is available as 100 mg/10 ml single-use and 500 mg/50 ml single-use vials. Each vial also contains sodium chloride 9 mg/ml, sodium citrate 7.35 mg/ml, polysorbate 80 0.7 mg/ml and water for injection. Rituximab for injection concentration must be diluted with 5% Dextrose or 0.9% NaCl prior to administration. After dilution, unused drug is stable for 24 hours when refrigerated (2-8 degrees Celsius) and 12 hours at room temperature. Vials should be protected from direct sunlight.

Supplier: commercially available

Toxicity: Hypersensitivity reactions may occur; therefore, premedication with acetaminophen and diphenhydramine should be considered before each infusion. The most common toxicities are infusion related and may include chills, fever, headache, nausea, vomiting, angioedema (13%), hypotension (10%), bronchospasm (8%), and arrhythmia. Other possible adverse reactions include thrombocytopenia, myalgias, arthralgias, asthenia, and throat irritation.

Guidelines for administration: Do not administer as an intravenous push or bolus. Infusions should be initiated at 50 mg/hour for 30 minutes. If hypersensitivity or infusion-related events do not occur, subsequent infusions can be administered at an initial rate of 100 mg/hour and increased by 100 mg/hour every 30 minutes as tolerated. Infuse at a maximum rate of 400 mg/hour. See section 5.2 (Remission Induction, Block I and Appendix II).

7. CLOFARABINE (Clolar™, Clofarex)

Source and pharmacology: Clofarabine is a purine nucleoside analog. It is intracellularly metabolized to the active metabolite clofarabine 5'-triphosphate which competes with deoxyadenosine triphosphate for binding to ribonucleotide reductase and DNA polymerase. It inhibits DNA synthesis, terminates DNA chain elongation and inhibits DNA repair. Clofarabine also disrupts the mitochondrial membrane which results in the release of proteins, cytochrome C and apoptosis-inducing factor leading to cell death. It is mainly excreted in the urine as unchanged drug.

Formulation and stability: Clofarabine is available in the parenteral form as a preservative-free solution that is 1 mg/mL. It is available in 20 mL vials. The undiluted drug should be stored at room temperature. The diluted solution is stable for 24 hours at room temperature. Clofarabine injection should be filtered through sterile 0.2 micrometer syringe filter and then further diluted with 5% dextrose or 0.9% NaCl containing solutions.

Supplier: The injection is commercially available.

Toxicity: The most common side effects are nausea, vomiting, diarrhea, headache, fever and pruritus. Also reported are pericardial effusion, tachycardia, hypotension, left ventricular systolic dysfunction, edema, flushing, hypertension, fatigue, anxiety, pain, dizziness,

depression, irritability. Patients who receive clofarabine are at risk for tumor lysis syndrome and need to be monitored closely.

Patients may also experience a systemic inflammatory response syndrome (SIRS) or capillary leak syndrome. Patients should be monitored for this during the infusion.

Dosage and route of administration: Intravenous, see section 5.2.1 (Remission induction, Block I) and section 5.4 (Re-induction)

8. CYCLOPHOSPHAMIDE (Cytoxan®)

Source and pharmacology: Cyclophosphamide is a nitrogen mustard derivative. It acts as an alkylating agent that causes cross-linking of DNA strands by binding with nucleic acids and other intracellular structures, thus interfering with the normal function of DNA. Cyclophosphamide is cell-cycle, phase non-specific. Cyclophosphamide is well absorbed from the GI tract with a bioavailability of > 75%. Cyclophosphamide is a prodrug that requires activation. It is metabolized by mixed-function oxidases in the liver to 4-hydroxycyclophosphamide, which is in equilibrium with aldofosfamide. Aldofosfamide spontaneously splits into cyclophosphamide mustard, which is considered to be the major active metabolite, and acrolein. In addition, 4-hydroxycyclophosphamide may be enzymatically metabolized to 4-ketocyclophosphamide and aldofosfamide may be enzymatically metabolized to carboxyphosphamide which are generally considered to be inactive. Cyclophosphamide and its metabolites are excreted mainly in the urine. Dosage adjustments should be made in patients with a creatinine clearance of < 50 ml/min.

Formulation and stability: Cyclophosphamide is available in 25 and 50 mg tablets. Cyclophosphamide is also available in vials containing 100, 200, 500, 1000 and 2000mg of lyophilized drug and 75 mg mannitol per 100 mg of cyclophosphamide. Both forms of the drug can be stored at room temperature. The vials are reconstituted with 5, 10, 25, 50 or 100 ml of sterile water for injection respectively to yield a final concentration of 20 mg/ml. Reconstituted solutions may be further diluted in either 5% dextrose or 0.9% NaCl containing solutions. Diluted solutions are physically stable for 24 hours at room temperature and 6 days if refrigerated, but contain no preservative, so it is recommended that they be used within 24 hours of preparation.

Supplier: commercially available

Toxicity: Dose limiting toxicities of cyclophosphamide are bone marrow suppression and cardiac toxicity. Cardiac toxicity is typically manifested as congestive heart failure, cardiac necrosis or hemorrhagic myocarditis and can be fatal. Hemorrhagic cystitis may occur and necessitates withholding therapy. The incidence of hemorrhagic cystitis is related to cyclophosphamide dose and duration of therapy. Forced fluid intake and/or the administration of MESNA decreases the incidence and severity of hemorrhagic cystitis. Other toxicities reported commonly include nausea and vomiting (may be mild to severe depending on

dosage), diarrhea, anorexia, alopecia, immunosuppression and sterility. Pulmonary fibrosis, SIADH, anaphylaxis and secondary neoplasms have been reported rarely.

Dosage and route of administration: Intravenous, see section 5.2.1 (Remission induction, Block I), section 5.3 (Interim continuation) and section 5.4 (Re-induction).

9. ETOPOSIDE (VP-16) (Vepesid®)

Source and pharmacology: Etoposide is an epipodophyllotoxin derived from *Podophyllum pelatatum.* It is thought to act mainly by inhibiting topoisomerase II, causing double and single strand DNA breaks. Etoposide is cell cycle, phase-specific, with activity in the G2 and S phases. Absorption of etoposide is approximately 30-40% and is highly variable and somewhat dose-dependent. It is extensively bound to serum proteins and is metabolized in the liver, including cytochrome P450 3A metabolism to several moieties that include a reactive oxidized species. Etoposide and its metabolites are excreted mainly in the urine with a smaller amount excreted in the feces. Dosage adjustments should be considered in patients with liver dysfunction, kidney dysfunction or hypoalbuminemia.

Formulation and stability: Etoposide is available in multi-dose vials containing 100mg, 150mg, 500mg and 1000mg of etoposide as a 20mg/ml solution and 30% alcohol. Etoposide is also available as a 50 mg capsule. The intact vials of etoposide solution should be stored at room temperature. The capsules should be stored under refrigeration. Etoposide solution should be diluted in D5W or 0.9% NaCl prior to administration. Solutions with a final concentration of 0.2 and 0.4 mg/ml are stable at room temperature for 96 hours and 24 hours respectively.

Supplier: commercially available

Toxicity: Dose limiting toxicity is myelosuppression. Nausea and vomiting (usually of low to moderate severity), diarrhea, mucositis (particularly with high doses), alopecia and anorexia are fairly common. Hypotension can occur with rapid infusions. Other side effects reported less commonly include hepatitis, fever and chills, anaphylaxis and peripheral neuropathy. Secondary leukemia has been reported.

Dosage and route of administration: Intravenous, see section 5.2.1 (Remission induction, Block I), section 5.3 (Interim continuation) and section 5.4 (Re-induction)

10. ETOPOSIDE PHOSPHATE (Etopophos®) - *To be used in case of etoposide reactions (see section 7.9).*

Source and pharmacology: Etoposide is an epipodophyllotoxin derived from *Podophyllum pelatatum.* It is thought to act mainly by inhibiting topoisomerase II, causing double and single strand DNA breaks. Etoposide is cell cycle, phase-specific, with activity in the G2 and S phases. Absorption of etoposide is approximately 30-40% and is highly variable and somewhat dose-dependent. It is extensively bound to serum proteins and is metabolized in the liver, including cytochrome P450 3A metabolism to several moieties that include a reactive

oxidized species. Etoposide and its metabolites are excreted mainly in the urine with a smaller amount excreted in the feces. Dosage adjustments should be considered in patients with liver dysfunction, kidney dysfunction or hypoalbuminemia.

Formulation and stability: Etoposide phosphate is a water-soluble ester of etoposide. The higher water solubility of etoposide phosphate than that of etoposide lessens the potential for precipitation following dilution and during administration. Etoposide phosphate is available in single-dose vials containing etoposide phosphate equivalent to 100mg etoposide. The intact vials of etoposide solution should be stored at 2 to 8 degrees Celsius. Etoposide phosphate solution should be diluted in D5W or 0.9% NaCl prior to administration. Solution is stable at room temperature for 24 hours.

Supplier: commercially available.

Toxicity: Dose limiting toxicity is myelosuppression. Nausea and vomiting (usually of low to moderate severity), diarrhea, mucositis (particularly with high doses), alopecia and anorexia are fairly common. Hypotension can occur with rapid infusions. Other side effects reported less commonly include hepatitis, fever and chills, anaphylaxis and peripheral neuropathy. Secondary leukemia has been reported.

Guidelines for administration: Intravenous, see section 5.2.1 (Remission induction, Block I), section 5.3 (Interim continuation) and section 5.4 (Re-induction).

11. CliniMACS™ System

This system is described fully in the Investigators Brochure and is briefly described here. The mechanism of action of the CliniMACS Cell Selection System is based on magnetic-activated cell sorting. The CliniMACS device is a powerful tool for the isolation of many cell types from heterogeneous cell mixtures. For example, apheresis products can be separated in a magnetic field using an immunomagnetic label specific for the cell type of interest, such as CD34+ human hematopoietic cells enrichment, CD3+ T cell depletion, or CD56+ NK cell enrichment.

The cells to be isolated or depleted are specifically labeled with super-paramagnetic particles by an antibody directed toward a cell surface antigen. After magnetic labeling, the cells are separated using a high-gradient magnetic separation column as described below. The magnetically labeled cells are retained in the magnetized column while the unlabeled cells flow through. The retained cells are eluted by removing the magnetic field from the column, washing the cells out and collecting them.

The super-paramagnetic particles are small in size (about 50 nm in diameter) and are composed of iron oxide/hydroxide and dextran conjugated to monoclonal antibodies. These magnetic particles form a stable colloidal solution and do not precipitate or aggregate in magnetic fields. The CliniMACS device incorporates a strong permanent magnet and a separation column with a ferromagnetic matrix to separate the cells labeled with the magnetic

particles. The high-gradient system allows the application of strong magnetic forces and a rapid demagnetization. Small ferromagnetic structures, such as the column matrix, placed in a magnetic field, concentrate this homogenous field and thereby produce high magnetic gradients. In their immediate proximity, the ferromagnetic structures generate magnetic forces 10,000-fold greater than in the absence of those structures enabling the retention of magnetically labeled cells. After removing the column from the magnet, the rapid demagnetization of the column matrix allows the release of retained cells.
The CliniMACS device is comprised of a computer controlled instrument incorporating a strong permanent magnet, a closed-system sterile tubing set containing columns with a coated ferromagnetic matrix and a paramagnetic, cell specific, labeling reagent. The instrument will separate the cells in a fully automated process

12. INTERLEUKIN-2 (IL-2, ALDESLEUKIN, PROLEUKIN®)

Source and pharmacology: Aldesleukin is a biosynthetic cytokine (a lymphokine) of recombinant DNA origin. It differs from human interleukin-2 by the absence of an N-terminal alanine, the replacement of cysteine with serine at position 125 of the sequence, and the absence of glycosylation. It is a biologic response modifier with complex antineoplastic and immunomodulating activities.

Formulation and stability: Aldesleukin vials contain 22 million units of lyophilized recombinant interleukin. Each single use vial is reconstituted with 1.2 ml of sterile water for injection to give 18 million units/ml. Aldesleukin should be admixed with D5W for infusion, with albumin 0.1% added to decrease adsorption. Do not use an in-line filter. Do not mix in saline.

Supplier: commercially available

Toxicities: Aldesleukin is a highly toxic drug. Most adverse effects are dose related and schedule dependent, with fewer toxicities associated with low dose, subcutaneous or continuous IV infusions as compared to high dose, rapid IV infusions. Most adverse effects are self-limiting and reversible within 2 to 3 days of drug discontinuance. Many of the adverse effects of aldesleukin are related to capillary leak syndrome, which has been associated with this drug. The most frequently reported serious adverse effects include hypotension, renal dysfunction with oliguria/anuria, dyspnea or pulmonary congestion, and mental status changes (lethargy, somnolence, confusion, agitation). Additional serious adverse effects reported include myocardial ischemia, myocarditis, gangrene, respiratory failure leading to intubation, GI bleeding, intestinal perforation, ileus, coma, seizures, sepsis, and renal impairment requiring dialysis. Most patients receiving aldesleukin develop some degree of a flu-like syndrome that may include fever, chills, rigors, fatigue, weakness, malaise, arthralgia and myalgia.

Guidelines for administration: Subcutaneous injection, for NK Cell transplant participants only, see section 5.2.1 (Remission induction, Block I).

13. PEG-L-ASPARAGINASE (Pegaspargase, Oncaspar®)

Source and pharmacology: PEG-asparaginase is a modified version of the enzyme, L-asparaginase. L-asparaginase is modified by covalently conjugating units of polyethylene glycol (PEG) to the enzyme. The asparaginase used in the manufacturing of PEG-asparaginase is derived from *Escherichia coli.* Asparaginase hydrolyzes serum asparagine (an amino acid required to synthesize proteins and DNA) to aspartic acid and ammonia, and is therefore lethal to cells that cannot synthesize asparagine. Asparaginase is active during all phases of the cell cycle. Asparaginase is not absorbed from the GI tract and must be given parenterally. PEG-asparaginase has a plasma half-life of approximately 6 days, but is measurable for at least 15 days following the initial treatment. It cannot be detected in the urine.

Formulation and stability: PEG-asparaginase is available in single-use vials containing 5 ml of PEG-asparaginase as a clear solution. Each vial contains 3750 units of drug at a concentration of 750 units/ml. The intact vials should be stored under refrigeration. Freezing destroys its activity, which cannot be detected visually. It should not be used if it is cloudy or a precipitate is present.

Supplier: commercially available

Toxicity: Acute toxicity includes anaphylactic reactions which occur most commonly when the drug is given IV. These can be characterized by laryngeal constriction, hypotension, diaphoresis, fever, chills, edema and loss of consciousness. Allergic reactions at the site of IM injection include pain, swelling and erythema. The incidence of hypersensitivity reactions to PEG-asparaginase may be less than with conventional E. coli derived asparaginase although cross-sensitivity can occur. Other adverse effects include neutropenia and associated immunosuppression, mild nausea and vomiting, malaise, anorexia, elevated LFT's, pancreatitis and hyperglycemia. A decrease in protein synthesis including albumin, fibrinogen and other coagulation factors may occur which can result in thrombosis or pulmonary embolism. Less common side effects include renal dysfunction and CNS complications, including somnolence, weakness, lethargy, coma and seizures.

Guidelines for administration: Intravenous or intramuscular injection, see section 5.2.1 (Remission induction) and 5.4 (Re-induction).

14. ERWINIA L-ASPARAGINASE (Erwinase®)

To be used in case of allergy or intolerance to PEG-Asparaginase.

Source and pharmacology: Erwinia asparaginase is an enzyme. It is derived from *Erwinia chrysanthemi* and may be useful in patients with an allergy to the *E. coli* derived product. Asparaginase hydrolyzes serum asparagine (an amino acid required to synthesize proteins) to aspartic acid and ammonia, and is therefore lethal to cells that cannot synthesize asparagine. Asparaginase is active during all phases of th cell cycle. Asparaginase is not absorbed from

the GI tract and must be given parenterally. Asparaginase does not cross into the CSF. The plasma half-life of Erwinia asparaginase when given IM is approximately 16 hours. Only minimal urinary and biliary excretion occurs. Clearance is unaffected by age, renal function or hepatic function.

Formulation and stability: Erwinia asparaginase is available in vials containing 10,000 units of lyophilized drug. Unused vials should be refrigerated. The contents of each vial should be diluted with 1 ml of preservative-free normal saline, giving a resultant solution of 10,000 units/ml. Once in solution, it is recommended that it be used within 8 hours as no preservative is added. Occasionally a small number of gelatinous-like fibers may develop upon standing. If this occurs, the solution can be filtered through a 5 micron filter to remove the particles with no change in potency.

Supplier: Commercially available

Toxicity: Acute toxicity includes anaphylactic reactions that occur most commonly when the drug is given IV. These can be characterized by laryngeal constriction, hypotension, diaphoresis, fever, chills, edema and loss of consciousness. Allergic reactions at the site of IM injection include pain, swelling and erythema. Other adverse effects include neutropenia and associated immunosuppression, mild nausea and vomiting, malaise, anorexia, elevated LFTs, pancreatitis and hyperglycemia. A decrease in protein synthesis including albumin, fibrinogen and other coagulation factors may occur which can result in hemorrhage. Thrombosis and/or pulmonary embolism can also occur. Less common side effects include renal dysfunction and CNS complications including somnolence, weakness, lethargy, coma and seizures.

Guidelines for administration: Intravenous or intramuscular injection, see section 5.2.1 (Remission induction) and 5.4 (Re-induction).

For additional information about this drug, please see package insert.

15. MERCAPTOPURINE (6-MP) (Purinethol®)

Source and pharmacology: Mercaptopurine is a purine antimetabolite. It must be converted intracellularly to 6-thioguanine nucleotides (6-TGNs), the active forms of the drug. The 6-TGNs are then incorporated into DNA and RNA and cause inhibition of DNA and RNA synthesis. Mercaptopurine is cell cycle, S phase specific. Absorption is variable and incomplete (5-37%) and is decreased by the presence of food in the gut. Mercaptopurine does distribute into the CSF, with CSF concentrations of ≈ 27% of plasma concentrations when given by continuous infusion. Mercaptopurine undergoes first pass metabolism in the GI mucosa and the liver. It is metabolized in hematopoietic tissues by HPRT to the active nucleotide forms. It is inactivated to methylated metabolites by TPMT (thiopurine methyl transferase) and to 6-thiouric acid by xanthine oxidase. TPMT is a genetically regulated, polymorphically distributed enzyme and is deficient in about 1 in 300 persons who cannot tolerate usual doses of 6-MP. Mercaptopurine is eliminated through the urine as both unchanged drug and metabolites.

Formulation and stability: Mercaptopurine is commercially available as a 50 mg tablet and 20 mg/mL oral suspension; store at room temperature and protect from light.

Supplier: Tablets are commercially available.

Toxicity: The dose-limiting toxicity of mercaptopurine is myelosuppression. Mercaptopurine can cause intrahepatic cholestasis and focal centralobular necrosis and is usually manifested by hyperbilirubinemia and increased liver function tests. Other toxicities include mild nausea and vomiting, skin rash, hyperuricemia, and mild diarrhea.
Guidelines for administration: oral tablets or suspension, see section 5.2.2 (Remission induction, Block II), section 5.3 (Interim continuation), section 5.5 (Continuation).

16. LEUCOVORIN (Folinic Acid)

Source and pharmacology: Leucovorin is a racemic mixture of tetrahydrofolic acid, which is involved as a cofactor for 1-carbon transfer reactions in the synthesis of purines and pyrimidines. Leucovorin is a potent antidote for both the hematopoietic and reticuloendothelial toxic effects of folic acid antagonists by replenishing reduced folate pools. It is postulated that in some cancers, leucovorin enters and "rescues" normal cells from the toxic effects of folic acid antagonists, in preference to tumor cells, because of differences in membrane transport and affinity for polyglutamylation. Leucovorin is converted in the intestinal mucosa and the liver to 5-methyl-tetrahydrofolate, which is also active as a reduced folate. It is excreted primarily in the urine with minor excretion occurring in the feces.

Formulation and stability: Leucovorin is supplied in 5, 15 and 25 mg tablets and vials containing 50, 100 or 350 mg of leucovorin as a lyophilized powder. The tablets and the lyophilized powder can be stored at room temperature. The 50 mg and 100 vials can be reconstituted by adding 5 or 10 ml of sterile water or bacteriostatic water for injection respectively to yield a final concentration of 10 mg/ml. The 350 mg vials can be reconstituted with 17 ml of sterile water or bacteriostatic water for injection to yield a final concentration of 20 mg/ml. The reconstituted solution is stable for at least 7 days at room temperature. Leucovorin may be further diluted in 5% dextrose or 0.9% NaCl containing solutions. Leucovorin is also available as a 1 mg/ml oral solution.

Supplier: commercially available

Toxicity: Leucovorin is generally well tolerated. Toxicities that have been reported uncommonly include rash, mild nausea, headache, and wheezing (possible allergic reaction). Intrathecal leucovorin is contraindicated and has caused neurotoxic deaths. There have been rare reports of leucovorin promoting seizures.

Guidelines for administration: oral or intravenous, see section 5.2.2 (Remission induction Block II after HDMTX, and section 5.7 (after ITHMA), section 5.7.2 (Down syndrome participants), and section 6.3.3.3 (ITHMA with CNS irradiation).

17. MITOXANTRONE (Novantrone®)

Source and pharmacology: Mitoxantrone is an anthracenedione that is structurally similar to the anthracyclines. It is thought to act by intercalating into DNA, causing template disorder, steric obstruction and inhibition of DNA and RNA synthesis. In addition, mitoxantrone inhibits the action of topoisomerase II. Mitoxantrone is active throughout the cell cycle. Mitoxantrone is about 78% protein bound and does cross the blood brain barrier. Mitoxantrone is metabolized in the liver to inactive metabolites. The parent drug and metabolites are excreted primarily via hepatobiliary excretion with small amounts excreted in the urine. Dosage adjustment is recommended for patients with severe hepatic dysfunction (total bilirubin > 3.4 mg/dl).

Formulation and stability: Mitoxantrone is available in multi-dose vials containing 20 mg, 25 mg or 30 mg of mitoxantrone as a dark blue, aqueous solution at a concentration of 2 mg/ml. The intact vials should be stored at room temperature. Refrigeration may result in precipitation of mitoxantrone, which will re-dissolve upon warming to room temperature. The drug should be further diluted to at least 50 ml in 5% dextrose or 0.9% NaCl prior to administration. These solutions are chemically stable for at least 7 days when stored at room temperature.

Supplier: commercially available

Toxicity: The major dose-limiting toxicity of mitoxantrone is leukopenia with thrombocytopenia and anemia occurring much less frequently. Nausea and vomiting are usually moderate in severity. Other side effects reported commonly include alopecia, diarrhea, headache, fever and stomatitis. Blue to green discoloration of urine and other body fluids occurs. Other side effects reported less commonly include elevated liver function tests, allergic reactions, seizures, jaundice and renal failure. Congestive heart failure has been reported, but is much less common than with doxorubicin. CHF has been reported primarily in patients receiving prior therapy with anthracyclines. Patients with an increased risk of cardiotoxicity include those having received prior therapy with anthracyclines, those with previous mediastinal radiotherapy and those with pre-existing cardiac conditions.

Guidelines for administration: Intravenous, see section 5.2.3 (Remission induction, Block III).

18. TENIPOSIDE (VM-26) (Vumon®)

Source and pharmacology**:** Teniposide is a semi-synthetic derivative of epipodophyllotoxin derived from *Podophyllum peltatum*. It is thought to act mainly by inhibiting topoisomerase II and thus causing double and single strand DNA breaks. Teniposide is cell cycle, phase-specific, with activity in the G2 and S phases. Teniposide is highly protein bound. It is extensively metabolized in the liver, including O-demethylation by cytochrome P450 3A4 to

reactive oxidative metabolites. It is excreted in the urine as parent drug and metabolite. Fecal excretion may account for a small amount of total excretion.

Formulation and stability: Teniposide is available in clear glass ampules containing 50 mg of teniposide. Each ml of teniposide solution contains 10 mg of teniposide, 30 mg benzyl alcohol, 500 mg Cremophor EL and ≈43% dehydrated alcohol. Intact ampules should be stored under refrigeration and protected from light. Freezing does not adversely affect the product. Teniposide must be diluted in 5% dextrose or 0.9% NaCl containing solutions prior to administration to a final concentration between 0.1-1mg/ml. At these concentrations, solutions are stable for up to 24 hours at room temperature. Solutions of 1mg/ml should be used within 4 hours of preparation. Refrigeration of diluted solutions is not recommended.

Supplier: Commercially available
Toxicity: The major dose-limiting effect of teniposide is bone marrow suppression. Nausea and vomiting (moderate in severity), diarrhea, alopecia and mucositis are reported commonly. Hypotension may occur if the drug is given too rapidly. Hypersensitivity reactions and secondary leukemias have been reported.

Guidelines for administration: Intravenous, see section 5.3 (Interim continuation) and section 5.5 (Continuation).

19. VINBLASTINE (Velban®)

Source and pharmacology: Vinblastine is an alkaloid extracted from the periwinkle plant (Vinca Rosea). It reversibly binds to microtubule and spindle proteins causing metaphase arrest. It may also block cellular utilization of glutamic acid, thereby inhibiting purine synthesis and urea formation via the citric acid cycle. Vinblastine is highly protein bound and poorly penetrates the CSF. Metabolism in the liver is extensive with one metabolite, deacetyl vinblastine, being more active than the parent drug. The major route of elimination is via the bile. Dosages should be adjusted for patients with impaired liver function (bilirubin > 3 mg/dl).

Formulation and stability: Vinblastine is available in 10 ml vials containing 1mg/ml of vinblastine in solution. Intact vials of vinblastine solution and lyophilized vinblastine should be stored under refrigeration.

Supplier: Commercially available

Toxicity: The dose limiting toxicity is myelosuppression. Other toxicities reported commonly include alopecia, mild nausea and vomiting and constipation. Vinblastine is a vesicant and may cause severe tissue damage if extravasation occurs. Vinblastine rarely produces neurotoxicity characterized by peripheral neuropathy, loss of deep tendon reflexes, weakness, jaw pain and “foot drop”. This toxicity is much less common than with vincristine. Acute shortness of breath and severe bronchospasm have been reported following the administration of vinca alkaloids.

Guidelines for administration: Intravenous, see section 5.3 (Interim continuation) and section 5.5 (Continuation).

The following tyrosine kinase inhibitors may be used for participants with Ph+ ALL and Ph-like ALL as part of standard clinical management, at the discretion of the treating investigator.

20. IMATINIB (Gleevec®, imatinib mesylate)

Source and pharmacology: imatinib mesylate is a phenylaminopyrimidine derivative and is a 4-[(4-Methyl-1-piperazinyl)methyl-N-[4-methyl-3-[[4-(3-pyridinyl)-2-pyrimid-inyl]amino]-phenyl]benzamidemethanesulfonate. It is a protein-tyrosine kinase inhibitor that inhibits the Bcr-Abl tyrosine kinase, the constitutive abnormal tyrosine kinase created by the Philadelphia chromosome abnormality.

Formulation and stability: Each film-coated tablet contains 100 mg or 400 mg of imatinib free base. The drug should be stored at 25°C (77°F); excursions permitted to 15°C-30°C (59°F - 86°F). The tablets should be dispensed in a tight container, USP, and protected from moisture.

The prescribed dose should be administered orally, with a meal and a large glass of water. In children, the daily dose may be split into two – once in the morning and once in the evening. For patients unable to swallow the film-coated tablets, the tablets may be dispersed in a glass of 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.

Supplier: Commercially available

Toxicity: Common toxicities include dyspepsia/heartburn, nausea/vomiting, headache, myelosuppression, and fatigue. Occasional toxicities include fever, edema in limbs, face, periorbital area, weight gain, increased SGOT/SGPT, alkaline phosphatase, bilirubin, abdominal pain and cramping, myalgia, arthralgia, decreased bone marrow cellularity, lymphopenia, eczema dermatitis, rash, muscle pain and cramping, anorexia, and pigmentation changes (hypo-vitiligo). Rare toxicities include cerebral edema, melena/GI bleeding, anemia, diarrhea, dysphagia, esophagitis, odynophagia, hemorrhage/bleeding without grade 3 or 4 thrombocytopenia, pneumonitis/pulmonary infiltrates, late hepatotoxicity and decrease in the heart's ability to pump blood.

21. DASATINIB (SPRYCEL®)

Source and pharmacology: Dasatinib (an aminothiazole analogue) is an inhibitor of multiple tyrosine kinases. It is approved for the treatment of chronic myelogenous leukemia (CML) and for the treatment of adults with Philadelphia chromosome-positive ALL with resistance or intolerance to prior therapy. It is being investigated as a broad-spectrum antitumor agent against solid tumors. Dasatinib is a potent, broad spectrum ATP-competitive inhibitor of 5 critical oncogenic tyrosine kinase families: BCR-ABL, SRC family kinases, c-KIT, ephrin (EP) receptor kinases, and PDGFβ receptor. Each of these protein kinases has been strongly linked

to multiple forms of human malignancies. In adults, the maximum plasma concentration of dasatinib after oral administration is observed between 0.5 and 6 hours. The observed effects from food were not clinically relevant. The overall terminal half-life is 3-5 hours. The drug exhibits dose proportional increases in AUC and linear elimination characteristics over the dose range of 15 mg to 240 mg/day in adults. Dasatinib is primarily metabolized in the liver by the human CYP3A4 enzyme, is a significant inhibitor of CYP3A4. Dasatinib may decrease the metabolic clearance of drugs that are significantly metabolized by the CYP3A4 enzyme. Due to the potential of dasatinib to prolong the QT/QTc, caution must be used when administering it with other potential QTc prolonging medications. Due to the possibility of gastrointestinal, cardiac, and cutaneous hemorrhage, avoid using medications that inhibit platelet function or anticoagulants in conjunction with Dasatinib. Dasatinib is not a p - glycoprotein inhibitor.

Formulation and stability: Dasatinib is available as tablets of 20 mg, 50 mg, or 70 mg. The core tablet is surrounded by a film coating to prevent exposure to the active drug substance during handling. If tablets are cut or crushed, procedures to prevent exposure to the active drug substance should be followed. Pregnant women or breastfeeding mothers should not handle crushed and/or broken dasatinib tablets.

The intact dasatinib tablets can be placed (and allowed to dissolve) in 1 ounce of lemonade (a double strength juice is recommended to obscure the bitter taste), or 1 ounce of preservative-free apple juice, or 1 ounce of preservative-free orange juice.

The following (i.e. steps 1 thru 7) is the procedure for the preparation of the lemonade dosing solution. For preservative-free apple juice or preservative-free orange juice, steps 2 thru 7 should be followed.

1. Mix the contents of one 12 ounce can of Minute Maid Premium Frozen Concentrate with 2 cans (i.e. the emptied lemonade container) of water. This will produce lemonade that is a little more than twice as concentrated as the instructions on the can with a sweeter taste. Please keep the lemonade solution refrigerated when not in use.
2. Place 1 ounce (30 mL) of this lemonade solution into a drinking glass.
3. Place the proper dose of intact tablets into the lemonade. Please be sure to always wear protective gloves when handling the medication. A mask is not required when handling the medication. Always use the 1 oz of lemonade. Do not increase the lemonade volume.
4. Start timing for 20 minutes. At approximately the 5 minute mark, swirl the contents of the glass well for about 3 seconds.
5. At approximately the 15 minute mark, swirl the contents of the glass a second time. At the 20 minute mark, swirl the contents of the glass one last time. Immediately administer the entire contents of the glass.
6. In order to ensure administration of the entire medication dose, a rinsing step is necessary. Add 0.5 ounce (15 mL) of lemonade into the same glass that has just been emptied. Swirl the contents to remove any remaining signs of tablets from the sides or bottom of the glass.
7. Administer the washing lemonade to the patient.

The intact bottles should be stored at controlled room temperature (15° - 25°C (59° - 77°F) and protected from light.

Supplier: Commercially available

Toxicity: The most frequently reported adverse events include fluid retention (pleural effusion), diarrhea, nausea, abdominal pain, rash, headache, fatigue, vomiting, bleeding events and myelosuppression. The most frequently reported serious adverse events include pyrexia, febrile neutropenia, gastrointestinal bleeding, pneumonia, thrombocytopenia, dyspnea, anemia and cardiac failure (3%). Less commonly reported are anorexia, dehydration, abdominal distension and flatulence, flushing, pruritis, elevated creatinine, and neuropathy. Elevations in transaminases and bilirubin can usually be managed with dose reductions or dose interruption. Hypocalcemia during dasatinib therapy can be managed with oral calcium supplementation.

22. NILOTINIB (Tasigna™)

Source and pharmacology**:** Nilotinib is a selective Bcr/Abl tyrosine kinase inhibitor that may be used in the treatment of chronic myeloid leukemia (CML) and Philadelphia chromosome acute lymphoblastic leukemia (Ph+ALL). Nilotinib binds to and stabilizes the inactive conformation of the kinase domain of Abl protein. It is considered to be a "second generation" tyrosine kinase inhibitor and may be efficacious in patients who have becomes resistant to "first generation" drugs such as imatinib. Nilotinib is an analog of imatinib and features a more energetically favorable binding mode that contributes to its enhanced potency (twenty times higher than imatinib). The increased potency is hoped to allow lower dosages, which may limit side effects that would otherwise make this medication intolerable.

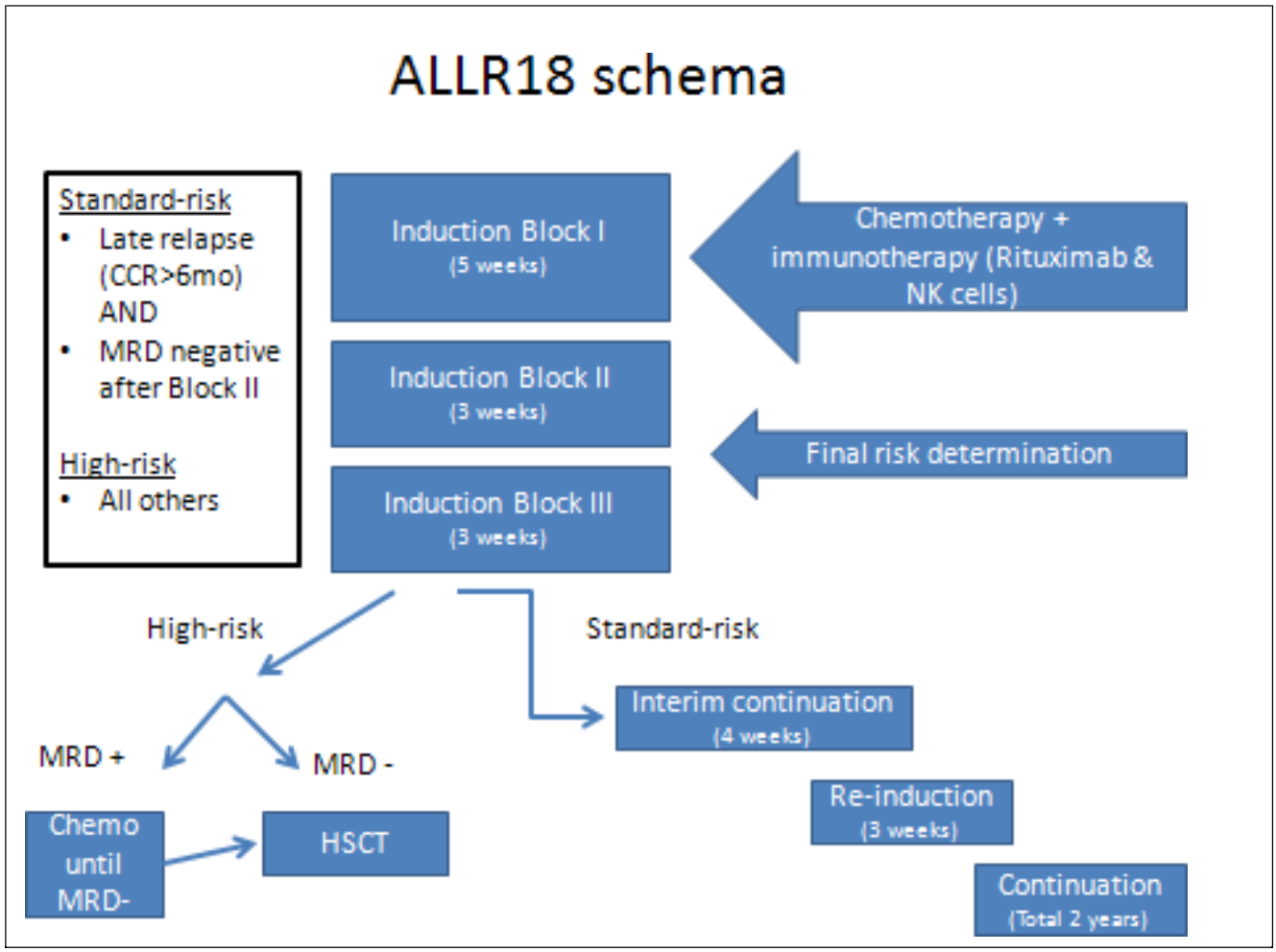
Formulation and stability**:** Tasigna (nilotinib) capsules, for oral use, contain 150 mg or 200 mg nilotinib base, anhydrous (as hydrochloride, monohydrate) with the following inactive ingredients: colloidal silicon dioxide, crospovidone, lactose monohydrate, magnesium stearate and polyoxamer 188. The capsules contain gelatin, iron oxide (red), iron oxide (yellow), iron oxide (black) and titanium dioxide. The capsules should be stored at 25°C, although excursions are permitted between 15-30°C.

Supplier**:** Commercially available

Toxicity**:** Its most common side effects, occurring in over 10% of patients, include peripheral edema, headache, rash, pruritis, fatigue, GI upset, increased lipids, neutropenia (grades 3/4, median duration 15 days), thrombocytopenia (grades 3/4, median duration 22 days), neuralgias, musculoskeletal pain, and upper respiratory tract infections. Other serious side effects include QT prolongation, depression, insomnia, electrolyte disturbances, pancytopenia, and elevated LFTs. Reports of sudden deaths from QTc prolongation has let the FDA to require a "Black Box" warning on Tasigna emphasizing the need to monitor electrolyte levels and co-administration of other medications in patients taking the drug. Nilotinib should not be

taken with drugs that prolong the QT interval or inhibitors or inducers of CYP3A4. It may induce other CYP enzymes, thus affecting the metabolism of other drugs. It also inhibits P-glycoprotein and is a substrate for its efflux transporter. The REMS (Risk Evaluation and Mitigation Strategy) approved in conjunction with the Black Box Warning requires that all patients receive specific oral and written counseling to take nilotinib on an empty stomach, time doses appropriately, get regular ECGs, avoid grapefruit products, and report all other medications to the prescribing physician. Nilotinib should be used with caution in patients with a history of pancreatitis or impaired hepatic function.

Dosage and route of administration**:** Nilotinib is available as oral capsules in 150 mg and 200 mg strengths. Nilotinib capsules should be taken twice daily (about 12 hours apart) with water. It must not be taken with food as this can significantly change adsorption; patients should not eat 2 hours prior or 1 hour after taking nilotinib. For patients unable to swallow capsules, the contents may be mixed with a small amount of pureed apple sauce and taken within 15 minutes.



APPENDIX V: TREATMENT AND EVALUATION CALENDARS

Pretreatment - See order sheets. Offer enrollment for institutional banking and pharmacogenetic protocols

Block I – must be given at St. Jude (including collaborating site participants)

1 **ITMHA*** **VCR** **DEX** *CSF cell count, diff and cytology	2 **DEX** #Peripheral blood CD20	3 **DEX** TPMT genotyping *(if not available from frontline therapy)* Germline DNA	4 **RITUX** **DEX** #Peripheral blood CD20	5 **(ITMHA*)** **DEX** (*CSF cell count, diff and cytology)	6 **CLO** **CYCLO** **VP16** **DEX** #Peripheral blood MRD and CD20	7 **CLO** **CYCLO** **VP16** **DEX**
8 **ITMHA*** **CLO** **CYCLO** **VP16** **DEX** *CSF cell count, diff and cytology	9 **CLO** **CYCLO** **VP16**	10 **CLO** **CYCLO** **VP16**	11 **(ITMHA*)** **IL-2** #Peripheral blood CD20 (*CSF cell count, diff and cytology)	12 **NK cell infusion**	13 **RITUX** **IL-2**	14 #Peripheral blood CD20
15 **IL-2**	16	17 **IL-2**	18	19 **IL-2** NK Pheno (patient)	20 **RITUX**	21 **PEG-ASP**** **VCR** **(ITMHA*)** **DEX** (*CSF cell count, diff and cytology) #Peripheral blood CD20 **Asp & antibodies ***TKI Ph+ ALL & Ph-like ALL
22 **DEX**	23 **DEX**	24 **DEX**	25 **DEX**	26 **DEX**	27 **RITUX** **DEX**	28 **ITMHA*** **VCR** **DEX** *CSF cell count, diff and cytology
29	30	31	32	33	34	35 **PEG-ASP**** **VCR** **Asp & antibodies BM/MRD and mutant cells after count recovery

Notes:

1. #Peripheral blood MRD and CD20 evaluations – will be discontinued following a negative MRD result (less than 0.01%)
2. *CNS1: ITMHA Days 1, 8, 28
   CNS2 and CNS3 and traumatic tap with blasts: Days 1, 5, 8, 21 and 28. If CNS blasts not cleared by Day 8, additional ITMHA on Day 11
3. **Asparaginase and anti-asparaginase antibodies: additional samples at time of reaction
4. H&P, CBC diff, CMP every 3-7 days
5. ***For participants with Ph+ ALL and Ph-like ALL (see section 6.1), tyrosine kinase inhibitor (TKI) may be added at the discretion of the treating physician and principal investigator (e.g., imatinib, dasatinib and nilotinib). TKI's should be introduced after day 20 of Induction Block I therapy to avoid drug interactions and interference with NK cell function. Treatment with TKI will be as per SOC or manufacturer's recommendation. See package insert for specific drug for details.

Block II – HDMTX and 6MP

1 ***ITMHA** **HDMTX** **6MP** *CSF cell count, diff Germline DNA	2 **6MP**	3 **6MP**	4 **6MP**	5 **6MP**	6 **6MP**	7 **6MP**
8 **HDMTX** **6MP**	9 **6MP**	10 **6MP**	11 **6MP**	12 **6MP**	13 **6MP**	14 **6MP**
15 **6MP**	16 **6MP**	17 **6MP**	18 **6MP**	19 **6MP**	20 **6MP**	21 **6MP**
22 BMA/MRD/mutant cells day 21	23	24	25	26	27	28

Notes:

1. H&P, CBC diff, CMP every 3-7 days

Block III – ARAC and MITO

1 **ARA-C** **ARA-C**	2 **ARA-C** **ARA-C**	3 **ARA-C** **ARA-C** **MITO**	4 **ARA-C** **ARA-C** **MITO**	5 **MITO**	6	7 ***ITMHA** *CSF cell count, diff
8	9	10	11	12	13	14
15	16	17	18	19	20	21 BMA/MRD/mutant after cell count recovery CT scan involved areas - lymphoma pts.

Notes:

1. H&P, CBC diff, CMP every 3-7 days

Interim Continuation

1 **ITMHA*** **VP16** **CYCLO** *CSF cell count, diff Asp & antibodies	2	3	4	5	6	7
8 **MTX** **6MP**	9 **6MP**	10 **6MP**	11 **6MP**	12 **6MP**	13 **6MP**	14 **6MP**
15 **VM26** **ARA-C**	16	17	18	19	20	21
22 **VINBLAST** **DEX**	23 **DEX**	24 **DEX**	25 **DEX**	26 **DEX**	27	28 BMA/MRD/mutant cells upon count recovery

Notes:

1. H&P, CBC diff every week, CMP as clinically indicated
2. Serum for asparaginase and anti-asparaginase antibodies

Re-Induction

1 **ITMHA*** **CLO** **CYCLO** **VP16** **DEX** *CSF cell count, diff, cytology	2 **CLO** **CYCLO** **VP16** **DEX**	3 **CLO** **CYCLO** **VP16** **DEX**	4 **CLO** **CYCLO** **VP16** **DEX**	5 **CLO** **CYCLO** **VP16** **DEX**	6 **PEG-ASP**** **VCR** **DEX** **Asp & antibodies	7
8	9	10	11	12	13 **VCR**	14
15 **(*ITMHA)** (*CSF cell count, diff, cytology)	16	17	18	19	20 **PEG-ASP**** **VCR** **Asp & antibodies	21 BMA/MRD/mutant cells after count recovery

Notes:

1. *CNS1: ITMHA on Day 1
   CNS 2, 3 and traumatic tap with blasts: ITMHA on Days 1 and 15
2. **Asp & antibodies: additional samples at time of reaction
3. H&P, CBC diff and CMP every 3-7 days

CONTINUATION THERAPY

Week	Treatment	Special Studies
1	MTX + 6MP + ITMHA	CSF cell count, diff
2	MTX + 6MP	
3	VM26 + ARA-C	
4	DEX + VCR	
5	MTX + 6MP (+ ITMHA)	(CSF cell count, diff)
6	MTX + 6MP	
7	VM26 + ARA-C	
8	DEX + VINBLAST	
9	MTX + 6MP + ITMHA)	CSF cell count, diff
10	MTX + 6MP	
11	VM26 + ARA-C	
12	DEX + VCR	
13	MTX + 6MP (+ ITMHA)	(CSF cell count, diff)
14	MTX + 6MP	
15	VM26 + ARA-C	
16	DEX + VINBLAST	
17	MTX + 6MP + ITMHA	CSF cell count, diff
18	MTX + 6MP	
19	VM26 + ARA-C	
20	DEX + VCR	
21	MTX + 6MP (+ ITMHA)	(CSF cell count, diff)
22	MTX + 6MP	
23	VM26 + ARA-C	
24	DEX + VINBLAST	
25	MTX + 6MP + ITMHA	CSF cell count, diff
26	MTX + 6MP	
27	VM26 + ARA-C	
28	DEX + VCR	
29	MTX + 6MP (+ ITMHA)	(CSF cell count, diff)
30	MTX + 6MP	
31	VM26 + ARA-C	
32	DEX + VINBLAST	
33	MTX + 6MP + ITMHA	CSF cell count, diff
34	MTX + 6MP	
35	VM26 + ARA-C	
36	DEX + VCR	
37	MTX + 6MP (+ ITMHA)	(CSF cell count, diff)
38	MTX + 6MP	
39	VM26 + ARA-C	
40	DEX + VINBLAST	
41	MTX + 6MP + ITMHA	CSF cell count, diff
42	MTX + 6MP	
43	VM26 + ARA-C	
44	DEX + VCR	
45	MTX + 6MP (+ ITMHA)	(CSF cell count, diff)
46	MTX + 6MP	
47	VM26 + ARA-C	
48	DEX + VINBLAST	
49	MTX + 6MP + ITMHA	CSF cell count, diff
50	MTX + 6MP	
51	VM26 + ARA-C	
52	DEX + VCR	
53	MTX + 6MP (+ ITMHA)	(CSF cell count, diff)

Week	Treatment	Special Studies
54	MTX + 6MP	
55	VM26 + ARA-C	
56	DEX + VINBLAST	
57	MTX + 6MP + ITMHA	CSF cell count, diff
58	MTX + 6MP	
59	VM26 + ARA-C	
60	DEX + VCR	
61	MTX + 6MP (+ ITMHA)	(CSF cell count, diff)
62	MTX + 6MP	
63	VM26 + ARA-C	
64	DEX + VINBLAST	
65	MTX + 6MP + ITMHA	CSF cell count, diff
66	MTX + 6MP	
67	VM26 + ARA-C	
68	DEX + VCR	
69	MTX + 6MP (+ ITMHA)	(CSF cell count, diff)
70	MTX + 6MP	
71	VM26 + ARA-C	
72	DEX + VINBLAST	
73	MTX + 6MP + ITMHA	CSF cell count, diff
74	MTX + 6MP	
75	VM26 + ARA-C	
76	DEX + VCR	
77	MTX + 6MP (+ ITMHA)	(CSF cell count, diff)
78	MTX + 6MP	
79	VM26 + ARA-C	
80	DEX + VINBLAST	*off therapy

Notes:

1. BMA/MRD/mutant cells every 16 weeks
2. CNS1: Cycles 1-10, day 1 of week 1
   CNS2 and CNS3 and traumatic tap with blasts:
   a. Cycles 1-5: day 1 of week 1 and day 1 of week 5
   b. Cycles 6-10: day 1 of week 1
3. H&P and CBC diff every week, CMP as clinically indicated
4. *Off therapy evaluations: H&P, CBC diff, CMP, BMA/MRD, ECHO/EKG, CT scan involved areas (lymphoma pts.)

APPENDIX VI: DEFINITIONS RELATED TO INFECTIOUS DISEASES

I. ASSESSMENTS

A. Fever: A single temperature of > 38.3ºC (101ºF) or ≥38.0ºC (100.4ºF) on two occasions within 12 hours. The measurement must be oral with glass or IVAC[7] thermometers or at the tympanic membrane by infrared instruments (Thermoscan[7]). The same method should be used throughout the febrile episode for each patient.

B. Neutropenia: Neutrophil count < 500/mm^3 or < 1,000/mm^3 with predicted decline to ≤500/mm^3.

C. Duration of Fever (for episodes of fever with neutropenia): The initial temperature is the one immediately before the first dose of antibiotics and GCSF. This is designated as zero hour. The end of a febrile period is at the time of the first temperature of 38.0ºC or less which is sustained at this level over a period of 24 hours or longer without antipyretic intervention. The duration of fever is the number of hours from zero hour to the end of the febrile period.

D. Antibiotic Therapy: Any systemic antibacterial drug will be considered as antibiotic therapy whether given orally or parenterally. Topical antibiotics will not be included.

E. Antifungal Therapy: Any antifungal drug administered orally or parenterally will be considered antifungal therapy. Antifungal therapy will be categorized for analysis as:

1. Treatment for oral candidiasis
   Nystatin
   Clotrimazole troches
   Micafungin
   Fluconazole
   Voriconazole
2. Treatment for systemic mycoses (includes empirical use)
   Systemic amphotericin B (standard and liposomal)
   Fluconazole
   Voriconazole
   Posiconazole
   Itraconazole
   Micafungin

II. BACTERIAL

1. Bacteremia only: Defined as a growth of an organism that is judged not to be a contaminant in a blood culture drawn during a febrile episode. Organisms are considered contaminants if they are part of skin flora (e.g., diphtheroids other than *Corynebacterium jeikeium*, *Bacillus spp. not B. cereus, Propionibacterium spp., coagulase-negative staphylococcus (CNS) or Micrococcus spp.*) and if they are isolated from only one culture receptacle. All other organisms are regarded as pathogens. (Specify catheter-related or not.)

2. Bacterial sepsis: positive blood culture for any bacterium plus clinical evidence of infection (fever, chills, hypotension, etc.). (Specify catheter-related or not.)
3. Urinary tract infection: urine colony count of 100,000 or greater of a single organism plus symptoms, dysuria, flank pain, etc. Asymptomatic bactiuria is the same colony count without symptoms.
4. Pneumonia (bacterial): radiographic discernible infiltrate plus isolation of potentially causative bacteria from bronchoalveolar lavage, blood or biopsy specimen. If positive blood culture, code as bacterial sepsis with pneumonia.
5. Meningitis: positive culture of causative bacteria from CSF plus symptoms compatible with meningitis.
6. Osteomyelitis: radiographic lesions plus positive blood or bone aspirate/biopsy cultures.
7. Acute Otitis Media: physician diagnosis plus antibiotic treatment.
8. Pharyngitis: only if group A beta hemolytic streptococcus is isolated from throat culture in patient with symptoms. A positive rapid streptococcal test is acceptable in place of culture. Other types of pharyngitis will not be considered.
9. Cellulitis: erythema and induration plus isolation of bacteria from aspirate/ drainage.

III. FUNGAL INFECTIONS

A. Candidiasis
   1. Oral: presence of typical whitish lesions on the mucosal surface with yeast or pseudomycelia on gram stain or KOH preparation or isolation of *Candida* species in culture from the mouth.
   2. Esophageal or urinary bladder: evidence of tissue involvement proven by endoscopy and biopsy plus isolation of fungus in culture.

B. Invasive fungal infections
   1. Proven invasive fungal infection (not endemic mycosis)

Analysis and specimen	Molds[a]	Yeasts[a]
Microscopic analysis: sterile material	Histopathologic, cytopathologic, or direct microscopic examination[b] of a specimen obtained by needle aspiration or biopsy in which hyphae or melanized yeast-like forms are seen accompanied by evidence of associated tissue damage	Histopathologic, cytopathologic, or direct microscopic examination[b] of a specimen obtained by needle aspiration or biopsy from a normally sterile site (other than mucous membranes) showing yeast cells – for example, *Cryptococcus* species indicated by encapsulated budding yeasts or *Candida* species showing pseudohyphae or true hyphae[c]

Culture		
Sterile material	Recovery of a mold or "black yeast" by culture of a specimen obtained by a sterile procedure from a normally sterile and clinically or radiographically abnormal site consistent with an infectious disease process, excluding bronchoalveolar lavage fluid, a cranial sinus cavity specimen, and urine	Recovery of a yeast by culture of a sample obtained by a sterile procedure (including a freshly placed [<24 h ago] drain) from a normally sterile site showing a clinical or radiographical abnormality consistent with an infectious disease process
Blood	Blood culture that yields a mold[d] (e.g. *Fusarium* species) in the context of a compatible infectious disease process	Blood culture that yields yeast (e.g. *Cryptococcus* or *Candida* species) or yeast-like fungi (e.g. *Trichosporon* species)
Serological analysis: CSF	Not applicable	Cryptococcal antigen in CSF indicates disseminated cyroptococcosis

[a] If culture is available, append the identification at the genus or species level from the culture results.
[b] Tissue and cells submitted for histopathologic or cytopathologic studies should be stained by Grocott-Gomorri methenamine silver stain or by periodic acid Schiff stain, to facilitate inspection of fungal structures. Whenever possible, wet mounts of specimens from foci related to invasive fungal disease should be stained with a fluorescent dye (e.g., calcofluor or blankophor).
[c] Candida, Trichosporon, and yeast-like Geotrichum species and Blastoschizomyces capitatus may also form pseudohyphae or true hyphae.
[e] Recovery of Aspergillus species from blood cultures invariably represents contamination

2. Probable invasive fungal infection (not endemic mycosis)

Host factors[a]

- Recent history of neutropenia (<0.5 x 10^9 neutropils/mm^3) for > 10 days) temporally related to the onset of fungal disease
- Receipt of an allogeneic stem cell transplant
- Prolonged use of corticosteroids (excluding among patients with allergic bronchopulmonary aspergillosis) at a mean minimum dose of 0.3 mg/kg/day of prednisone equivalent for > 3 weeks.
- Treatment with other recognized T cell immunusuppressants, such as cyclosporine, TNF-α blockers, specific monoclonal antibodies (such as alemtuzumab), or neucleoside analogues during the past 90 days.
- Inherited severe immunodeficiency (such as chronic granulomatous disease or severe combined immunodeficiency)

Clinical criteria[b]

- Lower respiratory tract fungal disease[c]
  - The presence of 1 of the following 3 signs on CT:
    - Dense, well-circumscribed lesion(s) with our without a halo sign
    - Air-crescent sign
    - Cavity
- Tracheobronchitis
  - Tracheobronchial ulceration, nodule, pseudomembrane, plaque, or eschar seen on bronchoscopic analysis
- Sinonasal infection
  - Imaging showing sinusitis plus at least 1 of the following 3 signs:
    - Acute localized pain (including pain radiating to the eye)
    - Nasal ulcer with black eschar
    - Extension from the paranasal sinus across bony barriers, including into the orbit
- CNS infection
  - 1 of the following 2 signs:
    - Focal lesions on imaging
    - Meningeal enhancement on MRI or CT
- Disseminated candidiasis[d]
  - At least 2 of the following 2 entities after an episode of candidemia within the previous 2 weeks:
    - Small, target-like abscesses (bull's eye lesions) in liver or spleen
    - Progressive retinal exudates on ophthalmologic examination

Mycological criteria

- Direct test (cytology, direct microscopy, or culture)
  - Mold in sputum, bronchoalveolar lavage fluid, bronchial brush, or sinus aspirate samples, indicated by 1 of the following
    - Presence of fungal elements indicating a mold
    - Recovery by culture of a mold (e.g. *Aspergillus, Fusarium, Zygomycetes, or Scedosporium* species)
- Indirect tests (detection of antigen or cell-wall constituents)[e]
  - Aspergillosis
    - Galactomannan antigen detected in plasma, serum, bronchoalveolar lavage, or CSF
  - Invasive fungal disease other than cryptococcosis and zygomycoses
    - β-$_D$-glucan detected in serum

NOTE: Probable IFD requires the presence of a host factor, a clinical criteriaon, and a mycological criterion. Cases that meet the criteria for a host factor and a clinical criterion but for which mycological criteria are absent are considered possible IFD.

[a] Host factors are not synonymous with risk factors and are characteristics by which individuals predisposed to IFDs can be recognized. They are intended primarily to apply to patients given treatment for malignant disease and to recipients of allogeneic hematopoietic stemm cell and solid-organ transplants. These factors are also applicable to patients who receive corticosteroids and other T-cell suppressants as well as to patients with primary immunodeficiencies.

[b] Must be consistent with the mycological findings, if any, and must be temporally related to current episode

[c] Every reasonable attempt should be made to exclude an alternative etiology

[d] The presence of signs and symptoms consistent with sepsis syndrome indicates acute disseminated disease, whereas their absence denotes chromic disseminated disease
[e] These tests are primarily applicable to aspergillosis and candidiasis and are not useful in diagnosing infections due to Cryptococcus species or Zygomycetes (e.g. Rhizopus, Mucor, or Absidia species). Detection of nucleic acid is not included, because there are as yet no validated or standardized methods.

3. Possible invasive fungal infection (not endemic mycosis)
In the table above, cases that meet the criteria for a host factor and a clinical criterion but for which mycological criteria are absent are considered possible IFD.

C. Histoplasmosis: a diagnosis of disseminated disease may be established by a positive *Histoplasma* antigen test on CSF, urine or serum by EIA, or the presence of characteristic intracellular yeast forms in a peripheral blood smear or in bone marrow.

Criteria for the diagnosis of endemic mycoses
Diagnosis and criteria
Proven endemic mycosis
In a host with an illness consistent with an endemic mycosis, 1 of the following • Recovery in culture from a specimen obtained from the affected site or from blood • Histopathologic or direct microscopic demonstration of appropriate morphologic forms with a truly distinctive appearance characteristic of dimporphic fungi, such as *Coccidiodes* species spherules, *Blastomyces dermatitis* thick-walled broad-based budding yeasts, *Paraccidioides brasilensis* multiple budding yeast cells, and, in the case of histoplasmosis, the presence of characteristic intracellular yeast forms in a phagocyte in a peripheral blood smear or in tissue macrophages • For coccidioidomycosis, demonstration of coccidioidal antibody in CSF, or a 2-dilution rise measured in 2 consecutive blood samples tested concurrently in the setting of an ongoing infectious disease process. • For paracoccidioidomycosis, demonstration in 2 consecutive serum samples of a precipitin band to paracoccidioidin concurrently in the setting of an ongoing infectious disease process
Probable endemic mycosis
• Presence of a host factor, including but not limited to those specified in #2 Table above, plus a clinical picture consistent with endemic mycosis and mycological evidence, such as positive Histoplasma antigen test result from urine, blood, or CSF

Note: Endemic mycosies includes histoplasmosis, blastomycosis, coccidioidomycosis, paracoccidioidomycosis, sporotrichosis, and infection due to Penicillium marneffei. Onset within 3 months after presentation defines a primary pulmonary infection. There is no category of possible endemic mycosis, as such, because neither host factors nor clinical features are sufficiently specific; such cases are considered to be of value too limited to include in clinical trials, epidemiological studies, or evaluations of diagnostic tests.

D. Others: the investigator may include certain other infectious diseases of unique nature. Acceptance requires decision before decoding occurs and with the agreement of the principal investigator and an attending from the Infectious Disease Department.

IV. PROTOZOAN

A. *Pneumocystis carinii* Pneumonia: discernible radiographic lesion plus identification of P. *carinii* in bronchoalveolar lavage fluid, biopsy or induced sputum.
B. *Cryptosporidiosis*: *C. parum* identified in stool plus diarrhea.
C. *Toxoplasmosis:* see ACTG protocol 254.

V. TOPOGRAPHICAL

A. The following diagnoses are acceptable for objectively identified infections without confirmation of etiology.
   1. Pneumonia: lesion on radiograph
   2. Osteomyelitis: radiological diagnosis
   3. Sinusitis: radiological diagnosis (x-ray, CT, MRI)
   4. Cellulitis: physician diagnosis

VI. FEVER OF UNDETERMINED ETIOLOGY

B. Fever without any focus or etiology identified by clinical history, physical examination, radiological or microbiologic finding. Identify as:
   1. neutropenic = ANC $<500/mm^3$
   2. non-neutropenic = ANC $\geq 500/mm^3$

VII. CULTURE NEGATIVE SEPSIS

In the absence of a positive culture, a systemic response to a possible infection by hemodynamic instability, focal or multiple organ involvement such as poor skin perfusion, oliguria, hypoxemia, and/or altered mental status or lethargy

APPENDIX VII: COMMON SUBSTRATES, INHIBITORS AND INDUCERS OF CYP3A4/3A5

The following lists describe medications which are common CYP3A4 substrates, inhibitors and inducers. This list should not be considered all inclusive. Consult Pharmaceutical Services for specific information on metabolism by CYP3A4/CYP3A5 and particularly for Ph+ participants receiving tyrosine kinase inhibitor.

substrates		
Macrolide antibiotics	clarithromycin erythromycin NOT azithromycin	
Anti-arrhythmics	quinidine	
Benzodiazepines	alprazolam diazepam midazolam triazolam	
Immune modulators	cyclosporine tacrolimus (FK506)	sirolimus
HIV antivirals	indinavir nelfinavir ritonavir saquinavir	
Antihistamines	astemizole chlorpheniramine terfenidine	
Calcium channel blockers	amlodipine diltiazem felodipine lercanidipine	nifedipine nisoldipine nitrendipine verapamil
HMG CoA reductase inhibitors	atorvastatin cerivastatin lovastatin NOT pravastatin simvastatin	
Steroid 6beta-OH	estradiol hydrocortisone progesterone testosterone	

Substrates - continued		
Other	alfentanyl buspirone cafergot caffeine=>TMU cocaine dapsone codeine-N-demethyl dextromethorphan eplerenone fentanyl finasteride imatinib haloperidol (in part) irinotecan	Lidocaine methadone ondansetron pimozide propranolol quinine salmetrerol sildenafil tamoxifen paclitaxel trazodone vincristine zaleplon zolpidem
inhibitors		
HIV antivirals	delaviridine indinavir nelfinavir ritonavir saquinavir	
Other	amiodarone NOT azithromycin cimetidine ciprofloxacin clarithromycin diethyl-dithiocarbamate diltiazem erythromycin fluconazole fluvoxamine gestodene	grapefruit juice itraconazole ketoconazole mifepristone nefazodone norfloxacin norfluoxetine mibefradil verapamil voriconazole
inducers		
HIV antivirals	efavirenz nevirapine	
Other	barbiturates carbamazepine glucocorticoids modafinil phenobarbital phenytoin	

APPENDIX VIII: HDMTX ADMINISTRATION AND MONITORING GUIDELINES INDUCTION BLOCK II

High-dose MTX (HDMTX) will be given at a dose of 5 g/m^2 over 24 hours or targeted to a Cpss of 65 μM.

The physician or pharmacist should review the past and current medication list for the patient prior to administration of HDMTX. Every effort should be made to avoid nephrotoxins (amphotericin, acyclovir) and drug that could compete with MTX (penicillins and proton pump inhibitors) on the day of and the day after HDMTX begins; use of NSAIDs and salicylates of any kind is contraindicated. HDMTX should not be given to patients with any residual mucositis without discussion with the PI of the protocol.

Standing orders should be in place that includes twice daily BMP, magnesium, and phosphorus. In addition, orders should include specific instructions to notify the physician of record or their designee. These include:

- Notification if HDMTX stopped for a cumulative time of 60 minutes or more
- Any increase in serum creatinine by $\geq$ 0.3 mg/dL or a doubling of creatinine from pre-HDMTX value
- 2 or more consecutive urine pH < 6.5 or 2 consecutive urine pH $\geq$ 9
- No urine output for 4 hours
- $\geq$ 2 episodes of diarrhea in a 24 hour period within first 48 hours after start of HDMTX
- $\geq$ 2 episodes of emesis in a 4 hour period within first 48 hours after start of HDMTX
- Any episode of hypotension or sepsis within first 48 hours after start of HDMTX

MTX plasma concentrations will be obtained at least 4 times with the HDMTX: prior to starting the infusion and at hours 6, 23, and 42 from the start of the infusion. Patients with MTX concentrations at 42 hours above 0.5 μM will be assessed at least daily until the concentration is < 0.1 μM. After the patient has cleared MTX, please fax copies of the MTX concentrations (from your lab system) to the SJ Pharmacokinetics Lab at [redacted] for inclusion into the SJ electronic medical record.

Ideally, each patient will receive IV hydration with Bicarb beginning the evening prior to scheduled admission for HDMTX with a rate of at least 125 mL/m^2/hr. Hydration should continue at this rate until the MTX concentration is < 0.5 μM or longer if the patient has a history of toxicity or delayed excretion. Patients with poor clearance are candidates for increased hydration and more aggressive alkalinization. Acetazolamide is an effective diuretic in patients with delayed MTX excretion. Patients with MTX concentrations > 1 μM benefit from increasing urine output.

MTX dosing may be individualized to achieve a target Cpss of 65 μM. The physician or their designee at the collaborating site should contact a SJ clinical pharmacist to discuss targeting and leucovorin rescue. (Please use the group email [redacted]).SJ will also provide contact information for help during the infusion at that time.

Leucovorin rescue should begin at 42 hour from the start of HDMTX. The dose is 15 mg/m^2/dose po or IV q6h x 3 doses. Patients with a history of delayed Grade 3 or 4 GI toxicity with prior HDMTX or a history of typhlitis with any chemotherapy should have leucovorin continue for 5 doses, rather than 3. Those with early toxicity should have leucovorin begin at hour 36. Patients with vomiting, poor oral intake, period (>4 hours) of no urine output should receive all leucovorin doses IV. All doses > 50 mg should be given IV.

MTX Concentration	**Threshold for Action**	**Recommended LV Rescue**
6 hr – 5 gm/m^2 or targeted to 65 µM	95 – 150 µM	Check that hydration and alkalinization are adequate; check for nephrotoxic drugs; check creatinine; notify the house officer and floor nurse to be particularly vigilant with urine output
	> 150 µM	All of above plus obtain another blood sample between 12 and 24 hrs for assay
Extra sample between 12 and 24 hr because of suspected delayed excretion - 5 gm/m^2 or targeted to 65 µM	95 - 150 µM	Check that hydration and alkalinization are adequate; check for nephrotoxic drugs; check creatinine; notify the house officer and floor nurse to be particularly vigilant with urine output
	> 150 µM	All of above plus stop MTX, continue monitoring MTX to estimate half-life.
23 hr - 5 gm/m^2 or targeted to 65 µM	95 - 150 µM	Check that hydration and alkalinization are adequate; check for nephrotoxic drugs; check creatinine; notify the house officer and floor nurse to be particularly vigilant with urine output
	> 150 µM	All of above plus obtain another blood sample for assay; consider possible early LV rescue;* consider obtaining glucarpidase, etc.
42 hr#	< 0.5 µM	Protocol rescue & no further concentrations indicated, unless clinically warranted
	0.5 – 1 µM	Protocol rescue, cont IVF with bicarb & check 66 hr conc
	1 – 2 µM	30 mg/m^2/dose po/IV q6h
	2 – 5 µM	50 mg/m^2/dose IV q6h
	5 – 10 µM	100 mg/m^2/dose IV q6h
	10 – 20 µM*	200 mg/m^2/dose IV q6h or continuous infusion
	>20 µM*	Individualized
66 hr#	< 0.1 µM	Per protocol and stop checking unless clinically indicated
	0.1 – 0.2 µM	5 mg/m^2-/dose po/IV q12h & continue checking until < 0.1
	0.2 – 0.5 µM	15 mg/m^2/dose po /IV q12h & continue checking until < 0.1
	0.5 – 1 µM	15 mg/m^2/dose po/IV q6h & continue checking until < 0.1
	1 – 2 µM	30 mg/m^2/dose po/IV q6h & continue checking until < 0.1
	2 – 5 µM	50 mg/m^2/dose IV q6h & continue checking until < 0.1
	>5 µM	Individualized

MTX Concentration	Threshold for Action	Recommended LV Rescue
90 hr and later#	< 0.1 µM	Stop LV & stop checking, unless clinically indicated
	0.1 – 0.2 µM	5 mg/m^2/dose po/IV q12h & continue checking until < 0.1
	0.2 – 0.5 µM	15 mg/m^2/dose po/IV q12h & continue checking until < 0.1
	0.5 – 1 µM	15 mg/m^2/dose po/IV q6h & continue checking until < 0.1
	>1 µM	As above under hr 66

*Early rescue should be initiated and glucarpidase availability should be checked whenever it is a possibility that the patient will have plasma MTX concentrations > 10 µM at 42 hours.

#Higher doses of leucovorin may be indicated based on the clinical condition of the patient. Patients with MTX concentrations > 1 µM benefit from increased urine output. Forced diuresis with acetazolamide, along with increased hydration may be necessary.

NOTE: Glucarpidase would not need to start before hr. 42, but availability should be checked 1 day prior. Nephrology should be consulted and hemoperfusion should be anticipated if glucarpidase will not be available by 42 hours. Ideally, leucovorin should NOT be given within 2 hours BEFORE or AFTER glucarpidase dosing because leucovorin is also a substrate for glucarpidase and will interfere with methotrexate hydrolyzation.

NOTE: Early leucovorin rescue should not need to start prior to 24 hours, regardless of the concentration. Although it is not likely to be very effective, early leucovorin should be instituted sometime between 24 and 42 hours at a dose of ~2000 – 2400 mg/m^2/day if the 24 hour concentration is > 300 µM or it is above 200 µM and the half-life is > 8 hours. Continuous infusions of leucovorin are slightly preferred over bolus infusions, on the off chance that high leucovorin concentrations could either compete with MTX for excretion or contribute to neurotoxicity, but doses as high as 500 mg/m^2 can be given safely if necessary.