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TOTAL THERAPY STUDY XVI FOR NEWLY DIAGNOSED PATIENTS WITH ACUTE LYMPHOBLASTIC LEUKEMIA

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TO: **IRB and CT-SRC**

FROM: **Sima Jeha, MD**

DATE: **April 15, 2016**

RE: **TOTXVI, Total Therapy Study XVI for Newly Diagnosed Patients with Acute Lymphoblastic Leukemia**

Re: Letter of Amendment (LOA) to continue enrollment

Amendment 6.0 was submitted on 4-8-2016, and is scheduled for review by the CT-SRC on 4-19-2016. This amendment is to continue enrollment beyond the current 531 targeted accrual to allow secondary objectives to be completed. Participants enrolled on Amendment 6.0 will contribute to several secondary and exploratory objectives; however they will not be randomized for the primary PEG-asparaginase objective, and will not be offered participation in the following research studies: NK cell receptor, neuroimaging, and intestinal microbiome objectives.

TOTXVI enrollment is now at 531 participants. With this letter of amendment, we are requesting that enrollment continue with the currently approved protocol until the new Amendment 6 is approved and activated noting the following specifications:

1. New patients will not be randomized to standard- versus high-dose PEG asparaginase; they will receive standard dose.

2. New patients will not be offered participation in NK cell receptor, neuroimaging or intestinal microbiome research.

3. Patients enrolled via the LOA will be re-consented with Amendment 6.0 at the next clinic visit after Amendment 6.0 is activated.

4. The above information will be explained to the patient/LAR and documented in the medical record.

The transition between Amendment 5 and Amendment 6 will allow participants to continue benefiting from protocolized complex therapy and contribute to answering important secondary objectives. Thank you for considering this request.

SYNOPSIS

ALL regimens consist of a combination of multiple agents delivered over a period of 2.5 to 3 years in 3 main phases (remission induction, consolidation, and continuation). Seemingly minor modifications in the administration of drugs developed over 30 years ago (such as mercaptopurine, methotrexate, vincristine, corticosteroids, anthracyclines, and asparaginase) have had significant impact on outcome. For example, changes in vincristine and asparaginase dosage following intensification, and sequential escalating-dose methotrexate followed by asparaginase, significantly improved outcome of high-risk patients in CCG trials[1]. Longer exposure to asparaginase during early consolidation had a significant impact on outcome in Dana Farber studies[2]. Recent large randomized trials were dedicated to compare mercaptopurine to thioguanine[3], prednisone to dexamethasone[4;5], single to triple intrathecal therapy[6] etc. The cumulative knowledge gathered from these and other trials form the basis of the impressive cure rates achieved in children with ALL today.

The primary objective of TOTXVI is to compare the clinical benefit, the pharmacokinetics, and the pharmacodynamics of polyethylene glycol-conjugated (PEG) asparaginase given in higher dose versus those of PEG-asparaginase given in conventional dose during the continuation phase. Asparaginase is one of the key components of ALL therapy but its administration is complicated by the availability of different preparations, each with a different pharmacokinetic profile[7]. The native E. coli asparaginase has been the most commonly used preparation. PEG- asparaginase, a long-acting and less allergenic form, is progressively replacing the native product and is being increasingly administered intravenously instead of intramuscularly[8]. Most studies are empirically adopting a PEG-asparaginase dose of 2,500 units/m^2, based on published reports comparing this dose to 6 doses of 6,000 units/m^2 of native E. coli asparaginase. However, this dose has failed to deplete CSF asparaginase in some studies, suggesting it might be inadequate for CNS control. This is of utmost importance in TOTXVI where we rely on systemic chemotherapy in addition to intrathecal therapy to avoid cranial irradiation. In a randomized study of 2,500 units/m^2 PEG-asparaginase intramuscularly every other week for 15 doses vs. native 25,000 units/m^2 E. coli asparaginase intramuscularly every week for 30 doses (DFCI 91- 01), although there was no significant difference in EFS (84% for native vs. 78% for PEG- asparaginase, $p = 0.29$), the trend is in the direction suggesting that 2,500 units/m^2 of PEG-asparaginase is slightly less effective than the 25,000 units/m^2 of native asparaginase. As it is the latter dose of native drug that has been used on TOTXV, we are comfortable replacing the more frequently administered intramuscular native E. coli asparaginase with the longer acting, less immunogenic intravenous PEG-asparaginase, but we propose to test the potential benefits of administering PEG-asparaginase at doses higher than the 2,500 units/m^2 during continuation.

Patients will be randomized at the completion of remission induction in TOTXVI to compare the safety, efficacy, and PK/PD between the "conventional" and a higher dose of PEG-asparaginase. During the remission induction phase, TOTXV used higher doses of native E. coli asparaginase (10,000 units/m^2 per dose) than other pediatric ALL protocols (6,000 units/m^2 per dose) into which PEG-asparaginase has been substituted at 2,500 units/m^2. We have chosen to give all children on TOTXVI 3,000 units/m^2 instead of the "conventional" dose of 2500 units/m^2 of PEG-asparaginase, because we do not want the intensity of asparaginase in this St. Jude trial to possibly be less than that on prior St. Jude trials, all of which used 10,000 units/m^2 of native E. coli asparaginase.

We have been leaders in reducing preventive cranial irradiation, and then eliminating it completely in TOTXV. This is the single most important modification to decrease long-term adverse effects and improve quality of life of survivors. This was achieved with the intensification of CNS-directed intrathecal and systemic chemotherapy. The risk of CNS relapse (about 3.5%) on TOTXV compares favorably with that of regimens still employing preventive cranial irradiation. Moreover, all patients with CNS relapse are alive in second remission with retrieval therapy including therapeutic CNS irradiation (as the first course of irradiation). However a subgroup of patients with T-cell lineage, t(1;19)/*E2A-PBX1*, and those with blasts in the CSF remain at highest risk of CNS relapse; these patients will receive 2 additional intrathecal treatments on day 4 and 11 of induction on TOTXVI to reduce their risk of CNS relapse.

In TOTXV we confirmed that the presence of minimal residual disease (MRD) on day 19 is a significant prognostic factor. Over 95% patients with negative MRD on day 19 remain in remission. In TOTXVI, these patients will receive less intensive Reinduction II, and a reduced cumulative dose of anthracyclines (80 mg/m^2 instead of 110 mg/m^2). In TOTXV, we observed that patients with day 19 MRD $\geq$ 5% had a dismal outcome: 34.5% have relapsed. In TOTXVI we will intensify induction therapy with fractionated cyclophosphamide in these patients to achieve maximal cytoreduction by the end of remission induction (a critical endpoint to maximize the chances of durable remissions).

The majority of patients will no longer have surveillance marrows post remission induction, as monitoring of MRD in marrow after remission induction will only be performed in patients with positive MRD on day 42 (or when clinically indicated). In TOTXV, we used flow cytometry and PCR in tandem to monitor MRD. Because of the very high concordance between the 2 methods, in TOTXVI we will use primarily flow cytometry, reserving PCR for cases where leukemic cells do not express suitable immunophenotypes.

Infants with MLL rearrangements have a dismal outcome despite use of intensive regimens including stem cell transplantation. TOTXVI will explore the benefit of adding a promising new therapy block developed by St Jude investigators[9]. The current dismal outcome in this age group makes the detection of potential benefit possible despite the expected small number of patients.

Other pharmacologic adjustments have been made to improve quality of life of our patients, decrease the risk of adverse events, and minimize inconvenience to patients and staff. Examples include:

a) asparaginase will be administered intravenously instead of intramuscularly;
b) there is no methotrexate window in TOTXVI as the study question has been answered on TOTXV;
c) high dose methotrexate will not be targeted in low risk patients (about half enrolled patients), except when clinically indicated;
d) cumulative anthracycline dose will be reduced by 30 mg/m^2 in half the patients;
e) duration of therapy had been reduced by 26 weeks in boys;
f) dexamethasone dose has been reduced after week 69 (for a cumulative reduction of 80 mg/m^2 for low-risk patients and 240 mg/m^2 for standard-risk patients)

All diagnostic samples will be examined for global gene expression using gene array chips. This will continue to enrich our unique database on the gene expression of ALL cells which has

become an invaluable resource (http://www.stjuderesearch.org/data/ALL1/index.html) for ALL investigators worldwide. In addition, this will provide us with an opportunity to validate prospectively the many retrospective observations made in past correlative studies including global gene expression. For example, the expression of several genes in leukemic cells at diagnosis was strongly associated with day 19 or day 46 (end of induction) MRD in TOTXV, and was found to be an independent predictor of overall treatment outcome in correlative studies of patients enrolled in Total XIII[10;11]. These genes include *CASP8AP2* (which encode a protein that promotes apoptosis and glucocorticoid signaling)[10], a set of genes associated with *in vitro* drug resistance in childhood ALL[11-13] and genes encoding proteins with known regulatory functions in cell cycle progression[11]. In TOTXVI, we will study the association of these genes' expression with MRD on days 15 and 42, and with incidence of relapse.

We will study the *in vitro* sensitivity to mercaptopurine and thioguanine in patients enrolled on TOTXVI, and simultaneously determine the sensitivity of these new cases to other anti-leukemic agents that we have previously studied (prednisone, vincristine, daunorubicin and asparaginase). This will permit us to elucidate genomic determinants of multi-drug cross resistance to all 6 anti-leukemic agents. We will also use other genome-wide approaches, including SNP and haplotype analyses, to identify genes and genetic polymorphisms significantly linked to resistance to each of these medications, and to the multi-drug cross resistance phenotype. Our prior work focused predominantly on B-lineage ALL, and we aim to extend this work to T- lineage ALL and investigate cases studied in TOTXV and TOXVI together. This subset has a worse prognosis in many treatment protocols, and therefore it is important that we understand the genomic basis of drug resistance in this subtype of childhood ALL.

We will study the germline genomic determinants of risk factors for relapse, poor response (MRD positivity), acute toxicities, pharmacologic phenotypes and biomarkers and long-term complications of therapy as outlined in section 2.2.1. These analyses in TOTXVI will be compared and interpreted in the context of ongoing and future genomic analyses in other ALL trials at St. Jude and elsewhere, with those findings replicated in clinical and laboratory settings becoming candidates for future use in individualizing therapy.

Protocol Summary

TOTAL THERAPY STUDY XVI FOR NEWLY DIAGNOSED PATIENTS WITH ACUTE LYMPHOBLASTIC LEUKEMIA
Principal Investigator: Sima Jeha, M.D.
IND holder: Not applicable, non-IND/IDE study
Brief overview: Treatment will consist of three main phases: Remission Induction, Consolidation, and Continuation. Total duration of therapy is 2½ to 3 years.
Intervention: Remission induction Drug: Prednisone Drug: Prednisolone Drug: Dexamethasone Drug: Vincristine Drug: Daunorubicin Drug: PEG-asparaginase Drug: Cyclophosphamide Drug: Cytarabine Drug: Thioguanine Drug: Mercaptopurine Drug: Dasatinib (Ph+ only) Drug: Clofarabine (*MLL+* infants only) Drug: Etoposide (*MLL+* infants only) Drug: Cyclophosphamide (*MLL+* infants only) Procedure: Intrathecal chemotherapy Consolidation treatment Drug: Methotrexate Drug: Mercaptopurine Procedure: Intrathecal chemotherapy Continuation treatment Risk assignment Assigned intervention *Low risk* Drug: Mercaptopurine Drug: Dexamethasone Drug: Vincristine Drug: Methotrexate Drug: Peg-asparaginase Drug: Methotrexate Drug: Doxorubicin Procedure: Intrathecal chemotherapy *Standard/high risk*: Drug: Mercaptopurine Drug: Dexamethasone Drug: Doxorubicin Drug: Vincristine Drug: Methotrexate Drug: Cytarabine Drug: Etoposide Drug: PEG-asparaginase Drug: Cyclophosphamide Procedures intrathecal chemotherapy, Procedures (high risk only): external beam RT (CNS relapse) and HSCT

Sample size: 420 evaluable participants in 8.6 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 intensive, multi- agent chemotherapy. Participants are informed of this and other potential side effects during informed consent. Adverse events will be monitored, reported and treated appropriately.

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1.0 OBJECTIVES

The overall aim of this study is to improve the cure rate of children with precursor B-cell and precursor T-cell acute lymphoblastic leukemia (ALL).

1.1 Primary Objective

The primary objective of this study is to compare the distributions of continuous complete remission of patients randomized on the first day of the continuation phase to receive a higher dose of PEG-asparaginase or to receive the conventional dose (2,500 units/m^2).

1.2 Secondary Therapeutic Objectives

1.2.1 To estimate the event-free survival and overall survival of children with ALL who are treated with risk-directed therapy.

1.2.2 To study whether intensifying induction, including fractionated cyclophosphamide and thioguanine, in patients with day 15 MRD $\geq$ 5%, will result in improved leukemia cytoreduction in this subgroup compared to TOTXV.

1.2.3 To assess whether intensification of CNS-directed intrathecal and systemic chemotherapy will improve outcome in patients at high risk of CNS relapse.

1.3 Exploratory Pharmacologic Objectives

1.3.1 To identify pharmacogenetic, pharmacokinetic and pharmacodynamic predictors for treatment-related outcomes in the context of the systemic therapy used in the protocol.

1.3.2 To compare the pharmacokinetics and pharmacodynamics of PEG-asparaginase given in higher dose (3,500 or 3,000 units/m^2) versus those of PEG-asparaginase given in conventional dose (2,500 units/m^2) in the continuation phase.

1.4 Exploratory Biologic Objectives

1.4.1 To determine the prognostic value of levels of minimal residual disease in peripheral blood at day 8 of remission induction.

1.4.2 To validate new markers and methods for MRD detection.

1.4.3 To genotype natural killer (NK) cell receptors and measure their expressions at diagnosis and before reinduction, and to associate these features with treatment outcome.

1.4.4 To identify new prognostic factors by applying new technologies to study patient material (e.g., stored plasma, serum, cerebrospinal fluid, and normal and leukemic cells).

1.5 Exploratory Neuroimaging Objectives

1.5.1 To use quantitative MR measures (Diffusion Tensor Imaging and high resolution volumetric imaging) to assess differences in myelin and cortical thickness development in patients treated for ALL relative to healthy controls matched for age and gender.
1.5.2 To assess the impact of folate pathway genetic polymorphisms on myelin and cortical thickness development and neurocognitive performance.
1.5.3 To assess the impact of frontal-parietal lobe myelin and cortical thickness development on neurocognitive performance in attention, working memory, fluency, visual-spatial reasoning and processing speed with both neurocognitive testing and functional magnetic resonance imaging.

1.6 Exploratory Intestinal Microbiome Objectives

1.6.1 To perform a comprehensive analysis of the intestinal microflora in patients with ALL prior to and subsequent to chemotherapy using next generation sequencing technologies as an exploratory approach.
1.6.2 To study any alteration in the composition and population dynamics of the resident intestinal microflora in patients with ALL after exposure to chemotherapy compared to prior to exposure to chemotherapy.
1.6.3 To describe the frequency and severity of gastrointestinal illnesses during induction chemotherapy and over the 6 month period after completion of induction in patients who did and those who did not have changes in intestinal microbiota.
1.6.4 To detect the presence of pathogens found to be abundant by the microbiome analysis in subsequent stool samples collected over the 6 month period after completion of induction chemotherapy.

2.0 BACKGROUND AND RATIONALE

2.1 Therapy

2.1.1 Risk assignment

Childhood ALL cure rates exceed 80%[14]. Further progress requires the early identification of leukemia subtypes that are resistant or responsive to current chemotherapy, so that patients are not under-or over-treated. The TOTXVI protocol applies the most comprehensive risk classification to date, combining blast cell immunophenotype and genotype, with presenting clinical features and early treatment response. Accordingly, patients 1-18 year-old are divided into three risk groups: low-, standard-, and high-risk (corresponding to standard-, high-, and very high-risk categories in other protocols)[15]. Infants constitute a separate risk group[16;17].

B-cell precursor ALL cases with age between 1 and 10 years and presenting leukocyte count $<50 \times 10^9$/L, leukemic cell DNA index ≥ 1.16 or *TEL-AML1* fusion have an overall excellent prognosis and are generally considered to have low-risk leukemia. However, as

many as 15% of these cases still relapse on antimetabolite-based therapy, for unknown reasons[18;19]. Recent studies showed that response to early treatment can identify patients who have good presenting features but nonetheless have a high risk of relapse. Interim analysis indicates that patients with no detectable blasts on day 19 of remission induction have an excellent prognosis in TOTXV, in agreement with previous findings with TOTXIII patients[20] The risk of relapse increases in patients with detectable blasts on day 19 of induction: those with 5% or more blasts among bone marrow mononuclear cells as measured by flow cytometry have a 5-year cumulative risk of relapse of 34% $\pm$ 22%. Poor cytoreduction on day 26 and detectable MRD ($\geq$0.01%) at the end of remission induction (~ day 46) are additional indicators of high risk of relapse. Hence, patients with favorable presenting features but poor early treatment response will be treated as having standard-risk ALL. The remaining patients with favorable presenting features and good response to remission induction therapy will be treated on the low-risk arm.

Because of their generally poor outcome, cases with CNS-3 status ($\geq$5 WBC/μL of cerebrospinal fluid with identifiable blasts or cranial nerve palsy), testicular leukemia, or hypodiploidy (<44 chromosomes), will be treated on the standard-risk arm[15;21]. Patients with T-cell ALL, pre-B-cell ALL with the t (1;19) and *E2A-PBX1* fusion or *MLL* rearrangement fare well provided that they receive intensive therapy[22;23]. Hence, they are also assigned to the standard-risk group in TOTXVI.

Patients with Philadelphia-chromosome (*BCR-ABL)*-positive ALL have a high relapse hazard. Several studies showed that their prognosis can be improved with allogeneic hematopoietic stem cell transplantation (HSCT)[24]. Tyrosine kinase inhibitors have lately been incorporated into frontline regimens for Ph+ ALL. TOTXV was amended in June 2004 to incorporate imatinib. The new tyrosine kinase inhibitor dasatinib has activity in *BCR-ABL*-positive leukemias that are resistant to imatinib[25-27]. Therefore, dasatinib will replace imatinib in TOTXVI (section 2.5).

Patients with $\geq$1% blasts at the end of remission induction and those with $\geq$0.1% at 16 weeks post remission induction also have very poor prognosis. Therefore, these patients are considered to have high-risk leukemia and are candidates for allogeneic HSCT during their first remission.

We recently identified a subset (12%-15%) of T-ALL characterized by an atypical immunophenotype consisting of low expression of T-cell markers (CD5, CD1, CD8) and expression of myeloid- and stem cell- associated markers (CD13, CD33, CD11b, CD34, CD117 and/or CD133). This early T-cell precursor (ETP) subtype has a distinct gene expression profile, a high prevalence of unusual karyotypes, and an increase in gene copy number changes and lesions[28]. These patients had a markedly inferior clearance of leukemic blasts during remission induction therapy and a dismal outcome: 10-year overall survival for the 17 patients with atypical T-ALL was 16.5% + 10.6% versus 84.7% + 6.8% for the 122 patients with typical T-ALL (P<0.0001). These patients will also be considered to have high-risk leukemia in TOTXVI.

2.1.2 Remission induction

Remission induction consists of 3 weeks of prednisone, vincristine, daunorubicin, and PEG-asparaginase, followed by 2 weeks of cyclophosphamide, cytarabine, and thioguanine combinations. This remission induction is similar to that used in TOTXV except for following modifications:

(a) The optional 4-day methotrexate window therapy will not be offered and standard induction will start on day 1.
(b) Intramuscular L-asparaginase will be substituted with intravenous PEG-asparaginase which has been safely and successfully used in the Dana- Farber Cancer Institute (DFCI) protocols. Early responders will receive a single dose of PEG-asparaginase (substituting for the 6 intramuscular doses of L-asparaginase on TOTXV), whereas patients with 1% or more blasts on day 15 will receive an additional dose of PEG-asparaginase (substituting for the additional 3 doses of L-asparaginase given on TOTXV for this subgroup). The rationale for this dose is indicated in the Synopsis for TOTXVI.
(c) Thioguanine will be given on days 22 to 35 instead of mercaptopurine. The 2 week block of cyclophosphamide, cytarabine and thioguanine has been widely used in the Children's Cancer Group (CCG) regimens. Randomized studies have suggested that thioguanine is associated with improved outcome. While intermittent pulses of thioguanine have been safely used in several regimens, prolonged use of thioguanine has been associated with increased incidence of venoocclusive disease of the liver and portal hypertension. Hence we will not use thioguanine beyond this 14 day course, and will continue to use mercaptopurine for prolonged administration during consolidation and continuation.
(d) Cyclophosphamide therapy will be intensified in patients with 5% or more blasts on day 15 of induction because of the high risk of relapse (34%) in this group of patients. Fractionated cyclophosphamide is widely used in adult ALL protocols, and has proven safe and effective in children with Burkitt leukemia/lymphoma and relapsed ALL.
(e) TOTXV has proven that preventive cranial irradiation can be eliminated, irrespective of risk group, with intensification of CNS- directed and systemic chemotherapy. Cumulative risk of CNS relapse (~ 3.5%) on TOTXV compares favorably with that of regimens still employing preventive cranial irradiation. Moreover, all patients with CNS relapse are alive in second remission with retrieval therapy including therapeutic CNS irradiation (as the first course of irradiation). Patients with T-cell lineage, t(1;19)/*E2A-PBX1,* and those with blasts in the CSF are at highest risk of CNS relapse, and these patients will receive 2 additional intrathecal treatments on day 4 and 11 of induction on TOTXVI to reduce their risk of CNS relapse.
(f) Patients with ETP immunophenotype will receive dexamethasone (10 mg/m^2 per day) instead of prednisone during remission induction. The use of dexamethasone during induction therapy is associated with superior outcome, but increased toxicity, in patients with T-ALL treated in the AIEOP-BFM ALL 2000 trial[29]. We feel that the potentially increased risk of treatment-related toxicity is justifiable in patients with the ETP immunophenotype, in view of their poor outcome when

treated with conventional chemotherapy.

(g) Patients who are TPMT heterozygote (about 10% of all patients) will receive mercaptopurine during induction instead of thiopurine. Total 16 induction therapy was complicated with reversible hepatic venoocclusive disease (VOD) in a patient who is TPMT heterozygote. Genetic polymorphisms that decrease TPMT activity are correlated with increased sensitivity and toxicity to thiopurine. In view of the lack of VOD on the BFM studies using thioguanine and in WT patients enrolled to-date on Total 16, along with the correlation between incidence of VOD and TPMT activity, Total 16 will be amended to have TPMT HET/deficient patients (about 10% of total) receive mercaptopurine as per Total 15 during induction instead of thioguanine. No changes will be made for WT patients; however, vigilant monitoring is recommended.

2.1.3 Consolidation therapy

Consolidation therapy consists of HDMTX (average of 5 gm/m^2 over 24 hours in standard-/high-risk or average of 2.5 gm/m^2 over 24 hours in low-risk cases) given every other week for 4 doses with leucovorin rescue, together with intrathecal treatment plus concomitant daily mercaptopurine (50 mg/m^2). The feasibility and effectiveness of HDMTX given at 5 gm/m^2 have been documented in large numbers of patients treated in TOTXV, the Berlin-Frankfurt-Münster (BFM)[30] and the Associazione Italiana Ematologia Oncologia Pediatrica (AIEOP) studies[31]. In fact, the improved treatment outcome of T-cell ALL cases in the BFM studies and of intermediate-risk B-lineage ALL cases in the AIEOP study was attributed in part to the use of HDMTX at 5 gm/m^2. Results of Total pharmacologic studies suggest that while HDMTX at 2.5 gm/m^2 is sufficient for most B-lineage ALL cases[32;33], non-hyperdiploid B-lineage blasts accumulate more methotrexate polyglutamates with high-dose than with low-dose methotrexate. In a DFCI study with vast majority of cases of B-lineage phenotype, children received a single up-front dose of 4 gm/m^2 had better overall leukemia-free survival than those receiving 40 mg/m^2.[34] In addition, in our study X, higher exposure to methotrexate was most important among the higher-risk B- lineage cases (p=0.007)[35;36]. Hence, in TOTXVI, HDMTX will be given similar to dosing on TOTXV. The goal is to provide average systemic exposure that is achieved at 5 gm/m^2 for T-cell and standard-/high-risk B-lineage ALL, and average exposure achieved at 2.5 gm/m^2 for low-risk B-lineage ALL, for a total number of 4 doses HDMTX.

Targeting systemic exposure in TOTXV appeared to be somewhat less important in the low-risk arm than in the standard-/high-risk arm. Inter-course targeting in the low-risk arm resulted in 12%, 70%, and 18% of courses being below, in, and above the 24 hr plasma concentration target range of 33μM +/- 20%. Additionally, there was an acceptably low proportion of courses (6%) with "delayed excretion" (42 hr plasma MTX concentration > 1.0μM). Targeting the low-risk arm did not have a substantial impact on avoiding extreme exposures relative to simulated conventional dosing: 2% vs. 3% steady-state-concentrations < 40% of target and 5% vs. 3% with steady-state-concentrations > 40% of target with targeted vs. simulated conventional dosing respectively.

Inter-course targeting in the standard-/high-risk arm resulted in 23%, 62%, and 15% of courses being below, in, and above the target range of 65uM +/- 20%. Additionally, there was an acceptably low proportion of courses (15%) with "delayed excretion" (42 hr plasma MTX concentration > 1.0 uM). Targeting the standard-/high-risk arm avoided extreme exposures relative to simulated conventional dosing: 3% vs 0% with steady-state concentrations < 40% of target and 3% vs. 10% with steady-state-concentrations > 40% of target with targeted vs. simulated conventional dosing, respectively. The median 42 hour concentration in the standard-/high-risk arm was 0.54 uM, which is quite similar to that reported (0.43 uM) by the BFM. Moreover, the leucovorin rescue administered by our groups was similar, but toxicity was substantially lower in TOTXV than that reported by BFM: ~4% vs. 44% grade 3 or 4 mucositis. Thus, it appears that there may be more benefit in avoiding the extremes of exposure in the standard/high than in the low-risk arms, although target concentrations were better achieved in the low-risk arm.

For TOTXVI, our decision is to continue targeting HDMTX in the standard-/high- risk arm but not in the low-risk arm, unless it is specifically requested due to clinician concerns regarding patient instability. The reason for this is that the dose of 2.5 g/m^2 given conventionally to the TOTXV low-risk group is generally adequate: it results in steady-state concentrations >21μM in 95% of courses; toxicity was low, with infrequent grade 3 or 4 mucositis (1.2%); and using a fixed 2.5 g/m2 dose, 95% of the steady-state concentrations would have been < 46μM while the highest steady-state concentration would have been 91μM. However, on TOTXVI, if renal dysfunction, patient instability, or use of concurrent drugs leads to concerns regarding MTX clearance for any particular course, the clinician can request targeting to be performed.

We will continue our targeting approach for standard-/high-risk patients on TOTXVI as we did on TOTXV. Our goals are to continue to avoid unacceptably low concentrations (albeit in a small % of courses), minimize unacceptably high concentrations, avoid GI toxicity, and maintain the majority of courses in the desired target range. 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).

One of the lessons of targeting inter-course HDMTX was that we were not as successful at keeping all courses within ±20% of the target as when we performed intra-course HDMTX targeting[37]. We modified TOTXV to allow intra-course targeting when possible but have targeted only a few cases due to the more complicated logistics of needing to start HDMTX early enough in the day to allow feasible intra-course targeting. However, based on simulations which used the previous MTX clearance along with a 2 hour MTX plasma concentration to target the steady-state concentration, 1%, 91%, and 8% of courses would be below, in, and above the target range. Thus, 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.

It should be noted that approximately 10% of provisional low-risk ALL will be changed to standard-/high-risk ALL because of positive minimal residual leukemia at the end of induction. In these cases, HDMTX dosage after the first dose will be changed to the higher average dose of 5 gm/m^2 (65uM).

2.1.4 Continuation treatment

After consolidation therapy, patients will receive risk-directed continuation treatment. Low-risk patients will receive daily mercaptopurine and weekly methotrexate with pulses of dexamethasone and vincristine every 3 to 5 weeks (for the first two years). In randomized trials, dexamethasone treatment was superior to prednisone in reducing CNS relapse and improving event-free survival[38] partly due to its superior CNS penetration. Mercaptopurine will be continued during the week of dexamethasone and vincristine treatment in this study to intensify systemic therapy, as the dose intensity of mercaptopurine was the most important pharmacologic variable in determining treatment outcome in TOTXII study[39]. Methotrexate will not be given during the week of dexamethasone plus vincristine to reduce the risk of typhlitis.

Continuation treatment for standard- and high-risk cases incorporates effective treatment components of the most successful clinical trials to date. Intensifying asparaginase therapy during the early phase of treatment improved outcome for all risk groups in the DFCI studies[2], for poor early responders (90% of whom had B- lineage ALL) in the CCG augmented therapy[1], and for T-cell cases in the Pediatric Oncology Group POG-8704 study[40]. Intensive L-asparaginase treatment failed to improve outcomes in a study of low-risk B-lineage ALL. Hence, intensive asparaginase therapy will continue to be given to patients with T-cell ALL as well as those with high-risk B-lineage ALL in TOTXVI as in TOTXV.

Intravenous PEG-asparaginase is increasingly used in frontline ALL regimen. In TOTXVI, patients with standard- or high-risk ALL will receive PEG-asparaginase every 2 weeks for a total of 15 doses during the first 30 weeks of continuation therapy. Low-risk patients will only receive 4 doses (2 in each re-induction).

Although the optimal dose of the different preparations of asparaginase is not firmly established, equivalent EFS has been reported with PEG-asparaginase 2,500 units/m^2 and native L-asparaginase at 6,000 units/m^2 x 6 doses[41]. Hence the dose of 2,500 units/m^2 for PEG- asparaginase has been widely adopted. However, PEG-asparaginase at 2,500 units/m^2 failed to deplete CSF asparagine in some studies, suggesting this dose is inadequate for CNS control. Moreover, L- asparaginase was given at 10,000 units/m^2/dose on TOTXV. Because asparaginase has been shown to be such an important component to modern ALL trials, and since TOTXV used higher than conventional L-asparaginase doses, on TOTXVI, patients will be randomized to receive the conventional PEG- asparaginase dose (2,500 units/m^2), or the higher PEG-asparaginase dose (3,500 or 3,000 units/m^2). The difference in pharmacokinetics and pharmacodynamic measures, and outcome, will be studied in the 2 arms and separately for 3,500 and 3,000 units/m^2 if both doses are used in the higher PEG-asparaginase arm.

As in TOTXV, use of epipodophyllotoxins will be limited to high-risk ALL in TOTXVI to decrease the risk of therapy-related AML[41]. We will use cyclophosphamide and anthracyclines to intensify treatment in standard-/high-risk cases. However, total cumulative dose of cyclophosphamide is limited to 4.6 gm/m^2 for the vast majority of the patients and to 5.4 gm/m^2 in about 7% patients who have 5% or more blasts on day

15 of induction to reduce the problem of sterility (especially in boys). Anthracycline cumulative dosage is limited to 230 mg/m^2 in standard/high-risk patients, and to 80 mg/m^2 in low-risk cases. These dosages of anthracyclines are associated with a very low risk of cardiomyopathy[42].

Reinduction treatment is an integral component of most contemporary trials, with benefits extending to low-risk cases. A CCG study showed that an augmented BFM regimen (equivalent to the use of double reinduction) can even abolish the adverse prognostic impact of poor early response[1]. Hence, in TOTXVI, all patients (regardless of risk status) will receive two courses of reinduction, albeit at different intensity as determined by risk group assignment. In low-risk patients, doxorubicin will be omitted from re-induction II, reducing the total cumulative anthracycline dose to 80 mg/m^2 (compared to 110 mg/m^2 on TOTXV) in this group. In standard- risk and high-risk groups, high-dose cytarabine (2 gm/m^2) is incorporated in re- induction II because TOTXII suggested that T-cell cases benefit from this treatment[37] and an adult BFM study showed that high-dose cytarabine can markedly improve outcome, even in patients with the t (4;11) ALL[43].

Gender has long been recognized as a prognostic factor in childhood ALL. Boys have consistently fared worse than girls, given equivalent therapy. Male patients treated on CCG, BFM, and TOTXV protocols received an extended duration of treatment (i.e. 3 years). However, in TOTXIIIB (in which the duration of chemotherapy for both males and females was 2.5 years) and in TOTXV, with overall 5-year event-free survival rates over 80%, male patients fared as well as female patients. Hence, in TOTXVI, the duration of therapy will be the same for both genders (2½ years). As in TOTXV, continuation treatment beyond 2 years in TOTXVI consists of mercaptopurine and methotrexate only (no dexamethasone and vincristine pulses). Moreover, dexamethasone dose will be decreased to 6 mg/m^2 between week 69 and week 101 to decrease the morbidity from dexamethasone therapy.

2.1.5 Sub-clinical CNS treatment

Since CNS irradiation can induce serious long-term sequelae, including growth retardation, intellectual impairment and brain tumors, and in view of the outstanding CNS control in TOTXIIIA & B, CNS irradiation was eliminated altogether in TOTXV, except in patients who developed CNS relapse.

The overall 5-year cumulative risk of CNS relapse was 3.7% $\pm$1.3% (SE) in TOTXV, which is comparable to results in studies still using preventive CNS irradiation. We attribute this success to early intensification of intrathecal treatment, especially in patients at high risk of relapse, and effective systemic treatment including the use of dexamethasone. Most CNS events on TOTXV occurred in patients with T-cell lineage (with cumulative risk of 11.1%$\pm$4.8%), or with any amount of blasts in the CSF (11%$\pm$4.3%). A recent analysis of Total Therapy studies XIIIA, XIIIB, XIV, and XV further indicated that patients with t(1;19)/*E2A-PBX1*have a lower cumulative incidence of any hematological relapse but a significantly higher incidence of CNS relapse (p=0.005;12.4%$\pm$5.9%vs. 2.7%$\pm$0.7% at 5 years)[44].

Notably, all patients with isolated CNS relapse were alive in second remission after receiving their first course of irradiation for treatment of CNS relapse, resulting in no significant difference in survival between patients with B-lineage or T-lineage ALL, or between those with or without blasts in CSF at diagnosis. Hence, isolated CNS relapse in patients who did not receive preventive CNS irradiation are curable with retrieval therapy. In TOTXVI, intrathecal treatment will be intensified during induction treatment with 2 additional intrathecal treatment on days 4 and 11 for patients with T-cell ALL, t(1;19)/*E2A-PBX1,* or any amount of leukemic blasts identifiable in the cerebrospinal fluid at diagnosis (including those with cerebrospinal fluid contaminated with leukemic-blast cells due to a traumatic lumbar puncture, because they are at higher risk of CNS relapse and have a poorer event-free survival if treatment is not intensified). These patients then receive additional intrathecal doses every 4 weeks during the first 56 weeks of continuation therapy, as do patients with no identifiable blasts in the cerebrospinal fluid who were judged to have an increased risk of CNS relapse based on other features (i.e., those with WBC $\geq$100 x 10^9/L, and Philadelphia- chromosome positive cases).

As in TOTXV, preventive CNS irradiation is eliminated altogether in this study, except in patients who during continuation treatment have morphologic evidence of lymphoblasts in the CSF, confirmed by immunological testing (i.e., TdT) on two separate occasions. We expect that these events should not occur in more than 5% of the patients. Patients are managed according to Section 9.4.

2.1.6 Rationale for modification of mercaptopurine administration (revision 5.5)

Prior to revision 5.5, this protocol stipulated that mercaptopurine should be taken on an empty stomach (i.e., > 2 hours after meal) at bedtime; patients should not drink milk or take dairy products together with mercaptopurine.

However, based on literature review summarized below, this changed with amendment 6.0 and now stipulates that 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.

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[45-47] 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[48;49] reported statistically significant differences in time of maximum drug concentration in plasma (tmax) and AUC in the fasting state of parent drug[48] or changes in ALL disease free survival[49] 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, et al. 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[50].

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[51]. 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.

2.2 Pharmacologic Studies

2.2.1 Germline genetic polymorphisms

Using candidate gene approaches, we and others have identified multiple polymorphisms associated with relapse risk, acute toxicity, and late effects (such as second cancers)[12;52-56]. Replication of associations from ongoing studies are required to use this information to individualize dosing for the majority of these associations, with the exception of thiopurine methyltransferase (TPMT), which is already being used to individualize therapy (Section 7.10).

Genome wide analyses are also being incorporated into studies of treatment-related outcomes for ALL[57;58]. The substrate for these genome wide studies can be DNA or RNA, and may involve the ALL blast material or germline material. Although the choice of tissue to serve as the substrate for genome-wide assessments of gene expression is not

clear, peripheral blood leukocytes are a reasonable and accessible source of "germline" RNA, and we have used this tissue for preliminary studies on TOTXV.

Because of the high false discovery rate inherent in genome-wide association studies, the critical concept in genetic studies is replication in multiple clinical trials. Thus, to some extent, genetic studies in TOTXVI will be repeating analyses on earlier St. Jude and other ALL studies. Those phenotype/genotype relationships that are replicated will have greater generalizability in the future; some associations that do not replicate are likely due to treatment differences among protocols, and some will have been due to false positive associations. Those associations of greatest importance are the subject of laboratory follow-up studies in the labs of Pharmaceutical, Oncology, and Pathology Department faculty, as well as other groups.

Two genetic polymorphisms that have been linked to outcome phenotypes by St. Jude investigators will be tested on TOTXVI, the *TYMS* enhancer repeat polymorphism and an *IL15* SNP[58]. The former comes from a candidate gene approach and has been linked to relapse risk[53] and to osteonecrosis risk[59]; the latter is based on our genome-wide analysis of germline polymorphisms that are significantly related to MRD status[60].

Given the pace with which genotyping technologies are evolving, it is not realistic to describe what genomic interrogations will actually be used to evaluate genomic determinants of efficacy and adverse events, but it is clear that genome-wide interrogations will continue to evolve to become less expensive and more feasible during the years that TOTXVI will accrue patients. Given the relatively low cost of prospectively obtaining genomic material for future studies, we will continue to collect peripheral blood for germline DNA and RNA isolation, as we have for TOTXV, and employ state-of-the-art techniques to assess whether genomic variation predicts risk factors for relapse, (hematologic or CNS), poor response (MRD positivity), acute toxicities (e.g. osteonecrosis, neurotoxicity, infection), intermediate pharmacologic phenotypes (e.g. plasma methotrexate clearance, serum levels of biomarkers such as serum cholesterol or cortisol), and long-term complications of therapy (e.g. second cancers, osteopenia). Reference serum at diagnosis will also be collected for the potential use to measure soluble factors in future research, as well as serum for "biomarkers" (as in 10.3 and 16.3) during therapy.

Examples of genotype/phenotype association studies that will be performed extend our earlier work in ALL trials to define germline genomic variation[53;59;61-64] that predicts toxic and therapeutic response; genome-wide gene expression that reflects germline variability and corresponds with response phenotypes[12;52;54;57] and acquired genomic changes that may affect ALL risk group and treatment response[65;66]. Sample power estimates are included in the Statistical Section 17.5.

TPMT exhibits genetic polymorphism, with <1% of the population homozygous deficient and 10% heterozygous[66]. We have been using information on patients' TPMT status to assist in dosage individualization for continuation therapy since 1991 in patients enrolled at St. Jude. We will continue to assess patients for TPMT phenotype and genotype, and use these data (along with data on clinical tolerance of chemotherapy and measurements of active thiopurine metabolites in red blood cells) as part of our algorithm for dosage adjustment during continuation therapy on TOTXVI. Patients who are TPMT

HET/deficient will receive mercaptopurine as per TOTXV during induction instead of thioguanine

2.2.2 Asparaginase

To address the randomized asparaginase questions, plasma asparaginase will be measured during induction and reinductions. There are substantial data from other investigations[67;68] supporting that a trough level at 14 days is in the elimination phase of the drug's concentration-times-time curves even when the drug is administered IM. With the first dose during induction, a total of 3-5 post-dose samples will be obtained, timed to coincide with clinic visits and blood draws, to better assess PK after IV dosing. Due to the very long half-life, there is flexibility as to when the samples are obtained, and Bayesian modeling permits limited sampling. The goals of these studies is to define the range of trough plasma asparaginase concentrations achievable with IV dosing of PEG-asparaginase, to compare concentrations in the 2,500 units/m^2 vs. 3,500 units/m^2 (and 3,000 units/m^2 if used) groups, and to explore whether asparaginase concentrations relate to asparagine depletion and anti-asparaginase antibodies.

Allergic reactions occur commonly with administration of asparaginase (an enzyme not present in humans) and can limit its use. More importantly, development of anti-asparaginase antibodies (with or without clinical hypersensitivity) may inactivate asparaginase and thus attenuate its ability to deplete asparagine systemically. Because asparaginase plays such a prominent role in TOTXVI, we will obtain samples for anti-asparaginase antibodies and plasma asparaginase (based on a biological assay of activity, which will thereby reflect any inactivating effects of concomitant antibodies), and CSF asparagine, a pharmacodynamic measure of asparaginase effect and one feasibly measured in the clinic.

Asparagine depletion is widely used as an index of asparaginase effect. CSF constitutes a convenient tissue for sampling, because CSF asparagine levels reflect systemic levels and are not subject to the difficulties in immediate sample processing caused by asparaginase in blood. In TOTXVI, intravenous PEG- asparaginase is replacing native E. coli L-asparaginase (Elspar), and will be administered in a randomized fashion at two dose levels post remission induction. We will measure anti-asparaginase antibodies, as well as CSF asparagine depletion in all patients at identical time periods during therapy, to explore whether more favorable results are associated with the higher dose of PEG- asparaginase, and whether these variables are related to treatment outcome, toxicities, and to each other.

In TOTXV, serum markers such as lipids, cholesterol, and cortisol were clearly influenced by both asparaginase and dexamethasone, and related to adverse events; moreover, asparaginase appeared to have a prominent effect on dexamethasone pharmacodynamics[69]. To establish this relation, serum samples timed relative to asparaginase and steroids will be obtained as was done on TOTXV (as detailed in Table 11.2).

2.2.3 Determination of ALL blast sensitivity to anti-leukemic agents, *in vitro*

We have previously studied the ex vivo sensitivity and resistance to anti-leukemic agents of primary leukemia cells isolated from newly diagnosed children with ALL[12;13]. *Ex vivo* drug sensitivity has predicted 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 in laboratory models. Our previous study identified 42, 59, 54 and 22 gene probes (corresponding to 19 to 40 known genes per drug) that were differentially expressed in B-lineage ALL cells that were either sensitive or resistant to prednisolone, vincristine, L-asparaginase or daunorubicin, respectively[12]. In addition, we have identified 45 genes differentially expressed in ALL exhibiting cross resistance to prednisolone, vincristine, asparaginase, and daunorubicin[13]. Our studies have identified genes not previously associated with drug resistance in childhood leukemia; discriminating genes belong to numerous functional groups, and specific functional categories were significantly over- represented for some anti-leukemic agents[12;13]. These findings document that resistance to mechanistically distinct anti-leukemic agents is associated with differential expression of different functional groups of genes, in support of combination chemotherapy as the paradigm for cancer treatment. Moreover, these findings point to previously unrecognized potential targets for developing new agents to augment the efficacy of current chemotherapy for acute lymphoblastic leukemia. This is illustrated by our finding of MCL1 over-expression in steroid resistant ALL, and the subsequent work of the Armstrong Lab at Harvard, who showed that rapamycin can down-regulate MCL1 protein, and enhance ALL sensitivity to steroids[70]. Our studies have also shown that there are drug-specific changes in gene expression in ALL, after in vivo treatment, and that drug combinations are distinct from the sum of single agent treatment[71]. In TOTXVI we will continue to study ex vivo drug sensitivity of primary ALL blasts to additional anti-leukemic agents (6MP, 6TG), in addition to extending our previous studies, and further investigate the nature of gene expression patterns associated with resistance to single anti-leukemic agents or to multi-drug cross resistance. Our hypothesis is that there is a definable set of genes that are differentially expressed in primary ALL cells exhibiting *de novo* resistance to chemotherapy and that identification of these genes will provide important new insights into the basis of ALL resistance and reveal potential new targets for overcoming resistance to these widely used medications. The work we propose in TOTXVI differs from prior work in that (1) we are prioritizing studies of mercaptopurine and thioguanine (drugs for which few patients were evaluable previously) and (2) we will simultaneously determine the sensitivity of these new cases to the anti-leukemic agents we have previously studied (prednisone, vincristine, daunomycin, asparaginase)[12] in order to elucidate genomic determinants of multi- drug cross resistance for all 6 medications. We have previously shown that gene expression patterns associated with multi-drug cross resistance are distinct from genes conferring resistance to single agents[13]. We will also use other genome-wide approaches, including SNP and haplotype analyses, to identify genes and genetic polymorphisms linked to resistance to each of these medications (not previously done), and to the multi-drug cross resistance phenotype. Our prior work was limited predominantly to B-lineage ALL. In TOTXVI, we aim to extend this work to T-lineage ALL. This subset has a worse prognosis in many treatment protocols, and therefore it is important that we understand the genomic basis of drug resistance in this common subtype of childhood ALL.

2.2.3 Plasma homocysteine levels

In TOTXIV and TOTXV studies, CSF and plasma homocysteine levels were measured in conjunction with HDMTX administration during Consolidation therapy. The rationale for assessing MTX-induced changes in homocysteine is based on evidence linking MTX exposure to neurotoxicities (e.g., seizures)[72;73], which have been hypothesized to be manifested, at least in part, by MTX-induced elevation in plasma homocysteine levels. An analysis of TOTXIV data revealed a trend in association between increased plasma homocysteine levels and incidence of seizures[63]. While no correlations were observed between plasma homocysteine (or seizures) with previously identified MTHFR genotypes as was postulated[74;75], it is likely that other genetic polymorphisms underlie the observed HDMTX- induced toxicities and interpatient variability in plasma homocysteine concentrations. We will continue to prospectively assess plasma homocysteine in HDMTX courses 1 and 2 of consolidation in Total XVI. Data will be used to pinpoint genetic biomarkers associated with differential plasma homocysteine levels both before, and following treatment with HDMTX, as well as those correlated with any observed toxicities.

2.3 Biologic Studies

2.3.1 Minimal residual disease

We and others have shown that detection of submicroscopic ("minimal") disease strongly correlates with leukemia relapse[76]. Using both flow cytometric detection of aberrant immunophenotypes and polymerase chain reaction (PCR) amplification of immunoglobulin and T-cell receptor genes, we were able to monitor minimal residual leukemia in all patients in TOTXV with highly concordant results. We also determined the degree of correlation between levels of residual leukemia in blood versus bone marrow by studying paired samples from patients enrolled in the study. MRD results in peripheral blood were concordant in T-cell disease, but not in B-*cell* lineage[20]. In TOTXVI we will monitor MRD using primarily flow cytometry; in patients whose leukemic cells lack a suitable immunophenotype (estimated to be 5% or less) we will use the PCR method applied in TOTXV. MRD can also be followed by the next generation deep sequencing of the IgH locus in B-ALL and TCR loci in T-ALL. The sensitivity and specificity are better than flow-based or PCR methods[77].

Patients who achieve negative MRD at the end of induction in TOTXV were unlikely to have reappearance of blasts on further follow up. Hence, post remission bone marrow samples will only be obtained for sequential MRD monitoring in patients with detectable MRD at the end of induction. However, we will continue to monitor MRD in all patients with T-cell ALL irrespective of their MRD status at the end of remission induction because T-cell cases have higher risk of early relapse compared to B-lineage cases. Because we showed that MRD in patients with T-ALL can be effectively monitored in peripheral blood, we will use this less-invasive procedure instead of bone marrow aspirates for this purpose. For B-ALL, we will also follow MRD of the bone marrow and peripheral blood by deep sequencing method to determine if this more sensitive and specific test will show concordance unlike the flow and PCR based MRD tests. If

concordant, then we can monitor B-ALL patient using a less invasive testing of the peripheral blood similar to T-ALL patients.

Investigators of the Children's Oncology Group (COG) demonstrated the prognostic value of determining MRD on day 8 of induction. In TOTXVI, we will explore whether day 8 MRD in peripheral blood is a practical approach that might potentially allow early therapeutic intervention in future studies.

In efforts to identify new markers of leukemia to use for MRD monitoring, we compared the gene expression profile of more than 300 diagnostic samples of B-lineage ALL to that of sorted CD19+ CD10+ cells from 4 normal bone marrow samples. Several genes were found to be differentially expressed in leukemic cells and antibodies suitable for flow cytometric staining are available for a subset of these. In TOTXVI, we will compare the usefulness of these new markers to monitor MRD by flow cytometry.

A rare blast identified by morphologic examination of the CSF at diagnosis currently upgrade the CNS relapse risk category resulting in extra intrathecal treatment. Of 349 patients enrolled on TOTXVI since 2007, only one has had a CNS relapse to date. MRD analysis of the CSF by flow or PCR based tests is not feasible because the CSF lacks sufficient cells. We will use a deep sequencing technique to evaluate MRD in CSF in order to study whether levels of MRD in the CSF at diagnosis and dynamics of early MRD reduction in CSF could be used to further refine our current classification for risk of CNS relapse. This might potentially allow the reduction of intrathecal therapy in future studies in children with rare identifiable blast by morphology but low levels of MRD.

2.3.2 NK cell receptor study

The normal physiological roles of NK cells are to control infection and prevent cancer. In fact, NK cells are the only immune cells that have been shown by prospective cohort study in healthy persons to have immunosurveillance capability against human cancer[78]. In a prospective study of more than 3000 healthy volunteers who were followed for 11 years, the risk of cancer was associated with decreased NK cell cytotoxicity against K562 leukemia cells, decreased expression of the NK receptor NKRP1, and decreased production of cytokines such as TNFα and IFNγ in NK cells.

Three recent studies of adult leukemia have demonstrated a direct link between leukemia and NK cell. In one study of patients with acute myeloid leukemia, the majority (16 of 18) of blood samples showed defective expression and function of NK cell–triggering receptors (NCRs) (dull)[79]. The expression of NK cell surface receptors was low, and the cytolytic activity against autologous leukemia cells, autologous B lymphoblasts, and NK cell–sensitive cell lines was weak. The abnormal NCR (dull) phenotype was confirmed in another study of 71 patients with acute myeloid leukemia and was found to be present in various morphologic and genotypic subtypes of leukemia[80]. In the third study of 25 AML and 14 ALL cases, the expression of HLA class I was frequently downregulated and that of the NK cell receptor ligands PVR and Nectin-2 were upregulated[81]. Together, these results suggest that NK receptor–ligand interaction may be crucial for the development of leukemia.

The normalization of NK cell receptor expression may be of prognostic value as NK cells may be important for the control of leukemia relapse and infection. These roles of NK cells have been shown by killer cell inhibitory receptor (KIR)-mismatched allogeneic stem cell transplantation[82]. Longitudinal study of adult AML patients showed that the NCR (dull) phenotype acquired during leukemia development was reversible in patients achieving complete remission after induction chemotherapy[80]. Reversibility of the NCR (dull) phenotype after complete remission suggested that leukemia cells might be involved in NCR down-regulation. Alternatively, the recovery of normal NCRs may allow the recovery of normal NK cell function that contributes to the acquisition of remission status. Interestingly, a correlation was found between the NCR (dull) phenotype and poor survival in AML patients after chemotherapy[80], suggesting that NK-deficient activation caused by NCR down-regulation could play a role in treatment outcome. Therefore, we will study NK cell receptor expression at diagnosis and before reinduction to elucidate the role of NK cell in the development and treatment response of childhood ALL.

2.3.3 Global gene expression

All diagnostic samples will be examined for global gene expression using microarray chips. This will continue to enrich our unique database on the gene expression of ALL cells which has become an invaluable resource for ALL investigators worldwide. In addition, this will provide us with an opportunity to validate prospectively the many retrospective observations made in past correlative studies including global gene expression. In addition to examples mentioned in Section 2.2, correlative studies performed with samples of patients enrolled in TOTXV identified several genes whose expression in leukemic cells at diagnosis was strongly associated with MRD during (day 19) or at the end of remission induction therapy (day 46)[10;11]. After excluding genes associated with genetic subgroups, we found that some of these genes were also strong predictors of overall treatment outcome in an independent cohort of 99 patients enrolled in TOTXIII[10;11]. A full listing of the genes can be found in the corresponding publications. One of the genes whose expression was most highly predictive for outcome was *CASP8AP2*, which encode a protein that promotes apoptosis and glucocorticoid signaling and was found to be expressed at lower levels in patients with MRD at the end of induction and a higher risk of relapse[10]. Another set of genes reported to be associated with in vitro drug resistance in childhood ALL[12;13], was also associated with MRD on day 19[11]. Genes found to be overexpressed in cases with in vitro drug resistance and with MRD on day 19 included *HNPRF, HMGB2*, *H2AFZ*, *KPNA2*, *SNRPG, KIAA0922*, *U2AF2* and *CCDC109B*. Genes with lower expression in patients with in vitro drug-resistance and with MRD on day 19 included *SLC2A3*, *MAFF*, *CD69*, *EGR1, CKS1B* and *OTUB1*. Finally, we identified genes that were strongly associated with MRD on day 19 or day 46, and were also independent predictors of outcome in an independent cohort, and encoded proteins with known regulatory functions in cell cycle progression: *CCNB2*, *CDC2*, *CKS1B*, *KLF7, BRRN1*, *TOP2A, MAD2L1*, *BUB3*, *NUSAP1*, *POLA2*, *EXO1*, *RNASAH2, MKI67, and ZWINT*[11]. In TOTXVI, we will study the association of these genes' expression with MRD on days 15 and 42, and with incidence of relapse. If there are antibodies available for the proteins encoded by the genes found to be associated with MRD and relapse in the previous studies, the expression of the proteins will be monitored by flow cytometry and their expression will be related to MRD on days 15 and 42, and

clinical outcome.

2.4 Neuroimaging Studies

2.4.1 Imaging studies

On TOTXIV, MR imaging of the brain was prospectively studied in all patients at week 6 of remission induction, week 7, week 31 and week 120 of continuation treatment. We evaluated the longitudinal prevalence of leukoencephalopathy in 45 subjects by treatment risk arm (low- and standard/high-risk)[83]. Higher doses and more courses of IV-MTX corresponded to a higher risk for leukoencephalopathy. However, many of the changes resolved after the completion of therapy and the effect of these changes on neurocognitive functioning and quality of life in survivors was not determined. A second study of this same patient cohort was conducted to assess the extent of LE (proportion of white matter impacted) through tissue segmentation and the relative intensity of LE through relative elevations in T1 and T2 relaxation rates[84]. The proportion of white matter affected in both treatment risk groups increased significantly with additional courses of IV-MTX, whereas the intensity of LE also increased steadily; however, both the intensity and extent of LE declined significantly by end of therapy. Increases in the T1 and T2 relaxation rates above normal-appearing white matter were significantly correlated with each other and were dependent on the proportion of white matter affected.

In TOTXV, MRI of the brain was prospectively studied in all patients at week 6 of remission induction, at first reinduction, at week 48 and week 120 of continuation treatment. Preliminary analysis of the first 229 patients to complete therapy showed that white matter changes were found in 29% of both low- and standard/high-risk patients at first reinduction. By week 120 of continuation, the prevalence in the low-risk patients had decreased to 24% while 29% of standard/high-risk patients continued to demonstrate white matter abnormality. The average proportion of white matter affected by these abnormalities was greater for standard/high-risk patients (9.2%) than for low-risk (6.6%) at first reinduction but decreased by more than half by week 120 of continuation for both groups (4.4% standard/high and 2.8% low).

New neuroimaging techniques and image processing methods now enable researchers to investigate the complex maturation of the human brain[85]. A longitudinal imaging study of 45 children between 5 and 11 years of age imaged twice, two years apart, was conducted and processed with the FreeSurfer software (http://surfer.nmr.mgh.harvard.edu/) to assess whole brain cortical thickness[86]. The brain grew at a rate of approximately 0.4 to 1.5 mm per year in many areas, prominently in the frontal and occipital regions. Cortical thinning was noted of approximately 0.15 to 0.3 mm per year in right dorsal frontal and bilateral parietal regions. These new technologies enable researchers to assess atypical cortical brain maturation across the entire brain to identify specific neuroanatomical regions which may be at increased risk of injury and relate these changes to specific neurocognitive domains which may be negatively impacted by therapy.

The use of Diffusion Tensor Imaging (DTI) in asymptomatic patients during or after treatment for ALL is a more recent development. Khong et al. prospectively tested the association of white matter fractional anisotropy (FA) and intelligence quotient (IQ) in 18

ALL survivors and age-matched healthy controls[87]. Among the ALL survivors, nine received HDMTX and nine underwent cranial irradiation with a trending difference between the two groups. The FA in the white matter of ALL survivors was often lower than that in the age-matched controls, and the differential between the FA in patients relative to that in controls was directly proportional to the full-scale, performance, and verbal IQ scores of the survivors. These preliminary findings suggested that white matter FA is a clinically useful biomarker for the assessment of treatment- related aberrant brain maturation.

As an ancillary measure of myelin degradation, quantitative multiple exponential T2 measurements have the advantages of providing both myelin water fraction (MWF) maps which are not sensitive to fiber crossings or density, and long-T2 water fraction (LWF) maps which are believed to suggest more severe damage. MWF maps have been shown to correlate strongly with both histological staining for myelin and with DTI measures of FA and radial diffusivity[88;89]. Signals on LWF maps suggest inflammation, extensive cellular destruction with increased extracellular water, and severe axonal damage in the white matter[90].

2.4.2 Genetic polymorphisms

Although several chemotherapeutic agents in ALL therapy may contribute to neurotoxicity and negative neurocognitive outcomes, methotrexate has been the primary focus of many studies[91-93]. The dose, cumulative exposure, and infusion rate of methotrexate have been associated with the degree of neurocognitive deficit[91;94]. These same factors have been associated with prevalence and intensity of white matter changes particularly in the frontal lobes which are often associated with attention processes[95;96]. Methotrexate and its metabolites inhibit folate pathway enzymes such as 5,10-methylenetetrahydroreductase (MTHFR)[97]. The MTHFR enzyme has two polymorphisms, characterized by C677T and A1298C, which alter the function of the enzyme, leading to increased homocysteine and reduced folate. Homocysteine is converted to methionine by methionine synthase (MS), and individuals with MS deficiency have elevated plasma homocysteine levels[98]. Additional key enzymes to consider include thymidylate synthase (TS) and cystathionine beta synthase (CBS). TS utilize circulating folate to convert deoxyuridine monophosphate to deoxythymidine monophosphate, which is essential for DNA synthesis. CBS deficiency results in classical homocysteinuria[99]. MS, TS, and CBS each have polymorphic variants capable of impacting levels of folate and/or homocysteine. Alterations in the function of folate pathway enzymes may impact chemotherapy-related toxic effects.

One recent study tested the hypothesis that these polymorphisms may partially explain the individual variation in children developing attention deficits after treatment for ALL[100]. Forty-eight survivors of ALL were evaluated for attention deficits and peripheral blood DNA was collected and genotyped for MTHFR polymorphisms. Patients with the C677T and A1298C genotypes were more likely to have attention deficits (P=0.07). While this study did provide strong preliminary evidence that MTHFR polymorphisms may play an influential role in determining which patients will develop attention deficits after therapy for ALL, the sample size was small and information on cumulative methotrexate exposure and steroid dose was not available. These results still need to be replicated in a larger

cohort, which would also permit the inclusion of prospective neuroimaging.

2.4.3 Imaging changes associated with neurocognitive performance

Evaluation of neuropsychological function of long-term survivors from TOTXIIIA showed that 41% (18/44) of the patients treated without cranial irradiation had Full-Scale IQ scores at least 1 standard deviation below normal but only 10% (4/44) had sufficient academic and attention deficits to qualify for an interventional feasibility study (MEMFIX). Nineteen of the 44 patients had MRI examinations suitable for segmentation analysis and three of these long-term survivors (16%) had leukoencephalopathy on the examinations. A direct relationship between normal appearing white matter volume and neurocognitive performance (Full-Scale IQ [r=0.33, p=0.165] and CPT Hits Reaction Time [r=0.44, p=0.059]) was also demonstrated in this pilot study.

To expand on these findings, we recently examined 112 ALL survivors treated with either chemotherapy alone (n=84) or in combination with irradiation (n=28)[101]. A group of 33 healthy sibling controls 6 to 16 years old was also recruited and imaged. Performance on most neurocognitive measures in ALL survivors demonstrated significant deficits from normative test scores, but only performance on the attention measures exceeded one standard deviation from the normative mean. Performance on measures of academic achievement differed between the two patient groups, and deficits exceeding one standard deviation from the normative mean were seen only in patients who received irradiation. Patients treated with chemotherapy alone had significantly smaller volumes of white matter than age- and sex-matched healthy sibling controls (P=0.03), and patients who received chemotherapy and irradiation had even smaller white matter volumes than those treated with chemotherapy alone (P=0.02). Moreover, smaller white matter volumes corresponded to larger deficits in attention, intelligence, and academic achievement.

In TOTXV, we prospectively tested the association of white matter abnormality with neurocognitive performance. We analyzed the association between the quantitative measures of normal appearing white matter volume, T1 and T2 of white matter, on week 120 continuation treatment and the neurocognitive performance of the first 229 patients at this same time point. Only 140 subjects had neurocognitive testing and corresponding neuroimaging performed. Lower volumes of normal appearing white matter were significantly associated with lower performance in math and spelling in low-risk patients. A substantial association also existed between normal appearing white matter volume and reading in these same patients. Elevated T1 relaxation rates in the standard/high risk patients were associated with lower full-scale, verbal and performance IQ as well as lower performance in reading and spelling. Elevated T2 relaxation rates in the standard/high risk patients were also associated with lower full-scale, verbal and performance IQ as well as lower performance in long term memory.

Differences in neurocognitive performance between risk arms for these patients can best be appreciated by examining the proportion of subjects at least one standard deviation below the normative mean on each measure. Low-risk subjects performed relatively well on intellectual measures of full-scale, performance, and verbal IQ (17%, 20%, and 11% more than one standard deviation below the normative mean) while greater proportions of

standard/high-risk subjects were more than one standard deviation below the normative mean (32%, 30%, and 26%). This same pattern can be seen in academic performance measures of math, reading, and spelling (5%, 2%, and 5% for low-risk; and 26%, 23%, and 26% for standard/high-risk).

Based on these associations between white matter volume and relaxation changes in these patients and the association of these changes with deficits in neurocognitive performance, we propose to continue prospective quantitative imaging of the development of patients treated on TOTXVI. To refine our understanding of the impact of therapy on the developing brain and the mechanisms that may be responsible, we have proposed to investigate the integrity of the white matter myelin using DTI and developmental changes in cortical thickness using high-resolution multispectral imaging. This will be the first opportunity to directly compare neuroimaging of patients during treatment for ALL with those of healthy children matched on selected characteristics. The neuropsychological battery has also been refined to be maximally sensitive to those brain regions identified as most vulnerable in recent neuroimaging studies.

We have recently implemented a prototypical N-back working memory task for fMRI, and have pilot studies with the task underway in 48 long-term survivors of ALL (>10 years post-therapy) and 25 age-similar healthy controls. Preliminary random effects group analysis has demonstrated activation in the typical network of brain regions that have been reported in fMRI studies of the N-Back task[102] including anterior cingulate/medial frontal, dorsolateral prefrontal, ventrolateral prefrontal, frontal polar and parietal areas. Activation in right dorsolateral prefrontal and right parietal increased with increasing working memory load, and bilateral activation in ventrolateral prefrontal areas was detected only in the 2- back condition. In the ALL survivor group, we have also acquired resting state fMRI data with acquisition parameters as reported by Sorg et al[103]. Independent component analysis (ICA) of the resting state data revealed neural networks with temporally correlated resting BOLD signal fluctuations, including the frontal- parietal and ventral frontal networks identified with the general linear model analysis of data from the N-back task. ICA analysis of the resting state and N- back data also revealed other networks that may be informative about the neural correlates of cognitive deficits in ALL survivors, including the primary sensory cortices and the default mode network[103]. The fMRI paradigm based on the N-back test of working memory was designed and the procedures were optimized specifically to address known neurocognitive deficits in the highly vulnerable young population of childhood cancer survivors. Functional MRI is an important complement to the structural imaging measures we propose that will help to characterize the functional consequences of therapy-induced changes in the gray matter and white matter that constitute the neural networks in the brain.

2.5 Philadelphia Chromosome (Ph)-Positive ALL

Patients with Ph+ ALL, a group which constitutes less than 5% of childhood ALL, have a dismal prognosis when treated with chemotherapy alone[24]. The t(9;22) translocation juxtaposes the breakpoint cluster region (BCR) gene with the Abelson leukemia virus proto-oncogene (ABL). The resultant fusion protein is a constitutively active tyrosine kinase that activates molecular cascades leading to cell proliferation, enhanced cell

survival, and alterations in cell adhesion. The tyrosine kinase activity of BCR-ABL is required for transformation in vitro and in murine models. Imatinib was the first tyrosine kinase inhibitor developed for treatment of Ph+ leukemia[104-108].

Between June 2000 and June 2004, five patients with Ph+ ALL were enrolled on TOTXV. One patient was refractory to treatment and 2 sustained a hematologic relapse. When imatinib became commercially available, TOTXV was consequently amended to incorporate imatinib in the treatment regimen of Ph+ ALL patients. All 4 Ph+ ALL patients enrolled after the amendment remain in continuous complete remission. Imatinib was well tolerated when incorporated into TOTXV backbone, with no significant added toxicity, making the risk- benefit of incorporating a tyrosine kinase inhibitor into the therapy of this high risk group worthwhile.

The recently approved dasatinib is a potent, broad spectrum ATP-competitive inhibitor of several tyrosine kinases including BCR-ABL and SRC. Dasatinib is ~500-fold more potent than imatinib in inhibiting BCR-ABL and binds to both active and inactive conformations of c-ABL, whereas imatinib only binds to the inactive state. Dasatinib demonstrated significant activity against several advanced human chronic myeloid leukemia (CML) xenografts, including an imatinib acquired-resistance model completely insensitive to imatinib[109;110]. Furthermore, early clinical studies of dasatinib have demonstrated its high activity and limited toxicity in treating patients with Ph+ leukemias, including patients with refractory Ph+ ALL[25-27]. Hematologic toxicities and pleural effusions are the most common adverse events associated with dasatinib. Adverse events are manageable with supportive care and dose reduction. Adult patients with Ph+ leukemia treated with dasatinib at 100 mg once daily had lower incidence of adverse events compared to those treated with 50 mg twice daily, 140 mg once daily, or 70 mg twice daily. Most importantly, efficacy was similar with all dose schedules[111]. M. D. Anderson Cancer Center investigators have incorporated dasatinib into frontline Ph+ ALL regimens, and the COG is incorporating it into their frontline Ph+ ALL protocol. The current standard adult dose is 70 mg given twice daily[25]. In the ongoing COG Phase I trial, a starting dose of 50 mg/m^2 twice daily was well tolerated in children with solid tumors.

In TOTXVI, we will build on the experience of TOTXV which safely incorporated imatinib into an intensive chemotherapy backbone for patients with Ph$^+$ ALL. Dasatinib will be substituted for imatinib in the chemotherapy backbone, starting at day 22 of induction until the end of therapy. We will use the equivalent of the standard adult dosage of 70 mg b.i.d., which is 40 mg/m^2 b.i.d. (see section 8.3 for dose modification). For patients who undergo hematopoietic stem cell transplantation, dasatinib will be held during the transplant period, and then resumed after recovery of marrow function until 1 year post transplant date or completion of 6 months dasatinib therapy post-transplant, whichever provides longer exposure to dasatinib post-transplant. The reason for starting dasatinib on day 22 of induction is that dasatinib is both an inhibitor and substrate for cytochrome P450 3A4. Therefore, it is best to avoid the use of this agent during prolonged treatment with prednisone and vincristine in the first three weeks of induction. Also, this will still allow us to assess early response to chemotherapy, which is an important factor in determining whether transplant is indicated or not for certain Ph+ ALL.

2.6 Infant ALL

Infant ALL constitutes about 4% of childhood ALL, and is associated with a high WBC at presentation, a high frequency of an immature precursor B-lineage characterized by the lack of CD10 expression and the presence of *MLL* gene (11q23) rearrangements. Whereas the outcome of infants with a *MLL* germline ALL (include 15-20% of infant ALL) is comparable to that of older children with ALL, infants with *MLL* rearranged ALL have a dismal prognosis, especially those with age < 6 months or WBC $\geq$ 300 at diagnosis[16;17].

Infants with ALL were included in TOTXII and TOTXIII. In 1999, a large international collaborative study group developed a common treatment protocol (Interfant-99) for infant ALL to try to improve the outcome for these very young children. St Jude joined that group, and infants were enrolled on Interfant-99 while TOTXV excluded children younger than 1 year of age.

Because the results of Interfant-99 protocol were not superior to that of TOTXIIIB, infants will be enrolled in TOTXVI. Those without germline *MLL* (15 to 20% of infant ALL) will receive the same risk-directed treatment plan as older children. The remaining 80 to 85% of infants with *MLL* rearrangement need innovative therapy to improve their dismal outcome. All infants with rearranged *MLL* will be treated on the standard-/high-risk arm of TOTXVI. Clofarabine is a recently approved novel nucleoside analog which has shown significant activity in children with ALL whose disease was refractory to standard agents[17;112;113]. When given in combination with cyclophosphamide and etoposide, clofarabine induced remissions in over 2/3 of relapsed and refractory children with ALL[114]. This combination will therefore be given to infants with *MLL* rearrangement on day 22 of remission induction (replacing the cyclophosphamide, cytarabine, thioguanine). It will also be administered in combination with dexamethasone and PEG- asparaginase on weeks 7 to 9 of continuation replacing reinduction I.

2.7 Background and Rationale for Intestinal Microbiome Objectives

Increased susceptibility to a number of pathogens in children with ALL can result in significant morbidity and treatment delays, thus compromising outcome[115;116]. Acute gastroenteritis is one of the most common diseases affects, particularly in children. Since the Norwalk virus was identified as a cause ting children worldwide. Viruses are recognized as a major cause of the gastroenteritis, the number of viral agents associated with diarrheal disease in humans has progressively increased. Until the release of a vaccine, rotavirus was the most common cause of severe diarrhea in children under 5 years of age. Human astroviruses, caliciviruses and enteric adenovirus are also important etiologic agents of acute gastroenteritis. Other viruses such as toroviruses, coronaviruses, picobirnaviruses, Aichi virus and human bocavirus are also increasingly being identified as causative agents of diarrhea. Little is known about the prevalence of enteric viruses in immunocompromised children. Several studies from the 1980's have shown that children with HIV or T-cell deficiencies can develop chronic infections with several enteric viruses leading to persistent diarrhea, which can cause considerable problems of management. By evaluating the population dynamics of these enteric viruses prior to and after chemotherapy, we will obtain a greater understanding of the prevalence of these viruses in

this patient population with the eventual goal of improved diagnostics and predictions of clinical complications.

We will study the impact of ALL therapy on the intestinal microbiome, and the relationship between the intestinal microbiome and the increased susceptibility of children with ALL to infections, including gasteroenteritis. The intestinal microbiome consists of all the intestinal micro-organisms and their associated genetic elements. Several lines of evidence suggest that the interactions between the host's enteric microbiota and the innate immune system are important in modulating the intestinal response to cancer therapy[117]. Alterations in the host microbiota can translate into alterations in the susceptibility of the host to infection[118]. *Clostridium difficile* is a common cause of antibiotic-associated diarrhea in this patient population. As perturbations of the normal gut microflora are thought to predispose patients to infections caused by *C. difficile*, these investigations will also address what alterations in the microflora correlate with the predominance of *C. difficile* in the gastrointestinal tract. The gut microbiota plays a crucial role in the development of an effective immune response.

Alterations in the gut microbiota can have profound consequences not only for intestinal diseases but also for mounting an effective immune response against invading pathogens at various sites in the human body[119]. Recent studies have shown that certain bacterial species in the gut can be utilized to estimate the risk of antibiotic-associated diarrhea with an error rate of 2%, emphasizing the relevant application of these data for improved prediction of clinical outcomes[120]. In summary, existing literature suggests that the human microbiome plays a variety of important roles in immunology and infectious diseases. Alterations in the host microbiota can have profound effects on the type and magnitude of the immune response against invading pathogens and thus on the infectious complications. We would like to investigate the effect of the chemotherapeutic regimens for pediatric ALL on the gut microbiome, and whether alterations of their microbiome have subsequent effects on infection-related clinical outcomes.

2.8 Inclusion of Women and Minorities

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.

3.0 ELIGIBILITY CRITERIA AND STUDY ENROLLMENT

3.1 Inclusion Criteria

3.1.1 Diagnosis of precursor B-cell or precursor T-cell ALL by immunophenotyping.

3.1.2 Age ≤18 years (inclusive).

3.1.3 Limited prior therapy, including systemic glucocorticoids for one week or less, one dose of vincristine, emergency radiation therapy to the

mediastinum and one dose of intrathecal chemotherapy. Other circumstances must be cleared by PI or co-PI.

3.1.5 Written, informed consent and assent following Institutional Review Board, NCI, FDA, and OHRP Guidelines.

3.2 Exclusion Criteria

3.2.1 Participants with prior therapy, other than therapy specified in 3.1.3.

3.2.2 Participants who are pregnant or lactating.

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

3.3 Research Participant Recruitment and Screening

Research participants will be recruited by study investigators through regular clinical practice.

3.4 Enrollment On Study

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 completed checklist will be faxed to the Central Protocol and Data Monitoring Office (CPDMO) at [redacted]; followed by a phone call to ext. [redacted] to ensure that the fax is received. Eligibility will be reviewed, and entered into the Patient Protocol Manager (PPM) system. A research participant-specific consent form and assent document, where applicable, will be generated. The protocol (if applicable), and consent/assent document will be delivered to the area designated on the checklist. The entire signed informed consent/assent form must be faxed to the CPDMO to complete the enrollment process.

The CPDMO is staffed 7:30 am-5:00 pm CST, Monday through Friday. A staff member is on call Saturday, Sunday, and holidays from 8:00 a.m. to 5:00 p.m.

4.0 RISK CLASSIFICATION

Patients are classified into one of three categories (low-, standard-, or high-risk) based on the presenting age, leukocyte count, presence or absence of CNS-3 status or testicular leukemia, immunophenotype, cytogenetics and molecular genetics, DNA index, and early response to therapy. Hence, definitive risk assignment (for provisional low-risk or standard-risk cases based on presenting features) will be made after completion of remission induction therapy. The criteria and the estimated proportion of patients in each category (based on data from TOTXV study) are provided below.

4.1 Criteria for Low-Risk ALL (approximately 48% of patients)

- B-cell precursor ALL with DNA index ≥1.16, *TEL-AML*1 fusion, OR age 1 to 9.9 years and presenting WBC <50 x 10^9/L.
- Must not have:
  - CNS 3 status (≥ 5 WBC/μL of cerebrospinal fluid with morphologically identifiable blasts or cranial nerve palsy).
  - Overt testicular leukemia (evidenced by ultrasonogram).
  - Adverse genetic features: t(9;22) or *BCR-ABL* fusion; t(1;19) with *E2A- PBX1* fusion; rearranged *MLL* (as measured by FISH and/or PCR); or hypodiploidy (<44 chromosomes).
  - Poor early response (≥1% lymphoblasts on day 15 of remission induction, ≥0.01% lymphoblasts by immunologic or molecular methods on remission date).

4.2 Criteria for Standard-Risk ALL (approximately 44% of patients)

- All cases of T-cell ALL and those of B-cell precursor ALL that do not meet the criteria for low-risk or high-risk ALL.

4.3 Criteria for High-Risk ALL (approximately 8% of patients)

- t(9;22) or *BCR-ABL* fusion.
- Infants with t(4;11) or *MLL* fusion.
- Induction failure or ≥1% leukemic lymphoblasts in the bone marrow on remission date.
- ≥ 0.1% leukemic lymphoblasts in the bone marrow in week 7 of continuation treatment (i.e. before reinduction I, ~14 weeks post remission induction).
- Re-emergence of leukemic lymphoblasts by MRD (at any level) in patients previously MRD negative.
- Persistently detectable MRD at lower levels
- Early T-cell precursor (ETP) ALL, defined by low expression of T-cell markers together with aberrant expression of myeloid markers (as described in section 2.1.1). The following features characterize ETP T-ALL:

  1) Levels of CD5 expression at least 10 fold lower than that of normal peripheral blood T-lymphocytes. In the study that identified this subset of T-ALL, we found that CD5 expression was 10 ->200-fold lower than that of normal lymphocytes and median percent leukemic cells expressing CD5 in the 17 atypical cases was 45%, in contrast to >98% for the 122 of the typical group.
  2) Absence (<10%) of CD1a and CD8 expression.
  3) Expression of cytoplasmic CD3 together with the expression of 1 or more markers associated with myeloid leukemia such as HLA-Dr, CD34, CD13, CD33 or CD11b, while myeloperoxidase is <3% by

cytochemistry and/or flow cytometry.

5.0 TREATMENT PLAN

Treatment will consist of three main phases: Remission Induction, Consolidation, and Continuation.

5.1 Intrathecal Chemotherapy

As a traumatic lumbar puncture at diagnosis may result in a poorer outcome and the need for extra intrathecal therapy subsequently, all diagnostic lumbar punctures will be performed by experienced personnel, preferably under general anesthesia or deep sedation. Triple intrathecal chemotherapy (MHA) will be administered immediately after cerebrospinal fluid is collected for diagnosis, and the dosage is age-dependent as following:

Age (months)	Methotrexate (mg)	Hydrocortisone (mg)	Cytarabine(mg)	Volume (ml)
<12	6	12	18	6
12-23	8	16	24	8
24-35	10	20	30	10
≥36	12	24	36	12

Frequency and total number of triple intrathecal treatments for Remission Induction is based on the patient's risk of CNS relapse, as follows:

- All patients will receive triple intrathecal treatment on days 1 and 15.
- Patients with any of the following features will receive additional triple intrathecal treatment on days 8 and 22:
  - Philadelphia chromosome
  - *MLL* rearrangement
  - Hypodiploidy (< 44)
  - WBC ≥100 x 10^9/L at presentation
- Patients with any of the following features will receive additional triple intrathecal treatment on days 4, 8, 11, and 22:
  - T-cell ALL
  - t (1;19)/*E2A-PBX1*
  - CNS-3 status (i.e., ≥5 WBC/μL of CSF with blasts or cranial nerve palsy)
  - CNS-2 status (<5 WBC/μL of CSF with blasts)
  - Traumatic LP (≥10 RBC/μL of CSF with blasts)

Leucovorin rescue (5 mg/m^2/dose, max 5 mg) PO will be given at 24 and 30 hours after each triple intrathecal treatment during induction. Follow plasma methotrexate levels (starting 24 hours after intrathecal therapy and until level becomes undetectable) in patients with renal dysfunction or extra fluid in third space, and rescue with leucovorin according to Pharm.D recommendation.

5.2 Remission Induction Chemotherapy (6 to 7 weeks)

Induction treatment will begin with prednisone, vincristine, daunorubicin, PEG-asparaginase and triple intrathecal treatment, followed by cyclophosphamide plus cytarabine plus thioguanine. Dexamethasone will be substituted for prednisone in patients with ETP immunophenotype. Mercaptopurine will be substituted for thioguanine in TPMT HET/deficient patients. Daunorubicin may be delayed in patients with febrile neutropenia, evidence of mucositis or increased hyperbilirubinemia (i.e., total bilirubin ≥2.0 mg/dl and direct bilirubin > 1.4 mg/dl). Patients with mucositis should be evaluated for herpes simplex infection and treated with acyclovir or famciclovir if work-up is positive.

5.2.1 Drug dosages (see section 5.2.1.1 for dosing of infants and Sections 7.0 and 8.0 for dose modification)

Participants	Agents	Dosages and routes	# Dose	Schedules
All participants				
	Prednisone[§] or Prednisolone[§]	40 mg/m^2/day PO (divided t.i.d.)	84	Days 1 – 28
	Dexamethasone (for ETP immunophenotype only)	10 mg/m^2/day PO divided t.i.d.) 4 mg/m^2/day PO (divided t.i.d) 2 mg/m^2/day PO(divided t.i.d)	63 9 12	Days 1-21 Days 22-24 Days 25-28
	Vincristine	1.5 mg/m^2 IV (max 2 mg)	4	Days 1, 8, 15, 22[α]
	Daunorubicin*	25 mg/m^2 IV	2	Days 1 and 8
	PEG-asparaginase	3,000 units/m^2 IV	1	Day 3
Day 15 MRD ≥ 1%				
	PEG-asparaginase	3,000 units/ m^2 IV	1	Day 15[Ω]
Day 15 MRD < 5% (excluding *MLL*+ infants)				
	Cyclophosphamide[†]	1000 mg/m^2 IV	1	Day 22
	Cytarabine	75 mg/m^2/dose IV	8	Days 23-26, 30-33
	Thioguanine Mercaptopurine (TPMT HET/deficient patients only)	60 mg/m^2/dose PO	14	Days 22-35
	Dasatinib[(‡)] (*Ph+ participants only*)	40 mg/m^2 b.i.d	Daily	Starting Day 22 of induction to continue until end of treatment

Day 15 MRD ≥ 5% (excluding *MLL*+ infants)				
	Cyclophosphamide†	300 mg/m^2 IV	4	q12 hrs, Days 22-23
	Cytarabine	75 mg/m^2/dose IV	8	Days 23-26, 30-33
	Thioguanine Mercaptopurine (TPMT HET/deficient patients only)	60 mg/m^2/dose PO	14	Days 22-35
	Dasatinib‡ (*Ph+ participants only*)	40 mg/m^2 b.i.d	Daily	Starting Day 22 of induction and continue until end of treatment
Participants	Agents	Dosages and routes	# Dose	Schedules
Infants with *MLL*+				
	Clofarabine	40 mg/m^2/dose IV	5	Days 22-26
	Etoposide	100 mg/m^2/dose IV	5	Days 22-26
	Cyclophosphamide	300 mg/m^2/dose IV	5	Days 22-26

**First dose of daunorubicin may be delayed until day 2 in patients with or at high risk of tumor lysis syndrome. Second dose of daunorubicin could be delayed up to one week if clinically indicated.*
ᵑSecond dose of PEG-asparaginase to be given as soon as MRD ≥=1% is confirmed and no later than day 21.
†See 5.2.3.2 for the need of bone marrow exam and the criteria of starting cyclophosphamide plus thioguanine on day 22. § Oral prednisone can be substituted with methylprednisolone at 20 mg/m^2/day IV (t.i.d.) for patients who cannot tolerate the oral medication.
ᵅDay 22 vincristine will be omitted for infants with MLL+ *(‡)Start dasatinib on day 22 for Ph+ cases. See Section 7.12.*

5.2.1.1 Dose adjustment for infants

With the exception of vincristine, 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, a 50% reduction in dosages of daunomycin, asparaginase, etoposide, methotrexate, thiopurines, cyclophosphamide, clofarabine, and cytarabine should be made. The vincristine dosage for patients < 12 months of age or < 10 kg weight is 0.05 mg/kg/dose.

5.2.2 Remission induction dose modifications

See Section 7.0 for treatment modifications for vincristine, daunorubicin, and PEG-asparaginase and section 7.10 for substitution of mercaptopurine and modification of thiopurine dosing based on TPMT status. Following Day 22 treatment, cytarabine and thioguanine may be held if patient develops febrile neutropenia or grade 3 or 4 mucositis. If C-reactive protein is normal, fever subsides, cultures are not significant and mucositis resolves, cytarabine and thioguanine may be resumed. Doses may be completely omitted if the patient is beyond day 30 of remission induction (i.e., half or more doses of thioguanine (or mercaptupurine) and cytarabine have been given), allowing early bone marrow recovery and early initiation of consolidation therapy.

5.2.3 Bone marrow evaluations

Day 15: A bone marrow aspirate will be done on day 15 of remission induction to assess anti-leukemic response. The presence of ≥1% of leukemic blasts in the bone marrow by morphologic exam or by MRD study is an indication for an additional dose of PEG-asparaginase to be administered between days 15 and 21 (for modifications due to PEG-asparaginase allergic reactions, see Section 7.9).

Day 22: Bone marrow aspirate on day 22 will be performed in patients who had received an extra dose of PEG-asparaginase due to the presence of 1% or more leukemic blasts in the bone marrow on day 15. Patients with residual leukemia identified on day 22 receive cyclophosphamide, thioguanine, and cytarabine as scheduled if their clinical condition permits, regardless of their ANC. For other patients, the treatment may be delayed for 3 to 7 days to allow some degree of hematopoietic recovery if APC (ANC + monocyte) $<300/mm^3$.

End of induction-MRD response: A bone marrow aspirate will be performed on day 38-42 of remission induction, depending on when ANC has recovered to $\geq 300/mm^3$, WBC to $\geq 1{,}000/mm^3$, and platelet count to $\geq 50 \times 10^9/L$. If the date falls on a week-end or holiday, the procedure may be performed on closest working day. MRD level will be determined in this bone marrow sample. Poor response will be defined as MRD level ≥0.01% (one or more lymphoblasts among 10^4 bone marrow mononuclear cells) by either immunologic or molecular assay. If the result of MRD is positive, provisional low-risk cases will then be re-classified as standard-risk (MRD ≥0.01% but less than 1%) or high-risk (MRD ≥1%), and will receive subsequent 3 doses of HDMTX at a higher dosage (i.e., 5 gm/m^2). (These patients would have received the first HDMTX of consolidation therapy at 2.5 gm/m^2.)

5.3 Consolidation Treatment (8 weeks)

5.3.1 Drug dosages

Agent	Dosage and Route	# Doses	Schedule
High Dose Methotrexate (HDMTX)	2.5 gm/m^2 (low risk), or targeted 65 µM (std/high-risk)	4	days 1, 15, 29 and 43
Mercaptopurine	50 mg/m^2/day	56	Days 1 to 56

When ANC $\geq 300/mm^3$, ≥WBC $1{,}000/mm^3$, and platelet count $\geq 50 \times 10^9/L$, consolidation treatment will be started, consisting of high dose methotrexate (every other week for 4 doses), daily mercaptopurine and IT chemotherapy on the same dates of high dose methotrexate (see section 5.3.4).

See section 5.3.5 (Interim Continuation Treatment) and section 7.0 for dose adjustments.

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. In patients for whom high dose methotrexate treatment is delayed, mercaptopurine may be continued until 14 days after the last course of high dose methotrexate. Mercaptopurine may be held in the presence of ANC $<300/mm^3$, WBC $<1,000/mm^3$, platelet count $<50,000/mm^3$ or grade 3 or 4 mucositis. Dosage of mercaptopurine in subsequent courses may be reduced to 25 mg/m^2/day in patients who have prolonged neutropenia after high dose methotrexate and mercaptopurine treatment. See section 7.10 for modifications of mercaptopurine based on TPMT status.

5.3.3 High-dose methotrexate (HDMTX) administration

The dosage of high dose methotrexate depends on the risk classification of individual patients. Patients with standard- or high-risk ALL will receive 5 gm/m^2, (or a dose targeted to achieve a steady-state plasma concentration of 65 μM) and those with low-risk ALL 2.5 gm/m^2, (or, when requested, a dose targeted to achieve a steady-state concentration of 33 μM) administered over 24 hr intravenously. The subsequent dose of high dose methotrexate, mercaptopurine and intrathecal treatment will be delayed if ANC $<300/mm^3$, WBC $<1000/mm^3$, platelet count $<50 \times 10^9$/L, SGPT >500 U/L, total bilirubin >2 mg/dl and direct bilirubin >1.4 mg/dl, or mucositis is present. Sodium bicarbonate may be given orally at 1 gm/m^2 every 6 hours or intravenously with pre-hydration fluid starting the day before high dose methotrexate. For patients with Down syndrome, high dose methotrexate administration will be modified (See Section 7.1.1). Based on prior experience in TOTXV, we anticipate that 2.5 g/m^2 should achieve the target concentration of 33 μM $\pm$ 20% in 70% of the LR courses (see Section 2.1.3 for details and rationale). Clearance is estimated from the plasma concentrations up through 48 hours from methotrexate infusion, using nonlinear curve fitting and a Bayesian estimation strategy as implemented in ADAPT. 10% of the total dose is administered as a loading dose over 1 hour and the remainder over 23 hours.

Dosage individualization may be clinically indicated in select LR patients. In these cases, the precise method for targeting will be documented in the research database for each dose of high dose methotrexate given, along with the reason(s) the dose was targeted.

Based on prior experience in TOTXV, we anticipate that 5 g/m^2 should achieve the targeted concentration of 65 μM $\pm$20% in only 62% of the courses (see Section 2.1.3 for details and rationale). Clearance is estimated from the plasma concentrations up through 48 hours from methotrexate infusion using nonlinear curves fitting and a Bayesian estimation strategy based on predictive characteristics (such as serum creatinine and SGPT) as implemented in ADAPT. Ten percent of the total dose is administered as a loading dose over 1 hour and the remainder over 23 hours.

5.3.3.1 Pre-hydration

At least two hours before high dose methotrexate, prehydration IV fluid (D5W + 40 mEq $NaHCO_3$/L + 20 mEq KCl/L) will be administered at the rate of 200 ml/m^2/hr. At start of prehydration, one IV dose of $NaHCO_3$ (unless otherwise clinically indicated, 12 mEq/m^2 for low-risk patients and 25 mEq/m^2 for standard-/high-risk patients) diluted in 50 ml D5W will be given over 15 minutes. Prehydration fluid may also be given overnight at a rate of at least 100 ml/m^2/hr. High dose methotrexate treatment will follow, provided that urinary pH is $\geq$6.5; exceptions must be cleared with the pharmacokinetics service and the attending physician.

5.3.3.2 High-dose methotrexate infusion

Methotrexate loading dose will be given over 1 hour, followed immediately by maintenance infusion over 23 hours. During the methotrexate infusion, patients should receive hydration fluid with D5W + 40 mEq/L $NaHCO_3$ + 20 mEq KCl/L at 100-150 ml/m^2/hr. Urine pH will be monitored with each void during infusion. An IV bolus of 12 mEq/m^2 $NaHCO_3$ 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. Patients 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 according to Section 10.1.

5.3.3.3 Leucovorin rescue

Leucovorin, 15 mg/m^2 (IV or PO) for standard-/high-risk or 10 mg/m^2 (PO or IV) for low-risk cases, will be started at 42 hours after the start of methotrexate and repeated every 6 hours for a total of three doses, as described by Reiter, et al[30]. The dosage of leucovorin will be increased in patients 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 patients with 42-hour methotrexate levels >10μM. Patients 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, rather than 3 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.

5.3.4 IT chemotherapy

All patients will receive triple intrathecal therapy every other week for four doses on Days 1, 15, 29, and 43 (dosages are based upon age, according to Section 5.1). The intrathecal treatment should be given on the same day of the high dose methotrexate administration. Consult the PI or Pharmacokinetics if the IT and high dose methotrexate become separated by more than 12 hours.

5.3.5 Interim continuation treatment

Interim continuation treatment will be given to the occasional patients who, upon attaining complete remission, are deemed unable to tolerate high dose methotrexate. Specific criteria to use interim continuation therapy include disseminated fungal infection requiring systemic antifungal therapy, recent development of cerebral thrombosis, or grade 3 or 4 renal or hepatic dysfunction. There may be other unforeseen reasons that warrant temporary withholding of high dose methotrexate.

Interim treatment will consist of oral mercaptopurine 75 mg/m^2 per day (50 mg/m^2 per day in those with ANC<500) and intravenous methotrexate 40 mg/m^2 per week; intrathecal therapy may be given every other week during this period of time and continued during the subsequent high dose methotrexate treatment for a total of 4 doses. High dose methotrexate will be started when the patient's physical condition allows. In the event that the interim therapy is longer than 4 weeks, an extra intrathecal therapy may be given with the last course of high dose methotrexate. Patients with a defective TPMT status may receive lower doses of mercaptopurine.

5.4 Reintensification Treatment (for High-Risk ALL)

Patients with high-risk leukemia may receive reintensification therapy and then will be offered the option of transplant. This treatment will attempt to maximize leukemic cell kill before allogeneic hematopoietic stem cell transplantation (HSCT). For patients with Philadelphia chromosome positive ALL, ETP T-ALL, and those with induction failure or ≥1% leukemic lymphoblasts (determined by MRD study) in bone marrow at the end of remission induction, treatment will be given after consolidation therapy. However, consolidation therapy may be shortened, depending on patient's response to therapy and on the timing of transplantation. For patients with ≥0.1% leukemic lymphoblasts (determined by MRD study) in bone marrow in week 7 of continuation treatment, this treatment will be given after the reinduction I. Upon marrow recovery (i.e., ANC ≥300/mm^3, WBC ≥1000/mm^3 and platelet count ≥50 x 10^9/L) after each course of reintensification, bone marrow examination with MRD study will be repeated.

This treatment course may be repeated only once if the patient still has persistently positive MRD (i.e. ≥0.01% blasts). Allogeneic hematopoietic stem cell transplantation may proceed after 1 course of the treatment if MRD becomes negative with the first course of treatment; otherwise, transplant will be performed after two courses of treatment. Patients deemed unsuitable for the transplant or who decline the procedure or whose donor has yet to be identified, will remain on study and receive subsequent chemotherapy as scheduled.

The treatment scheme and dosage of chemotherapy are summarized below.

Agent	Dosage and route	# Doses	Schedule
Dexamethasone	20 mg/m^2/day PO or IV (divided t.i.d)	18	Days 1-6
Cytarabine	2 grams/m^2, 3-hour IV infusion every 12 hours	4	Days 1-2
Etoposide	100 mg/m^2, 1-hour IV infusion every 12 hours	5	Days 3-5
ITMHA	See Section 5.1 for age-specific dose	1	Day 5
PEG-asparaginase	3,000 units/m^2 IV	1	Day 6

Patients with suboptimal response to reintensification may receive one to two cycles of clofarabine/cyclophosphamide/etoposide/dexamethasone:

Agent	Dosage and route	# Doses	Schedule
Clofarabine	40 mg/m^2/day, 2-hour IV infusion	5	Days 1-5
Etoposide	100 mg/m^2/day, 2-hour IV infusion	5	Days 1-5
Cyclophosphamide	300 mg/m^2/day, 30-60 minute IV infusion	5	Days 1-5
Dexamethasone	8 mg/m^2/day (divided t.i.d)	15	Days 1-5

See Section 5.2.1.1 for dosing for infants.

This clofarabine/cyclophosphamide/etoposide/dexamethasone cycle can substitute for the second reintensification in patients with high risk proceeding to transplant. Patients who are eligible to NKHEM could instead be transferred to transplant to receive NKHEM followed by allogeneic HSCT.

5.5 Continuation Treatment (120 weeks)

Post-remission continuation treatment begins after the completion of consolidation, provided that the ANC ≥300/mm^3, WBC ≥1000/mm^3 and platelet count ≥50 x 10^9/L as well as no evidence of grade 3 or 4 mucositis. Continuation treatment (120 weeks) differs according to the risk classification, as follows (abbreviations as defined below).

Week	Standard-/High-Risk (‡)	Low-Risk
1	DEX+DOX+VCR+MP + PEG-ASP	MP + DEX + VCR
2	MP	MP + MTX
3	¥MP + PEG-ASP	¥MP + MTX
4	DEX + DOX + VCR + MP	MP + DEX + VCR
5	MP + PEG-ASP	MP + MTX
6	MP	MP + MTX
7	*†Reinduction I§	*Reinduction I
8	Reinduction I	Reinduction I
9	Reinduction I	Reinduction I
10	MP	MP + MTX
11	DOX + VCR +MP + PEG-ASP	MP + MTX
12	*MP	*MP + MTX
13	MP + PEG-ASP	MP + MTX
14	DEX + DOX + VCR +6MP	MP + DEX + VCR
15	MP + PEG-ASP	MP + MTX
16	MP	MP + MTX
17	*†Reinduction II	*Reinduction II
18	Reinduction II	Reinduction II
19	Reinduction II	Reinduction II
20	No chemotherapy	MP + MTX

¥Triple intrathecal treatment will be given cases with WBC ≥100 x 10^9/L, T-cell ALL, t(1;19)/E2A-PBX, presence of Philadelphia chromosome, MLL rearrangement, hypodiploidy <44, or CNS-3 status, with CNS-2 or traumatic lumbar puncture with blasts at diagnosis..

**IT MHA (methotrexate + hydrocortisone + cytarabine; see section 5.1 for dosage)*

†MRD study in bone marrow for patients with positive MRD at end of remission induction, and for patients with atypical T-lineage ALL. For other T-lineage patients peripheral blood can be used instead.

§Patients with MRD ≥0.1% at week 7 receive reintensification treatment after Reinduction I (see Section 5.4)

See Section 5.5.2 for details on Reinduction treatment.

Dexamethasone, vincristine and PEG-asparaginase can be given regardless of blood counts, provided that the patient is not sick. Methotrexate, mercaptopurine and doxorubicin will be held if ANC <300/mm^3, WBC <1000/mm^3, or platelet count <50 x 10^9/L (see section 8.0 for other dose modifications).

(‡)Continue dasatinib in Ph$^+$ cases

5.5.1.1 Drug dosages, schedules and routes for continuation therapy weeks 1 to 6 and 10 to 16

DEX (dexamethasone)	12 mg/m^2 (std/high risk) or 8 mg/m^2 (low risk) PO daily (divided t.i.d.) for 5 days, Days 1-5
DOX (doxorubicin)	30 mg/m^2 IV, Day 1
VCR (vincristine)	2 mg/m2 IV push (max. 2 mg), Day 1 (0.05 mg/kg for patients <1 year of age or <10kg in weight)
MP (mercaptopurine)	50 mg/m^2 PO daily at consistent time* for 7 days (std/high risk), Days 1-7 75 mg/m^2 PO daily at consistent time* for 7 days (low risk), Days 1-7
PEG-ASP (PEG-asparaginase) randomization,	2,500 vs. 3,500 units/m^2 IV Day 1
MTX (methotrexate)	40 mg/m^2 IV Day 1

See Section 5.2.1.1 for dosing for infants.

Dexamethasone, vincristine, and asparaginase will be given regardless of blood counts, provided that the patient is clinically well. Doxorubicin, mercaptopurine and methotrexate will be held if WBC <1000/mm^3 or ANC<300/mm^3. See Section 8.0 for further details. *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.

5.5.2 Reinduction treatment

This phase of treatment will be started at weeks 7 and 17 after bone marrow examination confirms complete remission. Doxorubicin and HD- cytarabine will be held if ANC <300/mm^3 or WBC < 1000/mm^3. It is preferable to start HD-cytarabine when WBC $\geq$1800/mm^3 and ANC $\geq$300/mm^3

Reinduction treatment will be given twice: weeks 7 to 9 and weeks 17 to 19 for all patients.

Intrathecal treatment will be followed by leucovorin rescue (5 mg/m^2/dose PO, max 5 mg) at 24 and 30 hours only in patients with prior CNS toxicities or in patients with WBC <1500/mm^3, or ANC <500/mm^3

5.5.2.1 Reinduction I for standard/high-risk ALL excluding *MLL*+ infants(‡)

Agents	Dosages and routes	# Doses	Schedules
Dexamethasone	8 mg/m^2/day PO (divided t.i.d.)	45	Days 1-8, 15-21
Vincristine	1.5 mg/m^2/week IV 0.05mg/kg for infants <1yr or <10kg (max 2 mg)	3	Days 1, 8, 15
Doxorubicin	30 mg/m^2 IV	2	Days 1, 8
PEG-asparaginase	2,500 or 3,500 units/m^2 IV	2	Days 1, 15
Methotrexate + hydrocortisone + Ara-C	Age-dependent, IT (section 5.1)	1	Day 1

5.5.2.2 Reinduction II for standard/high-risk ALL including *MLL*+ infants (‡)

Agents	Dosages and routes	# Doses	Schedules
Dexamethasone	8 mg/m^2/day PO (t.i.d.)	45	Days 1-8, 15-21
Vincristine	1.5 mg/m^2/week IV 0.05mg/kg for infants<1yr or <10 kg (max 2 mg)	3	Days 1, 8, 15
PEG-asparaginase	2,500 or 3,500 units/m^2 IV	2	Days 1, 15
Methotrexate + hydrocortisone + Ara-C	Age-dependent, IT	1	Day 1
High-dose cytarabine	2 gm/m^2 IV q 12 hr	4	Days 15, 16

(‡)*Ph+ cases continue dasatinib*

5.5.2.3 Reinduction I for low-risk ALL

Agents	Dosages and routes	# Doses	Schedules
Dexamethasone	8 mg/m^2/day PO (divided t.i.d.)	45	Days 1-8, 15-21
Vincristine	1.5 mg/m^2/week IV 0.05mg/kg for <1yr or <10 kg (max 2 mg)	3	Days 1, 8, 15
PEG-asparaginase	2,500 or 3,500 units/m^2 IV	2	Days 1, 15
Doxorubicin	30 mg/m^2/ IV	1	Day 1
Methotrexate + hydrocortisone + Ara-C	Age-dependent, IT	1	Day 1

5.5.2.4 Reinduction II for Low Risk ALL

Agents	Dosages and routes	# Doses	Schedules
Dexamethasone	8 mg/m^2/day PO (divided t.i.d.)	45	Days 1-8, 15-21
Vincristine	1.5 mg/m^2/week IV 0.05mg/kg for <1yr or < 10 kg (max 2 mg)	3	Days 1, 8, 15
PEG-asparaginase	2,500 or 3,500 units/m^2 IV	2	Days 1, 15
Methotrexate + hydrocortisone +Ara-C	Age-dependent, IT	1	Day 1

5.5.2.5 Reinduction I for *MLL*+ infants

Agents	Dosages and routes	# Doses	Schedules
Dexamethasone	8 mg/m^2/day PO (divided t.i.d.)	45	Days 1-8 and 15-21
Clofarabine	40 mg/m^2/day, 2-hour IV	5	Days 1-5
Etoposide	100 mg/m^2/day, 2-hour IV	5	Days 1-5
Cyclophosphamide	300 mg/m^2/day, 1-hour IV	5	Days 1-5
PEG-asparaginase	2,500 or 3,500 units/m^2 IV	2	Days 1, 15
Methotrexate + hydrocortisone +Ara-C	Age-dependent, IT	1	Days 1

For infants <1 month of age, or for infants <3 months of age born significantly prematurely, a 50% reduction in dosages of asparaginase, etoposide, cyclophosphamide, doxorubicin, and clofarabine should be made.

5.5.3 Intrathecal chemotherapy

- Triple intrathecal treatment will be given to low-risk cases with CNS-1 status (no identifiable blasts in CSF) on weeks 7, 12, 17, 25, 33, 41, and 49.
- Triple intrathecal treatment will be given to low-risk cases with CNS- 2, traumatic CSF with blasts status, or WBC ≥100 x 10^9/L at presentation on weeks 3, 7, 12, 17, 25, 29, 33, 37, 41, 45 and 49.
- Triple intrathecal treatment will be given to standard/high-risk cases on weeks 7, 12, 17, 25, 29, 33, 37, 41, 45 and 49.
- Triple intrathecal treatment will be given to other standard/high-risk cases with WBC ≥100 x 109/L at presentation, , T-cell ALL, t (1;19)/*E2A-PBX1*, presence of Philadelphia chromosome, *MLL* rearrangement, hypodiploidy <44, CNS-2 or CNS-3 status, or traumatic lumbar puncture with blasts on weeks 3, 7, 12, 17, 25, 29, 33, 37, 41, 45, 49, 57, 65, 73, 81, 89 and 97.

TOTXVI Intrathecal therapy

		Induction		Consolidation		Continuation Week 1-20		Continuation Weeks 21-97		All Phases
Risk-group	CNS Status	Days	Total	Days	Total	Weeks	Total	Weeks	Total	**Total**
Low-risk, WBC <100 x 10^9/L	CNS 1	1, 15	2	1, 15, 29, 43	4	7, 12, 17	3	25, 33, 41, 49	4	**13**
Low-risk, WBC ≥100 x 10^9/L	CNS 1	1, 8, 15, 22	4	1, 15, 29, 43	4	3, 7, 12, 17	4	25, 29, 33, 37, 41, 45, 49	7	**19**
Low-risk	CNS 2 or Traumatic Blasts	1, 4, 8, 11, 15, 22	6	1, 15, 29, 43	4	3, 7, 12, 17	4	25, 29, 33, 37, 41, 45, 49	7	**21**
Std-/high-risk WBC x ≥100 x 10^9/L, hypodiploidy, Ph +,or MLL+	CNS 1	1, 8, 15, 22	4	1, 15, 29, 43	4	3, 7, 12, 17	4	25, 29, 33, 37, 41, 45, 49, 57, 65, 73, 81, 89, 97	13	**25**
Std-/high-risk, other	CNS1	1, 15	2	1, 15, 29, 43	4	7, 12, 17	3	25, 29, 33, 37, 41, 45, 49	7	**16**
Std-/high-risk	CNS 2 or Traumatic Blasts	1, 4, 8, 11, 15, 22	6	1, 15, 29, 43	4	3, 7, 12, 17	4	25, 29, 33, 37, 41, 45, 49, 57, 65, 73, 81, 89, 97	13	**27**
T-cell t(1;19)/*E2A-PBX1*	CNS 1, CNS 2, or CNS 3	1, 4, 8, 11, 15, 22	6	1, 15, 29, 43	4	3, 7, 12, 17	4	25, 29, 33, 37, 41, 45, 49, 57, 65, 73, 81, 89, 97	13	**27**
Std-/high-risk	CNS3	1, 4, 8, 11, 15, 22	6	1, 15, 29, 43	4	3, 7, 12, 17	4	25, 29, 33, 37, 41, 45, 49, 57, 65, 73, 81, 89, 97	13	**27**

Thus, the total numbers of intrathecal treatments for various subgroups of patients are 13 (low-risk cases with WBC <100 x 10^9/L at presentation and CNS1 status), 19 (low-risk cases with WBC ≥100 x 10^9/L at presentation), 21 (low-risk cases with CNS2 status or traumatic CSF with blast status), 16 (std-/high-risk cases with CNS1 status), 25 (std-/high-risk cases with WBC ≥100 x 10^9/L at presentation, presence of Philadelphia chromosome, MLL rearrangement, or hypodiploidy < 44), 27 (std-/high-risk cases with T-cell phenotype, t(1;19)/*E2A- PBX1,* or CNS-2 or CNS-3 status, or traumatic lumbar puncture with blast). The number of intrathecal treatments for high risk patients will depend on whether and when the patients will receive stem cell transplantation. Leucovorin will not be given after intrathecal treatment during continuation treatment unless the patient has an adverse reaction with previous intrathecal or methotrexate treatment, e.g., seizure or encephalopathy, has renal dysfunction resulting in high plasma methotrexate concentration, or is Down syndrome and has unacceptable toxicity despite dosage decreases. Leucovorin may be given when patient is neutropenic, at treating physician's discretion. Down syndrome patients will receive leucovorin with every LPIT. While intrathecal treatments may be given at SJCRH affiliate institutions, all patients must have intrathecal treatment given at St. Jude at least every 16 weeks, because close monitoring of cerebrospinal fluid is an essential component of therapy.

5.5.4 Treatment (weeks 21 to 29)

Week	Standard/High Risk	Low Risk
21	MP + PEG-ASP+‡Dasatinib	MP + MTX
22	MP +‡Dasatinib	MP + MTX
23	MP + PEG-ASP+‡Dasatinib	MP + MTX
24	Cyclo + Ara-C+‡Dasatinib	MP + MTX
25	*DEX + VCR+ PEG-ASP+‡Dasatinib	*MP + DEX + VCR
26	MP+‡Dasatinib	MP + MTX
27	MP + PEG-ASP+‡Dasatinib	MP + MTX
28	Cyclo + Ara-C+‡Dasatinib	MP + MTX
29	*DEX + VCR + PEG-ASP+‡Dasatinib	(*)MP + DEX + VCR

**IT MHA (methotrexate + hydrocortisone + cytarabine; see section 5.1 for dosage) (*)IT MHA for low-risk cases with WBC ≥100 x 10^9/L , CNS-2 or traumatic lumbar puncture with blast.*
‡Only for Ph+ patients
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.

5.5.5 Treatment (weeks 30 to end of therapy)

Week	Standard/High Risk	Low Risk
30	MP + MTX+‡Dasatinib	MP + MTX
31	MP + MTX+‡Dasatinib	MP + MTX
32	Cyclo + Ara-C+‡Dasatinib	MP + MTX
33	*DEX + VCR+‡Dasatinib	*MP + DEX + VCR
34	MP + MTX+‡Dasatinib	MP + MTX
35	MP + MTX+‡Dasatinib	MP + MTX
36	Cyclo + Ara-C+‡Dasatinib	MP + MTX
37	*DEX + VCR+‡Dasatinib	(*)MP + DEX + VCR

**IT MHA (methotrexate + hydrocortisone + cytarabine; see section 5.1 for dosage) (*)IT MHA for low-risk cases with WBC ≥100 x 10^9/L, CNS-2 or traumatic lumbar puncture with blast.*
‡Only for Ph+ patients.
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.

5.5.5.1 Drug dosages, schedules and routes for continuation therapy from week 21 to end of therapy

MP (mercaptopurine)	75 mg/m^2 PO daily for 7 days, Days 1-7
MTX (methotrexate)	40 mg/m^2 IV or IM, Day 1
Cyclo (Cyclophosphamide)	300 mg/m^2 IV, Day 1
Ara-C (Cytarabine)	300 mg/m^2 IV, Day 1
DEX (dexamethasone)	12 mg/m^2 (std/high risk) or 8 mg/m^2 (low risk) PO daily (divided t.i.d.) for 5 days, Day 1-5 between week 21 and week 68 6 mg/m^2 PO daily (divided t.i.d.) x 5 days, Day 1-5 between week 69 and week 101 for all risk groups
VCR (vincristine)	2 mg/m^2 IV push (max 2 mg), Day 1 (0.05mg/kg for patients <1yr or < 10 kg)
PEG-ASP	2,500 vs. 3,500 units/m^2 IV randomization (until week 30)

Dexamethasone, vincristine, and asparaginase will be given regardless of blood counts, provided that the patient is clinically well. Cyclophosphamide, cytarabine, mercaptopurine and methotrexate will be held if WBC <1000/mm^3, platelet count to <50 x10^9/L, or ANC <300/mm^3 (see Section 8.0 for further details). 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.

Mercaptopurine and methotrexate will be reduced for if WBC and ANC do not increase by at least 2 folds a week after the start date of dexamethasone pulse. Exceptions can be made in patients with WBC and ANC that does not double, but is over 3000 and 1200, respectively, with no history of prior chemotherapy interruption or myelosuppression (see Section 8.0 for further details).

Doses of cyclophosphamide and cytarabine may need to be reduced if patient misses 25% of chemotherapy and if the low counts deem to be related to this combination.

The same treatment (weeks 30-37) will be repeated for a total of 5 times, until week 69 (see Section 5.5.3 for intrathecal therapy). After week 69, all patients will receive daily mercaptopurine and weekly methotrexate interrupted with pulses of dexamethasone, vincristine, and mercaptopurine every 4 weeks.

The dose of dexamethasone will be decreased to 6 mg/m^2 between week 69 and week 101, after which only mercaptopurine and methotrexate will be given. Intrathecal treatment will be given every 8 weeks only to patients at risk of CNS relapse after week 49 and will be discontinued after week 97. Continuation therapy will be discontinued after 120 weeks.

5.6 Hematopoietic Stem Cell Transplantation

Patients who meet the criteria of high-risk ALL are candidates for allogeneic hematopoietic stem cell transplantation. However, if the option is declined by the patients or guardians, or the procedure is deemed unsuitable by the attending physician and the principal investigator, the patient will remain on study and continue to receive chemotherapy.

6.0 DRUG INFORMATION

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

6.1 Drug Shortages and Unavailability

In the case of drug shortages and unavailability of any agent used in this protocol, treating investigators are urged to consult with the PI or co-PI and 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. Most importantly, 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.

7.0 TREATMENT MODIFICATIONS

7.1 Down Syndrome Participants

Participants with Down syndrome are eligible for enrollment, with the following modifications.

7.1.1 HDMTX: Dosages of high-dose MTX will be modified due to their well-documented altered pharmacokinetics and enhanced tissue sensitivity to MTX's effects. Their hydration and alkalinization regimen should be the same as outlined in Section 5.3.3. However, the dose of HDMTX is 500 mg/m^2 (50 mg/m^2 over 1 hour and 450 mg/m^2 given over 23 hours).

The baseline leucovorin rescue will begin early (at hour 30 at 30 mg/m^2 IV q 6 hours x 2 doses, followed by 10 mg/m^2 IV q6 hours x 6 doses). If MTX plasma levels are elevated, increased leucovorin rescue will be recommended by the Pharmaceutical Department. Vigorous hydration should be assured until the 42 hour MTX level is known.

7.1.2 Continuation low-dose MTX: The low-dose weekly MTX (40 mg/m^2) dosage 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) following the dose of 40 mg/m^2, the dosage should be decreased to 30 mg/m^2. If that dosage is similarly not tolerated, then the dosage may be further decreased 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 hour for 4 doses starting 42 hours from the MTX dosage, with titration to acceptable toxicity.

7.1.3 Intrathecal therapy: Intrathecal treatment should be administered as outlined in Section 5.1.

7.1.4 PEG-asparaginase: Patients with Down's syndrome will not be randomized, and will all receive 2,500 units/m^2 PEG-asparaginase post remission induction.

7.1.5 Down syndrome patients should be closely monitored. Dose reduction (30% to 50%) should be applied as clinically indicated (especially to dexamethasone and high-dose cytarabine for the few patients who would be treated on the standard arm, and who are noted to have higher than expected toxicity in earlier phases)

7.2 Renal Dysfunction

Subclinical renal impairment (normal serum creatinine but decreased GFR) may be present in patients receiving concurrent nephrotoxic drugs (e.g. IV acyclovir) 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.

7.3 Hepatic Dysfunction

Anthracyclines and vincristine dosages should be modified in patients with elevated direct bilirubin concentrations or other evidence of biliary obstruction. (More conservative criteria will be used for anthracycline treatment during initial remission induction, See Section 5.2.)

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

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

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 not for other chemotherapy.

7.4 Obesity

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

7.5 Testicular Leukemia at Diagnosis

Overt testicular leukemia occurs in 2% of boys at diagnosis, generally in infants or adolescents with hyperleukocytosis. Ultrasonogram should be performed to differentiate testicular leukemia from hydrocele and to measure the testicular volume. Overt testicular leukemia at diagnosis per se is not an indication for testicular irradiation, as many patients can be successfully treated with chemotherapy, including high-dose methotrexate. Ultrasonogram should again be performed upon completion of remission induction. If testicular size is still abnormally enlarged, the sonogram should be repeated after consolidation treatment with high-dose methotrexate and mercaptopurine. Persistently enlarged testes after consolidation treatment will be biopsied. Testicular irradiation (24 Gy) will be administered in the rare patients with positive biopsies, after consultation with a radiation oncologist.

7.6 Vincristine Neurotoxicity

The maximum single dose of vincristine must not exceed 2 mg. Mild vincristine toxicities (jaw pain, constipation, and decreased deep tendon reflexes) are anticipated. Loss of voice due to vocal cord paralysis may be a complication of vincristine toxicity but it must be differentiated from pharyngitis or Candida infection of the cord. If persistent, severe abdominal cramps, gait impairment, severe pain (requiring narcotic treatment), or SIADH develop, the dose may be reduced to 1 mg/m^2. Only motor paralysis or typhlitis warrants withholding vincristine.

7.7 Venous Thromboembolism

Patients who develop cerebral or other venous thrombosis will receive low molecular weight heparin throughout treatment with Asparaginase (during Reinduction I and Reinduction II in low-risk patients, during the first 30 weeks of continuation treatment in standard-/high-risk cases, and during reintensification I in high-risk patients). Acute management of thrombosis will follow the St. Jude guidelines on the use of thrombolysis and anticoagulation; questions should be referred to the PI or co-PI.

7.8 Avascular Necrosis of Bone

MRI exams will be interpreted by the radiologist. If there is evidence of epiphyseal or metaphyseal hip lesions, knee epiphyseal or metaphyseal lesions, or lesions of talus consistent with avascular necrosis of the bone, the patient will be referred to the orthopedic surgeon, who will evaluate symptoms, and will assess the severity and estimated risk of progression. Physical therapy, activity modifications, and surgical procedures will be recommended as needed. Patients with hip epiphyseal lesions or talus lesions affecting > 30% of weight-bearing area will have an X-ray of the affected area, and will be assessed at higher risk for progression. Symptomatic patients with such findings will likely have their dexamethasone stopped, especially if they are past reinduction II in therapy.

Asymptomatic patients with such findings will likely have their dexamethasone dose halved, especially if they are past reinduction II in therapy. Any patients with X-ray findings of AVN are candidates for dexamethasone modification, regardless of symptoms. All modifications (or lack thereof) of dosage will be recorded in the research database. Patients with progression of any lesions or with worsening symptoms will be re-evaluated by imaging and, if appropriate, by additional orthopedic follow-up. If the dexamethasone is discontinued, the, first choice will be to replace each week's dosing with one dose of methotrexate (40 mg/m^2), if tolerated. See Section 13.4 for additional guidelines.

7.9 PEG-Asparaginase

Patients with allergic reactions or intolerance to PEG-asparaginase subsequently will be given *Erwinia* L-asparaginase intramuscularly or intravenously over 30-60 minutes duration. If allergy occurs with the first PEG dose and the patient requires a day 15 dose during remission induction, *Erwinia* L-asparaginase will be given at 20,000 units/m^2/dose

thrice weekly for 2 weeks (total of 6 doses). After consolidation, in patients randomized to 2,500 units/m^2, each dose of PEG will be replaced by Erwinia at 30,000 units/m^2/dose twice weekly (3 to 4 days apart) for 4 doses. In patients randomized to 3,500 units/m^2 or 3,000 units/m^2, each dose of PEG will be replaced by Erwinia at 42,000 units/m^2/dose or 36,000 units/m^2/dose, respectively, twice weekly (3 to 4 days apart) for 4 doses. During Reintensification, Erwinia will be given 36,000 units/m^2/dose, twice weekly (3 to 4 days apart) for 2 doses. Native E. coli asparaginase can also be given when clinically indicated, but this should be discussed and approved by the PI or co-PI.

Asparaginase treatment should be delayed until at least 2 hours after intrathecal treatment.

If significant increase in toxicity is noted in the arm randomized to 3,500 units/m^2 PEG-asparaginase compared to the conventional dose arm, PEG-asparaginase dose will be reduced to 3,000 units/m^2. If significant increase in toxicity is noted with the reduced dose, randomization will cease, and all patients will be treated at the conventional dose of 2,500 units/m^2 (see section 17.0 for details and statistical analysis).

Patients who are intolerant to all asparaginase formulations can have asparaginase substituted with methotrexate 40 mg/m2 IV on weeks 21, 22, 23, 26, and 27.

7.10 TPMT Status and Thiopurine Dosage

TPMT defects have been linked to acute myelosuppression and to the long-term risk of therapy-related AML (t-AML), even in the context of ALL therapy consisting primarily of antimetabolites. Studies reporting an association of TPMT defects with risk of t-AML incorporated mercaptopurine at a dosage of 75 mg/m^2/day, whereas many ALL treatment groups use a starting dosage of 60 mg/m^2/day and then titrate up. Although the mechanism is not known, patients with TPMT defects have higher concentrations of thioguanine nucleotides, and substitution of thioguanine for guanine in DNA can affect topoisomerase II- induced cleavage of DNA in the presence and absence of a topoisomerase II inhibitor. Together, the clinical and preclinical evidence suggest that the 10% of patients who carry a variant TPMT allele may be at higher risk for secondary tumors. Because such patients often require dosage decreases of thiopurines, and because (in a setting in which dosage was adjusted in about 1/3 of patients with TPMT defects) overall leukemia-free survival was outstanding among patients with TPMT defects, we are recommending that patients with phenotype or genotype consistent with at least one variant TPMT allele receive no more than 60 mg/m^2/day of mercaptopurine or thioguanine unless it is clearly documented that compliance has been good and metabolite levels remain low (< 100 pmol/^{8} x 108 red cells) (see sections 8.1 and 8.2). Further changes in mercaptopurine dose should be titrated based on WBC and ANC. Every effort should be made to keep other anticancer agent doses at protocol levels for these patients. A blood sample (5 ml) will be drawn along with routine lab work at Day 3 of remission induction to allow for timely TPMT genotyping.

Assessing TPMT status: TPMT genotype will be determined by allele-specific genotyping, directed against the 3 most common inactivating polymorphisms at positions 238, 460, and 719 of the cDNA. These 3 polymorphisms account for >90% of all variant alleles.

Genotyping will be evaluated to assign patients as having homozygous wild-type, heterozygous, or homozygous variant TPMT status. As specified in section 8.2, all patients also have 6TGN thiopurine metabolite measures at the start of reinduction I and TPMT activity in RBCs assessed at least by week 17. The threshold for on-therapy, non-transfused TPMT activity that is considered the upper bounds for heterozygous status (14 units/ml) is higher than that for off-therapy, non-transfused patients (10 units/ml). The ratio of methyl thiopurine metabolites to TGNs can also be used to assist in phenotyping the patient if genotype and activity phenotype data are conflicting. If TPMT genotype or activity or metabolite measures are consistent with heterozygous status, the patient will be considered heterozygote; using these criteria, approximately 10% of patients are expected to be heterozygote or homozygous variants, in agreement with population studies for TPMT status.

Thus, approximately 10% of patients are expected to require substitution of mercaptopurine for thioguanine on the basis of TPMT status. See Section 8.2 for additional information.

7.11 Pancreatitis

Acute hemorrhagic pancreatitis is a contraindication to continue asparaginase treatment. In the case of mild to moderate pancreatitis, asparaginase should be held until symptoms and signs subside, and amylase and lipase levels return to normal and then resumed. Any patients 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 level three times or more of the upper limit of normal, and sonographic or CT scan evidence of pancreatitis), 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), asparaginase should be held and resumed once symptoms and signs subsided. Call the PI or co-PI to discuss the management if the patient is asymptomatic (without abdominal pain) and has only elevated amylase or lipase levels. Consideration should also be given to dexamethasone- or mercaptopurine- related pancreatitis. Contact the PI or co- PI to discuss the management if there is a possibility that the pancreatitis is due to either of these two drugs.

7.12 Philadelphia Chromosome Positive (Ph+) ALL

Patients with Ph+ ALL will receive dasatinib (40 mg/m^2 b.i.d.), starting on day 22 of induction chemotherapy. Patients may receive 80 mg/m^2 daily if better tolerated. This is equivalent to the standard adult dosage of 70 mg b.i.d., and is equal to the proposed dasatinib dose in the upcoming frontline COG regimen for Ph+ ALL. If pleural effusion occurs, or if dasatinib induced myelosuppression (or other toxicity) interferes with the ability to administer it continuously with chemotherapy, the dasatinib dose will be decreased by 25% to 60 mg/m^2 daily which is equivalent to the 100 mg daily dose that has been associated with equal efficacy and better tolerance in adults[111;121]. If myelosuppression results in therapy interruption at the reduced dasatinib dose of 60 mg/m^2 daily, dasatinib will be replaced with imatinib (340 mg/m^2 daily as given in TOTXV).

8.0 DOSE MODIFICATIONS DURING CONTINUATION THERAPY

Dosage of continuation treatment should be titrated to keep WBC between 1800 and 3000/mm^3, ANC between 500 and 1200/mm^3 (with the exception of the count one week after dexamethasone treatment), and platelet count ≥50 x 10^9/L.

Full dose of treatment will be administered when WBC ≥1500/mm^3, ANC is ≥300/mm^3 and platelet count ≥50 x 10^9/L; except when dose reduction is clinically indicated. If patient is clinically well and ANC <300/mm^3, full dose of treatment can be administered if WBC ≥1500/mm^3, platelet count ≥50 x 10^9/L, and APC ≥ 500/mm^3.

Doxorubicin, cyclophosphamide, cytarabine, methotrexate and mercaptopurine may be reduced by 30 to 50% if WBC is between 1000 and 1500/mm^3 with ANC ≥300/mm^3 and platelet count ≥50 x 10^9/L. Disproportionate dose reduction of one agent compared to another should be avoided unless clinically indicated. Frequent (e.g., weekly) changes of mercaptopurine doses should be avoided. Patients who receive mercaptopurine at a reduced dose for longer than 2 weeks should have a TGN level checked after at least 14 days of uninterrupted mercaptopurine if the plan is to continue at reduced doses.

Mercaptopurine and methotrexate will be reduced if WBC and ANC do not increase by at least 2 folds a week after the start date of dexamethasone pulse. Exception can be made in patients whose WBC and ANC do not double but are over 3000 and 1200 respectively with no history of prior chemotherapy interruption or myelosuppression. In Standard Risk patients consider reducing cyclophosphamide and cytarabine dose if suspected to contribute to myelosuppression. Dexamethasone, vincristine, and asparaginase will be given regardless of blood counts, provided that the patient is clinically well. On the weeks of vincristine and dexamethasone pulses, mercaptopurine may be reduced by 30% to 50% in patients with WBC ≤1500/mm^3 the week after prior dexamethasone pulse. In Standard Risk patients consider reducing cyclophosphamide and cytarabine dose if suspected to contribute to myelosuppression. Adjustments of dosages should be made in the following circumstances, with re-evaluation of tolerance and toxicities every 8 to 16 weeks (See section 8.3 for dasatinib modification).

8.1 Dose Modifications for Inadequate Myelosuppression

Patients who miss less than 25% of therapy but have persistently (>50% of time; not counting the week after dexamethasone/vincristine) high WBC (≥3 x 10^9/L) and high ANC (≥1000/mm^3) should be counseled on compliance, particularly if 6TGN levels are <100 pmol/8 x 10^8 RBCs. If the WBC remains high, mercaptopurine and methotrexate dosages should be increased by 30% (e.g., to 100 mg/m^2 and 50 mg/m^2, respectively), using a stepwise approach if needed. If patients have a TPMT defect, mercaptopurine dosage should not be increased unless the TGN levels are < 100 pmol/8 x 10^8 RBCs.

8.2 Dose Decreases Based on Mercaptopurine Pharmacology

Low risk and standard/high risk

All patients on both arms will have 6TGN measured at the start of reinduction I (i.e., week 7). TPMT will be measured at the start of reinduction II (week 17) or earlier if TPMT defects are suspected. TPMT genotype may be used to identify TPMT variant alleles. 6TGN and TPMT will be measured subsequently in patients with high 6TGN level, suspected noncompliance, problems with toxicities or high blood counts.

Patients missing ≥25% of therapy who have 6TGN levels >1000 pmol/8 x 10^8 RBCs will have mercaptopurine dosage reduced to achieve a steady-state 6TGN level between 200 to 1000; those with 6TGN level <1000 will have both mercaptopurine and methotrexate dosages reduced by 30%.

Dosages will be re-evaluated every 8 to 16 weeks. Other causes of low blood counts should also be considered (see Section 13.5).

Patients missing less than 25% of therapy and with WBC <3 x 10^9/L and ANC <1000 do not need any change in dosage, regardless of 6TGN level. See section 7.10 for mercaptopurine dosage adjustments.

8.3 Dasatinib Dosage Adjustments (Ph+ ALL Patients Only)

Consider holding dasatinib for grade III-IV non-hematological toxicity until resolution to grade 1 or less. Hold dasatinib for pleural effusion. May consider resuming at reduced (60 mg/m^2 daily). Dose can be escalated back to 40 mg/m^2 b.i.d. if well tolerated. Patients may receive 80 mg/m^2 daily if better tolerated. Consider replacing with imatinib 340 mg/m^2 daily (as given on TXV) if toxicity persists at reduced dosage. All modifications should be discussed with PI or co-PI.

Consider reducing dasatinib dose to 60 mg/m^2 daily if myelosuppression interferes with the ability to administer therapy without interruption. Dose can be escalated back to 40 mg/m^2 BID if tolerated. Patients may receive 80 mg/m^2 daily if better tolerated. Consider replacing with imatinib 340 mg/m^2 (as given on TXV) if interruptions persist at reduced dosage. All modifications should be discussed with the Principal Investigator.

Consider holding dasatinib during periods of myelosuppression, until ANC ≥300/mm^3 and platelet count ≥50 x 10^9/L.

Avoid concurrent use of drugs that are generally accepted to have a risk of causing Torsades de Pointes (including: erythromycin, pseudoephedrine), and drugs known to be interfere with CYP3A4.

9.0 CONTINGENCY PLANS FOR REFRACTORY DISEASE OR RELAPSE

9.1 Induction Failures

Patients who do not attain complete remission (≥5% leukemic blasts in bone marrow) after remission induction, consolidation treatment and reintensification treatment will be removed from the protocol. Those who do not achieve a remission after induction therapy, but subsequently attain complete remission after consolidation or reintensification treatment, are candidates for allogeneic hematopoietic stem cell transplantation.

9.2 Hematologic Relapse

Patients with ≥25% lymphoblasts in marrow aspirate will become eligible for relapse protocols.

9.3 Extramedullary Relapse

Patients with any form of extramedullary relapse (testes, ovarian, etc.) except that of CNS will become eligible for relapse protocols. Patients with overt CNS relapse (i.e. ≥5 WBC/μL of CSF with blasts) will remain on study and receive treatment outlined in Section 9.4. Patients who have <5 WBC/μL of CSF with identifiable blasts are not considered to have overt CNS relapse and will be treated as outlined in Section 9.4.

9.4 Emergence of CSF Lymphoblasts during Remission Requiring CNS Radiation

Preventive cranial irradiation will not be given prophylactically to patients with CNS leukemia at diagnosis or to those with high-risk leukemia. Only patients with immunologically proven leukemic lymphoblasts in CSF (regardless of cell count) during hematologic remission on two occasions in the study will receive therapeutic CNS irradiation in consultation with radiotherapists and after receiving a second remission induction (as in Section 5.2) followed by 1-2 cycles of reintensification (as in Section 5.4) to consolidate bone marrow remission after induction. Whether 1 or 2 cycles are to be given will depend on risk group, time to emergence of blasts in CSF, immunophenotype of blasts, and individual patient tolerance. In general, standard/high risk participants or those with early occurrence (<18 months) will receive 2 cycles. This should be discussed with the principal investigator. Triple intrathecal therapy will be continued every 3 to 4 weeks.

CNS irradiation for patients with CNS relapse is indicated as follows: cranial irradiation (24 Gy in 16 fractions) for patients with <5 WBC/μL of CSF occurring within the first 18 months of remission; cranial irradiation (18 Gy in 12 fractions) for those with any number of leukemic lymphoblasts in CSF after 18 months of initial remission; and craniospinal irradiation (24 Gy cranial irradiation in 16 fractions plus 15 Gy spinal irradiation in 10 fractions) for patients with ≥5 WBC/μL of CSF occurring within the first 18 months of remission. Those patients receiving cranial irradiation only should 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. Continuation treatment will be given for at least one year from time of relapse (at least 2½ years including initial treatment).

10.0 PHARMACOKINETIC/PHARMACODYNAMIC STUDIES

10.1 High-Dose Methotrexate (HDMTX)

2-3 ml of blood will be obtained in EDTA tubes (purple top) pre-dose and between 0.5 and 6 hr, 23 hr, and 42 hr from the start of the infusion. Additional samples will be obtained in patients in whom there is clinical suspicion of poor clearance, or in those who have high plasma MTX concentration (e.g., >0.5 μM at 42 hours), to adjust leucovorin rescue. (Note: blood should be in purple top tube to facilitated nucleic acid recovery from the buffy coat).

10.2 Thiopurines

Blood (3 ml) will be obtained at week 7 and at week 17 for red blood cell thiopurine concentrations and TPMT activity, respectively. Additional samples may be obtained as indicated in Section 8.2.

10.3 Asparaginase, Dexamethasone and Biomarkers

Serum for antibodies (2 ml blood in red-top tube) will be obtained on Days 1, 8, and 15 of remission induction therapy, day 1 of consolidation, and at Day 1 of weeks 7, 8, 9, 17, and 19 according to our previously established methods. Antibodies against native E.Coli (Elspar), PEGylated E.Coli (Oncaspar), and Erwinia asparaginase are measured by an ELISA in our laboratory. Those with sagittal sinus thrombosis or any deep vein thrombosis will also have measurement of anti-asparaginase antibodies at the time of thrombosis.

Plasma for asparaginase (3 ml blood) will be obtained on Days 1, 8, and 15 of remission induction therapy (plus ~2-3 additional time points during the first 14 days), day 1 of consolidation, and at Day 1 of weeks 7, 8, 9, 17, and 19 according to our previously established methods. Asparaginase will be measured using a spectrophotometric assay based on conversion of asparagine to aspartic acid[122].

In patients with a suspected clinical allergic reaction to a dose of asparaginase, blood samples for research purposes (up to 5 samples over up to 2 weeks, nor more than 2 samples in any 24-hour time period; each to be no more than 3 ml of blood in purple-top EDTA or red-top tube may be collected if the patient has functioning IV access.

Plasma for measurement of dexamethasone concentrations (3 ml in green top tube) will be obtained on Day 8 of Reinductions I and II (Day 8 of weeks 7 and 17).

CSF from therapeutic lumbar punctures will be collected at SJCRH and placed into sulfosalicylic acid-containing tubes prior to induction therapy (with ITMHA) and on Days 8 (if applicable) and 15 of remission induction therapy, and on Day 1 of consolidation and day 1 of week 7 of Reinduction I. CSF asparagine levels will be measured according to

our previously established methods. An additional blood sample (3 ml green top tube) will be obtained on the day of diagnosis of sagittal sinus thrombosis or any deep vein thrombosis for measurement of asparaginase concentration.

Serum (3 ml blood in red-top tube) will be obtained at day 15 of induction, day 15 of consolidation, and at weeks 7, 8, and 17 of continuation for steroid/asparaginase biomarkers (e.g. serum lipids, cortisol).

10.4 Plasma Homocysteine

A 2 mL sample of blood will be placed in EDTA (purple-top) tubes collected pre-infusion and, 6, 23 and 42 hours post the first and second courses of HDMTX during consolidation. Samples will be sent to the Clinical Pharmacokinetics Lab and kept at 4°C until centrifugation. After centrifugation, samples will be frozen and assayed subsequently for homocysteine.

11.0 EVALUATIONS, TESTS, AND OBSERVATIONS

11.1 Pretreatment Evaluation

All participants should be invited to participate in the Tissue Banking Protocol (TBANK) at the time of study entry.

- Complete history and physical exam with careful notation and assessment of clinical signs relevant to leukemia.
- Complete blood count with differential
- Chemistry profile: glucose, electrolytes, BUN, creatinine, LDH, uric acid, bilirubin, SGOT, SGPT, calcium, phosphorous, magnesium, total protein and albumin.
- Lipid screen: Total cholesterol, triglycerides, free fatty acids and high-density lipoprotein cholesterol
- Plasma 1, 25-dihydroxyvitamin D, osteocalcin
- Thyroid function tests (free T4, T4, TSH)
- Chest x-ray
- Bone marrow evaluation: morphology, cytochemistry, immunophenotyping, cytogenetics, DNA index, molecular diagnosis, MRD studies
- Lumbar puncture with CSF examination (cell count with differential of cytospin preparation)
- NK cell receptor studies, except in patients receiving steroids.
- Other studies as clinically indicated, e.g. sickle cell prep, hemoglobin electrophoresis and G6PD screen for black children; varicella titer; Hepatitis B antigen; HIV, EBV, TOXO, CMV titers.

11.2 Data to be Obtained Serially While Onstudy

Test/evaluation	At diagnosis	Induction/Consolidation	Continuation	Reinduction I & II
Physical Exam	At diagnosis	q3-7 days	q4 week	q3-7 day
CBC with differential	At diagnosis	weekly	weekly	weekly
Coagulation screen	At diagnosis	---	---	---
Uric acid, electrolytes, calcium phosphorous, magnesium	At diagnosis	Before each HDMTX	As indicated	Day 1 (Weeks 7 & 17)
Serum glucose, urinalysis	At diagnosis	As indicated	As indicated	As indicated
Bilirubin, SGOT, SGPT, total protein, albumin, LDH, BUN, creatinine	At diagnosis	Before each HDMTX	As indicated	Day 1 of Weeks 7, 8, 9, 17, and 19
MRD* (See tables below)	At diagnosis	Day 8, Day 15 (± day 22)*, and End of induction	(Week 49)* Week 120	(Day 1 Weeks 7 & 17)*
Total cholesterol, triglycerides, free fatty acids, and high-density lipoprotein cholesterol	At Diagnosis	- - -	After completion of therapy (Week 120 or later)	Day 1 of each Re-Induction (Day 1 of Weeks 7 and 17)
CSF studies	At diagnosis (with first intrathecal treatment)	With each intrathecal treatment	With each intrathecal treatment; then ≈q16wk	With each Intrathecal treatment
Chest x-ray	At diagnosis	End of induction in cases with mediastinal mass at diagnosis, then as indicated. Before each HDMTX in patients on dasatinib and as indicated	As indicated	As indicated
MRI of hips and knees (≥ 9 year-old only)	- - -	- - -	Off therapy	After each reinduction phase (Weeks 12-14 and 22-24)
PT/OT Evaluation (≥ 9 year-old only)	- - -	- - -	Off therapy	After each Re-induction

Test/evaluation	At diagnosis	Induction/Consolidation	Continuation	Reinduction I & II
Germline DNA/RNA	- - -	Day 3 and during consolidation (with blood for HDMTX)	- - -	- - -
Psychology****		Week 7 Continuation (Week 1 Reinduction I)	Week 120	Two years post therapy
MRI Brain**		Week 7 Continuation (Week 1 Reinduction I)	Week 120	Two years post therapy***
Functional MRI*****			Week 120	
RBC TPMT and TGN	- - -	Day 3 (TPMT)	- - -	Day 1 of week 7 (TGN) and week 17(TPMT)
CYP2D6 genotyping		Day 15		
Plasma Homocysteine		Pre and 6, 23, 42 hours post HDMTX #1 and #2. Section 10.4		
MTX Pharmacokinetics	- - -	Each HDMTX	- - -	
asparaginase antibodies	Day 1	Days 1, 8, 15, and Day 1 of consolidation	- - -	Day 1 (weeks 7, 8, 9, 17, 19)
CSF asparagine	With first intrathecal	Days 8, 15 induction and week 1 consolidation	- - -	Week 7 (Day 1)
Serum biomarkers	- - -	Day 15 of induction and consolidation	- - -	weeks 7, 8, 17
Asparaginase activity	Day 1	Days 1, 8, and 15 plus 2-3 time points over the first 14 days of induction; Day 1 consolidation	- - -	Day 1 (weeks 7, 8, 9, 17, 19
Dexamethasone activity	Day 8	Day 8 during weeks 7 & 17		
QCT for bone density	Week 1	- - -	Weeks 120	- - -
Urinalysis	As needed	As needed	As needed	As needed
Thyroid function	At diagnosis	- - -	As clinically indicated	- - -
Ammonia level	- - -	Induction: Day 1, 5, 8, 15, and 22 Consolidation: Day 1	SR/HR participants only: Day 1 (Weeks 1, 13, 23, & 29)	Day 1: (Weeks 7, 8, 9, 17, 18, & 19)
NK Cell Receptor	At diagnosis	- - -	- - -	Week 7 (Day 1)
Stool samples for Microbiome research		1) pre-chemo 2) within 2 weeks completion Induction. See section 16.7		Day 1 (weeks 7 & 17)

**See tables below for samples required for MRD studies*
*** MR should be obtained before starting dexamethasone.*
****Patients with abnormal imaging evaluations at week 120 will have an extra exam at 2 years off therapy to reassess brain structure.*
*****Neuropsychological testing should be performed prior to initiation of dexamethasone on day 1 of Reinduction I. Testing may be completed up to 4 weeks prior to day 1 of Reinduction I to accommodate a patient's travel and treatment schedule. If unable to schedule prior to dexamethasone, contact Dr. Conklin for scheduling.*
******Functional MRI will be performed only on patients who qualify for this exam. For a list of eligibility requirements, see Section 12.0.*
*******NK Cell Receptor studies will not be done in patients receiving steroids*

Samples required for MRD studies during remission induction

Time point	B-lineage ALL	T-lineage ALL
Day 8	PB	PB
Day 15	BM	BM and PB
(Day 22 if MRD^+ on Day 15)	BM	BM and PB
Day 43	BM	BM and PB

PB=peripheral blood; BM=bone marrow

Samples required for MRD studies post-remission induction

Time point	B-lineage ALL		T-lineage ALL	
	Day 43 MRD+	Day 43 MRD-	Day 43 MRD+ (or "atypical" T-ALL)	Day 43 MRD-
Day 1 (Weeks 7, 17, 49)	BM	None unless clinically indicated, or MLL+	BM and PB	PB
Week 120 (off therapy)	BM	BM	BM and PB	BM and PB

PB=peripheral blood; BM=bone marrow

11.3 Off-Therapy Evaluations

It is recommended that patients 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-study.

Patients 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 deemed related to therapy by the site PI. After attaining continuous, complete remission for 6 years or more, SJCRH patients 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). 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.

12.0 EVALUATION CRITERIA

12.1 Response Criteria

12.1.1 Complete remission: M1 marrow status with restoration of normal hematopoiesis and normal performance status.

12.1.2 Induction failure: ≥5% leukemic blasts in marrow after 42 days of remission induction treatment.

12.1.3 Bone marrow relapse: ≥25% leukemic blasts in marrow

12.1.4 CNS relapse: ≥5 WBC/□L of CSF with definite blasts on cytospin preparation.

12.1.5 Testicular relapse: Isolated testicular relapse must be confirmed pathologically; in the event of bone marrow relapse, combined testicular relapse can be based on testicular enlargement (documented by sonogram) without biopsy.

12.2 Toxicity Evaluation Criteria

This study will utilize the CTCAE Version 3.0 for toxicity and performance reporting. A copy of the CTCAE version 3.0 can be downloaded from the CTEP home page (http://ctep.info.nih.gov). Additionally, the toxicities are to be reported on the appropriate data collection forms.

12.3 Functional MRI Evaluation Criteria

Participants/guardians will be given the option to agree to this additional research test either during the informed consent process for Consolidation and Continuation of Treatment or before the scan is performed at end of treatment for all three risk arms. Only those patients who have agreed to this additional test and meet the following eligibility criteria will be scheduled for this exam.

- Patient is at least 8 years old at Week 120.
- Patient's primary language is English.
- Patient does not have braces or orthodontic appliances that cause image distortion or signal loss outside of the mouth and sinus area.
- Patient does not have any major physical, neurological, or psychiatric condition that would preclude participation in the functional MRI.
- Patient does not have a documented learning disability or attention disorder that predates cancer diagnosis.

13.0 SUPPORTIVE CARE

13.1 Fever at Diagnosis

All patients with fever at diagnosis will be admitted for broad spectrum parenteral antibiotic treatment until an infectious etiology can be excluded.

13.2 Metabolic Derangements

It is important to prevent or treat hyperuricemia and hyperphosphatemia with secondary hypocalcemia resulting from spontaneous or chemotherapy-induced leukemic cell lysis, especially in T-cell ALL.

- Patients with large leukemic cell burden should receive hydration and oral phosphate binder.

- Patients with large leukemic cell burden with or without hyperuricemia (e.g., WBC $\geq 100 \times 10^9$/L, uric acid ≥ 7.5 mg/dl or ≥ 6.5 mg/dl in patients <13 years old) may be treated with rasburicase if they have no history of G6PD deficiency or ongoing pregnancy. For patients not at high risk of hyperuricemia, hydration, allopurinol, and judicious use of alkalinization (keeping urine pH between 6.5 and 7.4) may be sufficient.

13.3 Hyperleukocytosis

For patients with extreme hyperleukocytosis (i.e., WBC $\geq 300 \times 10^9$/L), leukapheresis or exchange transfusion (in small children) may be considered. The Director of the Blood Bank should be consulted for this procedure.

13.4 Avascular Necrosis of Bone

Osteonecrosis of the bone, a known complication of treatment with corticosteroids, can be expected to occur in approximately 10-15% of patients, especially in those older than nine years of age. This devastating complication may result in collapse of the articulating surface with subsequent pain and development of arthritis. Early detection of small lesions will permit intervention which may prevent pain and irreversible damage of the joints. In this study, all patients 9 years of age and older will have MRI scans of the pelvis/hips and knees after each reinduction phase, at off therapy date, and as needed thereafter.

Patients diagnosed with osteonecrosis (see section 7.8) will be referred to orthopedics. Any patient who develops symptoms of joint pain prior to or between scheduled MRI scans should have an MRI performed to rule out osteonecrosis or progression of this complication.

For patients who require surgical intervention, treatment will vary based on degree of progression, i.e., observation, core decompression, bone grafting and resurfacing hemiarthroplasty.

13.5 Pancytopenias

Patients with prolonged (>3 weeks) unexplained anemia (hemoglobin <7 g/dl) or neutropenia (ANC <300/mm^3) during remission should be evaluated for B19 parvovirus infection or hemolysis or toxicity from non-chemotherapeutic agents (e.g., TMP/SMZ).

13.6 Nutritional Supplementation

Nutritional or vitamin therapies should not result in patients receiving more than the RDA for folic acid with dietary and supplement intake, to prevent interference with the effectiveness of methotrexate.

13.7 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 anti-leukemic 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.

13.8 Down Syndrome

Patients with Down's syndrome should be closely monitored for toxicity, and offered aggressive supportive care. Methotrexate dosage will be reduced as described in section 7.1.1. Historically, most of these patients will be treated in the low-risk category, and should have their chemotherapy doses appropriately reduced to avoid undue toxicity. Oral leucovorin (5 mg/m^2 q 12 hr x 2) should be given 24 hrs after each IT MHA. All Down's patient will receive the conventional (2500 units/m^2) dose of PEG-asparaginase post remission induction and will not be included in the randomization to the higher dose of PEG-asparaginase. A 30% dose reduction of dexamethasone and/or high dose cytarabine should be considered for Down's syndrome patients treated on the standard/high-risk arm who experience higher than expected toxicity during earlier phases of therapy.

13.9 RSV Prophylaxis

All infants should receive RSV prophylaxis as per current institutional policy.

13.10 Prophylactic Antibiotics During Periods of Prolonged Neutropenia

Patients with expected periods of severe neutropenia (ANC<300) of 5 days or longer (e.g., during induction, reinduction, and intensification) should receive prophylactic antibiotics (vancomycin + ciprofloxacin or cefepime) and antifungals (micafungin if patient is receiving weekly vincristine, or voriconazole if patient has completed all vincristine doses in the treatment phase).

14.0 OFF-TREATMENT AND OFF-STUDY CRITERIA

14.1 Off Treatment Criteria

A patient is taken off TOTXVI therapy if any of the following occurs:

- Failure to achieve remission after remission induction, consolidation, and reintensification treatment
- Bone marrow or extramedullary (except CNS) relapse
- Second malignancy (e.g., therapy-induced AML or brain tumor)
- Development of unacceptable toxicity during treatment (with concurrence of the PI or co-PI)
- Patients with severe congenital immunodeficiency (e.g. ataxia telangiectasia) or HIV infection will be taken off TOTXVI therapy and will receive alternate therapy (to prevent excessive toxicities) with curative intent.
- Completion of all protocol-prescribed treatment
- Participant/family decision to withdraw from protocol treatment at any time for any reason
- Investigator decides continued protocol treatment is no longer in patient's best interest

14.2 Off Study Criteria

A patient is taken off TOTXVI study if any of the following occurs:

- Death
- Lost to follow-up
- Withdrawal of consent for continued follow-up
- Found to be ineligible (e.g., incorrect diagnosis)

Follow up will stop at the time the patient is off study. The follow up time of an off-study patient is censored at this point, and no outcome data beyond the off-study time will be used in analyses.

15.0 DATA SAFETY MONITORING BOARD

The protocol progress will be reviewed and monitored by the St. Jude Children's Research Hospital Data Safety Monitoring Board (DSMB). Data summaries will be provided to the DSMB by the Department of Biostatistics after review by the Principal Investigator. The data will include patient accrual, demographic summaries, grade 3/4 toxicities, major adverse events (i.e. deaths, relapses, second malignancies) and results of interim and final analyses as specified in the protocol. The data are retrieved from the database and are reviewed at every TOTXVI meeting, which is generally held twice a month, and are reflected in the minutes, which are provided to the biostatistician. When the appropriate number of patients have failed or accrued, triggering interim or final analysis, the biostatistician will be informed by the PI. Should a safety stopping rule be exceeded, the protocol will be temporarily closed until the DSMB can review the situation. Reporting will comply with institutional guidelines for monitoring.

16.0 BIOLOGIC STUDIES

16.1 Minimal Residual Disease

This study will apply immunologic and molecular methods as done in TOTXV. The leukemia markers commonly used to study minimal residual disease by 4- color flow cytometry are listed in the table below. During the course of TOTXVI, we will gradually increase the parameters to be studied simultaneously, taking advantage of novel instruments that allow the detection of 6 or more fluorochromes. This should allow a better discrimination of leukemia cells while reducing the number of cells required for the analysis.

Immunophenotypic markers most commonly used to study MRD in children with ALL

ALL lineage	Phenotype	Frequency (%)†
B-lineage	CD19/CD34/CD10/TdT*	30-50
	CD19/CD34/CD10/CD38*	30-50
	CD19/CD34/CD10/CD45*	30-50
	CD19/CD34/CD10/CD13	10-20
	CD19/CD34/CD10/CD15	5-10
	CD19/CD34/CD10/CD33	5-10
	CD19/CD34/CD10/7.1	3-5
	CD19/CD34/CD10/CD66c	10-20
	CD19/CD34/TdT/cytoplasmic μ*	10-20
T-lineage	TdT/CD3/CD5	90-95
	CD34/CD3/CD5	30-50

**The use of these immunophenotypes for MRD studies relies mainly on differences in intensities of expression between leukemic lymphoblasts and normal lymphoid progenitor cells. The remaining combinations rely mainly on the aberrant expression of one of the markers. †Proportion of childhood ALL cases in which one leukemic cell in 10^4 normal bone marrow cells can be detected with the listed immunophenotypic combination. Most cases express more than one combination suitable for MRD studies.*

Any participant registered in TOTXVI whose leukemic cells are amenable to our assays will be eligible for testing. Peripheral blood samples (5-10 ml) will be taken on day 8 in all patients. Bone marrow samples will be examined in all patients on day 15 (+/- 22) of remission induction therapy and at completion of induction therapy; patients with T-lineage ALL will also have their peripheral blood examined at these time points. All patients with B-lineage ALL with residual blasts at end of induction will have sequential MRD assays during remission (e.g. day 1 of reinduction I and II), and week 48 of continuation treatment. All patients with T-lineage ALL and residual blasts at the end of induction and those with "atypical" T-ALL will have sequential MRD assays in bone marrow and peripheral blood during remission (e.g. day 1 of reinduction I and II), and week 48 of continuation treatment. Patients with T-lineage ALL and no residual blasts at the end of induction will have MRD determined only in peripheral blood at the same time points. MRD will be also determined in all patients whenever clinically indicated.

We will use deep sequencing technique to evaluate MRD in CSF with the aim of determining whether levels of MRD in the CSF at diagnosis and dynamics of early MRD reduction in CSF could be used to further refine the risk of CNS relapse. This might potentially lead to improved classification for patients at risk of CNS relapse and allow the reduction of intrathecal therapy in future studies in children with rare identifiable blast by morphology but low levels of MRD. We will also follow MRD of the bone marrow and peripheral blood by deep sequencing technique to determine if this more sensitive and specific test will show concordance unlike the flow and PCR based MRD tests. If concordant, then we can monitor B-ALL patient using a less invasive testing of the peripheral blood similar to T-ALL patients.

16.2 Germline DNA/RNA

Peripheral blood (5 ml) at day 3 (for initial TPMT genotyping and as a back-up for any participants with early withdrawal or death) and concurrent with the blood collected for MTX plasma levels during consolidation. Leukocytes will be isolated, and cells will be cryopreserved or DNA and RNA will be isolated using standard techniques.

An aliquot of DNA from every patient who has signed TBANK (or its succeeding banking protocol) will be available from the pharmacogenetics DNA sample to the Cell Bank laboratory.

16.3 Dexamethasone/Asparaginase Serum Pharmacodynamic Biomarkers

Blood samples (3 ml each) will be drawn in a red top tube at day 15 of induction and day 15 of consolidation and at weeks 7, 8, and 17 of continuation, and a green top tube (3 ml each) will be drawn on Day 8 of weeks 7 and 17 of continuation.

In addition, heights are now recorded carefully monthly on a stadiometer (using the average of 3 replicate measures) and these data are entered in the AS400 and MILLI/Powerchart systems. Both cortisol and height change have been related to glucocorticoid plasma AUC previously.

16.4 Determination of ALL Blast Sensitivity to Anti-Leukemic Agents, *in vitro*

We will use a portion of the diagnostic bone marrow from each patient having an adequate number of cells in the same ex vivo drug sensitivity assay we have previously used to measure resistance to anti-leukemic agents, as we have previously described in detail[13]. Within hours of sampling, mononuclear cells are isolated from bone marrow aspirates by Ficoll-Hypaque gradient; if necessary, samples are further enriched to more than 90 percent leukemic blasts by removing non-malignant cells using magnetic activated cell sorting. Leukemic cells are then re-suspended in culture medium consisting of RPMI 1640 supplemented with 20 percent fetal calf serum, 2 mM L-glutamine, 200 µg/ml gentamicin, 100 IU/ml penicillin, 100 µg/ml streptomycin, 0.125 µg/ml fungizone, and 5 µg/ml insulin, 5 µg/ml transferrin and 5 ng/ml sodium selenite. These tumor cells are cultured in a 96-well micro culture plate in the presence of a range of drug concentrations.

After 4 days of culture, MTT (3-4,5-dimethylthiazol-2,5-diphenyl tetrazolium bromide) is added for several hours, as previously described in detail[13]. This method is used to determine drug sensitivity to mercaptopurine, thioguanine, prednisolone, dexamethasone, vincristine, L-asparaginase, cytarabine, and daunorubicin. These studies require $<2 \times 10^5$ cells per well, for 6 drug dilutions in duplicate plus 6 control wells, for a total of 3×10^6 cells per drug (If fewer than 2.4×10^7 cells are available, then we will work with the Pathology Department to prioritize which drugs can be studied based on the number of cells available. This is the same procedure we have had in place since 2000.) The drug concentration lethal to 50 percent of the leukemia cells (LC_{50}-value) is used as the measure of cellular drug resistance.

For each anti-leukemic agent, we will treat the *in vitro* individual drug and multi- drug cross sensitivities as continuous phenotypes in data analyses. In addition, we will separate patients into top 25%, middle 50% and bottom 25% groups based on their drug sensitivities, ranging from the most resistant to the most sensitive. We will also examine if patients can be separated into groups based on a model based clustering method (MCLUST) based on their drug sensitivities. We will explore whether individual drug sensitivities or multi-drug cross sensitivities differ among ALL subtypes (e.g. B-lineage vs. T-cell).

16.5 NK Cell Receptor Study

Peripheral blood (3 ml) will be obtained at diagnosis for immunophenotyping and (8.5 ml) before reinduction for immunophenotyping and genotyping for NK cell receptors as described previously[82;123]. Blood samples will be collected in ACD (yellow-top) tubes. We will assess NK cell receptors including KIRs, NCRs, NKG2D, DNAM-1, 2B4, and NTBA. The sample should be obtained before the administration of steroids.

16.6 Intestinal Microbiome Research

To participate in this research, participants cannot have received chemotherapy, for longer than 72 hours, and must not have received antimicrobial agents for $\geq$ 72 hours within one week prior to enrollment. Participants must also not have a clinical or microbiologically-

proven diarrheal illness.

Two stool specimens will be collected from research participants, one is during a time period before starting induction chemotherapy (or until the first 72 hours after starting induction chemotherapy), and the second sample will be collected within 2 weeks after completion of ALL remission-induction chemotherapy (before the HDMTX dose #2 of the consolidation phase) to investigate the changes in the gut microbiota. Paired stool samples will be batched to be analyzed simultaneously. Bacterial genomic DNA will be harvested from these samples and utilized for ribosomal DNA sequencing to determine the overall bacterial microflora prior to and following chemotherapy. These two methodologies will provide a preliminary investigation into any notable alterations in bacterial diversity resulting from therapeutic interventions. In tandem, we will also isolate RNA from these samples for sequencing in order to identify novel RNA viruses. Once notable differences in the microbiome before and after chemotherapy are determined, we will then attempt to describe the gastrointestinal complications during induction and over the next six months. In particular, we are interested in whether the abundance or absence of particular bacterial or viral pathogens will show association with subsequent gastrointestinal illnesses. Over the approximately 6 month period after the collection of the second stool sample, two follow-up stool samples will be prospectively collected at time points where patients are seen in Memphis: within 48 hours of Day 1 of Reinduction I and Reinduction II. These stool samples will be collected at the specified time points irrespective of intake of antimicrobial agents. In addition, a 1.0-2.0 mL volume of leftover stool specimens collected at SJCRH for clinical purposes will be prospectively obtained from the clinical bacteriology and virology laboratories within 48 hours of collection. These stool samples will be used for further testing of bacterial and viral DNA using PCR assays that will be developed to detect any viral or bacterial pathogen that was prominent by the gene sequencing analysis of the changing microbiome. The same approach will be used to investigate fungal pathogens. Medical records will be reviewed to collect data about infectious diseases, probiotics, antacids and antimicrobial use during the study period. Only descriptive statistics such as median, range and percentages will be used as appropriate. The proposed study is intended to be a pilot investigation to test feasibility of this approach and optimize experimental procedures. The data and information obtained in these investigations will be utilized as preliminary data for a larger scale study and future grant applications.

16.6.1 Visit A: Stool sample will be collected during a time period before starting induction chemotherapy (or until first 72 hours after starting induction chemotherapy). This specimen will be used to run the laboratory investigation in Dr. Tuomanen's and Dr. Schultz-Cherry's research laboratories as described in section 16.7.4. Visit A can occur on the same day as screening/enrollment.

16.6.2 Visit B: Stool sample will be collected within 2 weeks after completion of ALL remission- induction chemotherapy (before the HDMTX dose #2 of the consolidation phase). This specimen will be used to run the laboratory investigation in Dr. Tuomanen's and Dr. Schultz-Cherry's research laboratories as described in section 16.7.4.

16.6.3 Follow-up stool specimens: Over the approximately 6 month period after the collection of the second stool sample (Visit B), two follow-up stool samples will be prospectively collected at time points where patients are seen in Memphis: within 48 hours of Day 1 of Reinduction I and Reinduction II. These stool samples will be collected at the specified time points irrespective of intake of antimicrobial agents. In addition to these samples, 1.0-2.0 mL of left-over stool specimens collected at SJCRH for bacterial stool culture and *C. difficile* toxin detection will be prospectively obtained from the clinical bacteriology and virology laboratories, respectively, within 48 hours of collection. These stool samples will be used for further testing of bacterial and viral DNA using PCR assays that will be developed to detect any viral or bacterial pathogen that was prominent by the gene sequencing analysis of the changing microbiome. The same approach will be used to investigate fungal pathogens.

16.6.4 Laboratory methods and materials

Fecal samples will be collected and immediately placed into RNA Protect, a stabilization reagent. Tubes will be prepared in advance and stored until sample collection. Samples will then be processed within 6 hours of collection. Fecal samples will be stored at -80°C until processing at a later time in batched runs. DNA and RNA will be extracted from the sample by bead-beating followed by phenol chloroform extraction or column purification methods. Any left-over stool specimens after the completion of the DNA and RNA extraction will be discarded.

The gut microbiota will be sequenced using two independent methodologies. One methodology will utilize universal primers and subcloning to amplify the ribosomal sequences from the isolated bacterial DNA. These subclones will then be utilized to generate an Illumina genomic library. The other approach will utilize DNA and RNA that has been enriched from any eukaryotic contamination without any subcloning. Our methodology should yield approximately 100 µgs of DNA and 20 µgs of RNA, sufficient for approximately ten independent sequencing runs. This will allow for the determination of bacterial diversity in the gut as well as the identification of novel, gut associated viruses that correlate with downstream clinical outcomes.

For the sequencing, 3pM of each small library will be loaded onto a flow cell on the Illumina Cluster Station. A 3pM PhiX DNA sample was used as a cluster and sequencing control. The reads on the single read flowcell will then be subjected to 152 cycles of sequencing on the Illumina Genome Analyzer platform. The base calls were analyzed using Illumina's Pipeline version 1.4.0. This methodology allows for the generation of approximately 10 million reads of an average length of 100 base pairs, providing excellent genetic coverage.

The reads will then be analyzed by a number of readily available software programs specifically designed for metagenomic analysis and phylogenetic reconstruction. This will allow for the determination of changes in overall bacterial diversity and population dynamics. This methodology will also allow for the detection of any known or previously uncharacterized viruses in the sample.

17.0 NEUROIMAGING STUDIES

The neuroimaging aims are organized under the hypothesis that therapy for ALL can have a detrimental impact on the developing brain resulting in neurocognitive deficits, which will result in decreased quality of life in survivors. Therapy induced imaging changes in the developing brain (such as myelin and cortical thickness development) could partially be related to genetic polymorphisms associated with folate metabolism.

Study	Induction	Consolidation	Week 7, continuation (Week 1 Re-induction I)	Week 120, continuation	Two year post therapy
Psychology			X	X	X
MRI-brain			X*	X	X**

** MR should be obtained before starting dexamethasone.*
***Patients with abnormal imaging evaluations at week 120 will have an extra exam at 2 years off therapy to reassess brain structure.*

17.1 MR Imaging

MRI of the brain will be obtained at 2 time points: Week 1 of Reinduction I (Week 7 Continuation before starting dexamethasone) and week 120 of continuation therapy. These studies were designed to yield longitudinal measures of white matter integrity (myelination) and cortical thickness during treatment.

The first MRI examination at Reinduction I will provide a quantitative measure of myelin and cortical thickness development relative to a gender and age matched healthy control group, which may be disrupted by therapy. The second MRI examination is at week 120 of continuation (end of therapy). By assessing all patients at this time point, we can determine if the normal brain development has been affected by therapy.

These abbreviated clinical MRI scans will consist of T1 sagittal for localization followed by high-resolution T1, T2/PD, and FLAIR images covering the entire cerebrum acquired at 3 Tesla. These are followed by DTI and myelin water imaging. The full examination can be performed in approximately 45 minutes for children not requiring sedation and 75 minutes for children requiring sedation.

Most children six years of age and older can tolerate the procedure without sedation. All scans will be evaluated by a neuroradiologist for clinically relevant findings that will be captured in a data reporting form.

The T1, T2/PD, and FLAIR images will be analyzed with software developed and validated at our center specifically for quantification of white matter changes and brain tissue volumes. These high-resolution images will also be used to assess cortical thickness based on the segmented tissue maps using the FreeSurfer software. DTI acquires a set of diffusion images in six or more directions and uses them to fully define a diffusion tensor for each point in the image[124;125]. Once the tensors have been calculated, Eigen values can be derived and used to calculate apparent diffusion coefficient (ADC), perpendicular and parallel diffusion coefficients ($D_{\perp}$, $D_{\parallel}$), and fractional anisotropy (FA) maps for the whole

brain. These measures are related to the integrity of the myelin sheath which restricts the diffusion of water perpendicular to the axons within the white matter fiber tracts. Myelin water imaging will be analyzed using software developed and validated at our center specifically for quantification of multi-exponential T2 relaxation data to assess the integrity of the myelin sheath. Quantitative multiple exponential fittings of the T2 relaxation components in white matter can be used to generate MWF maps (from the T2 components less than 40-50 ms) and LWF maps (T2 components 200-800ms).

Imaging from a very large age-similar healthy control population has been collected under the "NIH MRI Study of Normal Brain Development" (http://www.bic.mni.mcgill.ca/nihpd/info/). The first phase of this project enrolled children ages 4 years, 6 months through 18 years (total N = 433). This sample was recruited across the six Pediatric Study Centers using community based sampling to reflect the demographics of the United States in terms of income, race, and ethnicity. The subjects are studied with both imaging and clinical/behavioral measures at two year intervals for three time points. Imaging collected included conventional T1-/T2-/PD-weighted MR imaging as well as diffusion tensor imaging. These imaging data sets will be analyzed with the same procedures used for the patient examinations.

17.2 Genetic Polymorphisms

All patients will be genotyped as described in section 2.2.1. We will focus our analysis on a theoretically justified class of polymorphisms related to the folate metabolism pathway. All relevant folate pathway polymorphisms will be included, given their relationship to MTX treatment and elevated homocysteine. Genes of interest include MTHFR, DHFR, TS, RFC, MS, MTRR, and CBS.

17.3 Psychological Testing

Age appropriate psychological testing will be performed in all participants at Week 1 of Reinduction I (Week 7 of Continuation), at week 120 of Continuation, and two years post therapy. Psychological measures were carefully selected based on psychology and MRI findings from the TOTXV protocol, as well as the available research literature[126-132]. Both lines of empirical support converge in identifying attention/executive functions, memory (particularly nonverbal) and processing speed as vulnerable cognitive functions in this population. By focusing the assessment battery on specific cognitive functions, rather than more traditional global measures (i.e., IQ and academic achievement), the proposed battery will have increased sensitivity to those deficits most likely to be demonstrated by ALL survivors. Further, a focused assessment will facilitate the identification of specific cognitive areas for early intervention and assist in the identification of underlying neurological mechanisms. In addition to behavioral specificity, measures were selected that have neuroanatomic specificity in keeping with TOTXV neuroimaging findings. Therefore, measures were selected that are putatively sub served by frontal brain regions (e.g., attention, fluency and working memory) and periventricular areas (e.g., verbal and nonverbal memory). Measures were also chosen that have been demonstrated in other clinical populations to be sensitive to white matter changes (i.e., processing speed and visual-spatial abilities).

All selected measures have age-specific norms from large, representative standardization samples. Measures also have appropriately demonstrated reliability and validity. Given the longitudinal study design, measures were chosen with appropriate test-retest reliability and negligible practice effects for the proposed interval between testing time points. An effort was made to select measures that could assess the widest age range possible given the likely age distribution at presentation and longitudinal design. Nonetheless, no single intelligence measure exists that covers this entire age spectrum, such that a separate IQ measure is needed for children less than three years of age. Children less than three years of age will be administered the Mullen Scales of Early Learning[133]. Parent questionnaires will be the same for patients to allow for longitudinal analyses including this young group. Please see Appendix VI for a complete listing of test measures, assessed abilities, administration age range and estimated testing time for children.

A primary assessment goal was to select a battery that could be administered in a circumscribed period of time to reduce the time burden for children, their parents and the research team. The administration time for the test battery is 1-3 hours, dependent on the age of the child and the speed with which they can complete tasks. It is anticipated that the average administration time will be 2 hours. The maximum administration time of 3 hours is only expected for older children (i.e., 7-8 years of age) or children that are notably slow (e.g., unusually slow speed of cognitive and/or physical processing). Therefore, the administration length of the current battery readily lies within the standard length of typical clinical and research batteries. It is anticipated that parents will spend on average of 45 minutes completing questionnaires while their child is being assessed in a separate room.

17.4 Functional MR Imaging

Blood Oxygenation Level Dependent Imaging (BOLD) is the 'standard technique' used to generate images in functional MRI studies, and relies on regional differences in cerebral blood flow to delineate regional activity during the performance of a specific neurocognitive task. BOLD imaging is acquired across the entire brain continuously during the performance of the task. The N-Back task is a prototypical working memory measure that requires a non-sedated participant to respond to a presented stimulus (either letters or objects in our implementation) only when it is the same as the one presented on a trial a predetermined number (N) prior to the current trial. For each letter participants need to hit a response button when the letter is identical to the letter presented one or two back in sequence for the 1-back and 2-back trials, respectively. A control condition (0-back trial) will be used during which the same continuous stream of single letters or object is presented but the participant need only decide whether each letter or object matches a single target communicated at the start of the task. Patients who meet eligibility for the functional MRI (see Section 12.3) will undergo this test at Week 120 of Continuation. In addition to the letter and object versions of the N Back task, the functional MRI will also include a task to identify the primary visual cortex and a task to identify resting networks.

Functional BOLD images will be analyzed with SPM (SPM5, University College London, UK) via 2-level analysis. Normalization of fMRI data from children and adults to the same stereotactic brain space has been validated for children older than 7 years[134;135].

Independent component analysis (ICA) of fMRI data will be conducted with the GIFT Toolbox for SPM. N-Back data will be analyzed according to a fixed-effect GLM. Task-induced activity will be represented by a boxcar function convolved with the canonical hemodynamic response function. After parameter estimation, the contrasts reflecting the effect of interest (e.g., 2- back >1-back) will be set in the model, and the corresponding contrast image will be generated. The contrast image from each subject will then be used as a variable in second-level, random-effect analysis to identify group patterns of activation and areas in which brain activity is associated with behavioral performance. Voxels will be considered active for $p<0.05$, corrected for multiple comparisons. Our primary analysis will focus on activation in the dorsolateral prefrontal cortex during the N-back task.

18.0 STATISTICAL CONSIDERATIONS

TOTXVI has a single primary objective to compare the distributions of continuous complete remission (CCR) of patients randomized on the first day of continuation phase to receive higher dose PEG-asparaginase or to receive conventional dosing (2,500 units/m^2). The stratified analysis of the primary objective will include all eligible patients as randomized and will be conducted consistent with the intent-to-treat hypothesis. Zelen's randomization algorithm, as implemented in the Department of Biostatistics since 1992, will be used with a blocking factor of 4 in each stratum. Details of the design, sample size and statistical methodology required for the primary objective is addressed in Section 17.2 and will be the defining sample size for TOTXVI.

The refusal of participation in any of the research aims will not render a patient ineligible for TOTXVI; patients who refuse to consent to some or all of the research objectives will still be evaluable for other study aims including estimation of the distributions of event-free survival and survival. Design parameters for the primary objective will not be adjusted for multiple tests. However, within a secondary objective it may be appropriate to apply methods to control the type I error rate in terms of the family-wise type I error probability or the false discovery rate, and these are spelled out below. All analyses of the randomized question will be consistent with the "intent to treat" hypothesis; that is, all eligible randomized patients will be followed for the primary outcome and included in analyses as randomized. To the extent possible, as indicated in the following sections, all eligible patients will be included in all analyses.

Methodology for analyzing data related to secondary objectives are presented, but in general power calculations are not provided since the sample size for the trial is governed by the primary objective. Moreover, if during the course of this trial more appropriate statistical methods are identified or developed, then those methods will be used to analyze the data.

Effect size: The intent-to-treat hypothesis being tested in TOTXVI is that doses of PEG-asparaginase higher than conventional doses being used by other ALL research groups will improve outcome of children with ALL. The trial will begin by randomizing patients to receive either 3,500 units/m^2 or 2,500I units/m^2 of PEG-asparaginase during continuation therapy and if 3,500 units/m^2 proves to be too toxic (see 17.1.2) the trial will

continue with patients being randomized to either 3,000 units/m^2 or 2,500 units/m^2. It should be noted that the intent of TOTXVI is to test whether higher-than-conventional doses of PEG-asparaginase improve outcome and not to determine the difference in efficacy or toxicity between 3,500 units/m^2 and 3,000 units/m^2 dosages. As discussed below, should the higher dose of 3,500 units/m^2 prove to be too toxic, we still will be able to generate new important information and interpretable results.

The conjectured effect size for higher doses of PEG-asparaginase represents risk-benefit considerations of what outcome is reasonable to expect and would justify accepting possibly slightly higher rates of manageable asparaginase toxicities. Whether the hypothesized effect size will be associated with exposure to 3,500 units/m^2 or 3,000 units/m^2 is not known and whether there will be unacceptable toxicities associated with the higher dose is also not known. What we do know is that the trial with traditional statistical design parameters provides power for detecting a difference in 5-year continuous complete remission (CCR) rates of 8.53% between the conventional versus the higher dose and final interpretations require that the overall effect size of 8.53% is maintained if toxicity requires that the dose of PEG-asparaginase be reduced form 3,500 units/m^2 to 3,000 units/m^2. Section 17.2 includes one of many possible realizations of the trial that would be consistent with an overall 8.53% improvement in the 5-year CCR rate. We do not know what deliverable dose of PEG-asparaginase is actually necessary to produce these results. We do believe doses higher than conventional doses can be safely given and that these higher doses will produce improved outcome, which remains an important goal for childhood ALL treatment as currently not all children are cured.

Interpretation: We believe that 3,500 units/m^2 of PEG-asparaginase can be delivered safely within the context of TOTXVI risk-directed regimens, and expect that the most likely realization of the trial is that a toxicity monitoring rule during continuation therapy will not be triggered and we will not have to dose-reduce PEG-asparaginase. However, we have planned a course of action if unacceptable toxicity were to materialize.

The question that remains is what inference will be drawn at the end of the trial if dose-reduction of PEG-asparaginase is required. If it is necessary to dose-reduce, and if the planned final analysis of the trial results in a p-value greater than 0.045, the primary conclusion will be that, as intended to be delivered in TOTXVI, the statistical evidence is not sufficient to conclude that the distributions of CCR between patients who would receive higher doses of PEG-asparaginase and those patients who would receive conventional dosing differ. Furthermore, we would have evidence that, at the *a priori* threshold set for toxicity, 3,500 units/m^2 of PEG-asparaginase was deemed too toxic. As with most amply powered negative trials, additional analyses will likely be attempted to elucidate the findings. These analyses would predominantly rely on estimation and not testing, albeit with limited power. We speculate on only a few of the many possible reasons why the trial may end with this result. It might be that the overall treatment results for both conventional and higher dose PEG-asparaginase regimens were so good that no difference could be detected; that had 3,500 units/m^2 been tolerated the hypothesized difference may have been detected; that there was evidence that patients randomized to the higher dose PEG-asparaginase treatment regimen did experience a better outcome but not as great as necessary for the number of patients randomized and events observed to

show a statistical difference; that patients randomized to conventional dosing had a much better outcome than expected. Publication of findings from this adequately powered negative study would include retrospective analyses exploring "why," as would be expected by journal reviewers and readers. We must add that if the trial were stopped due to futility, these analyses would be conducted with much less statistical power and would be less likely to yield useful information on which future investigations could be built.

If it is necessary to dose-reduce and the planned final analysis of the trial results in a statistically significant p-value (<0.045), the primary conclusion will be that, as intended to be delivered in TOTXVI, the statistical evidence is sufficient to conclude that the distributions of CCR between patients who would receive higher doses of PEG-asparaginase and those patients who would receive conventional dosing are different. Furthermore, we would have evidence that, at the *a priori* threshold set for toxicity, 3,500 units/m^2 of PEG-asparaginase was deemed too toxic. Additional analyses will be required to fully elucidate the findings and again we speculate on several possible reasons for the results. It might be that the outcome for patients randomized to 3,500 units/m^2 was so superior that differences for this stratum dominated the overall results; that outcome for patients randomized to both 3,500 units/m^2 and 3,000 units/m^2 were similar and superior to the outcome for patients who were randomized to 2,500 units/m^2; or that most patients randomized to high dose PEG-asparaginase were in the 3,000 units/m^2 regimen and the outcome was superior to the outcome for patient randomized to conventional doses of PEG-asparaginase. All three possibilities are easily interpreted and the first would stimulate research as to how 3,500 units/m^2 may be safely given.

We have purposefully not specified an accrual number at which the trial would be restarted using only the lower 3,000 units/m^2 PEG-asparaginase, if the higher dose is too toxic, nor have we suggested that outcome at that time could help making a decision regarding restarting the trial. There are simply too many speculative scenarios that could occur and in all likelihood there would be too little outcome information to shed light on the situation. However, TOTXVI will be monitored by the St. Jude DSMB that is composed of senior, experienced clinical trial experts. We have chosen to design the trial as we have, knowing and fully expecting that the DSMB will provide guidance in the unlikely event that 3,500 units/m^2 proves to be too toxic.

Sample size & study duration: The accrual goal of this study is 420 evaluable patients for the continuation phase randomization between higher dose PEG-asparaginase and standard dose PEG-asparaginase. This sample size is dictated by power considerations for the primary objective, as discussed in Section 17.2. Current TOTXV experience shows that about 5% of the patients failed to enter the continuation phase of treatment. Although every effort will be made to consent patients to the randomized study, we anticipate approximately 10% of patients will not consent to participate in the randomization; thus an attrition rate of 14.5% is expected and a total accrual of approximately 531 eligible patients to TOTXVI will be necessary. Historical data from TOTXIIIA, TOTXIIIB, and TOTXV (see Section 17.2) indicate that this accrual goal can be reached in about 8.6 years.

This sample size provides approximately 80% power to detect the hypothesized improvement in the distribution of CCR ($\alpha = 0.05$; two-sided test) for patients treated on the high dose PEG-asparaginase regimen. Due to two planned interim analyses, the final analysis, if all 420 patients are randomized, will be conducted at the 0.045 level. This final analysis will be conducted three years after the last patient is randomized. Thus, the total duration of the study could be approximately 11.6 years.

18.1 Safety Monitoring

The thresholds for safety monitoring were chosen after careful consideration of the potential risk/benefit ratio for the trial. All eligible patients who receive any TOTXVI induction therapy will be included in safety monitoring described below from the initiation of induction therapy to the beginning of continuation therapy. All eligible patients who are randomized and receive any continuation therapy will be included in safety monitoring described below for the interval from initiation of continuation therapy to the end of Week 35 of continuation. Monitored events that occur after week 35 of continuation therapy are not likely to be related to PEG-asparaginase and thus will not be considered in the formal safety monitoring plan.

Critical for the timely safety monitoring described in this section is up-to-date and accurate data for analysis. Each of the monitoring analyses, except for death during induction, will be conducted as follows:

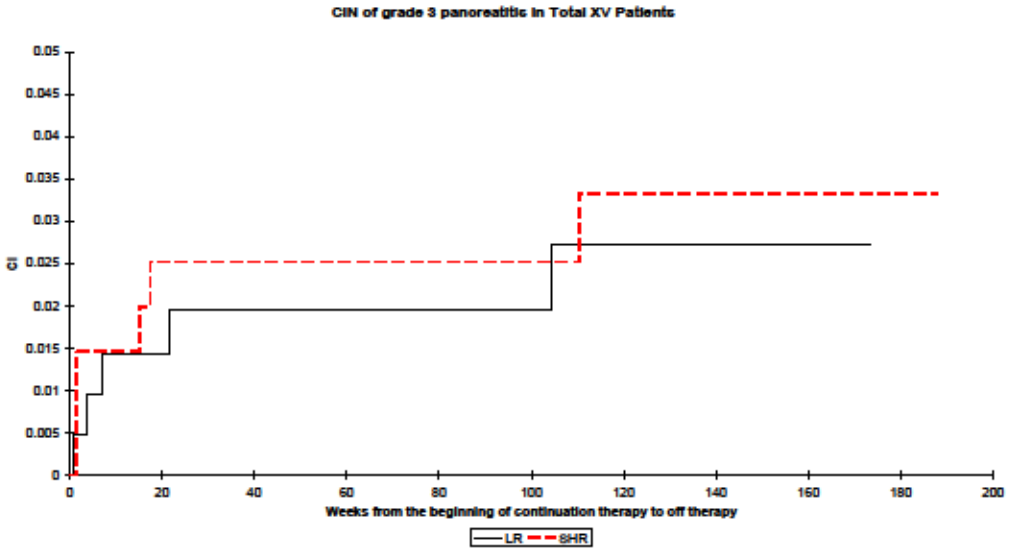
1. The triggering event will be confirmed by the attending physician and the protocol PI. Upon confirmation, all associated information for the event (dates, grade, etc) will be locked (frozen) in the TOTXVI database.
2. The protocol biostatistician will extract data from the TOTXVI database and conduct the analysis.
    a. If the safety stopping threshold is not reached, the trial continues unabated and this will conclude the analysis for this event.
    b. If the safety stopping threshold is exceeded, the analysis is repeated by assuming that patients who do not have data in the TOTXVI database current to the date of the triggering event are "safe" on that date.
        i. If the analysis of these data still exceeds the stopping threshold, then the appropriate action described below is executed.
        ii. If the analysis of these data does not result in the stopping threshold being exceeded, then the relevant data in the TOTXVI database is immediately (within several days) updated and the analysis repeated. This will constitute the final analysis triggered by the event and appropriate action will be taken pending on whether the stopping threshold is or is not exceeded.
3. Safety monitoring rules provide a statistical framework in which to consider whether observed toxicities are excessive. Upon completion of an interim analysis, the findings will be reported to the St. Jude DSMB that will make recommendations to the protocol PI and advise the St. Jude leadership on continuing, stopping or amending the trial.

For the monitored toxicities during TOTXV induction, the recent experience (as of 6/5/2007) shows that out of 453 patients, there are 2 (0.44%) deaths, 5 (1.1%) seizures (grade 2 or more), 11 (2.4%) mucositis, and 11 (2.4%) infections (grade 4). As of 5/23/2007, the cumulative incidence of disseminated fungal infections till the end of re-induction II is 4.6%±1.09%, and is 5.9%±1.24% over the entire treatment duration. As of 6/25/2007 there are 6 fatal chemotherapy toxicities on TOTXV (4 at SJ: 2 in induction, 1 in consolidation, and 1 in continuation; 2 at Cook's: 1 in re-induction I and 1 in re-Induction II). All non-fatal toxicities have been manageable.

Pancreatitis and deep vein thrombosis (DVT) are two asparaginase related toxicities that will be closely monitored in TOTXVI due to the increased exposure of patients to this agent. On TOTXV therapy, no patient experienced repeated episodes of pancreatitis or DVT in the interval between initiation of therapy and the beginning of continuation therapy. During continuation therapy one patient did experience two episodes of DVT but no patient experienced multiple episodes of pancreatitis.

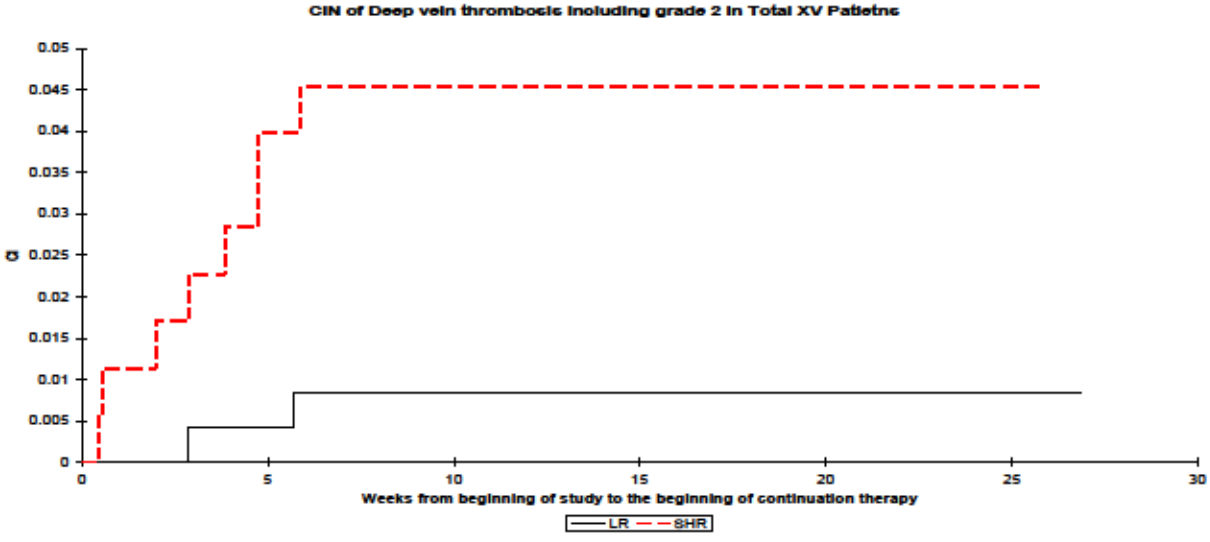
Pancreatitis is a toxicity that is associated with asparaginase treatment and thus will be carefully monitored during induction and continuation therapy. The cumulative incidence of pancreatitis from the initiation of induction therapy to the beginning of continuation therapy in TOTXV was 0.97% (SE = 0.48%) and there was no evidence that that the incidence was related to risk classification with 2 events in low risk patients and 2 episodes in standard/high risk patients who received more asparaginase. All four events occurred during the first four weeks of induction therapy. The 95% upper confidence bound just prior to the initiation of continuation therapy is 1.76%.

The cumulative incidence of pancreatitis from the initiation of continuation therapy to the end of re-induction II was 2.23% (SE = 0.74%) with the last episode being observed within two weeks of the last dose of asparaginase, which was given in week 21 of continuation therapy. The 95% upper confidence bound at the end of this period is 3.45%.Two other episodes were observed in weeks 105 and 111 but neither is thought to be related to asparaginase exposure and these are not used in the estimation. S/HR patients received more exposure to asparaginase during TOTXV continuation therapy than did low risk patients. However, as the following figure illustrates, there is no evidence of a dose-toxicity relationship (p=0.72). Thus, in TOTXVI, LR and S/HR patients will pooled to monitor for excessive episodes of pancreatitis.

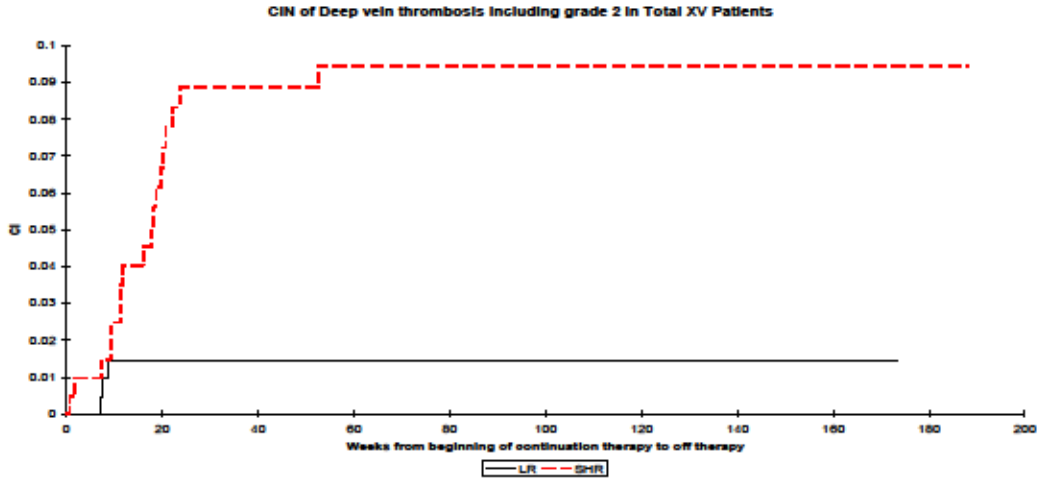


DVT, on the other hand, is a toxicity that does seem to be associated with the dose of asparaginase as evidenced by the cumulative incidences of DVT during induction and continuation therapy observed in TOTXV. The episodes of DVT may be observed 3-4 weeks after the last dose of asparaginase. We note that the increased incidence could also be related to exposure to asparaginase in the context of the more intense therapy that S/HR patients received during this period.

The following figure shows the cumulative incidence of DVT for the period from the initiation of induction therapy to the initiation of continuation therapy on TOTXV for low risk and standard & high-risk patients. The cumulative incidence for low risk patients (N=238) is 0.84% (SE = 0.59%) and for S/HR patients (N=176) is 4.55% (SE = 1.57%). The 95% upper confidence bounds at the end of this period are 1.81% and 7.13%, respectively. Gray's test comparing the two cumulative incidence functions is significant (p=0.015).



The following figure shows the cumulative incidence of DVT during TOTXV continuation therapy for low risk and standard & high-risk patients. The last dose of asparaginase is given week 21 of continuation therapy and episodes of DVT were seen in S/HR patients up to three weeks later. The cumulative incidence for low risk patients (N=209) is 1.44% (SE = 0.83%) and for S/HR patients (N=205) is 8.87% (SE = 2.06%). The 95% upper confidence bounds at the end of this period are 2.81% and 12.58%, respectively. Gray's test comparing the two cumulative incidence functions is significant (p=0.0006).



18.1.1 During induction and to the initiation of continuation therapy

For monitoring purposes we will count the first episode of a monitored toxicity should the same toxicity occurs multiple times on a single patient during induction. TOTXVI has more intense induction and IT therapy than TOTXV. Therefore we will closely monitor toxic death, pancreatitis, DVT and seizures during the induction phase.

Death: Based on the TOTXV data, the 95% upper confidence bound on the death rate during induction is 0.95, which implies that 99% of patients would be expected to survive induction. Beginning with the second death during induction, we will construct the 95% upper confidence bound of the true unknown probability of surviving induction therapy, using the Blyth-Still-Casella method, when a death occurs. If at any analysis this upper bound drops below 0.990, we will close the trial to accrual and consider changes to the induction therapy. Note that the denominator in these analyses will be the number of patients that have completed induction therapy at the time of the index death.

The following table illustrates the stopping characteristics.

Stopping Rule for Death During Induction				
N	**# Dth.**	**% Dth.**	**95% UB**	**Decision**
100	2	2.0	0.995	Go
100	3	3.0	0.989	Stop
200	4	2.0	0.991	Go
200	5	2.5	0.987	Stop
300	6	2.0	0.991	Go
300	7	2.33	0.988	Stop

Pancreatitis (Grade 3 or higher): The 95% upper confidence bound on the cumulative incidence of pancreatitis during the period from initiation of induction to initiation of continuation therapy is 1.76%. PEG-asparaginase has a longer half-life that the formulation of asparaginase used in TOTXV and due to the expected increased efficacy of Peg-asparaginase, we will tolerate a true cumulative incidence of 3% before recommending that accrual to the trial be stopped. Beginning with the fifth patient experiencing an episode of pancreatitis prior to initiation of continuation therapy we will estimate the cumulative incidence function and if the 95% lower confidence bound is greater than 3.00%, we will recommend that accrual cease. Few deaths or relapses (competing events) are expected from initiation of induction therapy to the beginning of continuation therapy so the following table based on the binominal distribution provides approximate stopping criteria.

NOTE: We will upgrade any pancreatitis resulting in discontinuation of PEG-asparaginase to Grade 3 (even if Grade 2 by CTCAE v3 criteria).

Approximate Stopping Rule for Episodes of Pancreatitis: Induction to Continuation Therapy				
N	**# Episodes**	**% Episodes**	**95% LB**	**Decision**
100	6	6.00%	2.65%	Go
100	7	7.00%	3.68%	Stop
200	10	5.00%	2.96%	Go
200	11	5.50%	3.12%	Stop
300	14	4.67%	2.84%	Go
300	15	5.00%	3.25%	Stop

Seizures (Grade 3 or higher): The 95% upper confidence bound for seizures during the period between initiation of induction therapy and the beginning of continuation therapy for TOTXV was 1.91%. TOTXVI has more intense IT therapy that is considered potentially more effective than TOTXV IT therapy, so we will tolerate a true seizure incidence of 3%. Beginning with the fifth patient experiencing a seizure prior to initiation of continuation therapy, we will estimate the cumulative incidence function and if the 95%

lower confidence bound is greater than 3.00%, we will recommend that accrual cease. We will use the same stopping criteria as for pancreatitis during induction (see above table).

DVT (Grade 3 or higher): S/HR patients will receive a higher cumulative dose of PEG-asparaginase during induction than LR patients and thus, will be monitored separately. Based on TOTXV, the 95% upper bound for DVT due to induction therapy for LR patients is 1.81% and for S/HR is 7.13%.

PEG-asparaginase has a longer half-life that the formulation of asparaginase used in TOTXV and due to the expected increased efficacy of PEG-asparaginase, we will tolerate a true cumulative incidence of 3% in LR patients before recommending that accrual to the trial be stopped. Beginning with the second patient experiencing an episode of DVT prior to initiation of continuation therapy, we will estimate the cumulative incidence function and if the 95% lower confidence bound is greater than 3.00%, we will recommend that accrual cease.

Few deaths or relapses (competing events) are expected from initiation of induction therapy to the beginning of continuation therapy so the following table based on the binominal distribution provides approximate stopping criteria.

Approximate Stopping Rule for Episodes of DVT LR Patients: Induction to Continuation Therapy				
N	**# Episodes**	**% Episodes**	**95% LB**	**Decision**
50	3	6.00%	2.22%	Go
50	4	8.00%	3.54%	Stop
100	6	6.00%	2.65%	Go
100	7	7.00%	3.68%	Stop
150	8	5.33%	2.83%	Go
150	9	6.00%	3.26%	Stop

For S/HR patients we will tolerate a true cumulative incidence of 10% before recommending that accrual to the trial be stopped. Beginning with the seventh patient experiencing an episode of DVT prior to initiation of continuation therapy, we will estimate the cumulative incidence function and if the 95% lower confidence bound is greater than 10.0%, we will recommend that accrual cease.

Few deaths or relapses (competing events) are expected from initiation of induction therapy to the beginning of continuation therapy so the following table based on the binominal distribution provides approximate stopping criteria.

Approximate Stopping Rule for Episodes of DVT S/HR Patients: Induction to Continuation Therapy				
N	**# Episodes**	**% Episodes**	**95% LB**	**Decision**
50	8	16.00%	8.22%	Go
50	9	18.00%	10.46%	Stop
100	15	15.00%	9.74%	Go
100	16	16.00%	10.5%	Stop
150	21	14.00%	9.91%	Go
150	22	14.67%	10.14%	Stop

18.1.2 During continuation through the end of Week 35 among all randomized participants

The randomization to two different dosing schedules of PEG-asparaginase during continuation therapy requires that we closely monitor for grade 3 or more pancreatitis and deep vein thrombosis. The monitoring period is from day 1 of continuation to the Week 35, which is approximately 4 weeks after the last PEG-asparaginase given to the S/HR patients. For monitoring purposes, we will count the first episode of a monitored toxicity should the same toxicity occurs multiple times in an individual patient during the monitoring period.

Monitoring for pancreatitis and DVT will be based on the combined treatment regimens. However, if monitoring suggests that accrual should be stopped, the cumulative incidence functions for the two treatment regimens will be compared to carefully assess whether the problem is only with the higher dose PEG-asparaginase regimen.

Pancreatitis (Grade 3 or higher): The 95% upper confidence bound of the cumulative incidence of pancreatitis on TOTXV is 3.45% during continuation therapy. (See section 17.1 above) We expect that the incidence will be greater on TOTXVI due to the use of PEG-asparaginase and are willing to accept a true rate as high as 7%. This increase risk of a manageable toxicity is clinically acceptable due to the expected benefits of using PEG-asparaginase. Beginning with the ninth patient experiencing an episode of pancreatitis prior to week 35 of continuation therapy, we will estimate the cumulative incidence function and if the 95% lower confidence bound is greater than 7.00%, we will recommend that accrual cease. Few deaths or relapses (competing events) are expected from initiation of continuation therapy to the end of week 35 so the following table based on the binominal distribution provides approximate stopping criteria.

NOTE: We will upgrade any pancreatitis resulting in discontinuation of PEG-asparaginase to Grade 3 (even if Grade 2 by CTCAE v3 criteria).

Approximate Stopping Rule for Episodes of Pancreatitis: Continuation Therapy through Week 35				
N	# Episodes	% Episodes	95% LB	Decision
100	11	11.00%	6.56%	Go
100	12	12.00%	7.07%	Stop
200	20	10.00%	6.83%	Go
200	21	10.50%	7.28%	Stop
300	28	9.33%	6.72%	Go
300	29	9.67%	7.002%	Stop

DVT (Grade 3 or higher): The data from TOTXV clearly shows that the S/HR patients experienced a higher cumulative incidence of DVT than did the LR patients.

As shown in section 17.1 above, the 95% upper confidence bound for the cumulative incidence of DVT in the LR patients during continuation therapy was 2.81%. Since low risk patients randomized to the high dose PET-ASP regimen are projected to experience only a 2-4% improvement in duration of CCR, we will not accept a rate of DVT much higher than that observed for LR patients in TOTXV. Beginning with the second patient experiencing an episode of DVT prior during continuation therapy we will estimate the cumulative incidence function and if the 95% lower confidence bound is greater than 3.00%, we will recommend that accrual cease.

Few deaths or relapses (competing events) are expected from initiation of continuation therapy to week 35 so the following table based on the binominal distribution shows approximate stopping criteria.

Approximate Stopping Rule for Episodes of DVT Low Risk: Continuation Therapy through Week 35				
N	# Episodes	% Episodes	95% LB	Decision
50	3	6.00%	2.22%	Go
50	4	8.00%	3.54%	Stop
100	6	6.00%	2.65%	Go
100	7	7.00%	3.68%	Stop
150	8	5.33%	2.83%	Go
150	9	6.00%	3.26%	Stop

As shown in section 17.1 above, the 95% upper confidence bound on the cumulative incidence of DVT in S/HR patients during continuation therapy in TOTXV is 12.52%. We would accept a slightly higher incidence, 15%, for the S/HR patients that received the low dose PEG-asparaginase regimen of TOTXVI. The S/HR patients randomized to the high dose PEG-asparaginase regimen are expected to derive considerable benefit in terms of improved distribution of CCR and so we are willing to accept a higher rate of DVT in

these patients, 20%. Beginning with the eleventh patient experiencing an episode of DVT prior during continuation therapy we will estimate the cumulative incidence function and if the 95% lower confidence bound is greater than 17.50%, we will recommend that accrual cease. Few deaths or relapses (competing events) are expected from initiation of continuation therapy to week 35 so the following table based on the binominal distribution shows approximate stopping criteria.

Approximate Stopping Rule for Episodes of DVT S/HR Risk: Continuation Therapy through Week 35

N	# Episodes	% Episodes	95% UB	Decision
50	13	26.00%	16.2%	Go
50	14	28.00%	18.6%	Stop
100	24	24.00%	17.13%	Go
100	25	25.00%	18.04%	Stop
150	34	22.67%	17.16%	Go
150	35	23.33%	17.76%	Stop

18.1.3 During entire therapy: disseminated fungal infections

The same as in TOT-XV trial, we will monitor the incidence of disseminated fungal infections. We will monitor against a baseline rate of 5% and will suggest modifying the study if there is statistical evidence that the disseminated fungal infection rate exceeds 10%. The overall significance level will be 0.0567 with a power of 0.9967 to detect the unacceptable higher level.

Only the first event in a given patient will be counted although we will closely monitor for repeated infections. Sample size (denominator) is the number of patients either who have recovered from the second re-induction or developed visceral fungal infection before recovery from second reinduction. The numerator is the number of the first events among all monitored patients (i.e. those in denominator). Accrual will be suspended if the number of events (numerator) is equal to or exceeds that corresponding to each sample size.

Interim analysis	Sample size (patients)	Modify if # patients with disseminated fungal infections ≥
1	30	6
2	60	9
3	120	14
4	180	18
5	240	21
6	300	28
7	360	31
8	420	33
9	480	35

18.1.4 Decrease of PEG-asparaginase dose in the Higher Dose PEG-asparaginase regimen.

Once any of the stopping conditions for the monitoring period in the Continuation phase is met, we will carefully examine and analyze all the toxicity incidences. Treatment outcome (CCR, EFS and survival) will **not** be analyzed simply due to a stopping criteria having been met. Certainly if there has been a recent planned analysis of outcome, the DSMB may consider that information in formatting their recommendation. Cumulative incidence functions of the concerning toxicity for the two treatment regimens will be compared for evident that the monitored toxicities are more prevalent in the 3500 units/m^2 regimen. We will also consider whether the upper confidence bounds for each the two regimens exceed what were postulated to be acceptable. If stopping criteria are exceeded for both regimens, which is not expected, consideration will be given to stopping the trial. If only the higher dose regimen has excessive toxicity, then consideration will be given to reducing the dose of PEG-asparaginase to 3000 units/m^2 and to continue the randomization.

Assuming that the protocol is amended to reduce the dose of PEG-asparaginase, the randomization would continue and the final analysis and any subsequent interim analyses of the primary study question would be stratified by patients randomized prior to and post the protocol amendment. All patients who were randomized at the time that the high dose of PEG-asparaginase was 3500 units/m^2 will be included in the in the "prior amendment" stratum; all patients randomized after the PEG-asparaginase dose was reduced to 3000 units/m^2 will be included in the "post amendment" stratum. Note that the five original strata are maintained within this umbrella stratification with no change in the randomized sequence of randomized blocks; so the randomization between higher dose PEG-asparaginase and conventional dosing will remain balanced. Zelen's randomization scheme with block sizes of 4 will be used. Thus, the absolute worst case for imbalance between high dose PEG-asparaginase and conventional dose PEG-asparaginase within either amendment defined strata is 10 patients. Certainly this imbalance is unlikely to occur as independently in each stratum, randomization is controlled by random sequences of random blocks of size 4. Thus any imbalance that may occur should be small and thus will have little effect on the statistical power of the trial.

The above plan admits the use of all data to address the primary study question and avoids having to made ad hoc decisions as to whether the trial should be amended to start all over again. Even patients who experience toxicities, which are manageable, may still benefit from the treatment and toxicities should not be a reason for excluding them from the primary analysis. Furthermore, the proposed futility analysis, discussed below, would likely stop the trial if, in fact, 3000 mg/m^2 is not as effective as has been hypothesized for the high dose PEG-asparaginase regimen. Furthermore, realistically if the 3500u/m2 dose is too toxic then the monitoring boundaries will be expected to be reached relatively early during the trial, at which time few CCR/EFS-related events will have occurred; thus the toxicity-stopping time point is not likely to occur at a time that statistically meaningful comparisons of outcome can be conducted and interpreted. Therefore, we do not plan to perform any analysis of outcome due to early excessive toxicity. Clearly if interim analyses of outcome have already been conducted, then those results would be available for the DSMB when they consider and advise regarding any observed statistical stopping criteria for toxicities.

Toxicity monitoring rules would be reinitialized with this major change in therapy and if 3000 units/m^2 subsequently proves to be too toxic then the trial would likely be closed, as the primary research question would no longer be viable.

If toxicity is excessive in both treatment regimens, serious consideration as to whether the study should continue using PEG-asparaginase. This event would likely result in permanent closure of the trial or at the very least a major change in the treatment regimens.

18.2 Primary Objective

The primary objective of this study is to compare the distributions of continuous complete remission of patients randomized on the first day of continuation phase to receive higher dose of PEG-asparaginase or to receive conventional dose (2,500 units/m^2).

Design: This study will be conducted by an open-label, randomized trial. Participating patients will be randomized to receive either high dose PEG-asparaginase or conventional dosing of 2,500 units/m^2 PEG-Asparaginase per dose during the continuation phase.

The randomization will be stratified by several factors based on the following consideration: Low risk and standard/high risk patients receive different treatments in the continuation phase; there have been reports that children with T-cell ALL benefit more from asparaginase treatment than children with B-lineage ALL; and Day-19 MRD positivity (≥0.01%) is an important prognostic factor on TOTXV.

The following table shows the 5-year CCR rate since continuation in each stratum on TOTXV (as of 4/5/2007). Patients with no Day-19 MRD results are excluded.

Strata	n	5-years CCR since continuation Estimate ± SE (%)
LR	152	96.1 ± 3.5
SR&T-cell&MRD19+	27	73.5 ± 21.8
SR&T-cell&MRD19-	19	88.9 ± 13.3
SR&B-cell&MRD19+	63	77.2 ± 11.1
SR&B-cell&MRD19-	27	76.4 ± 15.1
HR	14	39.3 ± 21.6

Thus, the randomization will be stratified first by the risk status for the continuation phase (Low, Standard, High; see Section 4.0), then within Standard risk by lineage (T-lineage, B-lineage) and further within the T- lineage by Day-15 MRD level (<0.01% vs. ≥0.01%), creating 5 strata.

The randomization will occur on the starting day of the continuation phase, at which time all information necessary for performing the randomization should be available. In the rare

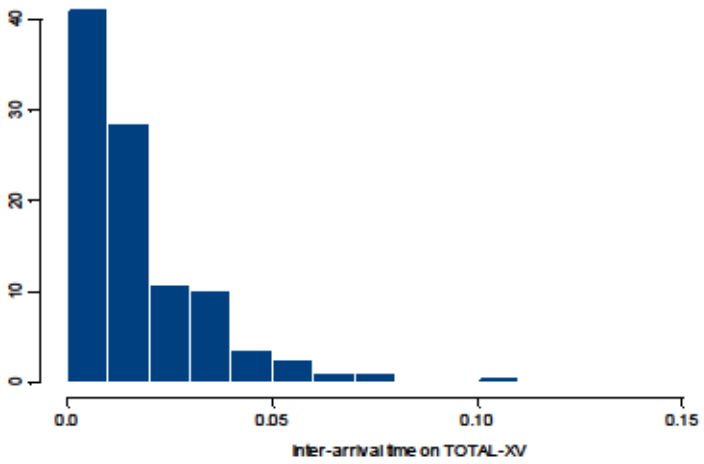
case that immunophenotype and/or Day-15 MRD is not available, we will make the following assumptions: If immunophenotype is unknown at the time the randomization is to be executed, then it will be assumed B-lineage. If Day-15 MRD is unknown at the time of randomization, then it will be assumed negative (<0.01%). Past experience indicate that few patients will fall into these unknown categories.

Note only eligible patients who consent and are randomized are evaluable for this objective. The study endpoint is duration of continuous complete remission post randomization. Duration of CCR will be measured from the randomization date to the date of initial failure for patients who fail. Failure includes the following endpoints:

- relapse in any site
- second malignancy
- death during remission

Duration of CCR will be measured to the date of last contact for patients who are failure free at the time of analyses.

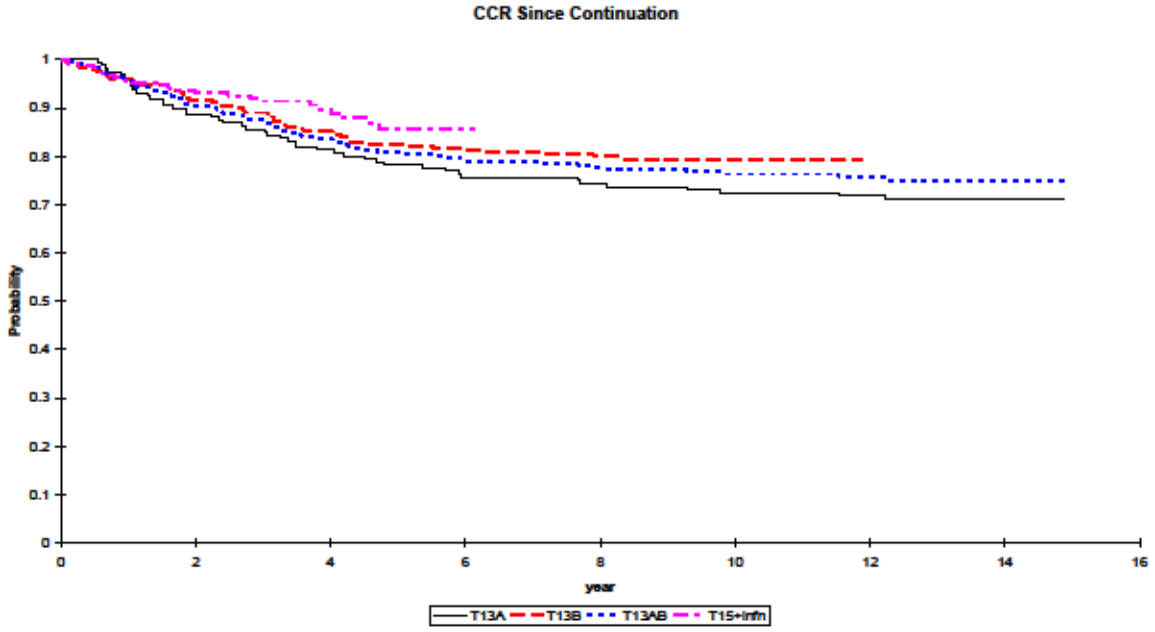
Accrual: The distribution of the length of the time interval between two consecutive enrollments (the inter-arrival time) on TOTXV (excluding patients treated at Cook's) is displayed in the following figure, in which the time unit is year. This distribution will be used in the Monte Carlo estimation of statistical power. The mean inter-arrival time on TOTXV is estimated 0.0181 year; the standard deviation of inter-arrival time is estimated 0.0195 year. Thus the estimated enrollment frequency is approximately 56 patients per year. We expect the enrollment frequency in TOTXVI will be similar and thus the accrual goal will be achieved in 8 to 9 years. The total accrual time will be assessed by the simulation study in the power consideration section.



Historical data: The following table and figure show respectively the 5-year KM estimates of duration of CCR from the beginning of continuation therapy in TOTXIII A, B, and TOTXV (excluding Cook's patients) studies. Because TOTXV did not include infants, outcome data of the infants on the INFANT99 protocol who enrolled after TOTXV was opened were added to TOTXV outcome to impute the scenario of including

infants on TOTXV. Patients who failed to enter the continuation phase were excluded. Note in this table there are 16 infants and 11 TOTXV patients without Day-19 MRD are included for "T15+Infn."

STUDY	Total Achieving CR	# Patients Entered Continuation	# Infants Entered Continuation	5-Year CCR Rate From Initiation of Continuation Estimate ± SE (%)
T13A	162	160	4	78.1 ± 3.3
T13B	242	237	10	82.6 ± 2.5
T13A & B combined	404	397	14	80.8 ± 2.0
T15+Infn (as of 4/5/07)	375	329	16	85.6 ± 4.4



The following table shows the 5-year duration of CCR rates from the beginning of continuation therapy of these previous studies by risk strata, where infants described above were added to the TOTXV Standard/High risk stratum.

RISK ARMS	n	5-Year CCR Rate From Initiation of Continuation Estimate ± SE (%)
T13A S/H	150	76.7 ± 3.4
T13B S/H	123	76.4 ± 3.8
T15 S/H with infants (as of 4/5/2007)	171	76.1 ± 7.3
T13A L	10	100 ± 0.0
T13B L	114	89.3 ± 2.9
T15 L (as of 4/5/2007)	158	96.2 ± 3.4

TOTXIII A and B combined provide an adequate description of the CCR distribution in recent studies and will be used as preliminary data for power consideration, as described below. Because TOTXV has not stopped accrual and has relatively short follow-up, it will be used secondarily for power consideration.

Analysis: The final analysis of the primary objective will be three years after the last patient is randomized. The primary analysis is a Mantel-Haenszel (stratified log-rank) test comparison of the distributions of CCR for patients randomized to the two treatment arms, using the strata defined for randomization. Power consideration for this analysis is given below. Kaplan-Meier (KM) estimates of the distribution of CCR for each treatment arm will be provided.

Two interim analyses, one at the end of year 3 and one at year 6.5 are planned. The analyses will perform two-sided Mantel-Haenszel (stratified log-rank) test comparison of the distributions of CCR for patients randomized to the two treatment arms, using the strata defined for randomization and the statistical significance level α=0.005. A futility analysis as detailed below will be conducted as part of the 6.5 year interim analysis if the comparison at the 0.005 level is not statistically significant. Due to the two planned interim analyses, the final analysis will be conducted at α=0.045.

Subset analyses comparing the two arms within strata may be conducted as well, using the log-rank test. These subset analyses will have limited statistical power to detect differences and will be interpreted with extreme caution as descriptive. KM estimates of the distribution of CCR by treatment arm within each stratum will be provided.

Power consideration: Here power consideration is developed based on the unstratified test. Overall, the 5-year CCR rate from initiation of continuation therapy in TOTXIII A & B combined and TOTXV plus infants are 80.8 ± 2.0% and 85.6 ± 4.4%, respectively; the

5-year CCR rate from initiation of continuation therapy in the TOTXV low risk stratum is 96.2 ± 3.4%. We believe that the effect size in each risk-based stratum as shown below is clinically meaningful, based on the considerations: (1) previous front-line ALL trials have shown that a therapy augmented with asparaginase could improve 5-year event-free survival by 20%;[1] and patients receiving less asparaginase therapy could have 5-year EFS reduced by 17%;[2] (2) males will not be receiving the additional 26 weeks of continuation therapy on TOTXVI; (3) TOTXVI will include approximately 20 infants and (4) the follow up time of TOTXV is still short.

Postulated effect size in each stratum based on TOTXV

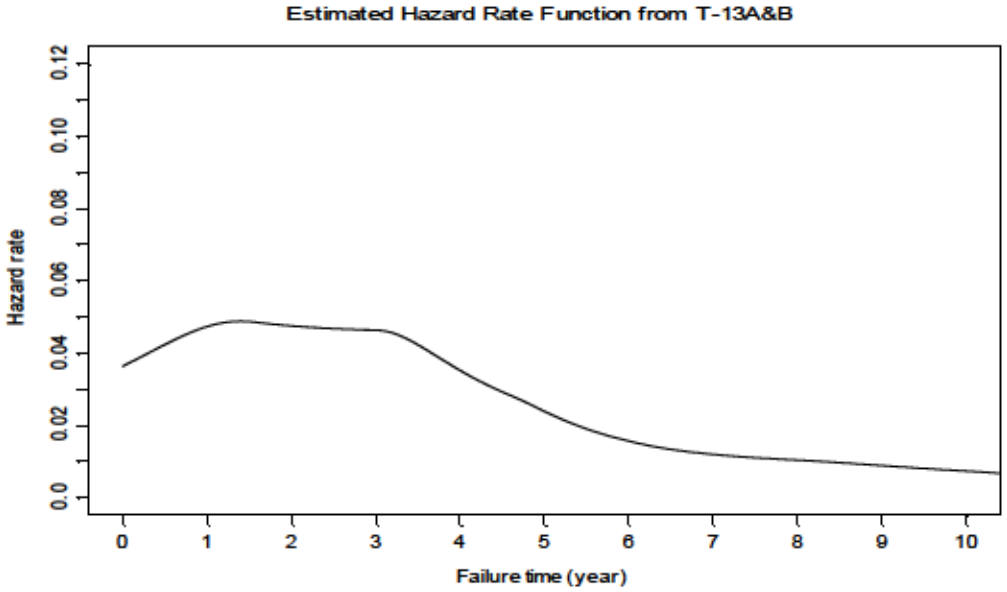
Stratum	n (%)	5-Y CCR since continuation Estimate ± SE (%)	Postulate 5-y CCR in the 2500u/m^2 arm (%)	Postulate 5-y CCR in the high-dose arm (%)	Weighted effect size
HR	14 (4.64)	39.3 ± 21.6	32	64	1.484
SR&T-cell&MRD19+	27 (8.94)	73.5 ± 21.8	66	92	2.324
SR&T-cell&MRD19-	19 (6.29)	88.9 ± 13.3	82	92	0.629
SR&B-cell	90 (29.80)	77.3 ± 9.2	73	92	5.662
LR	152 (50.33)	96.1 ± 3.5	94	98	2.012
Overall	302		81.579	93.722	12.021

The low risk group nonetheless contributes an appreciable amount of effect (2%, the third highest) to the overall effect size. Although the statistical power is mostly brought by the effects in the S/H risk groups, we choose to randomize the low risk patients as well in order to have an opportunity to fully understand PEG-asparaginase PK (in both intensively and less intensively treated patients), to evaluate their tolerance to higher dose of asparaginase, and to observe their long term outcome in the context of the TOTXVI therapy. As is shown below, we will have adequate statistical power to detect the postulated effect size in the entire cohort.

Thus for TOTXVI, we postulate overall the 5-year CCR rate post randomization will be approximately 82% in the 2500U/m^2 arm and 94% in the high dose PEG-asparaginase arm. The study will be powered to detect a 12% difference in the five-year CCR rate.

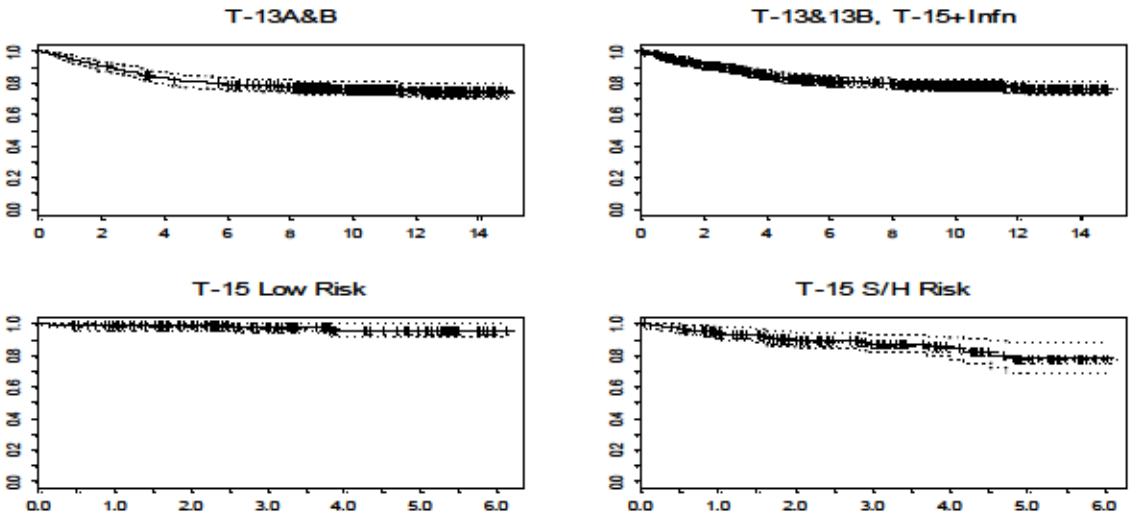
Considering the probability distribution of the failure times, the following figure shows a nonparametric estimate of its hazard rate function based on the CCR distribution from

TOTXIII A & B combined. This estimate is constructed via spline estimates of the quantile and quantile density functions of the CCR distribution and is a special case of the estimators investigated by Cheng.[136] The exponential distribution assumption of a constant hazard rate is clearly violated. As the hazard rate function is only approximately constant during years one and three, it is not really feasible to estimate this function with a sequence of piece-wise exponentials. We can estimate the statistical power for any given sample and effect sizes by simulation using nonparametric estimates obtained from the high quality preliminary data. **Here we estimate the power for 300 evaluable patients at the above-specified effect size.** Simulation also allows for obtaining estimates of other important trial parameters (e.g., the probability distribution of the total accrual time to reach the target of 300 evaluable patients) that are not available from conventional methods.

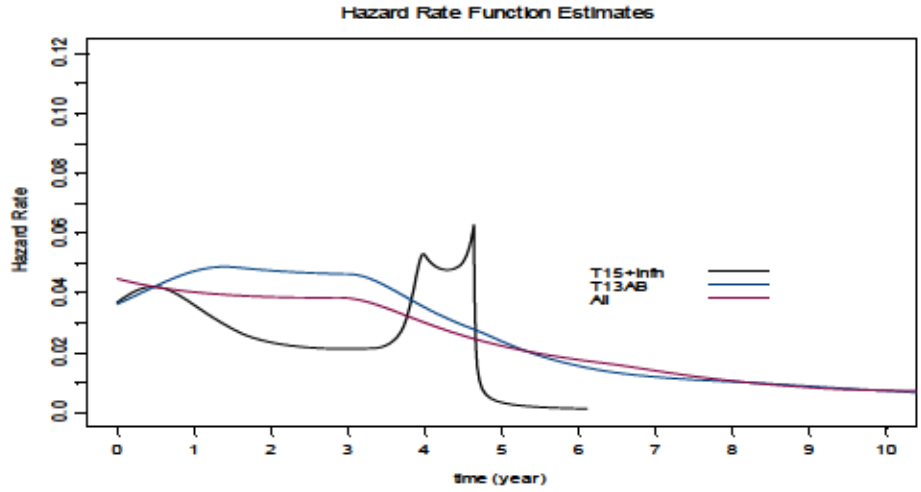


The statistical power of a two-sided log-rank test at the 5% significance level for the sample size of 300 and the postulated effect size is estimated by 1000 simulation runs. **The Monte Carlo estimate of the power is 0.85, with 95% confidence interval (CI) [0.83, 0.88]**. The estimated probability that more than 7 years of total accrual time will be required is 0.02 (95% CI [0.01, 0.03]), and the estimated probability of less than 6 years total accrual time is 0.24 (95% CI [0.21, 0.26]).

Considering to include the recent (4/5/2007) TOTXV outcome data as preliminary data for Monte Carlo power estimation as well, the following figures show KM estimates of the CCR survival curve with point-wise 95% CI (dotted lines) and tick marks indicating censoring, for TOTXIIIA and TOTXIIIB, TOTXV plus infants, TOTXV Standard/High risk, and TOTXV Low risk.



Excessive censoring within 6 years in TOTXV is noticeable, even in the low risk cohort; evident of relatively short follow up time. The following figure shows nonparametric hazard rate function estimates from TOTXIIIA, TOTXIIIB, and TOTXV with infants combined (All), TOTXV with infants, and TOTXIIIA&B. The irregular shape of the hazard rate function estimate for TOTXV is largely due to heavy censoring caused by lack of follow up.



Monte Carlo estimates of the statistical power of the log rank test based TOTXIIIA, TOTXIIIB, and TOTXV plus infants is 84%, with the 95% CI [0.81, 0.86].

Conservatively we next consider postulating the effect sizes based on the point estimates from the TOTXV data: 85.199% vs. 93.722% CCR at year 5 since continuation; see table below. **Based on TOTXIIIA and XIIIB preliminary data, the estimated power at 5% significance level is 0.791 (95% CI [0.766, 0.816]) if the sample size is increased to 420.** For this sample size the probability of more than 9.5 accrual is 0.054 (95% CI [0.040, 0.068]); the probability of less than 8.5 year accrual is 0.247 (95% CI [0.220, 0.274]).

Postulated effect size in each stratum based on point estimates from TOTXV

Stratum	n (%)	5-Y CCR since continuation Estimate ± SE (%)	Postulate 5-y CCR in the 2500u/m 2 arm (%)	Postulate 5-y CCR in the high-dose arm (%)	Weighted effect size
HR	14 (4.64)	39.3 ± 21.6	39	64	1.160
SR&T-cell&MRD19+	27 (8.94)	73.5 ± 21.8	73	92	1.601
SR&T-cell&MRD19-	19 (6.29)	88.9 ± 13.3	89	92	0.189
SR&B-cell	90 (29.80)	77.3 ± 9.2	77	92	4.470
LR	152 (50.33)	96.1 ± 3.5	96	98	1.007
Overall	302		85.199	93.722	8.533

Futility assessment at year 6.5: If the interim analysis at year 6.5 fails to detect a statistically significant difference at the 0.005 level (two-sided test) between the two PEG-asparaginase regimens, a futility analysis, as requested by the CT-SRC, will be conducted. We note that clinical trials that are designed with statistical power of 80% to detect a hypothesized effect have a probability of 0.20 (20%) of being "futile" even if the hypothesized effect size is true. In this context "futility" does not mean that the research hypothesis is not true but rather that at the final analysis no statistically significant difference is detected.

Thus, the goal of this futility analysis is to assess early evidence that the conjectured 8.53% effect size between high dose PEG-asparaginase and conventional dosing of PEG-asparaginase is too optimistic and continued randomization will with high probability result in a futile final analysis (i.e., final p-value>0.045). The simulation study indicates that at year 6.5 we will have observed approximately 37% of the total number of events expected at the planned final analysis. Given the goal and the limited amount of information expected to be available 6.5 year after initiation of randomization, we propose the following non-standard approach. This proposed futility analysis is not grounded in peer-reviewed published statistical research, but we believe that the method is valid and indeed at first glance it does have some necessary operating characteristics of a futility analysis. We fully expect to complete research on the proposed method within a year and at that time or with the first TOTXVI protocol amendment include the final futility analysis design and justification for CPSRMC consideration. If we are unable to justify the statistical validity of our method, we will switch to a standard approach.

We will conduct the futility analysis in a way that the final planned analysis will not be affected should the trial continue to that analysis. For this purpose we define "futility" as

the (conditional) event that the two-sided test in the final analysis is not statistically significant at the 0.045 level given that the null hypothesis of no difference is not rejected in favor of the one-sided alternative hypothesis that the high dose PEG-asparaginase regimen has superior distribution of CCR, at some yet to be defined significant level γ. We call the conditional probability of (failing to reject the null hypothesis by the two-sided test at the 0.045 level in the final analysis given failing to reject the null hypothesis in favor of the one-sided alternative at the significance level γ in the futility assessment) the *probability of futility* (PoFu(γ)), which we note is a function of γ.

After 6.5 years of randomization, we will declare the trial statistically futile, if we fail to reject, at the γ significance level, the null hypothesis of no difference against the one-sided alternative hypothesis that the high dose PEG-asparaginase regimen has the superior distribution of CCR.

The significance level γ is a parameter to be determined to ensure the futility assessment has the following desirable operating characteristics: (1) If the actual effect size is at least as postulated (i.e., 8.5%), then with probability greater than 0.80 the trial will continue; (2) If the actual effect size is very small (or even if the high dose PEG-asparaginase regimen actually is less effective), with high probability the trial will be declared futile and hence randomization is stopped. The value of γ_m that maximizes PoFu(γ) has these properties. Intuitively, the decision rule that stops the trial for failing to reject the null hypothesis at the γ_m significance level maximizes the probability of identifying when continued randomization and follow up is not likely to produce a statistically significant result.

The probability of futility and the probability of declaring the trial futile at various γ values and effect sizes are estimated by simulation using the same preliminary data as in the above power consideration. At the 8.5% effect size, the estimated probability of futility is maximized at γ=0.315 and PoFu(0.315)=0.539 (95% CI [0.517, 0.561]). This means that if the actual effect size is 8.5% and if we fail to reject the null hypothesis in favor of the one-sided alternative at the 31.5% level, then with 53.9% probability we will not reject the null hypothesis at the 0.045 level using the **two-sided** test in the final analysis. The probability of declaring futility (stopping the trial when it should have continued) when the true effect size is 8.5% for γ=0.315 is estimated to be 0.102 (95% CI [0.089, 0.115]). The following table shows the operating characteristic of the futility assessment using γ=0.315 at a few effect sizes (negative effect size means that the 5-year CCR of the high dose PEG-asparaginase regimen is actually lower by the given amount).

True effect size	Probability of futility PoFu(0.315) (95% CI)	Probability of declaring futility/stop (95% CI)
8.5%	0.539 ([0.517, 0.561])	0.102 ([0.089, 0.115])
4.0%	0.964 ([0.956, 0.972])	0.378 ([0.356, 0.399])
2.0%	0.908 ([0.895, 0.921])	0.773 ([0.755, 0.791])
-4.0%	0.820 ([0.803, 0.837])	0.879 ([0.865, 0.893])

So if the true effect size is 4.0% (2.0%) in favor of the high dose PEG-asparaginase regimen, then with probability 0.378 (0.773), the trial will stop at 6.5 years. With probability 0.622 (0.237) the trial will continue and the probability that the final two-sided

test will be statistically significant is 0.036 (0.092) given the trial continues to the point of final analysis. Note that the 2.0% effect size has a higher probability of resulting in a statistically significant finding due to the final test being two-sided; that is sometimes the conventional dose arm will have a superior distribution of CCR.

Thus, we will declare the trial statistically futile to continue and stop accrual based on the 6.5 year analysis, if the test of the null against the one-sided alternative that the high dose PEG-asparaginase regimen has the better distribution of CCR is not significant at the 31.5% level.

The above plan for the futility analysis will either be justified by solid statistical science including assessment of the operating characteristics within a year of protocol initiation or the design will be replaced with a more standard approach.

In summary, to have acceptable power to detect a reasonable and clinically meaningful improvement in the distribution of duration of CCR for the high-dose PEG-asparaginase regimen, we will accrue 531 patients with the expectation that 420 will be randomized. This number of patients will provide approximately 80% statistical power ($\alpha = 0.05$; two-sided test) to detect an improvement of 8.53 % in the 5-year CCR for the high-dose regimen as compared to the standard-dose regimen. Zelen's randomization algorithm, as implemented in the Department of Biostatistics since 1992, will be used with a blocking factor of 4 in each stratum. Two interim analyses for efficacy are planned after 3 and 6.5 years after initiation of randomization and a futility analysis will also be conducted at the 6.5 year mark. The study duration is estimated to require approximately 8.6 years of accrual and the final analysis of the primary objective will be 3 years after the last patient is randomized. Thus the total study duration will be 11-12 years.

18.3 Therapeutic Objectives

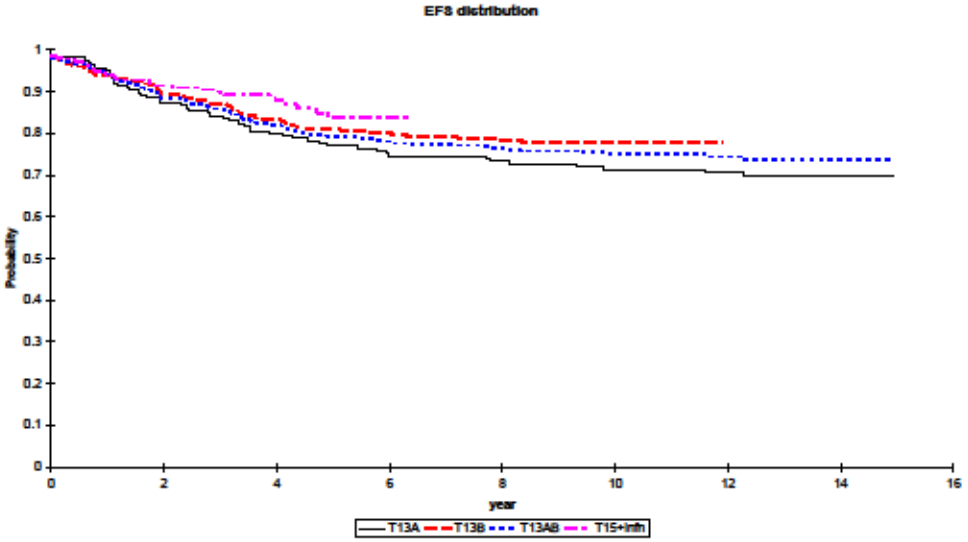
To estimate the event-free survival and overall survival of children with ALL who are treated with risk-directed therapy and to compare the EFS results of TOTXVI with that of TOTXV.

These analyses will only be conducted at the time the planned interim analyses comparing the two continuation regimens are completed. That is after 3 and 6.5 years of accrual.

EFS will be measured from the date of complete response to the date of initial failure for patients who fail. Failure includes the traditional endpoints of failure to achieve a complete remission, relapse in any site, secondary malignancy, and death during induction or remission. EFS time will be measured to the date of last contact for patients who are failure free at the time of analysis. The EFS time is defined to be zero (0) for patients who die during induction therapy or fail to achieve a complete remission.

The following table and figure show respectively the 5-year and EFS distribution estimates of TOTXIIIA, TOTXIIIB, and TOTXV plus infants.

STUDY	n Total	n Infants	5-Year EFS Estimate ± SE (%)
T13A	165	5	77.0 ± 3.3
T13B	247	10	80.9 ± 2.5
T13A & B combined	412	15	79.3 ± 2.0
T15+Infn (as of 4/5/07)	381	16	83.7 ± 4.2



The following table shows the 5-year EFS of these previous studies by risk strata, where infants described above were added to the TOTXV Standard/High risk stratum.

RISK ARMS	n	5-Year EFS Estimate ± SE (%)
T13A S/H	151	76.8 ± 3.4
T13B S/H	126	76.1 ± 3.8
T15 S/H with infants	194	75.8 ± 6.6
T13A L	11	100 ± 0.0
T13B L	116	89.5 ± 2.9
T15 L	181	95.7 ± 3.4

Kaplan-Meier estimates of EFS and survival will be provided and compared historically with those of TOTXV, TOTXIIIA and TOTXIIIB. All eligible patients entered on TOTXVI will be included in these analyses, as appropriate. Infants will be excluded from the Total XVI for comparisons with TOTXV as only children at least one year of age at diagnosis were treated on Total XV. Comparisons will be made both unstratified and stratified by TOTXVI defined risk groups. Some assumptions will be necessary as not all

factors defining risk on TOTXVI will be available for all patients treated on TOTXIIIA and B. The comparisons with TOTXV define the standards with which the final results of this study should be compared to assure continued progress towards the St. Jude goal of improving the cure rate of childhood ALL. The following table shows the recent EFS in TOTXV (Data frozen on 4/5/2007).

EFS in TOTAL- XV					
Year	Risk	Fail	Cens	Prob	SE
0	365	6	0	0.984	0.007
1	359	11	52	0.950	0.012
2	296	7	67	0.925	0.017
3	222	3	61	0.910	0.022
4	158	3	53	0.887	0.029
5	102	4	36	0.846	0.042
6	62	0	50	0.846	0.092
7	12	0	12	0.846	0.332

These comparisons are necessary to assess the improvement in outcome that the risk-based TOTXVI therapy for ALL has over the TOTXV therapy.

Since male patients will receive shorter durations of therapy on TOTXVI than on TOTXV, we will specifically compare the distributions of EFS for male patients between these two trials. Also we will compare the distributions of CCR from the point that TOTXVI therapy for male patients ceases.

To study whether intensifying induction, including fractionated cyclophosphamide and thioguanine, in patients with day 15 MRD $\geq$ 5%, will result in improved leukemia cytoreduction in this subgroup compared to TXV.

These analyses will only be conducted at the time the planned interim analyses comparing the two continuation regimens are completed. That is after 3 and 6 years of accrual.

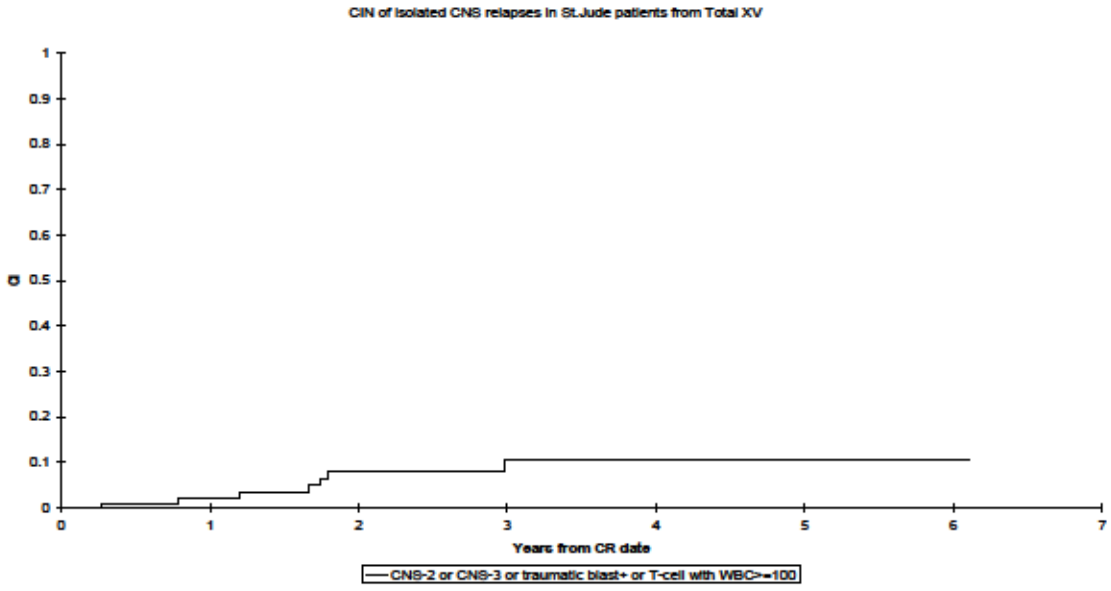
As of 3/21/07, TOTXV data indicate that 45/439=10.5% of patients had Day-19 MRD of at least 5%. Among this subgroup, 9/45=20% achieves negative MRD (<0.01%) at Day 46, and 80% remains MRD positive at the end of remission induction (95% CI [0.683, 0.9168]). The analysis will first compare the proportion of TOTXV patients who had Day-19 MRD$\geq$5% with the proportion of patients on TOTXVI with Day-15 MRD$\geq$5%. Next we will compare the proportion of patients with Day-15 MRD$\geq$5% on TOTXVI who are Day-42 MRD positive with the proportion of Day-46 MRD positives in the corresponding subgroup in TOTXV by Fisher's exact test. With the accrual of approximately 350 patients, we conservatively anticipate there will be at least 34 patients who have Day-15 MRD $\geq$5%. The following table shows the power of two-sided exact test at the 0.05 significance level for several postulated proportions of Day-42 MRD positive patients and sample sizes from each trial.

N.T15	Prop.T15	N.T16	Prop.T16	Power
45	0.68	34	0.50	0.34
45	0.80	34	0.50	0.77
45	0.92	34	0.50	0.99
45	0.80	34	0.55	0.62
45	0.92	34	0.55	0.97
45	0.80	34	0.60	0.44
45	0.92	34	0.60	0.92

To assess whether intensification of CNS directed intrathecal and systemic chemotherapy will improve outcome in patients at high risk of CNS relapse.

These analyses will only be conducted at the time the planned interim analyses comparing the two continuation regimens are completed. That is after 3 and 6 years of accrual.

The TOTXV experience has confirmed that CNS-2 and CNS-3 status, traumatic lumber puncture with blast, and T-lineage with WBC more than 100,000 remain highly associated with CNS relapse. On Total XV, 98 of 359 (23.3%) patients fell into this category of being high risk for CNS relapse. Of particular interest here is isolated CNS relapse. On TOTXV, as of 4/5/2007 all CNS relapses in this subgroup are isolated, the cumulative incidence of which is given below. The 5-year cumulative incidence of isolated CNS relapse is 10.4%±4%.



For TOTXVI, cumulative incidences of isolated CNS relapse as well as combined CNS relapse among all patients, and among the high-risk CNS patients (defined as having any of CNS-2 or 3, traumatic LP with blast, or T-lineage with WBC ≥100,000) will be estimated by the Kalbflisch-Prentice estimator.[137] The cumulative incidence functions of isolated and combined CNS relapse among these high-risk CNS patients on TOTXVI will be compared to the corresponding group on TOTXV using Gray's test.[138]

18.4 Exploratory Pharmacologic Objectives

To identify pharmacogenetic, pharmacokinetic and pharmacodynamic predictors for treatment-related outcomes in the context of the systemic therapy used in the protocol.

This exploratory objective is in concert with the biological objective "To identify new prognostic factors by applying developing technologies to study patient material (e.g., stored plasma, serum, cerebrospinal fluid, and normal and leukemic cells." Statistical considerations for this aim will be given in Section 17.5.

To compare the pharmacokinetics and pharmacodynamics of PEG-asparaginase given in higher dose of (3,500 units/m^2 or 3,000 units/m^2) versus those of PEG-asparaginase given in conventional dose (2,500 units/m^2) in the continuation phase.

Comparison of a PK/PD measurement at a fixed time point between the two PEG-asparaginase arms will be done by the Wilcoxon rank-sum test. Comparison of a longitudinal PK/PD measurement will be done by general linear models (GLM) for repeated measures to account for intra-patient correlation; such a model will include the treatment arm, a patient random effect, and possibly other covariates as main effects as well as interaction effects by these factors when deemed as appropriate. For comparison purposes symmetrizing and variance stabilizing transformations may be applied to the PK/PD measurements if necessary, to make the data better adhere to the model assumption of normal distribution for the response variable.

18.5 Exploratory Biological Objectives

To determine the prognostic value of day 8 levels of minimal residual disease in peripheral blood.

Cumulative incidence hazard regression modeling[139] will be used to model the cause-specific failures of hematological or combined hematological relapses (including failures to achieve remission, for which time to event is set to zero) as a function of Day-8 MRD level, along with other known prognostic factors as covariates. The Day-8 peripheral blood MRD levels will be treated in two ways: as a continuous variable, and categorized into two or three categories; for example 0% to <1% vs. 1% to <10% vs. 10% and more.

To genotype natural killer (NK) cell receptors and measure their expressions at diagnosis and before reinduction, and to associate these features with treatment outcome.

For this explorative objective, associations of genotypes (or gene expressions) with long-term treatment outcomes will be explored by failure time models[139]; associations of the genotypes with categorical clinical outcomes (e.g., MRD status at the end of induction) will be analyzed by contingency tables and Chi-square tests; associations of gene expressions with categorical clinical outcomes will be analyzed by rank-based procedures such as the Wilcoxon test.

To identify new prognostic factors by applying developing technologies to study patient material (e.g., stored plasma, serum, cerebro-spinal fluid, and normal and leukemic cells).

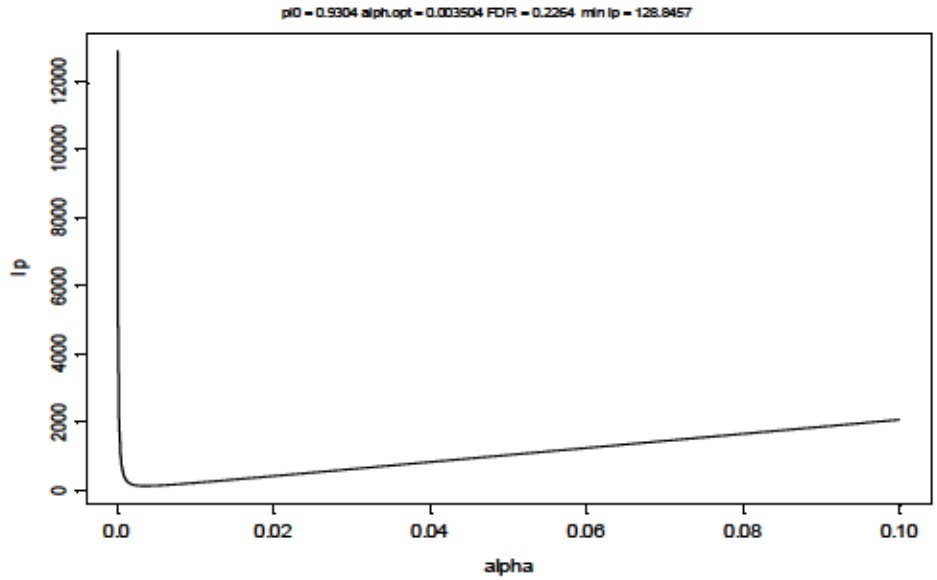
This is an exploratory objective. Biomarkers to be considered may come from several sources including (but not limited to) candidate genes and developing technologies such as mRNA expression arrays, exon expression arrays, SNP arrays, array CGH, and proteomics. The basic approach to this objective will be genotype-phenotype association studies and genome-wide association analysis (GWA). Usually, the genotype will reflect germline DNA variation, RNA transcript variation ("functional genomic variability), or acquired genomic variation (from comparisons of blast and germline DNA variation). Of course, both RNA variation and acquired genomic variation can also be considered phenotypes that are partly a product of germline variation themselves. We have ample experience in experimentation and analyses for genome-wide and candidate gene approaches[2;10;12;52-57;59;61-65;140]

Statistical methodologies for GWA and genotype-phenotype association analyses based on candidate genes recently developed in this institution and elsewhere will be applied to the data analyses for this aim.[141-143] Although our analyses carefully evaluate false discovery rate[144] and when necessary, use permutation techniques for assigning "p values" to associations, the primary "validation" of genotype/phenotype associations is dependent upon replication in multiple clinical studies, biological plausibility and/or laboratory model verification. Therefore, statistical tests are only a part of the evaluation of genotype/phenotype associations. We next illustrate the magnitude of false positive and false negative errors that we anticipate for these studies by two types of studies: haplotype-phenotype association and microarray gene expression profiling, after a consideration for power of testing candidate gene polymorphisms.

Power of testing candidate gene polymorphisms. An example for the power to evaluate a candidate genotype and a dichotomous phenotype (e.g. MRD vs. no MRD) is given here. An *IL15* SNP (rs ID # A-4264519) observed in one of our genome-wide analyses of germline polymorphisms related to end-of-induction MRD status has a minor allele frequency of ~ 10%. On the SJ cohort in the TT genotype 18.98% (0.1898) were MRD positive whereas in the TC/CC genotype 37.25% (0.3725) were MRD positive. We will test whether this polymorphism is similarly associated with MRD status (end of induction) in TOTXVI. With a sample size of 400, at the 0.05 significance level there will be 82.78% power to detect the difference of 0.1827 (18.98% vs. 37.25%) observed in the genome-wide study.

Haplotypes and a continuous variable phenotype. This is illustrated for a minimum sample size of 300, a total of 4-10 major haplotypes, and the assumption that the smallest number of individuals in the most rare genotype/haplotype be 10% or n = 30. For statistical power we conservatively consider two less favorable sample size and effect configurations. If there are ten groups with respective sample sizes 10, 10, 20, 20, 40, 40, 60, 60, 20, 20, and the means (for example, for a continuous variable reflecting toxicity, such as homocysteine level) in the first five haplotype groups differ from the overall mean by 2, 2, 2, 2, and 1 times within-group standard deviation, respectively, there will be 81% power to detect this difference at the 5% significance (one-way ANOVA).

Anticipated false discovery rate. To illustrate the magnitude of false positive and false negative errors we may encounter in a genome-wide association analysis (GWA) such as gene expression profiling, we show the average power (AP) and anticipated false discovery rate (aFDR) [145] based on a data set used in a recent study of gene expression, MRD and relapse of ALL.[12] This data set consists of log-transformed signal intensities from 99 Affymetrix U133A GeneChips run on the diagnostic bone marrow blasts of 99 TOTXIIIB patients and their time to ALL relapse (failure) or last follow up. Association between the expression of each probeset and failure time is tested by cumulative incidence hazard rate regression,[139] creating 22276 P values, one for each probeset. The following figure shows the profile information criterion[141] (Ip) computed for this set of P values.



The proportion of probesets (gene expressions) not associated with relapse is estimated pi0=93%, the "optimal" P-value cutoff (alpha.opt) by Ip that balances the levels of false positive and false negative errors is 0.0035, and at this alpha level the FDR is estimated 22.64%.

The following table shows, based on the above set of P values, the anticipated number of false positives (aNFP), true positives (aNTP), and aFDR in one gene expression profiling analysis for several combinations of the proportion of non-associative genes (π), number of non-associative genes (m0), number of associative genes (m1), and alpha levels. The total number of probesets is m0+m1=22276.

		α=0.001	α=0.003	α=0.005
π=90%	aNTP	146	227	292
m0=20048	aNFP	20	60	100
m1=2228	aFDR (%)	12.08	20.92	25.57
π=93%	aNTP	145	225	288
m0=20717	aNFP	21	62	104
m1=1559	aFDR (%)	12.48	21.62	26.43
π=99%	aNTP	144	222	223
m0=22053	aNFP	22	66	110
m1=223	aFDR (%)	13.28	23.02	28.13

Validation inference for gene expression. We plan to validate on TOTXVI a number of candidate genes identified from the previous gene expression profiling analyses of TOTXV and TOTXIII data. To validate these gene expressions for their association with the phenotypes of interest (e.g. gene expressions related to MRD and relapse; see Section 2), two methods will be applied. First a gene-by-gene examination will be performed: The same analyses in the previous publications will be applied to the validation data (data collected on TOTXVI), and the genes of interest will be examined for their association with the phenotypes according to the test P values and whether the same directions of association are observed in the validation data and the previous analyses. It is important to realize that due to uncontrollable random variations the **observed** statistical significance (P values) of these genes in the previous studies is unstable and is unlikely to replicate – the test P value of each gene computed on the validation data can be either greater or smaller than that in the previous analyses. For this reason, we do not insist that the tests of these genes on the validation data have to reach equal or higher significance levels than in the previous analyses. Instead, we will test the null hypothesis that the P values of these genes from the validation data are completely randomly (uniformly) distributed between 0 and 1 against the alternative that they tend to cluster toward 0. This will be accomplished by a permutation test. Second, a recently developed method[146] will be applied to validate gene co-expression clusters generated from these candidate genes. A co-expression cluster for a candidate gene is a set of genes whose expression is highly correlated with the candidate gene. This method will also help to discover interesting genes that potentially carry similar biological functions or are in the same pathway with the candidate genes but did not meet the purely statistical significance criteria used in the previous analyses.

18.6 Exploratory neuroimaging objectives

These exploratory objectives involve case-control comparisons, analyses of the associations between a number of neuroimaging measurements generated at two time points (week 1 of re-induction I and week 120 of continuation) and neurocognitive tests done at the same time points, and analyses of the associations between the

neuroimaging measurements and the folate pathway genetic polymorphisms. Therapy differences as indicated by risk (S/H vs. L) and the intensity of IT therapy (e.g., number of ITs received before the MRI) will be taken as covariates (adjusting factors) in the models. Subset analyses within certain patient groups (e.g., S/H and L risk) may be performed as well. The analyses for a similar study on TOTXV resulted in about 4,000 statistical tests. We anticipate that a large number of statistical tests will be produced for this aim on TOTXVI as well. Adjustments for multiple tests will be performed using recent methods for estimation and control of the false discovery rate.[142,143]

To use quantitative MR measures (Diffusion Tensor Imaging and high resolution volumetric imaging) to assess differences in myelin and cortical thickness development in patients treated for ALL relative to healthy controls matched for age and gender.

The comparison will be performed for each MR time point (Week 1 of re-induction I and week 120 of continuation) separately. Possible longitudinal change will be visualized by statistical graphics. Neuroimaging measurements of myelin and cortical thickness between patients and age and gender matched normal healthy controls will be compared by two-sample t tests. Subset analyses within certain patient groups (e.g., S/H and L risk) may be performed as well.

To assess the impact of folate pathway genetic polymorphisms on myelin and cortical thickness development and neurocognitive performance.

Associations between the genetic polymorphisms and the imaging measurements and the neurocognitive measurements will be analyzed by a number of general linear models. The imaging and neurocognitive variables will be taken as dependent variables; the polymorphisms and clinical factors (such as risk and IT intensity) will be taken as covariates. Subset analyses within certain patient groups (e.g., S/H and L risk) may be performed as well.

To assess impact of frontal-parietal lobe myelin and cortical thickness development on neurocognitive performance in attention, working memory, fluency, visual-spatial reasoning and processing speed.

Descriptive statistics of the imaging and the neuropsychological test results will be provided. Cross-sectional analyses will be done using rank-based procedures and general linear models. General linear models accounting for repeated measures (by a patient random effect for example) will be applied to analyze the longitudinal data (two time points). Therapy differences as indicated by risk (S/H vs. L) and the intensity of IT therapy (e.g., number of ITs received before the MRI) will be taken as covariates (adjusting factors) in the models. Subset analyses within certain patient groups (e.g., S/H and L risk) may be performed as well.

To investigate the relationship between the change in imaging measurements and the amplitude of BOLD signal change and the volume of activated cortex will use a simple linear regression model. We will test to assess whether there is a significant linear relationship between the myelin disruption and BOLD activation in specific cortical

regions. Similar analysis will be carried out with change of cortical thickness as covariates and either the BOLD signal amplitude or the activated volume as dependent variable.

18.7 Exploratory Intestinal Microbiome Objective

This is a prospective stool banking study. Stool samples will be collected from all eligible patients and stored for further testing. Descriptive statistics such as median, range and percentages will be used as appropriate to measure the overall alteration in composition and population dynamics of gut microflora and to describe the frequency and severity of gastrointestinal illnesses during induction phase of chemotherapy and the subsequent 6 months. To take into account the differences in the induction, consolidation and continuation phases of chemotherapy, analysis of the data will be stratified based on the ALL risk classification (Low risk, Standard risk, and High-risk).

19.0 SAFETY AND ADVERSE EVENT REPORTING REQUIREMENTS

19.1 Reporting Adverse Experiences (AEs) and Deaths

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 <u>unanticipated problem involving risks to subjects or others</u> 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:

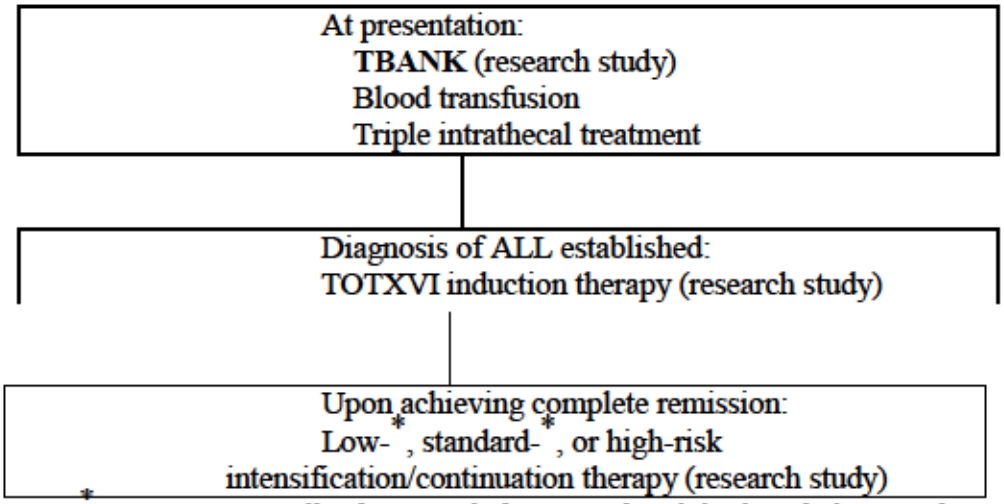
- Improperly staging a participant's tumor resulting in the participant being assigned to an incorrect arm of the research study;
- The theft of a research computer containing confidential subject information (breach of confidentiality); and
- The contamination of a study drug. Unanticipated problems 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.

20.0 INFORMED CONSENT PROCESS

20.1 Consent/assent at Remission Induction and Post Remission Therapies

The process of informed consent for TOTXVI 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 TBANK protocol (research study), blood transfusion (if needed), the first intrathecal therapy (if the diagnosis of leukemia is certain either by referral bone marrow smears or blood smears at SJCRH), and treatment with recombinant urate oxidase, if needed. After the diagnosis of ALL is established, we will invite the patient to participate in the TOTXVI protocol, as well as obtain standard medical consent for HIV testing. After the patient attains complete remission, we will seek informed consent for their participation in risk-directed intensification/continuation

therapy (low-, standard- or high-risk according to presenting features, treatment response and the level of minimal residual leukemia). Rare patients with low- or standard- risk leukemia may be reassigned to high-risk therapy due to persistently high minimal residual leukemia (see Section 5.3). These patients will be asked to re- consent with the high-risk therapy consent. The timeline for various informed consents is indicated in the figure below.



* *Rare patients will subsequently be treated with high-risk therapy due to the persistence of minimal residual disease, per protocol.*

Throughout the entire treatment period, participants and their parents receive constant education from health professionals at SJCRH 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 patients $\geq$14 years of age.

20.2 Consent at Age of Majority

The age of majority in the state of Tennessee is 18 years old. Research participants on active therapy must be consented at the next clinic visit after their 18th birthday. Participants, who have reached age of majority and have completed all protocol-directed therapy, will be re-consented with a separate consent specifically for this purpose (AOM consent). Participants, who reach age of majority after the 5 year protocol required follow-up, will be followed for survival and late effects as per the SJLTFU. A waiver for AOM consent will be requested for these participants at St. Jude.

If an affiliate site is located in a state where a different age of majority applies, that location must consent the participants according to their local laws.

20.3 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 will be 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.

21.0 DATA COLLECTION, MONITORING AND CONFIDENTIALITY

21.1 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 and her designees. 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 (bi-monthly) summaries of toxicity and protocol events will be generated for the PI and the department of Biostatistics to review.

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 patients 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 TOTXVI database). Missed doses do not include doses held or reduced for medical reasons (toxicity, illness) and will not be considered protocol deviations or violations.

21.2 Study Monitoring

Source document verification of eligibility for all SJCRH cases will be performed within two weeks of completion of enrollment. This will include verification of appropriate documentation of consent. Monitoring of timeliness of serious adverse event reporting will be done as events are reported in TRACKS.

Monitoring of this protocol is considered to be in the "moderate" risk category. The Monitoring Plan is outlined in a separate document from this protocol, but has been submitted for review and approval by the Clinical Trials Scientific Review Committee and the Institutional Review Board.

St. Jude affiliates and domestic collaborating study sites will be monitored on-site by a representative of St. Jude at intervals specified in the Data and Safety Monitoring Plan. International collaborators will be monitored according to the study-specific monitoring plan.

21.3 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.

22.0 REFERENCES

Reference List

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APPENDIX I: TREATMENT/EVALUATION CALENDARS

Remission Induction Pretreatment - See Pre-Printed Order sheets. Offer enrollment for institutional tissue banking protocol (TBANK).

1 LP, **ITMHA, LV rescue** **PRED (DEX for ETP¤)** **VCR** **DAUNO§** PE, CBC diff, Uric Acid, UA, Chem. 18[1, 2, 3, 4, 8]	2 **PRED (DEX for ETP¤)** PE, CBC diff, Uric Acid, lytes, Ca, P, Mg,	3 **PRED (DEX for ETP¤)** **PEG-ASP** PE, CBC diff, Uric Acid, lytes, Ca, P, Mg, [6, 7]	4 LP, **ITMHA(*), LV rescue** **PRED (DEX for ETP¤)** PE, CBC diff, Uric Acid, lytes, Ca, P, Mg CSF Studies*	5 **PRED (DEX for ETP¤)** PE, CBC diff, URA, lytes, Ca, P, Mg bili, SGPT	6 **PRED (DEX for ETP¤)** PE, CBC diff, URA, lytes, Ca, P, Mg,	7 **PRED (DEX for ETP¤)** CBC, diff, URA, lytes, Ca, P, Mg
8 **PRED (DEX for ETP¤)** **VCR** **DAUNO §** **(*)ITMHA**, LV rescue MRD CSF Studies*[3, 4]	9 **PRED (DEX for ETP¤)**	10 **PRED (DEX for ETP¤)**	11 **PRED (DEX for ETP¤)** **(*)ITMHA**, LV rescue CSF Studies*	12 **PRED (DEX for ETP¤)** CBC diff	13 **PRED (DEX for ETP¤)**	14 **PRED (DEX for ETP¤)**
15 LP, **ITMHA, LV rescue** †BM **PRED (DEX for ETP¤)** **VCR** **(Ω)PEG-ASP** **TMP-SMZ** MRD CSF Studies*[3, 4, 5]	16 **PRED (DEX for ETP¤)** **TMP-SMZ**	17 **PRED (DEX for ETP¤)** **TMP-SMZ**	18 **PRED (DEX for ETP¤)**	19 **PRED (DEX for ETP¤)** CBC diff	20 **PRED (DEX for ETP¤)**	21 **PRED (DEX for ETP¤)**
22 †BM **(*)ITMHA, LV rescue** **PRED (DEX for ETP¤)** **VCR** **CYCLO (α)** **TG (6MP for HET/deficiency)∞** **Clo/Cy/VP (ε)** **‡Dasatinib** TMP-SMZ CSF Studies* MRD	23 **PRED (DEX for ETP¤)** **ARA-C** **TG (6MP for HET/deficiency)** **CYCLO (α)** **Clo/Cy/VP (ε)** **‡Dasatinib** TMP-SMZ	24 **PRED (DEX for ETP¤)** **ARA-C** **TG (6MP for HET/deficiency)** **Clo/Cy/VP (ε)** **‡Dasatinib** TMP-SMZ	25 **PRED (DEX for ETP¤)** **ARA-C** **TG (6MP for HET/deficiency)** **Clo/Cy/VP (ε)** **‡Dasatinib**	26 **PRED (DEX for ETP¤)** **ARA-C** **TG (6MP for HET/deficiency)** **Clo/Cy/VP (ε)** **‡Dasatinib** CBC diff	27 **PRED (DEX for ETP¤)** **TG (6MP for HET/deficiency)** **‡Dasatinib**	28 **PRED (DEX for ETP¤)** **TG (6MP for HET/deficiency)** **‡Dasatinib**

29 Stop Prednisone (**stop Dex for ETP**) **TG (6MP for HET/deficiency)** **‡Dasatinib** TMP-SMZ	30 **ARA-C** **TG (6MP for HET/deficiency)** **‡Dasatinib** TMP-SMZ	31 **ARA-C** **TG (6MP for HET/deficiency** **‡Dasatinib** TMP-SMZ	32 **ARA-C** **TG (6MP for HET/deficiency)** **‡Dasatinib**	33 **ARA-C** **TG (6MP for HET/deficiency)** **‡Dasatinib** CBC diff, bili, 24-hour urine, Spot urine	34 **TG (6MP for HET/deficiency)** **‡Dasatinib**	35 **TG (6MP for HET/deficiency)** **‡Dasatinib**
36 **‡Dasatinib** TMP-SMZ	37 **‡Dasatinib** TMP-SMZ	38 **‡Dasatinib** TMP-SMZ	39 **‡Dasatinib**	40 **‡Dasatinib** CBC diff	41 **‡Dasatinib**	42 **‡Dasatinib** †BM (see consolidation calendar) MRD

Notes:

* IT methotrexate + hydrocortisone + cytarabine (ITMHA) all participants Day 1 and 15; (*) Participants with the following features will received additional triple intrathecal treatment on days 8 and 22: WBC $\geq$100 x 10^9/L at presentation, Presence of Philadelphia chromosome, *MLL* rearrangement or hypodiploidy. Participants with the following features will receive additional triple intrathecal treatment on days 4, 8, 11, and 22: T-cell ALL, t (1;19)/*E2A-PBX1*, CNS-3 status (i.e., $\geq$5 WBC/µL of CSF with blasts or cranial nerve palsy, CNS-2 status (< 5WBC/µL CSF with blasts, or traumatic status ($\geq$ 10 RBC/µL of CSF with blasts. Leucovorin rescue (5 mg/m^2/dose) PO will be given at 24 and 30 hours after each triple intrathecal treatment during induction. Follow plasma methotrexate levels (starting 24 hours after IT therapy and until levels become undetectable) in patients with renal dysfunction or extra fluid in third space, and rescue with leucovorin according to PharmD recommendation. CSF Studies to be done with each ITMHA treatment.

§ First dose of Daunorubicin may be delayed to day 2 in patients with or at high risk of tumor lysis syndrome. Second dose of daunorubicin could be delayed up to one week if clinically indicated.

Ω Second dose of PEG-asparaginase to be given as soon as MRD $\geq$/= 1% is confirmed and no later than day 21.

α Participants with Day 15 MRD <5%,will receive cyclophosphamide 1000 mg/m^2 IV Day 22 for 1 dose; Participants with Day 15 MRD $\geq$ 5% (excluding MLL+ Infants) will receive cyclophosphamide 300 mg/m^2 every 12 hours on Days 22-23 for a total of 4 doses.

ε Clofarabine/cyclophosphamide/etoposide in MLL+ Infants only. MLL+ Infants will continue prednisone but will not receive day 22 vincristine, thioguanine, or cytarabine in induction.

† All bone marrow samples for MRD; BM Evaluations required on Day 15, 22, and End of Induction (Day 38-42)

‡ Philadelphia positive (Ph+) participants only

π Dexamethasone for ETP immunophenotype only. See Section 5.2.1, 10 mg/m^2 PO days 1-21, 4 mg/m^2 days 22-24, and 2 mg/m^2 PO days 25-28

∞ TPMT HET/deficient patients will receive mercaptopurine as per TOTXV during induction instead of thioguanine. WT patients will receive TG, but will be closely monitored for signs or symptoms of VOD.

Special studies:

(1) CBC with diff required daily for first 7 days, and then at least weekly (2) blood chemistries (Chem 18 profile) (3)blood for asparaginase (obtain pre-asparaginase, day 8, day 15, and 2-3 additional samples after first dose but before any 2nd dose (4) CSF Asparagine – Section 10.3 collected prior to ITMHA treatments on Day 1 and 15 (and 8 if applicable). Additional sample required on day of diagnosis of sagital sinus thrombosis or DVT (5) Serum biomarkers, (e.g.., serum lipids and cortisol) (6) blood for DNA (%_DNA sample) (7) Red Blood cell Thiopurine concentration – Sections 8.0 and 10.2. (8) QCT for bone density

MRD studies:

Samples required for MRD studies during remission induction

Time point	B-lineage ALL	T-lineage ALL
Day 8	PB	PB
Day 15	BM	BM and PB
(Day 22 if MRD^+ on Day 15)	BM	BM and PB
Day 43	BM	BM and PB

PB; peripheral blood; BM: bone marrow

Consolidation

End of Induction-MRD response: BM aspirate will be performed around day 38-42 of remission induction when ANC has recovered to ≥300/mm^3 and platelet count to ≥50 x 10^9/L. If the date falls on a week-end or holiday, the procedure may be performed on the closest working day. MRD level will be determined in this bone marrow sample. Poor response will be defined as MRD level ≥ 0.01% lymphoblasts by either immunologic or molecular assay. If both assays are unsuccessful or the sample is inadequate, the MRD will be assumed to be negative. If the result should become available later and disclose a level ≥ 0.01%, the provisional low-risk case will then be classified as standard-risk or high-risk accordingly, and will receive subsequent HDMTX at a higher dosage (i.e., 5 gm/m^2).

1 ***ITMHA, LV rescue** **HDMTX #1** **6MP** **‡Dasatinib** CBC diff, [1, 2, 3, 4, 5, 6]	2 **6MP,** **‡Dasatinib**	3 **6MP** **LV ___ mg** **‡Dasatinib**	4 **6MP** **LV ___ mg** **‡Dasatinib**	5 **6MP** **‡Dasatinib**	6 **6MP** **‡Dasatinib**	7 **6MP** **‡Dasatinib**
8 **6MP** **‡Dasatinib** CBC diff	9 **6MP** **‡Dasatinib**	10 **6MP** **‡Dasatinib**	11 **6MP** **‡Dasatinib**	12 **6MP** **‡Dasatinib**	13 **6MP** **‡Dasatinib**	14 **6MP** **‡Dasatinib**
15 ***ITMHA, LV** **HDMTX #2** **6MP** **‡Dasatinib** CBC, diff, [2, 3, 7]	16 **6MP** **‡Dasatinib**	17 **6MP** **LV ___ mg** **‡Dasatinib**	18 **6MP** **LV ___ mg** **‡Dasatinib**	19 **6MP** **‡Dasatinib**	20 **6MP** **‡Dasatinib**	21 **6MP** **‡Dasatinib**
22 **6MP** **‡Dasatinib** CBC, diff	23 **6MP** **‡Dasatinib**	24 **6MP** **‡Dasatinib**	25 **6MP** **‡Dasatinib**	26 **6MP** **‡Dasatinib**	27 **6MP** **‡Dasatinib**	28 **6MP** **‡Dasatinib**
29 ***ITMHA, LV rescue** **HDMTX #3** **6MP** **‡Dasatinib** CBC, diff,[1, 2, 3]	30 **6MP** **‡Dasatinib**	31 **6MP** **LV ___ mg** **‡Dasatinib**	32 **6MP** **LV ___ mg** **‡Dasatinib**	33 **6MP** **‡Dasatinib**	34 **6MP** **‡Dasatinib**	35 **6MP** **‡Dasatinib**

36 **6MP** **‡Dasatinib** CBC, diff,	37 **6MP** **‡Dasatinib**	38 **6MP** **‡Dasatinib**	39 **6MP** **‡Dasatinib**	40 **6MP** **‡Dasatinib**	41 **6MP** **‡Dasatinib**	42 **6MP** **‡Dasatinib**
43 ***ITMHA, LV rescue** **HDMTX #4** **6MP** **‡Dasatinib** CBC, diff[1, 2, 3]	44 **6MP** **‡Dasatinib**	45 **6MP** **LV ___ mg** **‡Dasatinib**	46 **6MP** **LV ___ mg** **‡Dasatinib**	47 **6MP** **‡Dasatinib**	48 **6MP** **‡Dasatinib**	49 **6MP** **‡Dasatinib**
50 **6MP** **‡Dasatinib** CBC, diff	51 **6MP** **‡Dasatinib**	52 **6MP** **‡Dasatinib**	53 **6MP** **‡Dasatinib**	54 **6MP** **‡Dasatinib**	55 **6MP** **‡Dasatinib**	56 **6MP** **‡Dasatinib**

Notes:
**IT methotrexate + hydrocortisone + cytarabine, to be given on days 1, 15, 29, and 43; IT therapy should be given on same day as HDMTX administration (consult PI or PK if the IT and HDMTX become separated by more than 12 hours.*
‡ Only Ph+ patients
Physical Exam every 3-7 days during Consolidation. CBC with differential needed weekly.

Special studies:

(1) blood chemistries (Chem 18 profile); (2) CSF Studies with each ITMHA (3) HDMTX Pharmacokinetics – Section 10.1 (4) germline DNA/RNA (obtain sample at remission, before first and third HDMTX); (5) Asparaginase Antibodies/Activity – Section 10.3, serum and plasma obtained on days 1 consolidation (6) CSF Asparagine – day 1 consolidation (7) Serum biomarkers (e.g. lipids, cortisol) – Day 15 consolidation

TREATMENT SCHEMA and special laboratory tests during continuation therapy

	Standard/High Risk		Low Risk	
Week		Special Studies		Special Studies
1	DEX + DOXO + VCR + 6MP + PEG-ASP +‡Dasatinib		6MP + DEX + VCR	
2	6MP + ‡Dasatinib		6MP + MTX	
3	(*)6MP + PEG-ASP + ‡Dasatinib		(*)6MP + MTX	
4	DEX + DOX + VCR + 6MP + ‡Dasatinib		6MP + DEX + VCR	
5	6MP + PEG- ASP+‡Dasatinib		6MP + MTX	
6	6MP +‡Dasatinib		6MP + MTX	
7	*(†)Reinduction I (DEX + DOXO + VCR + PEG-ASP+‡Dasatinib)	[1, 2, 4, 5, 6, 7, 8, 9, 10, 11]	*(†)Reinduction I (DEX + VCR + DOXO + PEG-Asp)	[1, 2, 4, 5, 6, 9, 10, 11]
8	Reinduction I (DOXO + VCR ‡Dasatinib)	[1, 4, 6, 13, 14, 15]	Reinduction I (VCR)	[1, 4, 6,]
9	Reinduction I (DEX + VCR + PEG-ASP+‡Dasatinib)	[1, 4]	Reinduction I (DEX + VCR + PEG-ASP)	[1, 4]
10	6MP +‡Dasatinib		6MP + MTX	
11	DOX + VCR + 6MP + PEG-ASP+‡Dasatinib		6MP + MTX	
12	*6MP + ‡Dasatinib	[8]	*6MP + MTX	[8]
13	6MP + PEG-ASP+‡Dasatinib		6MP + MTX	
14	DEX + DOX + VCR + 6MP + ‡Dasatinib		6MP + DEX + VCR	
15	6MP + PEG-ASP+‡Dasatinib		6MP + MTX	
16	6MP + ‡Dasatinib		6MP + MTX	
17	*(†)Reinduction II (DEX + VCR + PEG-ASP+‡Dasatinib)	[1, 3, 4, 6, **11**]	*(†) Reinduction II (DEX + VCR + PEG-ASP)	[1, 3, 4, 6, **11**]
18	Reinduction II (VCR + ‡Dasatinib)		Reinduction II (VCR)	
19	Reinduction II (DEX + VCR + HD Ara-C + PEG-ASP+‡Dasatinib)	[1, 4]	Reinduction II (DEX + VCR + PEG-ASP)	[1, 4]
20	‡Dasatinib		6MP + MTX	
21	6MP + PEG-ASP+‡Dasatinib		6MP + MTX	
22	6MP+‡Dasatinib	[8]	6MP + MTX	[8]
23	6MP + PEG-ASP+‡Dasatinib		6MP + MTX	
24	CYCLO + ARA-C+‡Dasatinib		6MP + MTX	
25	*DEX + VCR + PEG-ASP+ Dasatinib		*6MP + DEX + VCR	
26	6MP + ‡Dasatinib		6MP + MTX	
27	6MP + PEG-ASP+‡Dasatinib		6MP + MTX	
28	CYCLO + ARA-C +‡Dasatinib		6MP + MTX	
29	*DEX +VCR+PEG-ASP+‡Dasatinib		(*)6MP+DEX + VCR	
30	6MP + MTX+‡Dasatinib		6MP + MTX	
31	6MP + MTX+‡Dasatinib		6MP + MTX	
32	CYCLO + ARA-C +‡Dasatinib		6MP + MTX	

	Standard/High Risk		Low Risk	
Week		Special Studies		Special Studies
33	*DEX + VCR+‡Dasatinib		*6MP +DEX + VCR	
34	6MP + MTX+‡Dasatinib		6MP + MTX	
35	6MP + MTX +‡Dasatinib		6MP + MTX	
36	CYCLO + ARA-C+‡Dasatinib		6MP + MTX	
37	*DEX + VCR+‡Dasatinib		(*)6MP + DEX + VCR	
38	6MP + MTX+‡Dasatinib		6MP + MTX	
39	6MP + MTX+‡Dasatinib		6MP + MTX	
40	CYCLO + ARA-C +‡Dasatinib		6MP + MTX	
41	*DEX + VCR+‡Dasatinib		*6MP +DEX + VCR	
42	6MP + MTX+‡Dasatinib		6MP + MTX	
43	6MP + MTX +‡Dasatinib		6MP + MTX	
44	CYCLO + ARA-C+‡Dasatinib		6MP + MTX	
45	*DEX + VCR+‡Dasatinib		(*)6MP + DEX + VCR	
46	6MP + MTX+‡Dasatinib		6MP + MTX	
47	6MP + MTX+‡Dasatinib		6MP + MTX	
48	CYCLO + ARA-C +‡Dasatinib		6MP + MTX	
49	*(†)DEX + VCR+‡Dasatinib		*6MP +DEX + VCR	
50	6MP + MTX+‡Dasatinib		6MP + MTX	
51	6MP + MTX +‡Dasatinib		6MP + MTX	
52	CYCLO + ARA-C+‡Dasatinib		6MP + MTX	
53	DEX + VCR+‡Dasatinib		6MP + DEX + VCR	
54	6MP + MTX+‡Dasatinib		6MP + MTX	
55	6MP + MTX+‡Dasatinib		6MP + MTX	
56	CYCLO + ARA-C +‡Dasatinib		6MP + MTX	
57	(*)DEX + VCR+‡Dasatinib		6MP +DEX + VCR	
58	6MP + MTX+‡Dasatinib		6MP + MTX	
59	6MP + MTX +‡Dasatinib		6MP + MTX	
60	CYCLO + ARA-C+‡Dasatinib		6MP + MTX	
61	DEX + VCR+‡Dasatinib		6MP + DEX + VCR	
62	6MP + MTX+‡Dasatinib		6MP + MTX	
63	6MP + MTX+‡Dasatinib		6MP + MTX	
64	CYCLO + ARA-C +‡Dasatinib		6MP + MTX	
65	(*)βDEX + VCR+‡Dasatinib		β6MP +DEX + VCR	
66	6MP + MTX+‡Dasatinib		6MP + MTX	
67	6MP + MTX +‡Dasatinib		6MP + MTX	
68	CYCLO + ARA-C+‡Dasatinib		6MP + MTX	
69	DEX + VCR+‡Dasatinib		6MP + DEX + VCR	
70	6MP + MTX+‡Dasatinib		6MP + MTX	
71	6MP + MTX+‡Dasatinib		6MP + MTX	
72	6MP + MTX+‡Dasatinib		6MP + MTX	
73	(*)6MP + DEX + VCR+‡Dasatinib		6MP +DEX + VCR	
74	6MP + MTX+‡Dasatinib		6MP + MTX	
75	6MP + MTX+‡Dasatinib		6MP + MTX	
76	6MP + MTX+‡Dasatinib		6MP + MTX	
77	6MP + DEX + VCR+‡Dasatinib		6MP +DEX + VCR	
78	6MP + MTX+‡Dasatinib		6MP + MTX	
79	6MP + MTX+‡Dasatinib		6MP + MTX	
80	6MP + MTX+‡Dasatinib		6MP + MTX	
81	(*)β6MP + DEX + VCR+‡Dasatinib		β6MP +DEX + VCR	

	Standard/High Risk		Low Risk	
Week		Special Studies		Special Studies
82	6MP + MTX+‡Dasatinib		6MP + MTX	
83	6MP + MTX+‡Dasatinib		6MP + MTX	
84	6MP + MTX+‡Dasatinib		6MP + MTX	
85	6MP + DEX + VCR+‡Dasatinib		6MP +DEX + VCR	
86	6MP + MTX+‡Dasatinib		6MP + MTX	
87	6MP + MTX+‡Dasatinib		6MP + MTX	
88	6MP + MTX+‡Dasatinib		6MP + MTX	
89	(*)6MP + DEX + VCR+‡Dasatinib		6MP +DEX + VCR	
90	6MP + MTX+‡Dasatinib		6MP + MTX	
91	6MP + MTX+‡Dasatinib		6MP + MTX	
92	6MP + MTX+‡Dasatinib		6MP + MTX	
93	6MP + DEX + VCR+‡Dasatinib		6MP +DEX + VCR	
94	6MP + MTX+‡Dasatinib		6MP + MTX	
95	6MP + MTX+‡Dasatinib		6MP + MTX	
96	6MP + MTX+‡Dasatinib		6MP + MTX	
97	(*)β6MP + DEX + VCR+‡Dasatinib		β6MP +DEX + VCR	
98	6MP + MTX+‡Dasatinib		6MP + MTX	
99	6MP + MTX+‡Dasatinib		6MP + MTX	
100	6MP + MTX+‡Dasatinib		6MP + MTX	
101	6MP + DEX + VCR+‡Dasatinib		6MP +DEX + VCR	
102	6MP + MTX+‡Dasatinib		6MP + MTX	
103	6MP + MTX+‡Dasatinib		6MP + MTX	
104	6MP + MTX+‡Dasatinib		6MP + MTX	
105	6MP + MTX ‡Dasatinib		6MP + MTX	
106	6MP + MTX+‡Dasatinib		6MP + MTX	
107	6MP + MTX+‡Dasatinib		6MP + MTX	
108	6MP + MTX+‡Dasatinib		6MP + MTX	
109	6MP + MTX ‡Dasatinib		6MP + MTX	
110	6MP + MTX+‡Dasatinib		6MP + MTX	
111	6MP + MTX+‡Dasatinib		6MP + MTX	
112	6MP + MTX+‡Dasatinib		6MP + MTX	
113	6MP + MTX +‡Dasatinib		6MP + MTX	
114	6MP + MTX+‡Dasatinib		6MP + MTX	
115	6MP + MTX+‡Dasatinib		6MP + MTX	
116	6MP + MTX+‡Dasatinib		6MP + MTX	
117	6MP + MTX ‡Dasatinib		6MP + MTX	
118	6MP + MTX+‡Dasatinib		6MP + MTX	
119	6MP + MTX+‡Dasatinib		6MP + MTX	
120	†6MP + MTX+‡Dasatinib	**[7, 8, 9, 10]**	6MP + MTX	**[7,8, 9, 10]**

Notes:

**IT methotrexate + hydrocortisone + cytarabine:*

- *(*)Triple intrathecal treatment will be given to low-risk cases with CNS-1 status (no identifiable blasts in CSF) on weeks 7, 12, 17, 25, 33, 41, and 49.*
- *Triple intrathecal treatment will be given to low-risk cases with CNS-2, traumatic CSF with blasts status, or WBC ≥ 100 x 10^9/L on weeks 3, 7, 12, 17, 25, 29, 33, 37, 41, 45 and 49.*
- *Triple intrathecal treatment will be given to standard/high-risk cases on weeks 7, 12, 17, 25, 29, 33, 37, 41, 45 and 49.*
- *Triple intrathecal treatment will be given to other standard/high-risk cases with WBC ≥100 x 10^9/L, T-cell ALL, , t (1;19)/E2A-PBX1, presence of Philadelphia chromosome, MLL rearrangement, hypodiploidy <44, CNS-2 or CNS-3*

status, or traumatic lumbar puncture with blasts on weeks 3, 7, 12, 17, 25, 29, 33, 37, 41, 45, 49, 57, 65, 73, 81, 89 and 97.

- *Surveillance cerebrospinal fluid examination with each ITMHA and then approximately every 16 weeks.*

†Bone marrow examination; sample for MRD (See Section 11.2)
CBC with differential is needed weekly, physican exam every 4 weeks, and chemistries as clinically indicated. During Re-Inductions I and II: PE required every 3-7 days, CBC weekly, chem 18 on Day 1 Weeks 7, 8, 9, 17. 19
‡ Only Ph+ patients
βSurveillance cerebrospinal fluid examination

Special studies:

1) Blood chemistries (Chem 18) (2) RBC TGN – Day 1 Week 7 (3) RBC TPMT – Day 1 week 17 (4) Asparaginase Antibodies/Activity – Day 1 Week 7, 8, 9, 17, and 19. (5) CSF Asparagine – Day 1 Week 7 (6) Serum biomarkers (e.g. lipids, cortisol) – Week 7, 8, and 17. (7) QCT for bone density – Week 120. (8) MRI hips/knees and PT/OT evaluation (participants ≥ 9 years of age) – After each Re-Induction (weeks 12-14 and 22-24, and Week 120 or later), (9) Psychological Testing, (10) MRI Brain. MR should be acquired before DEX. Participants with abnormal imaging evaluations at week 120 will have an extra exam at 2 years off therapy to reassess brain structure. (11) Plasma for measurement of dexamethasone concentrations to be obtained with Day 8 labs during Reinductions I and II (weeks 7 and 17).

Samples required for MRD studies post-remission induction

Time point	B-lineage ALL		T-lineage ALL	
	Day 43 MRD+	Day 43 MRD-	Day 43 MRD+ (or "atypical" T-ALL)	Day 43 MRD-
Day 1 (Weeks 7, 17, 49)	BM	None unless clinically indicated or MLL+	BM and PB	PB
Week 120 (off therapy)	BM	BM	BM and PB	BM and PB

PB; peripheral blood; BM: bone marrow

APPENDIX II: DRUG INFORMATION

1. PREDNISONE, PREDNISOLONE

Source and pharmacology: Prednisone is a synthetic congener of hydrocortisone, the natural adrenal hormone. Prednisone is a white or yellowish crystalline powder. It binds with steroid receptors on nuclear membranes, impairs cellular mitosis and inhibits protein synthesis. Prednisone also has potent anti-inflammatory effects and suppresses the immune system. Prednisone is well absorbed orally. It is converted to prednisolone, the pharmacologically active metabolite, in the liver. Prednisolone is further metabolized to inactive compounds in the liver. The metabolites are excreted mainly in the urine.

Formulation and stability: Prednisone is available as various strength tablets and oral solution from multiple manufacturers. All dosage forms can be stored at room temperature. At St. Jude Children's Research Hospital, prednisolone oral solution may be substituted for prednisone liquid at equal doses due to its superior palatability.

Supplier: commercially available

Toxicity: Side effects of prednisone vary depending on the duration of its use. Side effects that can occur with short term use include sodium and water retention with associated hypertension, peptic ulcer 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: See treatment and dose modification sections of the protocol.

2. VINCRISTINE (Oncovin®)

Source and pharmacology: Vincristine is an alkaloid obtained from the periwinkle *(Vinca rosea)* plant. It reversibly binds to microtubule and spindle proteins causing metaphase arrest. Vincristine has poor penetration into the CSF. It is approximately 75% protein bound. Extensive metabolism occurs in the liver. Excretion is primarily in the bile. A dosage decrease is recommended in patients 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: Commercially available

Toxicity: Dose limiting toxicity is neurotoxicity. This can be 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 bronchospasms has been reported following the administration of vinca alkaloids. Myelosuppression is rare at usual doses. Vincristine is a vesicant and may cause severe tissue damage if extravasation occurs. NOTE: dose reduction may be necessary in patients < 1 year of age, or < 10 kg 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: See treatment and dose modification sections of the protocol.

3. DAUNORUBICIN (Daunomycin, Cerubidine®)

Source and pharmacology: Daunorubicin is an anthracycline antibiotic derived from *Streptomyces coeruleorubidus*. Daunorubicin intercalates between base pairs of DNA causing steric obstruction, disruption of DNA function and inhibition of RNA synthesis. In addition, daunorubicin inhibits topoisomerase II, an enzyme responsible for allowing strands of DNA to pass through one another as they unwind. Even though daunorubicin exerts its major effects in the S phase, it is considered to be cell cycle phase non-specific. Daunorubicin is widely distributed in tissues but does not cross the blood brain barrier. It is metabolized to daunorubicinol, which is the major active metabolite and aglycones (inactive). The major route of elimination is through the bile (40%) with additional elimination through the urine. Dosages should be reduced in patients with liver dysfunction (as in section 7.3) or renal dysfunction (creatinine > 3 mg/dL).

Formulation and stability: Daunorubicin is supplied in vials containing 20 mg of reddish colored lyophilized powder and 100 mg of mannitol. The intact vials should be stored at room temperature. Each vial can be reconstituted with 4 ml of sterile water for injection to give a final concentration of 5 mg/ml. Reconstituted solutions are stable for 24 hours at room temperature and 48 hours if refrigerated.

Supplier: Commercially available

Toxicity: Dose-limiting toxicities of daunorubicin include myelosuppression and cardiotoxicity. Two forms of cardiac toxicity can occur. Acute toxicity may take the form of arrhythmias, heart block or pericarditis and may be fatal. The chronic form of cardiotoxicity is related to total cumulative dose and is characterized by heart failure. Mediastinal radiotherapy and/or other cardiotoxic drugs may increase the risk of cardiotoxicity. In general, total lifetime dosages of 450-550mg/m^2 should not be exceeded. Other toxicities include nausea and vomiting, mucositis, alopecia, diarrhea and red discoloration of the urine and other body fluids. Severe tissue damage and necrosis can occur upon extravasation. Radiation recall reactions can occur and can be severe. Rarely, allergic reactions have occurred. Typhlitis can occur when combined with cytarabine.

Guidelines for administration: See treatment and dose modification sections of the protocol.

4. 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: See treatment and dose modification sections of the protocol.

5. ERWINIA L-ASPARAGINASE (Erwinaze®)

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 the 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: See treatment and dose modification sections of the protocol.

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

6. DOXORUBICIN (Adriamycin®)

Source and pharmacology: Doxorubicin is an anthracycline antibiotic produced by *Streptomyces peucetius*. Doxorubicin exerts its anti-tumor effects in several different ways. Doxorubicin intercalates between base pairs of DNA causing steric obstruction, disruption of DNA function and inhibition of RNA synthesis. In addition, doxorubicin inhibits topoisomerase II, an enzyme responsible for allowing strands of DNA to pass through one another as they unwind. Lastly, doxorubicin undergoes enzymatic electron reduction to generate highly reactive species, including the hydroxyl free radical, which is thought to be responsible for the drug's cardiac toxicity, but may play a role in its anti-tumor activity as well. Doxorubicin is cell-cycle, phase non-specific. Doxorubicin is widely distributed in the tissues and plasma, but does not cross the blood brain barrier to an appreciable extent. It is metabolized to doxorubicinol, which is thought to be the major active metabolite, and aglycones. Doxorubicin and its metabolites are excreted mainly in the bile and feces (approximately 80%). The remainder is excreted in the urine. Dosage should be reduced in patients with liver dysfunction (section 7.3) or renal dysfunction (creatinine > 3mg/dl).

Formulation and stability: Doxorubicin is available in vials containing 10 mg, 20 mg, 50 mg and 200 mg as a 2mg/ml red-orange solution. It is also available in vials containing 10 mg, 20 mg, 50 mg, 100 mg and 150 mg of doxorubicin as a red-orange lyophilized powder. Intact vials of doxorubicin solution should be stored under refrigeration while the lyophilized product should be stored at room temperature. Both products should be protected from light. Lyophilized doxorubicin can be reconstituted by adding 5, 10, 25, 50 or 75 ml of 0.9% NaCl respectively to the 10, 20, 50, 100 and 150 mg vials to produce a final concentration of 2 mg/ml. Bacteriostatic diluents are not recommended. After reconstitution, the resultant solution should be protected from light and is stable for 7 days at room temperature and 15 days if refrigerated.

Supplier: Commercially available

Toxicity: Dose-limiting toxicities include myelosuppression and cardiotoxicity. Two forms of cardiac toxicity can occur. Acute toxicity may take the form of arrhythmias, heart block or pericarditis and may be fatal. The chronic form of cardiotoxicity is related to total cumulative dose and is characterized by heart failure. Mediastinal radiotherapy and/or other cardiotoxic drugs may increase the risk of cardiotoxicity. In general, total lifetime dosages of 450-550mg/m2 should not be exceeded. Other toxicities include nausea and vomiting, mucositis, alopecia, diarrhea and red discoloration of the urine and other body fluids. Severe tissue damage and necrosis can occur upon extravasation. Radiation recall reactions can occur and can be severe. Rarely, allergic reactions have occurred. Typhlitis can occur when combined with cytarabine.

7. 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 decrease 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.

Guidelines for administration: See treatment and dose modification sections of the protocol.

8. 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: See treatment and dose modification sections of the protocol.

9. CYTARABINE (High Dose ARA-C)

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: See treatment and dose modification sections of the protocol.

10. THIOGUANINE (6-TG)

Source and pharmacology: Thioguanine is a purine antimetabolite. It is intracellularly converted to ribonucleotides which are incorporated into DNA and RNA. Absorption of thioguanine is variable and poor and is decreased by food. Thioguanine undergoes extensive metabolism in the liver and other tissues to the inactive, methylated derivative and to 6-thiouracil by xanthine oxidase. Thioguanine is excreted in the urine almost completely as metabolites.

Formulation and stability: Thioguanine is available as a 40 mg scored tablet. It may be stored at room temperature.

Supplier: Tablets are commercially available.

Toxicity: The major dose-limiting toxicity is myelosuppression. Nausea and vomiting are usually mild. Other toxicities reported include diarrhea, rash, anorexia, stomatitis, and hyperuricemia. Jaundice and elevated liver function tests have been reported rarely

Guidelines for administration: See treatment and dose modification sections of the protocol.

11. CLOFARABINE (Cl-F-Ara-A, CAFdA, 2-Chloro-9-(2-deoxy-2-fluoro-beta-D-arabinofuranosyl)-9H-purin-6-amine, Clofarex, Clolar®)

Source and pharmacology: Clofarabine is sequentially metabolized intracellularly to the 5'-monophosphate metabolite by deoxycytidine kinase and mono- and di-phosphokinases to the active 5'-triphosphate metabolite. Clofarabine has high affinity for the activating phosphorylating enzyme, deoxycytidine kinase, equal to or greater than that of the natural substrate, deoxycytidine. Clofarabine inhibits DNA synthesis by decreasing cellular deoxynucleotide triphosphate pools through an inhibitory action on ribonucleotide reductase, and by terminating DNA chain elongation and inhibiting repair through incorporation into the DNA chain by competitive inhibition of DNA polymerases. The affinity of clofarabine triphosphate for these enzymes is similar to or greater than

that of deoxyadenosine triphosphate. In preclinical models, clofarabine has demonstrated the ability to inhibit DNA repair by incorporation into the DNA chain during the repair process. Clofarabine 5'-triphosphate also disrupts the integrity of mitochondrial membrane, leading to the release of the pro-apoptotic mitochondrial proteins, cytochrome C and apoptosis-inducing factor, leading to programmed cell death. Clofarabine is cytotoxic to rapidly proliferating and quiescent cancer cell types in vitro.

The population pharmacokinetics of clofarabine was studied in 40 pediatric patients aged 2 to 19 years (21 males/19 females) with relapsed or refractory ALL or AML. At the given 52 mg/m2 dose, similar concentrations were obtained over a wide range of BSAs. Clofarabine was 47% bound to plasma proteins, predominantly to albumin. Based on non-compartmental analysis, systemic clearance and volume of half-life was estimated to be 5.2 hours. No apparent difference in pharmacokinetics was observed between patients with ALL and AML or between males and females. Based on 24-hour urine collections in the pediatric studies, 49-60% of the dose is excreted in the urine unchanged. *In vitro* studies using isolated human hepatocytes indicate very limited metabolism (0.2%), therefore the pathways of non-renal elimination remain unknown.

Although no clinical drug-drug interaction studies have been conducted to date, on the basis of the *in vitro* studies, cytochrome p450 inhibitors and inducers are unlikely to affect the metabolism of clofarabine. The effect of clofarabine on the metabolism of cytochrome p450 substrates has not been studied. The pharmacokinetics of clofarabine has not been evaluated in patients with renal or hepatic dysfunction.

Formulation and stability: Clofarabine (1 mg/mL) is supplied in a 20 mL, single-use vial. The 20 mL vial contains 20 mg clofarabine formulated in 20 mL unbuffered normal saline (comprised of Water for Injection, USP, and Sodium Chloride USP). The pH range of the solution is 4.5 to 7.5. Store at 25°C (77°F); excursions permitted to 15-30°C (59-86°F).

Toxicity: The most common toxicities of clofarabine are vomiting, nausea, diarrhea, anemia, leukopenia, thrombocytopenia, neutropenia, febrile neutropenia, and infection. Greater than 10% of patients receiving clofarabine have the following adverse events: tachycardia, abdominal pain, constipation, gingival bleeding, sore throat, edema, fatigue, injection site pain, lethargy, mucosal inflammation, pain, pyrexia, rigors, hepatomegaly, jaundice, weight loss, anorexia, arthralgia, myalgia, back pain, limb pain, dizziness, headache, somnolence, tremor, anxiety, depression, irritability, hematuria, cough, dyspnea, epistaxis, pleural effusion. Respiratory distress, confusion, dermatitis, dry skin, erythema, palmar-plantar erythrodysesthesia syndrome, petechiae, pruritus, flushing, hypertension, hypotension, increases in ALT, AST, and bilirubin, transient left ventricular systolic dysfunction, and increased serum creatinine. Four of 113 pediatric patients experienced capillary leak syndrome or SIRS leading to multi-organ failure.

Fetal and teratogenic effects have been noted in animals. It is not known whether clofarabine or its metabolites are excreted in human milk.

Guidelines for administration: See treatment and dose modification sections of the protocol. Filter clofarabine through a sterile 0.2 µm syringe filter and then further dilute with 5% dextrose injection USP or 0.9% sodium chloride injection USP to a convenient volume and infuse over 2 hours. The resulting admixture may be stored at room temperature, but must be used within 24 hours of

preparation.

To reduce the effects of tumor lysis and other adverse events it is recommended that continuous IV fluids be given throughout the 5 days of clofarabine administration. See Appendix II for Supportive Care Guidelines.

Since clofarabine is primarily excreted through the kidneys, drugs with known renal toxicity should be avoided during the 5 days of clofarabine administration. In addition, since the liver is a known target organ for clofarabine toxicity, concomitant use of medications known to induce hepatic toxicity should be avoided.

Supplier: Commercially available

12. 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 $\geq$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 approximately 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 250mg 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 2.5mg 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 25mg/ml. The 1000mg vials containing lyophilized product are reconstituted to a final concentration of 50mg/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: See treatment and dose modification sections of the protocol.

13. 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 purine 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: See treatment and dose modification sections of the protocol.

14. 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 approximately 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 hematopoeitic 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 a 20 mg/mL oral suspension. The tablets should be stored at room temperature and protected from light.

Supplier: 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: See treatment and dose modification sections of the protocol.

15. DEXAMETHASONE (Decadron®)

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

Formulation and stability: Dexamethasone is available as various strength tablets 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 hours at room temperature.

Supplier: Commercially available

Toxicity: Side effects of dexamethasone vary depending on the duration of its use. Side effects that can occur with short term use include peptic ulcer 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: See treatment and dose modification sections of the protocol.

16. HYDROCORTISONE, systemic (Cortef, Solu-Cortef®)

Source and pharmacology: Hydrocortisone is a synthetic steroid akin to the natural adrenal hormone cortisol. Hydrocortisone decreases inflammation by suppression of migration of polymorphonuclear leukocytes and reversal of increased capillary permeability. It is excreted in the urine and catabolized in the liver.

Formulation and stability: Solu-Cortef sterile powder is supplied in the following packages: 100 mg plain, and 100 mg, 250 mg, 500 mg, and 1000 mg ACT-O-VIAL (MIX-O-VIAL). Store lyophilized 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.

Supplier: Commercially available

Toxicity: Hyperphagia, obesity, striae, acne, cataracts, immunosuppression, electrolyte disturbances, edema, hypertension, osteoporosis, personality changes, insomnia, headaches, diabetes and Cushingoid Syndrome. Pancreatitis, peptic ulcer and/or GI bleeding have also been noted.

Guidelines for administration: See treatment and dose modification sections of the protocol.

17. 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.

Guidelines for administration: See treatment and dose modification sections of the protocol.

18. DASATINIB (SPRYCEL®)

FOR PH+ ALL PATIENTS ONLY

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 through 7) is the procedure for the preparation of the lemonade dosing solution. For preservative-free apple juice or preservative-free orange juice, steps 2 through 7 should be followed.

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.

Place 1 ounce (30 mL) of this lemonade solution into a drinking glass.

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. Start timing for 20 minutes. At approximately the 5 minute mark, swirl the contents of the glass well for about 3 seconds.

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.

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.

Administer the washing lemonade to the patient. The intact bottles should be stored at controlled room temperature (15°- 25°C or 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.

19. IMATINIB (Gleevec®, imatinib mesylate, formerly known as STI571) For Ph+ ALL patients only. See package insert for additional information

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.

APPENDIX III: DEFINITIONS RELATED TO INFECTIOUS DISEASES

I. ASSESSMENTS

A. Fever: A single temperature of > 38.3°C (101°F) or ≥38.0°C (100.4°F) on two occasions within 12 hours. The measurement must be oral with glass or IVAC thermometers or at the tympanic membrane by infrared instruments (Thermoscan). 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 38.0°C 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
   Posaconazole
   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 or 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[147]

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/mm3) 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 leas 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 • Reocovery in culture froma 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 clincical 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. nonneutropenic = ANC $\geq 500/mm^3$

VII. CULTURE NEGATIVE SEPSIS

In the 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 IV: 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.

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

Substrates - continued	
Steroid 6 beta-OH	estradiol hydrocortisone progesterone testosterone
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 rifampin St. John's wort troglitazone pioglitazone rifabutin

APPENDIX V - TOTXVI RANDOMIZATION FORM

TOTXVI RANDOMIZATION FORM

Please fax the completed form to the St. Jude Central Pharmacy at [redacted].

Please call the St. Jude pharmacy at [redacted] to let them know you've faxed a request for TOTXVI randomization. If you are unable to send this form by **FAX**, the information may be given by phone and sent by **FAX** later.

MRN: ☐☐☐☐☐☐☐☐☐ Initials: ☐☐☐ Date: ☐☐ ☐☐ ☐☐
MM DD YY

Name: _____

Has participant/LAR signed informed consent, agreeing to participate in PEG-asparaginase randomization?

☐ Yes
☐ No (Participant will not be randomized-do NOT fax the form to pharmacy)

Note: Participants with Down syndrome will NOT be randomized - DO NOT fax the form to pharmacy.

Stratification factors (please check only one in each category)

1. Risk status at start of continuation phase (see section 4.0):

   ☐ Low ☐ Standard ☐ High

2. For **standard risk** participants only:

   ☐ T-Lineage ☐ B-Lineage ☐ Unknown

3. For **Standard risk, T-Cell lineage** participants only:

   ☐ Day 15 MRD negative ☐ Day 15 MRD positive ☐ Unknown

Physician: _____
(print name)

Contact name if not the physician: _____

Telephone contact number for questions: ☐☐☐ ☐☐☐ ☐☐☐☐

Pharmacist: Please email dose assignment to email group **TOTXVI Randomization Results**
Include patient's name, medical record number and PEG-asparaginase dose assignment.
Print a copy of your email message and place it in the notebook with this form

APPENDIX VI: PSYCHOLOGICAL TEST BATTERY FOR CHILDREN OVER THREE YEARS OF AGE

Domain	Measure	Broad skill	Task characteristics	Age range	Time
Intelligence	SB-V[1] Routing Subtests	Global Cognitive Function	Object Series/Matrices and Vocabulary subtests	3+	20
Attention & Executive Function	Digit Span[2,3]	Auditory Attention & Working Memory	Forward- repeat random digit strands of increasing length Backward- repeat random digit strands in reverse order	6+	5
	Spatial Span[2,3]	Spatial Attention & Working Memory	Forward- repeat random block taps of increasing length Backward- repeat random block taps in reverse order	6+	5
	Auditory Attention[4]	Auditory Selective Attention	Point to a picture indicated by a spoken word; background noise is increased over time	3+	5
	CPT[5,6]	Sustained Visual Attention	Selectively respond to targets on a computer screen, While inhibiting response to non-targets	4+	20
	Auditory Working[4] Memory	Auditory Working Memory	Repeat words and numbers after organizing based on rule	4+	5
	Retrieval Fluency[4]	Verbal Fluency	Generate name of foods, people & animals	3+	5
(Parent Measures)	BRIEF[6]	Executive Function	Questionnaire assessing executive dysfunction	2+	15
Memory	CVLT[8,9]	Verbal List Learning	Learn verbal list over with multiple presentations, interference, delay & recognition	5+	15
	Story Memory[10]	Story Recall	Recall stories after single presentation and following a delay; recognition cueing provided after free recall	5+	15
	Bead Memory[11]	Visual Memory	Recreate bead patterns of increasing length	3+	5
	Rey Figure[12]	Visual Memory & Organization	Copy a complex figure followed by drawing from memory after short and long delays	6+	20
Processing Speed	Visual Matching[4]	Processing Speed	Find matching pairs	3+	3
	Decision Speed[4]	Processing Speed	Finding two conceptually similar pairs	3+	3

Visual Spatial/ Visual Motor	Spatial Relations[4]	Mental Rotation & Spatial Integration	Pick object that matches puzzle pieces put together	3+	5
	VMI[13]	Visual Perception & Visual-Motor Integration	Match shapes of decreasing size; Draw shapes of increasing complexity	3-21	15
Motor	Purdue Pegboard[14]	Fine Motor Speed & Dexterity	Place pegs using dominant, nondominant & both hands	3+	3
Adaptive	ABAS-II[15]	Adaptive Functioning	Parent questionnaire of adaptive skills	Birth+	15
Social-Emotional	BASC-II[16]	Psycho-social Adjustment	Parent questionnaire of behavioral domains.	2-21	15

(1)Stanford-Binet, Fifth Edition; (2)Wechsler Intelligence Scale for Children, Fourth Edition; (3)Wechsler Adult Intelligence Scale, Third Edition; (4)Woodcock Johnson, Third Edition; (5)Conners' Continuous Performance Test, Second Edition; (6)Conners' Kiddie Performance Test; (7)Brief Rating Inventory of Executive Function; (8)California Verbal Learning Test-Children's Version; (9)California Verbal Learning Test, Second Edition; (10)Children's Memory Scale; (11)Stanford-Binet, Fourth Edition; (12)Rey-Osterrieth Complex Figure; (13)Beery-Buktenica Test of Visual-Motor Integration; (14)Purdue Pegboard Test; (15)Adaptive Behavior Assessment System-II; (16)Behavior Assessment System for Children-II.

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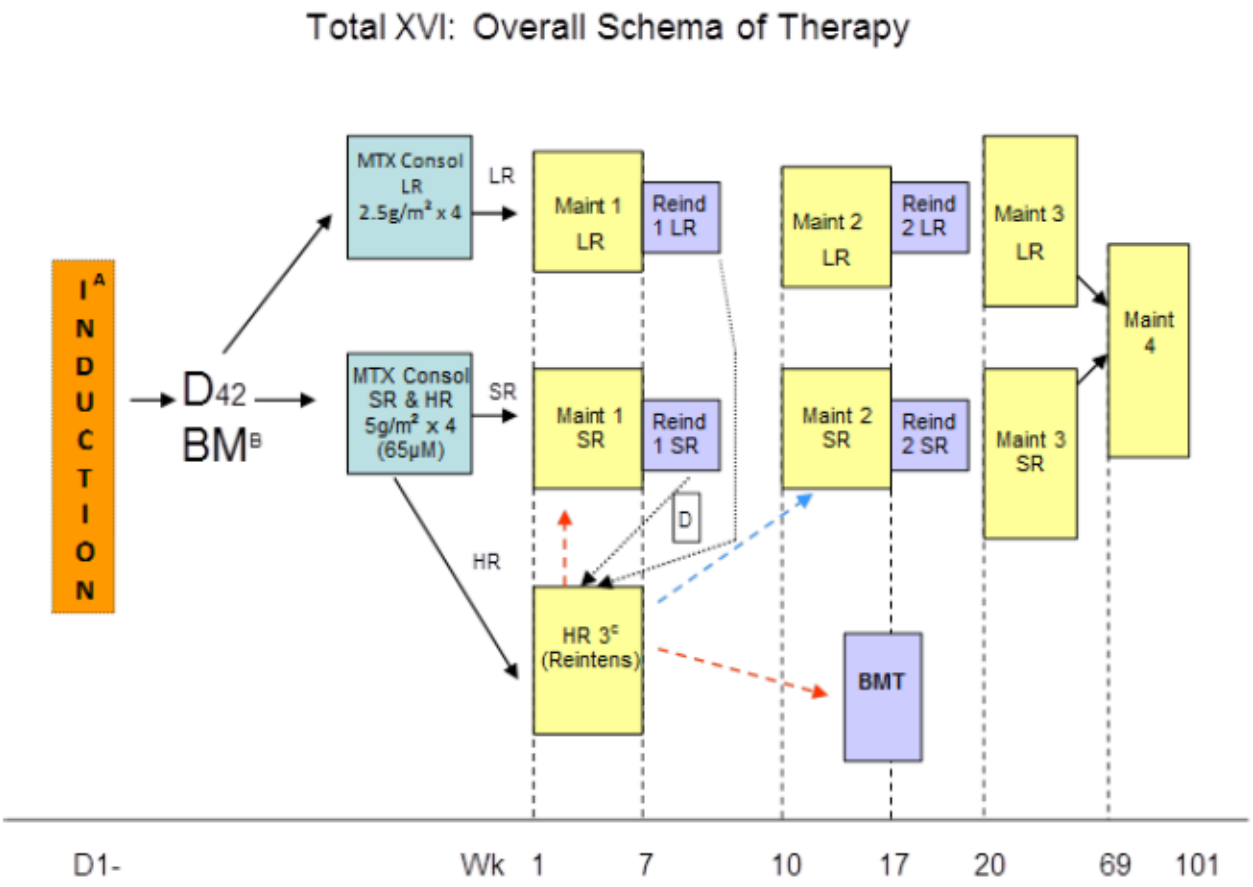
APPENDIX VII: SCHEMA



A = Day 15 MRD≥1% get 1 additional dose of PEG-aspar, D15 MRD≥1% also moves LR to SR; fractionated cyclophosphamide for Day15 MRD ≥5%; Clo/VP16/CTX for MLL+infants
B = MRD ≥0.01% but <1% moves LR to SR; MRD ≥1% moves LR or SR to HR
C = If MRD > 0.01% after one HR3 block, continue HR3 blocks; after HR3, go to BMT or to SR arm
D = MRD≥ 0.1% at week 7 moves LR or SR to HR after Reind 1

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

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

- History & physical exams
- Hematology: CBC with diff, coagulation screen
- All blood and urine chemistries, lipid screen, plasma 1, 25-dihydroxyvitamin D, osteocalcin, thyroid function tests
- Chest x-rays
- Bone marrow for morphology, cytochemistry, immunophenotyping, cytogenetics, DNA index, molecular studies, and MRD studies
- Peripheral blood for MRD
- Lumbar punctures with CSF examination and administration of intrathecal chemotherapy
- Other studies as clinically indicated (e.g. sickle cell prep, hemoglobin electrophoresis and G6PD screen for black children, varicella titer, hepatitis B antigen, HIV, EBV, TOXO, CMV titers)
- MRI of hips and knees and PT/OT evaluations
- Methotrexate pharmacokinetics
- QCT for bone density
- Urinalysis
- Ammonia levels
- TPMT DNA (TPMT genotyping)
- TPMT RBC activity and 6TGN RBC testing

The research services are as follows:

- Bone marrow sample for biology studies (taken at same time bone marrow procedure is done for routine care at diagnosis – pre-trial bone marrow)
- Germline DNA/RNA
- Neuropsychological testing
- MRI brain (includes sedation) and functional MRI brain
- Plasma homocysteine
- Serum and CSF for asparaginase and anti-asparaginase antibodies
- MRD – aliquot of CSF samples sent to TBANK
- Serum biomarkers
- Asparaginase and dexamethasone activity
- NK cell receptor studies
- Intestinal microbiome research – stool specimens