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NHL16 STUDY FOR NEWLY DIAGNOSED PATIENTS WITH ACUTE LYMPHOBLASTIC LYMPHOMA

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Protocol Summary

NHL16 STUDY FOR NEWLY DIAGNOSED PATIENTS WITH ACUTE LYMPHOBLASTIC LYMPHOMA
Principal Investigator: Hiroto Inaba, M.D., Ph.D.
IND holder: Not applicable, non-IND study. St. Jude is the study sponsor
Brief overview: This is a phase II clinical trial using risk-adapted therapy. The treatment is ALL-based therapy, using multi-agent regimens comprising of induction, consolidation, and continuation (maintenance) phases delivered over 24-30 months. Participants will be classified into 3 treatment stratums, based on bone marrow/peripheral blood lymphoma cells involvement at diagnosis and day 8 for T-lymphoblastic lymphoma and bone marrow/peripheral blood lymphoma cells involvement at diagnosis for B-lymphoblastic lymphoma.
Intervention: Multi-agent chemotherapy based on treatment stratum (Stratum 1, 2, or 3). Treatment will consist of 3 main phases: remission induction, consolidation (only for patients with any CNS disease and/or testicular involvement), and continuation
Objectives: Primary objective 1. To improve the outcome of children with lymphoblastic lymphoma (LL) who have minimal disseminated disease (MDD) equal to or more than 1% at diagnosis by using MDD- and minimal residual disease (MRD)- based risk-adapted therapy. Secondary objectives 1. To determine event-free and overall survival 2. To determine prognostic value of MDD at diagnosis and MRD on day 8 of remission induction Exploratory objectives 1. To estimate frequency of early T-cell progenitors in LL 2. To evaluate, copy number, gene expression, and mutations in tumor and normal tissues 3. To compare immunophenotyping, MRD, and genomic data between T-LL and T-ALL 4. To estimate and monitor bone mineral density and osteonecrosis 5. To establish prevalence of fertility and cardiac adverse late effects
Criteria for evaluation: The response evaluation will be based on the multidisciplinary (oncologist, pathologist and radiologist) interpretation of physical examination, laboratory and diagnostic imaging and response criteria listed in Section 12.1 of the protocol. Toxicity and performance reporting will be according to CTCAE Version 4.0.
Study design: Multi-center, non-randomized phase II study utilizing risk-adapted multi-agent chemotherapy.
Study population: Participants $\leq$ 21 years of age with newly diagnosed lymphoblastic lymphoma (LL)
Sample size: 72 participants in approximately 10.5 years.
Randomization: N/A

NHL16 STUDY FOR NEWLY DIAGNOSED PATIENTS WITH ACUTE LYMPHOBLASTIC LYMPHOMA
Data analyses: Outcome of this subset of patients will be analyzed in terms of overall survival (OS) and event-free survival (EFS) since diagnosis. Only death will be considered a failure for OS. For EFS, relapse and second malignancies will be considered as failures in addition to death in complete remission. The time to EFS will be set to 0 for patients who fail to achieve complete remission. Kaplan-Meier estimates of the OS and EFS curves will be computed, along with estimates of standard errors by the method of Peto. Analysis will begin after 2 years of follow up since the completion of therapy of the last enrolled patient.
Anticipated primary completion date: September 2023
Anticipated study completion date: September 2023
Time frame for primary outcome measure: September 2023
Data management: Data management and statistical analysis will be provided by the Comprehensive Cancer Center clinical research staff (Leukemia/Lymphoma Division) and Biostatistics Department at St. Jude Children's Research Hospital.
Human subjects: The risks to participants will be related to the toxicity of multi-agent chemotherapy. Participants will be informed of this and other potential side effects during informed consent. Adverse events will be monitored and reported and treated appropriately.

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Appendices

1.0 OBJECTIVES

1.1 Primary Objective

To improve the outcome of children with lymphoblastic lymphoma who have minimal disseminated disease (MDD) equal to or more than 1% at diagnosis by using MDD- and minimal residual disease (MRD)– based risk-adapted chemotherapy.

1.2 Secondary Objectives

1.2.1 To estimate the event-free survival and overall survival of children with lymphoblastic lymphoma who are treated with MDD- or MRD-based risk-directed therapy.

1.2.2 To evaluate the prognostic value of levels of MDD at diagnosis and MRD on day 8 of remission induction.

1.3 Exploratory Objectives

1.3.1 To estimate the frequency of early T-cell progenitors in lymphoblastic lymphoma.

1.3.1 To evaluate copy number, gene expression, and mutations in tumor and normal tissues of patients.

1.3.2 To compare the immunophenotyping, MRD, and genomic data between T-lymphoblastic lymphoma and T-acute lymphoblastic leukemia (ALL).

1.3.3 Bone mineral density (BMD) and osteonecrosis

1.3.3.1 To prospectively estimate BMD at diagnosis and at end of therapy and correlate with risk factors for potential BMD deficits in pediatric patients with NHL.

1.3.3.2 To prospectively monitor osteonecrosis and correlate with risk factors for potential osteonecrosis in pediatric patients with NHL.

1.3.4 Fertility late effects

To establish the prevalence of gonadal and germ cell dysfunction in children treated with this regimen.

1.3.5 Cardiac late effects

To establish the prevalence and severity of cardiac toxicity associated with this regimen.

2.0 BACKGROUND AND RATIONALE

2.1 Therapy

2.1.1 Patients with advanced-stage lymphoblastic lymphoma

The majority of successful regimens designed to treat advanced-stage lymphoblastic lymphoma are derived from regimens designed to treat ALL. They are multiagent regimens comprising induction, consolidation, and maintenance phases delivered over 18–30 months. Although involved field irradiation of the primary tumor site was included to treat residual mediastinal masses in earlier trials, it is generally not recommended in contemporary protocols due to associated late effects.

The Children Cancer Study Group (CCSG) performed an important randomized clinical trial to compare the cyclophosphamide-based 4-drug regimen COMP and the 10-drug LSA_2L_2 regimen.[1-3] COMP was a lymphoma-based regimen and LSA_2L_2 was designed for ALL therapy. Outcomes of patients with stage 3 or 4 disease were significantly related to treatment arm and histology. The LSA_2L_2 regimen (ALL approach) was superior to COMP for lymphoblastic lymphoma [5-year event-free survival (EFS) 64% vs. 35%], whereas the COMP regimen was superior to LSA_2L_2 for Burkitt lymphoma (5-year EFS 50% vs. 29%).

The early St. Jude study Total X-high risk demonstrated the survival efficacy of the addition of cytarabine and teniposide to an otherwise standard antimetabolite-based ALL regimen.[4] Patients enrolled on the more recent St. Jude study NHL 13, which was based on our regimen for childhood T-lymphoblastic leukemia, had a 5-year EFS of 82.9% and overall survival (OS) of 90.2%.[5] The regimen included induction, consolidation, continuation, and reinduction phases and used high-dose methotrexate (HD-MTX) and etoposide. Epipodophyllotoxins have also been used in other protocols,[6,7] but have been omitted from recent regimens because of the risk of secondary leukemia.

There has been continued controversy about the role of HD-MTX in the management of advanced-stage lymphoblastic lymphoma. Excellent results were achieved with use of the LMT81 regimen of the French cooperative group (SFOP) when multiple courses of HD-MTX ($3g/m^2$/course) were added onto an LSA_2L_2 backbone.[8] The BFM 90 regimen included 4 courses of HD-MTX ($5g/m^2$/dose) as a consolidation phase and produced one of the best outcomes to date (5-year EFS for stage III 90% and stage IV 95%).[9] The Pediatric Oncology Group (POG) 9404 regimen, which examined the role of HD-MTX in a backbone of intensive doxorubicin and weekly L-asparaginase, was terminated early because of an inferior outcome for children with T-ALL in the arm without HD-MTX.[10] Although the POG study was not powered to examine the impact of HD-MTX in advanced-stage precursor T-lymphoblastic lymphoma, the 5 year-EFS was higher among patients not receiving HD-MTX (87.8%) than those receiving it (78.1%) (p=0.1057). In contrast, a recently completed Children's Oncology Group (COG) study (A5971) examining the role of HD-MTX in a BFM backbone reported no significant difference

between 3-year EFS of patients receiving (84.5%) or not receiving HD-MTX (82.7%) (p=0.93).[11]

Asparaginase is thought to be an important component of effective lymphoblastic lymphoma therapy. The POG 8704 study demonstrated a survival advantage for patients who received an additional 20 weekly doses of L-asparaginase after consolidation therapy.[6] In the Dana-Farber Consortium Protocol 91-01 ALL study, patients who received at least 26 weeks of asparaginase had a significantly better outcome than those who received 25 or fewer weeks of asparaginase.[12] Anthracyclines are also thought to be important in the treatment of precursor T-cell ALL and lymphoblastic lymphoma,[13] and the use of anthracyclines in reinduction (delayed intensification) phases may also improve outcome, a strategy used in the BFM regimens, COG A5971, and St. Jude Total studies.[9,11,14]

Prophylactic cranial irradiation was not used in BFM 95, but the outcome of patients on BFM 95 was not inferior to that of patients on BFM90/86 therapies.[15] St. Jude NHL13 included intensive intrathecal (IT) chemotherapy for central nervous system (CNS) therapy and excluded prophylactic cranial radiation.[5] St. Jude TOTXV[14] and the Dutch Childhood Oncology Group (DCOG) ALL-9[16] studies showed that cranial irradiation can be safely eliminated from ALL treatment if effective risk-directed chemotherapy and IT therapy is given. The 5-year cumulative risk of isolated CNS relapse in patients in these studies was 2.7% and 2.6%, respectively, which is within the range (1.5% to 4.5%) of risk of CNS relapse observed in clinical trials that use prophylactic cranial irradiation.

The treatment regimen that we have developed for NHL16 is based on the knowledge acquired from previous trials and from new evidence indicating that minimal disseminated disease (MDD) at diagnosis as measured by flow cytometry is prognostically important. In NHL 16, we will adopt the modified St. Jude frontline ALL protocol TOTXVI approach. PEG-asparaginase will be given every 2 weeks for 30 weeks (15 doses) without interruption (36 weeks and 18 doses for patients who have CNS disease and/or testicular involvement and receive consolidation therapy). Fractionated cyclophosphamide will be given to patients with MDD ≥1% at diagnosis and any CNS and/or testicular involvement. Patients with T-lymphoblastic lymphoma who had MDD ≥1% at diagnosis and positive day 8 MRD (≥0.01%) will receive dexamethasone instead of prednisone during induction on days 15-28. HD-MTX will not be used for patients who do not have CNS disease and/or testicular involvement. For patients with CNS disease and/or testicular involvement, 4 doses of HD-MTX will be used after remission-induction therapy. No cranial or craniospinal radiation or residual mass irradiation will be used. Treatment will be completed in 24 months (98 weeks of continuation therapy) for patients with MDD <1% at diagnosis and in patients with MDD ≥1% but with negative MRD on day 8. Patients with MDD ≥1% and positive MRD on day 8 or those with CNS disease and/or testicular involvement will receive 22 additional weeks of continuation therapy for a total of 120 weeks.

2.1.2 Patients with limited-stage disease

Because of the rarity of early-stage lymphoblastic lymphoma, most of the published outcome data combine cases of precursor B- and precursor T-lymphoblastic lymphoma. In a study by Link et al., patients with limited-stage disease were randomized to receive the same 9 weeks of CHOP chemotherapy with or without the 24-week maintenance phase.[17] This study demonstrated that the maintenance phase could be safely eliminated for patients with Burkitt or diffuse large cell lymphoma, but that maintenance benefited those with lymphoblastic lymphoma. Patients with B-precursor lymphoblastic lymphoma on NHL-BFM 86 and 90 were treated with either an intensive "ALL-type" or shorter "Burkitt lymphoma–type" regimen.[18] The ALL-type approach, which was similar to that used in T-lymphoblastic lymphoma (BFM-90), was superior to the short-pulse Burkitt lymphoma–type strategy.

The COG A5971 study used uniform ALL-based therapy to treat patients with localized lymphoblastic lymphoma; most patients (79%) with localized disease had a pre-B immunophenotype.[19] With 24 months of CCG BFM therapy without the 4 additional day 28 IT therapies with methotrexate during maintenance, 5-year EFS and OS rates were 85% and 94%, respectively. EFS rates were similar to those of patients with advanced disease, which supports the need for intensive treatment in this population.

In NHL16, patients with limited-stage lymphoblastic lymphoma will be treated by the same strategy used for those with advanced-stage disease, except that high-dose cytarabine will not be given in reinduction II. PEG-asparaginase will be given every 2 weeks for 30 weeks (15 doses) without interruption. Fractionated cyclophosphamide will be given only to patients with MDD ≥1% at diagnosis. No high-dose MTX will be given. Treatment will be completed in 24 months (98 weeks of continuation therapy) in patients with MDD <1% at diagnosis and patients with MDD ≥1% and negative MRD on day 8. Patients with MDD ≥1% and positive MRD on day 8 will receive 120 weeks of continuation therapy.

2.1.3 Remission induction

Remission induction therapy will consist of 3 weeks of prednisone, vincristine, daunorubicin, and PEG-asparaginase, followed by 2 weeks of cyclophosphamide, cytarabine, thioguanine, and PEG-asparaginase combinations. This remission induction regimen is similar to that used in the St. Jude ALL TOTXVI study, except for the following modifications:

(a) Intramuscular PEG-asparaginase will be given every 2 weeks during induction without interruption.
(b) Cyclophosphamide therapy will be intensified in patients with MDD ≥1% or morphologic BM involvement at diagnosis. Fractionated cyclophosphamide is widely used in adult ALL protocols, and has proven safe and effective in children with Burkitt leukemia/lymphoma and relapsed ALL.

(c) Patients with MDD ≥1% and detectable MRD on day 8 will receive dexamethasone instead of prednisone during days 15–28 of remission induction therapy. The use of dexamethasone during induction therapy has been associated with superior outcome in patients with T-ALL in the ALL 2000 Berlin-Frankfürt-Münster/Associazione Italiana Ematologia Oncologia Pediatrica (BFM/AIEOP) study.[20]

2.1.4 Consolidation therapy

A recently completed COG study (A5971) examining the role of HD-MTX in a BFM backbone reported no significant difference between 3-year EFS of patients receiving (84.5%) or not receiving HD-MTX (82.7%) (p=0.93).[11] On this basis, patients in NHL 16 who do not have CNS disease and/or testicular involvement will not receive consolidation therapy with HD-MTX and will immediately proceed to continuation therapy.

For patients with CNS disease and/or testicular involvement, consolidation therapy will consist of HD-MTX (average of 5 g/m^2 over 24 h) given every alternate week for 4 doses with leucovorin rescue, together with IT treatment plus concomitant daily mercaptopurine (50 mg/m^2) as well as PEG-asparaginase every 2 weeks. The feasibility and effectiveness of HD-MTX at 5 g/m^2 have been documented in large numbers of patients treated in BFM and St. Jude Total studies.[14,21]

Intercourse targeting of HD-MTX in the standard- and high-risk arms in TOTXV resulted in 23% of courses being below, 62% being within, and 15% being above the target range of 65 µM ± 20%. Also, an acceptably low proportion of courses (15%) were associated with "delayed excretion" (42-h plasma MTX concentration >1.0 µM). Targeting the standard- and high-risk arms avoided extreme exposures relative to simulated conventional dosing: 3% versus 0% with steady-state concentrations <40% of the target and 3% versus 10% with steady-state-concentrations >40% of the target for targeted and simulated conventional dosing, respectively. The median 42-h concentration in the standard- and high-risk arms was 0.54 µM, which is close to that reported (0.43 µM) by the BFM group. Moreover, the leucovorin rescue administered in the TOTXV and the BFM studies was similar, but toxicity was substantially lower in TOTXV (~4% grade 3 or 4 mucositis) than the BFM study (44% grade 3 or 4 mucositis). Thus, it appears that there may be more benefit in avoiding extremes of exposure in the standard- and high-risk arms than the low-risk arm, even though target concentrations are better achieved in the low-risk arm.

For NHL16, HD-MTX will be targeted in order to continue to avoid unacceptably low concentrations (albeit in a small percentage of courses), minimize unacceptably high concentrations, avoid gastrointestinal (GI) toxicity, and maintain the majority of courses within the desired target range. By using this approach in TOTXV, only 5.1% of courses were associated with grade 3-4 mucositis (compared to 44% of courses with administration of 5 g/m^2 HD-MTX without targeting in the BFM study) in patients with ALL.

One of our earlier studies has shown that for targeting intercourse HD-MTX, it is not possible to keep all courses within ±20% of the target, as achievable for intra-course HD-MTX targeting.[22] We modified TOTXV to allow intra-course targeting of HD-MTX when possible, but have been able to target only a few cases because it is logistically difficult to start HD-MTX early enough in the day to allow feasible intra-course targeting. However, according to our calculations based on simulations that used the previous MTX clearance along with a 2-h MTX plasma concentration to target steady-state concentrations, 1% of courses would be below, 91% would be within, and 8% would be above the target range. Thus, for 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 HD-MTX.

2.1.5 Continuation treatment

Continuation therapy will start after remission induction therapy for patients without CNS and/or testicular involvement and after consolidation therapy for patients with CNS and/or testicular involvement.

The continuation treatment for NHL 16 will incorporate effective treatment components of the most successful clinical ALL trials to date. In randomized trials of ALL, dexamethasone was superior to prednisone in reducing CNS relapse and improving EFS, partly because it has a better CNS penetration.[23,24] Intensifying asparaginase therapy during the early phase of treatment improved outcome for all risk groups in the DFCI studies.[12]

PEG-asparaginase is increasingly used in frontline ALL regimens. In NHL16, patients without CNS disease and/or testicular involvement will receive PEG-asparaginase every 2 weeks for a total of 15 doses during the first 30 weeks (until week 23 of continuation therapy). We will evaluate the efficacy and feasibility of this approach. Patients who received consolidation therapy (patients with CNS and/or testicular disease) will receive PEG-asparaginase for a total of 18 doses for the first 36 weeks (until reinduction II, week 19). Although the optimal dose of different preparations of asparaginase is not firmly established, similar EFS has been reported with PEG-asparaginase 2500 units/m^2 and native L-asparaginase at 6000 units/m^2 for 6 doses. Hence, for PEG-asparaginase, the dose of 2500 units/m^2 has been widely adopted.

As in TOTXV,[14] use of epipodophyllotoxins will be limited to patients with high-risk lymphoma [biopsy-proven detectable disease by positron emission tomography / computed tomography (PET/CT) scan, bone marrow morphology, or MRD (≥0.01%) of bone marrow or peripheral blood at the end of induction or thereafter] in NHL16 to decrease the risk of therapy-related leukemia. Cyclophosphamide and anthracyclines will be used to intensify treatment. However, the total cumulative dose of cyclophosphamide will be limited to 4.0 (no fractionation) to 4.2 gm/m^2 (fractionation) for reducing the problem of sterility (especially in boys). Anthracycline cumulative dosage will be limited to 230 mg/m^2, as this dosage is associated with a very low risk of cardiomyopathy. After

week 61, all patients will receive daily mercaptopurine and weekly methotrexate until end of therapy.

Reinduction treatment is an integral component of most contemporary ALL 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.[25] Hence, in NHL16, patients will receive 2 courses of reinduction but those with limited-stage B-lymphoblastic lymphoma will not receive high-dose cytarabine.

The majority of patients will receive 98 weeks of continuation therapy. Patients with MDD ≥1% at diagnosis as well as the presence of *any* detectable lymphoma blasts by MRD study (≥0.01%) on day 8 or thereafter, any CNS involvement (CNS-2 and 3, and traumatic tap with blasts), and testicular involvement are an indication that 120 weeks of continuation therapy is required.

2.1.6 Rationale for modifications in mercaptopurine administration (Revision 3.1)

Prior studies assessing the pharmacological and therapeutic impact of giving oral mercaptopurine (MP) with food or with dairy products, or giving 6MP at different times of day have produced variable results. All of the early studies 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[26-28]. Other studies[29,30] reported statistically significant differences in time of maximum drug concentration in plasma (tmax) and AUC in the fasting state of parent drug[29] or changes in ALL disease free survival[30] 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[31].

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[32]. 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[26-34].

2.1.7 Subclinical CNS treatment

Since CNS irradiation can induce serious long-term sequelae such as growth retardation, intellectual impairment, and brain tumors, and in view of the excellent CNS control achieved in TOTXV and NHL 13,[5,14] CNS irradiation will be eliminated in NHL16.

The overall 5-year cumulative risk of CNS relapse was 2.7% in TOTXV, which is comparable to that reported by studies still using preventive CNS irradiation.[14] This success is attributed to early intensification of IT treatment (especially in patients at high risk of relapse) and effective systemic treatment, including the use of dexamethasone. In NHL16, IT treatment will be intensified during induction treatment, with 2 additional IT treatments on days 8 and 22 for patients with any T-lymphoblastic lymphoma, advanced stage (stages III and IV) B-lymphoblastic lymphoma, or with any amount of leukemic blasts identifiable in the cerebrospinal fluid at diagnosis (including those with cerebrospinal fluid contaminated with lymphoma blast cells due to a traumatic lumbar puncture). Patients with any CNS involvement and testicular involvement will receive consolidation therapy with 4 courses of HD-MTX and triple intrathecal chemotherapy (IT-MHA). During continuation therapy, patients with limited stage (stages I and II) B-lymphoblastic lymphoma with CNS-1 status will receive 14 (total 16) and those with T-lymphoblastic lymphoma and advanced stage (stages III and IV) B-lymphoblastic lymphoma with CNS-1 status will receive 19 (total 23) doses of IT-MHA. Patients with any CNS involvement (CNS-3 status, CNS-2 status, traumatic tap with blasts), or testicular involvement will receive 17 (total 25) doses of IT therapy.

As in TOTXVI, preventive CNS irradiation will be eliminated altogether in this study, except in rare cases in who during continuation treatment have morphologic evidence of lymphoblasts in the CSF, confirmed by immunologic testing (i.e., TdT) on 2 separate occasions. We expect these events to be very rare; no patient had slow clearance of CSF blasts during remission induction in TOTXV.[14]

Patients who during continuation treatment have morphologic evidence of lymphoblasts in the CSF, confirmed by immunologic testing (i.e., TdT) on 2 separate occasions will be managed according to guidelines given in section 9.3.

2.2 Pharmacologic Studies

2.2.1 Germline genetic polymorphisms

The thiopurine methyltransferase (*TPMT*) gene exhibits genetic polymorphism, with <1% of the population homozygous and 10% heterozygous for the deficiency. We have used *TPMT* status to individualize dosages for continuation therapy since 1991 in patients with ALL enrolled at St. Jude. For NHL 16, we will 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 to determine dosage adjustment during continuation therapy. Also, 6-mercaptopurine will be given instead of thioguanine during remission induction for patients with homozygous or heterozygous *TPMT* polymorphisms.

2.3 Biologic Studies

2.3.1 Minimal disseminated disease and minimal residual disease

Approximately 15% of children with T-lymphoblastic lymphoma can have disease dissemination at diagnosis.[35] Until recently, it was unclear whether the remaining patients have submicroscopic systemic disease, and, if so, the prognostic importance of this finding. We developed a flow cytometric method that allows the detection of 1 T-lymphoma cell in 10,000 normal cells and used it to examine bone marrow and peripheral blood samples collected at diagnosis from 99 children with T-LL enrolled in the COG A5971 study, as well as 174 peripheral blood samples collected from 48 patients during remission induction therapy.[35] We detected T-lymphoma cells in 71 of the 99 (71.7%) diagnostic samples, ranging from 0.01% to 31.6% (median, 0.22%) of bone marrow mononuclear cells. Studies in bilateral aspirates agreed well with these diagnostic results. High levels of disease dissemination by flow cytometry were significantly associated with a poorer outcome: 2-year EFS was 68.1% ± 11.1% for patients with ≥1% lymphoblasts versus 90.7% ± 4.4% for the remaining patients ($P = 0.031$). In this study, chemotherapy was not modified based on lymphoma cell involvement at diagnosis. T-lymphoma cells were as prevalent in peripheral blood as they were in bone marrow at diagnosis; monitoring of residual T-lymphoma cells in peripheral blood during remission induction therapy was able to identify patients with slow disease clearance and persistent disease. In summary, these results demonstrate that more than 66% of children with T-lymphoblastic lymphoma have systemic disseminated disease at diagnosis, which is much higher than previously thought. Therefore, measurements of disease dissemination at diagnosis might provide useful prognostic information, which can be further refined by monitoring response to therapy through peripheral blood testing. In NHL16, we will use the same flow cytometry–based methodology to measure MDD at diagnosis and monitor MRD on day 8 of remission induction therapy.

2.3.2 Early T-cell progenitor phenotype

We have hypothesized that T-ALL originating from early T-cell precursors (ETPs), a recently defined subset of thymocytes that retain stem cell–like features,[36-38] will respond poorly to lymphoid cell–directed therapy.[39] We studied leukemic cells collected at diagnosis from patients to identify cases with ETP features and determine their clinical outcome. Leukemic cells from 239 patients with T-ALL enrolled at St. Jude and in the Italian national study AIEOP ALL-2000 were examined by gene expression profiling, flow cytometry and, single nucleotide polymorphism array analysis.[39] Probabilities of survival and treatment failure were calculated for subgroups considered to have ETP-ALL or typical T-ALL. Of the 239 patients, 30 (12.6%) had leukemic lymphoblasts with an ETP-related gene expression signature or its associated distinctive immunophenotype (CD1a, $CD8^{-}$, $CD5^{weak}$ with stem-cell/myeloid markers). Cases of ETP-ALL showed increased genomic instability. Patients with this form of leukemia had very high rates of remission failure or hematologic relapse: 72% (95% confidence interval, 40%–100%) at 10 years versus 10% (4%–16%) for typical T-ALL patients treated at St. Jude and 57% (25%–89%) at 2 years versus 14% (6%– 22%) for patients treated in the AIEOP trial. These results indicate that ETP-ALL is a distinct, previously unrecognized, pathobiologic entity that confers a dire prognosis when standard intensive chemotherapy is used. On the basis of these results, ETP-ALL has been classified as "very-high-risk ALL" in TOTXVI.

While studying MDD in patients with T-lymphoblastic lymphoma enrolled in the COG A5971 study, we noticed that disseminated cells in 4 of 54 (7.4%) cases studied with a full panel of antibodies suitable to identify ETP-ALL expressed the ETP phenotype; that is, cells were CD1a and CD8 negative, had low expression of CD5, and expressed stem cell- and myeloid-associated antigens. Moreover, a survey of the limited immunophenotypic data collected on 134 primary tumors from patients enrolled in the same trial identified that 15 tumors were negative for CD1a and CD8 and are potential ETP candidates. These results suggest that ETP malignancy can present as T-lymphoblastic lymphoma, leading us to hypothesize that most systemic relapses in children and adolescents with this disease occur in those with the ETP phenotype. In NHL16, we will systematically study T-lymphoma cells at diagnosis for the cell marker panel for ETP definition and determine the response to therapy and clinical outcome of patients with ETP-phenotype.

2.3.3 Gene expression analysis, copy number alteration, and mutation analysis in T-lymphoblastic lymphoma and comparison with T-ALL

Data on genetic analyses, including gene expression, copy number alteration, and mutation analysis for T-lymphoblastic lymphoma, are very limited mainly because of the shortage of adequate vital tumor samples available.[40] The comparison of T-lymphoblastic lymphoma and T-ALL profiles is difficult because of differences in the microenvironment of cells. In T-ALL, samples for analysis are readily available because the bone marrow or even blood samples can be used to isolate RNA or DNA. In contrast, in T-lymphoblastic lymphoma, RNA or DNA from malignant cells must be extracted from rarely available tumor biopsies (lymph nodes or mediastinal mass) or malignant effusions.

Raetz et al. conducted a DNA microarray analysis of the gene expression profiles of T-lymphoblastic lymphoma and T-ALL samples obtained from Children's Oncology Group (COG) tumor banks.[41] Unsupervised hierarchic clustering of all samples showed complete segregation of T-lymphoblastic lymphoma and T-ALL into distinct clusters. Next, a significance analysis of microarrays (a supervised statistical approach), was used to identify the 201 genes that best differentiated T-ALL from T-lymphoblastic lymphoma. Sixty-eight of these genes upregulated in T-lymphoblastic lymphoma encoded extracellular matrix proteins and adhesion molecules, and it was therefore considered that these genes might represent gene expression in intervening stroma cells and not in tumor cells. After excluding these 68 genes, a reanalysis was performed and 133 genes were identified that were expressed differentially in T-lymphoblastic lymphoma and T-ALL samples. Genes representing several functional groups were differentially expressed in T-lymphoblastic lymphoma and T-ALL samples, but this set did not include classic T-cell differentiation genes, indicating that differences in the maturational stage were unlikely to explain the observed differences. In the T-lymphoblastic lymphoma samples, genes encoding adhesion molecules and extracellular matrix proteins were upregulated, which led the authors to suggest that the overexpression of adhesion molecules may – at least in part – occur within the lymphoma cells themselves. In addition, regulatory genes for proliferation and apoptosis were differentially expressed (e.g., overexpression of *CARD10* in T-lymphoblastic lymphoma, which is involved in apoptotic regulation and NF-κB activation via *BCL10*, or *MLL* overexpression).

In a second series, samples from 10 children with T-ALL and 8 with T-lymphoblastic lymphoma were available for comparative expressed sequence hybridization, which allowed identification of chromosomal regions corresponding to differential gene expression.[42] RNA was isolated from the bone marrow in T-ALL samples or lymph node biopsies in T-lymphoblastic lymphoma samples and cohybridized with a reference sample of a RNA mixture isolated from 5 reactive lymph node biopsies. A median of 75 differentially expressed chromosomal regions per case in T-ALL samples and a median of 37 regions per case in T-lymphoblastic lymphoma samples were obtained. In general, T-ALL samples showed more regions with downregulated expression (median, 39 regions) than with upregulated expression (median, 27 regions). However, in T-lymphoblastic lymphoma, there were fewer regions with downregulated expression (median, 6 regions) than with upregulated chromosomal expression (median, 27 regions). A significant number of genes overexpressed in T-lymphoblastic lymphoma samples were associated with cell adhesion functions, thereby possibly contributing to the differences in the clinical presentation of pediatric T-lymphoblastic lymphoma and T-ALL.

Schraders et al. used high-resolution single nucleotide polymorphism–based array comparative genomic hybridization to analyze copy number alterations in 12 children with T-lymphoblastic lymphoma and 7 with precursor B-cell lymphoblastic lymphoma.[43] For the 12 T-lymphoblastic lymphoma cases, a total of 44 abnormalities were identified, resulting in an average of 3.6 genomic abnormalities (2.3 losses and 1.3 gains) per case. As observed previously in T-ALL, 92% of cases of T-lymphoblastic lymphoma exhibited recurrent deletions of the *CDKN2A* locus. Also, deletions of *RB1* (16%), duplications of *MYB* (16%), and an amplification of *ABL1* 8% were detected. These results show that as

seen for T-ALL, genomic alterations in T-lymphoblastic lymphoma predominantly target genes involved in cell cycle progression. The majority (71%) of cases of precursor B-cell lymphoblastic lymphoma were characterized by high-hyperdiploidy and closely resembled cases of high-hyperdiploid precursor B-cell ALL.

NOTCH1 plays an essential role in T-cell maturation,[44] and activating *NOTCH1* mutations have been reported in patients with T-ALL.[45] Park et al. reported *NOTCH1* mutations in 6 of 14 (43%) children with pediatric T-lymphoblastic lymphoma, compared with 17 of 55 (31%) patients with T-ALL.[46] Baleydier et al. reported mutations in 22 of 41 (54%) patients with T-lymphoblastic lymphoma; of these, 9 pediatric patients were younger than 16 years, 6 (67%) of whom had *NOTCH1* mutations.[47] The prognostic relevance of *NOTCH1* mutations in the outcome of patients with T-ALL and T-lymphoblastic lymphoma is still open to debate, as the findings of different study groups have been inconclusive.[40] In addition to *NOTCH1* mutations, recent reports have shown that a significant number of patients with T-ALL (11% to 31%) harbor mutations of *FBXW7*.[48,49] The *FBXW7* locus encodes the E3 ubiquitin ligase FBXW7 (F-box and WD repeat domain containing 7), which is able to bind, ubiquitylate, and induce the proteasome-mediated degradation of intracellular NOTCH1. It was postulated that mutations of *FBXW7* lead to reduced protein function and in turn to reduce NOTCH1 degradation, resulting in increased *NOTCH1* signaling. The only study comparing *FBXW7* mutations in patients with T-lymphoblastic lymphoma and those with T-ALL showed that *FBXW7* mutations were present in 3 of 14 (21%) children with T-lymphoblastic lymphoma versus 8 of 55 (14.6%) children with T-ALL.[46] Even though data are limited, it can be concluded that activating *NOTCH1* mutations and *FBXW7* mutations can occur in T-lymphoblastic lymphoma, and their frequency of occurrence is likely similar to that of *NOTCH1* and *FBXW7* mutations in pediatric T-ALL.

In T-ALL, translocations involving the TCR genes at chromosome 7q34 or 14q11 led to the identification of different oncogenes, which become activated when juxtaposed with TCR genes.[40] These oncogenes include different types of transcription factors, such as basic helix-loop-helix (bHLH) genes (*MYC*, *TAL1*, *TAL2*, *LYL1* and *OLIG2*); cysteine-rich (LIM-domain containing) genes (*LMO1* and *LMO2*); homeodomain genes (*TLX1*, *TLX3*, or *HOXA* gene cluster); or the *MYB* oncogene.[40] Information on the presence of these oncogenes in T-lymphoblastic lymphoma is very limited. Baleydier et al. quantified oncogene expression in 41 patients with T-lymphoblastic lymphoma, including 9 patients younger than16 years.[47] The predominant oncogene transcripts identified were *HOXA9*, *LMO2*, *LYL1*, *TAL1* and *TLX1*, but none of the pediatric patients had *TAL1* or *TLX1* overexpression.

The very few cases of T-lymphoblastic lymphoma analyzed limits us from drawing conclusions on the differences in the gene expression profiles of children with T-lymphoblastic lymphoma and T-ALL. To date, no single biologic parameter has been identified that clearly distinguishes pediatric T-lymphoblastic lymphoma from T-ALL.

2.4 Bone Mineral Density (BMD)

Deficits in BMD have been well documented among childhood ALL survivors and arise from the interaction of multiple factors. Approximately 10% of children have BMD deficits at the time of diagnosis of ALL and more than 67% develop them during therapy. In a study by Strauss et al., BMD deficits were reported in up to 63% of survivors of pediatric ALL after completion of therapy, and were associated with a 5-year cumulative incidence of fracture of 28% ± 3%.[50] Risk factors for developing BMD deficits include age at diagnosis ≥9 years;[50] treatment with corticosteroids (both prednisone[51] and dexamethasone[50]) and methotrexate;[51,52] endocrinopathy associated with radiation (growth hormone deficiency, hypogonadism, and hyperthyroidism);[53,54] and health behaviors such as inadequate intake of calcium and vitamin D,[55] lack of weight-bearing exercise,[55,56] smoking and alcohol use.[57,58] However, BMD changes in patients with lymphoblastic lymphoma have not been extensively studied. Therefore, we will prospectively study BMD changes in this patient population.

Corticosteroids and cytostatic therapy are associated with reduced BMD.[59-62] Like postmenopausal women, children are particularly at risk for steroid-induced osteoporosis because of their more rapid bone turnover.[63-65] There are several mechanisms of glucocorticoid-induced osteoporosis. The greatest impact is on the vertebral trabecular bone.[66] Glucocorticoids decrease osteoblastic replication, differentiation, and lifespan by inhibiting genes for type 1 collagen, osteocalcin, insulin-like growth factors, bone morphogenetic proteins, receptor activator of nuclear factor kappa-B ligand (RANKL), and transforming growth factor β.[67] Glucocorticoids inhibit calcium absorption and prevent 1 α-hydroxylation of vitamin D in the kidney to form the active metabolite 1,25-dihydroxyvitamin D, further impairing calcium metabolism. Glucocorticoids directly inhibit many hormones and factors important in calcium accretion of bone.[59,64,65,68] They inhibit the expression of the vitamin D receptor in bone and the production of osteocalcin (the major bone matrix protein) and decrease local production of cytokines (which normally inhibit bone resorption).[64,65,68] Glucocorticoids inhibit osteoblastic activity and reduce the renal resorption of calcium. This effect can result in hypercalciuria, and, in turn, increase the parathyroid hormone, which further stimulates calcium loss from bone.

To further understand the pathophysiology of BMD loss in children undergoing NHL16 therapy, we will obtain serum calcium; serum magnesium; plasma 25-hydroxyvitamin D; osteocalcin N-MID; N-Telopeptide with creatinine; bone specific alkaline phosphatase; parathyroid hormone; and spot urine collection for magnesium, creatinine, calcium, and calcium/creatinine ratio. If the calcium:creatinine ratio exceeds 0.2, then a 24-h urine collection will be obtained for creatinine, calcium, and calcium/creatinine ratio.

2.5 Anthracycline Cardiotoxicity

Although anthracyclines (e.g., doxorubicin, daunorubicin) have contributed significantly to childhood cancer survival, they confer an excess risk of asymptomatic left ventricular dysfunction, cardiomyopathy, congestive heart failure, and death.[69-73] Anthracycline cardiotoxicity results in loss of cardiac myocytes, which impedes myocardial

development.[74] Unfortunately, myocardial effects can be harmful, asymptomatic,[71-75] and progressive.[76] Cardiotoxicity has been reported at all dose levels, but the risk increases with higher cumulative doses, younger age at first exposure, time from exposure, and female sex.[70-73,76] As many as 5% of at-risk survivors develop congestive heart failure 15 years after treatment.[70] The effects may be asymptomatic in up to 57% of survivors, becoming apparent only with other physiologic stressors such as infection or pregnancy.[71,73]

Children exposed to anthracycline agents are at increased risk for cardiac toxicity, which may become more clinically significant as they age. Late-onset anthracycline toxicity (occurring more than 1 year after therapy) generally manifests as left ventricular dysfunction. Although subclinical cardiac changes after anthracycline therapy are commonly reported, symptomatic disease is seen only occasionally, particularly in children treated on protocols that proactively restrict anthracycline cumulative doses.[70,73] Because most at-risk patients are asymptomatic, ongoing monitoring for late cardiac complications is critical. At a minimum, a baseline electrocardiogram and echocardiogram is recommended after the completion of therapy, with serial follow-up and cardiac referral as indicated based on age at treatment and cumulative cardiotoxic exposures.[77] The cumulative dosage of anthracycline in NHL 16 will be limited to 230 mg/m^2, as this dosage is associated with a very low risk of cardiomyopathy. Recommended off-therapy follow-up for late cardiac toxicity is as follows.

Recommended off-therapy follow-up for late cardiac toxicity

Age at Treatment	**Frequency of Echocardiographic Screening by Doxorubicin Dose**
<1 year	Every year
1 to 4 years	Every 2 years
≥5 years	Every 5 years

2.6 Gonadal and Reproductive Function

Among agents used to treat children with non-Hodgkin lymphoma, alkylating agents confer a dose-related risk of gonadal toxicity. Because they divide faster, male germ cells (and their supporting Sertoli cells) are more vulnerable to toxicity from cancer therapy than the testosterone-producing Leydig cells.[78] Leydig cell failure after radiotherapy is dose dependent and inversely related to age at treatment. Thus, infertility is more common than androgen insufficiency after treatment for cancer. Treatment with combinations of alkylating agents or cumulative doses of cyclophosphamide exceeding 7.5 g/m^2 increases the risk of germ cell dysfunction.[79-81] Contemporary treatment protocols for non-Hodgkin lymphoma attempt to restrict these exposures in an effort to preserve reproductive function. In the NHL 16 study, the total cumulative dose of cyclophosphamide will be limited to between 4.0 (no fractionation) and 4.2 g/m^2 (fractionation). Androgen insufficiency as a result of chemotherapy-related damage to Leydig cells occurs only occasionally. In fact, most males experience normal puberty and are capable of producing normal adult levels of testosterone. Appropriate screening for signs and symptoms of androgen insufficiency should be performed annually for

survivors of cancer. The Children's Oncology Group (COG) Long-Term Follow-Up Guidelines also recommend checking baseline serum luteinizing hormone (LH), follicle stimulating hormone (FSH), and testosterone levels at age 14 years and as clinically indicated in patients with evidence of delayed puberty or androgen insufficiency. In young men at risk for infertility, semen analysis remains the most accurate noninvasive method for evaluating germ cell function.

In contrast to male physiology, injury to the female germ cells results in both infertility and endocrine dysfunction.[78] Because females have a fixed number of primordial follicles at birth, which steadily declines with age, the risk for therapy-related ovarian failure and infertility is directly related to age. Thus, pre-pubertal girls, having a greater reserve of follicles, are at lowest risk for these complications.[78] Female children and adolescents treated with the cumulative doses of alkylators prescribed in regimens for non-Hodgkin lymphoma are at relatively low risk for both gonadal or germ cell dysfunction. Evaluation for gonadal failure and infertility centers on a good history (to identify irregular or absent menses, history of pregnancy, or difficulty conceiving) and an annual physical examination, with specific attention to Tanner staging. The COG currently recommends checking baseline serum LH, FSH, and estradiol levels at age 13 years and when clinically indicated for menstrual irregularity, delayed puberty, or symptoms of estrogen insufficiency. Although low cumulative doses of alkylators would most likely preserve fertility, risk and option for gamete preservation need to be discussed with patient and family.

2.7 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 newly diagnosed lymphoblastic lymphoma (patients must have <25% tumor cells in bone marrow by morphology)

3.1.2 Age ≤ 21 years

3.1.3 Limited prior therapy, including systemic glucocorticoids for 1 week or less, 1 dose of vincristine, emergency radiation therapy to the mediastinum, and 1 dose of IT chemotherapy. Other circumstances must be cleared by PI or co-PI.

3.1.4 Written, informed consent and assent following guidelines of the Institutional Review Board, National Cancer Institute (NCI), Food and Drug Administration (FDA), and Office of Human Research Protections (OHRP).

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 Recruitment and Screening of Research Participants

Research participants will be recruited by study investigators through regular clinical practice at St. Jude. It is anticipated that additional sites will be added as new collaborations are established.

3.4 Enrollment On Study at St. Jude

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

The CPDMO is staffed 7:30 am-5:00 pm CST, Monday through Friday. A staff member is on call Saturday, Sunday, and holidays from 8:00 am to 5:00 pm. Enrollments may be requested during weekends or holidays by calling the CPDMO "On Call" cell phone [redacted] or referencing the "On Call Schedule" on the intranet).

3.5 Enrollment On Study at Collaborating Sites

Collaborating Site research participants should be registered at St. Jude within 24 hours of enrollment at the site. The completed Eligibility Checklist and entire signed Informed Consent should be faxed to [redacted]. Please call [redacted] if confirmation of the enrollment information is needed. The Protocol Eligibility Coordinator will then register the research participant in the Patient Protocol Manager (PPM) system. 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 am to 5:00 pm.

4.0 RISK CLASSIFICATION

Patients will be classified into 1 of 3 categories on the basis of the bone marrow/peripheral blood lymphoma cells involvement at diagnosis and day 8 for T-lymphoblastic lymphoma and bone marrow/peripheral blood lymphoma cells involvement at diagnosis for B-lymphoblastic lymphoma as well as CNS and testicular involvement. The criteria and the estimated proportion of patients in each risk category (based on data from COG A5971 study) are provided below.

4.1 Criteria for Stratum 1 (approximately 70% of patients)

- Minimal disseminated disease (MDD) <1% at diagnosis in T-lymphoblastic lymphoma
- No bone marrow involvement microscopically at diagnosis in B-lymphoblastic lymphoma

Patients should **NOT** have:

- Any CNS involvement: 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) or traumatic LP (>10 RBC/μL of CSF with blasts)
- Overt testicular involvement (evidenced by ultrasonogram).

4.2 Criteria for Stratum 2 (approximately 15% of patients)

- MDD ≥1% and MRD negative (<0.01%) on day 8 in T-lymphoblastic lymphoma
- Bone marrow involvement microscopically present at diagnosis in B-lymphoblastic lymphoma
- Any CNS involvement: 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) or traumatic LP (>10 RBC/μL of CSF with blasts) but does not fulfill the criteria of stratum 3
- Overt testicular involvement (evidenced by ultrasonogram) but does not fulfill the criteria of stratum 3

4.3 Criterion for Stratum 3 (approximately 15% of patients)

- Any patients with MDD ≥1% and MRD positive (≥0.01%) on day 8 in T-lymphoblastic lymphoma

Any patient with detectable disease [MRD (≥0.01%), morphology and imaging (biopsy proven)] at the end of induction or thereafter may be considered for reintensification therapy and hematopoietic stem cell transplantation (HSCT) – See Section 5.4 and 5.6 for further details.

5.0 TREATMENT PLAN

Treatment will consist of 3 main phases: remission induction, consolidation (only for patients with any CNS disease and/or testicular involvement), and continuation.

5.1 Intrathecal Chemotherapy

As a traumatic lumbar puncture at diagnosis may result in a poor 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. IT-MHA will be administered immediately after cerebrospinal fluid is collected for diagnosis, as per the following age-dependent dosage chart:

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 IT-MHA treatments for remission induction are determined on the basis of patients' risk of CNS relapse as follows:

- All patients will receive IT-MHA treatment on days 1 and 15.
- Patients with any of the following features will receive additional IT-MHA treatment on days 8 and 22:
    - Any T-lymphoblastic lymphoma
    - Advanced stage (Stages III and IV) B-lymphoblastic lymphoma
    - Any CNS involvement: 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) or traumatic LP (>10 RBC/μL of CSF with blasts)
    - Testicular involvement (evidenced by ultrasonogram).

[Patients with limited stage (Stages I and II) B-lymphoblastic lymphoma will not receive IT-MHA on days 8 and 22.]

Leucovorin rescue (5 mg/m^2/dose, max 5 mg) PO will be given 24 and 30 h after each IT-MHA during induction.

Plasma methotrexate levels will be monitored (starting 24 h after IT therapy and until level becomes undetectable) in patients with renal dysfunction or extra fluid in third space, and rescue with leucovorin initiated according to pharmacist recommendation.

5.2 Remission Induction Treatment (6 to 7 weeks)

Remission induction treatment will begin with prednisone, vincristine, daunorubicin, PEG-asparaginase and IT-MHA treatment, followed by cyclophosphamide plus cytarabine plus thioguanine. 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 will be evaluated for herpes simplex infection and treated with acyclovir or famciclovir if infection is confirmed. Prednisone is replaced by dexamethasone on day 15 in patients with T-lymphoblastic lymphoma having MDD ≥1% at diagnosis and MRD positive (≥0.01%) on day 8 (If the day 8 MRD result is not available by day 15, change to dexamethasone as soon as positive MRD is reported). For patients with heterozygous or homozygous TPMT genotype, 6-mercaptopurine is used instead of thioguanine.

5.2.1 Drug dosages (See Section 5.2.1.1 for dosing of infants and sections 7.0 and 8.0 for treatment and dose modifications)

Patients with T-lymphoblastic lymphoma having MDD <1% at diagnosis or patients with B-lymphoblastic lymphoma with no bone marrow involvement microscopically (No CNS or testicular involvement) **(Stratum 1)**			
Agent(s)	Dosages and Routes	No. of Doses	Schedules
Prednisone[1] or prednisolone[1]	40 mg/m^2/day PO (divided t.i.d.)	84	Days 1–28
Vincristine	1.5 mg/m^2 IV (max 2 mg)	4	Days 1, 8, 15, 22
Daunorubicin[2]	25 mg/m^2 IV	2	Days 1, 8
PEG-asparaginase	2500 units/m^2 IM or IV	3	Day 3, 15, 29
Cyclophosphamide[3]	1000 mg/m^2 IV	1	Day 22
Cytarabine	75 mg/m^2/dose IV	8	Days 23–26, 30–33
Thioguanine (6-mercaptopurine for patients heterozygous or homozygous for *TPMT*)	60 mg/m^2/dose PO	14	Days 22–35

Patients with T-lymphoblastic lymphoma having MDD ≥1% at diagnosis and MRD negative on day 8 (<0.01%), patients with B-lymphoblastic lymphoma with bone marrow involvement microscopically at diagnosis, or patients with CNS involvement and/or testicular involvement (**Stratum 2**)			
Agent(s)	Dosages and Routes	No. of doses	Schedules
Prednisone[1] or prednisolone[1]	40 mg/m^2/day PO (divided t.i.d.)	84	Days 1–28
Vincristine	1.5 mg/m^2 IV (max 2 mg)	4	Days 1, 8, 15, 22
Daunorubicin[2]	25 mg/m^2 IV	2	Days 1, 8
PEG-asparaginase	2500 units/m^2 IM or IV	3	Day 3, 15, 29
Cyclophosphamide[3]	300 mg/m^2 IV	4	Q12 h on Days 22, 23
Cytarabine	75 mg/m^2/dose IV	8	Days 23–26, 30–33
Thioguanine (6-mercaptopurine for patients heterozygous or homozygous for *TPMT*)	60 mg/m^2/dose PO	14	Days 22–35

Patients with in T-lymphoblastic lymphoma having MDD ≥1% at diagnosis and MRD positive on day 8 (≥0.01%) (**Stratum 3**)			
Agents	Dosages and Routes	No. of Doses	Schedules
Prednisone[1] or prednisolone[1]	40 mg/m^2/day PO (divided t.i.d.)	24	Days 1–14[5]
Dexamethasone[4]	10 mg/m^2/day PO (divided t.i.d.)	42	Days 15[5]–28
Vincristine	1.5 mg/m^2 IV (max 2 mg)	4	Days 1, 8, 15, 22
Daunorubicin[2]	25 mg/m^2 IV	2	Days 1, 8
PEG-asparaginase	2500 units/m^2 IM or IV	3	Day 3, 15, 29
Cyclophosphamide[3]	300 mg/m^2 IV	4	Q12 h on Days 22, 23
Cytarabine	75 mg/m^2/dose IV	8	Days 23–26, 30–33
Thioguanine (6-mercaptopurine for patients heterozygous or homozygous for *TPMT*)	60 mg/m^2/dose PO	14	Days 22–35

1. *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.*
2. *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 by up to 1 week if clinically indicated.*
3. *See 5.2.3.2 for the criteria of starting cyclophosphamide plus thioguanine on day 22.*
4. *Oral dexamethasone can be substituted with IV dexamethasone.*
5. *If day 8 MRD result is not available by day 15, change to dexamethasone as soon as positive MRD is reported.*

5.2.1.1 Dose adjustment for infants

Exception for vincristine, doses of all other drugs given to infants (<1 year old) will be based on body surface area. For infants younger than 1 month or infants younger than 3 months who were born significantly prematurely, doses of daunomycin, asparaginase, methotrexate, thiopurines, cyclophosphamide, and cytarabine will be reduced by 50%. The vincristine dose for patients younger than 1 year or less than 10 kg in weight will be 0.05 mg/kg/dose.

5.2.2 Dose modifications for remission induction

See section 7.0 for treatment modifications for vincristine, daunorubicin, and PEG-asparaginase and section 7.10 for modification of thiopurine dosing based on TPMT status. After 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 and cytarabine have been given), allowing early bone marrow recovery and early initiation of consolidation therapy.

5.2.3 Bone marrow/peripheral blood evaluations

5.2.3.1 Day 8

A peripheral blood MRD will be done on day 8 of remission induction to assess response in T-lymphoblastic lymphoma. In patients with MDD ≥1% at diagnosis, the presence of detectable lymphoma blasts by MRD study (≥0.01%) on day 8 is an indication for Stratum 3 therapy and 120 weeks of continuation therapy.

5.2.3.2 Day 22

For neutropenic 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.

5.2.3.3 Disease response at end of remission induction

Imaging by PET/CT and morphologic evaluation of bone marrow as well as MRD studies of bone marrow and peripheral blood (T-lymphoblastic lymphoma only) will be performed on days 38–42 of remission induction, depending on when ANC has recovered to ≥300/mm^3, WBC to >1000/mm^3, and platelet count to ≥50×10^9/L. If the date falls on a weekend or holiday, the procedure will be performed on closest working day. Any patient with evidence of disease [imaging (biopsy proven) or BM morphology] or detectable MRD at the end of induction or thereafter may be considered for reintensification therapy and HSCT.

5.3 Consolidation Treatment (10 weeks)

Consolidation therapy will be considered only for patients with any positive CNS disease or testicular involvement.

5.3.1 Drug dosages

Agent	Dosage and Route	No. of Doses	Schedule
PEG-asparaginase	2500 units/m^2 IM or IV	5	Day 1, 15, 29, 43, 57
High-Dose Methotrexate (HD-MTX)	5000 mg/m^2 or targeted 65 μM	4	Days 8, 22, 36, 50
Mercaptopurine	50 mg/m^2/day	70	Days 1-70

When ANC of ≥300/mm^3, WBC of ≥ 1000/mm^3, and platelet count of ≥ 50×10^9/L are reached, consolidation treatment will be started, consisting of PEG-asparaginase (every alternate week for 5 doses), HD-MTX (every alternate week for 4 doses, not concurrent with PEG-asparaginase), daily mercaptopurine, and IT chemotherapy (on the same days as HD-MTX) (see section 5.3.4). See section 5.3.5 on 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. For patients in whom HD-MTX treatment is delayed, mercaptopurine may be continued until 14 days after the last course of HD-MTX. Mercaptopurine may be held in the presence of ANC <300/mm^3, WBC <1000/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 HD-MTX and mercaptopurine treatment. See section 7.10 for modifications of mercaptopurine based on TPMT status.

5.3.3 HD-MTX administration

HD-MTX at 5 g/m^2 (or a dose targeted to achieve a steady-state plasma concentration of 65 μM) will be administered IV over 24 h. The subsequent dose of HD-MTX, mercaptopurine, and IT treatment will be delayed if ANC is <300/mm^3, WBC is <1000/mm^3, platelet count is <50 × 10^9/L, SGPT is >500 U/L, total bilirubin is >2 mg/dL and direct bilirubin is >1.4 mg/dL, or mucositis is present. Sodium bicarbonate may be given orally at 1 g/m^2 every 6 h or IV with prehydration fluid starting the day before HD-MTX. For patients with Down syndrome, dosages of HD-MTX will be modified (see section 7.1.1).

The results from TOTXV suggest that a dose of 5 g/m^2 can achieve the targeted concentration of 65 μM ± 20% in only 62% of the courses (see section 2.1.4 for details and rationale). In NHL16, clearance will be estimated from the plasma concentrations up to 48 h 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. Of the total dose, 10% will be administered as a loading dose over 1 h and the remainder over 23 hours.

5.3.3.1 Pre-hydration

At least 2 h before HD-MTX administration, pre-hydration IV fluid (D5W + 40 mEq $NaHCO_3$/L + 20 mEq KCl/L) will be administered at the rate of 200 mL/m^2/h. At start of pre-hydration, 1 IV dose of $NaHCO_3$ (unless otherwise clinically indicated, 25 mEq/m^2) diluted in 50 mL D5W will be given over 15 min. Pre-hydration fluid may also be given overnight at a rate of at least 125 mL/m^2/h. HD-MTX treatment will follow if urinary pH is ≥6.5; exceptions must be cleared by the Pharmacokinetics Service and the attending physician.

5.3.3.2 HD-MTX infusion

Methotrexate loading dose will be given over 1 h, followed immediately by maintenance infusion over 23 h. During the methotrexate infusion, patients will receive hydration fluid with D5W + 40 mEq/L $NaHCO_3$ + 20 mEq KCl/L at 100–150 mL/m^2/h. 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 PO every 6 to 8 h) may be given 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 a methotrexate infusion of less than 24 hours. Blood samples for methotrexate pharmacokinetics will be drawn according to procedures in section 10.1.

5.3.3.3 Leucovorin rescue

Leucovorin (15 mg/m^2 PO or IV) will be started 42 h after beginning methotrexate and repeated every 6 h for a total of 3 doses, as described by Reiter et al.[82] The dosage of leucovorin will be increased in patients with high plasma methotrexate concentrations (>1 μM at 42 h) and continued until the methotrexate concentration is less than 0.10 μM. Additional measures, such as hydration, hemoperfusion, or glucarpidase will be considered in patients with 42-h methotrexate levels >10 μM. Patients with a history of delayed grade 3 or 4 gastrointestinal toxicity with prior methotrexate or a history of typhlitis with any chemotherapy should be continued on leucovorin for 5 (rather than 3) doses; for those with early toxicity, leucovorin should be initiated at 36 h with subsequent methotrexate; if toxicity recurs, the baseline leucovorin dosage should also be increased.

5.3.4 Intrathecal (IT) chemotherapy

All patients will receive IT-MHA therapy every alternate week for 4 doses on days 8, 22, 36, and 50 (dosages are based on age, according to guidelines in section 5.1). IT treatment should be given on the same day as HD-MTX. If there is more than a 12-h gap between IT and HD-MTX administration, the PI or the Pharmacokinetics Service should be contacted.

5.3.5 Interim continuation treatment

Interim continuation treatment will be given to the occasional patients who, after attaining complete remission, are deemed unable to tolerate HD-MTX. Specific criteria for the use of 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; other unforeseen reasons may also warrant temporary withholding of HD-MTX.

Interim treatment will consist of oral mercaptopurine 75 mg/m^2 per day (50 mg/m^2 per day in those with ANC <500/mm^3) and intravenous methotrexate 40 mg/m^2 per week; IT may be given every other week during this period of time and continued during the subsequent HD-MTX treatment for a total of 4 doses. HD-MTX will be started when the patient's physical condition allows. In the event that the interim therapy is longer than 4 weeks, an extra IT treatment may be given with the last course of HD-MTX. Patients with a defective TPMT status may receive lower doses of mercaptopurine. Interim continuation therapy can be considered in other phase of treatment if necessary.

5.4 Reintensification Treatment

Options for patient with detectable disease by PET/CT scan (biopsy proven), bone marrow morphology, or MRD of bone marrow or peripheral blood at the end of induction or thereafter.

Patients with detectable disease by PET/CT imaging (biopsy proven), MRD, or morphologic disease at the end of induction or thereafter may receive reintensification therapy and will then be offered the option of transplant. This treatment will attempt to maximize lymphoma cell kill before allogeneic HSCT. When marrow is recovered (i.e., ANC ≥300/mm^3, WBC ≥1000/mm^3 and platelet count ≥ 50×10^9/L) after a course of reintensification, imaging, MRD and morphologic studies will be repeated.

Reintensification may be repeated if there was apparent benefit with the first course. This decision will also depend on HSCT planning by primary MD and BMT division MD. Patients deemed unsuitable for the transplant, those who decline the procedure, or those for whom donors have not yet been 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	No. of Doses	Schedule
Dexamethasone	20 mg/m^2/day PO or IV (divided t.i.d)	18	Days 1–6
Cytarabine	2000 mg/m^2, 3-h IV infusion every 12 h	4	Days 1–2
Etoposide	100 mg/m^2, 1-h IV infusion every 12 h	5	Days 3–5
IT-MHA	See section 5.1 for age-specific dose	1	Day 5
PEG-asparaginase	3000 units/m^2 IM or IV	1	Day 6

Patients with suboptimal response to reintensification may receive 1 or 2 cycles of clofarabine/cyclophosphamide/etoposide/dexamethasone:

Agent	Dosage and Route	No. of Doses	Schedule
Clofarabine	40 mg/m^2/day, 2-h IV infusion	5	Days 1–5
Etoposide	100 mg/m^2/day, 2-h IV infusion	5	Days 1–5
Cyclophosphamide	300 mg/m^2/day, 30–60 min IV infusion	5	Days 1–5
Dexamethasone	8 mg/m^2/day (divided t.i.d)	15	Days 1–5

All dosages given to infants (<1 year old) will be based on body surface area. For infants <1 month old or for infants <3 months of age born significantly prematurely, a 50% reduction in dosages of asparaginase, cyclophosphamide, cytarabine, clofarabine and etoposide will be made.

5.5 Continuation Treatment

(98 Weeks for Stratums 1 and 2 and 120 Weeks for Stratum 3 and Patients with any CNS Disease and/or Testicular Disease). Post-remission continuation treatment will be initiated after the completion of remission induction (consolidation for patients with CNS and/or testicular disease) if ANC is ≥300/mm^3, WBC is ≥1000/mm^3, and platelet count is ≥50×10^9/L as well as no evidence of grade 3 or 4 mucositis.

Week	All Stratums
1	DEX+DOX+VCR+MP + PEG-ASP
2	MP
3	MP + PEG-ASP
4	DEX + DOX + VCR + MP
5	MP + PEG-ASP
6	MP
7[3]	[1]Reinduction I
8[3]	Reinduction I
9[3]	Reinduction I
10	MP
11	DOX + VCR +MP + PEG-ASP
12	MP
13	MP + PEG-ASP
14	DEX + DOX + VCR +6MP
15	MP + PEG-ASP
16	MP
17[3,4]	[1]Reinduction II
18[3,4]	Reinduction II
19[3,4]	Reinduction II
20[4]	No chemotherapy (MP + MTX for stages I and II B–lymphoblastic lymphoma)

Note: DEX, dexamethasone; DOX doxorubicin; VCR, vincristine; MP, 6-Mercaptopurine; PEG-ASP, PEG-asparaginase. See 5.5.3 for intrathecal therapy schedule.

1. *MRD study in peripheral blood.*
2. *Any patient with detectable MRD at week 17 can receive reintensification treatment after reinduction II and HSCT can be considered (see section 5.4).*
3. *See section 5.5.2 for details on reinduction treatment.*
4. *See 5.5.2.3 for patients with stages I and II B-lymphoblastic lymphoma.*

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. Doxorubicin, mercaptopurine and methotrexate will be reduced for WBC <1500/mm^3, or if WBC and ANC not increase by at least 2 folds a week after the start date of dexamethasone pulse (see section 7.0 for other dose modifications). *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.

5.5.1 Drug dosages, schedules, and routes for continuation therapy for Weeks 1 to 6 and Weeks 10 to 16

Agent	Schedule
Dexamethasone	12 mg/m^2 for 5 days, Days 1-5
Doxorubicin	30 mg/m^2 IV, Day 1
Vincristine	2 mg/m^2 IV push (max. 2 mg), Day 1(0.05 mg/kg for patients <1 year of age or <10 kg in weight)
Mercaptopurine	50 mg/m^2 PO daily for 7 days, Days 1-7
PEG-asparaginase	2500 units/m^2 IM or IV, Day 1

See section 5.2.1.1 for dosing for infants.

5.5.2 Reinduction treatment

Reinduction treatment will be started at weeks 7 and 17. 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 ≥1800/mm^3 and ANC >300/mm^3.

Reinduction treatment will be given twice: at weeks 7–9 and weeks 17–19 for patients with all T-lymphoblastic lymphoma and advanced-stage (stages III and IV) B-lymphoblastic lymphoma. For patients with limited-stage B-lymphoblastic lymphoma (stages I and II), reinduction II treatment will not include high-dose cytarabine.\
IT treatment will be followed by leucovorin rescue (5 mg/m^2/dose PO, max 5 mg) at 24 and 30 h only in patients with prior CNS toxicities or in those with WBC < 1500/mm^3, or ANC < 500/mm^3.

5.5.2.1 Reinduction I

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 0.05 mg/kg for infants <1 year old or <10 kg in weight (max 2 mg)	3	Days 1, 8, 15
Doxorubicin	30 mg/m^2 IV	2	Days 1, 8
PEG-asparaginase	2500 units/m^2 IM or 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 all T-lymphoblastic lymphoma and advanced-stage (stages III and IV) B-lymphoblastic lymphoma

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 0.05 mg/kg for infants <1year old or <10 kg in weight (max 2 mg)	3	Days 1, 8, 15
PEG-asparaginase	2500 units/m^2 IM or IV	2	Days 1, 15
Methotrexate + hydrocortisone +Ara-C	Age-dependent, IT	1	Day 1
High-dose cytarabine	2000 mg/m^2 IV q 12 h	4	Days 15, 16

5.5.2.3 Reinduction II for limited stage (stages I and II) B-lymphoblastic lymphoma

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 0.05 mg/kg for infants <1year old or <10 kg in weight (max 2 mg)	3	Days 1, 8, 15
PEG-asparaginase	2,500 units/m^2 IM or IV	2	Days 1, 15
Methotrexate + hydrocortisone +Ara-C	Age-dependent, IT	1	Day 1

Mercaptopurine (75 mg/m^2 PO daily for 7 days, Days 1–7) and methotrexate (40 mg/m^2 IV or IM, Day 1) will be given in week 20 in this group.

5.5.3 Intrathecal chemotherapy - IT-MHA treatment will be given as follows:

- Patients with *limited stage (stages I and II) B-lymphoblastic lymphoma with CNS-1 status (no identifiable blasts in CSF)* on weeks 1, 3, 5, 7, 12, 17, 25, 29, 33, 37, 41, 45, 49, 57, 65, and 73 (total number of treatments 18).

- Patients with *T-lymphoblastic lymphoma (any stage) and advanced stage (stages III and IV) B-lymphoblastic lymphoma with CNS-1 status* on weeks 1, 3, 5, 7, 12, 17, 25, 29, 33, 37, 41, 45, 49, 57, 65, 73, 81, 89, and 98 (total number of treatments 23).

- Any patient with *CNS-3 status, CNS-2 status or traumatic CSF with blasts status, or testicular involvement* on weeks 3**,** 7, 12, 17, 25, 29, 33, 37, 41, 45, 49, 57, 65, 73, 81, 89, and 98 (total number of treatments 25).

The number of IT treatments for patients with detectable disease after induction will depend on whether patients receive HSCT, and, if so, when they receive it. Leucovorin will not be given after IT treatment during continuation treatment unless the patient has an adverse reaction with previous IT or methotrexate treatment, e.g., seizure or encephalopathy, has renal dysfunction resulting in high plasma methotrexate concentration, has Down syndrome and has unacceptable toxicity despite decrease in dosage, or has WBC < 1500/mm^3 or ANC < 500/mm^3.

Intrathecal therapy in NHL16

Histology and stage	CNS Status	Induction		Consolidation		Continuation Week 1-20		Continuation Weeks 21-98		All Phases
		Days	**Total**	**Days**	**Total**	**Weeks**	**Total**	**Weeks**	Total	Total
Limited stage (stages I and II) B-lymphoblastic lymphoma	CNS-1	1, 15	2		0	1, 3, 5, 7, 12, 17	6	25, 29, 33, 37, 41, 45, 49, 57, 65, 73	10	18
T-lymphoblastic lymphoma (any stage) and advanced stage (stages III and IV) B-lymphoblastic lymphoma	CNS-1	1, 8, 15, 22	4		0	1, 3, 5, 7, 12, 17	6	25, 29, 33, 37, 41, 45, 49, 57, 65, 73, 81, 89, 98	13	23
Any	CNS-3, CNS-2 or traumatic CSF with blasts, or testicular involvement	1, 8, 15, 22	4	8, 22, 36, 50	4	3, 7, 12, 17	4	25, 29, 33, 37, 41, 45, 49, 57, 65, 73, 81, 89, 98	13	25

5.5.4 Treatment (weeks 21 to 23)

Week	Treatment
21	MP + PEG-ASP[1]
22	MP[1]
23	MP + PEG-ASP[1]

Note: MP, 6-mercaptopurine; PEG-ASP, PEG-asparaginase; Patients who receive consolidation therapy will not receive PEG-ASP and weekly MTX will be given instead for weeks 21–23.

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 24 to end of therapy)

Week	Treatment
24	Cyclo + Ara-C
25	[1]DEX + VCR
26	MP + MTX
27	MP + MTX
28	Cyclo + Ara-C
29	[1]DEX + VCR
30	MP + MTX
31	MP + MTX

Note: DEX, dexamethasone; MP, 6-mercaptopurine; Cyclo, cyclophosphamide; Ara-C, cytarabine; VCR, vincristine; MTX, methotrexate. IT MHA (methotrexate + hydrocortisone + cytarabine; see section 5.1 for dosage and 5.5.3 for schedule).

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

Agent	Schedule
Mercaptopurine	75 mg/m^2 PO daily for 7 days, Days 1–7
Methotrexate	40 mg/m^2 IV or IM, Day 1
Cyclophosphamide	300 mg/m^2 IV, Day 1 *between week 21 and week 61*
Cytarabine	300 mg/m^2 IV, Day 1 *between week 21 and week 61*
Dexamethasone	12 mg/m^2 PO daily (divided t.i.d.) for 5 days, Days 1–5 *between week 21 and week 61*
Vincristine	2 mg/m^2 IV push (max 2 mg), Day 1 *between week 21 and week 61* (0.05mg/kg for patients <1yr or < 10 kg)
PEG-asparaginase[1]	2500 units/m^2 IM, Day 1 in weeks 21 and 23

Patients who receive consolidation therapy will not receive PEG-ASP and weekly MTX will be given instead for weeks 21–23.

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.

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 or ANC ≤300/mm^3. Mercaptopurine and methotrexate will be reduced for WBC < 1500/mm^3, or if WBC and ANC do not increase by at least 2 folds a week after the start date of dexamethasone pulse. 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 4 times, until week 61 (see section 5.5.3 for IT therapy). After week 61, Stratums 1 and 2 patients will receive daily mercaptopurine and weekly methotrexate until week 98 and Stratum 3 patients and those with any CNS disease and/or testicular involvement until week 120.

5.6 Hematopoietic stem cell transplantation

Patients who have detectable disease by imaging (biopsy proven), MRD, or morphology at the end of induction or thereafter are candidates for allogeneic HSCT. However, if patients or guardians decline this option, or the procedure is deemed unsuitable by the attending physician and the PI, 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/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 Patients with Down Syndrome

Patients with Down syndrome can be treated on NHL 16, with the following modifications:

7.1.1 HD-MTX: Dosages of HD-MTX will be modified because patients with Down syndrome have altered MTX pharmacokinetics and enhanced tissue sensitivity to the effects of MTX. The hydration and alkalinization regimen should be the same as outlined in section 5.3.3. However, the dose of HD-MTX should be 500 mg/m^2 (50 mg/m^2 over 1 h and 450 mg/m^2 given over 23 h). Baseline leucovorin rescue will begin early (at hour 30 at 30 mg/m^2 IV q 6 h × 2 doses, followed by 10 mg/m^2 IV q6 h × 6 doses). If MTX plasma levels are elevated, increased leucovorin rescue will be recommended by the Pharmacokinetics Service. Vigorous hydration should be ensured until the 42-h MTX level is known.

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

7.1.3 IT therapy: IT should be administered as outlined in section 5.1.

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

7.2 Renal Dysfunction

Subclinical renal impairment (normal serum creatinine but decreased glomerular filtration rate) may be present in patients receiving concurrent nephrotoxic drugs (e.g., IV acyclovir), which should, if possible, be held during and for 20 h after HD-MTX infusions or until adequate MTX clearance has been documented. Delaying MTX should be considered 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% decrease in dosage
Direct bilirubin 4–6 mg/dL:	75% decrease in dosage
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.

HD-MTX should be withheld if there is evidence of existing mucositis or if total bilirubin is >2 mg/dL and direct bilirubin is >1.4 mg/dL.

Subclinical hypertransaminasemia (SGPT >500 IU/L) is an indication to delay only HD-MTX, but not other chemotherapy.

7.4 Obesity

Actual body weight will be used to calculate body surface area in all patients and used for dosage calculations (Exception: vincristine dosage will be capped at 2 mg).

7.5 Testicular Involvement at Diagnosis

An ultrasonogram should be performed to differentiate testicular lymphoma involvement from hydrocele and to measure the testicular volume. Overt testicular involvement at diagnosis *per se* is not an indication for testicular irradiation, as many patients can be successfully treated with chemotherapy, including HD-MTX. An ultrasonogram should again be performed after completion of remission induction. If testicular size is still

abnormally enlarged, the sonogram should be repeated after consolidation treatment with HD-MTX and mercaptopurine. Patients with persistently enlarged testes after consolidation treatment will undergo biopsy. Testicular irradiation (24 Gy) will be administered in 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 syndrome of inappropriate antidiuretic hormone secretion (SIADH) develop, the dose may be reduced to 1 mg/m^2. Vincristine should be withheld only if patients develop motor paralysis or typhlitis.

7.7 Venous Thromboembolism

For patients who develop cerebral or other venous thrombosis during remission induction, subsequent treatment will be modified. Dexamethasone will be omitted from weeks 1, 4, and 14 of continuation treatment, and will be given on days 7–14 instead of 1–8 and 15–21 during reinduction I and II. Low-molecular-weight heparin may be required during the first 23 weeks of continuation treatment. Acute management of thrombosis will be as per St. Jude guidelines on the use of thrombolysis and anticoagulation. Questions on steroid and asparaginase dosing should be referred to the PI or co-PI.

7.8 Osteonecrosis of Bone

Total joint MRI exams will be interpreted by the radiologist. If there is evidence of epiphyseal or metaphyseal lesions involving any joint or lesions of talus consistent with osteonecrosis, the patient will be referred to the orthopedic surgeon, who will evaluate symptoms and assess the severity and estimated risk of progression. Physical therapy, activity modifications, and surgical procedures will be recommended as needed. Patients with hip, knee and shoulder epiphyseal lesions or talus lesions affecting >30% of weight-bearing area will have an X-ray of the affected area, and will be assessed as being at higher risk for progression. Dexamethasone will be discontinued in symptomatic patients with such findings, especially if they are past reinduction II in therapy. Dexamethasone dose may be reduced by 50% in asymptomatic patients with such findings, especially if they are past reinduction II in therapy. Patients with X-ray findings positive for osteonecrosis 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 reevaluated by orthopedic follow-up and re-imaging if needed. If dexamethasone is discontinued, the

first choice will be to replace each week's dosing of dexamethasone with 1 dose of methotrexate (40 mg/m^2), if tolerated. See section 13.4 for additional guidelines.

7.9 PEG-asparaginase

Patients with allergic reactions to PEG-asparaginase will instead be given *Erwinia* L-asparaginase IM or IV. If allergy occurs with the first or second PEG dose and the patient requires a dose at day 15 and/or 29 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 remission induction, each dose of PEG will be replaced by *Erwinia* L-asparaginase at 30,000 units/m^2/dose, twice weekly (3 to 4 days apart) for 4 doses. During reintensification, *Erwinia* L-asparaginase will be given 36,000 units/m^2/dose, twice weekly (3 to 4 days apart) for 2 doses.

Asparaginase treatment should be delayed until at least 2 h after IT treatment. If patients become allergic to both forms of asparaginase, methotrexate 40 mg/m^2/week should be given instead during the week of continuation treatment.

7.10 TPMT Status and Thiopurine Dosage

Defects in the TPMT enzyme 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 used mercaptopurine at a dosage of 75 mg/m^2/day, whereas other studies on ALL used a starting dosage of 60 mg/m^2/day and then titrate up. Although how TPMT status affects 6-mercaptopurine dosage is not fully 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, clinical and preclinical evidence suggests that the 10% of patients who carry a variant *TPMT* allele may be at higher risk for secondary tumors. Because such patients often require a decrease in dosage of thiopurines, and because overall leukemia-free survival has been shown to be excellent among patients with TPMT defects (in a setting in which dosage was adjusted in about 1/3 of patients with TPMT defects), we recommend that in NHL 16, patients with a phenotype or genotype consistent with at least 1 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 × 10^8 red cells) (see sections 8.1 and 8.2). Further changes in mercaptopurine dose should be determined on the basis of WBC and ANC counts. As far as possible, other anticancer agents should be administered at protocol doses for these patients. A blood sample (5 mL) should be drawn along with routine lab work at day 3 of remission induction to allow for timely *TPMT* genotyping.

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, which account for >90% of all variant alleles. On the basis of genotyping, patients will be

assigned a homozygous wild-type, heterozygous, or homozygous variant TPMT status. As specified in section 8.2, levels of 6-thioguanine nucleotides (6-TGN) thiopurine metabolite should be measured at the start of reinduction I and TPMT activity in RBCs assessed at least by week 17 for all patients. 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 for 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 a heterozygote; using these criteria, approximately 10% of patients are expected to be heterozygous or homozygous variants, in agreement with population studies for TPMT status, and thus will require decrease in doses of thiopurine on the basis of TPMT status. See section 8.2 for additional information on dose modification.

7.11 Pancreatitis

Acute hemorrhagic pancreatitis is a contraindication to continue asparaginase treatment. In case of mild to moderate pancreatitis, asparaginase should be withheld until symptoms and signs subside and amylase and lipase levels return to normal. For patients with abdominal pain that may be due to pancreatitis, serum amylase and lipase levels should be measured and an abdominal sonogram or CT scan done. For patients with severe pancreatitis (i.e., abdominal pain of 72 h or more, amylase level 3 times or more of the upper limit of normal, and sonographic or CT scan showing evidence of pancreatitis), asparaginase may be discontinued permanently when the possibility of glucocorticoid- or mercaptopurine-induced pancreatitis is excluded. In patients with mild to moderate pancreatitis (abdominal pain less than 72 h and amylase and lipase level less than 3 times the upper limit of normal), asparaginase should be withheld and resumed once symptoms and signs subside. For asymptomatic patients (no abdominal pain, only elevated amylase or lipase levels), management should be discussed with the PI or co-PI. Also, management of patients with dexamethasone- or mercaptopurine- related pancreatitis should be discussed with the PI or co-PI.

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 $\geq 50\times10^9$/L. Full dose of treatment will be administered when WBC $\geq$1500/mm^3, ANC is $\geq$300/mm^3 and platelet count $\geq 50\times10^9$/L; 30 to 50% dose reduction of mercaptopurine, methotrexate, or both should be considered if WBC is between 1000 and 1500/mm^3 with ANC $\geq$300/mm^3 and platelet count $\geq 50\times10^9$/L.

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 $\leq$1500/mm^3 the week after prior dexamethasone pulse. Adjustments of dosages

should be made in the following circumstances, with re-evaluation of tolerance and toxicities every 8 to 16 weeks.

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 ($\geq 3\times10^9$/L) and ANC (>1000/mm^3) counts should be counseled on compliance, particularly if 6TGN levels are <100 pmol/8×10^8 RBCs. If the WBC remains high, mercaptopurine and methotrexate dosages should be increased by 30% (to 100 mg/m^2 for mercaptopurine and 50 mg/m^2 for methotrexate), using a stepwise approach if needed. If patients have a TPMT defect, mercaptopurine dosage should not be increased unless the 6TGN levels are <100 pmol/8×10^8 RBCs.

8.2 Dose Decreases Based on Mercaptopurine Pharmacology

6TGN levels will be measured at the start of reinduction I (i.e., week 7) in all patients. TPMT activity 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 *TPM*T variant alleles. 6TGN and TPMT activity will be measured subsequently in patients with high 6TGN levels, suspected noncompliance, toxicity-related problems, or high blood counts.

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

Dosages will be reevaluated every 8–16 weeks. Other causes of low blood counts will also be considered (see section 13.5).

Patients missing less than 25% of therapy and with WBC $<3\times10^9$/L and ANC <1000 will not need any change in dosage, regardless of 6TGN level. See section 7.10 for mercaptopurine dosage adjustments.

9.0 CONTINGENCY PLANS FOR REFRACTORY DISEASE OR RELAPSE

9.1 Induction Failures

Patients who do not attain complete remission (imaging, morphologic BM exam, or MRD studies of BM or PB) after remission induction, consolidation treatment and reintensification treatment will be removed from the protocol treatment. Patients who do not achieve a remission after induction therapy, but subsequently attain complete remission after consolidation or reintensification treatment, are candidates for allogeneic HSCT.

9.2 Relapse

Patients with any form of relapse (original site, lymph node, bone marrow, testes, ovarian, etc.) except that of CNS will be eligible for relapse protocols. Patients with overt CNS relapse (i.e. ≥ 5 WBC/μL of CSF with blasts) will remain on study and receive the treatment outlined in section 9.3. 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 9.3.

9.3 Emergence of CSF Lymphoblasts During Remission Requiring CNS Radiation

Therapeutic cranial irradiation will not be given consolidatively to patients with CNS disease at diagnosis. Patients with immunologically proven leukemic lymphoblasts in CSF (regardless of cell count) during remission on 2 occasions in the study will receive therapeutic CNS irradiation after consultation with radiation oncologists and after receiving a second remission induction (as detailed in section 5.2), followed by 1 or 2 cycles of reintensification (as detailed in section 5.4) to consolidate remission after induction. Whether 1 or 2 cycles will be given will depend on risk group, time to emergence of blasts in CSF, immunophenotype of blasts, and individual patient tolerance. In general, patients with early occurrence (<18 months) will receive 2 cycles, but this should be discussed with the PI. IT-MHA therapy will be continued every 3 to 4 weeks.

CNS irradiation for patients with CNS relapse will be 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. Patients receiving cranial irradiation only should receive 4 or 5 doses of IT-MHA therapy with leucovorin rescue during irradiation. Mercaptopurine and methotrexate will be withheld for at least 1 week before 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 1 year from time of relapse (at least 2.5 years, including initial treatment).

10.0 PHARMACOKINETIC AND PHARMACODYNAMIC STUDIES

10.1 High-Dose Methotrexate (HD-MTX)

Blood (2–3 mL) will be obtained in EDTA tubes (purple top) pre-dose and between 0.5 and 6 h, 23 h, and 42 h from the start of infusion. Samples at additional time points 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 h) in order to adjust leucovorin rescue. (Note: blood should be in purple top tube to facilitated nucleic acid recovery from the buffy coat). Collaborating sites should follow local practice for MTX monitoring.

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, and the following exams/tests will be conducted:

All pre-treatment tests are standard of care (except some fertility studies, see Section 11.4)

- Complete history and physical exam, with careful notation and assessment of clinical signs relevant to lymphoma
- Complete blood count with differential
- Chemistry profile: glucose, electrolytes, blood urea nitrogen (BUN), creatinine, lactate dehydrogenase (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 25-hydroxyvitamin D, osteocalcin N-MID, urine N-Telopeptide with creatinine, bone specific alkaline phosphatase, parathyroid hormone
- Thyroid function test (free T4, TSH)
- Chest X-ray
- Echo (echocardiogram)/EKG (electrocardiogram) and/or multiple gate acquisition scan (MUGA)
- Neck/chest/abdomen/pelvis CT
- Positron emission tomography (PET)/CT scan
- Biopsy/cytology
- Bone marrow evaluation: morphology, cytochemistry, immunophenotyping, cytogenetics, DNA index, molecular diagnosis, MRD studies
- Peripheral blood MRD study
- Lumbar puncture with CSF examination (cell count with differential of cytospin preparation)
- Other studies as clinically indicated, e.g. sickle cell prep, hemoglobin electrophoresis, and G6PD screen for Black children; varicella titer; Hepatitis B antigen; HIV, Epstein-Barr virus (EBV), toxoplasma (TOXO), cytomegalovirus (CMV), histoplasma, and *Bartonella* titers.

11.2 Required Evaluations, Tests, and Observations During Treatment

	At diagnosis	Induction & Consolidation	Continuation	Reinduction I & II
History and 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 HD-MTX	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 HD-MTX	As indicated	Day 1 of Weeks 7, 8, 9, 17, and 19
MRD (see tables below)	At diagnosis	Day 8 (± days 15 and 22)*, and end of induction	Week 49* Week 98 or 120**	Day 1 Weeks 7 & 17*
Total cholesterol, triglycerides, free fatty acids, and high-density lipoprotein cholesterol	At Diagnosis	- - -	After completion of therapy (Week 98 or 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 and Week 98 or 120**	With each intrathecal treatment
Chest X-ray	At diagnosis	As indicated	As indicated	As indicated
Biopsy/cytology	At diagnosis			
Bone marrow aspirate and biopsy	At diagnosis	End of induction†	Week 98 or 120**	Day 1 (Weeks 7, 17, 49)‡
Neck/chest/abdomen/pelvis CT	At diagnosis	End of induction	Week 98 or 120**	Prior to week 7§
NM PET/CTscan	At diagnosis	End of induction	Week 98 or 120**	Prior to week 7§
EKG and Echo and/or MUGA, Troponin T, Natriuretic hormone levels##	At diagnosis		1 year from enrollment Week 98 or 120**	
Total joint MRI (shoulders, elbows, hips, knees and ankles: ≥9 year olds only)	- - -	End of induction	After completion of therapy (Week 98 or 120**, or later)	After each reinduction phase (Weeks 12-14 and 22-30)
QCT for bone density	Week 1	- - -	After completion of therapy (Week 98 or 120**, or later)	- - -

Required Evaluations, Tests, and Observations During Treatment

	At diagnosis	Induction & Consolidation	Continuation	Reinduction I & II
Plasma 25-hydroxyvitamin D, osteocalcin N-MID, urine N-Telopeptide with creatinine, parathyroid hormone, bone specific alkaline phosphatase, serum calcium and serum magnesium	At diagnosis	- - -	After completion of therapy (Week 98 or 120**, or later)	- - -
Spot urine for magnesium, creatinine, calcium, and calcium/creatinine ratio¶	At diagnosis	- - -	After completion of therapy (Week 98 or 120**, or later)	- - -
PT/OT evaluation (≥9 year olds only)	- - -	- - -	After completion of therapy (Week 98 or 120**, or later)	After each reinduction
TPMT genotype		Day 3 (TPMT genotype)		
TPMT and TGN	- - -		- - -	Day 1 of Week 7 (TGN) and week 17(TPMT activity)
CYP2D6 genotyping		Day 3		
MTX pharmacokinetics	- - -	Each HD-MTX	- - -	
Thyroid function	At diagnosis	- - -	As clinically indicated	- - -
Fertility studies##	At diagnosis	- - -	- - -	- - -
Pregnancy test#	At diagnosis	- - -	- - -	- - -

Note: CBC, complete blood count; BUN, blood urea nitrogen; LDH, lactate dehydrogenase; CT, computed tomography; MRD, minimal residual disease; PET, positron emission tomography; Echo, echocardiogram; EKG, electrocardiogram; MUGA, multiple gate acquisition scan; PT/OT, physical therapy/occupational therapy; TGN, thioguanine nucleotide; TPMT, thiopurine methyltransferase; HD-MTX, high-dose methotrexate.

**See tables below for samples required for MRD studies.*

*** Patients with MDD ≥1% at diagnosis and day 8 MRD positive in T-lymphoblastic lymphoma (Stratum 3), CNS or testicular involvement.*

†For patients with positive bone marrow at diagnosis only.

‡ Bone marrow aspiration is to be performed if the patient has: 1) circulating blast cells; 2) unexplained organomegaly or lymphadenopathy; 3) unexplained bone pain; 4) suspected or documented extramedullary disease; or 5) 2 weeks after stopping chemotherapy because of a low ANC or platelet count, the ANC still is <750/μL or the platelet count < 100,000/μL.

§ Patients with abnormal imaging evaluations at end of induction.

¶If the calcium: creatinine ratio exceeds 0.2, then a 24-h urine will be collected for creatinine, calcium, and calcium/creatinine ratio.

 # Applies only to female patients of childbearing potential

See section 11.4 for details and the timing for long-term follow-up evaluations

Samples required for MRD studies during remission induction

Time point	T-lineage lymphoma
Diagnosis	BM and PB
Day 8	PB
(Day 15 if MRD^+ on Day 8)	PB
(Day 22 if MRD^+ on Day 15)	PB
Day 43 (End of induction)	PB

Note: PB, peripheral blood; BM, bone marrow

Samples required for MRD studies post-remission induction

Time point	T-lineage lymphoma
Day 1 (Weeks 7, 17, 49)	PB
Week 98 or 120 (off therapy)	PB

Note: PB, peripheral blood; ETP, early T-cell precursor.

11.3 Suggested Off-Therapy Evaluations

The recommended follow-up for patients is every 4 months for the first year, every 6 months for the second year, and then yearly until the patient is in remission for 10 years and is at least 18 years old. Thereafter, patients will become alumni and will be followed according to the institution's policy. During their follow-up visit, CBC with differential and other laboratory studies as clinically indicated will be performed. It is recommended that QCT for bone density be performed at 5 years off-study.

Patients will be considered off therapy 30 days after the 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, patients treated at St. Jude will be invited to participate in a long-term follow-up umbrella protocol and may be referred to the After Completion of Therapy (ACT) clinic.

When a patient treated at St. Jude has been in remission for 10 years and is at least 18 years of age, he/she will become St. Jude alumni and will be followed according to institutional policy.

11.4 Suggested Long-Term Follow-up Evaluations

11.4.1 Fertility studies

The following tests and evaluations are recommended to monitor the late effects on fertility at pretreatment, 2 years from enrollment, 5 years from enrollment, at Tanner III and V (if not already studied)

Males

Standard of care (SOC)

- Pubertal development evaluation (annually)
- FSH, LH, and testosterone (annually)

Research (R)

- Semen analysis (through Fertility Associates of Memphis)
- Inhibin B level

Females

Standard of care (SOC)

- Pubertal development evaluation (annually)
- FSH, LH, and Estradiol (annually)

Research (R)

- Anti-müllerian hormone (AMH)

11.4.2 Cardiac studies

The following tests and evaluations are recommended to monitor the cardiac late effects at pretreatment, off therapy, 1 year from enrollment, and 5 years from enrollment: *These tests are standard of care:*

- 2D-echocardiogram
- Troponin T levels
- Natriuretic hormone levels (NT-PRO BNP)

12.0 EVALUATION CRITERIA

12.1 Response Criteria

12.1.1 Complete Response

Complete Response-A (CR-A)

This includes PET negative patients with complete disappearance of all clinical evidence of disease by physical examination, by imaging studies, by bone marrow aspirate/biopsy (where indicated), and by CSF evaluation (where indicated). Bone marrow must contain <5% blasts. For patients with CNS disease, CSF WBC must be <5/μL with no blasts or lymphomatous cells present on 2 consecutive taps. MRD must be negative.

Complete Response-B (CR-B)

This includes patients who have a >55% decrease in a sum of the products of the two greatest perpendicular diameters (SPPD) of the 6 largest dominant nodes or nodal masses. These nodes or masses should be selected according to the following features:

a) they should be clearly measurable in at least 2 perpendicular dimensions
b) they should be from as disparate regions of the body as possible
c) they should include mediastinal and retroperitoneal areas of disease whenever these sites are involved.

PET must be negative. Bone marrow must contain <5% blasts. For patients with CNS disease, CSF WBC must be <5μL with no blasts or lymphomatous cells present on 2 consecutive taps. MRD must be negative.

Complete Response-C (CR-C)
This group includes those with residual diagnostic imaging abnormality that is biopsy negative for lymphoma. MRD must be negative.

Complete Response-D (CR-D)
CR-B criteria with residual PET uptake in a mass that is not safely assessable for biopsy in the judgment of clinicians (i.e. risk/benefit ratio is too high). MRD must be negative.

Complete Response-E (CR-E)
Includes patients who are CR-A, CR-B, CR-C, or CR-D but MRD positive.

12.1.2 Partial Response (PR)

This includes patients with a decrease of 30% or more of the SPPD of the 6 largest dominant nodes or nodal masses, but not satisfying the criteria for CR-B, CR-C or CR-D. Bone marrow must contain <5% blasts. For patients with CNS disease at diagnosis, CSF WBC must be less than 5/μL with no blasts or lymphomatous cells present.

12.1.3 Stable Disease (SD)

This includes patients not satisfying the definition of CR-A, CR-B, CR-C, CR-D, CR-E, PR, RL or PD.

12.1.4 Relapse (RL)

In patients who have responded completely to therapy (including surgery), RL is indicated by the appearance or reappearance of tumor at any site (biopsy proven). Reappearance of cranial nerve palsy alone without definitive evidence of CNS involvement is not sufficient evidence of CNS relapse.

12.1.5 Progressive Disease (PD)

In patients with detectable residual disease, PD is indicated by an increase of >25% of the SPPD at any site of residual disease compared with immediate prestudy SPPD or to the SPPD of best prior response at that site, or the reappearance of tumor in sites of involvement (including marrow or CNS) which had responded completely to therapy (including surgery), or the appearance of tumor in previously uninvolved sites. Reappearance of cranial nerve palsy alone without definitive evidence of CNS involvement is not sufficient evidence of CNS relapse.

12.1.6 Treatment Failure

1. Failure to achieve CR-A, CR-B, CR-C or CR-D at week 7 of continuation therapy (prior to reinduction I) or after reintensification treatment. There must be histopathologic confirmation of disease. (Contact the PI or co-PI if there are problems in this confirmation.)
2. Progressive disease at any time.
3. Relapse at any time. There must be histopathologic confirmation of disease (Contact the PI or co-PI if there are problems in this confirmation.)

12.2 Toxicity Evaluation Criteria

CTCAE Version 4.0 [available for download from http://ctep.info.nih.gov] will be used for toxicity and performance reporting in NHL 16. The toxicities will also be reported on the appropriate data collection forms.

12.3 Acceptable Percentage of Missed Doses for Commercially Available Drugs

It is understood that deviations from the protocol should be avoided, except when necessary to eliminate an immediate hazard to a research participant. In such case, the deviation must be reported to the IRB. However, it is expected that 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 NHL16 database). Missed doses do not include doses held or reduced for medical reasons (toxicity, illness) and will not be considered protocol deviations or violations

13.0 SUPPORTIVE CARE

13.1 Fever at Diagnosis

All patients with fever at diagnosis will be administered broad-spectrum parenteral antibiotics 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-lymphoblastic lymphoma.

- Patients with a large lymphoma cell burden should receive hydration and an oral phosphate binder (aluminum hydroxide in those with relatively high calcium levels and calcium carbonate in those with low calcium levels). Sevelamer (Renagel®) or lanthanum (Fosrenol®) can be used in patients who cannot tolerate aluminum hydroxide or calcium carbonate.

- Patients with a large lymphoma cell burden with or without hyperuricemia (e.g., uric acid ≥7.5 mg/dl or ≥ 6.5 mg/dl in patients younger than 13 years) may be treated with rasburicase if they have no history of G6PD deficiency or ongoing pregnancy. Patients with a history of severe allergy (e.g., bronchial asthma requiring bronchodilator, atopic eczema), may be enrolled on RASALL. For all other patients not at high risk of hyperuricemia, hydration, allopurinol, and judicious use of alkalinization (maintaining urine pH between 6.5 and 7.4) may be sufficient.

13.3 Management of Airway Obstruction and Superior Vena Cava Syndrome

Some of these patients will present as acute emergencies. In these patients, the pre-treatment evaluation will be necessarily curtailed or completed as soon as possible after initiation of treatment. Airway obstruction may require emergency institution of therapy to reduce obstruction. Two alternative methods may be used as emergency treatment. Irradiation to the mediastinal mass (180 rad/dose x 3 doses) may be used, or methylprednisolone 20 mg/m² in 4 divided doses for one day. The use of methylprednisolone may be accompanied by tumor lysis syndrome, and may cause alteration of tumor histology in patients not yet biopsied.

13.3.1 Mediastinal Mass

Attempted resection of a mediastinal mass is not recommended. In most of these patients the diagnosis may be established by biopsy of a lymph node in the cervical or supraclavicular regions or by cytologic assessment of the pleural fluid.

13.4 Osteonecrosis of the Bone

Osteonecrosis of the bone, a known complication of treatment with corticosteroids, can occur in approximately 10%–15% of patients, especially those older than 9 years. This devastating complication may result in collapse of the articulating surface, with subsequent pain and development of arthritis. Early detection of small lesions can permit intervention, which may prevent pain and irreversible damage of the joints. In NHL 16, all patients 9 years and older will undergo total joint MRI scans of the shoulders, elbows,

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 before or between scheduled MRI scans should undergo MRI to rule out osteonecrosis or progression of this complication.

For patients who require surgical intervention, treatment will vary, based on the 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

When patients are administered nutritional or vitamin therapies, they should not receive more than the recommended daily allowance (RDA) for folic acid with dietary and supplement intake, in order 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-lymphoma 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 azithromycin) 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. The pharmacokinetics Service should be consulted if the long-term use of these interacting drugs is unavoidable.

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

13.8 Down Syndrome

Patients with Down syndrome should be closely monitored for toxicity, and offered aggressive supportive care. Methotrexate dosage should be reduced as described in section 7.1.1. Chemotherapy doses may be appropriately reduced to avoid undue toxicity. Oral leucovorin (5 mg/m^2 q 12 h × 2) should be given 24 h after each IT MHA.

A 30% dose reduction of dexamethasone and/or high dose cytarabine should be considered for patients with Down syndrome who experience higher than expected toxicity during earlier phases of therapy.

13.9 Respiratory Syncytial Virus (RSV) Prophylaxis

All infants should receive prophylaxis for respiratory syncytial virus (RSV) as per current institutional policy.

14.0 OFF THERAPY AND OFF STUDY CRITERIA

14.1 Off-Treatment Criteria

Patients will be taken off NHL16 therapy if any of the following occurs:

- Failure to achieve CR-A, CR-B, CR-C or CR-D at week 7 of continuation therapy (prior to reinduction I) or after reintensification treatment
- Relapse (except CNS)
- Second malignancy (e.g., therapy-induced AML or brain tumor)
- Development of unacceptable toxicity during treatment (confirmation by PI or co-PI required)
- Patients with severe congenital immunodeficiency (e.g., ataxia telangiectasia) or HIV infection will be taken off NHL16 therapy and will receive alternative therapy (to prevent excessive toxicities) with curative intent.
- Completion of all protocol-prescribed treatment
- Decision of participant or family to withdraw from protocol treatment at any time for any reason
- PI decides that continued protocol treatment is no longer in the patient's best interest

14.2 Off-Study Criteria

A patient will be taken off NHL16 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 BIOLOGIC STUDIES

15.1 Minimal Disseminated Disease (MDD) and Minimal Residual Disease (MRD)

Bone marrow and peripheral blood samples, collected in preservative-free heparin, will be obtained at diagnosis. Peripheral blood will be used to monitor response to treatment. Mononuclear cells, separated on a density step (Accu-Prep, Accurate Chemical, Westbury, NY), will be labeled with phycoerythrin (PE)-conjugated anti-CD5 (Dako, Carpinteria, CA) and a mixture of allophycocyanin (APC)-conjugated anti-CD33 and anti-CD19 (Becton Dickinson, San Jose, CA). After permeabilization with reagent 8E (developed in our laboratory from a proprietary formula), cells will be stained with fluorescence isothyocyanate (FITC)-conjugated anti-TdT (Supertechs, Rockville, MD) and peridinin chorophyll protein (PerCP)-conjugated anti-CD3 (Becton Dickinson). Anti-TdT-FITC will be replaced by a FITC-conjugated isotype-matched non-reactive immunoglobulin (Becton Dickinson) in control tubes. We will also use 9-color staining including antibodies such as anti-CD34-FITC, anti-CD3-PerCP (cytoplasmic), anti-CD19- and anti-CD33-APC, anti-CD5-PECy7, anti-HLA-Dr-APCCy7, anti-CD4-Pacific Blue, anti-CD8-AmCyan, anti-CD3-Alexafluor 700 (surface; all from Becton Dickinson), and anti-CD1a-(Beckman Coulter, Miami, FL) or anti-CD99-PE (Becton Dickinson). Cells (2-5 x 10^5 in each sample) will be analyzed with a FACSCalibur, FACScanto or LSR II flow cytometers (all from Becton Dickinson) as previously described.[35]

15.2 Early T-cell Progenitor Phenotype

The early T-cell progenitor (ETP) phenotype is defined by the expression of CD7 and cytoplasmic (or surface) CD3, the lack of CD1a and CD8 expression, dim expression of CD5, and exprerssion of one or more stem cell/myeloid-associated markers such as CD117, CD34, HLA-DR, CD13, CD33, CD11b.[39] The expression of this phenotype will be determined by flow cytometry in cases with disseminated disease at diagnosis. To this end, bone marrow and/or peripheral blood mononucleated cells obtained for studies of disseminated disease described in 15.1 will be labeled with antibodies directed against the markers that define that ETP phenotype. Cell staining and analysis will be performed as described in 15.1 using multiparameter flow cytometry.

15.3 Gene Expression Analysis

Total RNA will be isolated from tumor tissue by using the TriZOL reagent (Invitrogen, Carlsbad, CA). RNA will be quantitated by spectrophotometry using a Nanodrop (ThermoScientific, Wilmington, DE), and integrity assessed using a bioanalyzer (Agilent Technologies, Palo Alto, CA) to calculate the RNA integrity index. Nondegraded RNA samples will be processed for gene expression profiling, using U133 Plus 2.0 gene expression arrays (Affymetrix, Santa Clara, CA) or gene and exon arrays, or their combination. Briefly, 100 ng of total RNA will be amplified, labeled, fragmented, hybridized to arrays, and scanned as per manufacturer's protocols by the Clinical Applications Core Technology (CACT) laboratory at the Hartwell Center for Bioinformatics and Biotechnology at St. Jude. Array data will be processed by using

Microarrays Suite v 5.0 (Affymetrix) or equivalent programs. Array quality will be assessed by using various metrics such as background, noise, scale factor, present calls, and GAPDH/ACTIN 3'/5' ratios.

15.4 Copy Number Alteration

Genome wide profiling of DNA copy number alterations and loss-of-heterozygosity (LOH) will be performed using Affymetrix SNP 6.0 arrays, or equivalent state-of-the-art arrays available at the time of the study. SNP array execution, data processing, and lesion calling will be performed by using an analysis pipeline established and subject to ongoing modification and refinement at St Jude. Briefly, high-molecular-weight DNA will be extracted from tumor and normal tissue (e.g., peripheral blood leukocytes or bone marrow aspirates free of tumor) by column-based methods (e.g., DNA blood mini, Qiagen) or by organic extraction. DNA will be quantified by spectrophotometry (Nanodrop), fluorescence (e.g., PicoGreen, Invitrogen), or their combination, and integrity assessed by electrophoretic analysis of DNA (50–100 ng) on a 0.8% agarose gel. Samples exhibiting degradation will not be processed further, and, if possible, extraction will be repeated. 500ng DNA will be processed for SNP arrays by the CACT laboratory. Restriction enzyme digestion, PCR amplification, cleanup, labeling, array hybridization, and scanning will be performed as per the manufacturer's instructions.

15.5 Mutation Analysis

Mutation analysis will be performed by genomic PCR and Sanger sequencing of whole-genome amplified DNA (RepliG, Qiagen). Sequence quality assessment and mutation detection will be performed as previously described.[83] Putative mutations will be validated by sequencing of tumor and matched normal DNA (including nonamplified DNA if available).

15.6 Genomic Comparison between T-Lymphoblastic Lymphoma and T-ALL

The investigators have access to SNP array and gene expression data for more than 100 St. Jude T-lineage ALL samples studied on protocols Total XII, XIII, XV and XVI. Copy number data will be directly compared to this data set for (1) lesion type; (2) lesion frequency; (3) association of lesions with tumor subtype (defined by clinical, pathologic, genetic or microarray analyses); and (4) effect on gene expression identified by local or global integrated analysis.

16.0 STATISTICAL CONSIDERATIONS

16.1 Primary Objective

Improve the outcome of children who have minimal disseminated disease (MDD) equal to or more than 1% at diagnosis by using MDD- and minimal residual disease (MRD)–based risk-adapted chemotherapy.

Accrual: The institutional trial NHL-13 ran 10 years and enrolled 41 evaluable patients. The PI has indicated that NHL-16 will be a multi-institution trial and predicts that the accrual rate will be at least double that of NHL-13. We plan to accrue patients for 5–11 years. Assuming that 7 evaluable patients are enrolled per year, we expect to accrue 72 evaluable patients in approximately 10.5 years. We expect that approximately 30% of patients will have MDD equal or more than 1% at diagnosis; hence, we expect to enroll 22 evaluable patients for the primary objective.

Analysis: This is a phase-II clinical trial. Outcome of this subset of patients will be analyzed in terms of overall survival (OS) and event-free survival (EFS) since diagnosis. Only death will be considered a failure for OS. For EFS, relapse and second malignancies will be considered as failures in addition to death in complete remission. The time to EFS will be set to 0 for patients who fail to achieve complete remission. Kaplan-Meier estimates of the OS and EFS curves will be computed, along with estimates of standard errors by the method of Peto.[84] Two-year EFS and OS, as well as longer term survival rates (5 year and 10 year) will be estimated with 95% confidence intervals.

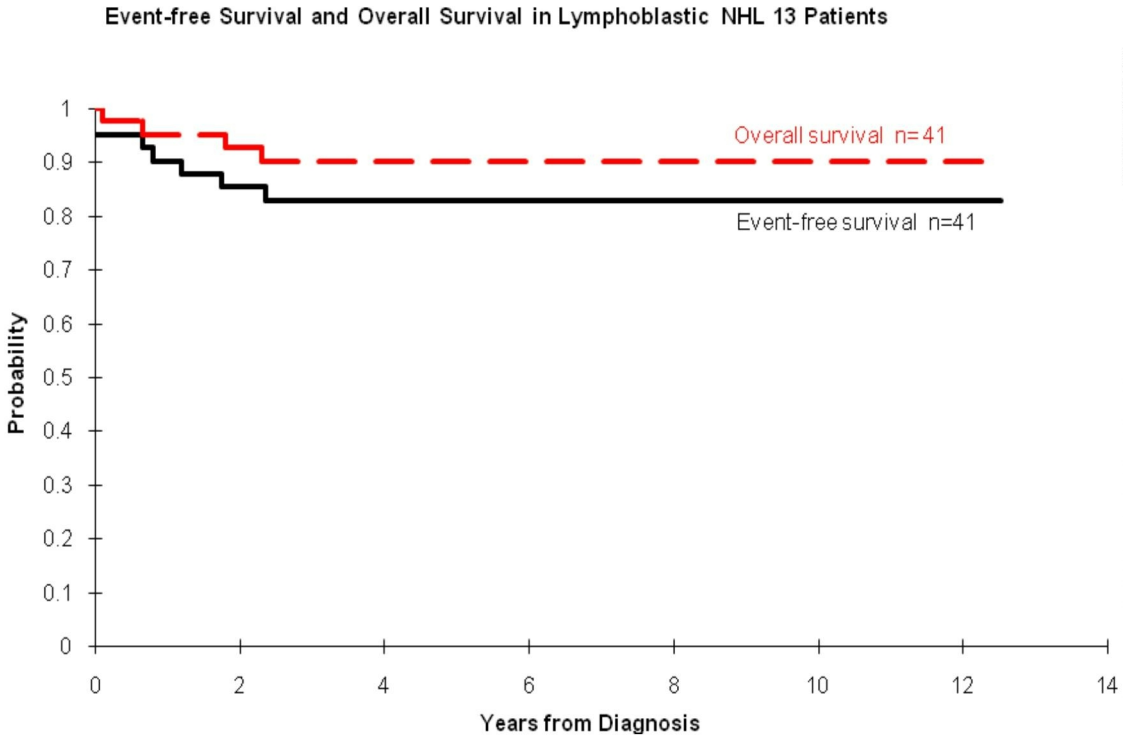
The objective here is estimation instead of comparison. Comparisons to historical controls are considered exploratory. The limited COG A5971 study data show that the 2 year EFS in this patient subset is 68.1% although chemotherapy was not modified based on lymphoma cell involvement at diagnosis.[35] The sample size n=22 provides 85% (71%) power for a one-sided log-rank test at the 5% level if the true 2 year EFS in the newly treated patients is 88.1% (83.1%) compared to the “historical” 68.1% in a simple null hypothesis.

Analysis will begin after 2 years of follow up since the completion of therapy of the last enrolled patient.

16.2 Secondary Objectives

16.2.1 *Estimate the event-free survival and overall survival of children with lymphoblastic lymphoma who are treated with MDD/MRD based risk-directed therapy.*

EFS and OS function estimates of NHL-13 is provided below for background information.



OS and EFS are defined as in Section 16.1; the survival functions will be estimated in the same way as described therein. Analysis will begin at the same time as the primary objective, i.e., after 2 years of follow up since the completion of therapy of the last enrolled patient.

16.2.2 *Evaluate the prognostic value of levels of MDD at diagnosis and MRD on day 8 of remission induction.*

Prognostic value of MDD and MRD on day 8 will be evaluated by their associations with the risk of relapse. MDD and MRD will be considered as either binary (negative vs. positive) or continuous/ordinal variables. Gray's test for comparing cumulative incidence functions (for binary MDD/MRD) and Fine and Gray hazard rate regression modeling (for continuous MDD/MRD) will be applied. Death in remission and second malignancy are considered as competing risks. Analysis for this aim will begin at the same time as the primary objective.

16.3 Exploratory Objectives

16.3.1 *Estimate the frequency of early T cell progenitors in lymphoblastic lymphoma*

The proportion of ETP will be estimated by the sample proportion along with the binomial distribution–based exact 95% confidence interval. Analysis will begin within six (6) months after the last enrollment.

16.3.2 *Evaluate copy number, gene expression, and mutations in tumor and normal tissues of patients*

DNA copy numbers and mutations in tumor cells will be detected using established methods.[85] Differential gene expression profiling between tumor and normal tissues will be performed by paired *t*-tests on log-transformed gene (RNA) expression signals. Levels of false-positive and false-negative errors will be assessed by established methods.[86-88]

Microarray data will be analyzed by using several complementary approaches. Gene expression data will be filtered by excluding probe sets called as absent across all arrays. Unsupervised hierarchical clustering will be performed for all probes remaining after this filtering, and after selection of probes exhibiting the greatest variation in intensity across the dataset (e.g., after using a coefficient of variation or minimal absolute deviation filter criterion). This approach will be used to identify subgroups of cases defined by gene expression profiling, and associations between these clusters and clinical, pathologic, and genetic variables will be examined. Integrated analysis of copy number alteration and local gene expression data will be performed by using a linear regression model to assess associations between copy number alterations and local gene expression. The similarity between the gene expression profile of T-lymphoblastic lymphoma and T-lineage acute lymphoblastic leukemia will be performed using Gene Set Enrichment Analysis or alternative statistical methods developed by the Departments of Biostatistics and Computational Biology during the course of the study.[89-91]

Array data will be processed using Genotyping Console (Affymetrix) or equivalent command-line/developer tools (Affymetrix). Contrast QC rates will be used to assess quality, arrays called "out-of-bounds" discarded, and arrays repeated. SNP genotyping will be performed for not less than 44 arrays per batch, using the Birdseed v2 or an equivalent algorithm. Probe-level intensity data will be summarized by using the model-based expression intensity algorithm implemented in dChip[92] or alternative approaches developed by Drs. Pounds and Cheng (Biostatistics).[85] Raw array data will be normalized by using reference normalization or alternative approaches that incorporate correction of signal intensity for aneuploidy. Inference of copy number alterations will be performed using circular binary segmentation (CBS) implemented in Bioconductor,[93,94] and raw segmentation outputs compared to array data in dChip. For samples with corresponding normal DNA also run on arrays, segmentation will be performed pairwise (tumor vs. matched normal). For tumors lacking matched normal samples, segmentation will be performed unpaired, in which data from each array will be compared to a pool of within-batch control samples. For unpaired analyses, somatic (tumor-acquired) lesions will be distinguished from inherited copy number variants by comparing lesions to online databases of CNVs[95] and in-house databases of CNVs acquired from several hundred normal/remission SNP arrays. LOH data will be analyzed by using Hidden Markov Models (HMM) or equivalent approaches.

Subsequent analysis of copy number data will include manual tallying of recurrent lesions and computational approaches to identify regions of significant recurrence (e.g.,

GISTIC, Beroukhim). Integrated analysis with copy number alterations will be performed as described above.

Analysis will begin within six (6) months after the last enrollment.

16.3.3 *Compare the immunophenotyping, MRD, and genomic data between T-lymphoblastic lymphoma and T-acute lymphoblastic leukemia (ALL)*

Frequencies of ETP and positive MRD between T-lymphoblastic lymphoma and T-ALL will be compared by Fisher's exact test. Large-scale comparisons of genomic features between the 2 diseases will be conducted by statistical tests (rank-sum test for continuous or ordinal features, Chi-square or Fisher's exact test for categorical features), and levels of false-positive and false-negative errors will be assessed by established methods.[86-88] Available methods [gene set enrichment analysis (GSEA)[89-91]] and those developed by the Department of Biostatistics and the Department of Computational Biology during the course of the study will be applied to detect and compare multigenetic and pathway differences between the 2 diseases. Analysis will begin within six (6) months after the last enrollment.

16.3.4 *Bone mineral density (BMD) and osteonecrosis*

16.3.4.1 *Prospectively estimate BMD at diagnosis and at end of therapy and correlate with risk factors for potential BMD deficits in pediatric patients with NHL.*

BMD levels at diagnosis and at the end of therapy will be analyzed by descriptive statistics, including mean, standard deviation, median, quartiles, and range. General linear models will be applied to assess the associations between BMD and potential risk factors for BMD deficits.

16.3.4.2 *Prospectively monitor osteonecrosis and correlate with risk factors for potential osteonecrosis in pediatric patients with NHL*.

Frequency of osteonecrosis will be estimated by using sample proportion and cumulative incidence function estimates. Logistic regression and Fine and Gray regression modeling will be applied to analyze the association between the risk of osteonecrosis and potential risk factors.

Analysis for this aim will begin at the same time as the primary objective.

16.3.5 *Fertility and cardiac late effects*

16.3.5.1 *Establish the prevalence of gonadal and germ cell dysfunction associated with NHL 16.*

The prevalence of gonadal and germ cell dysfunction will be estimated by the sample proportion and an exact 95% confidence interval.

16.3.5.2 *Establish the prevalence and* severity *of cardiac toxicity associated with NHL16.*

The same statistics in section 16.3.4.1will be applied to analyze this objective. Analysis for this aim will begin at the same time as the primary objective.

17.0 SAFETY AND ADVERSE EVENT REPORTING REQUIREMENTS

17.1 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, 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, 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.

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

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

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.

17.2 Recording Adverse Events and Serious Adverse Events

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

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

17.3 Process for Reporting AEs from Collaborating Sites to St. Jude and from St. Jude to Collaborating Sites

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

- Is the AE serious, unexpected, and related or possibly related to participation in the research?

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

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

At a minimum, Grade 3-5 AEs and expected/unrelated deaths that occur more than 30 days after last protocol treatment will be reported to all sites via a study progress report at the time of continuing review.

FURTHER GUIDELINES FOR REPORTING OF ADVERSE EVENTS AND UNANTICIPATED PROBLEMS

To determine whether an adverse event is an unanticipated problem, and therefore requires expeditious reporting under 45 CFR part 46, the PI will take into account the following:

1. The description of known or foreseeable adverse events and risks in the IRB-approved research protocol, any applicable investigator brochure, the current IRB-approved informed consent document, and other relevant sources of information, such as scientific literature, product labeling, and package inserts.

2. Any underlying diseases or conditions of the subject experiencing the adverse event.

3. A careful assessment of whether the adverse event is related or possibly related to the subject's participation in the research study.

Particular AEs and SAEs require more prompt reporting to the various governing regulatory authorities. The key to whether a SAE should be reported expeditiously or not to the St. Jude IRB is based on its expectedness of the event. Is this SAE an expected or unexpected event? Expeditious reporting to the various Federal governing regulatory authorities is based on the relationship of the unexpected event to the protocol treatment and the seriousness of the event.

Serious adverse events that are identified by the PI or designated sub-investigator as <u>expected</u>, regardless of causality are not subject to expedited reporting. All expected

SAEs will be reported to the St. Jude IRB and St. Jude Office of Regulatory Affairs in a summary type format on an annual or semi-annual basis or more frequently if requested by the IRB, FDA, or PI.

All **unexpected SAEs that are at least possibly related to the protocol treatment** need to be reported to the IRB's at all participating sites expeditiously. The nature and severity of the event will dictate the exact time frame for expeditious reporting (24 – 48 hours vs. 10 days).

Expeditious Adverse Event Reporting Requirements to the St. Jude IRB

- All deaths **that occur while participants are on active therapy or within 30 days of protocol therapy OR deaths that are deemed unexpected and at least possibly related to study treatment will be reported to the St. Jude IRB immediately (within 24-48 hours of notification) using the electronic TRACKS system**. Immediate reporting is required even if few event specific facts are available at the time of the initial report. In this case, a complete follow-up report detailing the event should be submitted to the IRB within 10 days of the event.

- All life threatening SAEs that are deemed **unexpected and at least possibly related** to study treatment will be reported to the St. Jude IRB as soon as possible using the electronic TRACKS system but no later than 24 - 48 hours and followed up in with a complete report within 10 working days of the occurrence of the event.

- All other **unexpected** SAEs (not fatal or life threatening) determined to be **at least possibly related** to study treatment will need to be reported to the St. Jude IRB within 10 working days of the occurrence.

Follow-up Reports

Follow-up reports for the expedited reports submitted to the St. Jude IRB are required for those that are checked as "unresolved" at the time of the report. Typically, follow-up reports should be submitted when the investigator becomes aware of significant new information regarding the initial event or subsequent patient status, or when the event has completely resolved and any sequelae have been identified and/or resolved.

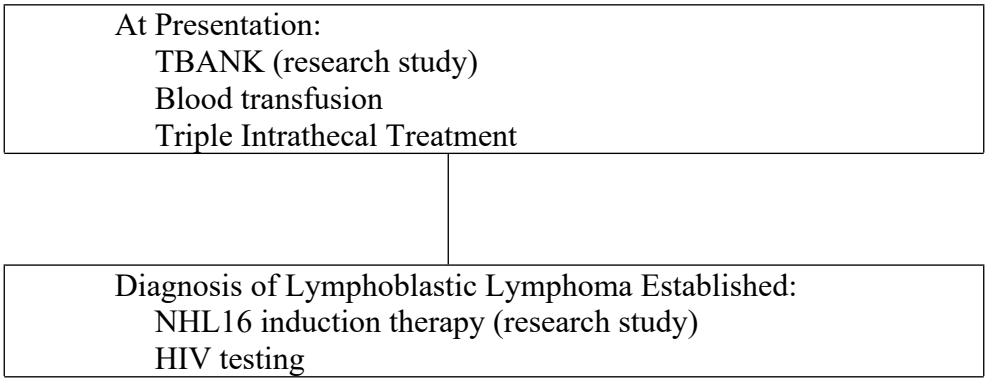
18.0 INFORMED CONSENT PROCESS

18.1 Consent at Enrollment and Post-Remission Therapy

The process of informed consent for NHL16 will follow institutional policy. The informed consent process 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 1 witness who is not a physician. Initially, informed

consent will be sought for the T BANK protocol (research study), blood transfusion (if needed), the first IT therapy (if the diagnosis of lymphoma is certain either by referral bone marrow smears or blood smears at St. Jude), and treatment with recombinant urate oxidase, if needed. After the diagnosis of lymphoblastic lymphoma is established, we will invite the patient to participate in the NHL16 protocol, as well as obtain standard medical consent for HIV testing.

The timeline for various informed consents is indicated in the figure below.



Throughout the treatment period, participants and their parents will receive constant education from health professionals at St. Jude 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 7–14 years old and written assent for all patients 14 years or older.

18.2 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 10 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.

18.3 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.

19.0 DATA COLLECTION, STUDY MONITORING AND CONFIDENTIALITY

19.1 Data Collection

Electronic case report forms (eCRFs) 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. Inaba or his designee. All protocol-specific data and all grade 3-5 adverse events will be recorded by the clinical research associates into the CRIS database, ideally within 2-4 weeks of completion of study phase. All questions will be directed to the attending physician and/or PI and reviewed at regularly-scheduled working meetings. The attending physicians (or their designees) are responsible for keeping up-to-date roadmaps in the patient's primary SJCRH medical chart.

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

19.2 Data Collection Instructions for Collaborating Sites

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

19.3 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.

19.4 Confidentiality

Study numbers will be used in place of an identifier such as a medical record number. No research participant names will be recorded on the data collection forms. The list containing the study number and the medical record number will be maintained in a locked file and will be destroyed after all data have been analyzed.

The medical records of study participants may be reviewed by the St. Jude IRB, FDA, and St. Jude clinical research monitors.

20.0 REFERENCES

1 Wollner, N., Exelby, P. R. & Lieberman, P. H. Non-Hodgkin's lymphoma in children: a progress report on the original patients treated with the LSA2-L2 protocol. *Cancer* **44**, 1990-1999 (1979).
2 Anderson, J. R. *et al.* Childhood non-Hodgkin's lymphoma. The results of a randomized therapeutic trial comparing a 4-drug regimen (COMP) with a 10-drug regimen (LSA2-L2). *The New England journal of medicine* **308**, 559-565, doi:10.1056/NEJM198303103081003 (1983).
3 Anderson, J. R. *et al.* Long-term follow-up of patients treated with COMP or LSA2L2 therapy for childhood non-Hodgkin's lymphoma: a report of CCG-551 from the Childrens Cancer Group. *Journal of clinical oncology : official journal of the American Society of Clinical Oncology* **11**, 1024-1032 (1993).
4 Dahl, G. V. *et al.* A novel treatment of childhood lymphoblastic non-Hodgkin's lymphoma: early and intermittent use of teniposide plus cytarabine. *Blood* **66**, 1110-1114 (1985).
5 Sandlund, J. T. *et al.* Effective treatment of advanced-stage childhood lymphoblastic lymphoma without prophylactic cranial irradiation: results of St Jude NHL13 study. *Leukemia* **23**, 1127-1130, doi:10.1038/leu.2008.400 (2009).
6 Amylon, M. D. *et al.* Intensive high-dose asparaginase consolidation improves survival for pediatric patients with T cell acute lymphoblastic leukemia and advanced stage lymphoblastic lymphoma: a Pediatric Oncology Group study. *Leukemia* **13**, 335-342 (1999).
7 Abromowitch, M. *et al.* Shortened intensified multi-agent chemotherapy and non-cross resistant maintenance therapy for advanced lymphoblastic lymphoma in children and adolescents: report from the Children's Oncology Group. *British journal of haematology* **143**, 261-267, doi:10.1111/j.1365-2141.2008.07320.x (2008).
8 Patte, C. *et al.* Results of the LMT81 protocol, a modified LSA2L2 protocol with high dose methotrexate, on 84 children with non-B-cell (lymphoblastic) lymphoma. *Medical and pediatric oncology* **20**, 105-113 (1992).
9 Reiter, A. *et al.* Intensive ALL-type therapy without local radiotherapy provides a 90% event-free survival for children with T-cell lymphoblastic lymphoma: a BFM group report. *Blood* **95**, 416-421 (2000).
10 Asselin BL *et al.* Improved event-free survival (EFS) with high dose methotrexate (HDM) in T-cell lymphoblastic leukemia (T-ALL) and advanced lymphoblastic lymphoma (T-NHL): a Pediatric Oncology Group (POG) study. *Proc ASCO* **20**, 367 (2001).
11 Abromowitch M *et al.* High-dose methotrexate and early intensification of therapy do not improve 3 year EFS in children and adolescents with disseminated lymphoblastic lymphoma. Results of the randomized arms of COG A5971. *Hematology Meeting Reports* **3**, 33 (2009).
12 Silverman, L. B. *et al.* Improved outcome for children with acute lymphoblastic leukemia: results of Dana-Farber Consortium Protocol 91-01. *Blood* **97**, 1211-1218 (2001).
13 Goldberg, J. M. *et al.* Childhood T-cell acute lymphoblastic leukemia: the Dana-Farber Cancer Institute acute lymphoblastic leukemia consortium experience. *Journal of clinical oncology : official journal of the American Society of Clinical Oncology* **21**, 3616-3622, doi:10.1200/JCO.2003.10.116 (2003).
14 Pui, C. H. *et al.* Treating childhood acute lymphoblastic leukemia without cranial irradiation. *The New England journal of medicine* **360**, 2730-2741, doi:10.1056/NEJMoa0900386 (2009).

15 Burkhardt, B. *et al.* Impact of cranial radiotherapy on central nervous system prophylaxis in children and adolescents with central nervous system-negative stage III or IV lymphoblastic lymphoma. *Journal of clinical oncology : official journal of the American Society of Clinical Oncology* **24**, 491-499, doi:10.1200/JCO.2005.02.2707 (2006).
16 Veerman, A. J. *et al.* Dexamethasone-based therapy for childhood acute lymphoblastic leukaemia: results of the prospective Dutch Childhood Oncology Group (DCOG) protocol ALL-9 (1997-2004). *The Lancet. Oncology* **10**, 957-966, doi:10.1016/S1470-2045(09)70228-1 (2009).
17 Link, M. P., Shuster, J. J., Donaldson, S. S., Berard, C. W. & Murphy, S. B. Treatment of children and young adults with early-stage non-Hodgkin's lymphoma. *The New England journal of medicine* **337**, 1259-1266, doi:10.1056/NEJM199710303371802 (1997).
18 Neth, O. *et al.* Precursor B-cell lymphoblastic lymphoma in childhood and adolescence: clinical features, treatment, and results in trials NHL-BFM 86 and 90. *Medical and pediatric oncology* **35**, 20-27 (2000).
19 Termuhlen A *et al.* Outcome of newly diagnosed children and adolescents with localized lymphoblastic lymphoma treated on COG A5971: a report from the children's oncology group. *Hematology Meeting Reports* **3**, 35 (2009).
20 Schrappe, M. *et al.* Dexamethasone in Induction Can Eliminate One Third of All Relapses in Childhood Acute Lymphoblastic Leukemia (ALL): Results of An International Randomized Trial in 3655 Patients (Trial AIEOP-BFM ALL 2000). *ASH Annual Meeting Abstracts* **112**, 7- (2008).
21 Conter, V. *et al.* Intensive BFM chemotherapy for childhood ALL: interim analysis of the AIEOP-ALL 91 study. Associazione Italiana Ematologia Oncologia Pediatrica. *Haematologica* **83**, 791-799 (1998).
22 Evans, W. E. *et al.* Conventional compared with individualized chemotherapy for childhood acute lymphoblastic leukemia. *The New England journal of medicine* **338**, 499-505, doi:10.1056/NEJM199802193380803 (1998).
23 Bostrom, B. C. *et al.* Dexamethasone versus prednisone and daily oral versus weekly intravenous mercaptopurine for patients with standard-risk acute lymphoblastic leukemia: a report from the Children's Cancer Group. *Blood* **101**, 3809-3817, doi:10.1182/blood-2002-08-2454 (2003).
24 Mitchell, C. D. *et al.* Benefit of dexamethasone compared with prednisolone for childhood acute lymphoblastic leukaemia: results of the UK Medical Research Council ALL97 randomized trial. *British journal of haematology* **129**, 734-745, doi:10.1111/j.1365-2141.2005.05509.x (2005).
25 Nachman, J. B. *et al.* Augmented post-induction therapy for children with high-risk acute lymphoblastic leukemia and a slow response to initial therapy. *The New England journal of medicine* **338**, 1663-1671, doi:10.1056/NEJM199806043382304 (1998).
26 Burton, N. K. *et al.* The effect of food on the oral administration of 6-mercaptopurine. *Cancer chemotherapy and pharmacology* **18**, 90-91 (1986).
27 Lafolie, P., Bjork, O., Hayder, S., Ahstrom, L. & Peterson, C. Variability of 6-mercaptopurine pharmacokinetics during oral maintenance therapy of children with acute leukemia. *Medical oncology and tumor pharmacotherapy* **6**, 259-265 (1989).
28 Lonnerholm, G., Kreuger, A., Lindstrom, B. & Myrdal, U. Oral mercaptopurine in childhood leukemia: influence of food intake on bioavailability. *Pediatric hematology and oncology* **6**, 105-112 (1989).

29 Riccardi, R. *et al.* Influence of food intake on bioavailability of oral 6-mercaptopurine in children with acute lymphoblastic leukemia. *Pediatric hematology and oncology* **3**, 319-324 (1986).
30 Rivard, G. E., Infante-Rivard, C., Hoyoux, C. & Champagne, J. Maintenance chemotherapy for childhood acute lymphoblastic leukaemia: better in the evening. *Lancet* **2**, 1264-1266 (1985).
31 Schmiegelow, K. *et al.* Impact of morning versus evening schedule for oral methotrexate and 6-mercaptopurine on relapse risk for children with acute lymphoblastic leukemia. Nordic Society for Pediatric Hematology and Oncology (NOPHO). *Journal of pediatric hematology/oncology* **19**, 102-109 (1997).
32 Lindsey Hageman WL, Y. C. H., K.; Kornegay, N. Evans, W.E.; Bostrom, B.; Casillas, J., Dickens, D., Agniolillo, A.; Lew, G.; Maloney, K.; Mascarenhas, L.; Ritchey, A.K.; Termuhlen, A.; Carroll, W.L., Relling, M.V.; Wong, F.L.; Bhatia, S. in *ASH.*
33 Arico, M. *et al.* The seventh international childhood acute lymphoblastic leukemia workshop report: Palermo, Italy, January 29--30, 2005. *Leukemia* **19**, 1145-1152, doi:10.1038/sj.leu.2403783 (2005).
34 Clemmensen, K. K. *et al.* The circadian schedule for childhood acute lymphoblastic leukemia maintenance therapy does not influence event-free survival in the NOPHO ALL92 protocol. *Pediatric blood & cancer* **61**, 653-658, doi:10.1002/pbc.24867 (2014).
35 Coustan-Smith, E. *et al.* Minimal disseminated disease in childhood T-cell lymphoblastic lymphoma: a report from the children's oncology group. *Journal of clinical oncology : official journal of the American Society of Clinical Oncology* **27**, 3533-3539, doi:10.1200/JCO.2008.21.1318 (2009).
36 Rothenberg, E. V., Moore, J. E. & Yui, M. A. Launching the T-cell-lineage developmental programme. *Nature reviews. Immunology* **8**, 9-21, doi:10.1038/nri2232 (2008).
37 Bell, J. J. & Bhandoola, A. The earliest thymic progenitors for T cells possess myeloid lineage potential. *Nature* **452**, 764-767, doi:10.1038/nature06840 (2008).
38 Wada, H. *et al.* Adult T-cell progenitors retain myeloid potential. *Nature* **452**, 768-772, doi:10.1038/nature06839 (2008).
39 Coustan-Smith, E. *et al.* Early T-cell precursor leukaemia: a subtype of very high-risk acute lymphoblastic leukaemia. *The Lancet. Oncology* **10**, 147-156, doi:10.1016/S1470-2045(08)70314-0 (2009).
40 Burkhardt, B. Paediatric lymphoblastic T-cell leukaemia and lymphoma: one or two diseases? *Br J Haematol* **149**, 653-668, doi:BJH8006 [pii] 10.1111/j.1365-2141.2009.08006.x.
41 Raetz, E. A. *et al.* Gene expression profiling reveals intrinsic differences between T-cell acute lymphoblastic leukemia and T-cell lymphoblastic lymphoma. *Pediatric blood & cancer* **47**, 130-140, doi:10.1002/pbc.20550 (2006).
42 Uyttebroeck, A. *et al.* Is there a difference in childhood T-cell acute lymphoblastic leukaemia and T-cell lymphoblastic lymphoma? *Leukemia & lymphoma* **48**, 1745-1754, doi:10.1080/10428190701509772 (2007).
43 Schraders, M. *et al.* High-resolution genomic profiling of pediatric lymphoblastic lymphomas reveals subtle differences with pediatric acute lymphoblastic leukemias in the B-lineage. *Cancer genetics and cytogenetics* **191**, 27-33, doi:10.1016/j.cancergencyto.2009.01.002 (2009).

44 Radtke, F. *et al.* Deficient T cell fate specification in mice with an induced inactivation of Notch1. *Immunity* **10**, 547-558 (1999).
45 Weng, A. P. *et al.* Activating mutations of NOTCH1 in human T cell acute lymphoblastic leukemia. *Science* **306**, 269-271, doi:10.1126/science.1102160 (2004).
46 Park, M. J. *et al.* FBXW7 and NOTCH1 mutations in childhood T cell acute lymphoblastic leukaemia and T cell non-Hodgkin lymphoma. *British journal of haematology* **145**, 198-206, doi:10.1111/j.1365-2141.2009.07607.x (2009).
47 Baleydier, F. *et al.* T cell receptor genotyping and HOXA/TLX1 expression define three T lymphoblastic lymphoma subsets which might affect clinical outcome. *Clinical cancer research : an official journal of the American Association for Cancer Research* **14**, 692-700, doi:10.1158/1078-0432.CCR-07-1927 (2008).
48 Asnafi, V. *et al.* NOTCH1/FBXW7 mutation identifies a large subgroup with favorable outcome in adult T-cell acute lymphoblastic leukemia (T-ALL): a Group for Research on Adult Acute Lymphoblastic Leukemia (GRAALL) study. *Blood* **113**, 3918-3924, doi:10.1182/blood-2008-10-184069 (2009).
49 Malyukova, A. *et al.* The tumor suppressor gene hCDC4 is frequently mutated in human T-cell acute lymphoblastic leukemia with functional consequences for Notch signaling. *Cancer research* **67**, 5611-5616, doi:10.1158/0008-5472.CAN-06-4381 (2007).
50 Strauss, A. J. *et al.* Bony morbidity in children treated for acute lymphoblastic leukemia. *Journal of clinical oncology : official journal of the American Society of Clinical Oncology* **19**, 3066-3072 (2001).
51 Mandel, K., Atkinson, S., Barr, R. D. & Pencharz, P. Skeletal morbidity in childhood acute lymphoblastic leukemia. *Journal of clinical oncology : official journal of the American Society of Clinical Oncology* **22**, 1215-1221, doi:10.1200/JCO.2004.04.199 (2004).
52 Holzer, G. *et al.* Bone mineral density in long-term survivors of highly malignant osteosarcoma. *J Bone Joint Surg Br* **85**, 231-237 (2003).
53 Brennan, B. M., Rahim, A., Adams, J. A., Eden, O. B. & Shalet, S. M. Reduced bone mineral density in young adults following cure of acute lymphoblastic leukaemia in childhood. *British journal of cancer* **79**, 1859-1863, doi:10.1038/sj.bjc.6690296 (1999).
54 Kaste, S. C. *et al.* Bone mineral decrements in survivors of childhood acute lymphoblastic leukemia: frequency of occurrence and risk factors for their development. *Leukemia* **15**, 728-734 (2001).
55 Nysom, K. *et al.* Bone mass after treatment of malignant lymphoma in childhood. *Medical and pediatric oncology* **37**, 518-524 (2001).
56 French, S. A., Fulkerson, J. A. & Story, M. Increasing weight-bearing physical activity and calcium intake for bone mass growth in children and adolescents: a review of intervention trials. *Preventive medicine* **31**, 722-731, doi:10.1006/pmed.2000.0758 (2000).
57 Kaste, S. C. *et al.* Changes in bone mineral density in survivors of childhood acute lymphoblastic leukemia. *Pediatric blood & cancer* **46**, 77-87, doi:10.1002/pbc.20553 (2006).
58 Wasilewski-Masker, K. *et al.* Bone mineral density deficits in survivors of childhood cancer: long-term follow-up guidelines and review of the literature. *Pediatrics* **121**, e705-713, doi:10.1542/peds.2007-1396 (2008).
59 Lukert, B. P. & Raisz, L. G. Glucocorticoid-induced osteoporosis: pathogenesis and management. *Annals of internal medicine* **112**, 352-364 (1990).
60 Lukert, B. P. & Raisz, L. G. Glucocorticoid-induced osteoporosis. *Rheumatic diseases clinics of North America* **20**, 629-650 (1994).

61 Bockman, R. S. & Weinerman, S. A. Steroid-induced osteoporosis. *The Orthopedic clinics of North America* **21**, 97-107 (1990).
62 Joseph, J. C. Corticosteroid-induced osteoporosis. *American journal of hospital pharmacy* **51**, 188-197; quiz 229-130 (1994).
63 Southard, R. N. *et al.* Bone mass in healthy children: measurement with quantitative DXA. *Radiology* **179**, 735-738, doi:10.1148/radiology.179.3.2027984 (1991).
64 Prince, R. L. *et al.* Prevention of postmenopausal osteoporosis. A comparative study of exercise, calcium supplementation, and hormone-replacement therapy. *The New England journal of medicine* **325**, 1189-1195, doi:10.1056/NEJM199110243251701 (1991).
65 Finkelstein, J. S., Neer, R. M., Biller, B. M., Crawford, J. D. & Klibanski, A. Osteopenia in men with a history of delayed puberty. *The New England journal of medicine* **326**, 600-604, doi:10.1056/NEJM199202273260904 (1992).
66 Heaney, R. P. *et al.* Ultrasound velocity, through bone predicts incident vertebral deformity. *Journal of bone and mineral research : the official journal of the American Society for Bone and Mineral Research* **10**, 341-345, doi:10.1002/jbmr.5650100302 (1995).
67 Epstein, S., Inzerillo, A. M., Caminis, J. & Zaidi, M. Disorders associated with acute rapid and severe bone loss. *Journal of bone and mineral research : the official journal of the American Society for Bone and Mineral Research* **18**, 2083-2094, doi:10.1359/jbmr.2003.18.12.2083 (2003).
68 Gilsanz, V., Carlson, M. E., Roe, T. F. & Ortega, J. A. Osteoporosis after cranial irradiation for acute lymphoblastic leukemia. *The Journal of pediatrics* **117**, 238-244 (1990).
69 Adams, M. J. & Lipshultz, S. E. Pathophysiology of anthracycline- and radiation-associated cardiomyopathies: implications for screening and prevention. *Pediatric blood & cancer* **44**, 600-606, doi:10.1002/pbc.20352 (2005).
70 Kremer, L. C., van Dalen, E. C., Offringa, M. & Voute, P. A. Frequency and risk factors of anthracycline-induced clinical heart failure in children: a systematic review. *Annals of oncology : official journal of the European Society for Medical Oncology / ESMO* **13**, 503-512 (2002).
71 van Dalen, E. C., van der Pal, H. J., Kok, W. E., Caron, H. N. & Kremer, L. C. Clinical heart failure in a cohort of children treated with anthracyclines: a long-term follow-up study. *European journal of cancer* **42**, 3191-3198, doi:10.1016/j.ejca.2006.08.005 (2006).
72 Sorensen, K., Levitt, G. A., Bull, C., Dorup, I. & Sullivan, I. D. Late anthracycline cardiotoxicity after childhood cancer: a prospective longitudinal study. *Cancer* **97**, 1991-1998, doi:10.1002/cncr.11274 (2003).
73 Kremer, L. C., van der Pal, H. J., Offringa, M., van Dalen, E. C. & Voute, P. A. Frequency and risk factors of subclinical cardiotoxicity after anthracycline therapy in children: a systematic review. *Annals of oncology : official journal of the European Society for Medical Oncology / ESMO* **13**, 819-829 (2002).
74 Berry, G. J. & Jorden, M. Pathology of radiation and anthracycline cardiotoxicity. *Pediatric blood & cancer* **44**, 630-637, doi:10.1002/pbc.20346 (2005).
75 Hudson, M. M. *et al.* Noninvasive evaluation of late anthracycline cardiac toxicity in childhood cancer survivors. *Journal of clinical oncology : official journal of the American Society of Clinical Oncology* **25**, 3635-3643, doi:10.1200/JCO.2006.09.7451 (2007).
76 Lipshultz, S. E. *et al.* Chronic progressive cardiac dysfunction years after doxorubicin therapy for childhood acute lymphoblastic leukemia. *Journal of clinical oncology : official*

journal of the American Society of Clinical Oncology **23**, 2629-2636, doi:10.1200/JCO.2005.12.121 (2005).
77 Roberson, J. R. *et al.* Clinical consequences of hyperglycemia during remission induction therapy for pediatric acute lymphoblastic leukemia. *Leukemia* **23**, 245-250, doi:10.1038/leu.2008.289 (2009).
78 Sklar, C. Reproductive physiology and treatment-related loss of sex hormone production. *Medical and pediatric oncology* **33**, 2-8 (1999).
79 da Cunha, M. F. *et al.* Recovery of spermatogenesis after treatment for Hodgkin's disease: limiting dose of MOPP chemotherapy. *Journal of clinical oncology : official journal of the American Society of Clinical Oncology* **2**, 571-577 (1984).
80 Meistrich, M. L., Wilson, G., Brown, B. W., da Cunha, M. F. & Lipshultz, L. I. Impact of cyclophosphamide on long-term reduction in sperm count in men treated with combination chemotherapy for Ewing and soft tissue sarcomas. *Cancer* **70**, 2703-2712 (1992).
81 Hobbie, W. L., Ginsberg, J. P., Ogle, S. K., Carlson, C. A. & Meadows, A. T. Fertility in males treated for Hodgkins disease with COPP/ABV hybrid. *Pediatric blood & cancer* **44**, 193-196, doi:10.1002/pbc.20172 (2005).
82 Reiter, A. *et al.* Chemotherapy in 998 unselected childhood acute lymphoblastic leukemia patients. Results and conclusions of the multicenter trial ALL-BFM 86. *Blood* **84**, 3122-3133 (1994).
83 Mullighan, C. G. *et al.* Deletion of IKZF1 and Prognosis in Acute Lymphoblastic Leukemia. *N Engl J Med* **360**, 470-480, doi:NEJMoa0808253 [pii] 10.1056/NEJMoa0808253 (2009).
84 Peto, R. *et al.* Design and analysis of randomized clinical trials requiring prolonged observation of each patient. II. analysis and examples. *British journal of cancer* **35**, 1-39 (1977).
85 Pounds, S. *et al.* Reference alignment of SNP microarray signals for copy number analysis of tumors. *Bioinformatics* **25**, 315-321, doi:10.1093/bioinformatics/btn624 (2009).
86 Benjamini, Y. & Hochberg, Y. Controlling the False Discovery Rate - a Practical and Powerful Approach to Multiple Testing. *Journal of the Royal Statistical Society Series B-Methodological* **57**, 289-300 (1995).
87 Storey, J. D., Taylor, J. E. & Siegmund, D. Strong control, conservative point estimation and simultaneous conservative consistency of false discovery rates: a unified approach. *Journal of the Royal Statistical Society Series B-Statistical Methodology* **66**, 187-205 (2004).
88 Cheng, C. *et al.* Statistical significance threshold criteria for analysis of microarray gene expression data. *Statistical applications in genetics and molecular biology* **3**, Article36, doi:10.2202/1544-6115.1064 (2004).
89 Subramanian, A. *et al.* Gene set enrichment analysis: a knowledge-based approach for interpreting genome-wide expression profiles. *Proceedings of the National Academy of Sciences of the United States of America* **102**, 15545-15550, doi:10.1073/pnas.0506580102 (2005).
90 Barry, W. T., Nobel, A. B. & Wright, F. A. Significance analysis of functional categories in gene expression studies: a structured permutation approach. *Bioinformatics* **21**, 1943-1949, doi:10.1093/bioinformatics/bti260 (2005).
91 Nettleton, D., Recknor, J. & Reecy, J. M. Identification of differentially expressed gene categories in microarray studies using nonparametric multivariate analysis. *Bioinformatics* **24**, 192-201, doi:10.1093/bioinformatics/btm583 (2008).

92 Lin, M. *et al.* dChipSNP: significance curve and clustering of SNP-array-based loss-of-heterozygosity data. *Bioinformatics* **20**, 1233-1240, doi:10.1093/bioinformatics/bth069 (2004).
93 Olshen, A. B., Venkatraman, E. S., Lucito, R. & Wigler, M. Circular binary segmentation for the analysis of array-based DNA copy number data. *Biostatistics* **5**, 557-572, doi:10.1093/biostatistics/kxh008 (2004).
94 Venkatraman, E. S. & Olshen, A. B. A faster circular binary segmentation algorithm for the analysis of array CGH data. *Bioinformatics* **23**, 657-663, doi:10.1093/bioinformatics/btl646 (2007).
95 McCarroll, S. A. *et al.* Integrated detection and population-genetic analysis of SNPs and copy number variation. *Nature genetics* **40**, 1166-1174, doi:10.1038/ng.238 (2008).

Appendix I: Treatment/Evaluation Calendars

Remission Induction Pretreatment

See order sheets. Offer enrollment for institutional tissue banking protocol.

1 LP**, ITMHA,** **LV rescue** **PRED** **VCR** **DAUNO**** CT/PET scan BM MDD CSF Studies* PE, CBC diff, Uric Acid, UA, Chem. 18 [1, 2, 3]	2 **PRED** PE, CBC diff, Uric Acid, lytes, Ca, P, Mg,	3 **PRED** **PEG-ASP** PE, CBC diff, Uric Acid, lytes, Ca, P, Mg, [4, 5]	4 **PRED** PE, CBC diff, Uric Acid, lytes, Ca, P, Mg	5 **PRED** PE, CBC diff, Uric Acid, lytes, Ca, P, Mg,bili, SGPT	6 **PRED** PE, CBC diff, Uric Acid, lytes, Ca, P, Mg,	7 **PRED** CBC, diff, Uric Acid, lytes, Ca, P, Mg
8 **PRED** **VCR** **DAUNO**** **(*)ITMHA**, LV rescue MRD CSF Studies*	9 **PRED**	10 **PRED**	11 **PRED**	12 **PRED** CBC diff	13 **PRED**	14 **PRED**
15 LP, **ITMHA**, **LV rescue** **PRED (DEX for Stratum 3†)** **VCR** **PEG-ASP** **TMP-SMZ** MRD‡ CSF Studies*	16 **PRED (DEX for Stratum 3†)** **TMP-SMZ**	17 **PRED (DEX for Stratum 3†)** **TMP-SMZ**	18 **PRED (DEX for Stratum 3†)**	19 **PRED (DEX for Stratum 3†)** CBC diff	20 **PRED (DEX for Stratum 3†)**	21 **PRED (DEX for Stratum 3†)**
22 (*)**ITMHA**, **LV rescue** **PRED (DEX for Stratum 3†)** **VCR**	23 **PRED (DEX for Stratum 3†)** **ARA-C**	24 **PRED (DEX for Stratum 3†)** **ARA-C**	25 **PRED (DEX for Stratum 3†)** **ARA-C** **TG (6MP#)**	26 **PRED (DEX for Stratum 3†)** **ARA-C** **TG (6MP#)**	27 **PRED (DEX for Stratum 3†)** **TG (6MP#)**	28 **PRED (DEX for Stratum 3†)** **TG**

CYCLO (§) **TG (6MP#)** TMP-SMZ CSF Studies* MRD‡	**TG (6MP#)** **CYCLO (§)** TMP-SMZ	**TG (6MP#)** TMP-SMZ		CBC diff		**(6MP#)**
29 Stop Prednisone (or Dexamethasone) **TG (6MP#)** **PEG-ASP** TMP-SMZ	30 **ARA-C** **TG (6MP#)** TMP-SMZ	31 **ARA-C** **TG (6MP#)** TMP-SMZ	32 **ARA-C** **TG (6MP#)**	33 **ARA-C** **TG (6MP#)** CBC diff, bili, 24-hour urine, Spot urine	34 **TG (6MP#)**	35 **TG (6MP#)**
36 TMP-SMZ	37 TMP-SMZ	38 TMP-SMZ	39	40 CBC diff	41	42 MRD CT/PET scan BM ¶ [6]

Notes:

* IT methotrexate + hydrocortisone + cytarabine (ITMHA) all participants Day 1 and 15; (*) Participants with the following features will receive additional triple intrathecal treatment on days 8 and 22: T-lymphoblastic lymphoma (any CNS status) and B-lymphoblastic lymphoma with advanced (stages III and IV) disease (including testicular involvement) or following CNS status: CNS-3 status (i.e., ≥5 WBC/μL of CSF with blasts or cranial nerve palsy), CNS-2 status (< 5WBC/μL CSF with blasts), or traumatic status (> 10 RBC/μL of CSF with blasts). [Patients with limited stage (Stages I and II) B-lymphoblastic lymphoma will not receive ITMHA on days 8 and 22.] 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.

† Patients with in T-lymphoblastic lymphoma having MDD ≥1% at diagnosis and MRD positive on day 8 (≥0.01%)

‡ Perform MRD tests if previous MRD is positive.

§ Participants with MDD <1% will receive cyclophosphamide 1000 mg/m^2 IV Day 22 for 1 dose; Participants with MDD ≥ 1% will receive cyclophosphamide 300 mg/m^2 every 12 hours on Days 22-23 for a total of **4** doses.
# For patients with heterozygous or homozygous TPMT genotype
¶ For patients with positive BM disease at diagnosis only

Special Studies:
(1) CBC with diff required at least every third day for first 7 days, and then at least weekly (2) blood chemistries (Chem 18 profile) (3) QCT for bone density (4) TPMT genotype– Sections 7.10 and 8.0. (5) CYP2D6 genotyping (6) Total joint MRI

MRD Studies:

Samples required for MRD studies during remission induction

Time point	T-lineage lymphoma
Diagnosis	BM and PB
Day 8	PB
(Day 15 if MRD$^+$ on Day 8)	PB
(Day 22 if MRD$^+$ on Day 15)	PB
Day 43 (End of induction)	PB

Note: PB, peripheral blood; BM, bone marrow

End of Induction response evaluation: Patients will be evaluated with neck/chest/abdomen/pelvis CT scan and PET scan. For patient with T-lymphoblastic lymphoma, peripheral blood MRD will be performed around day 38-42 of remission induction when ANC has recovered to ≥300/mm^3, WBC to ≥1000/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. For patients with initial bone marrow involvement, bone marrow aspirate and biopsy will be performed. Any patient with detectable disease [MRD (≥0.01%), morphology and imaging (biopsy proven)] at the end of induction or thereafter may be considered for reintensification therapy and hematopoietic stem cell transplantation (HSCT).

Consolidation

Consolidation therapy will be considered only for patients with positive CNS disease or testicular involvement.

1 **PEG-ASP** **6MP** CBC, diff,	2 **6MP**	3 **6MP**	4 **6MP**	5 **6MP**	6 **6MP**	7 **6MP**
8 ***ITMHA, LV** **rescue** **HDMTX #1** **6MP** CBC diff, [1, 2, 3]	9 **6MP,**	10 **6MP** **LV ___** **mg**	11 **6MP** **LV ___ mg**	12 **6MP**	13 **6MP**	14 **6MP**
15 **PEG-ASP** **6MP** CBC diff	16 **6MP**	17 **6MP**	18 **6MP**	19 **6MP**	20 **6MP**	21 **6MP**
22 ***ITMHA, LV** **HDMTX #2** **6MP** CBC, diff, [1, 2, 3]	23 **6MP**	24 **6MP** **LV ___** **mg**	25 **6MP** **LV ___ mg**	26 **6MP**	27 **6MP**	28 **6MP**
29 **PEG-ASP** **6MP** CBC, diff	30 **6MP**	31 **6MP**	32 **6MP**	33 **6MP**	34 **6MP**	35 **6MP**
36 ***ITMHA, LV** **rescue** **HDMTX #3** **6MP** CBC, diff, [1, 2, 3]	37 **6MP**	38 **6MP** **LV ___** **mg**	39 **6MP** **LV ___ mg**	40 **6MP**	41 **6MP**	42 **6MP**
43 **PEG-ASP** **6MP** CBC, diff,	44 **6MP**	45 **6MP**	46 **6MP**	47 **6MP**	48 **6MP**	49 **6MP**

50 ***ITMHA, LV rescue** **HDMTX #4** **6MP** CBC, diff [1, 2, 3]	51 **6MP**	52 **6MP** **LV ___ mg**	53 **6MP** **LV ___ mg**	54 **6MP**	55 **6MP**	56 **6MP**
57 **PEG-ASP** **6MP** CBC, diff	58 **6MP**	59 **6MP**	60 **6MP**	61 **6MP**	62 **6MP**	63 **6MP**
64 **6MP** CBC, diff, [1]	65 **6MP**	66 **6MP**	67 **6MP**	68 **6MP**	69 **6MP**	70 **6MP**

Notes:
*IT methotrexate + hydrocortisone + cytarabine, to be given on days 8, 22, 36, and 50; IT therapy should be given on the same day as HDMTX administration (consult PI or PK if the IT and HDMTX become separated by more than 12 hours).

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

(2) **TREATMENT SCHEMA and Special Laboratory Tests during Continuation Therapy**

Week		Special Studies
1	(*) DEX + DOXO + VCR + 6MP + PEG-ASP	
2	6MP	
3	* 6MP + PEG-ASP	
4	DEX + DOX + VCR + 6MP	
5	(*) 6MP + PEG- ASP	
6	6MP	
7	* Reinduction I**	1, 2‡, 3, 4, 5§, 6§
8	Reinduction I	
9	Reinduction I	
10	6MP	
11	DOX + VCR + 6MP + PEG-ASP	
12	* 6MP	7
13	6MP + PEG-ASP	
14	DEX + DOX + VCR + 6MP	
15	6MP + PEG-ASP	
16	6MP	
17	*Reinduction II**	1, 2‡, 3, 4,
18	Reinduction II	
19	Reinduction II	
20	No Chemotherapy (6MP + MTX for stages I and II B –lymphoblastic lymphoma)	
21	6MP + PEG-ASP†	
22	6MP†	7
23	6MP + PEG-ASP†	
24	CYCLO + ARA-C	
25	*DEX + VCR	
26	6MP + MTX	
27	6MP + MTX	
28	CYCLO + ARA-C	
29	*DEX +VCR	
30	6MP + MTX	
31	6MP + MTX	
32	CYCLO + ARA-C	
33	*DEX + VCR	
34	6MP + MTX	
35	6MP + MTX	
36	CYCLO + ARA-C	
37	*DEX + VCR	
38	6MP + MTX	

Week		Special Studies
39	6MP + MTX	
40	CYCLO + ARA-C	
41	*DEX + VCR	
42	6MP + MTX	
43	6MP + MTX	
44	CYCLO + ARA-C	
45	*DEX + VCR	
46	6MP + MTX	
47	6MP + MTX	
48	CYCLO + ARA-C	
49	*DEX + VCR	2‡
50	6MP + MTX	
51	6MP + MTX	
52	CYCLO + ARA-C	
53	DEX + VCR	
54	6MP + MTX	
55	6MP + MTX	
56	CYCLO + ARA-C	
57	*DEX + VCR	
58	6MP + MTX	
59	6MP + MTX	
60	CYCLO + ARA-C	
61	DEX + VCR	
62	6MP + MTX	
63	6MP + MTX	
64	6MP + MTX	
65	*6MP + MTX	
66	6MP + MTX	
67	6MP + MTX	
68	6MP + MTX	
69	6MP + MTX	
70	6MP + MTX	
71	6MP + MTX	
72	6MP + MTX	
73	*6MP + MTX	
74	6MP + MTX	
75	6MP + MTX	
76	6MP + MTX	
77	6MP + MTX	
78	6MP + MTX	
79	6MP + MTX	
80	6MP + MTX	
81	(*)6MP + MTX	

Week		Special Studies
82	6MP + MTX	
83	6MP + MTX	
84	6MP + MTX	
85	6MP + MTX	
86	6MP + MTX	
87	6MP + MTX	
88	6MP + MTX	
89	(*)6MP + MTX	
90	6MP + MTX	
91	6MP + MTX	
92	6MP + MTX	
93	6MP + MTX	
94	6MP + MTX	
95	6MP + MTX	
96	6MP + MTX	
97	6MP + MTX	
98¶	(*)6MP + MTX	1, 2, 4, 5, 6, 7, 8, 9, 10, 11 (Stratums 1 and 2)
99	6MP + MTX	
100	6MP + MTX	
101	6MP + MTX	
102	6MP + MTX	
103	6MP + MTX	
104	6MP + MTX	
105	6MP + MTX	
106	6MP + MTX	
107	6MP + MTX	
108	6MP + MTX	
109	6MP + MTX	
110	6MP + MTX	
111	6MP + MTX	
112	6MP + MTX	
113	6MP + MTX	
114	6MP + MTX	
115	6MP + MTX	
116	6MP + MTX	
117	6MP + MTX	
118	6MP + MTX	
119	6MP + MTX	
120¶	6MP + MTX	1, 2, 4, 5, 6, 7, 8, 9, 10, 11 (Stratum 3 & pts with CNS and/or Testicular disease)

DEX = dexamethasone; DOX = doxorubicin; VCR = vincristine; 6MP = mercaptopurine; PEG-ASP = PEG-asparaginase; CYCLO = cyclophosphamide; ARA-C = cytarabine

IT-MHA Treatment (*) Schedule During Continuation (See Section 5.5.2.3):

- Patients with *limited stage (stages I and II) B-lymphoblastic lymphoma with CNS-1 status (no identifiable blasts in CSF)* on weeks 1, 3, 5, 7, 12, 17, 25, 29, 33, 37, 41, 45, 49, 57, 65, and 73 (total number of treatments 18).

- Patients with *T-lymphoblastic lymphoma (any stage) and advanced stage (stages III and IV) B-lymphoblastic lymphoma with CNS-1 status* on weeks 1, 3, 5, 7, 12, 17, 25, 29, 33, 37, 41, 45, 49, 57, 65, 73, 81, 89, and 98 (total number of treatments 23).

- Any patient with *CNS-3 status, CNS-2 status or traumatic CSF with blasts status, or testicular involvement* on weeks 3**,** 7, 12, 17, 25, 29, 33, 37, 41, 45, 49, 57, 65, 73, 81, 89, and 98 (total number of treatments 25).

CBC with differential is needed weekly, Physical 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

Surveillance cerebrospinal fluid examination will be done with each IT treatment

Special Studies:

(1) Total cholesterol, triglycerides, free fatty acids, and high-density lipoprotein cholesterol
(2) Bone marrow aspirate and biopsy
(3) TPMT activity and TGN – Day 1 Week 7 (TGN) and Week 17 (TPMT activity)
(4) MRD studies; see below
(5) Neck/chest/abdomen/pelvis CT
(6) NM PET
(7) Total joint MRI (shoulders, elbows, hips and knees and PT/OT evaluation (participants ≥ 9 years of age) – After each Re-Induction (weeks 12-14 and 22-30, and at End of therapy – Weeks 98 or 120 or later)
(8) Surveillance cerebrospinal fluid examination (patients with T-lymphoblastic lymphoma, advanced stage B-lymphoblastic lymphoma and CNS/testicular involvement will receive IT-MHA treatment in week 98)
(9) QCT for bone density
(10) Plasma 1, 25-dihydroxyvitamin D, bone specific alkaline phosphatase, serum calcium and serum magnesium
(11) Spot urine for magnesium, creatinine, calcium, and calcium/creatinine ratio

MRD Studies – Samples required post remission

Time point	T-lineage lymphoma
Day 1 (Weeks 7, 17, 49)	PB
Week 98 or 120 (off therapy)	PB

Note: PB, peripheral blood; ETP, early T-cell precursor.
** See section 5.5.2 for reinduction treatment

† Patients who receive consolidation therapy will not receive PEG-ASP and weekly MTX will be given instead for weeks 21–23.

‡ Bone marrow aspiration is to be performed if the patient has: 1) circulating blast cells; 2) unexplained organomegaly or lymphadenopathy; 3) unexplained bone pain; 4) suspected or documented extramedullary leukemia; or 5) 2 weeks after stopping chemotherapy because of a low ANC or platelet count, the ANC still is <750/μL or the platelet count < 100,000/μL.
§ Patients with abnormal imaging evaluations at end of induction.
¶98 Weeks for Stratums 1 and 2 and 120 Weeks for Stratum 3 and Patients with CNS Disease and/or Testicular Disease

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 absorbed well 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 in various strengths as tablets and oral solution from multiple manufacturers. All dosage forms can be stored at room temperature. At St. Jude, prednisolone oral solution may be substituted for prednisone liquid at equal doses, as it is more palatable.

Supplier: The drug is commercially available.

Toxicity: The side effects of prednisone vary depending on the duration of its use. Short-term use can cause 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 to cause metaphase arrest. Vincristine has poor penetration into the CSF. It is extensively protein bound (~75%). Extensive metabolism occurs in the liver. Excretion is primarily in the bile. A dosage decrease is recommended in 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: The drug is commercially available.

Toxicity: Dose-limiting toxicity is neurotoxicity, which is characterized by constipation and/or paralytic ileus, ptosis, vocal cord paralysis, weakness, jaw pain, abdominal pain, peripheral

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

Guidelines for Administration: 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 (the major active metabolite) and aglycones (inactive metabolite). 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 (see section 7.3) or renal dysfunction (creatinine >3 mg/dL) (see section 7.2).

Formulation and Stability: Daunorubicin is supplied in vials containing 20 mg of a 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 h at room temperature and 48 h if refrigerated.

Supplier: The drug is 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. Chronic 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 450–550 mg/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 daunorubicin is 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: The drug is 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 (Erwinase®)

Source and Pharmacology: Erwinia asparaginase is an enzyme derived from *Erwinia chrysanthemi* and may be useful in patients who are allergic 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 h. Only minimal urinary and biliary excretion occurs. Clearance is not affected by age, renal function, or hepatic function.

Formulation and Stability: Erwinia asparaginase is available in vials containing 10,000 units of the 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 h, as no preservative is added. Occasionally, a small number of gelatinous-like fibers may develop upon standing, in which case the solution can be filtered through a 5 micron filter to remove the particles, with no change in potency.

Supplier: The drug is commercially available.

Toxicity: Acute toxicity includes anaphylactic reactions that occur most commonly with IV administration of the drug. 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 liver function tests (LFTs), pancreatitis, and hyperglycemia. A decrease in synthesis or proteins such as albumin, fibrinogen, and other coagulation factors may occur, which can result in hemorrhage. Thrombosis and pulmonary embolism can also occur. Less common side effects include renal dysfunction and CNS complications such as somnolence, weakness, lethargy, coma, and seizures.

Guidelines for Administration: See Treatment and Dose Modification sections of the protocol. For additional information about this drug, see package insert.

6. DOXORUBICIN (Adriamycin®)

Source and Pharmacology: Doxorubicin is an anthracycline antibiotic produced by *Streptomyces peucetius*. 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 may be responsible for the drug's cardiac toxicity but play a role in its antitumor activity as well. Doxorubicin is cell-cycle, phase non-specific. It 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 may be the major active metabolite) and aglycones. Doxorubicin and its metabolites are excreted mainly in the bile and feces (~80%), and the remainder in the urine. Dosage should be reduced in patients with liver dysfunction (section 7.3) or renal dysfunction (creatinine >3 mg/dL).

Formulation and Stability: Doxorubicin is available in vials containing 10 mg, 20 mg, 50 mg or 200 mg as a 2 mg/mL red-orange solution. Vials containing 10 mg, 20 mg, 50 mg, 100 mg, or 150 mg of the drug as a red-orange lyophilized powder are also available. Intact vials of doxorubicin solution should be stored under refrigeration, whereas 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: The drug is 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. Chronic cardiotoxicity is related to the 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 450–550 mg/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. Typhilitis 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. It is well absorbed from the GI tract and has 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 (considered 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 as 25 and 50 mg tablets. Cyclophosphamide is also available in vials containing 100, 200, 500, 1000, or 2000 mg of the 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 the 100, 200, 500, 1000, or 2000 mg vials 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 h at room temperature and 6 days if refrigerated, but as they contain no preservative, it is recommended that they be used within 24 h of preparation.

Supplier: The drug is 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 analog. It must be triphosphorylated 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, or 2000 mg of the 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 50 mg/mL. The 1000 and 2000 mg vials are reconstituted with 20 mL and 40 mL, respectively, resulting in a final concentration of 50 mg/mL. After reconstitution, the drug is stable for 8 days at room temperature.

Supplier: The product is commercially available.

Toxicity: The dose-limiting adverse effect is myelosuppression, 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 and 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 spectrum of CNS symptoms, such as 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, the syndrome of sudden respiratory distress progressing to pulmonary edema can occur.

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 analog. It must be triphosphorylated 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 multidose vials containing 100, 500, 1000 or 2000 mg of the 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 50 mg/mL. The 1000 and 2000 mg vials are reconstituted with 20 mL and 40 mL, respectively, resulting in a final concentration of 50 mg/mL. After reconstitution, the drug is stable for 8 days at room temperature.

Supplier: The drug is commercially available.

Toxicity: The dose-limiting adverse effect is myelosuppression, 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 and 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 spectrum 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, the syndrome of sudden respiratory distress progressing to pulmonary edema can occur.

Guidelines for Administration: See Treatment and Dose Modification sections of the protocol.

10. THIOGUANINE

Source and Pharmacology: Thioguanine is a purine antimetabolite. It is intracellularly converted to ribonucleotides, which are incorporated into the 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 get converted 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 diphosphokinases 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 by inhibiting 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 been shown 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 release of the proapoptotic 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 (21 males, 19 females) aged 2 to 19 years with relapsed or refractory ALL or AML. At the given 52 mg/m^2 dose, similar concentrations were obtained over a wide range of body surface areas. Clofarabine was 47% bound to plasma proteins, predominantly to albumin. Noncompartmental analysis showed that systemic clearance and volume of half-life was estimated to be 5.2 h. There was no apparent difference in pharmacokinetics between patients with ALL and AML or between males and females. In children, 24-hour urine collections show that 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 nonrenal elimination remain unknown. Although no clinical drug–drug interaction studies have been conducted to date, it can be inferred from *in vitro* studies that 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 (comprising water for injection, USP, and sodium chloride USP). The pH range of the solution is 4.5 to 7.5. The solution should be stored at 25°C; excursions are permitted to 15°C–30°C.

Toxicity: The most common toxicities of clofarabine are vomiting, nausea, diarrhea, anemia, leukopenia, thrombocytopenia, neutropenia, febrile neutropenia, and infection. More 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, and 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 have also been reported. In a study, 4 of 113 pediatric patients experienced capillary leak syndrome or SIRS, leading to multiorgan 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. Clofarabine should be filtered through a sterile 0.2 μm syringe filter and then further diluted with 5% dextrose injection USP or 0.9% sodium chloride injection USP to a convenient volume and infused over 2 h. The resulting admixture may be stored at room temperature, but must be used within 24 h 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.

Because 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: The drug is commercially available.

12. METHOTREXATE

Source and Pharmacology: Methotrexate is a folate analog 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, at an average of approximately 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 give hydroxymethotrexate. It is excreted unchanged mainly in the urine, with small amounts being excreted in the bile and feces. The percent recovered as unchanged drug in the urine is higher with short infusions than with prolonged infusions. Methotrexate has a biphasic elimination, with an initial half-life of 2–3 h and a terminal half-life of 10–12 h. 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 after being administered high-dose methotrexate.

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

Supplier: The drug is commercially available.

Toxicity: The dose-limiting toxicities of methotrexate are generally bone marrow suppression, ulcerative stomatitis, severe diarrhea, and 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 HD-MTX. Leucovorin rescue should be initiated within 48 h of starting HD-MTX and adjusted on the basis of MTX levels in order 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 acts as 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 rather than 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 some excretion occurring in the feces.

Formulation and Stability: Leucovorin is supplied as 5, 15, or 25 mg tablets and vials containing 50, 100, or 350 mg of leucovorin, respectively, 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: The drug is 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, which are then incorporated into the DNA and RNA and inhibit 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 hematopoietic tissues by HPRT to the active nucleotide forms. It is inactivated to methylated metabolites by TPMT and to 6-thiouric acid by xanthine oxidase. TPMT is a genetically regulated, polymorphically distributed enzyme and is deficient in approximately 1 in 300 individuals 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: Tablets are commercially available.

Toxicity: The dose-limiting toxicity of mercaptopurine is myelosuppression. Mercaptopurine can cause intrahepatic cholestasis and focal centralobular necrosis and is usually manifested by hyperbilirubinemia and increased liver function tests. Other toxicities include mild nausea and vomiting, skin rash, hyperuricemia, and mild diarrhea.

Guidelines for Administration: See Treatment and Dose Modification sections of the protocol.

15. DEXAMETHASONE (Decadron®)

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

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

Supplier: The drug is commercially available.

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

Guidelines for Administration: 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 suppressing the migration of polymorphonuclear leukocytes and reversing the 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). The lyophilized product should be stored at controlled room temperature (15°C–30°C). The reconstituted solution should be stored in the refrigerator and protected from light. Unused solution should be discarded after 3 days.

Supplier: The drug is commercially available.

Toxicity: The dose-limiting toxicities of hydrocortisone are 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 multidose vials containing 100 mg, 150 mg, 500 mg, or 1000 mg of etoposide as a 20 mg/mL solution and 30% alcohol. Etoposide is also available as a 50 mg capsule. 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 before administration. Solutions with a final concentration of 0.2 and 0.4 mg/mL are stable at room temperature for 96 hours and 24 h, respectively.

Supplier: The drug is commercially available.

Toxicity: The dose-limiting toxicity of etoposide 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.

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 2 occasions within 1 h. The measurement must be oral with glass or IVAC[7] thermometers or at the tympanic membrane by infrared instruments (Thermoscan[7]). The same method should be used throughout the febrile episode for each patient.

B. Neutropenia: Neutrophil count of <500/mm^3 or <1000/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 h 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 INFECTIONS

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 whether or not catheter related).

2. Bacterial sepsis: Blood culture positive for any bacterium, plus clinical evidence of infection (fever, chills, hypotension, etc.) (specify whether or not catheter related).
3. Urinary tract infection: A urine colony count of 100,000 or greater of a single organism plus symptoms such as dysuria and flank pain. Asymptomatic bacteriuria is the same colony count without symptoms.
4. Pneumonia (bacterial): A radiographically discernible infiltrate plus isolation of potentially causative bacteria from bronchoalveolar lavage, blood, or biopsy specimen. If blood culture is positive, should be coded as bacterial sepsis with pneumonia.
5. Meningitis: A positive culture of causative bacteria from the CSF plus symptoms consistent 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 patients 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. Systemic: Isolation of *Candida* species from an otherwise sterile body fluid or tissue (e.g., blood, CSF, aspirate or biopsy of tissue, bone marrow, joint fluid) along with clinically compatible illness, or histologic evidence of typical budding yeast and pseudohyphae in tissue biopsy plus isolation of *Candida* species in culture from the same tissue or otherwise sterile body fluid or tissue. Typical histopathology with pseudohyphae in pulmonary, hepatic, splenic, brain, or renal lesions is adequate since the organism often fails to grow from cultures of organs.
   2. 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.
   3. Esophageal or urinary bladder: Evidence of tissue involvement proven by endoscopy and biopsy plus isolation of fungus in culture.

B. Aspergillosis
   1. Systemic: Isolation of *Aspergillus* species from an otherwise sterile body fluid or tissue plus clinically compatible illness (e.g., pulmonary infiltrate, sinusitis, CNS lesion, hepatic or splenic lesion). Demonstration of typical branching septate hyphae in biopsies or aspirated tissue specimens plus isolation of the organism in culture also allows systemic diagnosis.
   2. Pulmonary: Typical cystic lesion with fungus ball ("half-moon sign") in chest radiograph and/or CT scan, and *Aspergillus* species isolated from sputum, tracheal aspirate, or bronchoalveolar lavage fluid.

C. Histoplasmosis: Isolation of *Histoplasma capsulatum* in culture from patient with compatible illness.

D. Presumptive Systemic Fungal Infection: Typical lesions in the liver, spleen, kidney, or brain on CT or MRI scans plus compatible clinical features and treated with systemic antifungal drugs.

E. Others: The investigator may include certain other infectious diseases of unique nature. Acceptance requires a decision to be made before decoding occurs and with the agreement of the PI and an attending physician from the Department of Infectious Diseases.

IV. PROTOZOAN INFECTIONS

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 stools plus diarrhea.

C. *Toxoplasmosis:* See ACTG protocol 254.

V. TOPOGRAPHIC DIAGNOSIS

The following diagnoses are acceptable for objectively identified infections without confirmation of etiology.

1. Pneumonia: Lesion on radiograph
2. Osteomyelitis: Radiologic diagnosis
3. Sinusitis: Radiologic diagnosis (X-ray, CT, MRI)
4. Cellulitis: Physician diagnosis

VI. FEVER OF UNDETERMINED ETIOLOGY

A febrile episode with no etiologic or topographic evidence of disease can be identified as being.

1. neutropenic if ANC is $<500/mm^3$
2. nonneutropenic if ANC is $\geq 500/mm^3$

VII. CULTURE NEGATIVE SEPSIS

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

APPENDIX IV: TESTS/EVALUATIONS/PROCEDURES PERFORMED FOR STANDARD OF CARE AND FOR RESEARCH

Standard of care

- Complete history and physical exam, with careful notation and assessment of clinical signs relevant to lymphoma
- Coagulation profile
- Complete blood count with differential
- Chemistry profile: glucose, electrolytes, blood urea nitrogen (BUN), creatinine, lactate dehydrogenase (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 25-hydroxyvitamin D, osteocalcin N-MID, urine N-Telopeptide with creatinine, bone specific alkaline phosphatase, parathyroid hormone
- Spot urine for magnesium, creatinine, calcium, and calcium/creatinine ratio
- Thyroid function test (free T4, TSH)
- Chest X-ray
- Echo (echocardiogram)/EKG (electrocardiogram) and/or multiple gate acquisition scan (MUGA), Troponin T, natriuretic hormone levels
- Neck/chest/abdomen/pelvis CT
- Positron emission tomography (PET)/CT scan
- Biopsy/cytology
- Bone marrow evaluation: morphology, cytochemistry, immunophenotyping, cytogenetics, DNA index, molecular diagnosis, MRD studies
- Peripheral blood MRD study
- Lumbar puncture with CSF examination (cell count with differential of cytospin preparation)
- Other studies as clinically indicated, e.g. sickle cell prep, hemoglobin electrophoresis, and G6PD screen for Black children; varicella titer; Hepatitis B antigen; HIV, Epstein-Barr virus (EBV), toxoplasma (TOXO), cytomegalovirus (CMV), histoplasma, and Bartonella titers.
- PT/OT evaluation; patients $\geq$ 9 year old
- Total joint MRI (shoulders, elbows, hips, knees and pelvis): patients $\geq$ 9 year old
- QCT for bone density
- TPMT genotype
- TPMT and TGN
- CYP2D6 genotyping
- Methotrexate pharmacokinetics
- Pregnancy testing

Research

- Semen analysis (through Fertility of Memphis)
- Inhibin B level
- Anti-müllerian hormone
- Biology and molecular genetic research, and storage for future research (pre-trial material obtained at diagnosis)