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Title: Randomized Study of Intravenous Calaspargase Pegol (SC-PEG asparaginase) and Intravenous Oncaspar in Children and Adolescents with Acute Lymphoblastic Leukemia or Lymphoblastic Lymphoma

Protocol Version Date: February 24, 2016

STATUS PAGE
PROTOCOL **11-001**

Closed To New Accrual

Closure Effective Date: *06/08/2015*
Reason: Study Accrual Goal Met

No new subjects may be enrolled in the study as described above. Any questions regarding this closure should be directed to the study's Principal Investigator

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Title: Randomized Study of Intravenous Calaspargase Pegol (SC-PEG asparaginase) and Intravenous Oncaspar in Children and Adolescents with Acute Lymphoblastic Leukemia or Lymphoblastic Lymphoma

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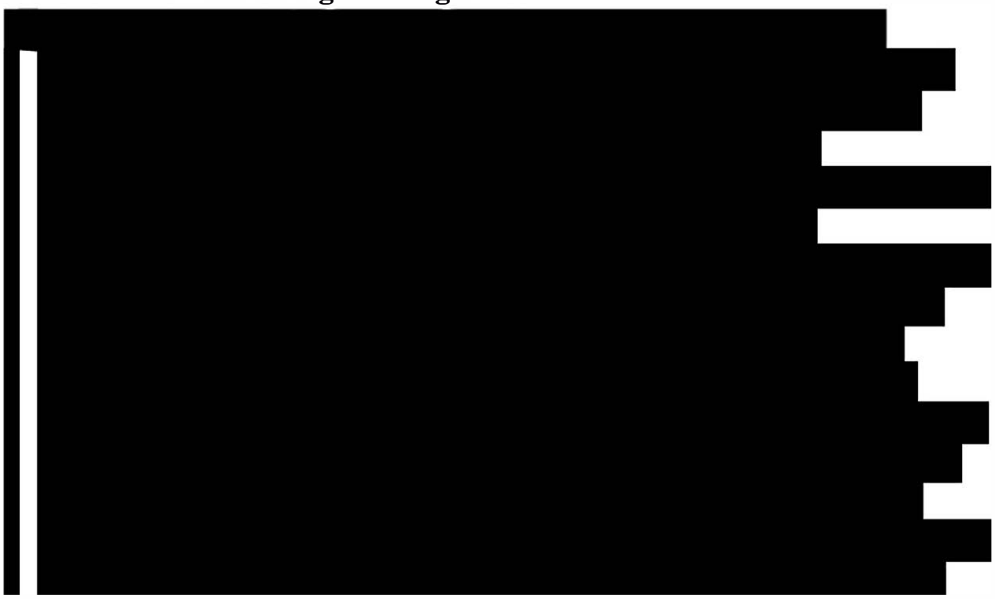

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OVERALL TREATMENT SCHEMA

Prophase	Induction	Consolidation I	CNS Therapy	Consolidation II	Continuation
3 days	4 weeks	3 weeks*	3 weeks	27 weeks	~70 weeks**

Randomization:

A. IV SC-PEG x 1 dose during induction, then every 3-weeks x 10 doses (30 weeks total) beginning at start of CNS Therapy Phase***
B. IV Oncaspar x 1 dose during induction, then every 2-weeks x 15 doses (30 weeks total) beginning at start of CNS Therapy Phase***

*~9 weeks for VHR patients
**Until 104 weeks (24 months) of CCR
***Post-induction Asparaginase begins during Consolidation I phase in VHR patients

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1. OBJECTIVES

1.1 Study Design

A randomized study to determine the safety and feasibility of administering Calaspargase Pegol (SC-PEG asparaginase), a new formulation of PEG-asparaginase, compared with Oncaspar (the currently available form of PEG-asparaginase), in children and adolescents with newly diagnosed acute lymphoblastic leukemia (ALL)and lymphoblastic lymphoma treated with a DFCI ALL Consortium therapeutic backbone.

1.2 Primary Objectives

To assess the safety and feasibility associated with the administration of IV Calaspargase Pegol 2500 IU/m^2 given as a single dose during Remission Induction and every 3-weeks for 30 weeks during post-induction therapy in children and adolescents with newly diagnosed ALL

1.2.1 To assess the toxicity associated with IV Calaspargase Pegol administration and determine if it is significantly different than toxicity associated with IV Oncaspar.

1.2.2 To determine the serum asparaginase activity (SAA) levels associated with IV Calaspargase Pegol administered as a single dose during remission induction and every 3-weeks for 30 consecutive weeks during post-induction therapy, and to compare them to the SAA associated with IV Oncaspar administered as a single dose during Remission Induction and every 2-weeks for 30 consecutive weeks during post-induction therapy.

1.3 Secondary Objectives

1.3.1 To assess the frequency of bacteremia, fungemia and invasive fungal infections during the remission induction phase in patients receiving antibiotic prophylaxis.

1.3.2 To describe the outcome of children and adolescents with lymphoblastic lymphoma treated with a DFCI ALL Consortium treatment regimen.

1.3.3 To explore the impact on outcome of changing therapy for very high risk patients, defined as: i)B-precursor ALL patients with high end-induction minimal residual disease (MRD); patients withMLL-gene rearrangements or low hypodiploidy; or iii) T-ALL/lymphoblastic lymphoma patients with ETP phenotype.

1.3.4 To assess the feasibility of vitamin D screening and supplementation in children and adolescents undergoing treatment for ALL in the context of a multi-institutional trial.

1.3.4.1 To determine the prevalence of vitamin D deficiency in children and adolescents with ALL at the following time-points: at diagnosis, at the end of remission induction, at the start of continuation, and at the conclusion of therapy.

1.3.4.2 To assess the feasibility of correcting vitamin D deficiency with supplementation of vitamin D and calcium in patients found to have vitamin D deficiency.

1.3.4.3 To explore the relationship between vitamin D status and skeletal toxicity (fracture and osteonecrosis) in children and adolescents undergoing therapy for ALL.

1.3.4.4 To explore risk factors for vitamin D deficiency, including demographic variables such as age, sex, and ethnicity, as well as geographic location (geographic latitude) and season of measurement.

1.3.5 To determine the feasibility of prospective screening for absence of biallelic TCRγ deletions (ABGD) via qPCR in patients with T-ALL

1.3.5.1 To determine the frequency of ABGD in patients with T-cell ALL at diagnosis

1.3.5.2 To explore the correlation between early T-cell precursor (ETP) phenotype and ABGD

1.3.6 To determine the feasibility of prospective screening for abnormalities (eg, mutations, deletions, rearrangements) of IKZF1, CRLF2 and JAK1/2 in patients with newly diagnosed B-ALL.

1.3.6.1 To determine the frequency of these abnormalities in standard-risk and high-risk patients

1.3.6.2 To explore the correlation between the presence of these abnormalities and end-induction MRD levels.

1.3.7 To explore the relationship between mitochondrial BCL-2 family preconditions and response to chemotherapy as measured by induction response, end-induction MRD level and event-free survival (EFS).

1.3.8 To obtain patient samples for the development of primary xenograft mouse models with the goal of identifying novel therapies for high-risk (including those with high end-induction MRD, T-ALL with ABGD) and relapsed patients.

2. BACKGROUND

2.1 Calaspargase Pegol (SC-PEG Asparaginase)

L-asparaginase, a bacterially-derived enzyme that catalazyes the hydrolysis of L-asparagine, is an important and universal component of therapy for childhood ALL. Lymphoblasts tend to have low intracellular levels of asparagine synthetase and so are dependent upon extracellular pools of asparagine to support their growth and proliferation. Treatment with asparaginase effectively starves lymphoblasts while normal cells, with higher intracellular levels of asparagine synthetase, remain relatively unaffected.

There are three major L-asparaginase preparations available in North America: native E.coli asparaginase (Elspar), PEG-asparaginase (Oncaspar), formed by covalently attaching polyethylene glycol to the native *E. coli* enzyme, and Erwinia (Erwinase), derived from *Erwinia chrysanthemi*. Of the three preparations, PEG asparaginase has the longest half-life and Erwinase the shortest.(1) Studies have suggested that PEG asparaginase may be less immunogenic than native E.coli asparaginase.(2, 3)

Calaspargase Pegol (SC-PEG-asparaginase) is a new pegylated asparaginase preparation which utilizes a novel linkage between the PEG-moiety and the native E.coli L-asparaginase protein. Calaspargase Pegol utilizes the same E.coli L-asparaginase and the same size PEG that is used in the currently available form of PEG asparaginase (Oncaspar), but the new linkage utilized in Calaspargase Pegol is felt to be more hydrolytically stable.

2.2 **Acute Lymphoblastic Leukemia/Lymphoblastic Lymphoma**

ALL is the most common cancer diagnosed in children, representing approximately 25% of cancer diagnoses among children younger than 15 years. There are approximately 2,400 children and adolescents up to age 20 years diagnosed with ALL each year in the United States.(4) ALL most commonly occurs in pre-school aged children (age 2-5 years); the incidence of ALL among children aged 2 to 3 years is approximately fourfold greater than that for infants and is nearly tenfold greater than that for adolescents 16 to 21 years of age.

Several clinical and biologic features have been found to have important prognostic significance in childhood ALL, including age, presenting leukocyte count, immunophenotype, recurrent chromosomal abnormalities, and response to initial therapy as measured by morphology and more sensitive techniques to assess minimal residual disease.(5) These prognostic factors have been used to stratify therapy; patients with “high risk” features have received more intensive treatments, whereas some of the more morbid components of therapy have been modified or eliminated for those children with more favorable presenting features. With current treatment regimens, event-free survival (EFS) rates for childhood acute lymphoblastic leukemia (ALL) approach or exceed 80%.(6, 7) (8)

Lymphoblastic lymphoma accounts for approximately 20% of childhood Non-Hodgkin’s lymphoma; there are about 150-200 cases diagnosed in the United States each year in children and adolescents younger than 20 years.(9) Although pediatric patients with lymphoblastic lymphoma are typically treated with ALL therapeutic regimens, there are relatively few published series reporting the outcomes of patients treated in this way. The BFM has reported long-term event-free survival rates of 80-90% for lymphoblastic lymphoma patients treated with their ALL regimens.(10, 11)

2.3 **Primary Objective: Rationale**

L-asparaginase is an essential component of treatment for ALL, but is associated with multiple toxicities. Because asparaginase preparations are bacterially-derived, they have the potential to be highly immunogenic. Hypersensitivity reactions to asparaginase occur in up to 30% of patients treated with native E. coli asparaginase.(2) Clinical allergy is frequently associated

with the development of neutralizing antibodies, so that even if symptoms are controlled, there is little therapeutic benefit after drug administration.(12) Other asparaginase-associated toxicities include pancreatitis (in 5-10% of patients) and thrombosis (in 2-5% of patients).(13)

Since 1977, the Dana-Farber Cancer Institute (DFCI) ALL Consortium has included 20-30 consecutive weeks of asparaginase during post-induction consolidation therapy, and has demonstrated that this treatment significantly improves long-term event-free survival.(14, 15) We also demonstrated that patients who received a truncated course of asparaginase because of intolerable side effects had an inferior outcome compared with those who receive all intended doses.(2) Thus, it is crucial to determine the optimal dosing and preparation of asparaginase in order to reduce a significant source of treatment-related morbidity and potentially improve cure rates.

Over the last two decades, we have performed sequential randomized studies to determine the optimal dosing and preparation of asparaginase.(2, 16) Between 1991 and 1995, we conducted a randomized clinical trial comparing intramuscular PEG-asparaginase and E.coli L-asparaginase in children with newly diagnosed ALL.(2) We found that PEG-asparaginase was associated with decreased frequency of asparaginase-related toxicities, primarily due to a lower incidence of allergic reactions, without any significant difference in event-free survival between the two preparations. We subsequently determined that Erwinia asparaginase, when dosed once weekly during a 20-week Consolidation phase, was less toxic but also less efficacious than native E.coli asparaginase (Protocol 95-01, 1996-2000).(16)

On Protocol 00-01 (2000-5), we compared conventional fixed dosing (25,000 IU/m^2/week) of E.coli asparaginase with individualized dosing (dosing adjusted based on patient's pre-dose nadir enzyme activity levels, with the goal of maintaining trough levels between 0.1-0.14 IU/mL). Nadir serum asparaginase activity (NSAA) was monitored in samples obtained before treatment and 7-days after the dose given on weeks 1, 3, 6, 9, 12, 15, 18, 21, 24, 27, and 29, just prior to administration of the next dose of asparaginase. 384 patients were randomized, and over 2,500 samples were collected and assayed in real-time (7-14 day turnaround time) for NSAA over the course of that study. We determined that there was large interpatient variability in NSAA for those receiving fixed dose asparaginase, and on the individualized dosing arm, adolescents (10-18 years of age) required a lower dose of E.coli asparaginase than younger children to maintain NSAA within the goal range. We observed no difference in the frequency of asparaginase-related toxicities when comparing individualized and fixed dosing. However, individualized dosing of asparaginase was associated with a superior event-free survival (5-yr EFS of 90 ± 2% compared with 82 ± 3% for fixed-dose asparaginase, p=0.04).(17)

Between 2005-2010, we conducted a Phase III randomized trial comparing IV Oncaspar with IM native E.coli asparaginase in children and adolescents (age 1-18 years) with newly diagnosed ALL (DFCI ALL Consortium Protocol 05-001; National Clinical Trial Reference Number 00400946: Pegasparaginase or Asparaginase and Combination Chemotherapy in Treating Young Patients With Newly Diagnosed Acute Lymphoblastic Leukemia).(18) All patients received IV Oncaspar 2500 IU/m^2 on Day 7 of multiagent remission induction. Patients then received 30 weeks of asparaginase commencing on week 7 of treatment. Patients were randomized to receive either IV Oncaspar 2500 IU/ m^2 every 2 weeks x 15 weeks or IM Elspar 25000 IU/ m^2

every week x 30 weeks. Serum samples from all patients are collected at several time points for analysis of asparaginase enzyme activity. As of March 1, 2010, 556 eligible subjects were enrolled on Protocol 05-001 (accrual rate of 116 patients/year), which met the accrual goal for the randomized comparison of IV PEG versus IM E.coli asparaginase. 506 patients (91% of enrolled subjects) agreed to participate in the asparaginase randomization.

We analyzed serum asparaginase activity levels weekly during the induction phase beginning 4 days after the dose of IV Oncaspar was administered. We observed that 88% of patients had serum asparaginase activity $\geq$ 0.1 IU/mL for 18-days after dosing, but only 7% maintained this level of activity after 25 days (Table 2.1), supporting the every 2-week dosing schedule during post-induction treatment phases.(18) The single dose of IV Oncaspar administered during induction on Protocol 05-001 was reasonably well-tolerated. Toxicities included pancreatitis (4.5% of patients), thrombotic complications (2%), and hypersensitivity reactions (1.5%). There were no asparaginase-related deaths during the induction phase.

Table 2.1. Serum asparaginase activity (IU/mL) after a single 2,500 IU/m^2 dose of PEG-asparaginase given as a 1-hour IV infusion in 186 children during Induction phase on DFCI Protocol 05-001.

Induction Day #	Days after IV PEG dose	No. of Patients	Serum asparaginase activity (IU/mL)		Proportion of patients with asparaginase activity (IU/mL)		
			Median (range)	Mean ± SD	$\geq$ 0.025	$\geq$ 0.10	$\geq$ 0.20
11	4	130	0.66 (<0.025, 2.34)	0.73 ± 0.29	96	96	96
18	11	133	0.48 (<0.025, 1.58)	0.49 ± 0.22	97	95	92
25	18	112	0.20 (<0.025, 1.24)	0.23 ± 0.14	96	88	52
32	25	113	0.04 (<0.025, 0.41)	0.07 ± 0.05	70	7	2

During the post-induction consolidation phase, samples for assessment of nadir serum asparaginase activity level were obtained every 6 weeks, just prior to the administration of an asparaginase dose (2-weeks after previous Oncaspar dose and 1-week after previous E.coli asparaginase dose. The median nadir serum asparaginase activity (NSAA) levels were higher with IV Oncaspar than with IM E.coli (Table 2.2). Nearly all patients treated with IV Oncaspar had NSAA $\geq$ 0.1 IU/mL (the goal therapeutic level previously associated with serum asparagine depletion) throughout the 30-weeks of post-induction asparaginase compared with fewer than half of patients treated with IM E.coli asparaginase (Table 2.3, $p<0.01$ at each timepoint), and significantly more IV Oncaspar-treated patients had NSAA levels exceeding 0.2 and 0.4 IU/mL than did IM E.coli asparaginase patients ($p<0.01$ at each time point). There was no significant difference in asparaginase-related toxicities (allergy, pancreatitis, thrombosis) between the two arms (Table 2.4), although there was a trend toward more thrombotic events with E.coli asparaginase ($p=0.07$).

Table 2.2 Median and mean nadir Serum asparaginase activity (IU/mL) during the Post-Induction Consolidation Phase on Protocol 05-001

	IV Oncaspar			IM E.coli		
Sample Time (weeks)	N	Nadir serum asparaginase activity (IU/mL) Median (range)	Mean ± SD	N	Nadir serum asparaginase activity (IU/mL) Median (range)	Mean ± SD
5	84	0.67 (<0.025, 1.99)	0.67 ± 0.32	92	0.094 (<0.025, 1.71)	0.15 ± 0.22
11	70	0.71 (<0.025, 1.30)	0.70 ± 0.26	74	0.094 (<0.025, 1.69)	0.15 ± 0.24
17	73	0.76 (<0.025, 2.53)	0.76 ± 0.32	86	0.092 (<0.025, 1.33)	0.15 ± 0.19
23	60	0.70 (0.21, 1.46)	0.72 ± 0.23	76	0.094 (<0.025, 1.72)	0.17 ± 0.26
29	68	0.70 (0.11, 2.42)	0.74 ± 0.31	63	0.095 (<0.025, 1.28)	0.13 ± 0.18
Mean per Patient	114	0.70 (0.22, 1.38)	0.72 ± 0.21	136	0.10 (<0.025, 1.17)	0.15 ± 0.19

Table 2.3 Proportion of patients with NSAA at or exceeding 0.025, 0.10, 0.20 and 0.40 IU/mL during the Post-Induction Consolidation Phase on Protocol 05-001 (lower limit of detection of assay: 0.025 IU/mL)

	IV Oncaspar					IM E.coli				
Sample time (weeks)		Proportion of patients with asparaginase activity (IU/mL)					Proportion of patients with asparaginase activity (IU/mL)			
	N	≥ 0.025	≥ 0.10	≥ 0.20	≥ 0.40	N	≥ 0.025	≥ 0.10	≥ 0.20	≥ 0.40
5	84	98	95	94	85	92	87	48	18	8
11	70	99	97	97	91	74	88	47	18	7
17	73	99	97	95	90	86	90	47	21	5
23	60	100	100	100	93	76	92	46	16	8
29	68	100	100	99	91	63	92	44	14	5

Table 2.4 Toxicities observed in 463 randomized patients enrolled on DFCI ALL Consortium Protocol 05-001 (2005-2010)

Toxicity	IM Ecoli N (%)	IV PEG N (%)	p-value
N	231	232	
Asparaginase Toxicity	58 (25)	59 (25)	>0.99
Allergy	20 (9)	26 (11)	0.44
Pancreatitis	21 (9)	25 (11)	0.64
Mild/Moderate	13 (6)	13 (6)	0.99
Severe	8 (3)	12 (5)	0.49
Thrombosis	21 (9)	14 (6)	0.22
Infection (bacteremia, invasive fungal)	46 (20)	35 (15)	0.18

Thus, the data from Protocol 05-001 indicates that every 2-week IV Oncaspar was no more toxic than weekly IM E.coli asparaginase during the 30-week post-induction consolidation phase. It was also associated with higher NSAA levels, which potentially could have a beneficial therapeutic effect. As of July 2011, median follow-up for randomized patients was 2.8 years. Longer follow-up is needed to determine the relative rates of EFS of the two treatment arms, but these toxicity and pharmacokinetic results justifies the continued study of intravenous pegylated asparaginase preparations during post-induction treatment phases.

It is expected that, over the next several years, Calaspargase Pegol will replace Oncaspar as the only available PEG-asparaginase preparation in North America, and so detailed pharmacokinetic and toxicity analyses of this preparation are essential.

Since 2008, The Children's Oncology Group (COG) has been conducting a randomized comparison of Calaspargase Pegol with Oncaspar in children with high-risk ALL (Protocol AALL07P4). The initial dose of Calaspargase Pegol and Oncaspar for all patients was 2500 IU/m^2. At the October 2009 COG meeting, preliminary data was presented regarding the toxicity and pharmacokinetics after the induction dose for the first patients enrolled on the study. There was no difference in the incidence of adverse events between the two preparations. It appeared that pharmacokinetics were similar through day 10, but enzyme activity levels were higher in Calaspargase Pegol-treated patients on Days 10-25 after the induction dose. Patients treated with Calaspargase Pegol maintained asparaginase enzyme activity levels $\geq$ 0.1 IU/mL for a longer duration than those who received Oncaspar:

Table 2.5: Percentage of patients with serum asparaginase activity level $\geq$ 0.1 IU/mL on COG Protocol AALL07P4

Day after PEG-ASP dose	Oncaspar	Calaspargase Pegol
15	100%	100%
22	85%	100%
29	22%	100%

Thus, all patients treated with 2500 IU/ m^2 Calaspargase Pegol maintained serum asparaginase activity levels $\geq$ 0.1 IU/mL for 4 weeks after a single dose. The COG study was subsequently amended so that patients randomized to Calaspargase Pegol received a dose of 2100 IU/m^2, which was felt to be more comparable pharmacokinetically to Oncaspar 2500 IU/m^2. However, the COG study was suspended on December 22, 2010 based on a higher rate of Day 29 end-induction MRD-positivity for patients treated with Calaspargase Pegol 2100 IU/m²/dose compared with Calaspargase Pegol 2500 IU/m²/dose or Oncaspar 2500 IU/m²/dose. The difference in frequency of MRD-positivity was statistically significant and crossed pre-defined safety monitoring boundaries. Subsequent analyses indicated that their was an imbalance in the randomization, with a greater proportion of higher risk patients randomized to Calaspargase Pegol 2100 IU/m^2 arm than to Oncospar 2500 IU/m^2: there were more patients on the Calaspargase Pegol 2100 IU/m2 arm who were older than 16 years at diagnosis, and more patients who were older than 10 years with presenting WBC counts $\geq$ 50K. This imbalance likely contributed to the differing rates in end-induction MRD rates between the two arms.

Data from the COG protocol suggests that a single dose of IV Calaspargase Pegol 2500 IU/m² is tolerable and likely to lead to asparagine depletion for up to 4 weeks after a single dose. Additionally, at this dose, there was no difference in end-induction MRD rates compared with Oncasspar 2500 IU/m^2. It still remains to be determined how best to dose Calaspargase Pegol during post-induction therapy. On COG Protocol AALL07P4, asparaginase is administered intermittently during the post-induction treatment phases (Days 15 and 43 of Consolidation, Days 2 and 22 of Interim Maintenance #1, Days 4 and 43 of Delayed Intensification #1, Days 2 and 22 of Interim Maintenance #1 and Days 4 and 43 of Delayed Intensification #2). On DFCI ALL Consortium protocols, asparaginase is given at regular intervals during the post-induction consolidation phase with the goal of maintaining therapeutically effective serum enzyme activity for 30 consecutive weeks. Additionally, the induction phase on DFCI ALL Consortium protocols is similar but not identical to COG high-risk ALL protocols, and so both the pharmacokinetics and toxicity of Calaspargase Pegol may be different during this phase as well. Thus, the toxicity and PK data derived from COG Protocol AALL07P4 may not fully inform how best to dose Calaspargase Pegol within the context of a DFCI ALL Consortium treatment regimen. The main objective of Protocol 11-001 is to determine the safety and feasibility of administering Calaspargase Pegol within the DFCI ALL Consortium regimen in anticipation of this formulation soon becoming the only available commercial PEG-asparaginase preparation.

Based on the initial data from the COG AALL07P4 protocol, we will plan on administering IV Calaspargase Pegol at a dose of 2500 IU/m^2. We will administer this dose once during the Remission Induction phase and then every 3 weeks for 30 weeks during post-induction treatment phases. The 3-week dosing interval has been chosen based on the results from COG AALL07P4 indicating that Calaspargase Pegol has a longer half-life than Oncaspar, with nearly all patients demonstrating levels $\geq$ 0.1 IU/mL 3-4 weeks after a single dose. Given that chemotherapy during post-induction therapy on DFCI ALL Consortium protocols is given in 3-week cycles, administration of PEG-asparaginase every 3-week is likely to be more convenient (requiring fewer clinic visits) for patients and families than every 2-week dosing (the Oncaspar dosing schedule on Protocol 05-001).

We will conduct this trial in a randomized fashion. Patients will be randomized to receive either Calaspargase Pegol (dosed as described above) or IV Oncaspar, dosed as on Protocol 05-001 (a single dose during induction and then every 2-weeks for 30 weeks during post-induction treatment phases). We will prospectively collect information regarding toxicity, and will assess enzyme activity weekly during the induction phase and prior to every post-induction asparaginase dose. Serum samples will also be collected to screen for anti-asparaginase antibodies. We will also closely monitor the frequency of high end-induction MRD (defined as $\geq$ 0.001 by PCR assay). The randomized design will allow us to directly compare serum asparaginase activity levels between the two preparations, and also ensure that asparaginase-related toxicities are not significantly different on either arm compared with those observed with IV Oncaspar on Protocol 05-001.

The administration of IV Calaspargase Pegol at 2500 IU/ m^2 as a single dose during induction and every 3 weeks for 30 weeks will be considered feasible if we determine that, when compared to every 2–week dosing of IV Oncaspar (from data collected both on this study and historic data from Protocol 05-001), it is no more toxic, is associated with NSAA $\geq$ 0.1 IU/mL in a similarly high proportion of patients during post-induction treatment phases, and is not associated with a higher frequency of high end-induction MRD in patients with ALL.

We will also monitor NSAA in real-time to identify any patients with extremely low asparaginase enzyme activity levels (<0.025 IU/mL). Results from DFCI ALL Consortium Protocol 00-01 indicate that sequential measurements of NSAA during the 30-week consolidation phase are feasible and identify patients maintaining inadequate serum enzyme activity levels. Moreover, individualized dose adjustment of native E.coli asparaginase on that trial (which included switching asparaginase preparations for patients with extremely low NSAA for presumed "silent" inactivation) was associated with superior EFS compared with standard dosing based on body surface area. In this pilot trial, patients on either arm (Oncaspar or Calaspargase Pegol) found to have non-detectable NSAA (<0.025 IU/mL) on two consecutive measurements will be switched to twice-weekly Erwinia asparaginase.

2.4 **Secondary Objectives: Rationale**

2.4.1 Antibiotic Prophylaxis during Remission Induction

Despite improvement in supportive care over the last several decades, infectious complications are a major cause of treatment-related morbidity and mortality, especially during the initial, more intensive treatment phases. On Protocol 05-001, approximately 25% of patients had at least one documented episode of infection during the induction phase, and 2% of patients died during this phase due to treatment complications (primarily infectious). The majority of these infectious episodes are bacteremias, with invasive fungal disease representing only a small fraction of cases. Episodes of bacteremia during remission induction frequently lead to prolonged hospitalization, as well as delays and/or dose reductions in chemotherapy, raising the question of whether antibacterial prophylaxis might be beneficial during this treatment phase.

The role of antibiotic prophylaxis in neutropenic cancer patients has been controversial for many years.(19) Meta-analyses of clinical trials have suggested that prophylaxis with fluoroquinolones or sulfamethoxazole may reduce gram negative bacteremias in neutropenic adult cancer

patients.(20, 21) However, these results have not been considered definitive because most of the analyzed trials included a limited number of patients, many of whom were of different risk categories, and only a few trials were randomized, placebo-controlled. In addition the recent emergence of fluoroquinolone-resistant bacterial strains is of increasing concern.(22, 23)

In 2005, the results of two large double-blind, placebo-controlled trials using levofloxacin as antibiotic prophylaxis in adult cancer patients with neutropenia were published.(24, 25) In one study, 760 adult cancer patients in whom chemotherapy-induced neutropenia was expected to occur for more than seven days were randomized to receive either oral levofloxacin or placebo from the start of chemotherapy until the resolution of neutropenia.(24) Patients randomized to prophylactic levofloxacin had fewer episodes of fever and a significantly lower rate of microbiologically documented infections. The effects of prophylaxis were also similar in patients with acute leukemia (who had more sustained and profound neutropenia) and those with solid tumors or lymphoma. In the second study, 1565 adult patients with lymphoma or solid tumors were randomized to receive oral levofloxacin or placebo during periods of chemotherapy-induced neutropenia, and, as in the first study, the incidence of fever and clinically documented infections was significantly lower in the group of patients receiving levofloxacin prophylaxis.(25) In 2008, based on these studies, the National Comprehensive Cancer Network (NCCN) recommended the use of fluoroquinolone prophylaxis in high risk cancer patients, including leukemia patients receiving induction chemotherapy.(26)

The role of antibiotic prophylaxis has not been as extensively studied in pediatric cancer patients. Studies evaluating the efficacy of trimethoprim-sulfamethoxazole (SMX-TMP) in preventing bacterial infections in ALL patients have had conflicting results,(27-30) although most of the published series suggest that SMX-TMP prophylaxis reduces the frequency of bacteremias. However, in randomized studies, the use of SMX-TMP has also been associated with a longer duration of neutropenia,(27) which potentially could lead to chemotherapy dose reductions or delays. In a non-randomized, retrospective study of 78 pediatric AML patients treated at St. Jude's Children's Hospital, antibiotic prophylaxis with either cefepime or vancomycin with ciprofloxacin (or a cephalosporin) were associated with lower rates of bacteremia (including lower rates of Strep viridans) compared with the use of an oral cephalosporin or no prophylaxis.(31) No change in the rate or type of fungal infections was observed based on the type of antibiotic prophylaxis.

Based on the high rate of bacteremia in patients during the induction phase of DFCI ALL Consortium studies, we will study the use of antibiotic prophylaxis during this phase during Protocol 11-001. All patients will receive a fluoroquinolone (either levofloxacin or moxifloxacin), or an alternative agent if unable to receive a fluoroquinolone, during the induction phase as a prophylaxis; antibiotic coverage will be extended for those who develop fever or documented infection. Rates of bacteremia will be compared to historic controls (25% incidence of bacteremia during the induction phase on Protocols 00-01 and 05-001). Fluoroquinolones offer several advantages over other antibiotics used as prophylaxis: broad antimicrobial spectrum, systemic bactericidal activity, good tolerability and lack of systemic myelosuppression.(32) The choice of a fluoroquinolone in our study is in accord with NCCN guidelines for leukemia patients during induction therapy.

2.4.2 Intensifying Therapy for B-precursor Patients with High End-Induction MRD

Multiple studies have demonstrated that end-induction MRD is an important, independent predictor of outcome in children with ALL. Patients with higher levels of end-induction MRD have a poorer prognosis than those with lower or undetectable levels. MRD at end-induction is used by almost all childhood ALL consortia as a factor to determine the intensity of post-induction treatment, with patients found to have higher levels allocated to more intensive therapies regardless of other presenting features.

We have assessed minimal residual disease levels (MRD) in all patients since 1996 by a polymerase chain reaction (PCR) technique targeting lymphoblast-specific immunoglobulin gene rearrangements. Using this technique, we demonstrated that MRD levels at the end of the remission induction phase significantly predicted subsequent risk of relapse for B-precursor patients treated between 1996-2000.(33) On Protocol 05-001, end-induction MRD was used to risk-stratify B-precursor patients, with those found to have levels $\geq$0.001 considered to be very high risk. We will continue to use this value as a VHR criterion on Protocol 11-001. Therefore, obtaining MRD levels at end of induction (Day 32) will be mandatory for all patients enrolled on this protocol. On Protocol 05-001, approximately 10% of enrolled subjects were classified as VHR and were treated with a more intensified post-induction regimen, consisting of two additional Consolidation I cycles and a high-risk Consolidation II backbone. Additionally, all VHR patients received cranial radiation. Treatment of VHR patients will be identical on Protocol 11-001. In this way, we will able to expand the cohort of patients treated with the VHR regimen first tested on Protocol 05-001 to explore how this intensified regimen impacts patient outcome.

MRD levels at later time points (e.g., day 78 after initiation of therapy) also have been shown to predict long-term outcome.(34-36) The BFM group uses MRD levels assessed at both end-induction and week 12 of treatment to risk-stratify patients.(36) They have demonstrated that, for patients with high MRD levels at week 4 (end-induction), those with persistently high MRD at week 12 have significantly worse outcomes than those whose MRD levels are low at this later timepoint.(36) Based on this finding, we will assess marrow MRD at the start of the CNS phase (week 13 of treatment, after completion of Consolidation IB and IC) in all patients with high end-induction MRD on Protocol 11-001. In addition, we will assess peripheral blood MRD prior to the start of Consolidation IB and IC. These results will be used to determine whether MRD at this timepoint has prognostic significance within the context of our VHR regimen, but will not be used to determine therapy for patients treated on Protocol 11-001.

Other groups utilize MRD values obtained from earlier timepoints (during remission induction) to determine final risk group. In addition to end-induction MRD, the Children's Oncology Group (COG) considers Day 8 MRD (as assessed in the peripheral blood) and the St. Jude Children's Research Hospital utilizes marrow MRD assessments from Day 19 when risk-stratifying patients.(34, 35) On Protocol 05-001, we collected MRD specimens at Day 18 of induction therapy from patients who consented to an additional, optional marrow specimen at that timepoint and will continue to do so on Protocol 11-001.

For patients with T-ALL, MRD assessed at later timepoint may be prognostically significant. In the AIEOP-BFM ALL 2000 trial, MRD status at day 78 (week 12) was the most important

predictor for relapse in patients with T-cell ALL.(37) A high MRD level at day 78 was associated with a significantly higher risk of relapse. Importantly, patients with detectable MRD at end-induction who had negative MRD by day 78 did just as well as patients who achieved MRD-negativity at the earlier end-induction time point. On DFCI ALL Consortium protocols, MRD has not been assessed at timepoints beyond the induction phase. In order to determine if MRD assessed at a later timpoint may have prognostic relevance for T-ALL within the context of our treatment regimen, we will be collecting MRD specimens at the start of the CNS phase (week 7 of therapy) in T-ALL patients who consent to an additional, optional marrow specimen at that timepoint.

Using the PCR-based assay, approximately 15% of results are "indeterminate", primarily due to failure to identify a clone. An alternative deep sequencing approach for MRD detection has been developed and validated.(67) Like our PCR-based assay, this methodology focuses on clonal rearrangements of IgH and TCR genes to identify residual disease. The deep sequencing methodology utilizes high-throughput sequencing to identify clonal rearrangements of these genes in diagnostic samples and to quantify MRD in follow-up samples. In a study conducted by St. Jude Children's Research Hospital (SJCRH), the deep sequencing methodology was compared with multiparameter flow cytometry and allele-specific oligonucleotide polymerase chain reaction (ASO-PCR) assays using diagnostic and follow-up samples from 106 patients with ALL. Deep sequencing detected MRD in all 28 samples shown to be positive by flow cytometry and in 35 of the 36 shown to be positive by ASO-PCR, and detected MRD in 10 and 3 additional samples that were negative by flow cytometry and ASO-PCR, respectively.(67)

In order to determine whether the deep sequencing methodology could be used for MRD assessment in those patients without an evaluable result using our standard ASO-PCR assay, we sent banked samples from patients treated on prior DFCI protocols to Sequenta, the biotechnology firm that performed the deep sequencing testing on samples from the SJCRH study. Initially we sent 7 samples, a mix of patients who did and did not relapse, and did and did not have high MRD in their end-of-induction samples. Results from DFCI ASO-PCR assay and Sequenta were concordant for all 7 samples. We subsequently sent 73 additional samples to Sequenta for MRD assessment. All samples were from patients with B-precursor phenotype. 27 of the patient samples had MRD results from the ASO-PCR assay, including 18 patients with low end- induction MRD, of whom 8 subsequently relapsed; 9 patients with high end-of-induction MRD, of whom 2 subsequently relapsed. Samples were also sent from 46 patients for whom ASO calibration could not provide an end- induction MRD assessment. Sequenta was able to identify leukemic clones and quantiate MRD in all 27 of the samples for which ASO-PCR assay had been successful, and for 44 of the 46 samples for which ASO-PCR assay had been unsuccessful. Among the latter, 3 patients had MRD levels at or above 0.1% of cells examined, the threshold used by the Consortium for ASO PCR. This is approximately 10% of the samples with "indeterminate" PCR-MRD assay results, which is the expected proportion of patients with high end-induction MRD.

Given these data, beginning in 2014, we will send diagnostic and Day 32 samples for all patients with ALL enrolled on Protocol 11-001 to Sequenta, whose deep sequencing assay is CLIA-approved. This will allow us to evaluate the feasibility of obtaining MRD results using deep sequencing methodology in real time and to more fully evaluate concordance between PCR and

deep sequencing results. For patients enrolled on Protocol 11-001, Day 32 MRD will still be primarily assessed based on PCR results; however, for cases in which MRD is indeterminate by PCR assay, deep sequencing results will be utilized for risk stratification purposes. No extra volume of sample will be obtained from patients; rather, the DFCI MRD lab will split the sample sent to them for MRD assessment, and forward some to Sequenta, so that MRD may be assessed in both labs simultaneously from the same sample.

2.4.3 Vitamin D Screening and Supplementation

Skeletal toxicities have been well-characterized as serious complications of therapy for childhood ALL.(38, 39) Children undergoing treatment for ALL are at increased risk for fracture during and immediately following the completion of therapy. On DFCI-ALL Consortium Protocol 00-01, which included a randomized comparison of dexamethasone and prednisone during post-induction treatment phases, dexamethasone was associated with superior event-free survival, but also more skeletal toxicities in older children/adolescents.(17) On that study, 14% of patients experienced a fracture, and older children and adolescents receiving dexamethasone appeared to be at increased risk for this toxicity. On Protocol 11-001, dexamethasone will again be utilized during post-induction therapy, given its superior anti-leukemia effect. Identifying interventions which will minimize skeletal complications would be of great benefit in reducing the treatment-related morbidity experienced by patients.

The potential role for supplementation with vitamin D and calcium in the context of ALL therapy has yet to be defined. Vitamin D deficiency has been recognized as an important concern with regard to bone health, even for healthy children, emphasized by the recent Institute of Medicine report and the American Academy of Pediatrics recommendation for increased minimal daily vitamin D intake.(40-42) Children undergoing leukemia treatment may be at increased risk for vitamin D deficiency.(43) The feasibility of assessing vitamin D status and of correcting vitamin D deficiency has not been previously evaluated in the context of multi-institutional clinical trial. In addition, the impact of vitamin D status with regard to acute and long-term skeletal morbidities has not been thoroughly explored in this population. Preliminary results from Protocol 05-001 investigating dietary micronutrient intake and treatment-related toxicity demonstrated that males undergoing treatment for ALL with low intakes of calcium are at increased risk for developing fractures, independent of age and leukemia risk group.(44) In addition, in a report of patients with ALL treated with DFCI ALL Consortium therapy, low calcium intake was a predictor of low bone mineral density (as assessed by dual energy X-ray absorptiometry) in children with ALL.(45)

Dietary interventions aimed at improving intake of calcium and vitamin D during treatment for ALL require further study. We plan on assessing the feasibility of screening for vitamin D deficiency in patients at several time points in order to determine the prevalence of this deficiency in ALL patients and to explore the relationship of vitamin D status and skeletal toxicity (fracture and osteonecrosis). We will also explore the feasibility of vitamin D and calcium supplementation in patients found to have vitamin D deficiency within the context of our multi-institutional clinical trial.

2.4.4 Prospective Screening of High-Risk Subsets of Patients

We will collect samples from patients at diagnosis to determine the feasibility of prospective screening for recently identified genetic predictors of outcome. Many of these factors have not yet been validated prospectively, and their true frequency is not known. Thus, one goal of prospective screening is to determine the frequency of these abnormalities in a population of newly diagnosed children and adolescents with ALL, and to explore their impact on end-induction MRD levels and rates of relapse. In addition, by demonstrating that it is possible to prospectively screen for these factors with a rapid turnaround of results, we will potentially be able to incorporate some or all of these factors into the risk classification scheme in the next DFCI ALL Consortium protocol, and, with a larger planned accrual on that trial, could test the impact of more intensive and/or novel therapies in patient populations with these abnormalities. The factors we plan to screen for include: absence of biallelic TCRγ deletions (ABGD) in patients with T-ALL, and IKZF1 deletions/mutations, CRLF2 over expression and JAK1/2 mutations in patients with B-ALL.

ABGD in T-ALL: There are few commonly accepted prognostic factors for patients with T-cell ALL. Using banked samples from 47 T-ALL patients treated either on DFCI Protocol 00-01 or COG Protocol 9404 (which was based on the high-risk DFCI Consortium treatment regimen), we were able to demonstrate that ABGD, a characteristic of early thymocyte precursors, was strongly associated with early treatment failure in patients with T-cell ALL.(46) This finding was confirmed in a validation cohort consisting of samples from T-ALL patients treated on St. Jude protocols. This initial study had a case-control design, with nearly half the samples coming from patients who either failed to achieve remission or experienced an early relapse. Additionally, the diagnosis of ABGD was initially made using array comparative genomic hybridization (CGH). A quantitative DNA-PCR assay for ABGD has been developed which may provide a reliable, more rapid screening of this abnormality.(46) On Protocol 11-001, we will screen all T-ALL patients for ABGD using this qPCR assay to assess the frequency of this abnormality in newly diagnosed patients. We will also study the correlation between ABGD and early T-cell phenotype (ETP), a recently characterized T-cell subset identified by gene expression profiling, flow cytometry and single nucleotide polymorphism array analyses.(47) This subset, characterized by a distinctive immunophenotype (CD1a and CD8 negativity, with weak expression of stem cell or myeloid markers and weak expression of CD5), has, like ABGD, been correlated with a poorer prognosis in retrospective analysis.(47)

IKZF1, CRLF2 and JAK mutations in B-ALL: Alterations (primarily deletions) of *IKZF1*, a gene that encodes the lymphoid transcription factor IKAROS, have recently been identified as a potentially significant predictor of outcome in B-ALL.(48, 49) In one series of 131 B-precursor ALL patients (including both standard and high-risk patients) with banked diagnostic samples, *IKZF1* deletions were identified in 14% of patients.(49) In another study, which included only high-risk Philadelphia chromosome-negative B-precursor ALL patients, 30% of 221 patients with assessable diagnostic samples were found to have *IKZF1* alterations.(48) In both of these reports, *IKZF1* alterations/deletions were associated with a high risk of relapse.

Elevated expression of CRLF2 (cytokine receptor-like factor 2) has been indentified in some patients with ALL, including subsets of high-risk patients and those with Down Syndrome.(50,

51) In one report, ~14% of high-risk, Philadelphia chromosome-negative B-ALL with assessable diagnostic samples had CRLF2 overexpression.(50) Nearly all of these cases were found to have either a translocation of the immunoglobulin heavy chain gene IGH to CRLF2 (in the pseudoautosomal region 1 of Xp22.3/Yp11.3) or to an interstitial deletion resulting in a P2RY8-CRLF2 fusion. CRLF2 rearrangements were significantly associated with IKZF1 abnormalities (~80% of CRLF2-altered cases also had an IKZF1 deletion or mutation), as well as with activating mutations of JAK1 or JAK2. Within this cohort, CRLF2 rearrangements were associated with a poor relapse-free survival, although on multivariate analysis, abnormality of IKZF1, and not CRLF2, was an independent predictor of outcome.(50)

Reports of the frequency and prognostic significance of IKZF1 and CRLF2 alterations have all been based on analyses of stored samples, and have not yet been evaluated prospectively. Because these factors may be used in the future to risk-stratify patients and potentially test novel targeted therapies (such as JAK inhibitors), we plan to prospectively screen all B-ALL patients for alterations of IKZF1 and CRLF2. CRLF2 overexpression will be screened using flow cytometry and CRLF2 rearrangements using FISH. All cases found to have CRLF2 abnormalities will be screened for JAK mutations (given that a previous report suggests that nearly all cases with JAK mutations are found in those with CRLF2-overexpression).(50) IKZF1 abnormalities were initially identified using single-nucleotide-polymorphism microarrays, transcriptional profiling, and resequencing,(48) but multiplex ligation dependent probe amplification (MLPA) has been shown to be a more rapid technique for accurate, high-throughput screening for IKZF1 deletions,(49) and so we will use this methodology.

2.4.5 BH3-profiling to identify patients at high risk of treatment failure

Nearly all chemotherapy agents can induce the hallmarks of apoptosis by the intrinsic apoptotic pathway. Therefore, cancer cells likely require a block in apoptosis in order to survive. For instance, overexpression of the antiapoptotic protein BCL-2 provides a block in apoptosis that is frequently observed in cancer cells, including leukemia.(52) The measurement of protein levels of all members of the BCL-2 family of apoptosis regulators is too complex, cumbersome, and difficult to be used as a clinical measure. The functional assay of BH3 profiling is an alternative methodology which has been used to identify the extent to which the blasts are 'primed' for apoptosis and also the specific block in the apoptotic pathway a cancer cell uses to resist the effects of chemotherapeutic agents.(53) In specimens taken from lymphoma patients, BH3-profiling was able to identify patient subsets with distinctive patterns of apoptotic defects which predicted response to conventional chemotherapy agents.(54) Furthermore, BH3 profiling permits evaluation of whether mitochondrial or extra-mitochondrial contributors dominate in causing chemotherapy resistance, which can facilitate identification of novel therapeutic targets.

As an initial test of the hypothesis that mitochondrial preconditions may be important determinants of chemotherapy response in ALL, BH3 profiling was performed on diagnostic samples taken from patients treated on Protocol 05-001. While only two samples from patients who subsequently relapsed were tested, their BH3 profiling indicated that they were significantly less 'primed' to undergo apoptotic cell death than any of the seven tested low-MRD patients who had not relapsed. Additionally, BH3 profiling of diagnostic samples from 10 adult patients with ALL showed that those patients who subsequently relapsed (N=7) were significantly less 'primed' to undergo apoptotic cell death than the patients who did not relapse (N=3). These

preliminary data support a more complete testing of the hypothesis that BH3-profiling of diagnostic specimens may predict subsequent response to chemotherapy. We will collect diagnostic specimens from patients enrolled on Protocol 11-001 to explore the association between BH3-profiling and subsequent treatment response, including peripheral blast response to the 3-day steroid prophase, end-induction marrow MRD level and occurrence of relapse. For those patients who experience a relapse, another sample will be obtained at relapse to compare the BH3-profile at diagnosis and relapse. The BH3-profile of pediatric specimens will also be compared to those taken from adults with ALL enrolled on DFCI ALL Consortium clinical trials for patients aged 18-50 years with newly diagnosed ALL.

2.4.6 Identification of new therapeutic targets

In order to facilitate the identification of new therapeutic targets for ALL, diagnostic specimens from patients who are subsequently found to have high risk features (including high end-induction MRD or T-cell phenotype with ABGD) and/or subsequently relapse will be used to develop primary xenografts in immunodeficient mice. Primary xenograft (primagraft) tumor models are established by direct inoculation of patient samples into immunodeficient mice. The rationale for this approach has been to remove the intermediary propagation of cells under artificial *ex vivo* conditions, which may alter the biology of the lymphoblast. Methods for engrafting primary ALL cells in immunodeficient mice (primagrafts) have been well established, and primagrafts have been successfully used for drug testing. We will plan on assessing novel therapies, including histone modification inhibitors and apoptosis-modulating therapies, in primagraft models derived from VHR and relapsed patients. Primagraft models derived from the lymphoblasts of standard risk patients will be developed to assess whether new therapies are specific for a given VHR subset or have more generalized anti-ALL activity. Additionally, whole genome sequencing in paired diagnostic/relapse specimens will be performed to screen for potential therapeutic targets.

2.4.7 Spinal Fluid Research Samples

We also plan to collect spinal fluid (CSF) specimens from patients who have consented to the use of leftover samples for research. Leftover CSF samples will be collected at the time the following therapeutic lumbar punctures are performed: Day 18, end-induction (Day 32), the fourth lumbar puncture during the CNS phase and the first lumbar puncture performed during the Consolidation II phase. These CSF samples will be collected to support studies seeking to define a biochemical profile predictive of increased risk for cognitive toxicity. Such a profile may help guide future therapeutic approaches to minimize neurocognitive late effects. Factors will be measured in CSF: total folate, homocysteine, homocysteic acid, homocysteine sulfinic acid, S-adenosyl-homocysteine, S-adenosyl-methionine, cysteine sulfinic acid, tau protein, and myelin basic protein.

2.5 **Treatment Regimen: Background**

The therapeutic backbone of this trial will be based upon previous DFCI ALL Consortium protocols for patients aged 1-18 years, specifically Protocol 05-001, which opened for enrollment in 2005. Outcome results for Protocols 91-01 (1991-5), 95-01 (1996-2000) and 00-01 (2000-4) are presented in Table 2.4:

Table 2.4: Outcome of patients enrolled on DFCI ALL Consortium protocols 91-01, 95-01 and 00-01 (1991-2004)

Protocol	91-01	95-01	00-01
N	377	491	492
Median f/u years	12.5	8.6	4.9
Remission (%)	370 (98)	480 (98)	473 (96)
Induction Failure (%)	5 (1.3)	7 (1.4)	16 (3.2)
Induction Death (%)	2 (0.5)	4 (0.8)	3 (0.6)
Remission Death (%)	12 (3.2)	3 (0.6)	5 (1.0)
Relapse (%)	53 (14)	79 (16)	66 (13)
EFS ± SE (%)*	81 ± 2	79 ± 2	80 ± 2
OS ± SE (%)*	86 ± 2	89 ± 2	91 ± 1

*10-year estimates for 91-01 and 95-01; 5-year estimates for 00-01.

2.5.1 *Risk Group Classification*:

Risk group classification will be similar to that on Protocol 05-001. All patients will initially be classified as standard risk or high risk based upon their presenting features, including age, white blood cell (WBC) count, immunophenotype, and presence or absence of CNS leukemia. By week 7 of treatment, a final risk group assignment will be made based upon the results of cytogenetics, FISH studies and end-induction minimal residual disease levels. As on Protocol 05-001, three groups of patients will be re-classified as very high risk: those with MLL gene translocation and/or hypodiploidy (<44 chromosomes) and B-precursor patients with high MRD levels at the end of remission induction (≥0.001 as assessed by polymerase chain reaction (PCR) targeting patient-specific immunoglobulin heavy chain (IgH) and/or T-cell receptor (TCR) gene rearrangements). We have previously published that B-precursor patients with high end-induction MRD whose treatment was not modified based on this result had a 5-year risk of relapse of 72%, significantly higher than patients with low MRD levels at this timepoint.(55) End-induction MRD has not been established as an independent prognostic factor for patients with T-cell ALL treated on DFCI ALL Consortium protocols, and so will not be used to risk-stratify patients on this trial.

An additional fourth group of patients will also be re-classified as very high risk: T-cell patients (either T-ALL or T-lymphoblastic lymphoma) with early T-cell precursor phenotype (ETP). This subset, identified in 10-15% of T- ALL cases, is characterized by a distinctive immunophenotype (CD1a and CD8 negativity, with weak expression of CD5 and co-expression of stem cell or myeloid markers.(47) Detailed molecular characterization of ETP ALL showed similarities to that of normal hematopoietic stem cells and myeloid leukemia stem cells.(56) The St. Jude Children's Hospital has reported that patients with ETP phenotype have a very high risk of early treatment failure, a result that was validated in an analysis of ETP patients treated by the Italian AIEOP clinical trials group.(47) These data would suggest that, with standard ALL therapy, patients with ETP phenotype may have EFS rates of 50% or lower.(47) Thus, on Protocol 11-001, we plan to treat all cases of ETP on the VHR arm of the protocol. Flow cytometry from all

patients with T-cell phenotype (T-ALL or T-lymphoblastic lymphoma) will be centrally reviewed at Boston Children's Hospital/ DFCI so that we may prospectively identify cases at the time of diagnosis in a uniform manner.

Since 2005, patients on the very high risk arm have been treated with an intensified regimen that includes two additional Consolidation I cycles (with agents including cyclophosphamide, cytarabine and etoposide) and a Consolidation II phase that is identical to that administered to high-risk patients. As of July 2011, 6 relapses and 1 case of secondary AML had been observed in 59 VHR patients for whom survival data was available. There had been no remission deaths. While these results are extremely premature, they are promising enough that we will continue to treat VHR patients using this intensified regimen. In this way, we will be able to expand the cohort of VHR patients treated in a nearly uniform way to better assess whether the treatment strategy had any beneficial impact in terms of relapse risk.

Philadelphia-chromosome-positive (Ph+) patients: On Protocol 05-001, children with Ph+ ALL were treated with imatinib mesylate, a selective inhibitor of the BCR-ABL protein kinase, commencing on Day 18 of the induction phase. All Ph+ patients with a suitable related or unrelated donor were taken to allogeneic stem cell transplant (SCT) within weeks of achieving complete remission (CR). This treatment strategy was based upon data generated in an era prior to the availability of imatinib and other similar tyrosine kinase inhibitors (TKIs) which demonstrated that allogeneic SCT in first CR was associated with superior outcome compared with chemotherapy. A study conducted by the Children's Oncology Group between 2002-2006 (COG AALL0031) evaluated whether imatinib mesylate could be incorporated into an intensive chemotherapy regimen for children with Ph+ ALL. Patients received imatinib mesylate in conjunction with chemotherapy during postinduction therapy. Some children proceeded to allogeneic stem cell transplant after two cycles of consolidation chemotherapy with imatinib mesylate, while other patients received imatinib mesylate in combination with chemotherapy throughout all treatment phases. The 3-year EFS for the 25 patients who received intensive chemotherapy with continuous dosing of imatinib was 87.7 + 10.9%, which was superior to historic controls treated with chemotherapy alone (without imatinib), and at least as favorable as the other patients on the AALL0031 trial who underwent allogeneic transplantation.(57) The follow-up COG study (AALL0622) is evaluating another TKI, dasatinib, given in conjunction with the same intensive chemotherapy backbone as the AALL0031 study. In order to allow possible enrollment on this COG study, all Ph+ patients will be removed from Protocol 11-001 by Day 15 of induction treatment.

2.5.2 ***Phases of Treatment***

2.5.2.1 **Steroid Prophase**

As on Protocol 05-001, therapy for all patients will commence with 3 days of methylprednisolone monotherapy. We had previously demonstrated on Protocol 91-01 (1991-5) that there was variability amongst patients in peripheral blood response to a 3-day prednisone prophase, with 57% of patients experiencing >85% decrease in absolute peripheral blast counts and the remaining patients a more modest decrease.(58) On Protocol 05-001, patients were treated with a 3-day prophase with the goal of correlating gene expression profiles with peripheral blood response to steroid, as assessing gene expression changes in leukemic blasts induced by corticosteroids. The 3-day prophase on this trial will allow us to continue these

research studies, as well as explore the correlation of BH3-profile at diagnosis with peripheral blood response to corticosteroids.

2.5.2.2 **Induction Therapy**

Beginning on Day 4 (after completing the steroid prophase), all patients will receive multi-agent remission induction with vincristine, prednisone, doxorubicin, methotrexate and a single dose of PEG-asparaginase. This regimen is identical to that used in our previous Protocol 05-001, with the exception of the asparaginase preparation. As noted above, patients will be randomized to receive either Calaspargase PEG (SC-PEG) or Oncaspar on Day 7 of induction. Because of concerns regarding adrenal suppression after 1 month of steroids, we will institute a short steroid taper at the end of the induction phase, in accord with the long-standing practice of the BFM and other Childhood ALL clinical trial groups.

2.5.2.3 **Consolidation I**

As on Protocol 05-001, this phase will commence at the end of the remission induction phase, after documentation of complete remission. For standard risk and high risk patients, this phase will involve one 3-week cycle consisting of one dose of high-dose methotrexate (5 gram/m^2 over 24 hours), vincristine (1 dose) and 6-mercaptopurine (14 days); high risk patients will also receive doxorubicin (with dexrazoxane) during this cycle. Early consolidation with high-dose methotrexate is a feature of many regimens for childhood ALL, and the results of randomized trials suggests that its addition may reduce both systemic and CNS relapses.(59),(60),(61)

Very high-risk patients will continue to receive an intensified Consolidation I phase, as they did on Protocol 05-001. After the first 3 weeks of Consolidation I, very high risk patients will receive 2 additional 3-week cycles consisting of i) cyclophosphamide, low-dose cytarabine and 6MP (Consolidation IB), and ii) high-dose cytarabine, etoposide, dexamethasone and asparaginase (Consolidation IC).

2.5.2.4 **CNS Treatment**

A major focus of our studies has been to improve the therapeutic index in the treatment of the CNS. Because of concerns regarding neurocognitive sequelae in younger patients, as well as the risk of radiation induced second malignancies, we have attempted, in successive protocols, to reduce exposure to radiation by lowering the dose (to 1200 cGy) or substituting it with intensive intrathecal chemotherapy.

Since 2000, all standard-risk patients have been treated without cranial radiation, instead receiving more frequent administration of intrathecal therapy during the first year of treatment, and will continue to be treated in this way on Protocol 11-001. On Protocol 05-001, we substituted intensive intrathecal chemotherapy for cranial radiation for a subset of high risk patients: B-precursor patients with presenting leukocyte counts less than 100,000/mm^3, and no CNS leukemia (that is, not CNS-3 and absence of cranial nerve palsy) at diagnosis. All other high risk patients and all very high risk patients received 1200 cGy cranial radiation (1800 cGy for CNS-3 patients). Thus, 71% of patients on Protocol 05-001 were treated without cranial radiation, compared with 24% on Protocol 91-01 (1991-5), 45% on Protocol 95-01 (1996-2000) and 57% on Protocol 00-01 (2000-2004).

On two recent studies, one conducted by the St. Jude Children's Research Hospital (SJCRH) and the other by the Dutch Childhood Oncology Group (DCOG), all patients were treated without cranial radiation (although, on the SJCRH trial, 7% of patients received an allogeneic stem cell transplant in first remission for very high-risk features, including high MRD). Each of these studies included four doses of high-dose methotrexate administered every 2 weeks during post-induction consolidation, as well as an increased frequency of IT chemotherapy and frequent vincristine/dexamethasone pulses during the first 1 to 2 years of therapy. The overall EFS for these studies was 85.6% (SJCRH) and 81% (DCOG) and the 5-year cumulative incidence of isolated CNS relapse on each trial was between 2% and 3%.(62, 63) However, some patient subsets had significantly higher rates of CNS relapse. On the SJCRH study, clinical features associated with a significantly higher risk of isolated CNS relapse included T-cell phenotype, the t(1;19) translocation, and the presence of blasts in the CSF at diagnosis.(62)

Other groups have also reported excessive relapses when cranial radiation has been eliminated from patients with T-ALL. Conter and colleagues reported the outcome of two groups of T-ALL patients (all steroid good responders) contemporaneously treated with nearly identical BFM-based systemic chemotherapy, but one group (in Italy) received triple intrathecal chemotherapy as CNS preventative therapy and the other (in Germany) received 12 Gy cranial radiation. The non-irradiated patients had a lower 3-year EFS (67% compared with 88% for irradiated patients), with a higher proportion of patients experiencing relapses involving the CNS (7.3% compared with 1.4%).(64)

The data supporting the administration of cranial radiation in B-precursor patients with high presenting leukocyte counts is based primarily on the results of POG Protocol 9906 for high risk B-precursor ALL. On that study, all non-CNS-3 patients were treated without cranial radiation. The 2-year cumulative incidence of CNS relapse was 12%. Univariate analysis revealed that presenting leukocyte count was the best predictor of subsequent CNS relapse: the 2-year cumulative incidence of CNS relapse was 24% for patients with presenting leukocyte counts > 100,000/mm^3 compared with 5% for those with presenting leukocyte counts < 100,000/mm^3 ($p<0.01$). However, on a study conducted by the Israel National Study Group (INS) on which only 14% of patients received cranial radiation, relapses involving the CNS occurred in only 6 of 189 non-irradiated patients (5-year isolated and combined CNS recurrence rates of 1.7% and 3.1%, respectively). There was only one CNS relapse observed in a non-irradiated, non-infant B-precursor patient with a presenting leukocyte count less than 100,000/mm^3.(65) On the BFM-95 protocol, 12 Gy cranial radiation was administered to all T-ALL patients, and B-precursor patients with either CNS-3 involvement at diagnosis or very high-risk features; high presenting leukocyte count was not an indication for cranial radiation in B-precursor patients. On that study, the 6-year cumulative incidence of isolated and combined CNS relapses were 1.8% and 2.2%, respectively.(66)

Based on these data, we will continue to administer cranial radiation to patients with any of the following features: T-cell phenotype, CNS-3 (or failure to clear CNS-2 status by Day 18 of the induction phase), or very high risk risk group classification. Because it is unclear that B-precursor patients with presenting leukocyte counts greater than 100,000/mm^3 benefit from cranial radiation, these patients will be treated without cranial radiation on Protocol 11-001. Based on the presenting characteristics of patients enrolled on prior protocols, it is expected that

approximately 20% of all patients enrolled on Protocol 11-001 will receive cranial radiation compared with 30% on Protocol 05-001.

2.5.2.5 **Consolidation II Therapy**

Since 1981, post-remission consolidation for all patients has included 20-30 consecutive weeks asparaginase, as well as every three-week cycles of vincristine, 6-MP and corticosteroid. Standard risk patients also have received weekly low-dose methotrexate during this treatment phase, while high risk patients received doxorubicin every three weeks. On Protocol 00-01, we randomized patients to receive either dexamethasone or prednisone during this phase of treatment, and demonstrated that dexamethasone was associated with significantly better EFS.(17) Dexamethasone will continue to be the steroid used during the Consolidation II and Continuation phases of treatment, as it was on Protocol 05-001.

On Protocol 11-001, patients will be randomized to receive either IV Calaspargase Pegol (SC-PEG asparaginase) 2500 IU/m2 every 3 weeks (10 total post-induction doses) or IV Oncaspar 2500 IU/m2 every 2 weeks (15 total post-induction doses). Other changes in the Consolidation II phase of treatment on Protocol 11-001 (compared with Protocol 05-001) include the following:

- For HR and VHR patients, there are a specified number of cycles (10) which should include high-dose dexamethasone. This is to ensure a more uniform exposure to high-dose dexamethasone for all HR/VHR patients, regardless of other treatment delays or side effects. In the past, some patients received high-dose dexamethasone for many more than 10 cycles when asparaginase administration exceeded 30 weeks due to hypertriglyceridemia or thrombotic complications.

- For SR patients (and HR/VHR patients after completion of doxorubicin), methotrexate will be administered on the same day as asparaginase, rather than 1 day after asparaginase.

2.5.2.6 **Continuation Therapy**

The continuation phase on Protocol 11-001 will be identical to that on Protocol 05-001. Therapy will be discontinued after 24 months of complete continuous remission.

3. PARTICIPANT SELECTION

3.1 Eligibility Criteria

Participants must meet the following criteria to be eligible to participate in the study:

3.1.1 Confirmed diagnosis of acute lymphoblastic leukemia or lymphoblastic lymphoma

3.1.1.1 **For acute lymphoblastic leukemia**: diagnosis should be made by bone marrow aspirate or biopsy demonstrating ≥ 30% involvement by lymphoblasts, with flow cytometry or immunohistochemistry confirming B-precursor or T-ALL phenotype. While myeloid co-expression on blasts determined to be primarily lymphoid is allowed, patients with leukemia of ambiguous lineage are not eligible.

3.1.1.1.1 For patients with circulating blasts in the peripheral blood, flow cytometry confirmation of B-precursor or T-ALL phenotype is sufficient for registration onto the study. Bone marrow aspirate and/or biopsy should be performed as soon as feasible, preferably prior to the initiation of any therapy.

3.1.1.1.2 Note: patients with mature B-cell (Burkitt's) ALL are excluded from study. Mature B-cell is defined by the presence of surface immunoglobulin and/or the t(8;14)(q24;q32), t(8;22), or t(2;8) translocation and/or *c-myc*-gene rearrangement by FISH or PCR.

3.1.1.2 **For lymphoblastic lymphoma**: diagnosis may be made by i) biopsy of involved site (eg, node, mediastinal mass), or ii) by cytology from pleural fluid or other fluid collection or iii) by marrow aspirate/biopsy demonstrating < 30% involvement by lymphoblasts (confirmed by flow cytometry, immunohistochemistry, cytogenetics and/or FISH studies) in a patient with evidence of lymphomatous masses by radiographic studies. Note: marrow aspirate and/or biopsy must be performed in patients with lymphoblastic lymphoma prior to study entry to confirm that patient does not meet definition of ALL. In rare circumstances, lymphoblastic lymphoma patients may be registered prior to marrow aspirate/biospy if it is felt that the procedure cannot be safely performed; permission in advance by principal investigator or designee is required.

3.1.2 Prior Therapy: No prior therapy is allowed except for the following:

- Corticosteroids: Short courses of corticosteroid (defined as ≤ 7 days of corticosteroids within the 4-weeks preceding registration) are allowed prior to registration. *Note*: If patient has received pre-treatment with corticosteroids, they should not receive steroid prophase, but should be treated as specified in Section 6.7.
    - Participants who have been on corticosteroids chronically (defined as more than 7 days of corticosteroids within the 4-weeks preceding registration or more than 28 days of corticosteroids over the preceding 6 months) are not eligible.
- IT Cytarabine: A single dose of intrathecal cytarabine (at the time of the diagnostic lumbar puncture) is allowed prior to registration. If patient has received IT cytarabine prior to registration, Day 1 IT cytarabine (Section 6.1) should not be administered.
- Emergent Radiation Therapy: Emergent radiation to the mediastinum or other life-threatening masses is allowed prior to registration.

3.1.3 Age: 365 days to < 22 years.

3.1.4 Laboratory Criteria: Participants must have direct bilirubin < 1.4 mg/dL (23.9 micromoles/L) Note: A total bilirubin of <1.4 mg/dL (23.9 micromoles/L) is acceptable to meet this requirement

3.1.5 Ability of parent or guardian to understand and the willingness to sign a written informed consent document.

3.2 Exclusion Criteria

Participants who exhibit any of the following conditions at screening will not be eligible for the study.

3.2.1 Participants who have received more than 7 days of corticosteroids in the preceding 4 weeks or more than 28 days of corticosteroids in the preceding 6 months are not eligible.

3.2.2 Participants who have received any chemotherapy or radiotherapy for previous malignancy are not eligible. Participants who have ever previously received any anti-neoplastic agent, including methotrexate, 6-mercaptopurine, 6-thioguanine, vincristine cyclophosphamide, cytarabine (except for IT cytarabine), or any anthracycline, for any reason (eg, rheumatologic or autoimmune condition) are not eligible.

3.2.3 Participants may not be receiving any other investigational agents.

3.2.4 Participants known to be HIV-positive are excluded (Note: HIV testing is not required prior to enrollment). HIV-positive individuals on combination antiretroviral therapy are ineligible because of the potential for

pharmacokinetic interactions with Calaspargase Pegol (SC-PEG asparaginase). In addition, these individuals are at increased risk of lethal infections when treated with myelosuppressive therapy. Appropriate studies will be undertaken in participants receiving combination antiretroviral therapy when indicated.

3.2.5 Uncontrolled intercurrent illness including, but not limited to ongoing infection with vital sign instability (hypotension, respiratory insufficiency), life-threatening acute tumor lysis syndrome (eg, with renal failure), symptomatic congestive heart failure, cardiac arrhythmia, intracranial or other uncontrolled bleeding, or psychiatric illness/social situations that would limit compliance with study requirements.

3.2.6 Pregnant women are excluded from this study because many of the agents used on this protocol have potential for teratogenic and/or abortifacient effects. Because there is an unknown but potential risk of adverse events in nursing infants secondary to treatment of the mother with these chemotherapy agents, breastfeeding should be discontinued if the mother is enrolled.

3.2.7 Individuals with a history of a previous malignancy are ineligible because cases of secondary leukemia/lymphoma arising after a previous malignancy are biologically distinctive and may require different therapies. Also, exposure to previous anti-neoplastic treatment may alter the ability to tolerate or respond to the agents utilized in this protocol. Exception: Individuals with a previous malignancy treated with surgery only (no chemotherapy or radiotherapy) more than 5 years prior to registration may be enrolled.

3.3 Inclusion of Women, Minorities and Other Underrepresented Populations

Individuals of both genders and all racial and ethnic backgrounds are eligible for this study.

4. REGISTRATION AND RANDOMIZATION PROCEDURES

4.1 General Guidelines

As soon as a potentially eligible patient is identified, anyone who wants to enroll the patient (at DFCI/Children's Hospital Boston or at an outside participating institution) should email [redacted] and/or page the Study Coordinators at [redacted].

Eligible participants will be registered with the DF/HCC Office of Data Quality (ODQ) central registration system. The registration process will be facilitated by the study staff at the Lead Site (DFCI) during weekday business hours (9am-5pm) and by each participating site outside of business hours, weekends, and holidays. **Registration must occur prior to the initiation of protocol therapy**. Any participant not registered to the protocol before treatment begins will be considered ineligible and registration will be denied.

The ODQ Registrar will send a fax confirmation of the registration to the person initiating the registration immediately following the registration. Once registration has been confirmed by ODQ, participants may begin protocol treatment. Issues that would cause treatment delays should be discussed with the Principal Investigator. If a participant does not receive protocol therapy following registration, the participant's protocol status must be changed. Notify the ODQ Registrar of participant status changes as soon as possible.

During ODQ business hours (Monday-Friday, 8:00 AM to 5:00 pm, EST, excluding holidays), randomized treatment will be assigned and communicated at the same time as registration confirmation. If registration occurs outside of ODQ business hours, randomized treatment will be assigned and communicated on the next business day.

4.2 Registration and Randomization Process

The ODQ registration staff is accessible on Monday through Friday, from 8:00 AM to 5:00 PM Eastern Standard Time.

In emergency situations when a participant must begin treatment during off-hours or holidays, call the ODQ registration line at [redacted] and follow the instructions for registering participants after hours. In the event of an off-hours or holiday registration, randomized treatment will be assigned and communicated on the next business day.

The registration procedures during business hours, Monday – Friday, from 8am – 5pm, are as follows:

1. Obtain written informed consent from the participant prior to the performance of any study related procedures or assessments.

2. Complete the protocol-specific eligibility checklist. **To be eligible for registration to the study, the participant must meet each inclusion and exclusion criteria listed on the eligibility checklist.**

3. **Fax or email** the eligibility checklist(s) and all pages of the consent form(s) to the DFCI Study Team at **Email** [redacted]

4. The DFCI Study Team will (a) review the eligibility checklist and consent and (b) register the participant on the study with ODQ.

5. The ODQ Registrar will send an email or fax confirmation of the registration and randomized treatment assignment to the DFCI Study Team immediately following the registration.

6. The DFCI Study Team will email the registration confirmation and randomized treatment assignment to the outsite site.

7. Treatment on study should only begin after confirmation of registration is received.

The registration procedures outside of business hours, on weekends, and holidays are as follows:

1. Obtain written informed consent from the participant prior to the performance of any study related procedures or assessments.

2. Complete the protocol-specific eligibility checklist. **To be eligible for registration to the study, the participant must meet each inclusion and exclusion criteria listed on the eligibility checklist.**

3. Call the ODQ registration line at [redacted] and follow the instructions for registering participants after hours. Leave detailed contact information for the ODQ registrar responsible for registering your participant.

4. The ODQ Registrar will (a) validate eligibility and (b) register the participant on the study.

5. ODQ will confirm registration by telephone, and email/fax confirmation will be sent on the next business day. Randomized treatment will be assigned and communicated on the next business day.

6. Treatment on study should only begin after confirmation of registration is received. On nights, weekends, and holidays, treatment may begin after

telephone confirmation (prior to communication of randomized treatment assignment).

7. **Fax or email** the eligibility checklist(s) and all pages of the consent form(s) to the DFCI Study Team at **Email** [redacted]

If you have any questions, the ODQ **can be reached at telephone** [redacted]

The following source documents need to be forwarded to the Coordinating Center (Dana-Farber/Children's Hospital Cancer Center) as soon as they are available. Final risk group classification will only be made once these documents are received by the Coordinating Center.

- Copy of pathology reports confirming diagnosis of leukemia (bone marrow aspirate or biopsy indicating ≥ 30% marrow involvement) or lymphoblastic lymphoma
- Copy of marrow pathology (for leukemia) and scan (for lymphoblastic lymphoma) reports confirming complete remission at end of remission induction phase.
- Copy of diagnostic flow cytometry reports (leukemia patients only)
- Copy of results of diagnostic cytogenetics, FISH and PCR (if performed)(leukemia patients only)
- CBC with differential from diagnosis and from end of induction

After receipt of source documents and review of MRD results (if applicable), Lead Institution will call or email the participating site to verify final risk group assignment.

5. RISK GROUP CLASSIFICATION

5.1 Risk Group Definitions

5.1.1 Standard Risk: All of the criteria must be met:

- Age: 365 days to < 10 years.
- WBC count: Highest pre-treatment WBC <50,000/mm^3 (prior to registration).
- CNS leukemia: No evidence of CNS leukemia by morphology, defined by meeting all of the following criteria:
    1. Diagnostic lumbar puncture (Day 1) without any CSF blast cells on cytospin (CNS-1) or fewer than 5 WBC/hpf in CSF with blast cells noted on cytospin (CNS-2). If traumatic tap (>10 RBC's on CSF cell count) with ≥ 5 WBC cells are seen, use Steinherz/Bleyer algorithm (see 5.1.6) to determine if patients should be considered CNS-2 or CNS-3 for purposes of risk group assignment.
    2. CNS-1 CSF on Days 18 and 32. SR patients with CNS-2 CSF on Days 18 or 32 will be re-classified and re-consented as high risk (Section 5.1.2). SR patients with CNS-3 CSF (≥ 5 WBC/hpf with blast cells) on Day 18 will be re-classified and re-consented as high risk and on Day 32 will be

considered Induction Failure (Section 6.8.4). If traumatic tap (>10 RBC's on CSF cell count) with ≥ 5 WBC cells are seen, use Steinherz/Bleyer algorithm (see 5.1.6) to determine if patients should be considered CNS-2 or CNS-3 for purposes of risk group assignment.

3. Absence of a cranial nerve palsy at diagnosis.

- Immunophenotype: Predominance of B-precursor cell surface antigens on lymphoblasts.
- Chromosomal abnormalities: Absence of t(9;22), MLL gene translocations and hypodiploidy < 44 chromosomes as determined by karyotype, PCR or FISH analysis. If chromosomal data unavailable, may continue to be treated as SR if all other risk group criteria are met.
- MRD (leukemia patients only): MRD level < 0.001 on a marrow sample obtained at end of remission induction therapy (Day 32). SR patients with MRD levels ≥ 0.001 at the end of remission induction therapy (Day 32) will be re-classified and re-consented as very high risk, and very high risk treatment will commence with Consolidation IB. SR patients whose end-of-induction MRD status cannot be determined will remain SR.

5.1.2 **High Risk: Any one of the following criteria (except patients who meet any VHR criteria)**

- Age: 10 to < 22 years
- WBC count: Highest pre-treatment WBC ≥50,000/mm^3 (prior to registration).
- CNS leukemia: Evidence of CNS leukemia by morphology, defined by meeting any of the following criteria:
  1. Diagnostic lumbar puncture (Day 1) with 5 or greater WBC/hpf and blast cells on cytospin (CNS-3). If traumatic tap (>10 RBC's on CSF cell count) with ≥ 5 WBC cells are seen, use Steinherz/Bleyer algorithm (see 5.1.6) to determine if patients should be considered CNS-2 or CNS-3 for purposes of risk group assignment.
  2. CNS-2 on Day 18 or 32. Patients with CNS-2 CSF (fewer than 5 WBC per hpf in CSF, with blasts cells on cytospin) on Day 18 or 32 will be re-consented as high risk if otherwise considered standard risk.
  3. CNS-3 on Day 18. Patients with CNS-3 CSF (≥ 5 WBC/hpf with blast cells) on Day 18 will be re-consented as high risk if previously considered standard risk. If traumatic tap (>10 RBC's on CSF cell count) with ≥ 5 WBC cells are seen, use Steinherz/Bleyer algorithm (see 5.1.6) to determine if patients should be considered CNS-2 or CNS-3 for purposes of risk group assignment.
  4. Presence of a cranial nerve palsy at diagnosis.
- Immunophenotype: Predominance of T-cell markers on lymphoblasts. Note: T-ALL and T-lymphoblastic lymphoma patients with immunophenotype consistent with early T-cell precursor (ETP) as determined by DFCI/CH central review will be considered VHR.
- MRD (leukemia patients only): B-precursor HR patients with MRD level < 0.001 on a marrow sample obtained at end of remission induction therapy (Day 32) will continue to be treated as HR. B-precursor HR patients with MRD

levels ≥ 0.001 at the end of remission induction therapy (Day 32) will be re-classified and re-consented as very high risk, and very high risk treatment will commence with Consolidation IB. HR patients whose end-of-induction MRD status cannot be determined will remain HR. MRD will not be used to change risk group of T-lineage patients.

5.1.3 **Very High Risk: Any of the following criteria**

- Chromosomal abnormalities:
  - Presence of MLL gene translocations [such as t(4;11)] by karotype or FISH or molecular analyses.
  - Presence of hypodiploidy < 44 chromosomes by karyotype or FISH analysis.
- ETP phenotype: For T-ALL/lymphoblastic lymphoma patients, presence of early T-cell precursor phenotype (ETP) as determined by DFCI/CH central review
- MRD status (leukemia patients only): B-precursor patients previously considered either standard or high risk with MRD ≥0.001 at end of remission induction therapy (Day 32).
- Patients who meet very high risk criteria will be re-consented as Very High Risk (VHR) if previously considered standard risk or high risk, and will commence with VHR treatment at the start of Consolidation IB.

5.1.4 **Special Situation: t(9;22)**

Patients found to have t(9;22) by cytogenetics, FISH and/or PCR will be removed from study by Day 15. These patients will be eligible to enroll on the open COG Protocol for Philadelphia chromosome-positive ALL.

5.1.5 **Lymphoblastic Lymphoma**: Lymphoblastic Lymphoma patients should be treated as Standard Risk, High Risk or Very High Risk based on age, immunophenotype, CNS status and cytogenetics, as specified above. MRD will not be used in lymphoblastic lymphoma for risk-group classification.

5.1.6 **Steinherz/Bleyer Algorithm to Interpret Traumatic LP's (>10 RBC per high power field) with ≥ 5 WBC/hpf**:

CSF specimens with > 10 RBC/hpf and ≥ 5 WBC/hpf should be treated as CNS-3 if: (CSF WBC/CSF RBC) > 2x (Blood WBC/Blood RBC).

All other CSF specimens with > 10 RBC/hpf and ≥ 5 WBC/hpf in CSF specimen should be treated as CNS2.

5.2 **Determination of Final Risk Group Status for patients with ALL**

- Patients should begin treatment as per appropriate risk group based on available data at the time of diagnosis (eg, age, presenting leukocyte count, immunophenotype, CNS status)

- Final risk group classification may differ from initial risk group based on results obtained during and immediately after remission induction therapy, based on:
    a) Presence of CNS blasts at Day 18 or 32
    b) Presence of MLL gene translocation or hypodiploidy (< 44 chromosomes) determined by karyotype, FISH or other molecular studies: all patients re-classified as VHR regardless of initial risk group classification. It is expected that re-classified patients will be able to begin VHR treatment at the end of remission induction treatment (beginning of Consolidation I)
    c) Presence of early T-cell precursor phenotype (ETP) in T-ALL patients as determined by DFCI/CH central review. It is expected that re-classified ETP patients will be able to begin VHR treatment at the end of remission induction treatment (beginning of Consolidation I).
    Note: See Section 5.1.3 for reclassification of lymphoblastic lymphoma patients.
    d) MRD level at end of remission induction therapy.
        i) SR patients: If MRD $\geq$ 0.001, will be re-classified as VHR, and begin VHR treatment at the start of the second cycle of Consolidation I therapy (Consolidation IB).
        ii) HR B-lineage patients: If MRD $\geq$ 0.001, will be re-classified as VHR and begin VHR treatment at the start of the second cycle of Consolidation I therapy (Consolidation IB).
        iii) MRD will not be used to change risk group classification of T-cell patients
    If end-of-induction MRD status cannot be determined, then patient will continue to be treated per initial risk group classification

- **Note: Final risk group status will be determined no later than 3 weeks from the date that morphologic complete remission is documented. If MRD results are not available when participant has recovered from the Consolidation I phase and meets criteria to begin the next treatment phase, then the final risk group status will be assigned at that time based on available data (presenting characteristics, CNS status during remission induction, cytogenetics, etc). Treatment should not be delayed while awaiting MRD results.**
- **Note: Patients with high Day 32 MRD ($\geq$0.001) must sign a new consent form for the VHR treatment arm prior to initiating their next phase of therapy. The newly signed VHR consent forms should be faxed to Study Coordinator at fax: (617) 632-3977 and to the** ODQ **fax: (617) 632-2295.**
- **Note: Patients with hypodiploidy (<44 chromosomes), MLL gene rearrangements, ETP or high Day 32 MRD ($\geq$0.001) who decline reassignment to VHR treatment arm will be removed from the study.**

Table 5.2: Determination of Final Risk Group based on MRD, Cytogenetics and CNS Status

INTIAL GROUP CLASSIFICATION (**Determined at diagnosis**)	**FINAL GROUP CLASSIFICATION** **(Determined after cytogenetics and Day 32 MRD result known)**
Standard Risk	***Very High Risk***: If MLL rearranged or hypodiploidy and/or MRD ≥ 0.001 at Day 32.
	High Risk*:* If: CNS-2 or CNS-3 CSF on Day 18 or CNS-2 on Day 32
	Off Study: Removed from protocol on Day 15 if found to have t(9;22)
	Standard Risk: If: None of the above
High Risk	***Very High Risk:*** If MLL rearranged or hypodiploidy or ETP and/or B-lineage with MRD ≥ 0.001
	Off Study: Removed from protocol on Day 15 if found to have t(9;22)
	High Risk: If: None of the above.

6. TREATMENT PLAN

Participants should begin treatment as soon as feasible after registration. Active issues related to fever, anemia, thrombocytopenia, coagulopathy, and acute tumor lysis syndrome should be addressed prior to the initiation of leukemia-directed therapy whenever possible. See Section 6.16 for Supportive Care Guidelines.

Participants will receive the following phases of treatment:

- Steroid Prophase (3 days)
- Remission Induction (29days)
- Consolidation I (3 weeks for SR/HR patients, 9 weeks for VHR patients)
- CNS Phase (3 weeks)
- Consolidation II (27 weeks)
- Continuation (until 24 months in complete continuous remission).

Drugs and dosages to be administered in each phase are detailed below. Allowable deviations to the schedule are outlined in Section 7.2.1. Dose modifications for toxicity are detailed in Sections 7.2.2 through 7.2.8. Laboratory studies and therapy that are allowed at satellite sites/affiliate hospitals and in the home are detailed in Section 15.

Additionally, all outpatient chemotherapy for each cycle must be prescribed by a physician, nurse practitioner or physician assistant, as per institutional standard, on the day that the patient is evaluated and found to meet criteria for that cycle. This means that only one cycle of outpatient chemotherapy may be prescribed at each visit, and refills for chemotherapy prescriptions are not allowed for study patients. Exception can be made for patients receiving Purixan® (6-MP oral suspension) who must be dispensed full bottles per medical insurance restrictions; in these cases, medical staff should review dosing with patient/family at the start of each cycle. Under no circumstances may other clinical staff call in prescriptions for chemotherapy for patients on study.

6.1 STEROID PROPHASE (All patients)

6.1.1 Starting Time: After confirmation of diagnosis, informed consent signed and patient registered

6.1.2 Starting Criteria: All patients who are eligible and enrolled on study should begin treatment with this phase, with the following exception: patients who have already received steroid pre-treatment prior to beginning protocol therapy (See Section 3.1.2 for allowable steroid pre-treatment). Such patients should undergo lumbar puncture with intrathecal cytarabine, as in Section 6.1.4, and then proceed directly to Remission Induction Phase (Section 6.4 for Standard Risk and Section 6.5 for High Risk and Very High Risk).

6.1.3 Steroid Prophase Therapy

Day	1	2	3
	P	P	P
	ITAra		

***Perform Therapeutic Lumbar Puncture on Day 1. Send CSF for cell count with differential and/or cytology.**
***Complete blood count with differential must be performed at diagnosis and after completion of 3 full days of methylprednisolone (Day 4). *Absolute blast count must be calculated at diagnosis and at Day 4 (Absolute blast count = peripheral blood WBC x % blasts)**
***All leukemia patients should have bone marrow and peripheral blood sample sent to develop MRD probe prior to starting systemic therapy (steroid prophase). See Section 9.0: CORRELATIVE/SPECIAL STUDIES.**

***Note: Antibiotic Prophylaxis should begin during the Prophase in all afebrile patients and continue until count recovery after expected hematologic nadir (ANC $\geq$ 200 or APC $\geq$ 500/mm^3). See Section 6.3.**

6.1.4 Steroid Prophase Dosages, Schedules & Routes

P Methylprednisolone 32 mg/m^2/day IV divided every 8 hours (or three times per day) on days1-3.

Note: Only IV Methyprednisolone should be administered during Prophase. Administration of oral prednisone (40 mg/m2/day divided into three daily doses) instead of intravenous methylprednisolone during prophase is not allowed, except if discussed in advance with Principal Investigator.

ITAra Intrathecal Cytarabine given on Day 1, dosed by age:

Age	1 – 1.99 yrs	2 - 2.99yrs	≥3 yrs
Cytarabine	20 mg	30 mg	40 mg

- Mix in 6 ml preservative-free normal saline (or use institutional guidelines for dilution).
- Intrathecal medications should be filtered prior to or during administration or according to institute policy for safe administration.

6.2 Additional Lumbar Punctures for Patients with CNS Leukemia

6.2.1 CNS blasts on Initial Lumbar Puncture (LP): Patients with CNS leukemia (CNS-2, CNS-3 or traumatic LP with blasts; regardless of RBC count) on initial Lumbar puncture (day 1) will receive additional IT Cytarabine (beginning on Days 4-6) twice-weekly until the CSF is clear, and then two additional doses will be given.

Dose of IT cytarabine according to age as indicated above (Section 6.1.4).

Note: 2 additional doses of IT chemotherapy (with clear CSF—no blasts) should be administered after first clear Lumbar Puncture. If patients are still receiving twice weekly lumbar punctures with IT therapy at Day18, IT chemotherapy should be changed from IT cytarabine to IT Methotrexate/Cytarabine/Hydrocortisone on Day 18 (as dosed in Section 6.4) and IT Methotrexate/Cytrabine/Hydrocortisone should be used for all subsequent lumbar punctures with IT therapy after Day 18, if any are still necessary (except if mucositis present, in which case IT Methotrexate may be held).

6.2.2 Cranial Nerve Palsy: Patients with cranial nerve palsy at diagnosis but with no leukemia blasts in the CSF will receive twice weekly IT cytarabine as above (Section 6.2.1) until Day 18.

Table 6.2: Change in Treatment and Risk Group based on CNS Status during Remission Induction

	Day of Induction		
	Day 1	**Day 18**	**Day 32**
CNS-1	No change in risk group	No change in risk group	No change in risk group
CNS-2	No change in risk group	SR changed to HR	SR changed to HR
CNS-3	SR changed to HR	SR changed to HR	Induction Failure

CNS-1: no blast cells in cytospin, regardless of CSF cell count
CNS-2: Fewer than 5 WBC on CSF cell count, with blasts on cytospin
CNS-3: 5 or more WBC on CSF cell count, with blasts on cytospin
Traumatic tap: > 10 RBC on CSF cell count. See Section 5.1.6 for interpretation of traumatic LP with blasts.

- Day 1, CNS-2 , CNS-3 or traumatic LP with blasts: treat with twice weekly IT cytarabine until 3 clear lumbar punctures in a row
- Day 18, CNS-2, CNS-3 or traumatic LP with blasts: treat with twice weekly IT MAH until 3 clear lumbar punctures in a row
- Day 32: CNS-2 (in patient with previous CNS-1 status): Treat as per Section 6.8.6.
- Day 32: CNS-3 (regardless of previous CNS status): Induction Failure (see Section 6.8.4)
- Patients with persistent CNS leukemia throughout remission induction (CNS-2, CNS-3 or traumatic LP with blasts) who still have CNS leukemia at Day 32 (blasts seen on cytospin, regardless of CSF WBC or RBC count) are considered Induction Failures (See Section 6.8.4)
- Standard Risk patients whose CNS status is CNS-3 anytime on or after Day 18 and prior to Day 32 should be reclassified as High Risk (HR consent required).

6.3 Antibiotic Prophylaxis during Remission Induction Phase

Antibiotic prophylaxis with a fluoroquinolone antibiotic (either levofloxaxin or moxifloxacin) will be initiated during the Steroid Prophase and continue throughout the Remission Induction phase until count recovery after expected hematologic nadir (ANC $\geq$ 200 or APC $\geq$ 500/mm^3). Patients unable to tolerate fluoroquinolones due to allergy or other toxicity should receive intravenous cefepime as prophylaxis. If a patient is unable to tolerate fluoroquinolone or cefepime, contact Principal Investigator.

Patients receiving broad-spectrum antibiotics for fever (See Section 6.16.2) or documented infection may discontinue prophylactic antibiotics. Broad spectrum antibiotics for febrile neutropenia should be continued until ANC > 500/mm^3, or per institutional guidelines.

Because of the high risk of infection, treatment should begin on an inpatient basis and hospitalization of participants is encouraged throughout the induction phase. Outpatient treatment during the induction phase may be considered after recovery of the absolute neutrophil count (ANC) after the expected hematologic nadir.

6.4 REMISSION INDUCTION PHASE (STANDARD RISK PATIENTS)

6.4.1 Remission Induction Phase for SR Patients

Day	4	5	6	7…	11 …	18*…	25…		32+
	P	→	→	→	→	→	→	→ →	begin taper
	V				V	V	V		
	D	D							
			m						
				ASP					
						IT MAH			ITM

***On Day 18, perform marrow aspirate (only in leukemia patients who have consented to optional Day 18 bone marrow procedure) and therapeutic lumbar puncture with intrathecal chemotherapy (all patients). Collect Day 18 blood only if patients consented to additional research peripheral blood samples.**

+ On Day 32, perform marrow aspirate/biopsy (if marrow involvement at diagnosis) and therapeutic lumbar puncture with intrathecal chemotherapy. It is suggested that the marrow sample be sent for cytogenetics/FISH (if abnormal at diagnosis).

+**For Leukemia Patients**: **Day 32 Marrow sample must be sent for MRD analysis to determine final risk group assignment. See Section 9.0: CORRELATIVE/SPECIAL STUDIES**.

See 6.8.3 if peripheral blood criteria for remission are not met by Day 32.

***Note: Antibiotic Prophylaxis should begin during the Prophase and continue through the Induction phase in all afebrile patients until count recovery after expected hematologic nadir (ANC ≥ 200 or APC ≥ 500/mm^3). See Section 6.3.**

See Section 7.2.3 for dose modifications. See Section 7.2.1 for allowable deviations in timing of medications/procedures and for allowable variation in dosing.

6.4.2 Starting Time: After completion of steroid prophase (Section 6.1)

6.4.3 Starting Criteria:

- Direct bilirubin ≤ 1.4 mg/dL (23.9 micromoles/L) If direct bilirubin > 1.4 mg/dL (23.9 micromoles/L), go to Section 6.6, "Special Circumstance 1: Hyperbilirubinemia"

6.4.4 Dosages, Schedules & Routes

P Prednisone Prednisone or Prednisolone 40 mg/m^2/day orally divided into two or three doses per day or methylprednisolone (solumedrol) 32 mg/m^2/day IV divided every 8 hours (or three times per day) on Days 4-32.

Note: A steroid taper (consisting of either prednisone or methylprednisolone) should be administered after completion of Induction course of

prednisone/methylprednisolone. Steroid taper should commence: 1) after completion of end-induction restaging procedures (marrow and/or scans) as long as the results of these studies are consistent with complete remission (including peripheral blood count recovery) and patient will be proceeding to Consolidation I phase, or 2) after last dose of steroid on Day 32, whichever comes first. Full-dose prednisone (40 mg/m2/day, or its methylprednisolone equivalent) should not be administered after Day 32. Duration of corticosteroid taper is up to discretion of treating physician but should not exceed 7 days (i.e., all corticosteroid should be completed by Day 39).

V Vincristine 1.5 mg/m^2 (maximum dose 2 mg) IV push or IV via minibag every week x 4 (on Days 4, 11, 18 and 25).

D Doxorubicin Doxorubicin 30 mg/m^2 IV on Days 4 and 5.

- Give as IV push via CVL or via freshly placed peripheral IV.
- May give as IV bolus over 15 minutes if administered via a central line.
- No Dexrazoxane

m Methotrexate Methotrexate 40 mg/m^2 IV push on Day 6. Give at least 8 hours but not more than 24 hours, after last Doxorubicin dose.
Note: Do not give methotrexate if effusions or ascites are present, or if creatinine is beyond upper limits normal for age. See Section 7.2.3.

ASP Asparaginase according to randomization. Give on Day 7. Mix in 100 mL Normal Saline and give IV over 1 hour.

A. SC-PEG Asparaginase (Calaspargase Pegol) 2500 IU/m^2/dose IV x 1 dose, or
B. Oncaspar 2500 IU/m^2/dose IV x 1 dose.

**All patients should have the following samples obtained:

- Asparaginase enzyme levels (PK samples): Obtain samples prior to Asparaginase dose on Day 7, 5-10 minutes after completion of Asparaginase infusion on Day 7, then every week x 4 on Days 11, 18, 25, and 32 (when vincristine is due to be administered) or within ±3 days of these days. (see Section 9.2)
- Asparaginase antibody levels: Obtain samples at Day 7 (pre-asparaginase dose) and at Day 32 (see Section 9.2), at the same time that Asparaginase enzyme level sample is obtained.

IT MAH Intrathecal Methotrexate/Cytarabine/Hydrocortisone
Give on Day 18. Dose according to age:

Age	1 – 1.99 yrs	2 - 2.99yrs	≥3yrs
Methotrexate	8mg	10mg	12mg
Cytarabine	20mg	30mg	40mg
Hydrocortisone	9mg	12mg	15mg

- Mix in 6 ml preservative-free normal saline (or use institutional guidelines for dilution).
- Intrathecal medications should be filtered prior to or during administration or according to institute policy for safe administration.

ITM Intrathecal Methotrexate on Day 32. If peripheral blood criteria for remission are met, perform bone marrow aspirate and lumbar puncture. Administer intrathecal methotrexate medications according to age:

Age	1 - 1.99yrs	2 - 2.99yrs	≥ 3yrs
Methotrexate	8mg	10mg	12mg

- Mix in 6 ml preservative-free normal saline (or use institutional guidelines for dilution).
- Intrathecal medications should be filtered prior to or during administration or according to institute policy for safe administration.

6.5 REMISSION INDUCTION PHASE (HIGH RISK AND VERY HIGH RISK PATIENTS)

6.5.1 Remission Induction Phase for HR/VHR patients

Day	4	5	6	7…		11 …	18*…		25…		32+
	P	→	→	→	→	→	→	→	→	→	begin taper
	V						V	V	V		
	Dd	Dd									
			m								
				ASP							
								IT MAH			ITM

***On Day 18, perform marrow aspirate (only in leukemia patients who have consented to optional Day 18 bone marrow procedure) and therapeutic lumbar puncture with intrathecal chemotherapy (all patients). Collect Day 18 blood only if patients consented to additional research peripheral blood samples. + On Day 32, perform marrow aspirate/biopsy** (if marrow involvement at diagnosis) and therapeutic lumbar puncture with intrathecal chemotherapy. It is suggested that the marrow sample be sent for cytogenetics/FISH (if abnormal at diagnosis).

+**For Leukemia Patients: Day 32 Marrow sample must be sent for MRD analysis to determine final risk group assignment. See Section 9.0: CORRELATIVE/SPECIAL STUDIES**.

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See 6.8.3 if peripheral blood criteria for remission are not met by Day 32.

***Note: Antibiotic Prophylaxis should begin during the Prophase and continue through the Induction phase in all afebrile patients until count recovery after expected hematologic nadir (ANC ≥ 200 or APC ≥ 500/mm^3). See Section 6.3.**

See Section 7.2.3 for dose modifications. See Section 7.2.1 for allowable deviations in timing of medications/procedures and for allowable variation in dosing.

6.5.2 Starting Time: After completion of steroid prophase (Section 6.1)

6.5.3 Starting Criteria:

- Direct bilirubin ≤ 1.4 mg/dL (23.9 micromoles/L). If direct bilirubin > 1.4 mg/dL (23.9 micromoles/L), go to Section 6.6, "Special Circumstance 1: Hyperbilirubinemia"

P Prednisone Prednisone or Prednisolone 40 mg/m^2/day orally divided into two or three doses per day or methylprednisolone (solumedrol) 32 mg/m^2/day IV divided every 8 hours (or three times per day) on Days 4-32.

Note: A steroid taper (consisting of either prednisone or methylprednisolone) should be administered after completion of Induction course of prednisone/methylprednisolone. Steroid taper should commence: 1) after

completion of end-induction restaging procedures (marrow and/or scans) as long as the results of these studies are consistent with complete remission (including peripheral blood count recovery) and patient will be proceeding to Consolidation I phase, or 2) after last dose of steroid on Day 32, whichever comes first. Full-dose prednisone (40 mg/m2/day, or its methylprednisolone equivalent) should not be administered after Day 32. Duration of corticosteroid taper is up to discretion of treating physician but should not exceed 7 days (i.e., all corticosteroid should be completed by Day 39).

V Vincristine 1.5 mg/m^2 (maximum dose 2 mg) IV push or IV via minibag every week x 4 (on Days 4, 11, 18 and 25).

D Doxorubicin 30 mg/m^2 IV preceded by Dexrazoxane 300 mg/m^2 IV on day 4 and day 5. Suggested administration for both drugs:

- Minute 0 Dexrazoxane over 15 minutes
- Minute 15 Doxorubicin IV push via central line or freshly placed peripheral IV. May give doxorubicin IV over 15 minutes if given via central venous line.
- Goal is no more than 30 minutes maximum elapsed administration time combined for both drugs
- May give doxorubicin prior to dexrazoxane if given via peripheral IV.

m Methotrexate Methotrexate 40 mg/m^2 IV push on Day 6. Give at least 8 hours but not more than 24 hours, after last Doxorubicin dose.
Note: Do not give methotrexate if effusions or ascites are present, or if creatinine is beyond upper limits normal for age. See Section 7.2.3.

ASP Asparaginase according to randomization. Give on Day 7. Mix in 100 mL Normal Saline and give IV over 1 hour.

A. SC-PEG Asparaginase (Calaspargase Pegol) 2500 IU/m^2/dose IV x 1 dose, or
B. Oncaspar 2500 IU/m^2/dose IV x 1 dose.

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**All patients should have the following samples obtained:

- Asparaginase enzyme levels (PK samples): Obtain samples prior to Asparaginase dose on Day 7, 5-10 minutes after completion of Asparaginase infusion on Day 7, then every week x 4 Days 11, 18, 25 and 32 (when vincristine is due to be administered) or within ±3 days of these dates. (see Section 9.2)
- Asparaginase antibody levels: Obtain samples at Day 7 (pre-asparaginase dose) and at Day 32 (see Section 9.2), at the same time that Asparaginase enzyme level sample is obtained.

IT MAH Intrathecal Methotrexate/Cytarabine/Hydrocortisone
Give on Day 18. Dose according to age:

Age	1 – 1.99 yrs	2 - 2.99yrs	≥3yrs
Methotrexate	8mg	10mg	12mg
Cytarabine	20mg	30mg	40mg
Hydrocortisone	9mg	12mg	15mg

- Mix in 6 ml preservative-free normal saline (or use institutional guidelines for dilution).
- Intrathecal medications should be filtered prior to or during administration or according to institute policy for safe administration.

ITM Intrathecal Methotrexate on Day 32. If peripheral blood criteria for remission are met, perform bone marrow aspirate and lumbar puncture. Administer intrathecal methotrexate medications according to age:

Age	1 - 1.99yrs	2 - 2.99yrs	≥ 3yrs
Methotrexate	8mg	10mg	12mg

- Mix in 6 ml preservative-free normal saline (or use institutional guidelines for dilution).
- Intrathecal medications should be filtered prior to or during administration or according to institute policy for safe administration.

6.6 Special circumstance: Induction Therapy for Patients with Hyperbilirubinemia on Day 4

If direct bilirubin >1.4 mg/dl (23.9 micromoles/L), on Day 4, timing of chemotherapy is as follows:

Prednisone	Give Days 4 through 32
Vincristine	Give Days 4, 11 18 and 25 if direct bilirubin ≤ 3.0 mg/dL (51.3 micromoles/L). If direct bilirubin is > 3.0 mg/dL (51.3 micromoles/L), hold dose of vincristine. With a bilirubin at this level, it may be allowable under certain circumstances to administer vincristine at 50% dose but only with prior approval of Principal Investigator. For dosing guidelines, see Section 6.4 for SR and 6.5 for HR/VHR.
Doxorubicin	Hold until direct bilirubin ≤1.4 mg/dL (23.9 micromoles/L), then give (according to risk group status) on 2 consecutive days (dosing as per Section 6.4 SR and Section 6.5 HR/VHR). HR/VHR should receive Dexrazoxane with doxorubicin as specified in Section 6.5.3. If patient has not received doxorubicin because direct bilirubin is not ≤ 1.4 mg/dL (23.9 micromoles/L), by Day 18 patient should be removed from study.
Methotrexate	Hold until direct bilirubin ≤ 1.4 mg/dL, then give 8-24 hours after 2nd dose of doxorubicin.
Asparaginase	Hold until direct bilirubin is ≤ 1.4 mg/dL (23.9 micromoles/L) ; Give 1 day after methotrexate dose is given at dose indicated in Section 6.4 for SR and Section 6.5 for HR/VHR.
IT MAH	Administer on Day 18 as indicated in Section 6.4 for SR and Section 6.5 for HR/VHR. Hold intrathecal methotrexate if direct bilirubin still > 1.4 mg/dL (23.9 micromoles/L), at that time.

Day 18 bone marrow aspirate should not be performed if any agents were delayed due to hyperbilirubinemia.

Perform marrow aspirate/biopsy (if marrow involvement at diagnosis) and therapeutic lumbar puncture (with intrathecal chemotherapy) at end of remission induction therapy when peripheral blood counts recover, at least 3 weeks from doxorubicin. It is suggested that the marrow sample be sent for cytogenetics/FISH (if abnormal at diagnosis).

For ALL patients: Day 32 Marrow sample must be sent for MRD analysis to determine final risk group assignment.

See Section 6.8.3 if peripheral blood criteria for remission are not met by Day 32.

Note: If direct bilirubin remains persistently elevated above 1.4 mg/dL (23.9 micromoles/L) at Day 18, then participant should be removed from study.

6.7 Special circumstance: Induction Therapy for Patients treated without Steroid Prophase

Note: Direct bilirubin must be ≤ 1.4 mg/dL (23.9 micromoles/L), if not, go to Section 6.6.
First day of chemo = Day 4
Doses of all agents the same as doses indicated in Section 6.4 for SR and Section 6.5 for HR/VHR. Treat without Steroid Prophase.

Prednisone	Give Days 4 through 32.
Vincristine	Give Days 4, 11, 18 and 25
Doxorubicin	Give on Days 4 and 5 according to risk group. Dexrazoxane given to HR/VHR patients.
Methotrexate	Give on Day 6.
Asparaginase	Give on Day 7, according to randomization.
IT cytarabine	Administer on Day 4.

IT methotrexate/cytarabine/hydrocortisone: Administer on Day 18
IT methotrexate Administer on Day 32, if peripheral blood criteria for remission all met.

On Day 18, perform therapeutic lumbar puncture (with intrathecal chemotherapy). Leukemia patients who have consented to Day 18 marrow should have this procedure performed at the same time as therapeutic lumbar puncture.
On Day 32, perform marrow aspirate/biopsy (if marrow involvement at diagnosis) and therapeutic lumbar puncture with intrathecal chemotherapy. It is suggested that the marrow sample be sent for cytogenetics/FISH (if abnormal at diagnosis).
For Leukemia Patients: Day 32 Marrow sample must be sent for MRD analysis to determine final risk group assignment.

See Section 6.8.3 if peripheral blood criteria for remission are not met by Day 32.

6.8 COMPLETION OF REMISSION INDUCTION THERAPY

6.8.1 Complete remission definition:

Leukemia: An interpretable bone marrow (a specimen with normal marrow elements present) with fewer than 1% malignant lymphoblasts by microscopic examination and peripheral blood without lymphoblasts, and an APC $\geq$ 1000/mm^3 and platelets $\geq$ 100,000/mm^3, and no evidence of extramedullary leukemia (no blasts in spinal fluid, at least 70% reduction in size of masses noted on imaging at diagnosis, as defined below for patients with lymphoblastic lymphoma). Note: Patients are allowed to have $\geq$ 1% normal myeloid or lymphoid blasts (hematogones) in their marrow consistent with recovery and still meet the definition of complete remission. Normal recovery blasts may be distinguished from persistent malignant lymphoblasts by flow cytometry, immunohistochemistry or other studies. Consult principal investigator with any questions.

Lymphoblastic Lymphoma: At least a 70% reduction in size of largest nodes or masses noted at diagnosis. Reduction may be measured as a decrease in a sum of the products of the two greatest diameters (SPD) of the 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 two perpendicular dimensions,
b) they should include mediastinal masses whenever this site was initially involved.

In addition to this radiographic criterion, there should also be complete disappearance of all clinical evidence of disease by physical examination and no evidence of disease in spinal fluid or in bone marrow (if involved at initial diagnosis). Definition of marrow remission for lymphoblastic lymphoma patients with initial marrow involvement is the same as that used for patients with leukemia (above).

When patient is documented to be in complete remission, proceed to Consolidation I (Section 6.9 for Standard Risk, Section 6.10 for High Risk, and Section 6.11 for Very High Risk).

6.8.2 Timing of Bone Marrow Examination and Therapeutic Lumbar Puncture

Leukemia patients and Lymphoblastic Lymphoma patients with marrow involvement at diagnosis: Bone marrow aspirate/biopsy should be performed on (or as close as possible) to Day 32 of remission induction, regardless of blood counts. Sample for MRD analysis should be sent for all leukemia patients. Therapeutic lumbar puncture (with intrathecal methotrexate) may be performed at the same time as this marrow aspirate/biopsy only if peripheral blood counts meet criteria for complete remission (APC $\geq$ 1000/mm^3 and platelets $\geq$ 100,000/mm^3) and there is no evidence of extramedullary leukemia. If patient is in documented complete remission on Day 32, proceed to Consolidation I (Section 6.9 for Standard Risk, Section 6.10 for High Risk, and Section 6.11 for Very High Risk). Note: For patients with leukemia, it is essential that bone marrow sample for MRD analysis be sent at Day 32.

Lymphoblastic Lymphoma patients without marrow involvement at diagnosis: Marrow aspirate/biopsy is not required at Day 32, but should be considered if there is delayed count recovery. Therapeutic lumbar puncture (with intrathecal methotrexate) should be performed at Day 32 (or as close as possible to this date) only if peripheral blood counts meet criteria for

complete remission (APC ≥ 1000/mm^3 and platelets ≥ 100,000/mm^3) and there is no evidence of extramedullary leukemia.

6.8.3 **Delayed Recovery**:
If complete remission is NOT achieved on Day 32 because either a) patient does not meet peripheral blood criteria, or b) marrow is too hypocellular to interpret (not enough marrow cells to perform adequate marrow differential), **and** <5% lymphoblasts are identified in the marrow (leukemia patients and lymphoblastic lymphoma patients with initial marrow involvement):

6.8.3.1 Patient does not meet peripheral blood criteria:

1) For leukemia patients and lymphoblastic lymphoma patients with initial marrow involvement: **perform bone marrow aspirate and biopsy on Day 32**. Day 32 marrow aspirate from leukemia patients should be sent for MRD testing, regardless of peripheral blood counts. Repeat marrow aspirate when peripheral blood counts meet criteria for remission, or by Day 53 if counts still not recovered. Additional bone marrow aspirates/biopsies may be performed between Days 32-53 at discretion of treating physician. For leukemia patients, send specimen from any bone marrow performed for MRD analysis, if possible.

2) **Do Not perform lumbar puncture**. **Administer vincristine 1.5 mg/m^2 (maximum dose 2 mg) IV push or IV via minibag weekly until CR is achieved**. When peripheral blood criteria met, lumbar puncture with intrathecal methotrexate should be performed (may be performed at same time as bone marrow aspirate for leukemia patients and lymphoblastic lymphoma patients with initial marrow involvement).

3) Patient will be removed from study if counts do not recover by Day 53

6.8.3.2 Hypocellular marrow *(peripheral blood criteria met but marrow uninterpretable)*--Leukemia patients and Lymphoblastic Lymphoma patients with initial marrow involvement only:

1) Continue vincristine 1.5 mg/m^2 (maximum dose 2 mg) IV push or IV via minibag weekly on Days 32, 39 and 46 or until CR achieved, whichever comes first.

2) Repeat marrow aspirate and/or biopsy weekly until complete remission is documented.

3) Complete remission must be documented by Day 53 for patients to remain on study.

6.8.4 **Induction Failure**
Leukemia: If ≥1% blasts (confirmed to be leukemic lymphoblasts by flow cytometry, immunohistochemistry or other studies) are identified in the marrow on Days 32, or on any marrow performed up through Day 53 (without a preceding marrow consistent with complete

remission), the patient will be classified as an Induction Failure and removed from protocol (contact Principal Investigator or designee prior to removing from protocol if patient is induction failure). Any patient with evidence of extramedullary leukemia on exam, in the spinal fluid or by radiographic imaging (<70% reduction in size of largest nodes or masses noted at diagnosis, or any new biopsy-proven masses) will also be considered an induction failure, regardless of marrow findings.

Lymphoblastic Lymphoma: If end-induction imaging reveals persistent nodal involvement or masses (< 70% reduction in size of largest nodes or masses noted at diagnosis), the patient will be classified as an Induction Failure and removed from protocol (call Principal Investigator or designee if patient is an induction failure). Any patient with new, biopsy-proven sites of disease will also be considered an induction failure. In addition, any patient with evidence of persistent disease in bone marrow (confirmed by flow cytometry, FISH or other studies) or spinal fluid will also be considered an induction failure.

6.8.5 **Indeterminate marrow**

If ≥ 5% blasts that are not clearly lymphoblasts based on morphology are identified in the marrow on Day 32 (or any marrow performed through Day 53), try to better characterize blasts by flow cytometry, FISH, immunohistochemistry or other studies. Repeat marrows can be performed weekly for questionable cases. Vincristine should be given if decision is made to repeat marrow in one week at a dose of 1.5 mg/m^2/week (maximum dose 2 mg) IV push or IV via minibag. If marrow results are indeterminate, call Principal Investigator or designee to discuss management.

6.8.6 **CNS blasts at the end of induction**:

If patient with ≥ 5 WBC/hpf in CSF with blast cells seen on cytospin (CNS-3) from lumbar puncture performed at end of induction (on Days 32, or after if delayed recovery), patient is considered Induction Failure, and is removed from protocol.

If patient has had CNS blasts present throughout Induction Therapy (CNS-2 or 3) without clearance of CSF, and blasts persist at Day 32, then patient is considered an Induction Failure at that time, and is removed from protocol. Note: If Day 32 lumbar puncture is clear of blasts, patient is not considered Induction Failure, even if patient has not yet had 3 negative lumbar punctures in a row by that time point.

If blasts are identified in the CSF from lumbar puncture performed at end of induction but CSF WBC < 5 cells/hpf (CNS-2) in a patient otherwise with previously clear CSF (no lymphoblasts) and in peripheral blood and bone marrow remission:

- SR patient reclassified to HR, and will be considered CNS-positive (to receive 1800 cGy during CNS Intensification Phase)
- If HR, will receive 1800 cGy during CNS Intensification Phase, regardless of prior radiation assignment.
- Twice-weekly lumbar punctures with intrathecal methotrexate, cytarabine and hydrocortisone. Dose IT chemo as per Section 6.5. Continue twice weekly lumbar punctures until CSF is clear, and then two additional doses will be given.

- Do not proceed to Consolidation I Phase. Instead, administer the following systemic chemotherapy (one cycle only):

  1) Vincristine 1.5 mg/m^2 (maximum dose 2 mg) IV push weekly or IV via minibag.

  2) Doxorubicin 30 mg/m^2 and dexrazoxane 300 mg/m^2. See Section 6.5 for administration guidelines. .

  3) 6-mercaptopurine 50 mg/m^2/day orally nightly x 14 consecutive days beginning on Day 1. (Suggested administration guidelines: Give on an empty stomach, at least 2 hours after and 1 hour before food and/or milk). Note: If patient taking tablets, daily dose may be adjusted to accommodate available tablet strengths to achieve total 14-day amount.

- If blasts have cleared by Day 53 patient should proceed to next phase of treatment (Consolidation I) when starting criteria for that phase are met.
- If patient with new CNS-2 status at Day 32 has persistent CNS blasts at Day 53 (regardless of CSF cell count), the patient will be classified as an Induction Failure and will be removed from protocol

If patient has scan evidence of CNS disease (eg, leukemic infiltrate of cranial nerve) at end of remission induction, then the patient will be considered an Induction Failure and be removed from protocol.

6.9 CONSOLIDATION I PHASE: STANDARD RISK PATIENTS

6.9.1 Starting Time: Patients must be in documented remission at all sites.

6.9.2 Starting Criteria: APC≥ 1,000, platelets ≥ 100,000, direct bilirubin ≤ 1.4 mg/dL (23.9 micromoles/L), SGOT ≤ 8 x normal, creatinine normal for age, no mucositis, no ascites/effusions/significant edema.

Suggested supportive care:

- Bactrim should be held beginning on day of HD MTX infusion, and not re-started until MTX level is non-detectable detectable or <0.1 micromolar (as per institutional standard).
- Nonsteroidal anti-inflammatory medications should also be held beginning on day of HD MTX infusion, and not re-started until MTX level is non-detectable or <0.1 micromolar (as per institutional standard).

See Section 7.2.4 for Dose Modifications. See Section 7.2.1 for allowable deviations in timing of medications/procedures and for allowable variation in dosing.

6.9.3 Consolidation I Phase for Standard Risk Patients

One 3-week cycle of therapy, administered as follows:

Days	1	8	15	22
	V			
	6MP----------			
	ITM			
	HDM			

Note: Patients should continue to receive tapering doses of prednisone or methylprednisolone (as specified in Section 6.4) at the same time that they begin Consolidation I Phase.

6.9.4 Dosages, Schedules & Routes

V Vincristine 2 mg/m^2 (maximum dose 2 mg) IV push or IV via minibag on Day 1

6MP 6-mercaptopurine 50 mg/m^2/day orally nightly x 14 consecutive days beginning on Day 1. (Suggested administration guidelines: Give on an empty stomach, at least 2 hours after and 1 hour before food and/or milk). Note: If patient taking tablets, daily dose may be adjusted to accommodate available tablet strengths to achieve total 14-day amount.

ITM Intrathecal Methotrexate. Administer on Day 1 (prior to starting methotrexate infusion, if possible) (**Note:** Induction Day 32 ITM can be considered to be Day 1 Consolidation I ITM if given within 72 hours of beginning Consolidation I systemic chemotherapy).

Dose according to age:

Age	1 - 1.99yrs	2 - 2.99 yrs	≥ 3 yrs
Methotrexate	8mg	10 mg	12 mg

- Mix in 6 ml preservative-free normal saline (or use institutional guidelines for dilution).
- Intrathecal medications should be filtered prior to or during administration or according to institute policy for safe administration.

HDM High dose methotrexate. Begin infusion on Day 1

- Prehydrate with IVF containing NaHCO3 until urine specific gravity is ≤ 1.010 and pH is ≥ 7.0 and ≤ 8.0. Suggested prehydration: D5W + 75 mEq NaHCO3/L at 125 mL/m^2/hour for 6 hours
- Hour 0: Total fluid while receiving methotrexate infusion: patient should receive total fluids of at least 125 mL/m^2/hour with NaHCO3-containing fluids beginning at Hour 0 and continuing until methotrexate level non-detectable or <0.1 micromolar (as per institutional standard). Fluid volume may be adjusted to maintain dilute urine (sg ≤ 1.010) until methotrexate non-detectable (or <0.1 micromolar). Fluid should contain NaHCO3, and amount of NaHCO3 should be adjusted to maintain urine pH ≥ 7 and ≤ 8. Suggested starting concurrent IVF: D5W + 75 mEq NaHCO3/L at 125 mL/ m^2/hour
- Hour 0: Methotrexate 0.5 grams/m^2 IV over 30 minutes. Suggested diluent: 10 mL D5W + 75 mEq NaHCO3/L to run at 20 mL/hour (along with concurrent IVF).
- Hour 0.5-24: Methotrexate 4.5 grams/m^2 continuous infusion IV over 23.5 hours. Suggested diluent: 470 mL D5W + 75 mEq NaHCO3/L to run at 20 mL/hour for 23.5 hours (along with concurrent IVF).
- Hour 24: Check MTX level. (**Note: check level at completion of infusion**)
- Hour 36: Leucovorin 75 mg/m^2 IV bolus x 1 dose, given 36 hours after start of MTX infusion
- Hour 42: Leucovorin 15 mg/m^2 IV bolus every 6 hours until methotrexate level is non-detectable or <0.1 micromolar (as per institutional standard). Once MTX level is non-detectable (or <0.1 micromolar), discontinue leucovorin and IV hydration/alkalinization.
- Hour 48: Check methotrexate level. Continue to check at least every 24 hours until methotrexate level non-detectable or <0.1 micromolar, as per institutional standard.

Dose adjustments for high methotrexate levels

- Hour 24: If level > 100 micromolar, increase total fluids to at least 200 mL/m^2/hour and start leucovorin 100 mg/m^2/dose IV bolus every 3 hours. Check MTX level every 12 hours, if possible. Continue at this dose of leucovorin until level is < 5 micromolar, then reduce dose of leucovorin to 15 mg/m^2 IV every 3 hours. Continue this dose of leucovorin until level is non-detectable or <0.1 micromolar (as per institutional standard).
- Hour 48: If level > 0.18 but < 5 micromolar, increase total fluids to at least 200 mL/m^2/hour and continue leucovorin at 15 mg/m^2 IV every 6 hours. If level > 5 micromolar, increase total fluids to at least 200 mL/m^2/hour and change leucovorin

dosing to 15 mg/m^2/dose IV every 3 hours. Continue this dose of leucovorin until level is non-detectable or <0.1 micromolar (as per institutional standard).

- Hour 72: If level > 0.1 but < 5 micromolar, ensure total fluids are at least 200 mL/m2/hour and continue leucovorin at 15 mg/m^2/dose IV every 6 hours. If level > 5 micromolar, ensure total fluids are at least 200 mL/m^2/hour and change leucovorin dosing to 15 mg/m^2/dose IV every 3 hours. Continue this dose of leucovorin until level is non-detectable or <0.1 micromolar (as per institutional standard).
  Note: Dosing guidelines for fluids and leucovorin (above) may be modified depending on patient's clinical status (such as rising creatinine, new effusions, etc). In rare circumstances, methotrexate infusion may be stopped early in the event of excessive toxicity (such as rising creatinine). Any modifications should be discussed in advance with Lewis Silverman, MD, or designee.

6.10 CONSOLIDATION I PHASE: HIGH RISK PATIENTS

6.10.1 Starting Time: Patients must be in documented remission at all sites.

6.10.2 Starting Criteria: APC≥ 1,000, platelets ≥ 100,000, direct bilirubin ≤ 1.4 mg/dL(23.9 micromoles/L), SGOT ≤ 8 x normal, creatinine normal for age, no mucositis, no ascites/effusions/significant edema.

Suggested supportive care:

- Bactrim should be held beginning on day of HD MTX infusion, and not re-started until MTX level is non-detectable or <0.1 micromolar (as per institutional standard).
- Nonsteroidal anti-inflammatory medications should also be held beginning on day of HD MTX infusion, and not re-started until MTX level is non-detectable or <0.1 micromolar (as per institutional standard).
- MTX infusion should be started at least 8 hours and not more than 24 hours following the dose of doxorubicin.

See Section 7.2.4 for Dose Modifications. See Section 7.2.1 for allowable deviations in timing of medications/procedures and for allowable variation in dosing.

6.10.3 Consolidation I Phase for High Risk Patients

One 3-week cycle of therapy, administered as follows:

Cycle 1

Days	1	8	15	22
	V			
	6MP----------			
	Dd			
	ITM			
	HDM			

Note: Patients should continue to receive tapering doses of prednisone or methylprednisolone (as specified in Section 6.5) at the same time that they begin Consolidation I Phase.

6.10.4 Dosages, Schedules & Routes

V Vincristine 2 mg/m^2 (maximum dose 2 mg) IV push or IV via minibag on Day 1

6MP 6-mercaptopurine 50 mg/m^2/day orally nightly x 14 consecutive days beginning on Day 1. (Suggested administration guidelines: Give on an empty stomach, at least 2 hours after and 1 hour before food and/or milk). Note: If patient taking tablets, daily dose may be adjusted to accommodate available tablet strengths to achieve total 14-day amount.

Dd Doxorubicin 30 mg/m^2 IV preceded by dexrazoxane 300 mg/m^2 IV on Day 1.
Suggested administration for both drugs:

- Minute 0 Dexrazoxane over 15 minutes
- Minute 15 Doxorubicin IV push via central line or freshly placed peripheral IV
- Goal is no more than 30 minutes maximum elapsed administration time combined for both drugs
- May give doxorubicin prior to dexrazoxane if given via peripheral IV
- May give doxorubicin IV over 15 minutes if given via central line

ITM Intrathecal Methotrexate. Adminster on Day 1 (prior to starting methotrexate infusion, if possible). (**Note:** Induction Day 32 ITM can be considered to be Day 1 Consolidation I ITM if given within 72 hours of beginning Consolidation I systemic chemotherapy).

Dose according to age:

Age	1 - 1.99yrs	2 - 2.99 yrs	≥ 3 yrs
Methotrexate	8mg	10 mg	12 mg

- Mix in 6 ml preservative-free normal saline (or use institutional guidelines for dilution).
- Intrathecal medications should be filtered prior to or during administration or according to institute policy for safe administration.

HDM High dose methotrexate. **Begin infusion at least 8 hours but no more than 24 hours following the dose of doxorubicin**

- Prehydrate with IVF containing NaHCO3 until urine specific gravity is ≤ 1.010 and pH is ≥ 7.0 and ≤ 8.0. Suggested prehydration: D5W + 75 mEq NaHCO3/L at 125 mL/m^2/hour for 6 hours
- Hour 0: Total fluid while receiving methotrexate infusion: patient should receive total fluids of at least 125 mL/m^2/hour with NaHCO3-containing fluids beginning at Hour 0 and continuing until methotrexate level non-detectable or <0.1 micromolar (as per institutional standard). Fluid volume may be adjusted to maintain dilute urine (sg ≤ 1.010) until methotrexate non-detectable (or <0.1 micromolar). Fluid should contain NaHCO3, and amount of NaHCO3 should be adjusted to maintain urine pH ≥ 7 and ≤ 8. Suggested starting concurrent IVF: D5W + 75 mEq NaHCO3/L at 125 mL/m^2/hour
- Hour 0: Methotrexate 0.5 grams/m^2 IV over 30 minutes. Suggested diluent: 10 mL D5W + 75 mEq NaHCO3/L to run at 20 mL/hour (along with concurrent IVF).
- Hour 0.5-24: Methotrexate 4.5 grams/m^2 continuous infusion IV over 23.5 hours. Suggested diluent: 470 mL D5W + 75 mEq NaHCO3/L to run at 20 mL/hour for 23.5 hours (along with concurrent IVF).
- Hour 24: Check MTX level. (**Note: check level at completion of infusion**)
- Hour 36: Leucovorin 75 mg/m^2 IV bolus x 1 dose, given 36 hours after start of MTX infusion

- Hour 42: Leucovorin 15 mg/m^2 IV bolus every 6 hours until methotrexate level is non-detectable or <0.1 micromolar (as per institutional standard). Once MTX level is non-detectable (or <0.1 micromolar), discontinue leucovorin and IV hydration/alkalinization.
- Hour 48: Check methotrexate level. Continue to check every 24 hours until methotrexate level non-detectable or <0.1 micromolar, as per institutional standard.

Dose adjustments for high methotrexate levels

- Hour 24: If level > 100 micromolar, increase total fluids to at least 200 mL/m^2/hour and start leucovorin 100 mg/m^2/dose IV bolus every 3 hours. Check MTX level every 12 hours if possible. Continue at this dose of leucovorin until level is < 5 micromolar, then reduce dose of leucovorin to 15 mg/m^2 IV every 3 hours. Continue this dose of leucovorin until level is non-detectable or <0.1 micromolar (as per institutional standard).
- Hour 48: If level > 0.18 but < 5 micromolar, increase total fluids to at least 200 mL/m^2/hour and continue leucovorin at 15 mg/m^2 IV every 6 hours. If level > 5 micromolar, increase total fluids to at least 200 mL/m^2/hour and change leucovorin dosing to 15 mg/m^2/dose IV every 3 hours. Continue this dose of leucovorin until level is non-detectable or <0.1 micromolar (as per institutional standard).
- Hour 72: If level > 0.1 but < 5 micromolar, ensure total fluids are at least 200 mL/m2/hour and continue leucovorin at 15 mg/m^2/dose IV every 6 hours. If level > 5 micromolar, ensure total fluids are at least 200 mL/m^2/hour and change leucovorin dosing to 15 mg/m^2/dose IV every 3 hours. Continue this dose of leucovorin until level is non-detectable or <0.1 micromolar (as per institutional standard).

Note: Dosing guidelines for fluids and leucovorin (above) may be modified depending on patient's clinical status (such as rising creatinine, new effusions, etc). In rare circumstances, methotrexate infusion may be stopped early in the event of excessive toxicity (such as rising creatinine). Any modifications should be discussed in advance with Lewis Silverman, MD, or designee.

6.11 CONSOLIDATION I: VERY HIGH RISK PATIENTS

Consolidation I Therapy for Very High Risk Patients consists of 3 phases

- Consolidation IA: vincristine, 6MP, doxorubicin/dexrazoxane, high dose methotrexate, ITM
- Consolidation IB: cyclophosphamide, low-dose cytarabine, 6MP, ITM
- Consolidation IC: high-dose cytarabine, etoposide, dexamethasone, asparaginase

6.11.1 Starting Time: Patients must be in documented remission at all sites.

6.11.2 Starting Criteria (Cycle IA): APC≥ 1,000, platelets ≥ 100,000, direct bilirubin ≤ 1.4 mg/dL (23.9 micromoles/L), SGOT ≤ 8 x normal, creatinine normal for age, no mucositis, no ascites/effusions/significant edema.

Suggested supportive care:

- Bactrim should be held beginning on day of HD MTX infusion, and not re-started until MTX level is non-detectable detectable or <0.1 micromolar (as per institutional standard).
- Nonsteroidal anti-inflammatory medications should also be held beginning on day of HD MTX infusion, and not re-started until MTX level is non-detectable or <0.1 micromolar (as per institutional standard).
- MTX infusion should be started at least 8 hours and not more than 24 hours following the dose of doxorubicin.

See Section 7.2.4 for Dose Modifications. See Section 7.2.1 for allowable deviations in timing of medications/procedures and for allowable variation in dosing.

6.11.3 **Consolidation IA (VHR patients):**

Days	1	15	22
	V		
	6MP--------		
	Dd		
	ITM		
	HDM		

Note: Patients should continue to receive tapering doses of prednisone or methylprednisolone (as specified in Section 6.5) at the same time that they begin Consolidation I Phase.

6.11.4 Dosages, Schedules & Routes

V Vincristine 2 mg/m^2 (maximum dose 2 mg) IV push or IV via minibag on Day 1

6MP 6-mercaptopurine 50 mg/m^2/day orally nightly x 14 consecutive days beginning on Day 1. (Suggested administration guidelines: Give on an empty stomach, at least 2 hours after and 1 hour before food and/or milk). Note: If patient taking tablets, daily dose may be adjusted to accommodate available tablet strengths to achieve total 14-day amount.

Dd Doxorubicin 30 mg/m^2 IV preceded by dexrazoxane 300 mg/m^2 IV on Day 1.
Suggested administration for both drugs:

- Minute 0 Dexrazoxane over 15 minutes
- Minute 15 Doxorubicin IV push via central line or freshly placed peripheral IV
- Goal no more than 30 minutes maximum elapsed administration time combined for both drugs
- May give doxorubicin prior to dexrazoxane if given via peripheral IV
- May give doxorubicin IV over 15 minutes if given via central line

ITM Intrathecal Methotrexate. Administer on Day 1, prior to starting methotrexate infusion if possible. (**Note:** Induction Day 32 ITM can be considered to be Day 1 Consolidation I ITM if given within 72 hours of beginning Consolidation IA systemic chemotherapy).
Dose according to age:

Age	1 - 1.99yrs	2 - 2.99 yrs	≥ 3 yrs
Methotrexate	8 mg	10 mg	12 mg

- Mix in 6 ml preservative-free normal saline (or use institutional guidelines for dilution).
- Intrathecal medications should be filtered prior to or during administration or according to institute policy for safe administration.

HDM High dose methotrexate. **Begin infusion at least 8 hours but no more than 24 hours following the dose of doxorubicin**

- Prehydrate with IVF containing NaHCO3 until urine specific gravity is ≤ 1.010 and pH is ≥ 7.0 and ≤ 8.0. Suggested prehydration: D5W + 75 mEq NaHCO3/L at 125 mL/m^2/hour for 6 hours
- Hour 0: Total fluid while receiving methotrexate infusion: patient should receive total fluids of at least 125 mL/m^2/hour with NaHCO3-containing fluids beginning at Hour 0 and continuing until methotrexate level non-detectable or <0.1 micromolar (as per institutional standard). Fluid volume may be adjusted to maintain dilute urine (sg ≤ 1.010) until methotrexate non-detectable (or <0.1 micromolar). Fluid should contain NaHCO3, and amount of NaHCO3 should be adjusted to maintain urine pH ≥ 7 and ≤ 8. Suggested starting concurrent IVF: D5W + 75 mEq NaHCO3/L at 125 mL/m^2/hour
  - Hour 0: Methotrexate 0.5 grams/m^2 IV over 30 minutes. Suggested diluent: 10 mL D5W + 75 mEq NaHCO3/L to run at 20 mL/hour (along with concurrent IVF).
  - Hour 0.5-24: Methotrexate 4.5 grams/m^2 continuous infusion IV over 23.5 hours. Suggested diluent: 470 mL D5W + 75 mEq NaHCO3/L to run at 20 mL/hour for 23.5 hours (along with concurrent IVF).
  - Hour 24: Check MTX level (**Note: check level at completion of infusion).**

- Hour 36: Leucovorin 75 mg/m^2 IV bolus x 1 dose, given 36 hours after start of MTX infusion
- Hour 42: Leucovorin 15 mg/m^2 IV bolus every 6 hours until methotrexate level is non-detectable. Once MTX level is non-detectable (or <0.1 micromolar), discontinue leucovorin and IV hydration/alkalinization or <0.1 micromolar (as per institutional standard).
- Hour 48: Check methotrexate level. Continue to check every 24 hours until methotrexate level non-detectable or <0.1 micromolar, as per institutional standard.

Dose adjustments for high methotrexate levels

- Hour 24: If level > 100 micromolar, increase total fluids to at least 200 mL/m^2/hour and start leucovorin 100 mg/m^2/dose IV bolus every 3 hours. Check MTX level every 12 hours. Continue at this dose of leucovorin until level is < 5 micromolar, then reduce dose of leucovorin to 15 mg/m^2 IV every 3 hours. Continue this dose of leucovorin until level is non-detectable or <0.1 micromolar (as per institutional standard).
- Hour 48: If level > 0.18 but < 5 micromolar, increase total fluids to at least 200 mL/m^2/hour and continue leucovorin at 15 mg/m^2 IV every 6 hours. If level > 5 micromolar, increase total fluids to at least 200 mL/m^2/hour and change leucovorin dosing to 15 mg/m^2/dose IV every 3 hours. Continue this dose of leucovorin until level is non-detectable or <0.1 micromolar (as per institutional standard).
- Hour 72: If level > 0.1 but < 5 micromolar, ensure total fluids are at least 200 mL/m2/hour and continue leucovorin at 15 mg/m^2/dose IV every 6 hours. If level > 5 micromolar, ensure total fluids are at least 200 mL/m2/hour and change leucovorin dosing to 15 mg/m^2/dose IV every 3 hours. Continue this dose of leucovorin until level is non-detectable or <0.1 micromolar (as per institutional standard).

Note: Dosing guidelines for fluids and leucovorin (above) may be modified depending on patient's clinical status (such as rising creatinine, new effusions, etc). In rare circumstances, methotrexate infusion may be stopped early in the event of excessive toxicity (such as rising creatinine). Any modifications should be discussed in advance with Lewis Silverman, MD, or designee.

6.11.5 **Consolidation IB (VHR patients)**

The second phase of VHR Consolidation I (Consolidation IB) may start approximately 21 days after the start of Consolidation IA, and only when there is no or nearly fully resolved mucositis and when APC ≥ 750, and platelets ≥ 75,000 and rising. Other starting criteria for starting Consolidation IB: creatinine normal for age, SGOT ≤ 8 x normal, direct bilirubin ≤ 1.4 mg/dL(23.9 micromoles/L).

Peripheral blood for MRD should be drawn prior to starting cycle. See Section 9.2.

Days	1	2…	9…	15
	Cyc			
		Ara	Ara	
	6MP----------			
	ITM			

6.11.6 Dosages, Schedules & Routes

Cyc Cyclophosphamide 1000 mg/m^2 IV over 1 hour on Day 1
Suggested administration:

- Prehdyration: NS 20 mL/kg IV over 1 hour, then D5 ½ NS at 125 ml/m^2/hour until urine s.g. < 1.010
- **Optional: Prior to hour 0 -** Mesna 360 mg/m^2 IV over 15 minutes in concurrent IVF just prior to Cyclophosphamide.
- Hour 0: Cyclophosphamide 1000 mg/m^2 IV in 125 mL/m^2 D5 ½ NS over 1 hour
- Posthydration (Begin Hour 1): D5 ½ NS at 125 mL/m2/hour x 4 hours
- **Optional:** Hour 3: Mesna 360 mg/m^2 IV over 15 minutes in concurrent IVF

Note: Use of Mesna is not required, and its administration is at the discretion of treating physician. Duration of pre- and post-hydration is also at the discretion of treating physician.

Ara Cytarabine (ara-C) 75 mg/m^2/day IV push on Days 2-5 and 9-12.

6MP 6-mercaptopurine 50 mg/m^2/day orally nightly x 14 consecutive days beginning on Day 1. (Suggested administration guidelines: Give on an empty stomach, at least 2 hours after and 1 hour before food and/or milk). Note: If patient taking tablets, daily dose may be adjusted to accommodate available tablet strengths to achieve total 14-day amount.

ITM IT methotrexate. Administer on Day 1.

Dose according to age:

Age	1 - 1.99yrs	2 - 2.99yrs	≥ 3yrs
Methotrexate	8mg	10mg	12mg

- Mix in 6 ml preservative-free normal saline (or use institutional guidelines for dilution).
- Intrathecal medications should be filtered prior to or during administration or according to institute policy for safe administration.

6.11.7 **Consolidation IC (VHR patients)**

The third phase of VHR Consolidation I (Consolidation IC) should start approximately 21 days after the start of Consolidation IB, and only when there is no or nearly fully resolved mucositis and when APC ≥ 750 and platelets ≥ 75,000, and rising.

Criteria to adnimister Day 8 Asparaginase: Direct bilirubin ≤ 1.4 mg/dL (23.9 micromoles/L). Suggestion: check amylase prior to administration of asparaginase.

Peripheral blood for MRD should be drawn prior to starting cycle. See Section 9.2.

Days	1	2	3	4	5…	8
	HDAra	HDAra				
			Etop	Etop	Etop	
	Dex----------					
						ASP

6.11.8 Dosages, Schedules & Routes

HDAra High-dose Cytarabine (ara-C) 2 grams/m^2/dose IV every 12 hours for a total of four doses beginning on Day 1. Suggested Administration Guidelines: Give each dose of cytarabine in 300 mL/m^2 D5 ¼ NS over 3 hours at 100 mL/m^2/hour

Suggested Supportive Care: Dexamethasone ophthalmic ointment/drops: apply to both eyes every 6 hours beginning with 1st dose of Cytarabine and continue until 48 hours after completion of Cytarabine.

Etop Etoposide (VP16) 100 mg/m^2/dose IV daily on Days 3, 4, and 5 (three doses total). Suggested Adminstration Guidelines: Give each dose in 250 mL/m^2 D5 1/2 NS over 1 hour.

Dex Dexamethasone 18 mg/m^2/day orally or IV divided twice daily (bid) on Days 1-5 (ten total doses)

ASP Asparaginase according to randomization, beginning on Day 8. Mix in 100 mL Normal Saline and give over 1 hour.

A. SC-PEG Asparaginase (Calaspargase Pegol) 2500 IU/m^2/dose IV every 3 weeks,
or
B. Oncaspar 2500 IU/m^2/dose IV every 2 weeks.

Note: Asparaginase should continue through CNS phase and Consolidation II phase for 30 total weeks (10 total post-induction doses of SC-PEG given every 3-weeks or 15 total post-induction doses of Oncaspar given every 2-weeks).

Note: All patients should have asparaginase enzyme (PK) samples obtained immediately prior to first dose of asparaginase and then prior to every subsequent dose of asparaginase.. Asparaginase antibody sample should also be drawn immediately prior to first dose of asparaginase (see Section 9.2.3).

6.12 CNS PHASE FOR STANDARD RISK PATIENTS

6.12.1 Starting time: 21 days from start of Consolidation I, as soon as starting criteria are met. See Section 7.2.1.2 for allowable deviations in timing.

6.12.2 Starting Criteria: APC≥ 1,000, platelets ≥ 100,000, direct bilirubin ≤ 1.4 mg/dL (23.9 micromoles/L), SGOT ≤ 8 x normal, no or nearly fully resolved mucositis. Suggestion: check amylase prior to administration of asparaginase).

Note: Asparaginase should only be administered on Day 1 for SR patients if all criteria for starting CNS phase are met.

See Section 7.2.7 for Dose Modifications. See Section 7.2.1 for allowable deviations in timing of medications/procedures and for allowable variation in dosing.

6.12.3 Dosages, Schedules & Routes

- Vincristine 2 mg/m^2 (maximum dose 2 mg) IV push or IV via minibag x 1 dose on Day 1.

- 6-mercaptopurine 50 mg/m^2/day orally nightly x 14 consecutive days beginning on Day 1. (Suggested administration guidelines: Give on an empty stomach, at least 2 hours after and 1 hour before food and/or milk). Note: If patient taking tablets, daily dose may be adjusted to accommodate available tablet strengths to achieve total 14-day amount.

- Dexamethasone 6 mg/m^2/day orally divided twice daily (bid) for 5 days beginning on Day 1.

- Asparaginase according to randomization on Day 1. Mix in 100 mL Normal Saline and give over 1 hour.

  A. SC-PEG Asparaginase (Calaspargase Pegol) 2500 IU/m^2/dose IV every 3 weeks,
  or
  B. Oncaspar 2500 IU/m^2/dose IV every 2 weeks.

Note: Asparaginase should continue through CNS phase and Consolidation II phase for 30 total weeks (10 total post-induction doses of SC-PEG given every 3-weeks or 15 total post-induction doses of Oncaspar given every 2-weeks). Direct bilirubin should be ≤ 1.4 mg/dL (23.9 micromoles/L) for all doses of asparaginase, including 2nd dose of asparaginase in the CNS phase.

Note: All patients should have asparaginase enzyme (PK) samples obtained immediately prior to first dose of asparaginase and then prior to every subsequent dose of asparaginase. Asparaginase antibody sample should also be drawn immediately prior to first dose of asparaginase (see Section 9.2.3).

- Intrathecal Methotrexate/Cytarabine/Hydrocortisone twice weekly x 4 doses

Age	1 - 1.99 yrs	2 - 2.99yrs	≥ 3yrs
Methotrexate	8mg	10mg	12mg
Cytarabine	20mg	30mg	40mg
Hydrocortisone	9mg	12mg	15mg

- Mix in 6 ml preservative-free normal saline (or use institutional guidelines for dilution).
- Intrathecal medications should be filtered prior to or during administration or according to institute policy for safe administration.

6.13 CNS PHASE: HIGH RISK AND VERY HIGH RISK PATIENTS

6.13.1 Starting time: 21 days from start of Consolidation I, as soon as starting criteria are met. For VHR patients, starting time is 21 days from start of **Consolidation IC**. See Section 7.2.1.2 for allowable deviations in timing.

6.13.2 Starting Criteria: APC≥ 1,000, platelets ≥ 100,000, direct bilirubin ≤ 1.4 mg/dL (23.9 micromoles/L), SGOT ≤ 8 x normal, no or nearly fully resolved mucositis. Suggestion: check amylase prior to administration of asparaginase.

Note: Asparaginase should only be administered on Day 1 for HR patients if all criteria for starting CNS phase are met.

For VHR leukemia patients and T-ALL patients: Bone marrow aspirate for MRD assessment should be performed at the same time as first lumbar puncture of CNS phase and blood for MRD should be drawn prior to starting cycle. **See Section 9.2. Note: This bone marrow aspirate is optional for non-VHR T-ALL patients, but required for VHR leukemia patients.**

See Section 7.2.7 for Dose Modifications. See Section 7.2.1 for allowable deviations in timing of medications/procedures and for allowable variation in dosing.

6.13.3 Dosages, Schedules & Routes

- Vincristine 2 mg/m^2 (maximum dose 2 mg) IV push or IV via minibag x 1 dose on Day 1.
- Doxorubicin 30 mg/m^2 IV bolus and Dexrazoxane 300 mg/m^2 IV bolus. Give on Day 1.

  Suggested administration for both drugs:
  - Minute 0 Dexrazoxane over 15 minutes
  - Minute 15 Doxorubicin IV push via central line or freshly placed peripheral IV
  - Goal no more than 30 minutes maximum elapsed administration time combined for both drugs
  - May give doxorubicin prior to dexrazoxane if given via peripheral IV
  - May give doxorubicin IV over 15 minutes if given via central line

- 6-mercaptopurine 50 mg/m^2/day orally nightly x 14 consecutive days beginning on Day 1. (Suggested administration guidelines: Give on an empty stomach, at least 2 hours after and 1 hour before food and/or milk). Note: If patient taking tablets, daily dose may be adjusted to accommodate available tablet strengths to achieve total 14-day amount.

- Dexamethasone 18 mg/m^2/day orally divided twice daily (bid) for 5 days beginning on Day 1.

- Asparaginase according to randomization on Day 1. Mix in 100 mL Normal Saline and give over 1 hour.

A. SC-PEG Asparaginase (Calaspargase Pegol) 2500 IU/m^2/dose IV every 3 weeks,
or
B. Oncaspar 2500 IU/m^2/dose IV every 2 weeks.

Note: Asparaginase should continue through CNS phase and Consolidation II phase for 30 total weeks (10 total post-induction doses of SC-PEG given every 3-weeks or 15 total post-induction doses of Oncaspar given every 2-weeks). Direct bilirubin should be ≤ 1.4 mg/dL (23.9 micromoles/L) for all doses of asparaginase, including 2nd dose of asparaginase in the CNS phase.Note: VHR patients who began asparaginase during the Consolidation IC phase should continue to receive asparaginase per randomization at the frequency indicated in Section 6.11.7: that is, during the CNS phase, asparaginase should only be administered 21 days after the previous dose of SC-PEG Asparaginase or 14 days after the previous dose of Oncaspar (and not necessarily on Day 1 of CNS phase).

Note: All patients should have asparaginase enzyme (PK) samples obtained immediately prior to first dose of asparaginase and then prior to every subsequent dose of asparaginase. Asparaginase antibody sample should also be drawn immediately prior to first dose of asparaginase (see Section 9.2.3).

- Intrathecal Methotrexate/Cytarabine/Hydrocortisone twice weekly x 4 doses

Age	1 - 1.99 yrs	2 - 2.99 yrs	≥ 3 yrs
Methotrexate	8 mg	10 mg	12 mg
Cytarabine	20 mg	30 mg	40 mg
Hydrocortisone	9 mg	12 mg	15 mg

  - Mix in 6 ml preservative-free normal saline (or use institutional guidelines for dilution).
  - Intrathecal medications should be filtered prior to or during administration or according to institute policy for safe administration.

6.13.4 **Cranial Radiation**:

To be administered only to:

- VHR patients and
- HR B-precursor ALL patients with any of the following criteria:
  - CNS-3 at diagnosis or at Day 18 (or any time after Day 18 but prior to Day 32)
  - CNS-2 at Day 18 or at end of remission induction
- T-ALL (all patients, regardless of CNS status)
- Lymphoblastic lymphoma patients (regardless of phenotype) who meet any of the following criteria:

- CNS-3 at diagnosis or at Day 18 (or any time after Day 18 but prior to Day 32)
- CNS-2 at Day 18 or at end of remission induction

- Dosage of Cranial Radiation: 1200 cGy cranial radiation delivered as 150 cGy daily fractions for 8 days. NOTE: Cranial radiation should start as close as possible to the administration of doxorubicin/vincristine.

  - Exception: Dosage of 1800 cGy (180 cGy fractions for 10 days) should be administered to patients with the following characteristics:
    - CNS-3 at diagnosis or at Day 18
    - CNS-2 at Day 18 or at end of remission induction

  See Table 6.13 (below) for summary of radiation dose for high risk/very high risk patients based on presenting characteristics, CNS status

TABLE 6.13: RADIATION DOSE FOR HIGH RISK/VERY HIGH RISK PATIENTS

PATIENT CHARACTERISTICS	Radiation Dose
High Risk B-precursor ALL ▪ CNS-1 at Days 1, 18 **and** 32, or ▪ CNS-2 at Day 1 but clear by Day 18	None
High-Risk B-precursor or T-cell Lymphoblastic Lymphoma ▪ CNS-1 at Days 1, 18 **and** 32, or ▪ CNS-2 at Day 1 but clear by Day 18	None
High-Risk T-cell ALL ▪ CNS-1 at Days 1, 18 **and** 32, or ▪ CNS-2 at Day 1 but clear by Day 18	1200 cGy
High Risk ALL or Lymphoblastic Lymphoma, any phenotype ▪ CNS-2 on Day 18 **or** 32 ▪ CNS-3 on Day 1 **or** 18, or ▪ Cranial nerve palsy at diagnosis	1800 cGy
Very High Risk ALL or Lymphoblastic Lymphoma ▪ CNS-1 at Days 1, 18 **and** 32, or ▪ CNS-2 at Day 1 but clear by Day 18	1200 cGy
Very High Risk ALL or Lymphoblastic Lymphoma ▪ CNS-2 at Days 18 or 32, or ▪ CNS-3 at Days 1 **or** 18, or ▪ Cranial nerve palsy at diagnosis	1800 cGy

CNS-1: no blast cells in cytospin, regardless of CSF cell count
CNS-2: Fewer than 5 WBC on CSF cell count, with blasts on cytospin
CNS-3: 5 or more WBC on CSF cell count, with blasts on cytospin
Traumatic LP: > 10 RBC on CSF cell count. See Section 5.16 for interpretation of traumatic LP with blasts.

6.14 CONSOLIDATION II PHASE

6.14.1 Starting Time for All Patients

Starting time: after recovery from CNS Therapy (21 days from start of systemic chemotherapy administered during CNS Therapy and as soon as starting criteria are met). See Section 7.2.1.2 for allowable deviations in starting time.

6.14.2 Criteria to begin Consolidation II Cycles

APC = (Total WBC) x %(Monos + Polys + Bands)

- To begin 1st week of each 3-week chemotherapy cycle:
  - APC $\geq$ 750/mm^3
  - Platelets $\geq$ 75,000/mm^3
  - Mucositis: none or mild (defined in Section 7.2.8.3).
  - SGOT $\leq$ 8 x normal
  - Direct bilirubin $\leq$ 1.4 mg/dl (23.9 micromoles/L)

- If criteria are not met, hold chemo for 1-7 days, and then re-check.
- In unusual circumstances, chemotherapy cycles may be started even when not all of these criteria have been met if approved in advance by Principal Investigator or designee.
- Note: These criteria do not apply to scheduled doses of asparaginase.

6.14.3 Criteria to continue cycle (Days 2 - 21):

- APC $\geq$ 500/mm^3
- Platelets $\geq$ 50,000/mm^3
- Mucositis: none or mild
- SGOT $\leq$ 8 x normal
- Direct bilirubin $\leq$ 1.4 mg/dl (23.9 micromoles/L)

- Note: These criteria do not apply to scheduled doses of asparaginase

6.14.4 Criteria to administer asparaginase

- No clinical pancreatitis
- No new, untreated deep venous thrombosis
- Direct bilirubin $\leq$ 1.4 mg/dL (23.9 micromoles/L)
- Note: Asparaginase should be administered regardless of blood counts, mucositis, or SGOT.

See Section 7.2.8 for Dose Modifications. See Section 7.2.1 for allowable deviations in timing of medications/procedures and for allowable variation in dosing.

6.14.5 CONSOLIDATION II THERAPY FOR STANDARD RISK PATIENTS

Week	1*	2	3	4	5	6 . . . .
	V Dex 6MP m	6MP m	m	V Dex 6MP m	6MP m	m
Arm A ASP	SC-PEG			SC-PEG		
Arm B ASP	Oncaspar		Oncaspar		Oncaspar	

Continue Asparaginase (according to randomization) for 30 weeks (10 post-induction doses of SC-PEG or 15 post-induction doses of Oncaspar). Proceed to Continuation Therapy after 30 post-remission weeks of asparaginase have been administered.

*** IT MAH - to be given every 9 weeks x 6 doses. First lumbar puncture with IT MAH during Consolidation II is 9 weeks after start of CNS treatment. After 6 doses of IT MAH (given every 9 weeks) completed, change to IT MAH every 18 weeks until end of therapy. IT medications should only be given at the start of a chemotherapy cycle.**

All outpatient chemotherapy for each cycle must be prescribed by a physician, nurse practitioner or physician assistant, as per institutional standard, on the day that the patient is evaluated and found to meet criteria for that cycle. This means that only one cycle of outpatient chemotherapy may be prescribed at each visit, and refills for chemotherapy prescriptions are not allowed for study patients. Exception can be made for patients receiving Purixan® (6-MP oral suspension) who must be dispensed full bottles per medical insurance restrictions; in these cases, medical staff should review dosing with patient/family at the start of each cycle. Under no circumstances may other clinical staff call in prescriptions for chemotherapy for patients on study.

See Section 7.2.8 for Dose Modifications. Allowable delays in chemotherapy/procedures and allowable variation in dosing specified in Section 7.2.1.

Dosages, Schedules & Routes

V	Vincristine	2 mg/m^2 (maximum dose 2 mg) IV push or IV via minibag on Day 1 of cycle
Dex	Dexamethasone	6 mg/m^2/day orally divided twice daily (bid) for 5 days on beginning on Day 1 of cycle
6MP	6-mercaptopurine	50 mg/m^2/day orally nightly x 14 consecutive days beginning on Day 1. (Suggested administration guidelines: Give on an empty stomach, at least 2 hours after and 1 hour before food and/or milk). Note: If patient taking tablets, daily dose may be adjusted to

accommodate available tablet strengths to achieve total 14-day amount.

m Methotrexate 30 mg/m^2 IV push or IM once a week (Days 1, 8 and 15 of each cycle). Hold systemic methotrexate on the week IT Methotrexate is given.

ASP Asparaginase according to randomization. Mix in 100 mL Normal Saline and give over 1 hour.

A. SC-PEG Asparaginase (Calaspargase Pegol) 2500 IU/m^2/dose IV every 3 weeks,
or
B. Oncaspar 2500 IU/m^2/dose IV every 2 weeks.

Note: Administer asparaginase only if direct bilirubin ≤ 1.4 mg/dL (23.9 micromoles/L). Asparaginae may be administered regardless of blood counts (see Section 6.14.4 for full criteria).

Note: Asparaginase should continue through Consolidation II phase for 30 total weeks (10 total post-induction doses of SC-PEG given every 3-weeks or 15 total post-induction doses of Oncaspar given every 2-weeks).

Note: All patients should have asparaginase enzyme (PK) samples obtained immediately prior each dose of asparaginase. Asparaginase antibody samples should be drawn during the Consolidation II phase at two time points as indicated in Section 9.2.3.

IT MAH Intrathecal Methotrexate/Cytarabine/Hydrocortisone

Give as close to every 9 weeks as possible for 6 doses, then give as close to every 18 weeks. Always give IT medications at the start of a chemo cycle. First dose is given 9 weeks after first lumbar puncture administered during CNS treatment.

Age	1 - 1.99yrs	2 - 2.99 yrs	≥ 3 yrs
Methotrexate	8 mg	10 mg	12 mg
Cytarabine	20 mg	30 mg	40 mg
Hydrocortisone	9 mg	12 mg	15 mg

- Mix in 6 ml preservative-free normal saline (or use institutional guidelines for dilution).
- Intrathecal medications should be filtered prior to or during administration or according to institute policy for safe administration.
- Hold systemic methotrexate on the week IT Methotrexate is given

6.14.6 CONSOLIDATION II THERAPY FOR HIGH RISK AND VERY HIGH RISK PATIENTS

Week	1 V Dex 6MP Dd (m)**	2 6MP	3	4 V Dex 6MP D(d)	5 6MP	6 . . . .
Arm A ASP	SC-PEG			SC-PEG		
Arm B ASP	Oncaspar		Oncaspar		Oncaspar	

Continue Asparaginase (according to randomization) for 30 weeks (10 post-induction doses of SC-PEG or 15 post-induction doses of Oncaspar).

- **Repeat this 3 week sequence of treatment until ready for next phase of therapy. Proceed to Continuation Therapy after all of the following have been administered:**
  - **Cumulative doxorubicin dosage of 300 mg/m^2 +/- 15 mg/m^2**
  - **30 post-remission weeks of asparaginase**
  - **10 cycles with high-dose dexamethasone (18 mg/m^2/day), including dexamethasone administered during the CNS phase (but, for VHR patients, not including dexamethasone administered during Consolidation IC phase).**

IT MAH – frequency of administration depends on whether or not patient received cranial radiation. If no cranial radiation, first IT dose is given 9 weeks after first lumbar puncture during CNS Therapy. If patient received cranial radiation, first IT dose is given 18 weeks after first lumbar puncture during CNS Therapy. IT medications should only be given at the start of a chemotherapy cycle.

All outpatient chemotherapy for each cycle must be prescribed by a physician, nurse practitioner or physician assistant, as per institutional standard, on the day that the patient is evaluated and found to meet criteria for that cycle. This means that only one cycle of outpatient chemotherapy may be prescribed at each visit, and refills for chemotherapy prescriptions are not allowed for study patients. Exception can be made for patients receiving Purixan® (6-MP oral suspension) who must be dispensed full bottles per medical insurance restrictions; in these cases, medical staff should review dosing with patient/family at the start of each cycle. Under no circumstances may other clinical staff call in prescriptions for chemotherapy for patients on study.

Allowable delays in chemotherapy/procedures specified in Section 7.2.

See Section 7.2.8 for Dose Modifications. Allowable delays in chemotherapy/procedures and allowable variation in dosing specified in Section 7.2.1.

Dosages, Schedules & Routes

V Vincristine 2 mg/m^2 (maximum dose 2 mg) IV push or IV via minibag on Day 1 of cycle

Dex Dexamethasone 18 mg/m^2/day orally divided twice daily (bid) for 5 days beginning on Day 1 of cycle.

Note: Patients should receive a total of 10 cycles with high-dose dexamethasone (18 mg/m2/day), including the CNS phase (but not including the Consolidation IC phase for VHR patients). After 10 cycles have been administered at this dose, the dose of dexamethasone for all subsequent cycles should be 6 mg/m^2/day orally divided twice daily (bid) for 5 days beginning on Day 1 of chemotherapy cycle, even if patientis still reciving Asparaginase and/or Doxorubicin.

6MP 6-mercaptopurine 50 mg/m^2/day orally nightly x 14 consecutive days beginning on Day 1. (Suggested administration guidelines: Give on an empty stomach, at least 2 hours after and 1 hour before food and/or milk). Note: If patient taking tablets, daily dose may be adjusted to accommodate available tablet strengths to achieve total 14-day amount.

Dd Doxorubicin 30 mg/m^2 IV preceded by Dexrazoxane 300 mg/m^2 IV on Day 1 of cycle.

Suggested administration for both drugs:

- Minute 0 Dexrazoxane over 15 minutes
- Minute 15 Doxorubicin IV push via central line or freshly placed peripheral IV
- Goal: no more than 30 minutes maximum elapsed administration time combined for both drugs
- May give doxorubicin prior to dexrazoxane if given via peripheral IV
- May give doxorubicin IV over 15 minutes if given via central line

****Stop doxorubicin when total cumulative dose is 300 mg/m^2 ± 15mg/m^2 (cumulative dose of 300 mg/m^2+ 15 mg/m^2 is to allow for variability in cumulative drug dosing; for those patients receiving 24 mg/m^2 as a result of dose reduction it is not possible to receive exactly 300 mg/m^2). After achieving cumulative dose of 300 mg/m^2 ± 15 mg/m^2 of doxorubicin, begin weekly methotrexate at the start of the next cycle as indicated below.**

When doxorubicin completed and weekly methotrexate started, give as follows:

m Methotrexate 30 mg/m^2 IV push or IM once a week, beginning on Day 1 of a cycle. Maximum dose for previously irradiated patients is 40 mg/m^2. Hold systemic methotrexate on the week IT Methotrexate is given.

ASP Asparaginase according to randomization. Mix in 100 mL Normal Saline and give over 1 hour.

A. SC-PEG Asparaginase (Calaspargase Pegol) 2500 IU/m^2/dose IV every 3 weeks,
or
B. Oncaspar 2500 IU/m^2/dose IV every 2 weeks.

Note: Administer asparaginase only if direct bilirubin ≤ 1.4 mg/dL (23.9 micromoles/L) . Asparaginae may be administered regardless of blood counts (see Section 6.14.4 for full criteria).

Note: Asparaginase should continue through Consolidation II phase for 30 total weeks (10 total post-induction doses of SC-PEG given every 3-weeks or 15 total post-induction doses of Oncaspar given every 2-weeks).

Note: All patients should have asparaginase enzyme (PK) samples obtained immediately prior each dose of asparaginase. Asparaginase antibody samples should be drawn once mid-Consolidation II and once a the end of Consoldiation II (see section 9.2.3).

IT MAH Intrathecal Methotrexate/Cytarabine/Hydrocortisone

No prior cranial radiation: Give as close to every 9 weeks as possible for 6 doses, then give as close to every 18 weeks. Always give IT medications at the start of a chemo cycle. First dose is given 9 weeks after first lumbar puncture administered during CNS treatment.

Prior cranial radiation: Give as close to every 18 weeks as possible - at the start of a chemo cycle. First dose is given 18 weeks after first lumbar puncture administered during CNS treatment.

Age	1 - 1.99 yrs	2 - 2.99 yrs	≥ 3 yrs
Methotrexate	8 mg	10 mg	12 mg
Cytarabine	20 mg	30 mg	40 mg
Hydrocortisone	9 mg	12 mg	15 mg

- Mix in 6 ml preservative-free normal saline (or use institutional guidelines for dilution).
- Intrathecal medications should be filtered prior to or during administration or according to institute policy for safe administration
- Hold systemic methotrexate on the week IT Methotrexate is given

6.15 CONTINUATION PHASE (for Standard, High Risk and Very High Risk Patients)

6.15.1 Starting Time: Completion of all components of Consolidation II Phase
For SR: Completion of all post-induction doses of asparaginase
For HR/VHR: Completion of all post-induction doses of asparaginase, administration of 10 cycles high-dose dexamethasone (during CNS and Consolidation II phase), and administration of total cumulative dose of doxorubicin of 300 mg/m^2 ± 15 mg/m^2

6.15.2 Starting Criteria for Chemotherapy for All Patients

APC = (Total WBC) x %(Monos + Polys + Bands)

- To begin 1st week of each 3-week chemotherapy cycle:
  - APC ≥ 750/mm^3
  - Platelets ≥ 75,000/mm^3
  - Mucositis: none or mild (defined in Section 7.2.8.3).
  - SGOT ≤ 8 x normal
  - Direct bilirubin ≤ 1.4 mg/dl (23.9 micromoles/L)

- If criteria are not met, hold chemo for 1-7 days, and then re-check.
- In unusual circumstances, chemotherapy cycles may be started even when not all of these criteria have been met if approved in advance by Principal Investigator or designee.

6.15.3 Criteria to continue cycle (Days 2 - 21):
- APC ≥ 500/mm^3
- Platelets ≥ 50,000/mm^3
- Mucositis: none or mild
- SGOT ≤ 8 x normal
- Direct bilirubin ≤ 1.4 mg/dl (23.9 micromoles/L)

See Section 7.2.8 for Dose Modficiations. Allowable delays in chemotherapy/procedures and variation in dosing specified in Section 7.2.1.

6.15.4 **Continuation Phase Treatment Schema** (all patients)

Week	1	2	3
	V Dex 6MP m	6MP m	m

Repeat this 3-week sequence of treatment until 2 years of CCR.

Intrathecal medications at the start of cycles every 9-18 weeks, depending on prior cranial radiation. IT medications should only be given at the start of a chemotherapy cycle. Allowable delays in chemotherapy/procedures specified in Section 7.2.1.2.

All outpatient chemotherapy for each cycle must be prescribed by a physician, nurse practitioner or physician assistant, as per institutional standard, on the day that the patient is evaluated and found to meet criteria for that cycle. This means that only one cycle of outpatient chemotherapy may be prescribed at each visit, and refills for chemotherapy prescriptions are not allowed for study patients. Exception can be made for patients receiving Purixan® (6-MP oral suspension) who must be dispensed full bottles per medical insurance restrictions; in these cases, medical staff should review dosing with patient/family at the start of each cycle. Under no circumstances may other clinical staff call in prescriptions for chemotherapy for patients on study.

See Section 7.2.8 for Dose Modifications.

6.15.5 Dosages, Schedules & Routes

V	Vincristine	2 mg/m^2 (maximum dose 2 mg) IV push or IV via minibag on Day 1 of cycle
Dex	Dexamethasone	6 mg/m^2/day orally divided twice daily (bid) for 5 days beginning on Day 1 of cycle
6MP	6-mercaptopurine	50 mg/m^2/day orally nightly x 14 consecutive days beginning on Day 1. (Suggested administration guidelines: Give on an empty stomach, at least 2 hours after and 1 hour before food and/or milk). Note: If patient taking tablets, daily dose may be adjusted to accommodate available tablet strengths to achieve total 14-day amount.
m	Methotrexate	30 mg/m^2 IV push or IM once a week (Days 1, 8 and 15 of each cycle). Maximum dose for previously irradiated patients is 40 mg/m^2. Hold systemic methotrexate on the week IT Methotrexate is given.

IT MAH: Intrathecal Methotrexate/Cytarabine/Hydrocortisone

- **No prior Cranial Radiation**:
  Give as close to every 9 weeks as possible for 6 doses (post-CNS therapy), then give as close to every 18 weeks.

- **Previous Cranial Radiation:**
  Give as close to every 18 weeks as possible.

  Always give IT medications at the start of a chemotherapy cycle

Doses of IT medication:

Age	1 - 1.99 yrs	2 - 2.99 yrs	≥ 3 yrs
Methotrexate	8 mg	10 mg	12 mg
Cytarabine	20 mg	30 mg	40 mg
Hydrocortisone	9 mg	12 mg	15 mg

- Mix in 6 ml preservative-free normal saline (or use institutional guidelines for dilution).
- Intrathecal medications should be filtered prior to or during administration or according to institute policy for safe administration.
- Hold systemic methotrexate on the week IT Methotrexate is given

6.16 General Concomitant Medication and Supportive Care Guidelines

No investigational or commercial agents or therapies other than those described in this protocol document may be administered with the intent to treat the participant's leukemia.

6.16.1 Supportive Care at Diagnosis

6.16.1.1 IV Hydration and Alkalinization: Prior to the initiation of therapy, hydration with or without alkalinization (depending on institutional guidelines) to prevent acute tumor lysis syndrome is suggested. Institutional guidelines may be used to achieve adequate hydration and alkalinization. Suggested hydration: 1.5-2x maintenance IV hydration. If alkalinization is used, suggested goal urine pH 7-8 specific gravity < 1.010. Alkalinization may be omitted if patient is receiving rasburicase. IV hydration may be discontinued at the discretion of the treating physician when patient is considered no longer at risk for developing acute tumor lysis syndrome.

6.16.1.2 Allopurinol or Rasburicase: Patients should have uric acid levels closely monitored, and should begin either allopurinol or urate oxidase (rasburicase) to treat or prevent elevated uric acid levels. If allopurinol is used, the suggested dose is 200-400 mg/m^2/day. Intravenous rasburicase may be used, especially if there is evidence of ongoing significant tumor lysis syndrome, markedly elevated uric acid levels, hyperleukocytosis, inability to tolerate oral medications, or inability to tolerate vigorous IV fluid hydration. Rasburicase should not be administered to patients with known or suspected G6PD deficiency. Allopurinol and/or rasburicase may be discontinued at discretion of treating physician when patient is considered no longer at risk for developing acute tumor lysis syndrome.

6.16.1.3 Gastritis Prophylaxis: It is recommended that gastritis prophylaxis be instituted at onset of corticosteroid treatment to minimize gastric irritation. Options include oral antacids (eg, calcium carbonate), sucralfate or proton pump inhibitors. H_2 blockers are not prohibited, but are not recommended as they may contribute to myelosuppression.

6.16.1.4 Transfusions: Patients should receive transfusions of packed red blood cells and platelets if significant anemia or thrombocytopenia is present at diagnosis. Transfusion threshold may be per institutional standards. In general, it is suggested that platelet counts be at or above 50,000/uL at the time of a lumbar puncture or as per institutional guidelines.

Note that packed red blood cells may contribute to hyperviscosity, so red blood cell transfusion should be administered with caution in the setting of hyperleukocytosis. All blood products should be leukodepleted and irradiated.

6.16.1.5 Coagulopathy: It is suggested that a screen for disseminated intravascular coagulation (DIC) be sent at diagnosis, and abnormalities corrected with fresh frozen plasma and cryoprecipitate prior to undergoing invasive procedures, if possible.

6.16.1.6 Leukemic Eye Infiltrates: It is suggested that patients have an ophthalmologic evaluation at or soon after diagnosis to screen for leukemic infiltrates or retinal hemorrhages that may be impacting vision. It is not necessary to delay treatment prior to an ophthalmologic evaluation. If initial ophthalmologic examination positive for infiltrates, it is suggested that follow-up examinations be obtained to document improvement/resolution and no visual compromise. Patients with progressive infiltrates or visual impairments may receive emergent radiation to eyes.

6.16.1.7 No added salt diet: Consider prescribing "no added salt" diet at diagnosis and during the remission induction phase because of fluid retention associated with corticosteroids.

6.16.1.8 Stool softeners and Laxatives: Stool softeners and laxatives should be used prophylactically during remission induction to prevent vincristine-associated constipation.

6.16.2 Infection Prophylaxis and Management of Fever During Steroid Prophase and Remission Induction Phase (See Section 6.3)

6.16.2.1 Antibiotic prophylaxis

Antibiotic prophylaxis with a fluoroquinolone antibiotic (either levofloxaxin or moxifloxacin) should be initiated during the Steroid Prophase and continue throughout the Remission Induction phase until count recovery after expected hematologic nadir (ANC $\geq$ 200 or APC $\geq$ 500/mm^3). Patients unable to tolerate fluoroquinolones due to allergy or other toxicity should receive intravenous cefepime as prophylaxis. If a patient is unable to tolerate fluoroquinolone or cefepime, contact Principal Investigator. Patients receiving broad-spectrum antibiotics for fever or documented infection may discontinue prophylactic antibiotics.

Because of the high risk of infection, treatment should begin on an inpatient basis and hospitalization of participants is encouraged throughout the induction phase.

Outpatient treatment during the induction phase may be considered after recovery of the absolute neutrophil count (ANC) after the expected hematologic nadir.

6.16.2.2 Fever

All patients with fever (defined as ≥ 38.5°C on a single occasion, or > 38°C on two occasions within 12 hours, or as per institutional guidelines) should be hospitalized and treated immediately with broad spectrum antibiotics, regardless of absolute neutrophil count. It is recommended that empiric coverage consist of broad-spectrum antibiotic(s) (eg, cefepime or ceftazidime, or an aminoglycoside plus extended-spectrum penicillin, or as per institutional standard). Once initiated, empiric antibiotics should be continued until ANC recovery past expected hematologic nadir, although the exact agents used may be modified per discretion of treating clinician. Antifungal therapy should be started for the persistence of fever, or emergence of new fever in neutropenic patients after 3 days of broad-spectrum antibiotic coverage, or sooner based on clinical status as determined by treating clinician, or per institutional guidelines. Radiographic studies should be considered for patients with persistent or recurrent fever to screen for invasive fungal disease, bacterial abcesses, or other infections.

6.16.3 Suggested Supportive Care During Treatment

6.16.3.1 Pneumocystis Prophylaxis:

Trimethoprim/sulfamethoxazole (Bactrim) prophylaxis should be instituted for all children from the time they enter complete remission and continued until 6 months after the cessation of treatment. Prophylactic trimethoprim/sulfamethoxazole (Bactrim) dosage is 5 mg/kg/day TMP 2-3 days per week, with a maximum total dose of 320 mg/day. Doses may be given once or twice daily. If unable to take trimethoprim/sulfamethoxazole, alternative PCP prophylaxis (such as Dapsone or Atovaquone) should be instituted. Bactrim may be held for a short period of time if blood counts are low, but should be reinstated once counts have recovered and appropriate adjustments in chemotherapy dosing have been made. Try to avoid permanently discontinuing or holding Bactrim for prolonged periods because of myelosuppression.

6.16.3.2 Management of Febrile Neutropenia: For patients with ANC < 500/μL and fever (defined as temperature between 38.0° and 38.5°C twice in 12 hours, or ≥ 38.5°C, or per institutional guidelines), empiric parenteral broad spectrum antibiotics should be instituted after obtaining appropriate cultures. The specific antibiotics may be per institutional standards. It is recommended that vancomycin or other appropriate coverage for strep viridans be included in empiric

antibiotics for fever occurring in VHR patients after high-dose cytarabine (Consolidation IC). If fever persists, systemic antifungal therapy and radiographic studies should be considered, as described in Section 6.16.2.2.

6.16.3.3 Blood Transfusions: All transfused blood products should be irradiated and leukodepleted.

6.16.3.4 Growth Factors: Filgastrim (G-CSF), GM-CSF and granulocyte transfusions shall be considered individually for patients with protracted granulocytopenia and/or severe infection, and may be administered at discretion of treating physician. Erythopoeitin may also be administered at discretion of treating physician.

6.16.3.5 Use of Corticosteroids other than as anti-leukemic agent: The use of corticosteroids to treat other conditions (eg, premedication for blood products, treatment of allergic reactions, reactive airways disease) is allowed, and may be instituted at the discretion of the treating physician. Prolonged use of corticosteroids (more than a few days at a time) beyond that which is outlined in this protocol as part of anti-leukemic treatment should be discussed in advance with Principal Investigator or designee, before being instituted. The use of corticosteroids as antiemetic agents is discouraged, but not prohibited.

6.16.3.6 Leucovorin after Intrathecal Chemotherapy: Leucovorin may be given after intrathecal chemotherapy containing methotrexate for patients with Down Syndrome or those who have experienced excessive toxicity with prior intrathecal methotrexate. Leucovorin may be added at discretion of treating physician. Suggested dosing (if given): Give leucovorin beginning 24 hours after lumbar puncture at 15 mg/m^2/dose orally three times a day for 3 doses only.

6.16.3.7 Vitamins: Try to avoid Folic Acid supplements so as to not interfere with efficacy of methotrexate (a folate antagonist).

6.17 Duration of Therapy

Therapy for all patients should be discontinued 104 weeks (+/- 2 weeks) after complete remission has been documented, even if there have been significant delays or dose modifications during treatment. Therapy should not be stopped in the middle of a 3-week continuation cycle. Participants will be considered "off treatment" Day 21 days after the start of the last cycle they receive during the continuation phase of treatment.

Protocol treatment will be discontinued if any of the following occur:

- Failure to achieve remission at the end of the Remission Induction Phase
- Patient found to have Ph+ (BCR/ABL) ALL
- Disease recurrence
- Development of a second malignancy
- Participant decides to withdraw from the study
- General or specific changes in the participant's condition or compliance with protocol therapy render the participant unacceptable for further treatment in the opinion of the treating investigator

6.18 Duration of Follow Up

All participants (including those who stop treatment early) will be followed indefinitely for disease and survival status, except if a participant withdraws from the study and specifically declines such follow-up.

6.19 Criteria for Removal from Study

- Death
- Lost to follow-up
- Withdrawal of consent for any further data submission

7. EXPECTED TOXICITIES AND DOSING DELAYS/DOSE MODIFICATIONS

Dose delays and modifications will be made using the recommendations listed below. Toxicity assessments will be done using the NCI Common Terminology Criteria for Adverse Events (CTCAE) Version 4.0 which is identified and located on the CTEP website at: http://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm.

7.1 Anticipated Toxicities

A list of the adverse events and potential risks associated with the agents administered in this study appear below.

7.1.1 Adverse Event List for Calaspargase Pegol (SC-PEG-asparaginase), Oncaspar, Erwinia Asparaginase

	Common Happens to 21-100 children out of every 100	**Occasional** Happens to 5-20 children out of every 100	**Rare** Happens to < 5 children out of every 100
Immediate: Within 1-2 days of receiving drug	Pain at IM injection site, fatigue	Rash	Allergic reaction/anaphylaxis (possibly severe and life-threatening), nausea, vomiting, abdominal pain, drowsiness, lethargy, headache
Prompt: Within 2-3 weeks	Hypoalbuminemia, prolonged partial thromboplastin time (PTT), prothrombin time (PT), and bleeding time (secondary to decreased synthesis of fibrinogen, antithrombin III & other clotting factors),	hyperglycemia, loss of appetite, abnormal liver function tests, pancreatitis, asymptomatic elevation of amylase/lipase, elevated serum ammonia	seizures (L), peripheral edema, ascites, azotemia and decreased renal function, coagulopathy leading to hemorrhage or thrombosis, disseminated intravascular coagulation (DIC) myelosuppression, infections, depression, confusion, hallucinations, EEG changes, hypertriglyceridemia,, hyperlipidemia
Delayed/Late: occurring anytime later during therapy or after the completion of treatment			elevated blood urea nitrogen and creatinine, fatty liver deposits, hepatomegaly

7.1.2 **Adverse Event List for Cyclophosphamide**:

	Common Happens to 21-100 children out of every 100	**Occasional** Happens to 5-20 children out of every 100	**Rare** Happens to < 5 children out of every 100
Immediate: Within 1-2 days of receiving drug	Nausea and vomiting	Hemorrhagic cystitis (may be prevented with extra intravenous fluid or Mesna)	Transient blurred vision, nasal stuffiness with rapid administration, arrhythmias (rapid infusion), skin rash, anaphylaxis, SIADH
Prompt: Within 2-3 weeks	Hair Loss, Myelosuppression, Mucositis		Cardiac toxicity with high dose (CHF, hemorrhagic myocarditis, hyperpigmentation, nail changes
Delayed/Late: occurring anytime later during therapy or after the completion of treatment	Mouth sores		Infertility (very unlikely at the doses to be used in this protocol), Second malignancy (leukemia) Lung damage (pulmonary fibrosis)

7.1.2 **Adverse Event List for Cytarabine (Ara-C)-Intravenous**

	Common Happens to 21-100 children out of every 100	**Occasional** Happens to 5-20 children out of every 100	**Rare** Happens to < 5 children out of every 100
Immediate: Within 1-2 days of receiving drug	Nausea, vomiting Flu-like symptoms: fever, aches, fatigue, Rash	Conjunctivitis*	Acute Neurologic toxicity*: dizziness, ataxia, slurred speech, altered mental status, coma, seizures (rarely can be irreversible)
Prompt: Within 2-3 weeks	Hair Loss, Mucositis, Myelosuppression		Pulmonary edema
Delayed/Late: occurring anytime later during therapy or after the completion of treatment			

*High-dose cytarabine only

7.1.3 **Adverse Event List for Dexrazoxane**

	Common Happens to 21-100 children out of every 100	**Occasional** Happens to 5-20 children out of every 100	**Rare** Happens to < 5 children out of every 100
Immediate: Within 1-2 days of receiving drug			Inflammation of the vein into which it was injected/pain during infusion, Headaches
Prompt: Within 2-3 weeks			Myelosuppression, Hair Loss, Changes in the liver function tests/irritation of the liver
Delayed/Late: occurring anytime later during therapy or after the completion of treatment			

7.1.4 **Adverse Event List for Doxorubicin**

	Common Happens to 21-100 children out of every 100	**Occasional** Happens to 5-20 children out of every 100	**Rare** Happens to < 5 children out of every 100
Immediate: Within 1-2 days of receiving drug	nausea, vomiting, loss of appetite, pink/red color to urine, sweat, tears, saliva	diarrhea	allergic reaction, rash, if extravasation occurs can lead to severe tissue injury.
Prompt: Within 2-3 weeks	myelosuppression, hair loss	mucositis, liver function test abnormalities	colitis
Delayed: occurring anytime later during therapy or after the completion of treatment		Asymptomatic changes on echocardiogram (thin left ventricular wall, increased afterload)	Discoloration of hands, feet and fingernails, secondary malignancy (L), Arrhythmia, acute CHF, progressive ventricular dysfunction leading to CHF

7.1.5 **Adverse Event List for Etoposide**

	Common Happens to 21-100 children out of every 100	**Occasional** Happens to 5-20 children out of every 100	**Rare** Happens to < 5 children out of every 100
Immediate: Within 1-2 days of receiving drug	Nausea and vomiting		Transient hypotension during infusion; anaphylaxis-including chills, fever, tachycardia, dyspnea, bronchospasm, hypotension
Prompt: Within 2-3 weeks	Hair Loss, Myelosuppression,	Diarrhea	Peripheral neuropathy
Delayed/Late: occurring anytime later during therapy or after the completion of treatment			Secondary cancer (leukemia, myelodysplasia)

7.1.6 **Adverse Event List for Intrathecal Cytarabine**

	Common Happens to 21-100 children out of every 100	**Occasional** Happens to 5-20 children out of every 100	**Rare** Happens to < 5 children out of every 100
Immediate: Within 1-2 days of receiving drug	nausea, vomiting	headache, CSF pleocytosis	rash, fever, drowsiness (L), meningisimus, seizures (L), paresis
Prompt: Within 2-3 weeks			
Delayed/Late: occurring anytime later during therapy or after the completion of treatment			learning disability

7.1.7 **Adverse Event List for Intrathecal Hydrocortisone**

	Common Happens to 21-100 children out of every 100	**Occasional** Happens to 5-20 children out of every 100	**Rare** Happens to < 5 children out of every 100
Immediate: Within 1-2 days of receiving drug		headache, CSF pleocytosis, nausea, vomiting, fever	rash, somnolence, meningisimus, seizures (L), paresis
Prompt: Within 2-3 weeks			somnolence, ataxia
Delayed/Late: occurring anytime later during therapy or after the completion of treatment		learning disability (L)	progressive CNS deterioration

7.1.8 **Adverse Event List for Intrathecal Methotrexate**

	Common Happens to 21-100 children out of every 100	**Occasional** Happens to 5-20 children out of every 100	**Rare** Happens to < 5 children out of every 100
Immediate: Within 1-2 days of receiving drug		headache, CSF pleocytosis	vomiting, fever, rash, somnolence, meningisimus, paresis
Prompt: Within 2-3 weeks		seizures, mucositis	myelosupperssion, somnolence, ataxia
Delayed/Late: occurring anytime later during therapy or after the completion of treatment		learning disability	Leukoencephalopathy, progressive CNS deterioration

7.1.9 **Adverse Event List for Mercaptopurine**

	Common Happens to 21-100 children out of every 100	**Occasional** Happens to 5-20 children out of every 100	**Rare** Happens to < 5 children out of every 100
Immediate: Within 1-2 days of receiving drug			Nausea and vomiting
Prompt: Within 2-3 weeks	Myelosuppression, Abnormal liver function tests (transaminities, hyperbilirubinemia)	Hair Loss, Mucositis	
Delayed/Late: occurring anytime later during therapy or after the completion of treatment			

7.1.10 **Adverse Event List for Methotrexate (Intravenous)**

	Common Happens to 21-100 children out of every 100	**Occasional** Happens to 5-20 children out of every 100	**Rare** Happens to < 5 children out of every 100
Immediate: Within 1-2 days of receiving drug	Nausea/Vomiting (frequent with high dose, uncommon with low dose)		Seizures (convulsions) Blurred vision, Headache, altered mental status
Prompt: Within 2-3 weeks	Mucositis, Myelosuppression, Liver function test abnormalities (transaminitis)	Hair Loss, Abnormal kidney function	Rash, photophobia
Delayed/Late: occurring anytime later during therapy or after the completion of treatment			Learning disabilities, Leukoencephalopathy, leading to confusion, seizueres, mental deterioration (can be irreversible)

7.1.11 Adverse Event List for Prednisone, Prednisolone, Methylprednisolone, Dexamethasone

	Common Happens to 21-100 children out of every 100	**Occasional** Happens to 5-20 children out of every 100	**Rare** Happens to < 5 children out of every 100
Immediate: Within 1-2 days of receiving drug	insomnia, hyperphagia	gastritis, hyperglycemia	
Prompt: Within 2-3 weeks	personality changes, adrenal axis suppression, acne	facial erythema, poor wound healing, infections (bacterial, fungal, parasitic, viral), edema	pancreatitis, increased intraocular pressure, hypertension, psychosis, vertigo, headache
Delayed/Late: occurring anytime later during therapy or after the completion of treatment	Cushing syndrome (moon facies, truncal obesity)	striae, easy bruising, muscle weakness, osteopenia, cataracts	fractures (L), growth suppression, peptic ulcer and GI bleeding, pseudotumor cerebri, fatty infiltration of liver, osteonecrosis

7.1.12 Adverse Event List for Leucovorin

	Common Happens to 21-100 children out of every 100	**Occasional** Happens to 5-20 children out of every 100	**Rare** Happens to < 5 children out of every 100
Immediate: Within 1-2 days of receiving drug			rash
Prompt: Within 2-3 weeks			
Delayed/Late: occurring anytime later during therapy or after the completion of treatment			

7.1.13 **Adverse Event List for Vincristine**

	Common Happens to 21-100 children out of every 100	**Occasional** Happens to 5-20 children out of every 100	**Rare** Happens to < 5 children out of every 100
Immediate: Within 1-2 days of receiving drug		jaw pain, headache	extravasation (rare) but if occurs can lead to local ulceration
Prompt: Within 2-3 weeks	alopecia, constipation	weakness, abdominal pain, mild brief myelosuppression	paralytic ileus, ptosis, diplopia, night blindness, hoarseness, vocal cord paralysis, SIADH, seizures, defective sweating
Delayed/Late: occurring anytime later during therapy or after the completion of treatment	loss of deep tendon reflexes	peripheral parasthesias (including numbness, tingling and pain), clumsiness, wrist drop, foot drop, abnormal gait	difficulty walking or inability to walk, sinusoidal obstruction syndrome (SOS, also known as VOD), blindness, optic atrophy, urinary tract disorders (including bladder atony, dysuria, polyuria, nocturia and urinary retention), autonomic neuropathy with postural hypotension, 8th cranial nerve (dizziness, nystagmus, vertigo and hearing loss)

7.2 Dose Modifications/Delays and Toxicity Management

7.2.1 Allowable Dosing Variations/Delays (All Phases)

7.2.1.1 Allowable Dosing Variation

Variations in drug dosages within 10% of calculated doses are acceptable to allow for ease of drug administration (rounding) and minor changes in BSA/weight.

7.2.1.2 Allowable Deviations in Timing of Therapy

All attempts should be made to administer therapy and perform procedures on days indicated in protocol. Therapy/procedures may be delayed due to toxicity, as indicated in Dose Modification sections. Delays due to intercurrent illnesses are allowed, but therapy should be re-started as soon as possible. Changes in times of therapy/procedures up to +/- 3 days during Induction, Consolidation I, CNS therapy and Consolidation II, and up to +/- 7 days during Continuation, are allowed for scheduling reasons (weekends, holidays, and patient or provider availability). If longer delays are anticipated for reasons other than severe illness or toxicity, contact Principal Investigator or designee for prior approval.

For medications to be given multiple times per day (for example, IV methylprednisolone during the Induction Phase), all attempts should be made to administer medications at the intervals specified in the protocol. Variations of +/- 3 hours from the specified intervals are acceptable to allow some flexibility in medication administration given the complex care needs of many patients.

If doxorubicin is given by peripheral IV, dexrazoxane may be given immediately after rather than prior to doxorubicin. Goal: no more than 30 minutes duration for administration of both doxorubicin and dexrazoxane, regardless of the order in which the medications are given.

High-dose methotrexate infusion is intended to be given over 24 hours. Variation of +/- 1 hour in duration of infusion is allowed to account for variability in pumps and other unavoidable factors. For all other chemotherapy infusions (IV Oncaspar, IV SC-PEG Asparaginase, Cyclosphosphamide, Etoposide), variation in protocol-specified infusion duration of +/- 15 minutes is allowed.

7.2.1.3 Allowable Deviation in Route of Administration

For patients who are unable to receive IV or IM methotrexate during the continuation phase due to travel or other unusual circumstances, a dose of methotrexate may be given orally, but no more than 3 times over the total duration of treatment. If it is necessary to give methotrexate by mouth more than 3 times, the Principal Investigator must be contacted in advance for approval.

For patients unable to tolerate oral dexamethasone during post-induction treatment phases, IV dexamethasone may be used (same dose as oral dosing).

During all phases of therapy, vincristine may be given either by IV push or IV via minibag per institutional standard.

7.2.1.4 **Allowable Deviation for Bilirubin**

A total bilirubin of < 1.4 mg/dL (23.9 micromoles/L) is sufficient to meet the bilirubin criteria for enrollment and starting or continuing a cycle during all phases of treatment. For patients receiving Erwinia asparaginase, direct bilirubin may be checked once per week (ie, does not need to be checked prior to each dose).

7.2.2 **Allowable Deviations In Therapy Due to Significant Illness**

7.2.2.1 **Significant Illness at the end of Induction Therapy**

In some circumstances when patients have severe life-threatening illness (e.g.disseminated candidiasis) and are not expected to tolerate myelosuppressive chemotherapy for an extended period of time, but are in documented CR at end of remission induction therapy, Consolidation I and CNS therapy may be delayed and patients may proceed with less intensive chemotherapy, such as vincristine (as dosed in Section 6.8.3.2) or some or all of the agents included in Consolidation II or Continuation cycles (Sections 6.14 and 6.15). Additional post-induction agents (e.g. steroid, methotrexate, 6MP, doxorubicin with dexrazoxane) may be added in step-wise fashion as tolerated. All such changes must be discussed with and approved by Lewis Silverman, MD or designee. If CNS phase is to be significantly delayed, patients should receive IT medications every 9 weeks (if possible) until patient is able to commence with CNS phase. Patients should receive Consolidation I and CNS therapy as soon as possible.

7.2.2.2 **Significant Illness at the end of Consolidation I**

In some circumstances when patients have severe life-threatening illness and are not expected to tolerate intensive chemotherapy for an extended period of time, CNS therapy may be delayed and patients may proceed with modified Consolidation II or Continuation cycles, including some or all of the agents typically included in those cycles (including vincristine, dexamethasone, 6MP, methotrexate, and for HR/VHR patients, doxorubicin/dexrazoxane) as dosed in Sections 6.14 and 6.15. Agents may be added in subsequent cycles in a step-wise fashion as tolerated. Asparaginase may be given, according to randomization; once started it should be continued for 30 consecutive weeks if tolerated. All such changes must be discussed with and approved by Lewis Silverman, MD or designee. Patients should receive IT medications every 9 weeks (if possible) until able to commence with CNS phase. Patients should begin CNS phase as soon as possible

7.2.2.3 **Significant Illness During Consolidation I for VHR patients**

In circumstances when patients have severe illnesses, and are not expected to tolerate intensive chemotherapy for an extended period of time, Cycle IB or IC may be held even if other starting criteria are met. Patients may proceed with modified Consolidation II or Continuation cycles,

including some or all of the agents typically included in those cycles (including vincristine, dexamethasone, 6MP, methotrexate, and doxorubicin/dexrazoxane) as dosed in Sections 6.14 and 6.15. Agents may be added in subsequent cycles in a step-wise fashion as tolerated. In general, asparaginase should not be given until Consolidation IC is administered, but exceptions can be made on an individual basis after discussion with Principal Investigator. All modifications must be discussed with and approved by Lewis Silverman, MD or designee. Patients should receive IT medications every 9 weeks (if possible) until able to commence with Consolidation IB/IC. All attempts should be made to proceed with Consolidation IB and IC as soon as possible.

7.2.2.4 **Significant Illness During Consolidation II/Continuation**

In circumstances when patients have severe illnesses, and are not expected to tolerate myelosuppressive chemotherapy for an extended period of time, chemotherapeutic medications can be held even if other starting criteria are met. During Consolidation II, all attempts should be made to continue asparaginase, unless otherwise contraindicated (See Section 7.2.8.5); if held for life-threatening illness, asparaginase should be resumed as soon as possible. Other agents (vincristine, steroid, 6MP, methotrexate, doxorubicin/dexrazoxane, IT chemotherapy) may be added back in stepwise fashion as tolerated. Weekly methotrexate may be substituted for doxorubicin in HR/VHR patients, but doxorubicin should be restarted in place of methotrexate as soon as possible. All such modifications must be discussed with and approved in advance by Lewis Silverman, MD, or designee.

If a patient has protracted myelosuppression during Consolidation II/Continuation (start of cycle delayed > 3 weeks due to low blood counts), vincristine and dexamethasone pulses can be given every 3-weeks while awaiting count recovery only with prior approval of Lewis Silverman, MD, or designee. In cases of protracted myelosuppression, a diagnostic marrow aspirate/biopsy is recommended prior to initiation of vincristine/dexamethasone pulses.

7.2.3 **Remission Induction: Dose Modifications**

Doxorubicin: If hyperbilirubinemia at Day 4, see **Special Circumstances** (Section 6.6)

Prednisone: All attempts should be made to avoid modifying or holding any doses. Medically treat hypertension and ketotic hyperglycemia.

Vincristine: All attempts should be made to avoid delaying or modifying dose. A dose of vincristine may be delayed for up to 3 days for ileus, SIADH, vocal cord paralysis, cranial neuropathy or life-threatening illness; if vincristine dose is held more than 3 days for toxicity, that week's dose should be held altogether and not made up. After delay or missed dose, vincristine should be given at 100% dose (no dose reduction); dose reductions are allowed if discussed in advance with Principal Investigator or designee. If days of administration are changed, the interval between any two vincristine doses should not be less than 6 days. If direct bilirubin > 3.0 mg/dl (51.3 micromoles/L), hold dose of vincristine. With a bilirubin at this level, it may be allowable under certain circumstances to administer vincristine at 50% dose but only with prior approval of Principal Investigator.

Methotrexate Do not give dose if patient has effusions, ascites, other fluid collections, or creatinine above normal limits for age. If hyperbilirubenima at Day 4, see Section 6.6 regarding the timing of dose.

Asparaginase:

- Hold for clinical pancreatitis, deep venous thrombosis.
- Allergic Reaction to Induction Dose of PEG asparaginase (Oncaspar or SC-PEG): Stop infusion for clinical hypersensitivity reaction. Patient should be switched to Erwinia asparaginase for all future doses of asparaginase (See Section 7.2.8.5)
  - If hypersensitivity reaction occurs within first 30 minutes of asparaginase infusion during induction phase, then patient should receive four doses of Erwinia twice-weekly during the induction phase. Dose of Erwinia: 25000 IU/m2, given IM. First dose of Erwinia should be given sometime between Days 8-11 of induction, if possible.
  - If hypersensitivity reaction to PEG asparaginase occurs in the at or after 30 minutes of the asparaginase infusion has been completed, no doses of Erwinia should be given during induction phase; Erwinia asparaginase should commence with 1st post-induction asparaginase dose (CNS Phase for SR/HR patients, Consolidation IC for VHR patients).

Intrathecal Therapy:

- If patient has moderate/severe mucositis on Day 18, omit IT Methotrexate (give IT cytarabine/hydrocortisone only)
- Do not hold or modify doses of Intrathecal Cytarabine for mucositis or blood counts. If patient had previous neurologic toxicity attributed to intrathecal cytarabine, subsequent doses may be held after discussion with Lewis Silverman, MD, or designee.
- If patient has any mucositis on Day 32, do not perform lumbar puncture and do not administer ITM (or proceed to Consolidation I) until recovered. BM aspirate and biopsy may still be performed.

Mucositis Criteria:

- Mild = red mucosa - no open ulcers
- Moderate = open sores, but taking p.o. normally
- Severe = open sores associated with decreased p.o. and/or increased salivation

7.2.4 **Consolidation I (SR/HR) and Consolidation IA (VHR): Dose Modifications**

7.2.4.1 **Mucositis**:

- **Start of cycle**: If mucositis evident prior to beginning Consolidation I, hold start of cycle until mucositis has significantly improved or resolved. If anticipated delay due to mucositis is likely to be 7 or more days, may give vincristine 1.5 mg/m^2/dose (maximum dose 2 mg) IV push or IV via minibag weekly until mucositis resolved (as long as no constipation or significant neuropathy).
- **During cycle**: Hold 6 MP for moderate/severe mucositis developing during Consolidation I/IA and do not re-start within the cycle.

7.2.4.2 **Creatinine elevated above normal for age**:

If creatinine elevated above upper limit of normal for age prior to start of cycle, hold high dose methotrexate. Proceed with remainder of chemotherapy. Attempt to give full cycle of Consolidation I/IA in 3 weeks if creatinine level has normalized. If still not normalized, proceed to next scheduled phase of chemotherapy. For patients who do not receive high-dose methotrexate during Consolidation I/IA, it may be given during Consolidation II (weekly MTX should not be given during a Consolidation II cycle in which HD MTX is given). Note: High dose methotrexate should not be given during Consolidation II if patient has received cranial radiation during the CNS phase.

7.2.4.3 **Edema/ascites/effusions**:

If present at start of cycle, and considered significant by treating physician, then high dose methotrexate should be held. Follow guidelines for elevated creatinine (Section 7.2.4.2).

7.2.4.4 **Low Blood Counts**:

Hold 6MP if APC < 500 or platelets < 50K during cycle and do not re-start within the cycle.

7.2.4.5 **Abnormal Liver Function Tests**:

Hold 6MP if SGOT> 8x normal or direct bilirubin >1.4 mg/dL (23.9 micromoles/L), during cycle.

7.2.5 **Consolidation IB: Dose Modifications (VHR Patients)**

7.2.5.1 **Low Blood Counts**:

Hold 6MP if APC < 500 or platelets < 50K during cycle and do not re-start. Delay cytarabine if APC < 500 or platelets < 50K on Day 9 (when 2nd 4-day course is due to begin), and begin this 4-day course once APC $\geq$ 500 and platelets $\geq$ 50K. Once a 4-day course of cytarabine commences, give all 4 doses of that course regardless of blood counts. If Day 9 cytarabine cannot be given within 14 days from when it was initially due because of low blood counts, it should be omitted from cycle.

7.2.5.2 **Abnormal Liver Function Tests**:

Hold 6MP if SGOT> 8x normal or direct bilirubin >1.4 mg/dL (23.9 micromoles/L) during cycle and do not restart within the cycle. Hold Day 9 cytarabine if direct bilirubin >1.4 mg/dL (23.9

micromoles/L) on the day it is due; administer at full dose once direct bilirubin is ≤ 1.4 mg/dL (23.9 micromoles/L). If Day 9 cytarabine cannot be given within 14 days from when it was initially due because of hyperbilirubinemia, it should be omitted from cycle.

7.2.5.3 **Mucositis**:

Hold 6MP for moderate/severe mucositis and do not restart within the cycle. Hold Day 9 cytarabine if moderate/severe mucositis on the day it is due; administer at full dose once mucositis improves. If Day 9 cytarabine cannot be given within 14 days from when it was initially due because of mucositis, it should be omitted from cycle.

7.2.6 Consolidation IC (VHR Patients)

7.2.6.1 **Etoposide:** Slow infusion rate in case of hypotension. Premedication with diphenydramine, corticosteroids, and acetaminophen allowed if hypersensitivity reaction. Doses can only be held if discussed in advance with Lewis Silverman, MD or designee.

7.2.6.2 **Asparaginase**: For dose modifications due to toxicity, refer to Section 7.2.8.5.

7.2.7 CNS Treatment Phase: Dose Modifications

7.2.7.1 **Intrathecal medications**:

- **Attempt to avoid dose reduction by re-evaluating patient in 2-3 days**
- If APC <500 or PLT <50K and/or moderate mucositis (open sores-red mucosa, but taking p.o. normally), decrease methotrexate dose in IT therapy by 50%.
- If APC <300 or PLT <25K and/or severe mucositis (decreased p.o. and increased salivation, mucosal ulcers), omit IT methotrexate and administer IT cytarabine and hydrocortisone
- If SGOT > 8 x normal and/or direct bilirubin > 1.4 mg/dL (23.9 micromoles/L): omit IT methotrexate and administer IT cytarabine and hydrocortisone.

7.2.7.2 **6- mercaptopurine**:

Hold for APC <500 or PLT <50K, SGOT > 8 x normal, direct bilirubin > 1.4 mg/dL (23.9 micromoles/L) and/or moderate/severe mucositis (defined in Section 7.2.3). Do not restart within cycle once held.

7.2.7.3 **Asparaginase**: Refer to Section 7.2.8.5.

7.2.7.4 **Vincristine:**

- Hold vincristine for severe constipation or neuropathy that is not responsive to maximum supportive care.
- Hold vincristine for cranial nerve abnormalities (such as ptosis).

7.2.8 **Consolidation II and Continuation Therapy: Dose Modifications**

7.2.8.1 **Modifications for Blood Counts**

Desired Hematologic Nadirs:

- APC 500 - 750/mm^3, and
- Platelets 50,000 - 100,000/mm^3.

Dose intensity is important and any delays in chemotherapy should be as short as possible.

If APC < 500 or Platelets < 50,000 during cycle (weeks 2 and 3):

- Consider holding Trimethoprim/sulfamethoxazole (Bactrim) and/or other potentially myelosuppresive, non-chemotherapeutic agents prior to reducing doses of any chemotherapy.

- Hold methotrexate and 6-MP for remainder of cycle if APC <500 or platelets < 50,000. Do not make up missed doses of methotrexate and 6-MP. At the start of next cycle, consider reducing doses of myelosuppresive chemotherapy. Suggested adjustments: reduce both agents by 20% at start of the next cycle.

- Consider testing for TPMT (thiopurine methyltransferase) activity and/or mutation status for patients with excessive or protracted myelosuppression. If low TPMT activity and/or mutation identified, 6MP should be dose-reduced preferentially over methotrexate.

- It is suggested that Doxorubicin doses should be reduced by 20% only if an initial reduction of 6-MP does not prevent further cycles with nadirs that are too low. If doxorubicin is dose-reduced, dexrazoxane should be dose-reduced to keep ratio of dexrazoxane:doxorubicin = 10:1. **Doxorubicin Dose may be reduced by 20% only once!** There are no further dose reductions of doxorubicin if patient is already receiving 80% dose doxorubicin.

- Asparaginase should be given regardless of blood counts.

If APC<750 or platelets <75 K when cycle is due:

- Consider holding Trimethoprim/sulfamethoxazole (Bactrim) and/or other potentially myelosuppresive, non-chemotherapeutic agents prior to reducing doses of any chemotherapy.

- Hold all chemotherapy except asparaginase until criteria are met to start cycle. At the start of next cycle, consider reducing the doses of myelosuppressive chemotherapy. Suggested adjustments: reduce methotrexate, 6MP, and/or doxorubicin as above.

- It is suggested that Doxorubicin doses should be reduced by 20% only if appropriate reduction of 6-MP does not prevent delayed cycles or nadirs that are too low. If doxorubicin is dose-reduced, dexrazoxane should be dose-reduced to keep ratio of dexrazoxane: doxorubicin = 10:1. **Doxorubicin Dose may be reduced by 20% only once!** There are no further dose reductions of doxorubicin if patient is already receiving 80% dose doxorubicin.

- Asparaginase should be given regardless of blood counts.

If nadirs too high: Consider dose escalation of 6MP/methotrexate (doxorubicin should not be dose-escalated).

- Suggested dose escalation: Increase dosages of methotrexate and 6-mercaptopurine in 20% increments according to patient tolerance. Consider escalating only agent per cycle, alternating which agent is escalated in successive cycles.
- Dosages of 6-MP should not exceed 150% (75 mg/m^2/day for 14 days).
- Maximum weekly dose of methotrexate:
  - Standard Risk and non-irradiated High Risk: 150% dose (45 mg/m^2/dose)
  - High Risk with prior cranial radiation and all Very High Risk: 133% dose (40 mg/m^2/dose)
- If Doxorubin was previously reduced, dose may be escalated back to 100% if nadirs exceed goal nadirs. Doxorubicin dose should not exceed 100% (30 mg/m^2/dose).

7.2.8.2 **Abnormal LFT's**:

- Hold start of cycle if SGOT (AST) >8 times normal or direct bilirubin >1.4mg/dl (23.9 micromoles/L). Begin cycle when levels have fallen below these values. (SGPT/ALT is not a criterion)
- Suggested dose adjustment of chemotherapy for abnormal LFT's:
  - high risk/very high risk patients (Consolidaton II): reduce 6-MP by 20%. If elevation of SGOT/direct bilirubin recurs, then reduce doxorubicin by 20%. If doxorubicin is dose-reduced, dexrazoxane should be dose-reduced to keep ratio of dexrazoxane: doxorubicin = 10:1. **Doxorubicin Dose may be reduced by 20% only once!** There are no further dose reductions of doxorubicin if patient is already receiving 80% dose doxorubicin. If elevation of SGOT/direct bilirubin recurs with 80% dose doxorubicin, continue to reduce doses of 6-MP with subsequent cycles by 20% each cycle.
  - standard risk patients (Consolidation II): reduce methotrexate by 20%. If elevation of SGOT/direct bilirubin recurs, then reduce 6-MP by 20% with

subsequent cycles. Alternate 20% reductions of methotrexate and 6-MP with subsequent cycles if elevation of SGOT/direct bilirubin recurs.
- continuation therapy (all patients): same as standard risk patients during intensification (above).
- Consider Re-escalation of chemotherapy as tolerated if LFTs remain within normal range on dose-reduced chemotherapy after 1 or more cycles (suggested escalation by 20% increments).

- If SGOT (AST) >8 times normal or a direct bilirubin >1.4mg/dl (23.9 micromoles/L), during cycle, hold 6-MP and methotrexate. These drugs may be resumed during the same cycle or at the start of the next cycle when levels have fallen below these values. Do not make up missed doses. Consider dose reductions when agents are resumed. Suggested adjustments: reduce doses of these agents as suggested above when they are resumed (and reduce doxorubicin at the start of then next cycle if patient receiving this agent). Do not make up missed doses of 6MP/methotrexate.
- Asparaginase should be given regardless of SGOT (AST). **Hold asparaginase if direct bilirubin is > 1.4 mg/dL (23.9 micromoles/L). Restart asparaginase without dose modification once direct bilirubin is ≤ 1.4 mg/dL (23.9 micromoles/L).**

7.2.8.3 Mucositis

mild = red mucosa - no open ulcers
moderate = open sores, but taking p.o. normally
severe = open sores associated with decreased p.o. and/or increased salivation

- Hold start of cycle for moderate/severe mucositis. When mucositis resolves, begin cycle, consider reducing doses of methotrexate/6-MP/doxorubicin as suggested above for abnormal LFTs (depending on risk group and phase of therapy).
- Hold week 2 or 3 methotrexate for moderate/severe mucositis. Restart when mucositis resolves. May restart during the same cycle or at the next of the cycle. Consider reducing doses when methotrexate restarted (suggested: 20% dose reduction). Do not make up missed doses of methotrexate.
- Hold 6-MP for severe mucositis. Restart when mucositis resolves. May restart during the same cycle or at the start of the next cycle. Consider reduced doses when restarting (suggested: 20% dose reduction). Do not make up missed doses of 6-MP.
- If patient has recurrent mouth sores, consider evaluation for HSV and prophylactic acyclovir.

7.2.8.4 Cardiac Studies

- Hold doxorubicin if patient has symptomatic congestive heart failure. Do not restart.
- Asymptomatic echocardiographic changes (eg, decreased shortening fraction or ejection fraction): Call Principal Investigator, Lewis Silverman, MD or designee to discuss management. Doxorubicin may be held for asymptomatic abnormal echocardiogram after discussion with Principal Investigator or designee, and may be restarted if shortening fraction or ejection fraction improves.

7.2.8.5 Asparaginase

- Patient should be observed after asparaginase dose as per institutional practice for possible allergic reaction.
- Clinical Hypersensitivity: Symptoms may include rash, urticaria, lip/tongue swelling, respiratory symptoms, wheezing, hypotension

For any clinical hypersensitivity reaction to SC-PEG asparaginase or Oncaspar:
Switch to twice-weekly Erwinia IM (intramuscular). Dose of Erwinia: 25,000 IU/m^2 IM twice weekly.

- If a patient is unable to tolerate Erwinia IM due to behavioral issues or pain related to frequent IM injections, Erwinia IV (intravenous) may be administered. **Dose of IV Erwinia: 25,000 IU/m^2 IV 3x/ week** (Monday, Wednesday, Friday), infused in 100 mL of normal saline over 1 hour. Once a patient has switched to IV Erwinia, they may not be switched back to IM Erwinia **The first dose of IM Erwinia should be administered approximately one week (or as soon as feasible) after the dose of Oncaspar or SC-PEG that caused the hypersensitivity reaction.**
- A PK sample should be obtained on the day of the first Erwinia dose, just prior to administration. Additional PK samples and antibody samples will not be collected for patients once they switch to Erwinia.
- If Erwinia dose is held or delayed, the PK should be collected prior to the correct corresponding asparaginase dose number (not by the week number).
- Direct bilirubin should be checked once per week in patients receiving Erwinia asparaginase. Bilirubin does not need to be checked prior to each dose of Erwinia.
- Calculation of number of doses of Erwinia to be given:
    - For patients randomized to IV SC-PEG (intended to receive 10 post-induction doses of SC-PEG): 6 doses (3 weeks) of IM Erwinia (given twice-weekly) replaces each dose of IV SC-PEG
        - Total # IM Erwinia doses to be administered= 6 x total # of remaining post-induction doses of SC-PEG the patient would have received (ie, 10 minus the # doses of post-induction SC-PEG doses that a patient has already received)
            - For IV Erwinia: 9 doses given 3 x weekly in 100 mL of normal saline over 1 hour (3 weeks) replace each dose of IV SC-PEG. Total # IV Erwinia doses to be administered= 9 x total # of remaining post-induction doses of SC-PEG the patient would have received (ie, 10 minus the # doses of post-induction SC-PEG doses that a patient has already received)
            - Note: Be sure to take into account the IM Erwinia doses and subtract them when doing the calculation of number of doses of IV Erwinia to be given.

- For patients randomized to IV Oncaspar (intended to receive 15 post-induction doses of Oncaspar): 4 doses (2 weeks) of IM Erwinia (given twice-weekly) replaces each dose of IV Oncaspar
    - Total # IM Erwinia doses= 4 x total # of remaining post-induction doses of Oncaspar the patient would have received (15 minus the # doses of post-induction Oncaspar doses that a patient has already received)
        - For IV Erwinia: 6 doses give 3x weekly in 100 mL of normal saline over 1 hour (2 weeks) replace each dose of IV Oncaspar. Total # Erwinia doses= 6 x total # of remaining post-induction doses of Oncaspar the patient would have received (15 minus the # doses of post-induction Oncaspar doses that a patient has already received)
        - Note: Be sure to take into account the IM Erwinia doses and subtract them when doing the calculation of number of doses of IV Erwinia to be given.

- If hypersensitivity reaction to SC-PEG or Oncaspar occurred within first 30 minutes of infusion, that dose is not included in the total # of post-induction asparaginase doses a patient has already received
- If hypersensitivity reaction to SC-PEG or Oncaspar occurred 30 minutes or more after the infusion began, then that dose should be counted as an administered post-induction dose when calculating the # of post-induction asparaginase doses a patient has already received.

- If patient develops clinical hypersensitivity to Erwinia, asparaginase is discontinued
- If Erwinia is unavailable, asparaginase is discontinued.

Silent Allergy: If after the first post-induction dose of asparaginase is administered (during Consolidation IC, CNS or Consolidation II), patient has 2 consecutive NSAA levels that are non-detectable (<0.025 IU/mL), then patient will be considered to have silent inactivation and will be switched to twice-weekly IM Erwinia asparaginase. IM Erwinia should be administered as described above for patients with clinical hypersensitivity. The first dose of Erwinia IM should be administered approximately one week (or as soon as feasible) after the last dose of asparaginase.

Note: A non-detectable pre-dose level will only be considered to be consistent with silent inactivation if the level was obtained prior to an asparaginase dose which had been administered without any delay from protocol-proscribed intervals (ie, 2-weeks between Oncaspar doses and 3-weeks between SC-PEG doses). Levels that are obtained more than 3 days after proscribed intervals may not be used for determination of silent inactivation.

Inform pharmacy at time of allergic reaction that preparation will be switched with the next dose of asparaginase. A PK sample should be obtained on the day of the first IM Erwinia dose, just prior to administration. Additional PK samples will not be collected for patients once they switch to Erwinia.

Calculation of number of doses of Erwinia to be given for patients with silent allergy:

- The goal is for patients to receive a total of 30 weeks of asparaginase depletion during post-induction therapy.
- If patients have <u>never</u> had any detectable nadir asparaginase activity levels after post-induction treatment doses, then they should receive 30 weeks of Erwinia asparaginase after silent allergy/inactivation is identified.
- If patient had detectable nadir asparaginase activity levels after post-induction doses of either Oncaspar or SC-PEG prior to the development of silent allergy/inactivation, then those doses may be counted as "effective" asparaginase doses, and the number of weeks following those doses should be taken into account when determining how many weeks of Erwinia asparaginase should be given to total 30 weeks. For instance:
    - If 2 weeks after the 1st post-induction dose of Oncaspar, patient had a detectable nadir asparaginase activity level, but then did not have any detectable nadir levels after subsequent doses, then the patient should receive 28 weeks of Erwinia asparaginase (30 total weeks-2 weeks of "effective" asparaginase after the first dose of Oncaspar)
    - If 3 weeks after the 1st post-induction dose of SC-PEG, patient had a detectable nadir asparaginase activity level, but then did not have any detectable nadir levels after subsequent doses, then the patient should receive 27 weeks of Erwinia asparaginase (30 total weeks-3 weeks of "effective" asparaginase after the first dose of SC-PEG)

If Erwinia is used after determination of silent inactivation, give 25,000 IU/m^2 IM twice weekly to complete 30 weeks of asparaginase therapy.

- If clinical hypersensitivity reaction develops to Erwinia, asparaginase is discontinued.
- If Erwinia is unavailable, asparaginase is discontinued.

IV Erwinia (3x/week) may be used instead of IM Erwinia if patient unable to tolerate IM injections to due behavioral problems or pain, as described above for clinical hypersensitivity. Give 25,000 IU/ m^2 IV 3x/week to complete 30 weeks of asparaginase therapy. Infuse IV Erwinia in 100 mL of normal saline over 1 hour.

- <u>CNS events</u> (bleed, thrombosis, infarction): Hold asparaginase. Resume when all clinical symptoms have resolved (and evidence of recanalization on CT/MRI in case of thrombus). In cases of significant CNS events, asparaginase may be permanently held after discussion with Dr. Lewis Silverman or designee. See Section 19.2.
- <u>Deep vein thrombosis</u>: Hold asparaginase. Begin treatment with anticoagulant medication. Resume asparaginase after coagulation status stabilized and clinical symptoms have resolved. If recurrent DVT on anticoagulation after restarting asparaginase, hold permanently. See Section 19.2.

- Lipemic Blood: Triglyceride levels do not need to be routinely followed for patients receiving asparaginase. If triglyceride levels are monitored, fasting levels are preferable. Hold asparaginase if a patient is found to have a triglyceride level >2000 mg/dl (>22.8 mmol/L). When triglyceride level drops below this level, resume asparaginase therapy without dose modification. For recurrent hypertriglyceridemia, lipid-lowering agents may be used at discretion of treating clinician.
- Severe pancreatitis (abdominal pain with amylase or lipase elevation above institutional normal limits for ≥72 hours duration and/or development of pancreatic pseudocyst or life-threatening complication): Permanently stop asparaginase.
- Mild to moderate pancreatitis (defined as abdominal pain of <72 hours duration with amylase and/or lipase elevation): Hold asparaginase. Resume when signs, symptoms and laboratory values return to baseline. Asparaginase may be discontinued after recurrent episodes of mild/moderate pancreatitis. Please call Principal Investigator, Lewis Silverman, MD or designee to discuss.
- Asymptomatic hyperamylasemia (for patients with amylase elevation ≥3x upper limit of normal, but no abdominal pain): If no concurrent cranial radiation, hold asparaginase for elevated amylase level (≥3 X normal), and resume when level normalizes. For patients receiving cranial radiation, check lipase level if amylase is elevated but patient is asymptomatic; if lipase elevated above normal, hold asparaginase. Resume when laboratory values normalize. If lipase within normal limits when patient is receiving cranial radiation, do not hold asparaginase.
- Elevated Direct bilirubin: Hold asparaginase if direct bilirubin is > 1.4 mg/dl (23.9 micromoles/L). Restart asparaginase without dose modification once direct bilirubin is ≤1.4 mg/dl (23.0 micromoles/L).
- Asparaginase Intolerance: For patients who are unable to receive more than 10 weeks of asparaginase (due to allergy to all preparations, severe or recurrent pancreatitis, other severe toxicities):

  1. Standard Risk patients at the start of the next 3-week cycle, patient should receive 3 cycles of High Risk Consolidation II chemotherapy (that is, three cycles with doxorubicin + dexrazoxane instead of weekly methotrexate, higher dose dexamethasone, as well as vincristine and 6MP), so that cumulative dose doxorubicin is 150 mg/m^2 +/- 15 mg/m^2 then proceed to Continuation Therapy. Dexrazoxane should be given prior to doxorubicin, as specified in High risk Consolidation II chemotherapy.
  2. High Risk/Very High Risk patients: Hold asparaginase. Continue Consolidation II chemotherapy until completion of doxorubicin (300 mg/m^2 +/- 15 mg/m^2) and 10 post-induction chemotherapy cycles with high dose dexamethaone (18 mg/m^2/day x 5 days), then proceed to Continuation Therapy.

7.2.8.6 **Vincristine**

- Hold vincristine for severe constipation or neuropathy that is not responsive to maximum supportive care. Suggested guidelines for reintroducing vincristine: Re-introduce at 50% dose when patient is improved, and escalate in 25% increments to 100% dose as tolerated.

- Hold vincristine for cranial nerve abnormalities. Reintroduce when improved or resolved. Suggested guidelines for reintroducing vincristine as above.
- For mild neuropathy (such as gait abnormalities or parasthesias), vincristine may be dose-reduced instead of held, as per discretion of treating physician. Attempt to dose escalate when symptoms improve.
- If direct bilirubin > 3.0 mg/dl (51.3 micromoles/L), hold dose of vincristine. With a bilirubin at this level, it may be allowable under certain circumstances to administer vincristine at 50% dose but only with prior approval of Principal Investigator.

7.2.8.7 **Dexamethasone**

- Severe steroid withdrawal symptomatology (myalgias, arthralgias, etc) may be treated with a taper. Total dose of steroid received per cycle (including taper) should not exceed total dose indicated in protocol (that is 5-day total of steroid). Suggested initial taper: use a 4-day full course followed by 2-3 day rapid taper. For severe withdrawal symptomatology in HR/VHR patients that persists despite taper and pain medications, lower (SR) dosing of dexamethasone (6 mg/m^2/day, with ot without taper) during Consolidation II may be used at discretion of treating clinician.
- For symptomatic avascular necrosis (osteonecrosis), permanently stop steroids. Steroids should only be discontinued in patients who have both symptoms (eg, bone or joint pain) with confirmatory radiographic findings (eg, plain film, MRI). Steroids should not be permanently discontinued for asymptomatic radiographic changes found incidentally on radiographic studies unless approved in advance by Principal Investigator.
- For bone fractures, hold corticosteroid until fractures are healed, then resume without dose modification. Do not make up missed doses. Healing may be documented by radiographic changes, symptomatic improvement or clearance by an orthopedist. For vertebral compression fractures or other fractures for which healing is not easily documented by radiographs, resolution of symptoms (eg, pain) may be used as a sign of healing.
- Steroids may be held at discretion of treating physician after episode of pancreatitis. All attempts should be made to reintroduce steroids as soon as possible. Do not make up missed doses.
- For HR/VHR patients who experience severe behavioral or mood changes during Consolidation II, dose reduction to SR dose is allowed. For severe behavioral or mood changes for patients receiving SR dose of corticosteroids, corticosteroids may be stopped or given less frequently only after discussion with Lewis Silverman, MD or designee.
- Rarely, preparation may be changed to prednisone for intolerable side effects. If changed, change preparation as follows:
    - If patient receiving dexamethasone 6 mg/m^2/day, change to prednisone 40 mg/m^2/day divided twice daily for five days on week 1 of each cycle.
    - If patient receiving dexamethasone 18 mg/m^2/day, change to prednisone 120 mg/m^2/day divided twice daily for five days on week 1 of each cycle.

Principal Investigator or designee must be contacted for prior approval of such a change.

7.2.8.8 **IT Chemotherapy**

- IT chemotherapy may be modified or held in case of seizures (attributable to prior IT chemotherapy), leukoencephalopathy, or other significant neurologic complications. IT methotrexate may be held (IT ara-C/hydrocortisone given instead) or all IT chemotherapy may be delayed or held for such conditions. All such changes in IT chemotherapy must be discussed in advance with Principal Investigator, Lewis Silverman, MD, or designee.

8. DRUG FORMULATION AND ADMINISTRATION

8.1 Asparaginase: Calaspargase Pegol (SC-PEG *E. coli* L-asparaginase, EZN-2285) IND# 100594 (05/19/10)

Source and Pharmacology: Calaspargase Pegol is a modified version of the enzyme *E. coli*-L-asparaginase. EZN- 2285 is a conjugate of L-asparaginase with numerous molecules of SC monomethoxypolyethylene glycol (SC-PEG) with a molecular weight of approximately 5,000 daltons. For Calaspargase Pegol, the PEG is covalently attached to the epsilon-amino acid side chains of the lysines in L-asparaginase. L-asparaginase is an enzyme composed of four identical subunits with one active site per tetramer. For Calaspargase Pegol, the linkage is a urethane bond, which is a more stable linkage compared with the less stable ester bond found in Oncaspar®. L-asparagine is a nonessential amino acid synthesized by the transamination of L-aspartic acid by a reaction catalyzed by the enzyme L-asparagine synthetase. The ability to synthesize asparagine is notably lacking in malignancies of lymphoid origin. Asparaginase depletes L-asparagine from leukemic cells (especially lymphoblasts) by catalyzing the conversion of L-asparagine to aspartic acid and ammonia. Recently, an additional indirect mechanism of action of L-asparagine has been suggested. According to this mechanism asparaginase may reduce the levels of plasma albumin and other serum proteins which are responsible for clearance of lipids and therapeutic corticosteroids. This may in turn result in enhanced corticosteroid exposure.

There is limited information from pharmacokinetic studies of Calaspargase Pegol in humans. Animal data show comparable kinetics to pegaspargase (Oncaspar®). The following information pertains to pegaspargase (Oncaspar®) for reference: In predominately L-asparaginase naive adult patients with leukemia and lymphoma, initial plasma levels of L-asparaginase following intravenous administration of pegaspargase were determined. Apparent volume of distribution was equal to estimated plasma volume. L-asparaginase was measurable for at least 15 days following the initial treatment with pegaspargase. The approximate t½ in adult patients is 5.73 days. The enzyme could not be detected in the urine. The half-life is independent of the dose administered, disease status, renal or hepatic function, age, or gender. In a study of newly diagnosed pediatric patients with ALL who received either a single intramuscular injection of pegaspargase (2,500 IU/m2), *E. coli* L-asparaginase (25,000 IU/m2), or *Erwinia* (25,000 IU/m2), the plasma half-lives for the three forms of L-asparaginase were: 5.73 ± 3.24 days, 1.24 ± 0.17 days, and 0.65 ± 0.13 days, respectively. The plasma half-life of pegaspargase is shortened in patients who are previously hypersensitive to native L-asparaginase as compared to non-hypersensitive patients. L-asparaginase is cleared by the reticuloendothelial system and very little is excreted in the urine or bile. Cerebrospinal fluid levels are < 1% of plasma levels.

Children's Oncology Group Study AALL07P4 compared the pharmacokinetics of Calaspargase Pegol and Oncaspar®. Data from the first 18 pharmacokinetics-evaluable patients that received Calaspargase Pegol at 2500 IU/m2 (n=11) or Oncaspar® at 2500 IU/m2 (n=7) showed a similar Cmax for Calaspargase Pegol and Oncaspar® (1604 ± 257 mIU/mL and 1406 ± 220 mIU/mL, respectively). The half-life of Calaspargase Pegol (12.65 ± 2.9 days [results from only 9 patients]) and the half-life of Oncaspar® (4.85 ± 1.1 days) suggested that at the same dose of 2500 IU/m2 for both drugs, Calaspargase Pegol is associated with a more prolonged exposure to asparaginase than Oncaspar®.

Formulation and Stability: Calaspargase Pegol is supplied as an isotonic sterile solution in phosphate-buffered saline, pH 7.3, for injection only. The solution is clear, colorless, and contains no preservatives. Calaspargase Pegol is supplied in single-use vials. Calaspargase Pegol activity is expressed in international units (IU). One IU of L-asparaginase is defined as that amount of enzyme required to generate 1 μmol of ammonia per minute at pH 7.3 and 37^{o}C. Each single-use vial contains 3,750 IU/5 mL (750 IU/mL). Each milliliter of Calaspargase Pegol contains: SC-PEG *E. coli* L-asparaginase 750 IU, Monobasic sodium phosphate, USP,1.29 mg, Dibasic sodium phosphate, USP, 5.58 mg, Sodium chloride, USP 8.5 mg, Water for injection, USP qs to1 mL. Store refrigerated at +2°C to +8ºC (36°F to 46°F). Use only one dose per vial; do not re-enter the vial.
Discard unused portions. Do not save unused drug for later administration.

Do not use Calaspargase Pegol (SC-PEG asparaginase) if the drug:

- Has been frozen.
- The infusion bag has been stored at room temperature for more than 48 hours or at 4°C
- (39°F) for more than 72 hours.
- Has been shaken or vigorously agitated.
- Is cloudy or discolored, or precipitate is present.

Do not use Calaspargase Pegol if the vial has been stored at room temperature (+15°C to +25°C; 59°F to 77°F) for more than 48 hours. If, during refrigerated storage, the storage temperature deviates from +2°C to +8ºC (36°F to 46°F), the affected product should be immediately removed from inventory and placed into refrigerated quarantine. The sponsor or its designee should be contacted immediately and provided with the storage temperature records covering the entire period of time the storage temperature deviated outside the prescribed range. Upon review of the storage temperature records provided by the study site, a determination of Calaspargase Pegol stability disposition will be made by the sponsor or its designee. Quarantined Calaspargase Pegol must not be administered to participants until such time that the sponsor or its designee provides written documentation that the product's stability was not affected by the temperature deviation. If it is determined that the stability of the quarantined product was affected, arrangements must be made to replace the inventory at the site and for the return of the quarantined product for destruction.

Prior to administration, dilute Calaspargase Pegol by adding the dose to a 100 mL bag of 0.9% Sodium Chloride Injection, USP (normal saline, NS). Calaspargase Pegol should not be prepared in Dextrose 5% in Water (D5W). Avoid excessive agitation; do not shake. Calaspargase Pegol diluted in NS is stable for up to 48 hours at room temperature and for up to 72 hours at 4oC. Calaspargase Pegol vial contains no preservatives and as with all parenteral drug products, aseptic technique should be used during the preparation of the diluted solution. The expiration time of Calaspargase Pegol should be determined by the local institution guidelines for compounding sterile products and may be shorter, but must not exceed, the times listed for stability.
Guidelines for Administration: See Treatment and Dose Modifications sections of the protocol. For IV administration: Calaspargase Pegol, diluted in a 100 mL NS bag, should be given through a running infusion line over a period of 1 hour. Avoid excessive agitation. DO NOT SHAKE. Do

not use if cloudy or if precipitate is present. Although Calaspargase Pegol should be prepared in an infusion bag containing NS, Calaspargase Pegol may be administered through an intravenous line that is running D5W or NS. While compatibility with a combination of NS and D5W has not been tested, the combination is expected to be acceptable as well. Calaspargase Pegol is also compatible with lines containing sodium bicarbonate. No compatibility studies of Calaspargase Pegol and total parenteral nutrition (TPN) or antibiotics have been conducted, and therefore Calaspargase Pegol should not be mixed with TPN or antibiotics. Also, co-administration with allopurinol or rasburicase should not occur as there have been no compatibility studies conducted with Calaspargase Pegol. No in-line filter is required for Calaspargase Pegol administration. Serious allergic reactions can occur in patients receiving Oncaspar® and are presumed to occur with Calaspargase Pegol. The risk of serious allergic reactions is higher in patients with known hypersensitivity to other forms of L-asparaginase. Have available during and after the infusion: Antihistamine, Epinephrine, Oxygen, IV Corticosteroids and IV Fluids. Observe patient for ONE hour after administration for signs of hypersensitivity reactions.
Supplier: To be supplied by Sigma-Tau Pharmaceuticals, Inc. Order Forms, Return Forms, and Drug Accountability Forms and directions are available in the Pharmacy Manual.

8.2 Asparaginase: Pegasparagase (PEG-asparaginase, Oncaspar®,) NSC #624239

Source and Pharmacology: Pegaspargase is a modified version of the enzyme L-asparaginase. Lasparaginase is modified by covalently conjugating units of monomethoxypolyethylene glycol (PEG), molecular weight of 5,000, to the enzyme, forming the active ingredient PEG-L-asparaginase. The Lasparaginase (L-asparagine amidohydrolase, type EC-2, EC 3.5.1.1) used in the manufacture of Pegaspargase is derived from *Escherichia coli*. L-asparagine is a nonessential amino acid synthesized by the transamination of L-aspartic acid by a reaction catalyzed by the enzyme L-asparagine synthetase. The ability to synthesize asparagine is notably lacking in malignancies of lymphoid origin. Asparaginase depletes L-asparagine from leukemic cells (especially lymphoblasts) by catalyzing the conversion of L-asparagine to aspartic acid and ammonia. In predominately L-asparaginase naive adult patients with leukemia and lymphoma, initial plasma levels of L-asparaginase following intravenous administration of pegaspargase were determined. Apparent volume of distribution was equal to estimated plasma volume. L-asparaginase was measurable for at least 15 days following the initial treatment with Pegaspargase. The approximate t½ in adult patients is 5.73 days. The enzyme could not be detected in the urine. The half-life is independent of the dose administered, disease status, renal or hepatic function, age, or gender. In a study of newly diagnosed pediatric patients with ALL who received either a single intramuscular injection of pegaspargase (2,500 IU/m2), *E. coli* L-asparaginase (25,000 IU/m2), or *Erwinia* (25,000 IU/m2), the plasma half-lives for the three forms of L-asparaginase were: 5.73 ± 3.24 days, 1.24 ± 0.17 days, and 0.65 ± 0.13 days, respectively. The plasma half-life of pegaspargase is shortened in patients who are previously hypersensitive to native L-asparaginase as compared to non-hypersensitive patients. L-asparaginase is cleared by the reticuloendothelial system and very little is excreted in the urine or bile. Cerebrospinal fluid levels are < 1% of plasma levels.
Formulation and Stability: Each milliliter of pegaspargase contains: PEG-L-asparaginase 750 IU ± 20%, monobasic sodium phosphate, USP 1.20 mg ± 5 % dibasic sodium phosphate, USP 5.58 mg ± 5%, sodium chloride, USP 8.50 mg ± 5%, water for injection, USP qs to 1.0 mL. The specific activity of pegaspargase is at least 85 IU per milligram protein. Available in 5 mL vials as sterile solution for injection in ready to use single-use vials, preservative free. Keep

refrigerated at 2°C to 8°C (36°F to 46°F). Do not use if stored at room temperature (+15°C to +25°C; 59°F to 77°F) for more than 48 hours. **DO NOT FREEZE.** Do not use product if it is known to have been frozen. Freezing destroys activity, which cannot be detected visually. If, during refrigerated storage, the storage temperature deviates from +2°C to +8°C (36°F to 46°F), the affected product should be immediately removed from inventory and should not be used. Prior to administration, dilute pegaspargase by adding the dose to a 100mL bag of 0.9% Sodium Chloride Injection, USP (normal saline, NS). Avoid excessive agitation; do not shake. Pegasparagase diluted in NS is stable for up to 48 hours at room temperature (15°C to 25°C; 59°F to 77°F) and for up to 72 hours at 4oC (39°F). Pegaspargase vial contains no preservatives and as with all parenteral drug products, aseptic technique should be used during the preparation of the diluted solution. The expiration time of pegaspargase should be determined by the local institution guidelines for compounding sterile products and may be shorter than the times listed for stability.
Guidelines for Administration: For IV administration: pegaspargase, diluted in a 100 mL NS ba, should be given through a running infusion line over a period of 1 hour. Avoid excessive agitation. DO NOT SHAKE. Do not use if cloudy or if precipitate is present. Although pegaspargase should be prepared in an infusion bag containing NS, it may be administered through a venous line that is running D5W or NS. While compatibility with a combination of NS and D5W has not been tested, the combination is expected to be acceptable as well. Pegaspargase is also compatible with lines containing sodium bicarbonate. No compatibility studies of pegaspargase and total parenteral nutrition (TPN) or antibiotics have been conducted, and therefore pegaspargase should not be mixed with TPN or antibiotics. Also, co-administration with allopurinol or rasburicase should not occur as there have been no compatibility studies conducted with pegaspargase.

Have available during and after the infusion: Antihistamine, Epinephrine, Oxygen, IV Corticosteroids and IV Fluids. Because pegaspargase is long acting, hypersensitivity reactions may not appear for hours after drug administration. Observe patient after administration for signs of hypersensitivity reactions.
Supplier: Pegaspargase (Oncaspar®) is commercially available from Sigma-Tau Pharmaceuticals, Inc. (Sigma-Tau) and can be purchased through the usual ordering channels (wholesalers).

8.3 Asparaginase: Erwinia
(Erwinaze®,Erwinia *chrysanthemi*, Erwinase®, Crisantaspase)
Source and Pharmacology: L-asparagine is a nonessential amino acid synthesized by the transamination of L-aspartic acid by a reaction catalyzed by the enzyme L-asparagine synthetase. Erwinia L-asparaginase is asparaginase obtained from cultures of Erwinia chrysanthemi (E. carotovora). Lymphoblastic neoplastic cells are asparagine-dependent. Reduction of plasma asparagine levels achieved by administration of L-asparaginase produces an anti-neoplastic effect. Peak levels of Erwinia L-asparaginase are achieved in the blood 1 to 2 hours after IM administration. The fall in enzyme levels follows first order kinetics with a half-life of 7 to 13 hours. L-asparaginase is cleared by the reticuloendothelial system and very little is excreted in the urine or bile. Cerebrospinal fluid levels are < 1% of plasma levels.

Erwinia L-asparaginase is immunologically distinct from E. coli L-asparaginase and may allow

continued asparaginase therapy when a hypersensitivity reaction occurs to the Escherichia coli source of asparaginase. Patients may still sustain a hypersensitivity reaction to the substitute; however, most (more than 75%) do not and can complete the planned therapy.

Formulation and Stability: Erwinaze® is supplied as a sterile, white lyophilized powder for reconstitution. Each single vial contains 10,000 International Units (IU) of asparaginase Erwinia chrysanthemi in Type 1 clear neutral glass vials of 3 mL nominal capacity, closed with 13 mm halobutyl freeze-drying stoppers and aluminum over seals. Unopened vials should be protected from light and stored between 2°C and 8°C (36°-46ºF). The product is stable for 4 hours after reconstitution either into a syringe for IM administration or in an IV bag.

Guidelines for Administration: The contents of each vial should be reconstituted by adding 1 mL or 2 mL of preservative free 0.9% sodium chloride to the vial containing 10,000 IU of asparaginase Erwinia chrysanthemi for Injection. The contents of the vial should be dissolved by gentle mixing or swirling. When the 10,000 International Unit vial is reconstituted with 1 mL of 0.9% sodium chloride, the resulting solution concentration is 10,000 International Units per mL. When the 10,000 International Unit vial is reconstituted with 2 mL of 0.9% sodium chloride the resulting solution concentration is 5,000 International Units per mL.

The calculated dose of solution should be withdrawn from the vial into a polypropylene syringe within 15 minutes of vial reconstitution. The reconstituted solution should not be refrigerated or frozen. The dose must be administered to the patient within 4 hours of initial reconstitution. For intravenous use, slowly inject the reconstituted asparaginase Erwinia chrysanthemi into an IV infusion bag containing 100 mL of normal saline acclimatized to room temperature. Do not shake or squeeze the IV bag.

Asparaginase Erwinia chrysanthemi when administered intramuscularly,no more than 2 mL should be given at any one injection site. For intravenous use, infuse asparaginase Erwinia chrysanthemi in 100 mL of normal saline over 1 hour. Do not infuse other intravenous drugs through the same intravenous line while infusing asparaginase Erwinia chrysanthemi.

Drug Ordering: Erwinaze® (asparaginase Erwinia chrysanthemi) is a commercially available product manufactured by EUSA Pharma (USA), INC. Erwinaze® is approved by the U.S. Food and Drug Administration (FDA) to treat patients with acute lymphoblastic leukemia, who have developed hypersensitivity to E.coli derived L-asparaginase and pegaspargase.

8.4 Cyclophosphamide (Cytoxan) NSC #26271

Source and Pharmacology: Cyclophosphamide is an alkylating agent related to nitrogen mustard. Cyclophosphamide is inactive until it is metabolized by P450 isoenzymes (CYP2B6, CYP2C9, and CYP3A4) in the liver to active compounds. The initial product is 4-hydroxycyclophosphamide (4-HC) which is in equilibrium with aldophosphamide which spontaneously releases acrolein to produce phosphoramide mustard. Phosphoramide mustard, which is an active bifunctional alkylating species, is 10 times more potent in vitro than is 4-HC and has been shown to produce interstrand DNA cross-link analogous to those produced by mechlorethamine. Approximately 70% of a dose of cyclophosphamide is excreted in the urine as

the inactive carboxyphosphamide and 5-25% as unchanged drug. The plasma half-life ranges from 4.1 to 16 hours after IV administration.

Formulation and Stability:

Cyclophosphamide for injection is available as powder for injection or lyophilized powder for injection in 500 mg, 1 gm and 2 gm vials. The powder for injection contains 82 mg sodium bicarbonate/100 mg cyclophosphamide and the lyophilized powder for injection contains 75 mg mannitol/100 mg cyclophosphamide. Storage at or below 25°C (77°F) is recommended. The product will withstand brief exposures to temperatures up to 30°C (86°F).

Guidelines for Administration: See Treatment and Dose Modifications sections of the protocol. Cyclophosphamide for Injection: Reconstitute with Sterile Water or Bacteriostatic Water for Injection (paraben preserved only) to a concentration of 20 mg/mL. Solutions reconstituted with preservative should be used within 24 hours if stored at room temperature or within 6 days if stored under refrigeration. If administered as undiluted drug at the 20 mg/mL concentration, reconstitute with NS only to avoid a hypotonic solution. Cyclophosphamide may be further diluted in dextrose or saline containing solutions for IV use.

Supplier: Commercially available from various manufacturers. See package insert for further information

8.5 **Cytarabine** (cytosine arabinoside, Ara-C, Cytosar®) NSC # 63878

Source and Pharmacology: Cytarabine appears to act through the inhibition of DNA polymerase. A limited, but significant, incorporation of cytarabine into both DNA and RNA has also been reported. It exhibits cell phase specificity, primarily killing cells undergoing DNA synthesis (S-phase) and under certain conditions blocking the progression of cells from the G1 phase to the S-phase. Cytarabine is metabolized by deoxycytidine kinase and other nucleotide kinases to the nucleotide triphosphate (Ara-CTP), an effective inhibitor of DNA polymerase. Ara-CTP is inactivated by a pyrimidine nucleoside deaminase, which converts it to the nontoxic uracil derivative (Ara-U). It appears that the balance of kinase and deaminase levels may be an important factor in determining sensitivity or resistance of the cell to cytarabine. It has an initial distributive phase t½ of about 10 minutes, with a secondary elimination phase t½ of about 1 to 3 hours. Peak levels after intramuscular or subcutaneous administration of cytarabine occur about 20 to 60 minutes after injection and are lower than IV administration. Intrathecally administered doses are metabolized and eliminated more slowly with a t½ of about 2 hours.

Formulation and Stability: Cytarabine for Injection, *USP*, is available in vials of 100 mg, 500 mg, 1 g, and 2 g containing a sterile powder for reconstitution. It is also available at a 20 mg/mL concentration with benzyl alcohol or as a preservative free solution, and at a 100 mg/mL concentration as preservative free solution. Hydrochloric acid and/or sodium hydroxide may be added to adjust the pH. Store at 25°C (77°F); excursions permitted to 15° to 30°C (59° to 86°F). Cytarabine solutions should be protected from light.

Stability: When reconstituted with Bacteriostatic Water for Injection, cytarabine is stable for 48 hours at room temperature. Solutions reconstituted without a preservative should be used immediately. Discard if solution appears hazy. Diluted solutions in dextrose 5% in water or 0.9% sodium chloride are stable for 8 days at room temperature; however, the diluted cytarabine should be used within 24 hours for sterility concerns

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

Intrathecal Administration: For intrathecal administration, dilute with 5-10 mL preservative free 0.9% sodium chloride injection,lactated Ringer's injection or Elliot's B solution or as per

institutional standard of practice. The volume of CSF removed should be equal to at least ½ the volume delivered. Intrathecal cytarabine mixed in 0.9% sodium chloride injection, lactated Ringer's injection, or Elliot's B solution is stable for 24 hours at 25ºC but contains no preservative and should be administered as soon as possible after preparation.
IV Infusion**:** Reconstitute the lyophilized powder with Bacteriostatic Water for Injection or 0.9% sodium chloride injection. Solution containing a bacteriostatic agent should not be used for the preparation of doses > 200 mg/m2. May be further diluted with dextrose or sodium chloride containing solutions. May give by IV push injection, by IV infusion, or by continuous infusion.
High Dose (>1000 mg/m²/dose) IV Infusion**:** Dilute in D5W or NS to a convenient volume and infuse over 3 hours. Administer steroid eye drops (dexamethasone or prednisolone), 2 drops each eye q6h beginning immediately before the first dose and continuing 24 hours after the last dose. If patient does not tolerate steroid eye drops may administer artificial tears on the same schedule.
Supplier: Commercially available from various manufacturers. See package insert for further information.

8.6 **Dexamethasone**
(Decadron®, Hexadrol®, Dexone®, Dexameth®) NSC #34521
Source and Pharmacology: Dexamethasone is a synthetic fluorinated glucocorticoid devoid of mineralocorticoid effects. Dexamethasone 0.75 mg has potent anti-inflammatory activity equivalent to approximately 5 mg of prednisone. Glucocorticoids produce widespread and diverse physiologic effects on carbohydrate, protein, and lipid metabolism, electrolyte and water balance, functions of the cardiovascular system, kidney, skeletal muscle, and the nervous systems. Glucocorticoids reduce the concentration of thymus-dependent lymphocytes (T-lymphocytes), monocytes, and eosinophils. Glucocorticoids selectively bind to the cortisol receptors on human lymphoid cells which are found in larger numbers on leukemic lymphoblasts. They also decrease binding of immunoglobulin to cell surface receptors and inhibit the synthesis and/or release of interleukins, thereby decreasing T-lymphocyte blastogenesis and reducing expansion of the primary immune response. The specific cellular mechanisms that act to halt DNA synthesis are thought to be related to inhibition of glucose transport or phosphorylation, retardation of mitosis, and inhibition of protein synthesis. Elimination half-lives for the following age groups have been reported to be: infants and children under 2 years of age: 2.3 to 9.5 hours; 8 to 16 years: 2.82 to 7.5 hours; and adults (age not specified): 3 to 6 hours. The biologic half-life is 36- 72 hours. It is primarily metabolized in the liver and excreted by the kidneys.
Formulation and Stability: Available in 0.25 mg, 0.5 mg, 0.75 mg, 1 mg, 1.5 mg, 2 mg, 4 mg, and 6 mg tablets; liquid formulations are available in 0.5 mg/5 mL and 0.5 mg/0.5 mL concentration. Inactive ingredients vary depending on manufacturer but tablet formulations may include: calcium or magnesium stearate, corn starch, lactose, and various dyes. Liquid formulations may include: 5%-30% alcohol, benzoic acid, sorbitol, sodium saccharin, glycerin, purified water, and various dyes.
Dexamethasone Sodium Phosphate Solution for Injection is available as 4 mg/mL, 10 mg/mL, 20 mg/mL, and 24 mg/mL. Four milligrams of dexamethasone sodium phosphate is equivalent to 3.33 mg of dexamethasone. Vial sizes include 1 mL, 5 mL, 10 mL, 25 mL, and 30 mL and are available in multi-dose vials as well as unit of use vials and syringes. Inactive ingredients vary depending on manufacturer but include creatinine, sodium citrate, sodium hydroxide to adjust

pH, Water for Injection, sodium sulfite, bisulfite and metabisulfite, methyl and propyl paraben, benzyl alcohol, and EDTA.
Guidelines for Administration: See Treatment and Dose Modifications section of the protocol.
In general, all doses to be given orally using tablets or liquid formulations. If IV administration is used, Dexamethasone Sodium Phosphate for Injection may be given IV, or IM undiluted. For IV use, it may be further diluted in dextrose or saline containing solutions. Avoid benzyl alcohol containing dexamethasone solutions for use in neonates. Diluted solutions that contain no preservatives should be used within 24 hours, but maintain stability for at least 14 days in PVC bags at room temperature protected from light. If taken orally, repeat oral dose if vomiting occurs within 30 minutes. If one dose is missed, this dose can be made up at the end of a cycle at the discretion of the treating physician. If more than one dose is missed, those doses should be skipped and not be made up.
Supplier: Commercially available from various manufacturers. See package insert for further information

8.7 **Dexrazoxane (**Zinecard®), NSC# 169780

Source and Pharmacology: Dexrazoxane is a synthetic chemical, a cyclic derivative of EDTA that readily penetrates cell membranes. Results of laboratory studies suggest that dexrazoxane is converted intracellularly to a ring opened chelating agent that interferes with iron mediated free radical generation thought to be responsible, in part, for anthracycline-induced cardiomyopathy. The disposition kinetics of dexrazoxane are dose-dependent with administered doses from 60 to 900 mg/m2. The plasma half-life is 2 to 2.5 hours. Qualitative metabolism studies have confirmed the presence of unchanged drug, a diacid-diamide cleavage product, and two monoacid-monoamide ring products in the urine of animals and man. Metabolite levels were not measured in the pharmacokinetics studies. Urinary excretion plays an important role in the elimination of dexrazoxane: 42% of the drug (500 mg/m2) was excreted in the urine. *In vitro* studies have shown that dexrazoxane is not bound to plasma proteins. The pharmacokinetics of dexrazoxane have not been evaluated in patients with hepatic or renal insufficiency. There was no significant effect of dexrazoxane on the pharmacokinetics of doxorubicin (50 mg/m2) or its predominant metabolite, doxorubicinol, in a crossover study in cancer patients.
Formulation and Stability: Dexrazoxane for Injection is available as a sterile, pyrogen-free lyophilized powder in the following strengths: 250 mg single dose vial packaged with a 25 mL vial of 0.167 Molar (M/6) Sodium Lactate Injection, *USP,* and 500 mg single dose vial packaged with a 50 mL vial of 0.167 Molar (M/6) Sodium Lactate Injection, *USP*. Store at 25°C (77°F); excursions permitted to 15°-30°C (59°-86°F). Dexrazoxane must be reconstituted with 0.167 Molar (M/6) Sodium Lactate Injection, *USP*, to give a concentration of 10 mg dexrazoxane for each mL of sodium lactate. Reconstituted dexrazoxane, when transferred to an empty infusion bag, is stable for 6 hours from the time of reconstitution when stored at controlled room temperature, 15°-30°C (59°-86°F), or under refrigeration, 2°- 8°C (36°-46°F). The reconstituted dexrazoxane solution may be further diluted with either 0.9% Sodium Chloride Injection, *USP* or 5.0% Dextrose Injection, *USP,* to a concentration range of 1.3 to 5.0 mg/mL in intravenous infusion bags. The resultant solutions are stable for 6 hours when stored at controlled room temperature, 15°-30°C (59°-86°F), or under refrigeration, 2°-8°C (36°-46°F).
Guidelines for Administration: See Treatment and Dose Modifications sections of the protocol.

Administer IV push immediately prior to anthracycline dose (the elapsed time from the beginning of the dexrazoxane dose to the end of the anthracycline infusion should be 30 minutes or less).
Supplier: Commercially available. See package insert for further information.

8.8 **Doxorubicin** (Adriamycin®) NSC #123127

Source and Pharmacology: An anthracycline antibiotic isolated from cultures of *Streptomyces peucetius*. The cytotoxic effect of doxorubicin on malignant cells and its toxic effects on various organs are thought to be related to nucleotide base intercalation and cell membrane lipid binding activities of doxorubicin. Intercalation inhibits nucleotide replication and action of DNA and RNA polymerases. The interaction of doxorubicin with topoisomerase II to form DNA-cleavable complexes appears to be an important mechanism of doxorubicin cytocidal activity. Doxorubicin cellular membrane binding may affect a variety of cellular functions. Enzymatic electron reduction of doxorubicin by a variety of oxidases, reductases and dehydrogenases generate highly reactive species including the hydroxyl free radical (OH•). Free radical formation has been implicated in doxorubicin cardiotoxicity by means of Cu (II) and Fe (III) reduction at the cellular level. Cells treated with doxorubicin have been shown to manifest the characteristic morphologic changes associated with apoptosis or programmed cell death. Doxorubicin-induced apoptosis may be an integral component of the cellular mechanism of action relating to therapeutic effects, toxicities, or both. Doxorubicin serum decay pattern is multiphasic. The initial distributive t½ is approximately 5 minutes, suggesting rapid tissue uptake of doxorubicin. The terminal t½ of 20 to 48 hours reflects a slow elimination from tissues. Steady-state distribution volumes exceed 20 to 30 L/kg and are indicative of extensive drug uptake into tissues. Plasma clearance is in the range of 8 to 20 mL/min/kg and is predominately by metabolism and biliary excretion. The P450 cytochromes which appear to be involved
with doxorubicin metabolism are CYP2D6 and CYP3A4. Approximately 40% of the dose appears in the bile in 5 days, while only 5 to 12% of the drug and its metabolites appear in the urine during the same time period. Binding of doxorubicin and its major metabolite, doxorubicinol to plasma proteins is about 74-76% and is independent of plasma concentration of doxorubicinHydrocortisone sodium succinate.
Formulation and Stability: Doxorubicin is available as red-orange lyophilized powder for injection in 10 mg[1], 20 mg[1], 50 mg[1], 150 mg[2] vials and a preservative free 2 mg/mL solution in 10 mg[1], 20 mg[1], 50 mg[1], 75 mg[1], 200 mg[2] vials.
[1] Contains lactose monohydrate, 0.9 NS, HCl to adjust pH to 3. The Adriamycin RDF˜ (rapid dissolution formula)
also contains methylparaben 1 mg per each 10 mg of Doxorubicin to enhance dissolution.
[2] Multiple dose vial contains lactose, 0.9% NS, HCl to adjust pH to 3.
Aqueous Solution: Store refrigerated 2°-8°C (36°-46°F). Protect from light. Retain in carton until contents are used.
Powder for injection: Store unreconstituted vial at room temperature 15°-30°C (59°-86°F). Retain in carton until contents are used. Reconstitute with preservative-free normal saline to a final concentration of 2 mg/mL. After adding the diluent, the vial should be shaken and the contents allowed to dissolve. The reconstituted solution is stable for 7 days at room temperature under normal room light (100 footcandles) and 15 days under refrigeration 2°-8°C (36°-46°F). Protect from exposure to sunlight. Doxorubicin may be further diluted in 0.9% NaCl or dextrose containing solutions and administered by infusion.

Guidelines for Administration: Administer IV through the tubing of rapidly infusing solution of D5W or 0.9% NaCl preferable into a large vein. Protect final preparation from light. To avoid extravasation, the use of a central line is suggested.
Supplier: Commercially available from various manufacturers. See package insert for further information.

8.9 Etoposide (VePesid®, Etopophos®, VP-16) NSC #141540

Source and Pharmacology: A semisynthetic derivative of podophyllotoxin that forms a complex with topoisomerase II and DNA which results in single and double strand DNA breaks. Its main effect appears to be in the S and G2 phase of the cell cycle. The initial t½ is 1.5 hours and the mean terminal half-life is 4 to 11 hours. It is primarily excreted in the urine. In children, approximately 55% of the dose is excreted in the urine as etoposide in 24 hours. The mean renal clearance of etoposide is 7 to 10 mL/min/m² or about 35% of the total body clearance over a dose range of 80 to 600 mg/m². Etoposide, therefore, is cleared by both renal and non renal processes, i.e., metabolism and biliary excretion. The effect of renal disease on plasma etoposide clearance is not known. Biliary excretion appears to be a minor route of etoposide elimination. Only 6% or less of an intravenous dose is recovered in the bile as etoposide. Metabolism accounts for most of the non renal clearance of etoposide. The maximum plasma concentration and area under the concentration time curve (AUC) exhibit a high degree of patient variability. Etoposide is highly bound to plasma proteins (~94%), primarily serum albumin. Pharmacodynamic studies have shown that etoposide systemic exposure is related to toxicity. Preliminary data suggests that systemic exposure for unbound etoposide correlates better than total (bound and unbound) etoposide. There is poor diffusion into the CSF < 5%. Etoposide phosphate is a water soluble ester of etoposide which is rapidly and completely converted to etoposide in plasma. Pharmacokinetic and pharmacodynamic data indicate that etoposide phosphate is bioequivalent to etoposide when it is administered in molar equivalent doses.
Formulation and Stability: Etoposide for Injection is available in sterile multiple dose vials. The pH of the clear, nearly colorless to yellow liquid is 3 to 4. Each mL contains 20 mg etoposide, 2 mg citric acid, 30 mg benzyl alcohol, 80 mg modified polysorbate 80/tween 80, 650 mg polyethylene glycol 300, and 30.5 percent (v/v) alcohol. Vial headspace contains nitrogen.
Unopened vials of etoposide are stable until expiration date on package at controlled room temperature (20°-25°C or 68°-77°F).
Etoposide phosphate for injection is available for intravenous infusion as a sterile lyophilized powder in single-dose vials containing etoposide phosphate equivalent to 100 mg etoposide, 32.7 mg sodium citrate *USP*, and 300 mg dextran 40. Etoposide phosphate must be stored under refrigeration (2°-8°C or 36°-46°F). Unopened vials of etoposide phosphate are stable until the expiration date on the package.
Guidelines for Administration: See Treatment and Dose Modification sections of the protocol.
Etoposide: Dilute Etoposide to a final concentration < 0.4 mg/mL in 5% Dextrose Injection, *USP* or 0.9% Sodium Chloride Injection, *USP*. Etoposide infusions are stable at room temperature for 96 hours when diluted to concentrations of 0.2 mg/mL; stability is 24 hours at room temperature with concentrations of 0.4 mg/mL. The time to precipitation is highly unpredictable at concentrations > 0.4 mg/mL. Use in-line filter during infusion secondary to precipitate formation risk. However, the use of an in-line filter is not mandatory since etoposide precipitation is unlikely at concentrations of 0.1-0.4 mg/mL. Do not administer etoposide by rapid intravenous

injection. Slow rate of administration if hypotension occurs. Leaching of diethylhexyl phthalate (DEHP) from polyvinyl chloride (PVC) bags occurred with etoposide 0.4mg/mL in 0.9% sodium chloride solution. To avoid leaching, prepare the etoposide solution as close as possible, preferably within 4 hours, to the time of administration or alternatively as per institutional policy, glass or polyethylene-lined (non-PVC) containers and polyethylene-lined tubing may be used.

Etoposide Phosphate: Dilute the 100 mg vial with 5 or l0 mL of Sterile Water for Injection, *USP*; 5% Dextrose Injection, *USP*; 0.9% Sodium Chloride Injection, *USP*; Bacteriostatic Water for Injection with Benzyl Alcohol; or Bacteriostatic Sodium Chloride for Injection with Benzyl Alcohol for a concentration equivalent to 20 mg/mL or 10 mg/mL etoposide (22.7 mg/mL or 11.4 mg/mL etoposide phosphate) respectively. Use sterile water for injection without benzyl alcohol for neonates and infants < 2 years of age or patients with hypersensitivity to benzyl alcohol. When reconstituted as directed, etoposide phosphate solutions can be stored in glass or plastic containers under refrigeration for 7 days. When reconstituted with a diluent containing a bacteriostat, store at controlled room temperature for up to 48 hours. Following reconstitution with Sterile Water for Injection, USP, 5% Dextrose Injection, or 0.9% Sodium Chloride Injection store at controlled room temperature for up to 24 hours. Following reconstitution, etoposide phosphate may be further diluted to concentrations as low as 0.1 mg/mL etoposide with 5% Dextrose Injection or 0.9% Sodium Chloride Injection. The diluted solution can be stored under refrigeration or at controlled room temperature for 24 hours.
Supplier: Commercially available from various manufactures. See package insert for more detailed information.

8.10 Hydrocortisone

Commercially available for injection in vials containing 100 mg of the drug in powdered form. To prepare for intrathecal use, reconstitute each vial with 2 mL of preservative-free 0.9% sodium chloride injection to yield a solution containing 50 mg of hydrocortisone/mL. Prepare just prior to use, and discard unused solution to avoid the risk of bacterial contamination. Commercial hydrocortisone injections which contain paraben or benzyl alcohol should not be used to prepare intrathecal dose forms.

8.11 Leucovorin Calcium (LCV, Wellcovorin®, citrovorum factor, folinic acid), NSC #003590

Source and Pharmacology: Leucovorin is a mixture of the diastereoisomers of the 5-formyl derivative of tetrahydrofolic acid (THF). The biologically active compound of the mixture is the (-)-l-isomer, known as Citrovorum factor or (-)-folinic acid. Leucovorin does not require reduction by the enzyme dihydrofolate reductase in order to participate in reactions utilizing folates as a source of "one-carbon" moieties. Administration of leucovorin can counteract the therapeutic and toxic effects of folic acid antagonists such as methotrexate, which act by inhibiting dihydrofolate reductase. In contrast, leucovorin can enhance the therapeutic and toxic effects of fluoropyrimidines used in cancer therapy, such as 5- fluorouracil. Leucovorin is readily converted to another reduced folate, 5,10-methylenetetrahydrofolate, which acts to stabilize the binding of fluorodeoxyuridylic acid (an active metabolite of 5-FU) to thymidylate synthase and thereby enhances the inhibition of this enzyme. Peak serum levels of 5-methyl THF (an active metabolite) were reached at approximately 1.3-1.5 hours (IV/IM) and 2.3 hours for the oral form. The terminal half-life of total

reduced folates was approximately 6.2 hours. Following oral administration, leucovorin is rapidly absorbed and expands the serum pool of reduced folates. At a dose of 25 mg, almost 100% of the l-isomer (the biologically active form) but only 20% of the d-isomer is absorbed. Oral absorption of leucovorin is saturable at doses above 25 mg. The apparent bioavailability of leucovorin was 97% for 25 mg, 75% for 50 mg, and 37% for 100 mg doses. Both oral and parenteral leucovorin raise the CSF folate levels.

Formulation and Stability: Leucovorin calcium for injection is supplied as a sterile ready to use liquid and a sterile powder for injection. The 10 mg/mL preservative free liquid is available in 50 mL vials containing sodium chloride 400 mg/vial. Store preservative free liquid in the refrigerator at 2°-8°C (36°-46°F) protected from light. The powder for injection is available in 50 mg, 100 mg, 200 mg, 350 mg and 500 mg vials. Store at room temperature 15° to 25°C (59° to 77°F) protected from light. Reconstitute the sterile powder with sterile water for injection or bacteriostatic water for injection to a concentration of 10 mg/mL leucovorin calcium. Do not use diluents containing benzyl alcohol for doses > 10mg/m² or in infants < 2 years of age or patients with allergy to benzyl alcohol. When Bacteriostatic Water is used, the reconstituted solution is good for 7 days. If reconstituted with sterile water, use solution immediately as it contains no preservative. One milligram of leucovorin calcium contains 0.004 mEq of leucovorin and 0.004 mEq of calcium. The oral form of leucovorin is available as 5 mg, 10 mg, 15 mg, and 25 mg tablets. Inactive ingredients vary depending on manufacturer but tablet formulations may include: corn starch, dibasic calcium phosphate, magnesium stearate, pregelatinized starch, lactose, microcrystalline cellulose, and sodium starch glycolate.

Guidelines for Administration: See Treatment and Dose Modifications sections of the protocol. Because of the calcium content of the leucovorin solution, no more than 160 mg of leucovorin should be injected intravenously per minute (16 mL of a 10 mg/mL solution per minute). IV leucovorin and sodium bicarbonate are incompatible. Oral leucovorin should be spaced evenly (e.g. every six hours) throughout the day and may be taken without regard to meals. Doses > 25 mg should be given IV due to the saturation of absorption. Leucovorin should not be administered < 24 hours after intrathecal injections which contain methotrexate unless there are special circumstances.

Supplier: Commercially available from various manufacturers. See package insert for further information.

8.12 Mercaptopurine (6-MP, Purinethol®, 6-mercaptopurine, Purixan®) NSC #000755

Source and Pharmacology: Mercaptopurine is an analogue of the purine bases adenine and hypoxanthine. The main intracellular pathway for 6-MP activation is catalyzed by the enzyme hypoxanthine-guanine phosphoribosyl transferase (HGPRT) which catalyzes the conversion of 6-MP to several active nucleotide metabolites including thioinosinic acid, a ribonucleotide which can interfere with various metabolic reactions necessary for nucleic acid (RNA and DNA) biosynthesis. It can also cause pseudofeedback inhibition of the first step in de novo purine biosynthesis or convert to another ribonucleotide which can cause feedback inhibition. Mercaptopurine can be incorporated into DNA in the form of 6-TG nucleotides as well and thus produce toxicity. The absorption of an oral dose of 6-MP is incomplete and variable, with only about 16%-50% of an administered dose reaching the systemiccirculation secondary to a first pass metabolism in the liver. Food intake and co-administration with cotrimoxazole (TMP/SMX) significantly reduces absorption of 6-MP. After IV administration, 6-MP has a plasma half-life of 21 minutes in children and 47 minutes in adults. Approximately 19% is bound to protein.

Mercaptopurine is well distributed into most body compartments except the CSF (with high dose IV 6-MP the CSF to plasma ratio is 0.15). Mercaptopurine is metabolized by xanthine oxidase in the liver to 6-Thiouric acid an inactive metabolite. In patients receiving both 6-MP and allopurinol (a xanthine oxidase inhibitor) the dose of 6-MP must be reduced by 50-75%. Since TPMT, 6-thiopurine methyltransferase, is also one of the enzymes involved in the metabolism of 6-MP, those individuals who have an inherited deficiency of the enzyme may be unusually sensitive to the myelosuppressive effects of 6-MP and prone to developing rapid bone marrow suppression following the initiation of treatment. 6-MP is excreted in urine as metabolites and some unchanged drug; about half an oral dose has been recovered in 24 hours. A small proportion is excreted over several weeks.
Formulation and Stability: Available as a 50 mg tablet containing the inactive ingredients corn and potato starch, lactose, magnesium stearate, and stearic acid. Store at 15°-25°C (59°-77°F) in a dry place.

PURIXAN® (mercaptopurine) oral suspension is supplied as a commercially available 2000 mg/100 mL (20 mg/mL) pink to brown viscous liquid in amber glass multiple-dose bottles. The suspension contains the following inactive ingredients: xanthan gum, aspartame, concentrated raspberry juice, sucrose, methyl parahydroxybenzoate, propyl parahydroxybenzoate and purified water. Once opened, PURIXAN® should be used within 6 weeks. Store between 15 to 25ºC (59º to 77ºF) in a dry place.

Guidelines for Administration: See Treatment and Dose Modifications sections of the protocol. Do not give oral mercaptopurine with food or milk. Concurrent milk products can decrease absorption and mercaptopurine's effect is enhanced if given at bedtime on an empty stomach. Oral mercaptopurine should be given, if possible, at least two hours after and at least one hour before food or milk. If allopurinol is also given, the oral dose of mercaptopurine should be reduced by 65-75%. Patients with severe myelosuppression should have their thiopurine S-methyltransferase (TPMT) status and/or their thiopurine metabolite concentrations evaluated, so that the dose of mercaptopurine can be reduced in patients with a TPMT defect. Patients with the rare homozygous deficient TPMT phenotype may tolerate only 1/10th to 1/20th the average mercaptopurine dose. TPMT testing and thiopurine metabolite measurements are commercially available.

For children unable to swallow the tablets whole, a 10mg/mL oral suspension can be compounded. Suspensions prepared in this manner are stable at room temperature or refrigerated (preferred) for at least 14 days following compounding. The suspension should be shaken well before each use. Procedures for proper handling and disposal of cytotoxic drugs should be used when preparing the suspension. (Aliabadi HM, Romanick M, Desai S et al. Effect of buffer and antioxidant on stability of mercaptopurine suspension. *Am J Heath-Syst Pharm.* 65:441-7, 2008).

PURIXAN® suspension (20 mg/mL) may also be used for patients unable to swallow tablets. Prior to each administration of PURIXAN®, the bottle should be shaken vigorously for at least 30 seconds to ensure the oral suspension is well mixed.

Repeat oral dose if vomiting occurs within 30 minutes. If one dose is missed, this dose can be made up at the end of a cycle at the discretion of the treating physician. If more than one dose is missed, those doses should be skipped and not be made up.

Supplier: Commercially available from various manufacturers. See package insert for further information.

8.13 **Methotrexate** (MTX, amethopterin, Trexall®) NSC #000740

Source and Pharmacology: A folate analogue which reversibly inhibits dihydrofolate reductase, the enzyme that reduces folic acid to tetrahydrofolic acid. Inhibition of tetrahydrofolate formation limits the availability of one carbon fragments necessary for the synthesis of purines and the conversion of deoxyuridylate to thymidylate in the synthesis of DNA and cell reproduction. The polyglutamated metabolites of MTX also contribute to the cytotoxic effect of MTX on DNA repair and/or strand breaks. MTX cytotoxicity is highly dependent on the absolute drug concentration and the duration of drug exposure. MTX is actively transported across cell membranes. At serum methotrexate concentrations exceeding 0.1 μmol/mL, passive diffusion becomes a major means of intracellular transport of MTX. The drug is widely distributed throughout the body with the highest concentration in the kidney, liver, spleen, gallbladder and skin. Plasma concentrations following high dose IV MTX decline in a biphasic manner with an initial half-life of 1.5-3.5 hours, and a terminal half life of 8-15 hours. About 50% is bound to protein. After oral administration, approximately 60% of a 30 mg/m2 dose is rapidly absorbed from the GI tract, with peak blood levels at 1 hour. At doses >30 mg/m² absorption decreases significantly. Even at low doses absorption may be very erratic, varying between 23% and 95%. The elimination of MTX from the CSF after an intrathecal dose is characterized by a biphasic curve with half-lives of 4.5 and 14 hours. After intrathecal administration of 12 mg/m², the lumbar concentration of MTX is ~ 100 x's higher than in plasma. (Ventricular concentration is ~ 10% of lumbar concentration). MTX is excreted primarily by the kidneys via glomerular filtration and active secretion into the proximal tubules. Renal clearance usually equals or exceeds creatinine clearance. Small amounts are excreted in the feces. There is significant entero-hepatic circulation of MTX. The distribution of MTX into third-space fluid collections, such as pleural effusions and ascitic fluid, can substantially alter MTX pharmacokinetics. The slow release of accumulated MTX from these third spaces over time prolongs the terminal half-life of the drug, leading to potentially increased clinical toxicity.

Formulation and Stability: Methotrexate for Injection is available as a lyophilized powder for injection in 20 mg and 1 g vials. The powder for injection contains approximately 0.14 mEq sodium in the 20 mg vial; 7 mEq sodium in the 1 g vial. Methotrexate for Injection is also available as a 25 mg/mL solution in 2, 4, 8, 10, 20 and 40 mL preservative free vials and 2 and 10 mL vials with preservative. The 2, 4, 8, 10, 20, and 40 mL solutions contain approximately 0.43, 0.86, 1.72, 2.15, 4.3, and 8.6 mEq sodium per vial, respectively. The preserved vials contain 0.9% benzyl alcohol as a preservative**.** Sterile methotrexate powder or solution is stable at 20 to 25ºC (68 to 77ºF); excursions permitted to 15°-30ºC (59°-86ºF). Protect from light.

Guidelines For Administration:

Intravenous Administration: Powder for injection: Dilute 1 g vial with 19.4 mL of non-preserved SWFI, Dextrose 5% in water or 0.9% Sodium Chloride Injection for a 50 mg/mL concentration.

Dilute the 20 mg vial to a concentration ≤ 25 mg/mL with above diluents. The powder for injection may be further diluted in 0.9% Sodium Chloride or dextrose containing solutions to a concentration of ≤ 25 mg/mL for IV use. The 25 mg/mL solution may be further diluted in Saline or Dextrose containing solutions for IV use. Do not use the preserved solution due to the risk of benzyl alcohol toxicity. Methotrexate dilutions are chemically stable for at least 7 days at room temperature but contain no preservative and should be used within 24 hours. Diluted solutions especially those containing bicarbonate exposed to direct sunlight for periods exceeding 4 hours should be protected from light.
High dose Methotrexate requires alkalinization of the urine, adequate hydration and leucovorin rescue. Avoid probenecid, penicillins, cephalosporins, aspirin, and NSAIDS as renal excretion of MTX is inhibited by these agents.
Intrathecal Administration: Use preservative free 25 mg/mL solution or 20 mg lyophilized powder for injection. For intrathecal administration, dilute with 5-10 mL preservative free 0.9% Sodium Chloride Injection, lactated Ringer's, or Elliot's B solution, or as per institutional standard of practice. The volume of CSF removed should be equal to at least half the volume delivered.
Supplier: Commercially available from various manufacturers. See package insert for further information

8.14 Prednisone (Deltasone, Meticorten, Orasone®, Liquid Pred, Pediapred®, Sterapred®) NSC #010023

Source and Pharmacology: Prednisone is a synthetic compound closely related to hydrocortisone. Glucocorticoids produce widespread and diverse physiologic effects on carbohydrate, protein, and lipid metabolism, electrolyte and water balance, functions of the cardiovascular system, kidney, skeletal muscle, and the nervous systems. Glucocorticoids reduce the concentration of thymus-dependent lymphocytes (Tlymphocytes), monocytes, and eosinophils. Glucocorticoids selectively bind to the cortisol receptors on human lymphoid cells which are found in larger numbers on leukemic lymphoblasts. They also decrease binding of immunoglobulin to cell surface receptors and inhibit the synthesis and/or release of interleukins, thereby decreasing T-lymphocyte blastogenesis and reducing expansion of the primary immune response. The specific cellular mechanisms that act to halt DNA synthesis are thought to be related to inhibition of glucose transport or phosphorylation, retardation of mitosis, and inhibition of protein synthesis. Peak blood levels occur within 2 hours of oral intake. Prednisone is approximately 75% protein bound with a plasma t½ of 3.2 to 4 hours. (Biologic half-life is 12-36 hours.)
Formulation and Stability:Available in 1 mg, 2.5 mg, 5 mg, 10 mg, 20 mg, 25 mg, and 50 mg tablets; liquid, 5 mg/5 mL or 5 mg/mL. Inactive ingredients vary depending on manufacturer but tablet formulations may include: calcium or magnesium stearate, corn starch, lactose, erythrosine sodium, mineral oil, sorbic acid, sucrose, talc and various dyes. Liquid formulations may include: 5-30% alcohol, fructose, sucrose, saccharin, and sorbitol.
Guidelines for Administration: See Treatment and Dose Modifications sections of the protocol. If taken orally, repeat oral dose if vomiting occurs within 30 minutes. If one dose is missed, this dose can be made up at the end of a cycle at the discretion of the treating physician. If more than one dose is missed, those doses should be skipped and not be made up.

Supplier: Commercially available from various sources. See package insert for further information

8.15 **Vincristine** (Oncovin®, VCR, LCR) NSC #67574

Source and Pharmacology: Vincristine is an alkaloid isolated from Vinca rosea Linn (periwinkle). It binds to tubulin, disrupting mircotubules and inducing metaphase arrest. Its serum decay pattern is triphasic. The initial, middle, and terminal half-lives are 5 minutes, 2.3 hours, and 85 hours respectively; however, the range of the terminal half-life in humans is from 19 to 155 hours. The liver is the major excretory organ in humans and animals; about 80% of an injected dose of vincristine sulfate appears in the feces and 10% to 20% can be found in the urine. The P450 cytochrome involved with vincristine metabolism is CYP3A4. Within 15 to 30 minutes after injection, over 90% of the drug is distributed from the blood into tissue, where it remains tightly, but not irreversibly bound. It is excreted in the bile and feces. There is poor CSF penetration.

Formulation and Stability: Vincristine is supplied in a vial each mL of which contains vincristine sulfate, 1 mg (1.08 μmol); mannitol, 100 mg; sterile water for injection; Acetic acid and sodium acetate are added for pH control. The pH of Vincristine Sulfate Injection, USP ranges from 3.5 to 5.5. This product is a sterile solution. Store refrigerated at 2°-8°C or 36°-46°F. Protect from light and retain in carton until time of use. Do not mix with any IV solutions other than those containing dextrose or saline.

Guidelines for Administration: The delivery of vincristine via either IV slow push or minibag is acceptable. Injection of vincristine sulfate should be accomplished within 1 minute. Vincristine sulfate must be administered via an intact, free-flowing intravenous needle or catheter. Care should be taken to ensure that the needle or catheter is securely within the vein to avoid extravasation during administration. The solution may be injected either directly into a vein or into the tubing of a running intravenous infusion. When dispensed the container or syringe containing vincristine must be enclosed in an overwrap with warnings that it is for intravenous use only and is fatal if given intrathecally or similar wording per institutional standard.

Supplier: Commercially available from various manufacturers. See package insert for more detailed

9. CORRELATIVE/SPECIAL STUDIES

9.1 Asparaginase Enzyme Activity/Pharmacokinetic (PK) Samples (Required of all participants)

9.1.1 Asparaginase PK Sample Acquisition and Handling

1. Draw 3 mL of blood into a red top Vacutainer tube (no anticoagulant). Record the date and time that the sample was collected.

2. Allow the tube to stand at room temperature for 30 min to clot (do not place the tube in ice).

3. Centrifuge the clotted blood sample at 1,100-1,300 x g for 10 minutes at 25º C. Record the time that the sample was centrifuged.

4. Using a disposable pipette, carefully remove the serum (uppermost clear layer) without disturbing the pelleted blood cells and buffy coat, and transfer it into a 2 mL self-standing polypropylene cryogenic tube with external threads (ex: Fisher Scientific Item# 09-761-71 or Corning Life Sciences Item # 430659).

5. Print vial labels using temperature-resistant labels (ex: USA Scientific Cryotags Item # 9187-1100). Each label must include the protocol #, specimen type (serum), PK#, study site and patient study ID # and initials (see example below). Affix the label onto the cryotube. Do not cover the cryovial cap with freezer tape.

- Label Example:

Protocol #:11001
Serum
Sample #: PK-05
Study Site: DFCI
Patient #: 11-099/XX

6. Immediately place the cryotube in a freezer maintained at -70ºC or lower until packaged for shipment. Record the time that the sample was placed in the freezer.

9.1.2 Shipment of Asparaginase PK Samples

Complete sets of samples from one or more patients should be sent by overnight mail to the address listed below. The sample tubes should be placed within a zip lock plastic bag and packaged in a seamless styrofoam container with 3-4 inches of dry ice on the bottom, and completely covered with an additional 3-4 inches or more of dry-ice. Seal the styrofoam container within a tight-fitting cardboard shipping box. Insert copies of the Pharmacokinetic Data Form for each set of samples into a separate zip-lock plastic bag placed on top of the styrofoam container before the external shipping box is sealed. Send the samples from Monday to Wednesday by overnight courier for delivery by 10 a.m. on the following day. Samples should not be shipped on a Thursday or Friday. Samples collected Thursday – Sunday should be shipped the following Monday. Please provide notification of the sample shipment by e-mail prior to shipping to both [redacted] ([redacted]).

Shipping Address:

[redacted]

9.1.3 Schedule

Obtain Asparaginase PK Samples at the Following Time Points:

- Induction Phase: Days 7 (two samples: one prior to IV asparaginase dose and one 5-10 minutes after completion of infusion), 11, 18, 25 and 32
- Post-Induction: Just prior to each dose of asparaginase administered during post-induction treatment phases (ie, every 3-weeks for patients randomized to SC-PEG asparaginase and every 2-weeks for patients randomized to Oncaspar)
- For patients switched to Erwinia: Just prior to first dose of Erwinia

Note: Induction Day 7 sample should be obtained 5-10 minutes after completion of IV asparaginase infusion.

Note: If Day 7 asparaginase is delayed in induction, shift the days that PK samples are collected accordingly. *For example: If asparaginase is given on Day 10 instead of Day 7, the PKs should be collected on Days 14, 21, 28 and 35 instead of Days 11, 18, 25 and 32.*

Note: Post-induction asparaginase samples will be run in real time. These samples should be shipped to MGH at least once per week.

Note: PK samples should be obtained on the day the asparaginase is scheduled to be administered (pre-dose) even if the asparaginase ends up being held. If the asparaginase dose is held and is not given within 3 days, then a repeat PK sample should be drawn just prior to the actual dose.

9.2 **Antibody Samples (Required of all participants)**

9.2.1 Antibody Sample Acquisition and Handling

1. Draw at least 2 mL of venous blood into a dark green-top (sodium heparinized) collection tube. Invert the tube gently 4 times to mix the blood with the heparin then **place tube immediately on ice** (no longer than one minute after mixing).

2. Record the sample date and time of collection.

3. Centrifuge the blood sample immediately (within 30 minutes of collection) in a refrigerated centrifuge, at approximately 3,000 rpm for 5 minutes.

4. Using a disposable pipette, transfer the plasma fraction (within 5 minutes of centrifugation) into each of *three* specimen shipping tubes. At least 0.25 mL (250 µL) of plasma should be in each tube. Sample tubes should be labeled with the pre-printed bar coded sample labels provided in the specimen kits.

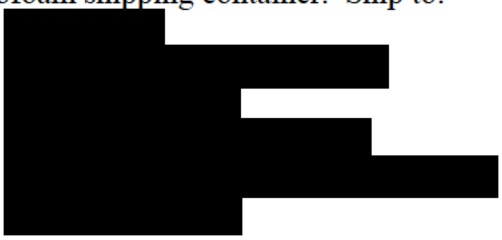
Each label must be completely filled out; information on the label should correspond to the information entered on the associated Sample Collection Form.

5. Within 10 minutes of plasma separation, place all three plasma samples in a -20 +/-5 ° C freezer (or at < -65° C if available) until shipping. *NOTE: The maximum total processing time from collection to freezing should be less than 60 minutes.*

9.2.2 Shipment of Antibody Samples

1. Complete a Sample Collection Form (included with the kits) for each patient for each treatment phase. Put a line through each section not being used (e.g., if Induction samples, cross out Consolidation section on form). Special circumstances/errors--clearly indicate if any of the following occurred: specimen hemolyzed during processing; any unusual appearance or occurrence during processing; processing time exceeded 60 minutes; missed time points.

2. Place labeled specimens and absorbent sheet in larger pocket of specimen bag and place copy of Sample Collection Form in the smaller pocket. Place specimen bag in the box and cover with dry ice. Indicate the approximate pounds of dry ice on the affixed dry ice label. Complete the airbill and affix to the Styrofoam shipping container. Ship to:



To facilitate tracking, email the Fed Ex tracking # to both:

3. Samples should be batched and shipped at the end of each month by overnight delivery on a Monday through Thursday. Please alert the Study Director if Saturday delivery is required.

9.2.3 Schedule

Obtain Antibody Samples at the Following FIVE Time Points:

- Day 7: Prior to first dose of asparaginase
- Day 32/End of Induction
- CNS Phase (or Consolidation I for VHR): Just prior to 1st post-induction dose of asparaginase
- Mid-Consolidation II: Week 13 of post-induction asparaginase (SC-Peg dose #5 or Oncaspar dose #7)
- End of Consolidation II: Just prior to last dose of asparaginase

Note: Antibody samples should be obtained at the same time as the PK samples (just prior to dose of asparaginase). Allowable deviations in timing of Induction Day 7 and Day 32 samples are +/- 3 days; for CNS Phase (or Consol I for VHR), Mid-Consolidation II and End of Consolidation II it is +/- 7 days. If asparaginase is held or delayed, the antibody sample should be collected prior to the correct corresponding asparaginase dose number (not by the week number).

9.3 **Note: If Day 7 asparaginase is delayed in induction, shift the days that antibody samples are collected accordingly.** ***For example: If asparaginase was given on Day 10 instead of Day 7, the antibody should be collected on Day 10 instead of Day 7.*** **Minimal Residual Disease (MRD) Samples (Leukemia patients only)**

9.3.1 Sample Acquisition and Handling

- Obtain
  - **Marrow: One purple top (EDTA) tubes**, with 2-3 mL of bone marrow aspirate
  - **Blood: One purple top (EDTA) tubes**, with 2-3 mL of peripheral blood

9.3.2 Shipment

- Outside Sites: Send with a cold pack (*not* on dry ice), same day overnight to DFCI (see section 9.6)
- DFCI: Place in dirty utility refrigerator on 6 North and page Study [redacted]

9.3.3 Schedule

- **At time of study entry (pre-treatment): Required of all leukemia patients**
- Induction Phase, Day 18 *optional* (Marrow: collect if consent is obtained for Day 18 bone marrow study; Blood: collect if consent is obtained to collect additional research specimens)
- **Induction Phase, Day 32: Required of all leukemia patients**

- **End of Treatment *optional* bone marrow (collect only if consent is obtained for end of treatment marrow);** Must be completed within 30 days before or 60 days after the off treatment date.
- VHR patients only:
    - Conslidation IB, Day 1: Peripheral blood
    - Consolidation IC, Day 1: Peripheral blood
    - CNS Phase, Day 1 (coincide with 1st LP): Bone Marrow and Peripheral blood
    - Consolidation II, Day 1 (ETP patients only): Peripheral blood
- T-ALL patients:
    - CNS Phase, Day 1 (coincide with 1st LP): Bone Marrow (if consent is obtained for CNS phase bone marrow study) and Peripheral blood
    - Consolidation II, Day 1: Peripheral blood
- At time of suspected relapse

9.4 Biology Research Samples—Peripheral Blood and Marrow (Optional)

9.4.1 Sample Acquisition and Handling

- Obtain
    - Marrow: 1-2 purple top (EDTA) tubes each with 2-3 mL of bone marrow aspirate in purple top (EDTA) tube
    - Peripheral Blood: 1-2 purple top (EDTA) tubes, each with 2-3 mL of blood

9.4.2 Shipping

- Outside Sites: Send with a cold pack (*not* on dry ice), same day overnight to DFCI (see section 9.6)

- DFCI: Place in dirty utility refrigerator on 6 North and page Study Team Sample [redacted]

9.4.3 Schedule

- At time of study entry (pre-treatment)
- At time of suspected relapse

9.5 CSF Research Samples (Optional)

9.5.1 Sample Acquisition and Handling

- Obtain 1-3 mL of leftover CSF (collected at the time of a therapeutic lumbar puncture, just prior to the administration of intracthecal chemotherapy) and immediately place specimen on ice.

- Within 1 hour of collection, centrifuge specimen for 5 minutes then aliquot into cryovial and immediately freeze at -80 degrees.

9.5.2 Shipping

- Supernatant should be shipped on dry ice to Peter Cole, MD (see Section 9.6)
- Please include the following information with each CSF specimen: Patient study ID # and initials (no names), date of collection and timepoint (Day 18, End of Induction, CNS Phase LP4, or Consolidation II). Please send an email at the time of shipping to [redacted]
- Sample may be batched and shipped approximately once a month

9.5.3 Schedule:

- Day 18 Lumbar Puncture
- Day 32 (or end-induction) Lumbar puncture
- 4th Lumbar puncture during the CNS phase
- 1st Lumbar puncture performed during the Consolidation II phase

9.6 Sample Shipment of MRD, Biology and CSF Research Samples

All MRD and Biology Research samples (**except** CSF samples) should be shipped to:

[redacted]

All CSF samples should be shipped to:

[redacted]

10. STUDY CALENDAR

Baseline evaluations, including radiographic imaging, are to be conducted within 7-days prior to start of protocol therapy. All study assessments should be performed within ± 3 days of the timepoints indicated below during the induction phase and within ± 7 days of the timepoints during post-induction phases, unless otherwise noted.

10.1 Study Assessements: Study Entry and Induction Phase

	Study Entry	Day 1	Day 4	Day 7	Day 11	Day 18	Day 25	Day 32
Physical Exam	X							X
CBC w/differential and platelets	X	X	X					X
Serum chemistry [a]	X							X
Bone Marrow aspirate/biopsy for morphology	X							X[b]
Bone Marrow for Immunophenotype	X[q]							
Bone Marrow for Cytogenetics, FISH and/or PCR	X[c]							
MRD (Marrow/Blood)	X					X[d]		X[b]
Biology Research Studies (Marrow/Blood)	X[e]							
Asparaginase PK samples (blood) [m]				X[n]	X[o]	X[o]	X[o]	X[o]
Asparaginase Antibody samples (blood)				X[p]				X
Vitamin D 25 OH level (blood)		X[f]						X[f]
CSF for cell count, cytospin						X[k]		X[k]
Chest x-ray	X							X[g]
CT scans (Neck, chest, abdomen, pelvis)	X[h]							X[i]
Echocardiogram (recommended, not required)	X							

B-HCG (urine or serum)	x[j]							
AE/Toxicity Assessment [L]	x	x	x		x	x	x	x

[a] Chemistries should include BUN, Creatinine, AST, direct bilirubin

[b] Required only if marrow involvement documented at diagnosis; Day 32 MRD studies from marrow/blood only for leukemia patients.

[c] FISH and/or PCR should be sent in for all patients at diagnosis to screen for TEL/AML1, BCR-ABL and MLL rearrangements (Note: when possible, FISH is preferred over PCR as screening test for TEL/AML1). Additional FISH/PCR tests (to screen for trisomies, E2A/PBX1, 9p deletions and other abnormalities) may be sent at discretion of treating clinician and are encouraged in patients with normal or uninterpretable karyotype. Reports from all karyotype, FISH and PCR studies should be sent to DFCI and will be reviewed by Principal Investigator or designee to determine final risk group status.

[d] Marrow specimen only for leukemia patients who have consented to optional Day 18 marrow; peripheral blood to be sent for any leukemia patient who has consented to optional additional research specimens

[e] Only for patients who have consented to optional research specimens

[f] Obtain Vitamin D level at study entry and at the end of the induction phase for patients who have consented to collection of these research specimens. The first Vitamin D sample may be collected within 3 days of beginning treatment.

[g] Required only if chest x-ray was positive at diagnosis

[h] Required only for lymphoblastic lymphoma patients. A chest CT for leukemia patients with mediastinal mass on pre-study chest x-ray is suggested but not required.

[i] CT of involved sites required for remission assessment only for lymphoblastic lymphoma patients. A chest CT scan is suggested for leukemia patients with mediastinal mass at diagnosis, but is not required.

[j] Only for female patients of childbearing potential prior to initiation of therapy.

[k] Day 18 and Day 32: CSF is collected for clinical purposes. Leftover CSF obtained at these timepoints may be sent for research purposes if patient has consented to optional research studies. If end-induction LP is delayed, should still collect CSF even if LP occurs more than 3 days past Day 32.

[L] AE assessment should be performed on an ongoing basis (at least once per month) throughout the time a participant is on study. Note: SAE's need be reported in real time.

[m] Days 25 and 32 Asparaginase PK samples will be run in real time. These samples should be shipped to laboratory within one week of the Day 32 sample being drawn.

[n] Day 7 Asparaginase PK samples should be collected prior to first dose of Asparaginase and 5-10 minutes after completion of Asparaginase infusion.

[o] Day 11, 18, 25 and 32 PK samples can be drawn $\pm$ 3 days from timepoint

[p] Day 7 asparaginase antibody sample should be collected prior to first dose of asparaginase (at same time first PK sample is drawn)

[q] Copies of flow histograms for all patients with T-ALL or T-lymphoblastic lymphoma should be sent to DFCI for central review.

Note: For lymphoblastic lymphoma patients without marrow involvement, MRD samples (bone marrow/blood) are not required.

10.2 Study Assessments: Post-Induction Treatment Phases

	Consolidation I	CNS Phase	Consolidation II	Continuation	End of Therapy
Physical Exam		X		X	
CBC w/differential and platelets	X[l]	X	X[a]	X[a]	
Serum chemistry[b]	X[l]	X	X[a]	X[a]	
Bone Marrow aspirate for morphology		X[c]			
MRD (Marrow/Blood)	X[c]	X[c]	X[k]		X[n]
Asparaginase PK samples (blood)	X[m]	X[d]	X[d]		
Asparaginase Antibody samples (blood)	X[m]	X[e]	X[f]		
Vitamin D 25 OH level (blood)				X[g]	X[g]
Echocardiogram					X[h]
CSF		X[i]	X[i]		
AE/Toxicity Assessment[j]	X	X	X	X	X

[a] Obtain CBC and chemistries at the start of each 3-week chemotherapy cycle
[b] Chemistries prior to each 3-week cycle should include AST and direct bilirubin.
[c] VHR Leukemia & T-ALL patients only. Blood for MRD should be obtained at the start of Consolidation IB and IC (for VHR leukemia patients only). Marrow should be performed at time of 1st LP of CNS phase. Marrow is required of all VHR leukemia patients and is optional for non-VHR T-ALL (should be performed only in T-ALL patients who have consented to marrow at this timepoint). Blood for MRD should be obtained at the start ofthe CNS phase (for VHR leukemia patients & T-ALL).
[d] Asparaginase PK samples should be obtained just prior to each post-induction dose of asparaginase (during CNS and Consolidation II phases, and Consolidation IC phase for VHR patients). All post-induction asparaginase PK samples will be run in real time. Samples should be shipped to laboratory for analysis as soon as they are obtained. PK samples should be obtained on the day the asparaginase is

scheduled to be administered (pre-dose) even if the asparaginase ends up being held. If the asparaginase dose is held and is not given within 3 days, then a repeat PK sample should be drawn just prior to the actual dose. If patient is switched to IM Erwinia, PK sample should be obtained just prior to first dose of Erwinia then every 3 weeks through Consolidation II, just prior to an Erwinia dose.

[e] Draw antibody sample just prior to 1st dose of post-induction asparaginase (CNS Phase for SR/HR; Consolidation IC for VHR)

[f] Draw 2 antibody samples: 1) Mid-Consolidation II-just prior to Week 13 dose of post-induction asparaginase (SC-Peg dose #5 or Oncaspar dose #7); 2) End of Consolidation II-just prior to last dose of asparaginase

[g] Obtain Vitamin D level at the start of the 1st or 2nd cycle of Continuation phase and at the 1st day of the last continuation cycle or the 1st off treatment visit for patients who have consented to collection of these research specimens.

[h] Echocardiogram is suggested for all patients, but is not required.

[i] Optional collection of leftover CSF during lumbar puncture performed as part of treatment. CSF is collected during the 4th Lumbar puncture during the CNS phase and 1st Lumbar puncture performed during the Consolidation II phase. Leftover CSF is only collected with consent.

[j] AE assessment should be performed on an ongoing basis throughout the time a participant is on study (at least once per month through the end of the Consolidation II phase and at least once very 3 months during the Continuation phase). Note: SAE's need be reported in real time.

[k] T-ALL patients only: obtain a blood sample at the start of their first cycle of Consolidation II

[l] Day 32 labs can be used if Consolidation I begins within 3 days of Day 32

[m] Only for VHR patients, on Day 8 of Consolidation IC

[n] Marrow specimen only for leukemia patients who have consented to optional End of Treatment marrow; must be collected within 30 days before or 60 days after the off treatment date.Peripheral blood not necessary at this timepoint.

Note: For lymphoblastic lymphoma patients without marrow involvement, MRD samples (bone marrow/blood) are not required at any time points.

10.3 Assessment Schedule after Completion of Therapy

Suggested:

- Physical exam/history and CBC with differential/platelets
  - Monthly x 6 months, then
  - Every 2 months x 6 months, then
  - Every 4 month x 1 year, then
  - Every 6 months x 1 year, then
  - Annually.
- Echocardiograms
  - SR patients: At the completion of therapy, and then 3-5 years after completion of therapy. May by obtained more frequently as clinically indicated

  - HR/VHR patients: Annually x 5 years, then every other year x 5 years, or as clinically indicated.
- Ophthalmologic Evaluation
  - At the completion of therapy and 1-year post-therapy (to rule out steroid-associated cataracts)
- Dental Evaluation
  - At the completion of therapy, then every 6 months, or as clinically indicated.

11. CLINICAL RESPONSE CRITERIA

11.1 Definition of CNS Status

CNS-1 = CSF without blasts on cytospin, regardless of CSF WBC count
CNS-2 = CSF WBC <5 with blasts present on cytospin
CNS-3 = CSF WBC ≥5 with blasts present on cytospin

Steinherz/Bleyer Algorithm to Interpret Traumatic LP's (>10 RBC per high power field) with ≥ 5 WBC/hpf:

- CSF specimens with > 10 RBC/hpf and ≥ 5 WBC/hpf should be treated as CNS-3 if : (CSF WBC/CSF RBC) > 2x (Blood WBC/Blood RBC).
- All other CSF specimens with > 10 RBC/hpf and ≥ 5 WBC/hpf in CSF specimen should be treated as CNS2.

11.2 Definition of Complete Remission

11.2.1 Leukemia:

An interpretable bone marrow (a specimen with normal marrow elements present) with fewer than 1% malignant lymphoblasts by microscopic examination and peripheral blood without lymphoblasts, and an APC ≥ 1000/mm^3 and platelets ≥ 100,000/mm^3, and no evidence of extramedullary leukemia (no blasts in spinal fluid, at least 70% reduction in size of masses noted on imaging at diagnosis, as defined below for patients with lymphoblastic lymphoma). Note: Patients are allowed to have ≥ 1% normal myeloid or lymphoid blasts (hematogones) in their marrow consistent with recovery and still meet the definition of complete remission. Normal recovery blasts may be distinguished from persistent malignant lymphoblasts by flow cytometry, immunohistochemistry or other studies. Consult principal investigator with any questions.

11.2.2 Lymphoblastic Lymphoma:

At least a 70% reduction in size of largest nodes or masses noted at diagnosis. Reduction may be measured as a decrease in a sum of the products of the two greatest diameters (SPD) of the 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 two perpendicular dimensions,
b) they should include mediastinal masses whenever this site was initially involved.

In addition to this radiographic criterion, there should also be complete disappearance of all clinical evidence of disease by physical examination and no morphologic evidence of disease in bone marrow (if involved at initial diagnosis) and spinal fluid.

11.3 Failure to Achieve Remission (Induction Failure)

11.3.1 Leukemia:

If ≥1% blasts (confirmed to be leukemic lymphoblasts by flow cytometry, immunohistochemistry or other studies) are identified in the marrow on Days 32, or on any marrow performed up through Day 53 (without a preceding marrow consistent with complete remission), the patient will be classified as an Induction

Failure and removed from protocol (contact Principal Investigator or designee prior to removing from protocol if patient is induction failure). Any patient with evidence of extramedullary leukemia on exam, in the spinal fluid or by radiographic imaging (<70% reduction in size of largest nodes or masses noted at diagnosis, or any new biopsy-proven masses) will also be considered an induction failure, regardless of marrow findings.

11.3.2 Lymphoblastic Lymphoma:
If end-induction imaging reveals persistent nodal involvement or masses (< 70% reduction in size of largest nodes or masses noted at diagnosis), the patient will be classified as an Induction Failure and removed from protocol (Notify Principal Investigator or designee if patient is an induction failure). Any patient with new, biopsy-proved sites of disease will also be considered an induction failure. In addition, any patient with evidence of persistent disease in bone marrow (≥5% blasts confirmed by flow cytometry, FISH or other studies) or spinal fluid (CNS-3) will also be considered an induction failure.

11.4 **Criteria for relapse**

11.4.1 Greater than 5% lymphoblasts identified morphologically in bone marrow aspirate/biopsy identified to be leukemic by flow cytometry, cytogenetics, FISH, immunohistochemistry, or other tests.

11.4.2 Development of biopsy-proven extramedullary site of disease (eg, CSF, testicle, lymph node)

11.4.2.1 CNS relapse: CSF sample with ≥ 5 WBC per high power field and lymphoblasts on cytospin, or 2 consecutive CSF specimens meeting the definition of CNS-2 (< 5 WBC/hpf with lymphoblasts seen) obtained at least 3 weeks apart.

12. ADVERSE EVENT REPORTING REQUIREMENTS

12.1 Definitions

12.1.1 Adverse Event (AE)

An adverse event (AE) is any undesirable sign, symptom or medical condition or experience that develops or worsens in severity after starting the first dose of study treatment or any procedure specified in the protocol, even if the event is not considered to be related to the study.

Abnormal laboratory values or diagnostic test results constitute adverse events only if they induce clinical signs or symptoms or require treatment or further diagnostic tests.

By protocol definition, the following expected toxicities related to the diagnosis and treatment of ALL and considered to be unrelated to asparaginase do not need to be recorded as AEs, unless they meet definition of an SAE in section 12.1.2:

- Blood count abnormalities (any grade)
- Electrolyte abnormalities (any grade)
- Uric Acid (any grade)
- Transaminitis without clinical symptoms
- Mucositis (grade 2 or below)
- Nausea (any grade)
- Vomiting (any grade)
- Fatigue (any grade)
- Anorexia (any grade)
- Fever (any grade)
- Constipation (any grade)
- Diarrhea (any grade)
- Hypertension thought to be related to steroids (grade 1)
- Hyperglycemia related to steroids that does not require the use of insulin
- Neuropathy – sensory, motor, or cranial (grade 3 or below and thought to be related to Vincristine)
- Vascular access complications (grade 2)
- Allergic reactions that are not related to asparaginase (grade 2 or below) **(Note: All asparaginase-related allergic reactions must be reported, regardless of grade.)**
- Any grade 1 or grade 2 expected toxicities that are not related to asparaginase

**Following approval of DFCI IRB amendment number 31, additional adverse events related to asparginase will be collected retrospectively on all participants through 30 days after their final dose of asparaginase. Additional events being retrospectively collected will not be considered new SAEs and

will not require reporting to the DFCI IRB, unless they are events which meet the protocol definition of SAE (Section 12.1.2). These additional adverse events are as follows:

- Febrile Neutropenia (grade 3 and 4 only)
- Seizure (grades 1 – 2)
- Intracranial Hemorrhage (grades 1 – 2)
- Injection site reactions related to Erwinia (all grades)
- The following abnormal laboratory values (only if ≥ grade 2):
    - Serum Amylase Increased
    - Lipase Increased
    - Alanine Aminotransferase Increased
    - Aspartate Aminotransferase Increased
    - Creatinine Increased
    - Blood Bilirubin Increased
    - Alkaline Phosphatase Increased
    - Hypoalbuminemia (low blood albumin levels)
    - Hypertriglyceridemia
    - Abnormal Sodium Levels (hypernatremia & hyponatremia)
    - Abnormal Potassium Levels (hyperkalemia & hypokalemia)
    - Abnormal Glucose Levels (hyperglycemia & hypoglycemia)
    - Activated Partial Thromboplastin Time Prolonged
    - INR Increased
    - Fibrinogen Decreased

12.1.2 Serious adverse event (SAE)

A serious adverse event (SAE) is any adverse event, regardless of causality that:

- Results in death
- Is life-threatening. Life-threatening means that the person was at immediate risk of death from the reaction as it occurred, i.e., it does not include a reaction which hypothetically might have caused death had it occurred in a more severe form.
- Requires intensive inpatient medical interventions, including mechanical ventilation, pressor support, and/or fluid resuscitation, or emergent surgical operations. Unplanned hospital admissions for febrile neutropenia, bacteremia or other documented infections, seizure, pancreatitis, thrombosis, nausea/vomiting, diarrhea, and other expected toxicities from the study treatment will not be considered a serious adverse event unless the severity of the condition is considered life-threatening and/or lead to intensive medical interventions or surgical procedures.
- Hospital admissions and/or surgical operations scheduled to occur during the study period, but planned prior to study entry are not considered SAEs if the illness or disease existed before the person was enrolled in the trial, provided that it did not deteriorate in an unexpected manner during the trial (e.g., surgery performed earlier than planned).

- Results in persistent or significant disability/incapacity. Disability is defined as a substantial disruption of a person's ability to conduct normal life functions.
- Is a congenital anomaly or birth defect; or
- Is an important medical event when, based upon appropriate medical judgment, it may jeopardize the participant and require medical or surgical intervention to prevent one of the outcomes listed above. An example of such a medical events is allergic bronchospasm requiring intensive treatment in an emergency room or in the hospital.

Events **not** considered to be serious adverse events are hospitalizations for:

- routine treatment or monitoring of the studied indication, not associated with any deterioration in condition, or for elective procedures
- elective or pre-planned treatment for a pre-existing condition that did not worsen
- toxicities that are expected from the study treatment and are not considered life-threatening (ie, do not require intensive medical or surgical management, as defined above).
- emergency outpatient treatment for an event not fulfilling the serious criteria outlined above and not resulting in inpatient admission
- respite care

12.1.3 Expectedness

Adverse events can be 'Expected' or 'Unexpected.'

12.1.3.1 Expected adverse event

Expected adverse events are those that have been previously identified as resulting from administration of the agent(s). For the purposes of this study, an adverse event is considered <u>expected</u> when it appears in the current adverse event list, the Investigator's Brochure, the package insert or is included in the informed consent document as a potential risk.

Refer to Section 7.1 for a listing of expected adverse events associated with the study agent(s).

12.1.3.2 Unexpected adverse event

For the purposes of this study, an adverse event is considered <u>unexpected</u> when it varies in nature, intensity or frequency from information provided in the current adverse event list, the Investigator's Brochure, the package insert or when it is not included in the informed consent document as a potential risk.

12.1.4 Attribution

Attribution is the relationship between an adverse event or serious adverse event and the study treatment. Attribution will be assigned as follows:

- Definite – The AE is clearly related to the study treatment.
- Probable – The AE is likely related to the study treatment.
- Possible – The AE may be related to the study treatment.
- Unlikely - The AE is doubtfully related to the study treatment.
- Unrelated - The AE is clearly NOT related to the study treatment.

12.2 Serious Adverse Event Reporting Requirements and Criteria

For multi-site trials where a DF/HCC investigator is serving as the principal investigator, each participating investigator is required to abide by the reporting requirements set by the DF/HCC. The study must be conducted in compliance with national regulations, local safety reporting requirements, and reporting requirements of the principal investigator.

Each investigative site will be responsible to report SAEs that occur at that institution to their respective IRB. It is the responsibility of each participating investigator to report serious adverse events to the study sponsor and/or others as described below.

12.2.1 Reporting to the Sponsor

All serious adverse events that occur after the time of enrollment, during treatment, or within 60 days of the first day of the last cycle of chemotherapy must be reported to the DF/HCC Overall Principal Investigator, Lewis Silverman, MD, **within 24 business hours of learning of the occurrence**. This includes events meeting the criteria outlined in section 12.1.2 as well as the following:

Reporting requirements:

- Grade 2 (moderate) and Grade 3 (severe) events that are unexpected and possibly, probably or definitely related to or associated with the study intervention

- All Grade 4 (life-threatening or disabling) events, except those that are expected AND specifically listed in the protocol as not requiring reporting

- All Grade 5 (fatal) events while the participant is enrolled and actively participating in the trial OR when the event occurs within 60 days of the first day of the last cycle of chemotherapy.

  Note: If the participant is in long term follow up, report the death at the time of continuing review.

Investigative sites within DF/HCC will report all serious adverse events directly to the DFCI Office for Human Research Studies (OHRS).

Outside investigative sites should report serious adverse events to their respective IRB according to the local IRB's policies and procedures in reporting adverse events.

The DF/HCC Study Team will submit SAE reports from outside institutions to the DFCI Office for Human Research Studies (OHRS) according to DFCI IRB policies and procedures in reporting adverse events.

12.2.2 Reporting to Regulatory Agencies

All SAEs must be reported to the Overall PI, Lewis Silverman, MD, per the reporting process outlined in 12.3. The DF/HCC Overall Principal Investigator, Lewis Silverman, MD, as holder of the IND and CTA, will be responsible for all communication with the FDA and with Health Canada. The Overall Principal Investigator will report to the FDA and Health Canada, regardless of the site of occurrence, any adverse event that is serious, unexpected and reasonably related (i.e., possible, probable, definite) to the investigational agent. All events will be reported on a DFCI Adverse Event form and/or MedWatch 3500 or 3500A form.

Unexpected fatal or life-threatening experiences associated with the use of the investigational agent will be reported to FDA and Health Canada as soon as possible but in no event later than 7 calendar days after initial receipt of the information.

All other serious unexpected experiences associated with the use of the investigational agent will be reported to the FDA and Health Canada as soon as possible but in no event later than 15 calendar days after initial receipt of the information.

12.2.3 Reporting to Baxalta US Inc.

The DF/HCC Overall Principal Investigator, Lewis Silverman, MD, will be responsible for all communication with Baxalta. The DF/HCC Overall Principal Investigator will report Baxalta, regardless of the site of occurrence, all serious

adverse events that occur after the time of enrollment, during treatment, or within 60 days of the first day of the last cycle of chemotherapy. Serious adverse events will be reported to Baxaltawithin 72 hours of becoming aware of the event.

12.3 Procedures for Adverse Event Recording and Reporting

Participating investigators must report each serious adverse event to Lewis Silverman, MD, within 24 business hours (1 business day) of learning of the occurrence. This can be done via phone or email. In addition, a DFCI Adverse Event Form and/or MedWatch 3500 or 3500A form must be submitted to DFCI within 24 hours of learning of the occurrence. In the event that the participating investigator does not become aware of the serious adverse event immediately (e.g., participant sought treatment elsewhere), the participating investigator is to report the event within 24 business hours after learning of it and document the time of his or her first awareness of the adverse event. Report serious adverse events by telephone or email to:

[redacted]

Within the following 24-48 business hours, the participating investigator must provide follow-up information on the serious adverse event. Follow-up information should describe whether the event has resolved or continues, if and how the event was treated, and whether the participant will continue or discontinue study participation.

Participating investigators will assess the occurrence of AEs and SAEs during the course of the study. All AEs and SAEs whether reported by the participant, discovered during questioning, directly observed, or detected by physical examination, laboratory test or other means, will be recorded in the participant's medical record and on the appropriate study-specific case report forms.

The descriptions and grading scales found in the NCI Common Terminology Criteria for Adverse Events (CTCAE) Version 4.0 will be utilized for AE reporting. Version 4.0 of the CTCAE is identified and located on the CTEP website at:
http://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm
All appropriate treatment areas should have access to a copy of Version 4.0 of CTCAE.

Non-serious adverse events will be reported to the DF/HCC Overall Principal Investigator, Lewis Silverman, MD, on the toxicity Case Report Forms.

12.4 Reporting to Hospital Risk Management

Participating investigators will report to their local Risk Management office any subject safety reports or sentinel events that require reporting according to institutional policy.

12.5 Monitoring of Adverse Events and Period of Observation

All adverse events, both serious and non-serious, and deaths that are encountered from initiation of study intervention, throughout the study, and within 60 days of the first day of the last cycle of chemotherapy should be followed to their resolution, or until the participating investigator assesses them as stable, or the participating investigator determines the event to be irreversible, or the participant is lost to follow-up. The presence and resolution of AEs and SAEs (with dates) should be documented on the appropriate case report form and recorded in the participant's medical record to facilitate source data verification.

For some SAEs, the study sponsor or designee may follow-up by telephone, fax, and/or monitoring visit to obtain additional case details deemed necessary to appropriately evaluate the SAE report (e.g., hospital discharge summary, consultant report, or autopsy report).

Participants should be instructed to report any serious post-study event(s) that might reasonably be related to participation in this study. Participating investigators should notify the DF/HCC Overall Principal Investigator, Lewis Silverman, MD, and their respective IRB of any unanticipated death or adverse event occurring after a participant has discontinued or terminated study participation that may reasonably be related to the study.

13. DATA AND SAFETY MONITORING

13.1 Data Reporting

13.1.1 Method

The ODQ will collect, manage, and monitor data for this study.

13.1.2 Data Submission

The schedule for completion and submission of case report forms (paper or electronic) to the ODQ is as follows:

Forms	Submission Timeline
Eligibility Checklist	Complete prior to registration with ODQ
On Study Forms	Within 14 days of registration
Induction Data Forms	Within 14 days of Induction Completion
Consolidation I Data Forms	Within 14 days of the end of Consolidation 1
CNS Data Forms	Within 14 days of the end of the CNS phase
Consolidation II Data Forms	Every 3 months
Continuation Data Forms	Every 3 months
Adverse Event Data Forms	Within 14 days of the end of each phase OR during the 3 month reporting period
Off Treatment/Off Study Form	Within 14 days of completing treatment or being taken off study for any reason
Follow up/Survival Forms	Within 14 days of the protocol defined follow up visit date or call
Serious Adverse Event Report Forms	Within 24 business hours of notification of the event

13.2 **Safety Meetings**

The existing DFCI/ALL Consortium Data and Safety Monitoring Committee (DMC) will review and monitor toxicity and accrual data from this trial. The committee is composed of clinical specialists with experience in oncology and who have no direct relationship with the study. Information that raises any questions about participant safety will be addressed with the Principal Investigator and study team.

The DFCI/ALL Consortium DMC will meet every six months and/or more often if required to review toxicity and accrual data. Information to be provided to the committee may include: up-to-date participant accrual; toxicity information as specified in Section 16; all grade 3 or higher unexpected adverse events that have been reported; summary of all deaths occurring during protocol therapy; any response information (response to induction therapy, relapse and second malignant neoplasm events); audit results, and a summary provided by the study team. Other information (e.g. scans, laboratory values) will be provided upon request.

The DFCI/ALL Consortium DMC will supply information to the DF/HCC Data Safety Monitoring Committee (DSMC) upon request.

13.3 Monitoring

Involvement in this study as a participating investigator implies acceptance of potential audits or inspections, including source data verification, by representatives designated by the DF/HCC Overall Principal Investigator, Lewis Silverman, MD, or DF/HCC. The purpose of these audits or inspections is to examine study-related activities and documents to determine whether these activities were conducted and data were recorded, analyzed, and accurately reported in accordance with the protocol, institutional policyand any applicable regulatory requirements.

All data will be monitored for timeliness of submission, completeness, and adherence to protocol requirements. Monitoring will begin at the time of participant registration and will continue during protocol performance and completion.

14. REGULATORY CONSIDERATIONS

14.1 Protocol Review and Amendments

This protocol, the proposed informed consent and all forms of participant information related to the study (e.g., advertisements used to recruit participants) and any other necessary documents must be submitted, reviewed and approved by a properly constituted IRB governing each study location.

Any changes made to the protocol must be submitted as amendments and must be approved by the IRB prior to implementation. Any changes in study conduct must be reported to the IRB. The DF/HCC Overall Principal Investigator, Lewis Silverman, MD, will disseminate protocol amendment information to all participating investigators.

All decisions of the IRB concerning the conduct of the study must be made in writing.

14.2 Informed Consent

All participants must be provided a consent form describing this study and providing sufficient information for participants to make an informed decision about their participation in this study. The formal consent of a participant, using the IRB approved consent form, must be obtained before the participant is involved in any study-related procedure. The consent form must be signed and dated by the participant or the participant's legally authorized representative, and by the person obtaining the consent. The participant must be given a copy of the signed and dated consent document. The original signed copy of the consent document must be retained in the medical record or research file.

14.3 Ethics

This study is to be conducted according to the following considerations, which represent good and sound research practice:

- US Code of Federal Regulations (CFR) governing clinical study conduct and ethical principles that have their origin in the Declaration of Helsinki
    - Title 21 Part 50 – Protection of Human Subjects www.access.gpo.gov/nara/cfr/waisidx_02/21cfr50_02.html
    - Title 21 Part 54 – Financial Disclosure by Clinical Investigators www.access.gpo.gov/nara/cfr/waisidx_02/21cfr54_02.html
    - Title 21 Part 56 – Institutional Review Boards www.access.gpo.gov/nara/cfr/waisidx_02/21cfr56_02.html
    - Title 21 Part 312 – Investigational New Drug Application www.access.gpo.gov/nara/cfr/waisidx_02/21cfr312_02.html
- State laws
- DF/HCC research policies and procedures http://www.dfhcc.harvard.edu/clinical-research-support/clinical-research-unit-cru/policies-and-procedures/

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 according to the local reporting policy.

14.4 Study Documentation

The investigator must prepare and maintain adequate and accurate case histories designed to record all observations and other data pertinent to the study for each research participant. This information enables the study to be fully documented and the study data to be subsequently verified.

Original source documents supporting entries in the case report forms include but are not limited to hospital records, clinical charts, laboratory and pharmacy records, recorded data from automated instruments, microfiches, photographic negatives, microfilm or magnetic media, and/or x-rays.

14.5 Records Retention

All study-related documents must be retained for the maximum period required by applicable federal regulations and guidelines or institutional policies.

14.6 Multi-center Guidelines

This protocol will adhere to the policies and requirements of the Dana-Farber/Harvard Cancer Center. The specific responsibilities of the DF/HCC Overall Principal Investigator, Lewis Silverman, MD, Coordinating Center, and Participating Institutions are presented in the Dana-Farber/Harvard Cancer Center Multi-Center Data and Safety Monitoring Plan (see Appendix III).

- The DF/HCC Overall Principal Investigator, Lewis Silverman, MD, is responsible for distributing all IND Action Letters or Safety Reports to all participating institutions for submission to their individual IRBs for action as required.

- Mechanisms will be in place to ensure quality assurance, protocol compliance, and adverse event reporting at each site.

- Except in very unusual circumstances, each participating institution will order the agent(s) directly from the supplier. A participating site may order the agent(s) only after the initial IRB approval for the site has been forwarded to the Coordinating Center.

15. Laboratory Evaluation and Therapy at Outside Affiliate Sites

Some standard-of-care laboratory studies and treatments may be performed at satellite sites/ hospitals that are affiliated with the Lead Insitution or a Participating Site. It is the responsibility of the Lead Institution or Participating Site to which the satellite/affiliate hospital is affiliated to monitor all care that is performed at the satellite/affiliate hospital in order to ensure protocol compliance and to screen for any adverse events.

15.1 **Laboratory Studies**

15.1.1 Laboratory studies that can be peformed at satellite sites/affiliate hospitals: Standard-of care labs (complete blood count, liver function tests, amylase, lipase, triglyceride levels) used to determine whether starting criteria for chemotherapy are met and/or to monitor for side effects.

15.1.2 Laboratory Studies that can only be obtained at Lead or Participating Instution:

- All Research Laboratories, including asparaginase enzyme levels
- Methotrexate levels (Consolidation I)

15.2 **Therapy**

15.2.1 Chemotherapy that may be administered at satellite sites/affiliate hospitals or at home:

- Standard-of-care chemotherapy agents (eg, vincristine, doxorubicin/dexrazoxane) administed during Consolidation II or Continuation phases may be administered at a satellite site/affiliate hospital.
- Intramuscular Erwinia asparaginase (for patients who develop allergy to PEG asparaginase) may be administered at a satellite site/affiliate hospital.
    - Intravenous Erwinia asparaginase (for patients who is unable to tolerate IM Erwinia) may not be administered at a satellite site/affiliate hospital.
- Oral agents (eg, prednisone, dexamethasone, 6-mercaptopurine) may be administered in the home or at a satellite site/affiliate hospital.
- Intravenous/Intramuscular methotrexate (Consolidation II or Continuation phase only) may be administered in the home or at a satellite site/affiliate hospital.
- Low-dose intravenous cytarabine during the Consolidation IB phase may be administered the home or at a satellite site/affiliate hospital.

15.2.2 Therapy that can only be administered at Lead or Participating Instution:

- **All doses of Oncaspar or Calaspargase Pegol (SC-PEG)**
- All intravenous chemotherapy administered during the Steroid Prophase, Remission Induction, Consolidation I, and CNS phases (exception: low-dose cytarabine during Consolidation IB for VHR patients)
- All intrathecal chemotherapy

15.2.3 Procedures and Radiographic Studies

- Diagnostic bone marrow, nodal and/or mass biopsies and radiographic studies that have already been performed at an outside institution do not have to be repeated prior to study entry, but the pathologic/radiographic interpretation of these studies confirming protocol eligibility must be performed at Lead Institution or Participating Institution.

- All subsequent protocol-required or optional bone marrow tests (eg, end-induction, Day 18 or end-of-therapy), restaging radiographic studies (to assess remission status) and lumbar punctures with intrathecal chemotherapy may only be performed at Lead Institution or Participating Institution

16. STATISTICAL CONSIDERATIONS

Patients will be randomized to receive either IV Calaspargase Pegol (SC-PEG) 2500 IU/m^2 (arm A) or IV Oncaspar 2500 IU/m^2 (arm B), both administered as a single dose during induction and then for 30 weeks during post-induction treatment phases (IV SC-PEG given every 3 weeks and IV Oncaspar every 2 weeks). Samples to assess serum asparaginase enzyme activity will be obtained weekly (for 4 weeks) after the induction dose (days 4, 11, 18, and 25 post-asparaginase dose). In the post-induction treatment phases, IV SC-PEG 2500 IU/m^2 will be administered every 3 for 30 weeks (weeks 1, 4, 7, 10, 13, 16, 19, 22, 25 and 28, a total of 10 doses in 30 weeks) with samples obtained prior to every dose. IV Oncaspar 2500 IU/m^2 will be administered every two weeks on weeks 1,3,5,…29 (a total of 15 doses in 30 weeks) and asparaginase enzyme samples will be obtained prior to every dose. Both IV Oncaspar and IV SC-PEG asparaginase enzyme level samples will be taken at the same time point on weeks 7, 13, 19 and 25.

Safety data will be monitored on each arm and compared to that of the historical control rate observed on DFCI protocol 05-001. On DFCI Protocol 05-001, 7% of enrolled subjects were not randomized due to ineligibility, induction failure, induction death, early toxicity, early withdrawal, and the presence of the Philadelphia chromosome. Additionally, some patients will be unable to complete 30 weeks of post-induction therapy. Therefore, we assume that approximately 7% of patients who enroll on this randomized pilot trial will not be evaluable for post-induction toxicity assessment and the post-induction pharmacokinetic analyses.

16.1 **Primary Objectives: Safety and Feasibility of SC-PEG asparaginase**

A total of 240 patients will be enrolled on this trial to evaluate the primary endpoint. We plan to enroll 120 patients on each arm to have 112 patients on each arm evaluable for the monitoring of asparaginase toxicity in Consolidation I, CNS and Consolidation II phases of treatment and the pharmacokinetic secondary endpoint. The randomization will be stratified into four groups based on disease type, age and risk group classification: ALL patients under 10 years of age and SR, ALL patients under 10 years of age and HR, ALL patients 10 years and older (and by definition HR), and patients with lymphoblastic lymphoma. We estimate that of the 240 patients enrolled, 34 will have lymphoblastic lymphoma.

16.1.1 **Safety**

16.1.1.1 Safety Monitoring

One primary objective of this study is to compare the asparaginase-related toxicity rate of IV SC-PEG with that of IV Oncaspar on Protocol 05-001. The toxicity data from the IV Oncaspar arm of this study will be monitored to ensure that the rate of asparaginase-related toxicities with IV Oncaspar is not dissimilar between Protocol 05-001 and Protocol 11-001, thus allowing for the toxicity comparison between 11-001 IV SC-PEG- and 05-001 IV Oncaspar-treated patients . Toxicity rates from the induction phase and post-induction phases of treatment for both arms will be monitored.

We will carefully monitor and prospectively collect data relating to asparaginase-related toxicities of any grade. On DFCI Protocol 05-001, the three most common asparaginase-related toxicities were pancreatitis, hypersensitivity and thrombotic complications. The rates of these toxicities on each arm will be compared at the DMC meeting approximately every six months. We will also monitor other severe toxicities, defined as any grade 4 or higher toxicity (excluding asymptomatic laboratory abnormalities, and noting that elevations of amylase and lipase are markers of pancreatitis and will be included in the rate of asparaginase-related toxicity) occurring during the induction phase and during the 30 weeks of post-induction treatment including asparaginase. Severe pancreatitis will be defined as an elevation in serum amylase greater than three times normal with clinical signs and symptoms consistent with that diagnosis persisting for more than 72 hours. Mild to moderate pancreatitis will be defined as above with signs and symptoms lasting for less than 72 hours. All other toxicity grading will be per CTC version 4.0.

On DFCI Protocol 05-001, the overall asparaginase-related toxicity rate for patients randomized to IV Oncaspar was 27% for the Consolidation I, CNS and Consolidation II phases of treatment. Safety data will be subsequently monitored approximately every 6 months by the DMC. The safety analysis will not be a comparison of randomized arms, but each arm will be monitored by comparing toxicity rates to the historical control arm of IV Oncaspar on 05-001. We will monitor the proportion of patients experiencing asparaginase-related toxicity to ensure that this rate does not significantly differ from the 27%, observed on 05-001 in either arm. Assuming we have approximately 30 patients enrolled on each arm (60 total), who have adequate toxicity data (Consolidation I-Consolidation II phases of treatment) at the time of the first interim analysis, if 12 or more of the 30 (40%; exact two-sided 80% CI: 28%-53%) patients experience asparaginase-related toxicity, we would consider this unacceptable, as the expected rate of 27% is excluded from the lower bound of the exact one-sided binomial 90% confidence interval (or exact two-sided 80% CI). The trial would stop accrual and the safety data would be carefully reviewed by the DMC. Similar assessments will be made at each interim analysis for each arm. Based on an accrual rate of 10 patients per month and a DMC meeting occurring every six months we plan on three interim looks at the data and a final analysis at 100% information when all patients are enrolled have adequate toxicity follow-up data (approximately 10 months post consolidation I). We assume that the asparaginase-related toxicity rate will be similar for lymphoblastic lymphoma patients. With 112 evaluable patients on each arm at the final analysis, if 38 or more (34%; exact two-sided 80% CI: 28%-40%) patients experience asparaginase-related toxicity, we would consider this unacceptable. The upper bound of the exact one-sided 90% CI (or exact two-sided 80% CI) for the true unknown rate of asparaginase-related toxicity would be no more than 40%.

At each DMC meeting, we will also consider asparaginase-related toxicities separately and consider stopping if the incidence of pancreatitis, allergy, and/or thrombosis for the SC-PEG arm are significantly higher than that of IV-Oncaspar. Similarly, the induction toxicity rate for pancreatitis, allergy, thrombosis, bacterial and fungal infection and the rate of excessive toxicity (all other grade 4 or higher toxicities) will also be compared to that of the historical control rate and monitored at each DMC meeting. Since the study is not powered to detect differences in toxicity rates between arms, only to monitor rates versus historical controls, comparison of the

toxicity rates between arms will only be made at the request of the DMC at each interim analysis. The rate of asparaginase-related toxicity will be reported for each arm along with the exact binomial 90% confidence intervals with a maximum width no wider than 16%.

Toxicity grading using CTC version 4.0 will also be reported for all patients who received therapy. The number and proportion of patients with each toxicity type will be reported. The worst grade toxicity will be determined for each patient and the proportions will be reported with the 90% confidence intervals for each arm.

16.1.1.2 Minimum Residual Disease (MRD) Monitoring

COG Protocol AALL07P4 tested SC-PEG asparaginase during the induction phase. Patients were initially randomized to receive either SC-PEG or Oncaspar at 2500 IU/m^2. After a protocol amendment, patients randomized to SC-PEG received a dose of 2100 IU/m^2. The study was suspended on December 22, 2010 based on a higher rate of Day 29 end-induction MRD-positivity (MRD+) for patients treated with SC-PEG 2100 IU/m²/dose compared with SC-PEG 2500 IU/m²/dose or Oncaspar 2500 IU/m²/dose. The difference in frequency of MRD-positivity was statistically significant and crossed pre-defined safety monitoring boundaries.

Based on this finding, we will monitor the frequency of high end-induction MRD (defined as > 0.001 by PCR assay) in patients with B-precursor ALL at each interim analysis. Of the first 432 B-ALL patients treated on Protocol 05-001, end-induction MRD was successfully assayed in 92% of patients and 12.5% were found to be MRD-high. We will monitor the proportion of patients with MRD levels >0.001 in each arm at the end of remission induction to ensure that this rate does not significantly differ from 12.5%. Assuming we have 30 patients per arm (60 in total) at the first interim analysis and 92% have informative end-induction samples (N=28), if 7 or more of the 28 (25%; two-sided 80% CI: 14%-38%) patients have high MRD samples, we would consider this unacceptable, as the expected rate of 12% is excluded from the lower bound of the exact one-sided 90% confidence interval. The trial would stop accrual and the data would be reviewed by the DMC. Similar assessments will be made at each interim analysis. Comparisons between arms will be made at the request of the DMC at each interim analysis. The rate of high end-induction MRD will also be reported for each arm along with the 90% confidence intervals with a maximum width no wider than 16%.

16.1.2 Feasibility and Pharmacokinetic Endpoints

16.1.2.1 Feasibility of Every 3-Week Dosing of IV SC-PEG

The feasibility of administering IV SC-PEG at a dose of 2500 IU/m^2 every 3-weeks will be assessed by calculating the proportion patients who achieve a nadir serum asparaginase activity (NSAA) level ≥0.10 IU/mL (considered therapeutic) at any post-induction time point in each arm. We will compare the proportion of patients who have at least 1 NSAA measure ≥0.10 IU/mL between arms. In the first 114 analyzed on DFCI Protocol 05-001, 100% of patients treated with IV Oncaspar had at least 1 NSAA level ≥ 0.10 IU/mL during the post-induction asparaginase consolidation. With 112 evaluable patients on each arm and assuming 99% of patients on the IV Oncaspar arm have at least 1 NSAA level ≥ 0.10 IU/mL during the post-induction asparaginase consolidation, we will have 89% power to detect a 10% reduction in the proportion of patients who have at least 1 NSAA level ≥ 0.10 IU/mL during the post-induction

asparaginase consolidation, using a one-sided Fisher exact test at the 0.025 significance level. If there is no difference in the proportion of patients who have at least 1 NSAA level ≥ 0.10 IU/mL during the post-induction asparaginase consolidation, we will consider the administration of every 3-week IV SC-PEG feasible.

16.1.2.2 Asparaginase Enzyme Activity: Induction Phase – Early Stopping

We will also compare the proportion of patients with serum asparaginase enzyme activity of at least 0.10 IU/mL at 4, 11, 18 and 25 days after a single dose of either IV Oncaspar (2500 IU/m^2) or IV SC-PEG (2500 IU/m^2) is administered during the induction phase.

At the Day 25 time point (18 days after the dose of IV Oncaspar) on Protocol 05-001, 88% patients in induction had a serum asparaginase enzyme activity level ≥0.10 IU/mL, however only 7% achieved this level at the Day 32 time point (Table 2.4 of the Background section). On the COG Protocol AALL07P4, 100% of patients had a serum asparaginase enzyme activity level ≥0.10 IU/mL at both 22 and 29 days after the dose of IV SC-PEG (Table 2.5 of the Background section). Asparaginase enzyme activity will be run in real time from samples obtained at Induction Days 25 and 32 (18 and 25 days after asparaginase dose) for all patients enrolled on Protocol 11-001. Assuming we have approximately 30 evaluable patients enrolled on each arm (60 total) at the time of the first interim analysis, if 23 or fewer of the 30 (77%; exact two-sided 80% CI: 64%-87%) patients have an asparaginase enzyme activity level ≥0.10 IU/mL, at the Day 25 time point (on either arm), we would consider this unacceptable, as the expected rate of 88% is excluded from the upper bound of the exact one-sided binomial 90% confidence interval. Similarly, at the Day 32 time point (25 days after the dose of IV SC-PEG) if 23 or fewer of the 30 patients (77%; exact two-sided 80% CI: 64%-87%) on the IV SC-PEG arm have an asparaginase enzyme activity level ≥0.10 IU/mL, we would also consider this unacceptable. This stopping rule will ensure that the proportion of patients who achieve a target therapeutic level with IV SC-PEG is comparable to that of IV Oncaspar at Day 25 (18-days post-dose) and is maintained at Day 32 (25-days post-dose), which will justify the every 3-week dosing schedule during Consolidation for patients randomized to received IV SC-PEG. Similar comparisons will be made at each interim analysis and at the final analysis.

Descriptive statistics including the median, range, mean and standard deviation of asparaginase enzyme level will be reported and compared as appropriate at each induction day that samples are collected for each arm. We will analyze and compare the data between arms longitudinally as described for the post-induction time points.

16.1.2.3 Asparaginase Enzyme Activity – Consolidation Phase (post-Induction)

Both IV Oncaspar and IV SC-PEG asparaginase enzyme level samples will be collected in real time at the same post-induction time points on weeks 7, 13, 19 and 25. Descriptive statistics including the median, range, mean and standard deviation of serum asparaginase activity will be reported for each post-induction week that samples are collected for each arm. To ensure that asparaginase levels are being maintained at the ≥0.10 level, the proportion of samples with levels

≥ 0.025, ≥ 0.10, ≥ 0.20, and ≥ 0.40 IU/mL at each time point for both arms will also be reported and reviewed at each DMC meeting.

We will directly compare asparaginase enzyme activity levels between the two arms. With a total of 224 evaluable patients (112 on each arm), we would have 96% power to detect a mean difference of 0.15 in asparaginase enzyme activity level at the week 7 time point (estimated to be steady-state based on data from Protocol 05-001) using a two-sided t-test and assuming a standard deviation of 0.30. This is equivalent to an effect size ($\delta=\Delta/\sigma$) of 0.5.

In addition to week 7 post-induction, we will also collect asparaginase enzyme levels at weeks 13, 19, and 25 for every 2-week IV Oncaspar and compare them to every 3-week IV SC-PEG. We will compare the asparaginase enzyme levels between arms longitudinally with a random effects mixed model as appropriate. A log-10 transformation will be used for the asparaginase levels if the distribution of asparaginase enzyme levels warrant. These models will consider additional baseline patient characteristics including age sex, as well as the stratification factors of risk group and disease type, while testing for a difference in asparaginase enzyme activity between arms. Generalized estimating equation (GEE) models will be explored using cut-offs including ≥ 0.025, ≥ 0.10, ≥ 0.20, and ≥ 0.40 IU/mL. Tables 2.1, 2.2 and 2.3 in the Background section provide descriptive statistics for the induction and post-induction time-points in for IV Oncaspar on Protocol 05-001.

16.2 **Secondary Objectives**

16.2.1 Antibiotic Prophylaxis during Induction Phase

Based on historical rate of bacterial infection in the induction phase of treatment on 05-001 of 25%, we will also test the hypothesis that antibiotic prophylaxis will significantly reduce the rate of bacterial infection. We will compare the rate of infection during the induction phase in patients with ALL enrolled on Protocol 11-001 with those who were treated on Protocol 05-001. The induction regimen of the two protocols is nearly identical, with the following exceptions: 1) patients on Protocol 11-001 will receive either a single dose of IV SC-PEG or IV Oncaspar while all patients on Protocol 05001 received a single dose of IV Oncaspar and 2) patients on 11-001 will receive antibiotic prophylaxis while those on Protocol 05-001 did not. For the 11-001 patient cohort, we will only consider the infections of patients with ALL (not lymphoblastic lymphoma) in this comparison, because only patients with ALL were treated on Protocol 05001 and the frequency and severity of infections may be different in patients with lymphoblastic lymphoma who do not have marrow involvement at diagnosis (and thus may have a higher neutrophil count for longer during the induction phase).

Because of potential interaction between asparaginase type and risk of infection, we will analyze infection rates separately in each randomized arm. With approximately 103 patients on each arm (excluding lymphoma patients), we would have 82% power to detect a 12% reduction in the rate of bacterial infection in the induction phase of treatment based on a one-sample, 1-sided exact binomial test with a significance level of 0.025. Each arm will be compared to that of the historical control rate of 25%.

16.2.2 Efficacy

Although not sufficiently powered to detect difference between the arms, event-free survival (EFS) and overall survival (OS) will be calculated using the method of Kaplan and Meier for each arm. Event-free survival will be defined as the time from achievement of complete remission to the time of relapse, death or second malignancy. Patients not achieving complete remission will be considered events at time zero. Overall survival will defined as the time from diagnosis until death. Five-year estimates of EFS and OS will be provided for each arm along with the 90% confidence intervals estimated using the formula of Greenwood once sufficient follow-up data is obtained. Additionally, we will report EFS and OS estimates for children (<10 years) and adolescents (≥10 years) and for lymphoblastic lymphoma patients separately for each arm along with the appropriate confidence intervals.

Since 2005, we have treated patients with VHR ALL (B-precursor patients with high end-induction MRD and/or high-risk cytogenetics) with a novel chemotherapy regimen including two additional cycles of consolidation therapy. Treatment for VHR patients on Protocol 11-001 will be identical to that on Protocol 05-001, except for the asparaginase preparation in some patients. We will combine outcome data for the VHR cohort on Protocol 11-001 with those treated on Protocol 05-001 to describe the outcome of this patient subset with the intensified VHR treatment. We estimate that approximately 70 patients will have been treated on the VHR arm on Protocol 05-001 at the time of its closure and anticipate that approximately 20 ALL patients on Protocol 11-001 will be classified as VHR for a total of 90 patients combined. Preliminary estimates of EFS and OSwill be reported for this cohort of patients.

16.2.3 Vitamin D Screening

Patients will have vitamin D levels will be prospectively screened (to determine the frequency of vitamin D deficiency) and patients will begin vitamin D supplementation at any time point after completion of the induction phase when low vitamin D levels are identified. The time points of assessment will include pre-treatment, end of induction therapy, the start of continuation phase, and at the conclusion of therapy. At each of these time points, patients will be classified based on the measurement of 25-hydroxy vitamin D level as: deficient <20 ng/mL, insufficient 20-29 ng/mL, sufficient 30-79 ng/mL, or having possible toxicity ≥80 ng/mL. Based on these assessments, patients will be assigned a recommended dose of vitamin supplementation if deemed deficient or insufficient. At each time point we will record the level of 25-hydroxy vitamin D and the classification of each patient (determines the age-adjusted recommended daily allowance or RDA). At each time point, we will calculate descriptive statistics including 95% confidence intervals based on these measures. Pre- and post-measurements will be compared at the end of induction and the start of Continuation using a two-sided paired t-test at the 0.05 level. For example, if we estimate the 10% of the 240 patients have insufficient or deficient levels at baseline, we have at 94% power to detect a difference (a potential increase) in these levels assuming an effect size ($\delta=|\mu_1- \mu_2|/\sigma$) of 0.75 or >99% power assuming an effect size of 1.0. Similar assessments will be made at subsequent time points.

16.2.4 Prospective Screening of TCRγ Deletions in T-ALL Patients and Abnormalities of IKZF1, CRLF2 and JAK 1/2 in B-ALL Patients

Because these abnormalities will be screened only in diagnostic marrow samples, only patients with leukemia will be included in these analyses. The proportion of these abnormalities will be recorded along with 90% confidence intervals of these estimates. The association of early T-ALL precursor phenotype and ABGD status will be tested. The association of each B-ALL abnormality with end-induction MRD both categorized as high (>0.001) and as a continuous measurement. It is understood that the proportion of these patients with deletions or abnormalities may be small, and thus we would be underpowered to detect differences, so this testing will be considered as exploratory. It is estimated that approximately 10% of ALL patients will be T-cell and 90% of patients will be B-cell. Assuming we have successful screening results on 206 patients (excluding lymphoma patients), the exact two-sided 90% confidence interval will be no wider than 40% for proportion of T-ALL patients with ABGD and no wider than 13% for the proportion of patients with each abnormality screened for B-ALL patients.

16.2.5 BH3-Profiling to Identify Patients at High Risk of Treatment Failure

The loss of mitochondrial membrane potential will be measured in lymphoblasts taken at the time of diagnosis to identify which anti-apoptotic BCL-2 family members are present and potentially primed for death. We will test for an association between the loss of membrane potential and chemotherapy response as measured by induction response, end-induction MRD level and EFS. Because BH3-profiling studies will be performed using diagnostic marrow samples, only patients with leukemia will be included in these analyses. Assuming we have 206 patients (excluding lymphoma patients) and approximately 90% enter complete remission, we would have 90% power to detect a difference in the loss of membrane potential between those who did not enter a complete remission versus those who entered a complete remission assuming an effect size of 0.75 using a two-sided t-test with a significance level of 0.05 and >99% power assuming an effect size of 1.0. The correlation of the loss of mitochondrial potential with end-induction MRD levels will also be explored. Preliminary estimates of EFS categorized based on the loss of mitochondrial membrane potential will be recorded using recursive partitioning based on the rpart package in R.

16.3 **Accrual Rate**

Based on accrual rates from Protocol 05-001, we expect to accrue approximately 10 patients per month to this trial. Assuming 240 patients are enrolled on the trial (120 per arm), accrual will be completed in approximately 2 years (24 months).

17. PUBLICATION PLAN

The results will be made public within 24 months of the end of data collection. The initial plan for release would be an abstract that meets the requirements of the International Committee of Medical Journal Editors. Once analyses are completed, results will be submitted to a peer-reviewed journal.

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19. APPENDICES

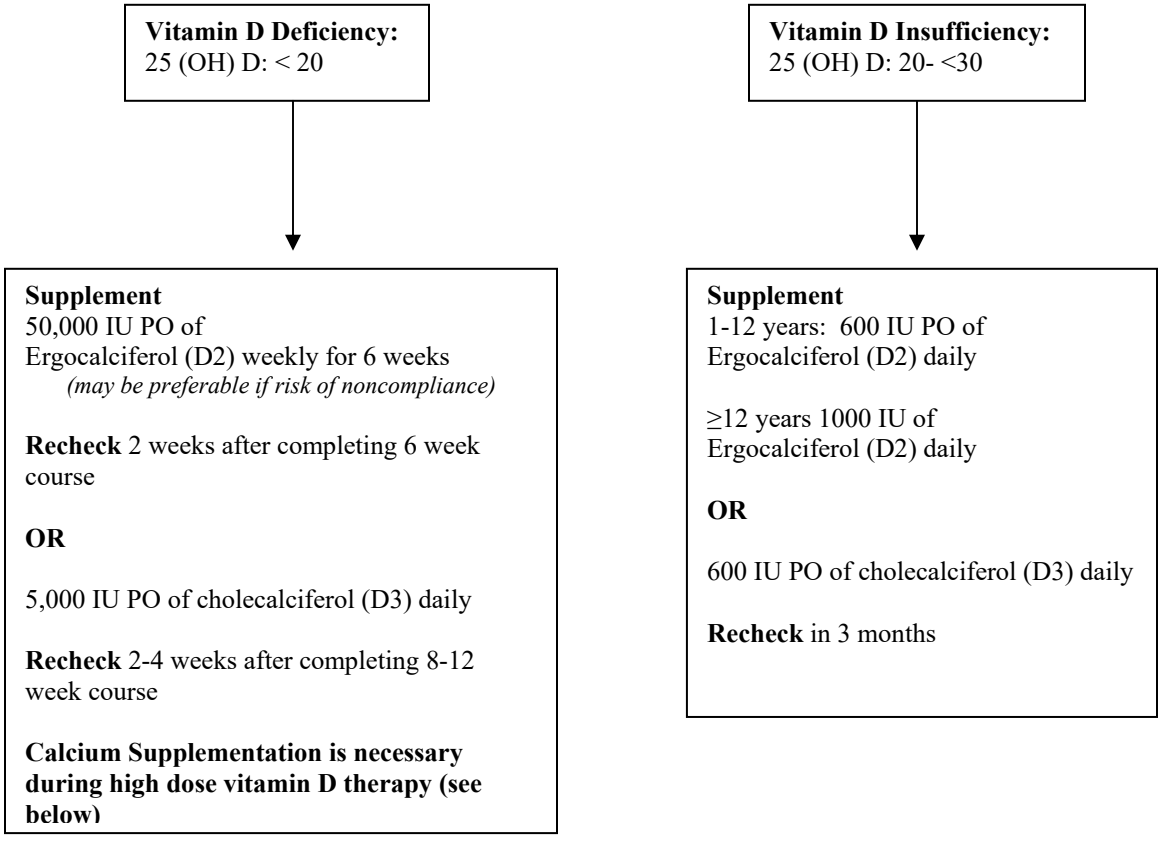
19.1 Appendix I: Vitamin D and Calcium Supplementation Guidelines

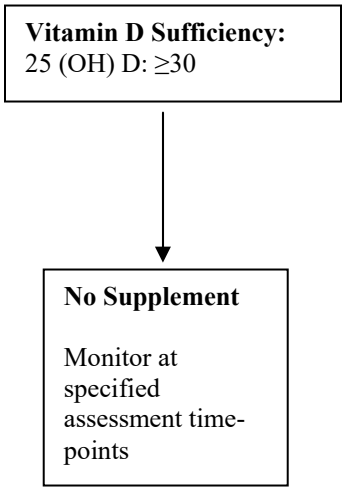
Vitamin D levels will be assessed in all patients at study entry, at the end of induction, at the start of continuation phase, and at the end of therapy. Patients are recommended to begin vitamin D supplementation at any time-point when low vitamin D levels are identified, beginning with the assessment at the end of the induction phase. Vitamin D supplementation during the induction phase is not recommended, even in patients with low levels identified at the time of diagnosis.

Vitamin D levels will be classified as follows:

- Vitamin D **deficiency**: 25-hydroxy vitamin D level less than 20 ng/mL
- Vitamin D **insufficiency**: 25-hydroxy vitamin D level 20-<30 ng/mL
- Vitamin D **sufficiency**: 25-hydroxy vitamin D level 30-80 ng/mL
- Possible toxicity: 25-hydroxy vitamin D level greater than 80 ng/mL

For patients with vitamin D deficiency (especially with levels less than 10 ng/ml) in whom high dose vitamin D supplementation is recommended, calcium supplementation is necessary.





Calcium Supplementation Guidelines

In all patients:

- Assess dietary calcium intake by estimating whether average intake meets age appropriate RDA (table).
- If inadequate dietary calcium intake, additional calcium should be administered with the use of supplements with the dose adjusted to the age associated RDA.
- If vitamin D deficient or insufficient, ensure adequacy of calcium intake.

Calcium Guidelines

Age	RDA (mg/day)	UL Intake (mg/day)
1-3 years	700 mg	2500 mg
4-8 years	1000 mg	2500 mg
9-13 years	1300 mg	3000 mg
14-18 years	1300 mg	3000 mg
19-30 years	1000mg	2500mg
31-50 years	1000mg	2500mg

Calcium Supplementation Guidelines for Vitamin D Deficient Patients

In patient with vitamin D deficiency (especially levels less than 10 ng/mL), calcium levels should be maintained with oral calcium supplements. Even for children who are not frankly hypocalcemic, calcium supplements are important for avoiding subsequent hypocalcemia from a decrease in bone demineralization and an increase in bone mineralization as PTH levels normalize ("hungry-bone" syndrome). Recommended doses of elemental calcium are 30 to 75 mg/kg per day in 3 divided doses. In addition to calcium supplements, calcitriol may be necessary in doses of 20 to 100 ng/kg per day in 2 to 3 divided doses until calcium levels normalize. High doses of calcium are necessary early in the course of

therapy, after which doses are reduced by half for the next 1 to 2 weeks. Once vitamin D supplementation has been reduced to 600-1000 IU/day, calcium supplementation is usually not necessary.

19.2 Appendix II: Guidelines for the Management of Thrombosis

- **Prevention of VTE:**
  - The risk of VTE in similar previous protocols is age-related with a known incidence of 2-36% based on age (highest risk at oldest age). The peak of this risk is during asparaginase therapy from induction through consolidation.
  - In patients with previously identified risk factors for VTE (ex., Factor V Leiden mutation, prior VTE, immobilization), consider prophylactic anticoagulation (+/- antithrombin infusions) during asparaginase therapy.

- **Diagnosis of VTE:**
  - All suspected VTE should be diagnosed/confirmed by radiologic imaging before stopping asparaginase and initiating anti-coagulation..

Thrombosis Type	Symptoms	Suggested Imaging
Non-CNS DVT	Limb swelling, pain, warmth, discoloration	• Doppler Ultrasound (US) • Contrast line study (may be falsely negative) • If high clinical suspicion in upper extremity and US is negative, consider MRV or contrast CT.
Pulmonary Embolism	Chest pain, shortness of breath, cough/hemoptysis	• CTA/spiral CT • V/Q scan
Sinus Venous Thrombosis	Headache, seizures, vision changes, mental status changes	• CTV • MRI/MRV (if high clinical suspicion and CTV is negative, consider MRV).

  - At the time of VTE, send a platelet count, antithrombin functional level, PT, PTT, and fibrinogen level.
  - Family history of thrombosis should be obtained. Based on family history and/or whether it will impact the decision of length of anticoagulation, consider sending tests of inherited thrombophilia[1] *after completing asparaginase therapy*. Please note: if these tests are sent during asparaginase therapy, the tests measuring protein levels may be falsely abnormal due to asparaginase and will need to be repeated when asparaginase is complete. The genetic tests (PT, FVL and MTHFR) are not affected by asparaginase.

- **Management of Asparaginase after VTE:**
  - Hold Asparaginase and start anticoagulation (see below).

[1] Thrombophilias: Activated Protein C resistance (APCR), Factor V Leiden (send if APCR is positive), prothrombin mutation, fasting homocysteine, MTHFR mutation (if homocysteine is positive), Protein C activity level, Protein S level, fasting lipoprotein(a), Lupus anticoagulant, Factor VIII activity level, D-Dimer

- Central line removal should be considered if the line is nonfunctional or causing symptoms, such as extremity swelling or pain, which do not resolve with anticoagulation. Consider 2-3 days of anticoagulation prior to line removal.
- Resume asparaginase when symptoms have resolved and there is evidence of clot stabilization and/or recanalization/improvement on repeat imaging. The typical time to stabilization/improvement is 3-4 weeks. If symptoms are not improved and/or imaging has not stabilized after 6-8 weeks, discuss with the study PI or designee.
- *Repeat imaging is necessary before asparaginase is resumed*.
- With adequate anticoagulation, permanent discontinuation of asparaginase due to thrombosis is typically not necessary. In cases of significant CNS events or recurrent clot, asparaginase may be permanently held only after discussion with the study PI or designee.

- **Anticoagulation:** Based on the safety and efficacy of LMWH in oncology patients, consider using LMWH for all acute thrombotic events.

- **Guidelines for Supportive Care to Start and Continue Anticoagulation**:
  - **Platelet count** ≥ 50 kcells/ul or per institutional guidelines. During anticoagulation, transfuse and/or modify anticoagulation based on institutional guidelines. *After initial diagnosis of thrombosis, during the first few weeks of anticoagulation, consider transfusing platelets to maximize anticoagulation dosing (and anti-Xa levels).*
    Consider guidelines in table below:

Platelet count (kcells/uL)	Anti-Xa level (IU/ml)	Transfuse Platelets
≥ 50	0.5-1	No
>20 but <50	Consider lowering goal anti-Xa level to 0.1-0.6 OR using half LMWH dose	Consider
<20	Hold LMWH	Consider

  - **Anti-Xa levels: Suggested guidelines**
    - **Anti-Xa levels** should be followed regularly (every 1-2 days) until on a stable LMWH dose and dose achieves goal level. Once on a stable LMWH dose, levels should be monitored at least monthly.
    - When asparaginase is restarted, anti-Xa levels will need to be followed at least weekly again until levels appear stable on asparaginase (typically takes 4-6 weeks).
      - Typical therapeutic range = 0.5 - 1 IU/ml (checked 4 hours after 2nd or 3rd dose). Must be checked by peripheral stick.
      - Levels may need weekly monitoring with fluctuating renal function.
      - *Please note*: Contact your coagulation laboratory and learn if they add antithrombin to samples prior to measuring anti-Xa levels. If they do not, then the anti-Xa level measured in your lab is accurate for patients on asparaginase. If antithrombin is added, the assay is not a good

measurement in patients on asparaginase, and you should ask your lab if they can run these samples without adding antithrombin.

- **Antithrombin functional levels: Suggested guidelines**
  - At the time of diagnosis of VTE, AT functional levels should be checked and repleted for a goal AT nadir > 50% (or per institutional standard).
  - If your lab does not add antithrombin to anti-Xa assays, AT deficiency should be suspected if unable to achieve goal anti-Xa levels with reasonable LMWH dosing.
  - During asparaginase therapy, AT levels can be followed/repleted as needed for a selected goal nadir *or* can be checked only in those patients with subtherapeutic anti-Xa levels.
    - AT units = [(desired AT% - baseline AT %) x weight (kg)] / 1.4
      (ex., where a reasonable AT% = 100 %)
    - *Please note*: Heparin efficacy requires that the AT functional level is adequate. AT replacement is often required during LMWH therapy while on asparaginase. If AT levels are followed and repleted for a selected goal nadir, then check AT levels twice weekly and replete as needed. Once a trend in AT levels is established, the schedule for checking AT may be altered.
  - When asparaginase is restarted, either anti-Xa levels or AT levels need to be followed at least weekly as AT levels will drop with asparaginase, and LMWH at previously adequate doses may not anticoagulate the patient. 4-6 weeks after asparaginase has been resumed, anti-Xa or AT levels can be followed less frequently depending on the patient's previous patterns of monitoring.

  - Consider transfusing cryoprecipitate to keep fibrinogen > 100 mg/dl while anticoagulating during asparaginase therapy

  - PT/PTT do not require routine monitoring except if bleeding or prior to surgery

  - For invasive procedures (ex. LP), hold anticoagulation at least 24 hours (or per institutional standard).

- **Anticoagulation Dosing: Suggested Guidelines**
  - *Treatment* anticoagulant dose is based on patient weight/age.
    Enoxaparin: 1 mg/kg subcutaneously q12 hrs (*recommended in pediatric patients*)
    Dalteparin: 200 IU/kg/d SC x 4 weeks, then 150 IU/kg/d until complete
    Fondaparinux: <50 kg: 5 mg SC qday
    50-100 kg: 7.5 mg SC qday
    >100 kg: 10 mg SC qday
  - *Prophylactic* anticoagulation dose is also based on patient weight/age.
    Enoxaparin: 0.5 mg/kg SC q12 hrs or 1 mg/kg SC daily (40 mg maximum)

Dalteparin: 5000 IU SC daily (based on adult dosing)
Fondaparinux: 2.5 mg SC daily (based on adult dosing)

- **Guidelines for Discontinuing Anticoagulation:**
  - After receiving a treatment course of anticoagulation (3-6 months), continue treatment dose anticoagulation until at least 6 weeks after the final asparaginase dose.
  - A prophylactic dose or treatment dose of anticoagulation can be considered between asparaginase and the end of ALL therapy. Consider prophylactic or treatment dose anticoagulation in patients with recurrent thrombosis or with inherited thrombophilias.

20. DANA-FARBER/HARVARD CANCER CENTER MULTI CENTER DATA AND SAFETY MONITORING PLAN

20.1 INTRODUCTION

The Dana-Farber/Harvard Cancer Center Multi-Center Data and Safety Monitoring Plan (DF/HCC DSMP) outlines the procedures for a DF/HCC Multi-Center research protocol.

20.1.1 Purpose

To establish standards that will ensure that a Dana-Farber/Harvard Cancer Center (DF/HCC) Multi-center protocol will comply with Federal regulationsand Health Insurance Portability and Accountability Act (HIPAA) requirements in accordance with the CTEP Multi-center Guidelines.

20.1.2 Multi-Center Data and Safety Monitoring Plan Components

The Multi-Center Data and Safety Monitoring Plan includes the following components:

DF/HCC Multi-center Protocol: One or more outside institutions collaborating with Dana-Farber/Harvard Cancer Center on a research protocol where DF/HCC is the Lead Institution. DF/HCC includes Dana-Farber/Partners Cancer Care (DF/PCC) Network Clinical Trial Affiliates.

Lead Institution: Dana-Farber/Children's Hospital Caner Center will be the Lead Institution and will be responsible for the coordination, development, submission, and approval of a protocol as well as its subsequent amendments per the DFCI IRB and applicable regulatory guidelines (i.e. the FDA). The Lead Institution is the home of the Overall PI.

DF/HCC Principal Investigator: Investigator located at the Lead Institution who will be charged with the responsibility of the administration of the DF/HCC Project. This most often will be the Protocol Chair, but occasionally this may be the overall grant or contract holder, as applicable.

Protocol Chair: The Protocol Chair is the Principal Investigator for the DF/HCC protocol submitted as the Lead Institution. For applicable protocols, the Protocol Chair will be the single liaison with any regulatory agencies (i.e. the FDA).

Participating Institution: A Participating Institution is an institution that desires to collaborate with DF/HCC and commits to accruing participants to a DF/HCC protocol. The Participating Institution acknowledges the Protocol Chair as having the ultimate authority and responsibility for the overall conduct of the study.

Coordinating Center: The entity (i.e. Lead Institution, Medical Monitor, Contract

Research Organization (CRO), etc) that provides administrative support to the DF/HCC Sponsor in order that he/she may fulfill the responsibilities outlined in the protocol document and DSMP, and as specified in applicable regulatory guidelines (i.e. CTEP Multi-Center Guidelines). In general, the Lead Institution is the Coordinating Center for the DF/HCC Multi-Center Protocol. Should the DF/HCC Sponsor decide to use a CRO, the CRO will be deemed the Coordinating Center.

Clinical Trials Office: The clinical trials offices of the DF/HCC consortium members support investigators and their study teams with the coordination, submission and ongoing conduct of research protocols involving human subjects. Specifically, these offices support four core service areas including; pre-review of PI initiated protocols; assistance in the preparation and management of Investigational New Drug (IND) applications and subsequent required reporting to the FDA; regulatory consultation and guidance in the interpretation of local, federal, and ICH/GCP guidelines and policies; and the orientation and ongoing training support of clinical research personnel.

DF/HCC Office of Data Quality: A group within DF/HCC responsible for registering human subjects for trials, ensuring high-quality standards are used for data collection and the ongoing management of clinical trials, auditing, and data and safety monitoring. ODQ also coordinates quality assurance efforts related to multi-center clinical research.

20.2 GENERAL ROLES AND RESPONSIBILITIES

In accordance with the CTEP Multi-center Guidelines, the Protocol Chair (DF/HCC Principal Investigator, Lewis Silverman, MD), Coordinating Center (Dana-Farber Children's Hospital Cancer Center), and the Participating Institutions will all agree to the general responsibilities as follows (specific procedures for these general responsibilities are detailed in the DSMP):

20.2.1 Protocol Chair (DF/HCC Principal Investigator)

The Protocol Chair, Dr. Lewis Silverman, will accept responsibility for all aspects of the Multi-Center Data and Safety Monitoring Plan to:

- Oversee the coordination, development, submission, and approval of the protocol as well as subsequent amendments.
- Ensure that the investigators, study team members, and Participating Institutions are qualified and appropriately resourced to conduct the protocol.
- Submit the Multi-Center Data and Safety Monitoring Plan as an inclusion to the protocol.
- Assure all Participating Institutions are using the correct version of the protocol.
- Ensure that each participating investigator and study team receives adequate protocol training and/or a Site Initiation Visit prior to enrolling subjects.
- For international trials, assure that the protocol is provided to Participating Institutions in the primary language spoken at the site.
- Monitor progress and overall conduct of the study at all Participating Institutions.
- Ensure all DFCI IRB, DF/HCC and other applicable (i.e. FDA) reporting

requirements are met.
- Review data and maintain timely submission of data for study analysis.
- Act as the single liaison with the FDA (investigator-held IND trials), as applicable.
- Identify participating institutions and obtain accrual commitments. The title page of the protocol will include the names and contact information for all participating institutions that perform the function of recruiting, enrolling, and treating participants for the protocol. The Coordinating Center (Dana-Farber/Children's Hospital Cancer Center) must be designated on the title page.

20.2.2 Coordinating Center (Lead Institution)

The Coordinating Center is the DF/HCC Lead Institution's study team or designee. The DF/HCC Lead Institution, Dana-Farber/Children's Hospital Cancer Center, will ensure that all Participating Institutions within the Multi-Center Protocol demonstrate their intent and capability of complying with Federal Regulations, GCPs and HIPAA requirements. To assist the Protocol Chair in meeting his/her responsibilities as required by the DSMP, the DF/HCC Lead Institution's study team or designee will assume the following general responsibilities:

- Assist in protocol review.
- Maintain copies of FWA and Institutional Review Board (IRB) approvals from all Participating Institutions.
- Maintain FDA correspondence, as applicable.
- Maintain updated roster of participants.
- Verify eligibility.
- Verify response.
- Collect data on protocol specific CRFs.
- Prepare all submitted data for review by the Protocol Chair.
- Maintain documentation of Serious Adverse Event (SAE) reports submitted by Participating Institutions and submit to Protocol Chair for timely review.
- Distribute Serious Adverse Event safety reports (both IND Safety reports and protocol specific SAEs).
- Monitor at Participating Institutions either by on-site inspection of selected participant records and/or with source documents and research records submitted to the Lead Institution.

In addition to the Lead Institution, the DF/HCC Quality Assurance Office for Clinical Trials provides the following support services to assist the Protocol Chair:

- Develop protocol specific case report forms (CRF/eCRFS).
- QA/QC data of protocol specific CRFs.
- Provide Central Participant Registration.
- Verify that eligibility has been confirmed by the investigator and that appropriate consent has been obtained.
- Provide auditing services (funding and ODQ approval required).

20.2.3 **Participating Institution**

Each Participating Institution will provide to the Coordinating Center a list of the key personnel assigned to the role for oversight of data management at their site. All sites must have office space, office equipment, and internet access that meet HIPAA standards.
The general responsibilities for each Participating Institution are as follows:

- Commit to accrual to the Lead Institution's (Dana-Farber/Children's Hospital Cancer Center) protocol.
- Submit protocol and/or amendments to their local IRB.
- Maintain a regulatory binder.
- Update Coordinating Center (Dana-Farber/Children's Hospital Cancer Center) with research staff changes on a timely basis.
- Register participants through the Coordinating Center (Dana-Farber/Children's Hospital Cancer Center).
- Submit source documents, research records, and CRFs per protocol specific submission guidelines to the Coordinating Center (Dana-Farber/Children's Hospital Cancer Center).
- Submit Serious Adverse Event reports to local IRB and directly to the Coordinating Center (Dana-Farber/Children's Hospital Cancer Center).
- Submit deviations and violations to local IRB and the Coordinating Center (Dana-Farber/Children's Hospital Cancer Center).
- Secure investigational agents per federal guidelines and protocol requirements.
- For protocols using investigational agents, the Participating Institution will order their own investigational agents regardless of the supplier (i.e. NCI, pharmaceutical company)

Some laboratory studies and treatments are allowed in outside satellite institutions/ affiliate hospitals that are affiliated with a Participating institution (Section 15). It is the responsibility of the Participating institution to monitor laboratory results, treatment and toxicities occurring at its affiliate sites, and to collect all required source documentation and data from that site for submission to the Coordinating Center.

20.3 **PROTOCOL DEVELOPMENT**

20.3.1 **Activation of a Protocol**

The Overall Principal Investigator, Dr. Lewis Silverman, is responsible for the coordination, development, and approval of the protocol as well as its subsequent amendments, and reporting SAEs, violations and deviations per DFCI IRB guidelines and if applicable CTEP Multi-center, FDA or OBA Guidelines. Further, the Protocol Chair will be the single liaison with the FDA.

To meet these requirements, the Overall Principal Investigator will be responsible for the following minimum standards:

- Inclusion of the DF/HCC Multi-Center Data and Safety Monitoring Plan in the protocol as an appendix.
- Identify, qualify and initiate Participating Institutions and obtain accrual commitments.
- Commit to the provision that the protocol will not be rewritten or modified by anyone other than the Protocol Chair.
- Ensure that there is only one version of the Protocol and that all Participating Institutions use the correct version.
- Oversee the development of data collection forms (case report forms) that are of common format for use at all the Participating Institutions.

20.3.2 **Coordinating Center Support Function**

The DF/HCC Lead Institution's study staff or designee will provide administrative and clerical support to the Protocol Chair for the development and distribution of the protocol.

The tasks to be performed by the DF/HCC Lead Institution's study staff or designee include:

- Maintain Regulatory documents for all Participating Institutions.
- Review of the protocol and consent to check for logistics, spelling, and consistency.
- Provide the Protocol Chair a list of queries related to any inconsistencies.
- Provide necessary administrative sections, including paragraphs related to registration logistics, data management schedules, and multi-center guidelines.
- Maintenance of contact list of all Participating Institutions in the DF/HCC Multi-center Protocol and the distribution of updates to the sites as needed.
- Derivation of the study calendar, if applicable.
- Assistance in preparation and maintenance of case report forms.
- Conduct regular communications with all Participating Institutions (conference call, emails, etc)
- Maintain documentation of all communications.
- Conduct and maintain documentation of the site initiation training via teleconference / webex with all participating sites as a condition and prior to study activation at each site.

20.4 **PROTOCOL MANAGEMENT**

The Coordinating Center (Dana-Farber/Children's Hospital Cancer Center) is responsible for assuring that each Participating Institution has the appropriate assurance on file with the Office of Human Research Protection (OHRP). Additionally, the Coordinating Center (Dana-Farber/Children's Hospital Cancer Center) must maintain copies of all IRB approvals, for each Participating Institution.

20.4.1 **Protocol Distribution**

The Coordinating Center (Dana-Farber/Children's Hospital Cancer Center) will distribute the final approved protocol and any subsequent amended protocols to all Participating Institutions.

20.4.2 **Protocol Revisions and Closures**

The Participating Institutions will receive phone, fax, mail or e-mail notification of protocol revisions from the Lead Institution or designee. It is the individual Participating Institution's responsibility to notify its IRB of these revisions.

Non life-threatening revisions: Participating Institutions will receive written notification of protocol revisions regarding non life-threatening events from the Lead Institution or designee. Non-life-threatening protocol revisions should be IRB approved and implemented within 90 days from receipt of the notification.

Revisions for life-threatening Causes: Participating Institutions will receive telephone notification from the Lead Institution or designee concerning protocol revisions required to protect lives with follow-up by fax, mail or e-mail. Life-threatening protocol revisions will be implemented immediately followed by IRB request for approval

Protocol Closures and Temporary Holds: Participating Institutions will receive fax, e-mail, or phone notification of protocol closures and temporary holds from the Lead Institution or designee. Closures and holds will be effective immediately. In addition, the Lead Institution or designee will update the Participating Institutions on an ongoing basis about protocol accrual data so that they will be aware of imminent protocol closures.

20.4.3 **Informed Consent Requirements**

The DF/HCC approved informed consent document will serve as a template for the informed consent from participating institutions. As best as possible, the template should be followed with the specifications outlined in the DF/HCC guidance document on Model Consent Language.

Participating sites are to send their version of the informed consent document and HIPAA authorization, if a separate document, to the Lead Site for their revision prior to submission to the participating site's IRB.

The Principal Investigator (PI) at each Participating Institution will identify the physician members of the study team who will be obtaining consent and signing the consent form for therapeutic protocols. **It is DF/HCC policy that only attending physicians can obtain informed consent and re-consent to drug and/or device trials.**

20.4.4 **IRB Documentation**

The following must be on file with the DF/HCC Lead Institution or designee and must be submitted and approved by the DFCI IRB prior to participant registration:

- Approval Letter of the institution's IRB
- Copy of the Informed Consent Form approved by the Participating Institution's IRB
- IRB approval for all amendments

It is the Participating Institution's responsibility to notify its IRB of protocol amendments. Participating Institutions will have 90 days from receipt to provide the DF/HCC Lead Institution their IRB approval for Amendments to a protocol.

20.4.5 **IRB Re-Approval**

Annual IRB re-approval from the Participating Institution is required in order to continue research and register participants onto a protocol. There is no grace period for continuing approvals.

Protocol registrations will not be completed if a re-approval letter is not received by the DF/HCC Lead Institution from the Participating Institutions on or before the anniversary of the previous approval date.

The Coordinating Center (DFCI) will notify the participating institutions when the continuing review is due for the DFCI IRB. The DFCI study team will ask for information regarding all subjects participating in the study as well as any subjects that may have consented to the study and never started treatment. Follow up information will also be requested on any subject that has come off study.

20.4.6 **Participant Confidentiality and Authorization Statement**

The HIPPA of 1996 contains, as one of its six major components, the requirement to create privacy standards for health care information that is used or disclosed in the course

of treatment, payment or health care operations. The original Privacy Rule, as it has come to be known, was published in December 2000. The Final Rule was published on August 14, 2002, which modified the privacy rule in significant ways vis-à-vis research.

In order for covered entities to use or disclose protected health information during the course of a DF/HCC Multi-Center Protocol, the study participant must sign an Authorization. This Authorization may or may not be separate from the Informed Consent. The DF/HCC Multi-Center Protocol, with the approval from the DFCI IRB and if applicable NCI/CTEP, will provide an Informed Consent template, which covered entities (DF/HCC Multi-Center Protocol Participating Institutions) must use.

The DF/HCC Multi-Center Protocol will use all efforts to limit its use of protected health information in its trials. However, because of the nature of these trials, certain protected health information must be collected per National Cancer Institute requirements. These are the primary reasons why DF/HCC has chosen to use Authorizations, signed by the participant in the trial, rather than limited data sets with data use agreements.

20.4.7 **Participant Registration and Randomization**

The ODQ registration staff is accessible on Monday through Friday, from 8:00 AM to 5:00 PM Eastern Standard Time.

In emergency situations when a participant must begin treatment during off-hours or holidays, call the ODQ registration line at 617-632-3761 and follow the instructions for registering participants after hours. In the event of an off-hours or holiday registration, randomized treatment will be assigned and communicated on the next business day.

The registration procedures during business hours, Monday – Friday, from 8am – 5pm, are as follows:

1. Obtain written informed consent from the participant prior to the performance of any study related procedures or assessments.

2. Complete the protocol-specific eligibility checklist. **To be eligible for registration to the study, the participant must meet each inclusion and exclusion criteria listed on the eligibility checklist.**

3. **Fax or email** the eligibility checklist(s) and all pages of the consent form(s) to the DFCI Study Team at
   Email: [redacted]

4. The DFCI Study Team will (a) review the eligibility checklist and consent and (b) register the participant on the study with ODQ.

5. The ODQ Registrar will send an email or fax confirmation of the registration and randomized treatment assignment to the DFCI Study Team immediately following the registration.

6. The DFCI Study Team will email the registration confirmation and randomized treatment assignment to the outsite site.

7. Treatment on study should only begin after confirmation of registration is received.

The registration procedures outside of business hours, on weekends, and holidays are as follows:

1. Obtain written informed consent from the participant prior to the performance of any study related procedures or assessments.

2. Complete the protocol-specific eligibility checklist. **To be eligible for registration to the study, the participant must meet each inclusion and exclusion criteria listed on the eligibility checklist.**

3. Call the ODQ registration line at 617-632-3761 and follow the instructions for registering participants after hours. Leave detailed contact information for the QACT registrar responsible for registering your participant.

4. The ODQ Registrar will (a) validate eligibility and (b) register the participant on the study.

5. ODQ will confirm registration by telephone, and email/fax confirmation will be sent on the next business day. Randomized treatment will be assigned and communicated on the next business day.

6. Treatment on study should only begin after confirmation of registration is received. On nights, weekends, and holidays, treatment may begin after telephone confirmation (prior to communication of randomized treatment assignment).

7. **Fax or email** the eligibility checklist(s) and all pages of the consent form(s) to the DFCI Study Team at **Email** [redacted]

If you have any questions, the ODQ can be reached at telephone [redacted]

The following source documents need to be forwarded to the Coordinating Center (Dana-Farber/Children's Hospital Cancer Center) as soon as they are available. Final risk group

classification will only be made once these documents are received by the Coordinating Center.

- Copy of pathology reports confirming diagnosis of leukemia (bone marrow aspirate or biopsy indicating ≥ 30% marrow involvement) or lymphoblastic lymphoma
- Copy of marrow pathology (for leukemia) and scan (for lymphoblastic lymphoma) reports confirming complete remission at end of remission induction phase.
- Copy of diagnostic flow cytometry reports (leukemia patients only)
- Copy of results of diagnostic cytogenetics, FISH and PCR (if performed)(leukemia patients only)
- CBC with differential from diagnosis and from end of induction
- Copy of CSF cell count/cytospin and/or cytopathologic report from diagnosis and from end of induction

After receipt of source documents and review of MRD results (if applicable), Lead Institution will call or email the participating site to verify final risk group assignment.

20.4.8 **DF/HCC Multi-center Protocol Case Number**

Once eligibility has been established and the participant successfully registered, the participant is assigned a five digit protocol case number. This number is unique to the participant on this trial and must be used for ODQ CRF/eCRF completion and written on all data and ODQ correspondence for the participant.

20.4.9 **DF/HCC Multi-center Protocol Registration Policy**

20.4.9.1 Initiation of Therapy: Participants must be registered with the DF/HCC ODQ before receiving treatment. Treatment may not be initiated until the Participating Institution receives a faxed or e-mailed copy of the participant's Registration Confirmation memo from the DF/HCC ODQ. If registration occurs outside of business hours, on weekends, or on a holiday, treatment may not be initiated until ODQ has confirmed registration by telephone. An email/fax confirmation will be sent on the next business day. Therapy must be initiated per protocol guidelines. The Protocol Chair and DFCI IRB must be notified of any exceptions to this policy**.**

20.4.9.2 Eligibility Exceptions: The DF/HCC ODQ will make no exceptions to the eligibility requirements for a protocol

without DFCI IRB approval. The DF/HCC ODQ requires each institution to fully comply with this requirement.

20.4.9.3 Verification of Registration: A registration confirmation memo for participants registered to DF/HCC Multi-Center Protocol will be faxed or emailed to the registering institution within one working day of the registration. Treatment may not be initiated until the site receives a faxed or e-mailed copy of the registration confirmation memo. If a participant is registered outside of business hours, on weekends, or on a holiday, ODQ will confirm registration by telephone, and email/fax confirmation will be sent on the next business day.

20.4.9.4 Confidentiality: All documents, investigative reports, or information relating to the participant are strictly confidential. Whenever reasonably feasible, any participant specific reports (i.e. Pathology Reports, MRI Reports, Operative Reports, etc.) submitted to the Lead Institution must have the participant's full name & social security number "blacked out" and the assigned DF/HCC ODQ case number and protocol number written in (with the exception of the signed informed consent document). Participant initials may only be included or retained for cross verification of identification.

20.4.10 **Schedule of Data Submission**
An Electronic Data Capture system will be used for this trial. The DF/HCC ODQ develops a set of electronic case report forms, (eCRFs) for use with the DF/HCC Multi-Center Protocol. ODQ provides a web based training for eCRF users. These forms are designed to collect data for each study.

Forms	**Submission Timeline**
Eligibility Checklist	Complete prior to registration with ODQ
On Study Form	Within 14 days of registration
Induction Data Forms	Within 14 days of Induction Completion
Consolidation 1 Data Forms	Within 14 days of the end of Consolidation 1
CNS Data Forms	Within 14 days of the end of the CNS phase

Consolidation II Data Forms	Every 3 months
Continuation Data Forms	Every 3 months
Adverse Event Data From	Within 14 days of the end of each phase OR during the 3 month reporting period
Off Treatment/Off Study Form	Within 14 days of completing treatment or being taken off study for any reason
Follow up/Survival Forms	Within 14 days of the protocol defined follow up visit date or call
Serious Adverse Event Report Forms	Within 24 business hours of notification of the event

20.4.10.1 Eligibility Checklist

Purpose - Outlines protocol-specific eligibility criteria and includes the following:

Participant Demographics (address, zip code, sex, race, ethnicity, initials, date of birth)
1) Parameters for eligibility
2) Parameters for exclusion
3) Parameters for stratifications

- If a time frame is not specified in the protocol, all test results used to confirm eligibility must be completed within 14 days prior to study enrollment by the ODQ.

Schedule for Submission – Completed prior to participant registration. The Informed Consent/Participant Authorization for the Release of Personal Health Information should be submitted with the Eligibility Checklist at the time of registration.

20.4.10.2 On-study Form(s)

Purpose - documents the following items:

- Demographic data
- Disease characteristics

20.4.10.3 Adverse Event Report Form(s)

Purpose – Documents adverse events that occur while the participant is receiving treatment and for up to 60 days following the first day of the last cycle of chemotherapy. All adverse events are to be graded by number using the toxicity grading scale required by the protocol. *This form is not for IRB submission, but for recording the AE in the research database.*

20.4.10.4 Off Treatment and Off Study Form(s)

Purpose - The Off Treatment and Off Study Forms are submitted when the participant is removed from the study or has completed all protocol treatment. Participants will complete all protocol treatment and be considered "off treatment" Day 21 days after the start of the last cycle they receive during the continuation phase of treatment. Note: If the participant dies while on protocol**,** the Off Study Form is the last form submitted.

20.4.10.5 Follow up / Survival Form

Purpose - Summarizes participant status at a given point in time after being removed from treatment.

20.4.11 **Data Form Review**

When data forms arrive at the DF/HCC ODQ, they are reviewed for:

Completeness:
Is all the information provided as required per protocol?

Protocol Treatment Compliance:
Are the body surface area (BSA) and drug dosage calculations correct? The dose must be within 10% of the calculated protocol dose.

Adverse Events (Toxicities):
Did the participant experience adverse events (toxicities or side effects) associated with the treatment? Was the treatment delayed due to the adverse event? What was the most severe degree of toxicity experienced by the participant?

Notations concerning adverse events will address relationship to protocol treatment for each adverse event grade. All adverse events encountered during the study will be evaluated according to the NCI Common Toxicity Criteria assigned to the protocol and all adverse events must be noted on the participant's Adverse Event (Toxicity) Forms.

Response:
Did the participant achieve a response? What level of response did they achieve? On what date did the participant achieve the response and how was the response determined?

Response criteria are defined in the protocol. A tumor assessment must be performed prior to the start of treatment and while the participant is on treatment as specified by the protocol.

Objective responses must have documentation such as physical measurements, x-rays, scans, or laboratory tests.

20.4.12 **Missing and Deficient Memorandum**

Data submissions are monitored for timeliness and completeness of submission. Participating Institutions are notified of their data submission delinquencies in accordance with the following policies and procedures:

Incomplete or Questionable Data

If study forms are received with missing or questionable data, the submitting institution will receive a query within the EDC from the DF/HCC ODQ Data Analyst. Responses to the query should be completed and returned within 14 days. Responses may be returned within the EDC or by updating the data point.

Missing Forms

If study forms are not submitted on schedule, the Participating Institution will receive a Missing Form Report from the DF/HCC ODQ noting the missing forms. These reports are compiled by the DF/HCC QACT and distributed a minimum of three times a year.

20.5 **REQUISITIONING INVESTIGATIONAL DRUG**

The ordering of investigational agent (Calaspargase Pegol) is specified in the protocol (Section 8.1).

Participating Institutions should order their own supply from Sigma Tau.

The local IRB should be kept informed of who will supply the agent (i.e., the pharmaceutical company) so that any regulatory responsibilities can be met in a timely fashion, and the pharmacy will be able to receive and store the investigational agent.

20.6 **SAFETY ASSESSMENTS AND TOXICITY MONITORING**

All participants receiving investigational agents will be evaluated for safety. The safety parameters include all laboratory tests and hematological abnormalities, physical examination findings, and spontaneous reports of adverse events reported to the

investigator by participants. All toxicities encountered during the study will be evaluated according to the NCI criteria specified in the protocol (CTCAE version 4.0) and recorded prior to each course of therapy. Life-threatening toxicities should be reported immediately to the Protocol Chair and Institutional Review Board (IRB).

Additional safety assessments and toxicity monitoring will be outlined in the protocol.

20.6.1 **Serious Adverse Events**

A serious adverse event (SAE) is any adverse drug experience at any dose that results in any of the following outcomes: death, a life-threatening adverse drug experience, a persistent or significant disability/incapacity, or a congenital anomaly/birth defect. Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered serious when, based upon appropriate medical judgment, they may jeopardize the participant or may require medical or surgical intervention to prevent one of the outcomes listed in this definition. Examples of such medical events include allergic bronchospasm requiring intensive treatment in an emergency room or at home, blood dyscrasias or convulsions in a participant who has never had seizure activity in the past that do not result in inpatient hospitalization, or the development of drug dependency or abuse. A serious adverse event will also include any adverse drug experience that results in intensive inpatient medical interventions (i.e. intensive care unit level of care), including mechanical ventilation, pressor support, and/or fluid resuscitation, or emergent surgical operations. Unplanned hospital admissions for febrile neutropenia, bacteremia or other documented infections, seizure, pancreatitis, thrombosis, nausea/vomiting, diarrhea, and other expected toxicities from the study treatment will not be considered a serious adverse event unless the severity of the condition is considered life-threatening and/or lead to intensive medical interventions or surgical procedures.

The NCI Common Terminology for Adverse Events (CTCAE), Version 4.0 will be utilized for AE reporting.

20.6.2 **Guidelines for Reporting Serious Adverse Events**

Guidelines for reporting Serious Adverse Events (SAEs) will be followed as is delineated in the protocol section 12.

The Lead Institution will maintain documentation of all Participating Institution Adverse Event reports and be responsible for communicating all SAEs to all sites conducting the trial.

Participating Institutions must report the AEs to the Protocol Chair and the Coordinating Center (Dana-Farber/Children's Hospital Cancer Center) following the DFCI IRB SAE Reporting Requirements.

- When an event occurs that meets the protocol definition for SAE reporting (refer to Section 12 of the protocol), the Overall PI, Lewis Silverman, MD and the DFCI study team much be notified within 24 business hours of discovery of the event. An SAE report must be completed on a DFCI Adverse Event Form and/or a MedWatch 3500 or 3500A form.

- This report must be completed and emailed or faxed to the Overall PI, Lewis Silverman, MD, and study coordinator (Samantha Kay-Green, Liza Duble or Katharine Majewski).

- SAE reports **MUST** include the following information within the narrative (which can be attached if it does not fit on the Medwatch form):

    1. CTCAE V4 Event Name (for every serious event reported)
    2. Grade of Event
    3. Investigator Relationship (not related, unlikely, possibly, probably, definitely)
    4. Expectedness (is the event listed in the consent or IB as risk?)
    5. **Date** of study team notification of event

Note: SAEs on study will be collected from the time of enrollment to 60 days following the first day of the last cycle of chemotherapy.

In addition, all sites are required to track all SAE events in an SAE log. Prior to each monthly telecom (see Section 20.8.1) the study team at DFCI will request that the SAE log is updated and signed by the Site PI for tracking and regulatory purposes.

20.6.3 **Guidelines for Processing IND Safety Reports**

The U.S. Food and Drug Administration (FDA) regulations require sponsors of clinical studies to notify the FDA and all participating investigators of any serious and unexpected adverse experiences that are possibly related to the investigational agent. The Protocol Chair will review all IND Safety Reports and is ultimately responsible for forwarding the IND Safety Reports to the Participating Institutions. The Participating Institution investigators are to review, send a copy to their IRB according to their local IRB's policies and procedures, and file a copy with their regulatory documents.

20.7 **PROTOCOL VIOLATIONS AND DEVIATIONS**

Neither the FDA nor the ICH GCP guidelines define the terms "protocol violation" or "protocol deviation." All DF/HCC Protocol Chairs must adhere to those policies set by the DFCI IRB, the definitions for protocol violation and deviation as described by the DFCI IRB will be applied for reporting purposes for all Institutions Participating in the DF/HCC Multi-center Protocol.

20.7.1 **Definitions**

Protocol Deviation: *Any departure from the defined procedures set forth in the IRB-approved protocol which is* prospectively approved *prior to its implementation.*

Protocol Exception: Any protocol deviation that relates to the eligibility criteria, e.g. enrollment of a subject who does not meet all inclusion/exclusion criteria.

Protocol Violation: *Any protocol deviation that was not* prospectively approved *by the IRB prior to its initiation or implementation.*

Please Note:

- If 3 for more minor deviations for the same subject (or of the same type) are found on the minor deviation log to impact the safety of participants, compromise the integrity of the study data and / or affect subject's willingness to participate in the study the events must be submitted to the DFCI IRB as MAJOR deviation/violation.

***Any questions about a major vs. minor deviation/violation should be reviewed with the PI and or study team at the lead institution.

20.7.2 **Reporting Procedures**

The Protocol Chair: is responsible for ensuring that clear documentation is available in the medical record and/or regulatory documents to describe all protocol exceptions, deviations and violations.
The Protocol Chair will also be responsible for ensuring that all protocol violations/deviations are promptly reported per DFCI IRB guidelines.

Participating Institutions: Protocol deviations require prospective approval from DFCI IRB. The Participating institution must submit the deviation request to the Protocol Chair or designee, who will submit the deviation request to the DFCI IRB. Upon DFCI IRB approval the deviation should be submitted to the Participating Institution's own IRB, per its institutional policy.
A copy of the Participating Institution's IRB report and determination will be forwarded to the DF/HCC Lead Institution or designee by mail, facsimile, or via e-mail within 10 business days after the original submission.

All protocol violations must be sent to the DF/HCC Lead Institution Protocol Chair or designee in a timely manner.

All sites are required to keep a minor deviation log. This is to be completed as minor deviations/violations occur (see definition above). Please submit an updated log to the DFCI study team monthly and maintain a copy in the regulatory file. At each annual

continuing review, minor deviation logs from all participating sites will be submitted to the DFCI IRB along with local continuing review approval.

Coordinating Center: Upon receipt of the violation/deviation report from the Participating Institution, the DF/HCC Lead Institution or designee will submit the report to the Protocol Chair for review. Subsequently, the Participating Institution's IRB violation/deviation report will be submitted to the DFCI IRB for review per DFCI IRB reporting guidelines.

20.8 MONITORING: QUALITY CONTROL

The quality control process for a clinical trial requires ongoing monitoring to ensure all participating sites are compliant with the most current protocol and that the study is being conducted according to all pertinent regulatory requirements. As the Coordinating Center, the DF/HCC Lead Institution or designee provides quality control oversight for the DF/HCC Multi-center Protocol.

20.8.1 Ongoing Monitoring of Protocol Compliance

The Participating Institutions will be required to submit subject source documents to the DF/HCC Lead Institution or designee for monitoring. Also, the Participating Institution will be subject to on-site monitoring conducted by the DF/HCC Lead Institution or designee.

The DF/HCC Lead Institution will implement monitoring activities ongoing to ensure that Participating Institutions are complying with regulatory and protocol requirements, data quality, and subject safety. Additional monitoring practices will include but are not limited to; source verification, review and analysis of the following: eligibility requirements of all participants , informed consent procedures, adverse events and all associated documentation, study drug administration / treatment, regulatory records and site trial master files, protocol deviations, pharmacy records, response assessments, and data management.

Monitoring will occur before the clinical phase of the protocol begins and will continue during protocol performance through study completion.

All data submitted to the DF/HCC ODQ will be monitored for timeliness of submission, completeness, and adherence to protocol requirements. The Lead Institution or designee and if applicable ODQ Data Analysts assigned to the Protocol will perform the ongoing protocol data compliance monitoring with the support of the Participating Institution's Coordinators, the Principal Investigators, and the Protocol Chair.

Site Qualification:
Prior to participating in this trial, all sites must complete a Site Selection and Feasibility Questionnaire. This questionnaire will ask co-investigators to consider DF/HCC policies regarding informed consent, serious adverse event reporting and

documentation, and pharmacy guidelines. The questionnaires will also require site consideration of IRB procedures, subject enrollment, appropriateness of his/her facility for carrying out the proposed protocol, plans for data submission and monitoring, and the identification of staff availability and credentials. Co-investigators will be offered opportunity to discuss the questionnaire and accompanying checklist with the DF/HCC Lead Institution's study staff and/or DFCI Overall Principal Investigator, Lewis Silverman, MD as appropriate.

The DF/HCC Lead Institution's study staff will review the Site Selection and Feasibility Questionnaire and determine whether the site is qualified to participate. Sites believed to be qualified to participate will be invited to submit the protocol to their institutional IRB for review.

Regulatory Documents:

Once a proposed Participating Institution receives institutional IRB approval, all approval documentation and IRB-approved related documents will be emailed to the Coordinating Center study staff and an amendment to add a site will be submitted to the DF/HCC IRB. Once approval is received to add the Participating Institution study materials will be sent to the Participating Institution. Study materials will include, but are not limited to, regulatory documents and participant tracking forms.

DFCI will collect all required regulatory documentation prior to study activation. This includes but, is not limited to 1572, Site PI and Co-Investigator CVs, Financial Disclosure Forms, IRB approval documentation. The study team will work with all participating sites to ensure all updated / revised regulatory documentation is maintained at each site and forwarded to DFCI and maintained in the Trials Master File.

All Participating Institutions must maintain and update all essential regulatory documents in a regulatory binder. A designated member of the DF/HCC Lead Institution will be responsible for maintaining the Trial Master File which will include copied of all regulatory documentation for the Lead Site and all Participating Institutions.

All Participating Institutions are required to submit all institutional IRB correspondences and approvals to be retained in the Trial Master File at the Lead Institution. The Lead Institution will review all working study documents and ensure that most current IRB-approved protocol and study documents are being used by the Participating Institutions.

Site Initiation Visit:

A Site Initiation Visit (SIV) will be conducted with all participating sites via teleconference prior to enrolling participants. The SIV will cover study objectives and rationale, history of Calaspargase Pegol (SC-PEG asparaginase), study design, eligibility, registration, required data, treatment schedule, side effects/AEs, SAE reporting, and dose modification. It will be lead by the DFCI Overall Principal

Investigator, Lewis Silverman, MD. It is expected that the Site PI and all study staff will attend.

Delegation of Authority/Responsibility:
Participating Institutions will be instructed to complete a Delegation of Responsibility/ Authority Log which will be reviewed/approved by the DFCI Overall Principal Investigator, Lewis Silverman, MD. A copy of this document will be retained in the Trial Master File. Participating Institutions will be instructed to inform the DF/HCC Lead Institution immediately should there be a change in personnel. An updated training and Delegation of Authority/Responsibility log will need to be completed as soon as possible.

Ongoing Communication and Training:
Regularly scheduled teleconferences will occur between the DF/HCC Lead Institution and Participating Institutions every one to two months. It is expected that Site PIs and study staff will attend. This will be a forum to discuss study related issues not limited to the following: study accrual, SAE's/AE's, clinical response and deviations/violations. SAE trackers and minor deviation/violation logs will be requested prior to each monthly teleconference.

An agenda will be sent to all sites at the time the teleconference is scheduled. Minutes will be taken and distributed to the participating institutions after each teleconference and will maintained in the regulatory file. Calls will be held more regularly as needed based upon study accrual or necessary study updates. The DF/HCC Lead Institution will be available to all Participating Institutions in order to discuss questions and concerns on an as-needed basis.

Study related documents and amendment information will be posted on the ALL Consortium Website (http://allconsortium.dfci.harvard.edu/)

In addition, the DF/HCC Lead Institution will hold on site consortium meetings at least annually. These meetings will include all site investigators and study staff, as necessary.

Source Documentation Submission and Review via Virtual and On-Site Monitoring:
All data collected on all participants will be monitored for accuracy. Participating institutions will be required to submit source documents to the Lead Institution for virtual monitoring. The required source documentation for virtual monitoring of this study includes, but is not limited to:

- Pathology reports confirming diagnosis
- All screening laboratory reports and scans.
- All results of the "Required Assessments".
- All results of the "Required Assessments" at **study entry** and at the **date of remission assessments**
- Any extra source documentation (i.e., admission notes, scans, culture results) pertaining to a serious adverse event (SAE)

At the time of registration, all points of eligibility must be found in either source documentation or if eligibility points can not be found in source documentation (ie lab reports etc.), they **must be included in an MD note**. Eligibility source documentation must be submitted to DFCI and will be reviewed via virtual monitoring on an on-going basis.

Registration documents (consent form and eligibility checklist) will also be reviewed via virtual monitoring on an on-going basis. At the time of registration, the eligibility checklist(s) and all pages of the consent form(s) must be faxed or emailed to the DFCI Study Team for review (refer to sections 4 and 20.4.7 for participant registration process). The DFCI Study Team will review each consent form to ensure 1) it is the most appropriate and current version, 2) that the participant information is included on all pages of the consent and that all pages of the consent have been submitted, 3) that the participant has signed and dated the consent form, 4) that a physician has reviewed and signed the consent form on the same day/time as the participant, and that 5) all appropriate items have been completed.

In addition, on-site monitoring will occur at each site and will include review of regulatory documentation and source documentation. If sub-standard performance is discovered during routine monitoring (virtual or on-site), more frequent on-site monitoring visits will be made. Sub-standard performance includes, but is not limited to, data not entered on time, unreported adverse events, or enrolling ineligible participants.

Adverse Event Reporting:
Documentation of all adverse events must be included as part of source documentation. Documentation that a physician determined attribution of each event must also be included. AEs and SAEs must be reported as instructed in the protocol with the log serving as record for all events at each Participating Institution. The DFCI Overall Principal Investigator, Lewis Silverman, MD, will be notified/report all AEs SAEs as per protocol. Each Participating Institution will be instructed to keep an SAE log and submit it to the DF/HCC Lead Institution prior to each monthly teleconference. The DF/HCC Lead Institution will maintain a master SAE list.

Drug Accountability:
Participating pharmacies will be required to submit Drug Accountability Logs at the time of monitoring documenting receipt and shipment of drug supply, dispensing/ordering of supply, and destruction of unused study medication and/or damaged or expired drug. Participating Institutional Pharmacies should destroy drug as per their institutional policy.

20.8.2 **Evaluation of Participating Institution Performance**

20.8.2.1 Eligibility Checklist:

Eligibility criteria are checked on a protocol-specific eligibility checklist and faxed to the DF/HCC ODQ prior to registration on protocol. The checklist and informed consent document are reviewed by a DF/HCC ODQ Protocol Registrar before the participant can be registered on a protocol. The DF/HCC ODQ cannot make exceptions to the eligibility requirements.

20.8.2.2 Accrual of Eligible Participants:

Annual accrual rates for eligible participants enrolled onto therapeutic clinical trials are calculated for each institution. Participating Institutions are expected to maintain the minimum annual average accrual as defined by the protocol grant or contract.

20.9 AUDITING: QUALITY ASSURANCE

Auditing is a method of Quality Assurance. The main focus in auditing is to measure if the standards and procedures set are being followed. Auditing is the systematic and independent examination of all trial related activities and documents. Audits determine if evaluated activities were appropriately conducted and the data were generated, recorded and analyzed, and accurately reported per the protocol, Standard Operating Procedures (SOPs) and the Code of Federal Regulations.

20.9.1 DF/HCC Sponsored Trials

One on-site audit will be schedule by the ODQ at each site after the first 10 subjects are accrued and then annually if 10 subjects accrue since the time of the last audit. If a site enrolls less than 10 participants, they will undergo one audit over the course of the study. Approximately 3-4 subjects would be audited at the site over a 2 day period. If violations which impact subject safety or the integrity of the study are found, more subject records may be audited.

20.9.2 Participating Institution Responsibilities

It is the Participating Institution's responsibility to notify the DF/HCC Lead Institution of all scheduled audit dates (internal or NCI) and re-audit dates (if applicable), which involve the DF/HCC Multi-Center Protocol. All institutions will forward a copy of final audit and/or re-audit reports and corrective action plans (if applicable) to the DF/HCC Lead Institution or designee within 12 weeks after the audit date.

20.9.3 Coordinating Center (Lead Institution) Responsibilities

The Protocol Chair will review all DF/HCC Multi-Center Protocol Final Audit reports and corrective action plans if applicable. The Lead Institution or designee must forward these reports to the DF/HCC ODQ per DF/HCC policy for review by the DF/HCC Audit Committee. Based upon the audit assessments the DF/HCC Audit Committee could accept or conditionally accept the audit rating and final report. Conditional approval could require the Protocol Chair to implement recommendations or require further follow-up. For unacceptable audits, the Audit Committee would forward the final audit report and corrective action plan to the DFCI IRB as applicable.

20.9.4 **Sub-Standard Performance**

The Protocol Chair, DFCI IRB and the NCI for CTEP trials, is charged with considering the totality of an institution's performance in considering institutional participation in the DF/HCC Multi-Center Protocol.

20.9.4.1 Corrective Actions

Participating Institutions that fail to meet the performance goals of accrual, submission of timely accurate data, adherence to protocol requirements, and compliance with state and federal guidelines will be recommended for a six-month probation period. Such institutions must respond with a corrective action plan and must demonstrate during the probation period that deficiencies have been corrected, as evidenced by the improved performance measures. Participating Institutions that fail to demonstrate significant improvement will be considered by the Protocol Chair for revocation of participation.

DANA-FARBER Boston Children's

CANCER AND BLOOD DISORDERS CENTER

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