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Revision 5.1 date 10-21-2021 (IRB approved)

TOTAL THERAPY FOR INFANTS WITH ACUTE LYMPHOBLASTIC LEUKEMIA (ALL) I

IND 127,270 (St. Jude Children's Research Hospital)

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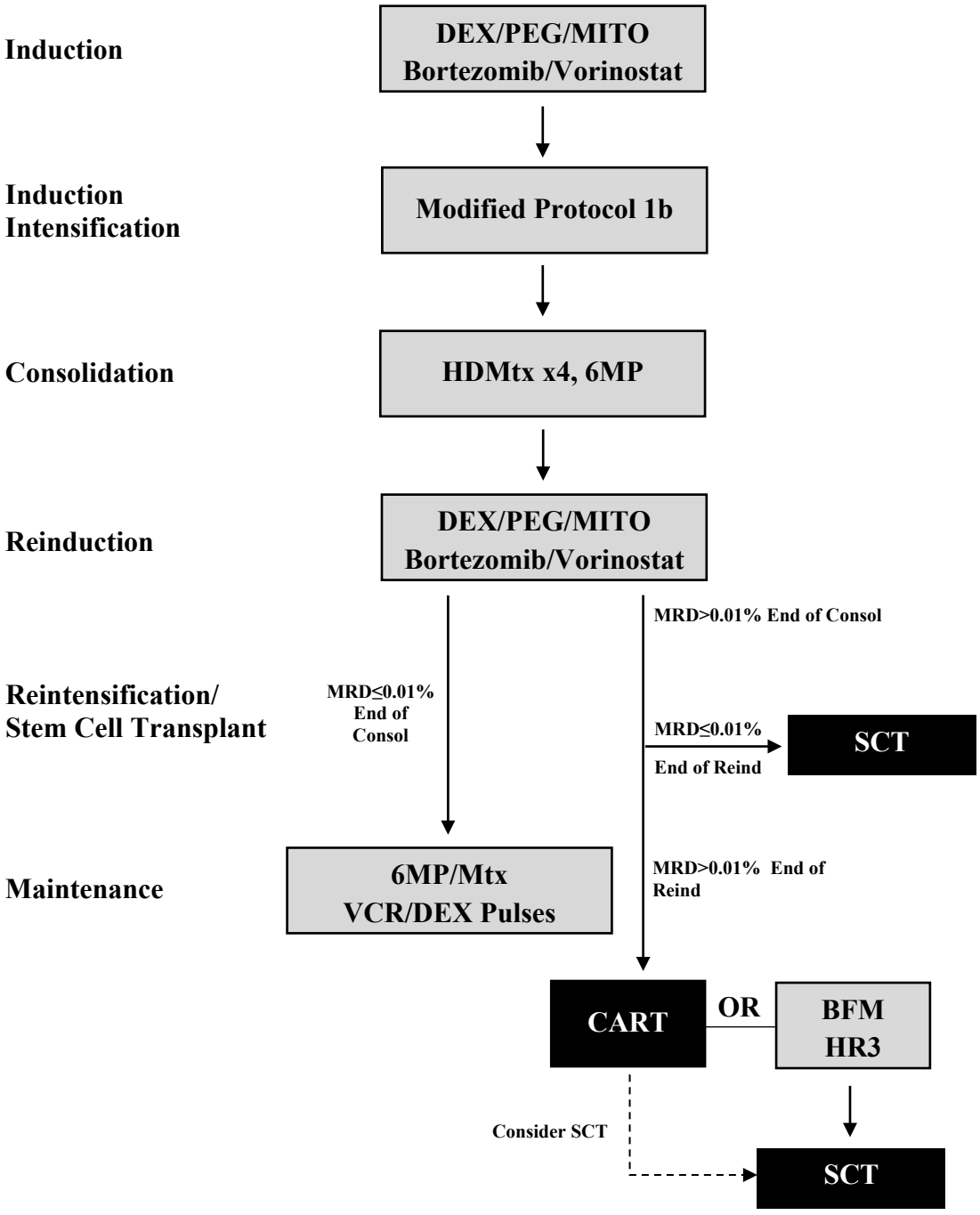
Protocol summary

TINI: Total Therapy for Infants with Acute Lymphoblastic Leukemia (ALL) I
Principal Investigator: Tanja A. Gruber, MD, PhD
IND holder: St. Jude Children's Research Hospital, #127,270
Brief overview: Treatment will consist of 4 main phases: Remission Induction, Consolidation, Reinduction, and Maintenance. High risk patients will receive a Reintensification phase prior to transplant in first remission. Total duration of therapy is 22 months.
Intervention: Remission Induction Drugs: Dexamethasone, mitoxantrone, PEG-asparaginase, bortezomib, vorinostat, cyclophosphamide, methotrexate, cytarabine, hydrocortisone, mercaptopurine Procedure: Intrathecal chemotherapy Consolidation Treatment Drugs: Methotrexate, mercaptopurine, cytarabine, hydrocortisone Procedure: Intrathecal chemotherapy Reinduction Drugs: Dexamethasone, mitoxantrone, PEG-asparaginase, bortezomib, vorinostat, methotrexate, cytarabine, hydrocortisone Procedure: Intrathecal chemotherapy Reintensification Drugs: Dexamethasone, cytarabine, etoposide, PEG-asparaginase, hydrocortisone, methotrexate Procedure: Intrathecal chemotherapy Maintenance Drugs: Dexamethasone, vincristine, methotrexate, mercaptopurine, cytarabine, hydrocortisone Procedure: Intrathecal chemotherapy
Study design: Non-randomized, multi-center feasibility/tolerability study.
Sample size: 50 evaluable participants in 7 years.
Data management: Data management and statistical analysis will be provided respectively by Clinical Research Informatics and the Comprehensive Cancer Center Hematological Malignancies Program and the Biostatistics Department at St. Jude Children's Research Hospital.
Human subjects: The primary risk to participants in this research study is toxicity from the intensive, multi-agent chemotherapy. Participants are informed of this and other potential side effects during informed consent. Adverse events will be monitored, reported and treated appropriately.

TINI Schema


Induction
DEX/PEG/MITO
Bortezomib/Vorinostat
Induction
Intensification
Modified Protocol 1b
Consolidation
HDMtx x4, 6MP
Reinduction
DEX/PEG/MITO
Bortezomib/Vorinostat
MRD>0.01% End of Consol
Reintensification/
Stem Cell Transplant
MRD≤0.01%
End of
Consol
MRD≤0.01%
End of Reind
SCT
Maintenance
6MP/Mtx
VCR/DEX Pulses
MRD>0.01% End of
Reind
CART
OR
BFM
HR3
Consider SCT
SCT

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APPENDICES

1.0 OBJECTIVES

The overall aim of this study is to improve the cure rate of infants with acute lymphoblastic leukemia (ALL).

1.1 Primary Objectives

The primary objective is to determine the tolerability of incorporating bortezomib and vorinostat into an ALL chemotherapy backbone for newly diagnosed infants with ALL.

1.2 Secondary Objectives

1.2.1 To estimate the event-free survival and overall survival of infants with ALL who are treated with bortezomib and vorinostat in combination with an ALL chemotherapy backbone.
1.2.2 To measure minimal residual disease (MRD) positivity using both flow cytometry and PCR.
1.2.3 To compare end of induction, end of consolidation, and end of reinduction MRD levels to Interfant99.

1.3 Exploratory Objectives

1.3.1 To measure histone acetylation, ubiquitination, and methylation in leukemic blasts pre and post treatment with bortezomib and vorinostat.
1.3.2 Assess NF-kB activity and proteasome inhibition pre and post treatment with bortezomib.
1.3.3 To assess the prognostic value of MRD by deep sequencing.
1.3.4 Identify all genomic lesions by comprehensive whole genome, exome and transcriptome sequencing on all patients.
1.3.5 Evaluate the sensitivity of patient blasts *in vitro* to a panel of highly active agents identified by a high throughput drug screen on primary infant ALL specimens.
1.3.6 Identify subclones in patients with detectable minimal residual disease by next generation deep sequencing.
1.3.7 Determine clonal evolution of relapsed patients by next generation sequencing.
1.3.8 Study immune repertoire diversity over the course of treatment by deep sequencing of lymphocyte variable regions.
1.3.9 Describe methotrexate clearance in infants with ALL.

2.0 BACKGROUND AND RATIONALE

2.1 Background

Acute lymphoblastic leukemia (ALL) in infants less than 1 year of age is a rare, but deadly disease. While pediatric ALL patients greater than 1 year of age achieve a five year event free survival (EFS) of 85.6%, infants have a much poorer prognosis with a four year EFS of only 47% on the recent Interfant99 study.[1,2] Importantly, outcomes for infant leukemia patients have not improved significantly over the last decade. The poor outcomes seen in this

subset of patients are believed to be a result of the unique biology of the leukemia cells, with approximately 80% of cases carrying a translocation that results in the rearrangement of MLL (MLLr) and its fusion to one of more than 40 different partner genes.[3] Indeed, infants carrying MLLr had a significantly worse four year EFS in contrast to those lacking MLLr (36.9% vs. 74.1% *P*=0.0001).[1] The presence of the MLLr has been documented in the neonatal blood spot prior to the development of overt leukemia, suggesting that this molecular event initiates disease and occurs *in utero*.[4] Moreover, recent data have demonstrated that infant MLLr ALL cases have less than one copy number alterations (CNAs) / case, indicating that few secondary mutations are required for the full development of leukemia.[5] Consistent with this interpretation, we recently completed whole genome DNA sequence analysis on leukemic blasts and matched non tumor tissue samples from 22 infant MLLr cases and identified a mean of only 3.5 structural variations (SVs) and 2.2 somatic single nucleotide variations (SNVs) and/or insertions/deletions (indels) per case - the lowest number of somatic mutations observed in 21 different pediatric cancers.[6,7] These data suggest that the major driver of transformation in this leukemia subtype is the translocation encoded MLL fusion protein. Despite recent progress in defining the epigenetic alterations that result from the expression of the MLL fusion protein, these insights have only recently begun to be extrapolated into the development of new therapeutic approaches whose benefits have yet to be defined.[8-11] Thus, there remains an urgent need for the development of alternative approaches to improve outcomes in these patients.

Due to this need for innovative therapy to improve outcomes, newly diagnosed infants carrying MLLr presenting to St. Jude Children's Research Hospital (SJCRH) have been enrolled in the Total XVI (TOTXVI) protocol and given an intensified treatment regimen. This study incorporates clofarabine, a nucleoside analog, into a high-risk chemotherapy backbone. Clofarabine in combination with cyclophosphamide and etoposide is given twice: towards the end of induction and as part of reinduction I. Six infants have received this therapy thus far; in all patients the courses of clofarabine were well tolerated (Table 1; Jeha et al., unpublished). Based on presenting features, these patients would all be classified as Group 2 (Intermediate Risk) with a four year EFS of 44.8% on the Interfant99 protocol.[1] Five patients remain in continuous complete remission; three of them have completed therapy. One patient died secondary to a severe RSV infection following a course of high dose cytarabine as part of reinduction II. While these numbers are small, the MRD data are extremely encouraging, as on Interfant 99, 70% of MLLr infants had MRD >0.01% after five weeks of therapy, and 40% continued to have >0.01% disease after nine weeks of therapy.[12] Similar to childhood ALL, MRD in infants has been shown to be a significant prognostic indicator on the Interfant99 trial.[12] Consistent with their CCR status, all infants achieved MRD negative status within seven weeks of therapy (Table 1).

Infants presenting at less than six months of age with WBC >300/mm^3 carried the worst prognosis on Interfant99, with a four year EFS of only 19.8%. Although none of the patients enrolled on TOTXVI have met this high-risk definition, we have had three relapsed/refractory infants referred to SJCRH. All three of these patients carried high risk features at diagnosis and had ≥5% leukemic blasts at the end of a five week induction course. These refractory patients received clofarabine containing courses at the time of relapse and failed to achieve durable responses (Table 2). This suggests that additional novel therapeutic approaches are necessary to target these high-risk patients. Based on

encouraging data in Phase I and II pediatric ALL trials, we treated these infants with the proteasome inhibitor bortezomib, in combination with a four drug chemotherapy backbone.[13-15] Surprisingly, bortezomib demonstrated significant activity in all three of these high risk patients (Table 2; Gruber et al., unpublished).

Table 1. MLLr Infant Outcomes TOTXVI

Age	WBC (mm^3)	MLLr	CNS status	Induction MRD[1]			Clinical status[2]
				Day 15	Day 22	Day 49	
2 mo	54.4	MLL-AF4	CNS3	Neg	ND	Neg	Died in CR1 from RSV
5 mo	52	MLL-AF4	Traumatic Tap	Neg	ND	Neg	Alive CCR Off Therapy 3/14
6 mo	411.9	MLL-EPS15	Traumatic Tap Facial Palsy	1.274%	0.158%	Neg	Alive CCR Continuation Wk 21
8 mo	31.1	MLL-AF10	CNS3+ Cranial Lesions	1.38%	0.45%	Neg	Alive CCR Off Therapy 12/10
8 mo	450	MLL-ENL	CNS2	0.01%	ND	Neg	Alive CCR Continuation Wk 98
10 mo	32.9	MLL-AF10	CNS1	13.7%	0.02%	Neg	Alive CCR Off Therapy 9/13

[1]MRD as determined by flow cytometry, with a sensitivity of 0.01%; ND = Not Determined
[2]Duration of therapy is 2.5 years, consisting of induction (7 weeks), consolidation (8 weeks), and continuation (120 weeks).

Table 2. Response to Salvage Regimens in Relapsed/Refractory ALL Infants[1]

Patient	Age[2]	WBC[2] (mm^3)	Pre-Clofarabine	Post-Clofarabine[3]	Pre-Bortezomib	Post-Bortezomib[4]
1	3 mo	600	56%	75%	75%	3.23%
2	2 mo	1300	7.93%	85%	95%	7.93%
3	2 mo	264	97.57%	86%[5]	86%	1.384%

[1]As measured by flow cytometry on bone marrow aspirates
[2]At diagnosis
[3]Patients 1 and 3 received clofarabine in combination with high dose cytarabine and sorafenib; Patient 2 received clofarabine in combination with etoposide, cyclophosphamide, and Natural Killer Cell infusions.
[4]All patients received Bortezomib in combination with the MRC R3 relapsed induction chemotherapy backbone. Patients 1 and 2 received four doses of bortezomib, patient 3 received 6.
[5]Patient 3 received two courses of clofarabine, after the first she decreased to 2.885% however following the second she recovered with 86% blasts in the marrow.

To investigate the activity of bortezomib in this leukemia subset further, we established *in vitro* and *in vivo* assays to evaluate drug sensitivity of primary infant ALL patient samples (Figure 1a; Gruber et al., unpublished). We established 15 primary human infant MLLr leukemias as xenografts in NOD/SCID/ IL2Rγnull (NSG) mice. These samples have undergone whole genome sequencing so that the presence or absence of genetic lesions can be correlated with response to targeted agents. All samples engrafted and expanded in NSG mice, leading to overt leukemia with a latency of 49 to 276 days. Purification of leukemic blasts from a single moribund mice yield on average 10^8 cells, providing sufficient material to screen large numbers of compounds. Moreover, we have established *in vitro* conditions that support growth in six of the patient specimens, allowing for a more accurate determination of drug sensitivity. Growth *in vitro* correlated with early onset of disease in NSG xenografts as well as younger age at presentation, allowing us to evaluate patient samples that represent aggressive high-risk disease (Figure 1b; Gruber et al., unpublished).

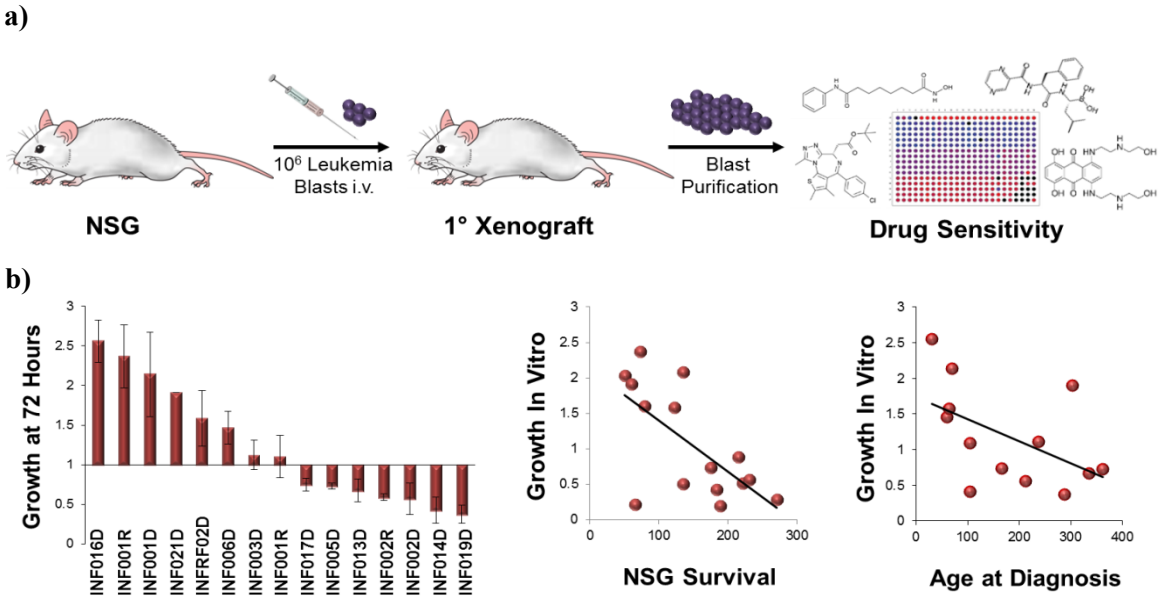

Figure 1. Platform for High Throughput Drug Screening. a) Study Design. Primary patient samples are injected into NSG recipient mice for expansion. Moribund mice are sacrificed, and human leukemia cells are purified for subsequent *in vitro* sensitivity studies. **b)** Growth Characteristics of Study Samples. Patient blasts purified from mice were grown *in vitro* in serum free media supplemented with cytokines on a collagen matrix and assessed for proliferation at 72 hours as determined by intracellular ATP concentrations. Correlation with disease latency in NSG and the age of the patient at presentation are shown.

Using this system, we tested bortezomib in addition to 29 other drugs, including standard ALL therapeutic agents as well as targeted kinase inhibitors and inhibitors of epigenetic marks (see Appendix II). Three classes of agents were active in this system: anthracyclines, histone deacetylase inhibitors (HDAC), and the proteasome inhibitor bortezomib (Table 3; Gruber et al., unpublished). In contrast to anthracyclines and HDAC inhibitors where IC50 values were on par with those reported in the literature for primary childhood ALL samples, MLLr infant samples required 10-100 fold less bortezomib to induce cytotoxicity. These data suggest an enhanced sensitivity of MLLr infant ALL cells to bortezomib.

Table 3. Drugs with Activity in Primary Infant ALL Samples*

Class	Drug	IC50 (uM)	Literature
Anthracycline	Doxorubicin	0.41±0.38	0.054-4.35uM (90 1° ALL Cases)[16]
	Daunorubicin	0.08±0.07	0.0053-1.05uM (157 1° ALL Cases)[16]
	Mitoxantrone	0.35±0.34	0.0015-0.444uM (112 1° ALL Cases)[16]
HDAC Inhibitor	Vorinostat	3.11±1.29	0.55-1.78uM (5 ALL Cell Lines)[17]
	Panobinostat	0.63±1.46	0.0077-0.092uM (9 ALL Cell Lines)[18]
Proteasome Inhibitor	Bortezomib	0.002±0.0008	0.046-1.342uM (106 1° ALL Cases)[19]

*Only agents active against all 15 patient samples are shown. IC50 values are from the 6 samples that proliferate *in vitro* (Figure 1b).

Bortezomib has been shown to mediate responses through several mechanisms including NFkB inhibition, stabilization of cell cycle regulatory proteins, and induction of apoptosis.[20] Recently, proteasome inhibition has been demonstrated to lead to accumulated MLL fusion protein levels, triggering apoptosis and cell cycle arrest in MLL rearranged cell lines.[21] To determine if NFkB inhibition also plays a role we evaluated cellular concentrations of the

activated NFkB transcription factor, but failed to see decreased levels when cells were treated with bortezomib (data not shown). Bortezomib has also been shown to deregulate ubiquitin stores and deplete histone H2B ubiquitination (H2Bub), an epigenetic mark that is linked to histone methylation and expression.[22] Recently, several groups have published reports demonstrating H2Bub is required for DOT1L activity and HOX gene expression.[23-25] We therefore evaluated histone H2B levels in MLLr cell lines and patient samples in the presence of bortezomib and confirmed depletion of this epigenetic mark (Figure 2a; Gruber et al., unpublished). Furthermore, genome wide chromatin immunoprecipitation sequencing (ChIPseq) of bortezomib treated cells demonstrated a decrease in histone 3 methylation at lysine residue 79 (H3K79me2) both genome wide and at MLL-AF4 target genes, the epigenetic mark imparted by DOT1L (Figure 2b; Gruber et al., unpublished). Consistent with these data, patient samples treated with bortezomib downregulated both the MLL gene expression signature and signatures of downstream targets such as cMYC (Figure 2b; Gruber et al., unpublished), suggesting that the MLL transcriptional program is inhibited in the presence of bortezomib.

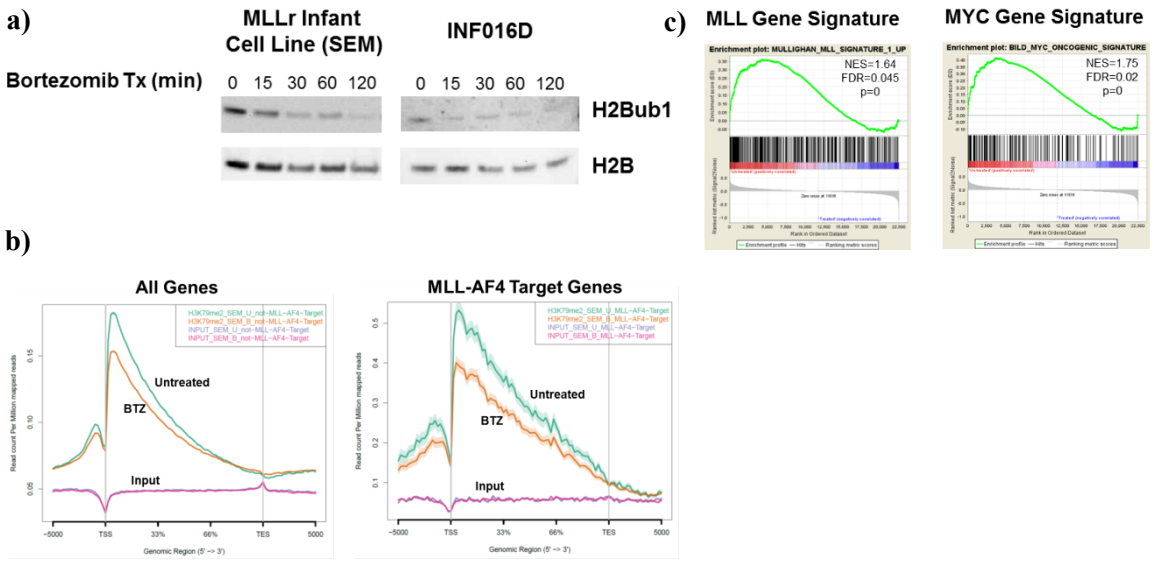

Figure 2. Bortezomib Depletes Histone H2B Ubiquitination and Downregulates the MLL gene expression program. a) H2Bub levels. Shown is an MLLr cell line and a representative primary patient sample treated with bortezomib. Histones were extracted, run on a gel, and blotted for total H2B and ubiquitinated H2B. b) ChIPseq of H3K79me2 in untreated and bortezomib (BTZ) treated MLL-AF4 positive SEM cells. Read counts per million mapped reads are shown at all genes and MLL-AF4 target genes as previously described. TSS - transcriptional start site; TES – transcriptional end site. **c)** GSEA of bortezomib treated patient samples. Patient samples were treated with bortezomib and subsequently underwent gene expression profiling. Gene set enrichment analysis was done on differentially expressed transcripts between treated and untreated cells. Shown are MLL and MYC gene signatures. NES, normalized enrichment score; FDR, false discovery rate.

All three relapsed/refractory infants receiving bortezomib in combination with a four drug regimen had responses and were subsequently transplanted with either haploidentical or match unrelated donor transplants (Table 2). While the first two patients died of treatment related mortality, patient 3 achieved MRD negative status thirty days post-transplant following total body irradiation (TBI) conditioning with a cord blood donor. She remained in remission for 210 days post-transplant but subsequently relapsed, presenting with hyperleukocytosis. At that time she was reinduced with a five drug regimen including bortezomib, vorinostat, PEG asparaginase, dexamethasone, and mitoxantrone (Patient 3b, Table 4; Gruber et al., unpublished). With this single course she achieved MRD negative status by flow as well as deep sequencing (sensitivity 1:10,000 and 2:1,000,000 respectively) following count recovery. She has subsequently been consolidated, undergone a second reinduction and is currently in maintenance. She remains MRD negative at greater than one year from the time of her relapse.

We have subsequently treated eight additional relapsed MLLr leukemia patients that have failed one or more chemotherapy regimens with the combination of bortezomib, vorinostat, and mitoxantrone. Patients included ALL, AML and mixed lineage immunophenotypes. Combined, eight of nine MLLr patients have responded to this combination for an overall response rate of 88.9% (Table 4). This compares very favorably with recent relapsed ALL and AML trials that have reported CR rates between 40-65% using the same criteria of <5% leukemic blasts. [13,15,2613,15,26] Furthermore, the regimen was tolerated in this highly pretreated cohort that included patients post-transplant (Table 5). There was one Grade 5 toxicity secondary to a systemic adenoviral infection that was present prior to treatment. The patient tolerated the chemotherapy, however upon signs of count recovery, the patient worsened clinically and died secondary to cardiac failure.

Table 4. Clinical Activity of Bortezomib and Vorinostat in MLLr Leukemia

			Chemo Doses		Pre Treatment								Post Treatment								
					PBL					BMA[1]			PBL					BMA			Response[2]
Case	IP[3]	Age[4]	BTZ[5]	SAHA[5]	WBC	Blast	APC	Plt	Hgb	Blast	MRD	PCR[6]	WBC	Blast	APC	Plt	Hgb	Blast	MRD	PCR	
1	ALL	7m	4	0	2.9	14%	2233	250	10.5	75%	94.9%	+++	0.3	0%	100	26	8.6	1%	3.23%	10^{-1}	CRi
2	ALL	5m	4	0	92.9	95%	4400	63	8.5	ND	ND	ND	0.5	0%	300	45	7.4	12%	7.93%	10^{-3}	PRi
3a[7]	ALL	10m	6	0	1.4	44%	182	22	8.3	83%	86%	10^{-2}	2.7	0%	2619	209	10.2	0%	1.38%	10^{-3}	CR
3b[7]	ALL	1.5y	8	12	83.5	73%	15865	255	10.9	83%	ND	10^{-1}	2	0%	1320	60	11.1	2%	Neg	Neg	CRi
4	ALL	11y	8	12	3.1	4%	2046	52	9.3	74%	67%	+++	0.6	0%	90	30	9.3	60%	48.14%	+++	NR
5	AML	3.6y	6	12	0.8	0%	112	141	8	52%	68%	+++	1.9	0%	1121	99	8.9	0%	2%	10^{-1}	CR
6[8]	AML	1.6y	8	12	0.3	0%	100	73	10.4	46%[6]	23%	++	0.3	0%	100	28	9.6	26%	Neg	10^{-5}	CRi
7	AML	9.25y	6	12	1.9	0%	798	175	10.2	8%	5.87%	ND	3.9	0%	3159	75	11.2	2%	Neg	10^{-4}	CR
8	AML	2.3y	8	16	1.3	0%	1261	118	8.9	8%	12.8%	10^{-4}	8.1	0%	8100	24	10.7	0%	0.28%	ND	CRi
9	Mixed	4.1y	6	12	1.6	3%	144	46	9.5	86%	78%	+++	0.4	0%	0	62	9.2	3%	0.997%	10^{-1}	CRi
10	ALL	1.5y	8	16	15.6	2.3%	13104	204	10.3	46%	30.8%	++	3.1	0%	2852	183	8.1	0%	Neg	Neg	CR
11	ALL	17y	8	16	9.7	0%	7469	57	11.8	84%	ND	+++	0.1	0%	0	129	7.6	0%	Neg	Neg	CRi

[1]ND, not determined.

[2]Response criteria:

CR <5% blasts by flow cytometry, ANC>500, Plts>75; CRi <5% blasts by flow cytometry, ANC<500, Plts<75

PR 5-25% blasts by flow cytometry and a reduction of at least 50%, ANC>500, Plts>75; PRi 5-25% blasts by flow cytometry and a reduction of at least 50%, ANC<500, Plts<75

[3]Immunophenotype by flow cytometry.

[4]Age at the time the chemotherapy course containing bortezomib and vorinostat were given.

[5]BTZ, bortezomib; SAHA, vorinostat. Total number of doses given over the course of the treatment are indicated. Patient 5 had two doses of bortezomib held secondary to neuropathy.

[6]PCR for the MLLr. +++ strongly positive, ++positive.

[7]Patient 3 course a was pre-transplant, course b was post-transplant.

[8]Patient 6 had a severe systemic adenoviral infection prior to and post chemotherapy. At the time of evaluation post treatment, while blasts were elevated, this was felt to be a recovering marrow given the negative MRD result by flow and PCR.

Table 5. Toxicity in MLLr Leukemia Patients Receiving Bortezomib and Vorinostat Containing Regimen

			Chemo Doses			
Case	IP	Age	BTZ	SAHA	Toxicity	Grade
1	ALL	7m	4	0	None	NA
2	ALL	5m	4	0	Fever and Neutropenia	3
3a	ALL	10m	6	0	VCR induced vocal cord paralysis	2
3b*	ALL	1.5y	8	12	Gram negative rod sepsis	4
					Human metapneumovirus pneumonia	2
4	ALL	11y	8	12	None	NA
5	AML	3.6y	6	12	Urinary retention	2
6	AML	1.6y	8	12	Systemic adenovirus (pre-existing prior to initiation of treatment)	5
7	AML	9.25y	6	12	None	NA
8	AML	2.3y	8	16	None	NA
9*	Mixed	4.1y	6	12	Fungal infection (lungs and CNS)	4
					VRE catheter related infection	2
10	ALL	1.5y	8	16	Upper respiratory infection	2
					Urinary retention	2
11*	ALL	17y	8	16	Neutropenic colitis	3
					Enterococcus bacteremia	3
					Fungal pneumonia	3

Patients receiving treatment upon relapse following one or more hematopoietic stem cell transplants are indicated with an asterisk (*).

2.2 Rationale

2.2.1 Induction

Pre-clinical laboratory data and treatment of a small number of relapsed/refractory patients with MLLr demonstrates significant activity of bortezomib. H2Bub depletion, which silences the MLL transcriptional complex is reversible. Accordingly, responses in our first two patients that only received four doses of bortezomib were transient. We observed a more robust response with patient three who received six doses with her initial course. Vorinostat is synergistic with bortezomib in multiple models and demonstrated activity in our preclinical data.[27] The addition of this drug in combination with bortezomib and standard chemotherapeutic agents led to MRD negativity by flow cytometry in five patients (Table 4). We therefore will incorporate both bortezomib and vorinostat into an induction backbone. The standard ALL induction regimen contains four drugs: a steroid, an anthracycline, vincristine, and asparaginase. Due to overlapping side effects with bortezomib and the under-recognized toxicity of vocal cord paralysis seen in infants, we have eliminated vincristine from our backbone. We do not feel this will compromise outcomes as it has been omitted in our ALL relapsed patients, 80% of whom (4/5) achieved CR/CRi. Furthermore, bortezomib has been shown to successfully replace vincristine in other hematologic malignancies.[28] Bortezomib in combination with the standard induction backbone has been evaluated in the relapsed setting, however the addition of vorinostat to this combination has not.[14,15] Therefore, we will use a 3+3 dose escalation phase for vorinostat, starting at 100mg/m^2 per dose which is 55% of the defined pediatric maximum tolerated dose of 180mg/sqm.[17]

Throughout treatment, patients will receive dexamethasone during times of steroid administration instead of prednisone. While evidence suggests that dexamethasone has superior cytotoxicity and CNS penetration compared to prednisone, prolonged continuous administration increases toxicity.[29] We are therefore limiting the drug to 12 days total during induction. The schedule of dexamethasone has been altered to coincide with vorinostat/bortezomib administration. The alteration of dexamethasone will both limit dosing as well as provide synergy which has been demonstrated for these three classes of agents in laboratory studies.[30] We have eliminated the prednisone prephase present in Interfant99 to bring up bortezomib and vorinostat on day 1 of treatment such that biologic correlate studies on the peripheral blood could be obtained that will be informative. Furthermore, patients with hyperleukocytosis will not run the risk of increasing WBC on prednisone monotherapy as they will receive additional chemotherapy upfront.

Mitoxantrone has been chosen as our induction anthracycline based on data from the Medical Research Council (MRC) which found that it conferred a significant benefit in progression-free and overall survival in children with relapsed acute lymphoblastic leukemia as compared to idarubicin.[13] Due to the high frequency of infants that present with hyperleukocytosis, mitoxantrone will be delayed until day 8 of our induction to allow for an initial reduction in tumor burden prior to its administration such that tumor lysis can be controlled.

Although induction intensification with clofarabine was well tolerated on TOTXVI, we will replace this with a modified BFM based Protocol IB due to toxicity of clofarabine seen in the COG AALL1131 trial leading to closure of the clofarabine containing arm. Protocol 1B has been modified in that our patients will receive two weeks of this regimen as opposed to four. Furthermore, cyclophosphamide will be fractionated rather than administered as a single dose. Giving cyclophosphamide in this manner has improved end of induction MRD levels in TOTXVI when compared to TOTXV patients that received cyclophosphamide as a single dose (data not shown). Response to therapy will be assessed on day 22 to assess response to our study drugs, and at the end of induction intensification upon count recovery.

TOTXV has proven that preventive cranial irradiation can be safely eliminated in childhood ALL irrespective of risk group with intensification of CNS-directed and systemic chemotherapy[2]. As the side effects of cranial irradiation are more profound in younger infants and patients with MLLr have a higher propensity of CNS relapse, we have continued to treat infants with intensified intrathecal (IT) chemotherapy on TOTXVI. Patients with CNS1 disease will receive weekly IT for a total of four doses while those with CNS2/3 disease will get two additional doses on days 4 and 11. The administration of leucovorin rescue at 24 and 30 hours post each IT is critical to prevent unwanted side effects such as seizures.

We recognize that the induction regimen is intensive. As such all patients are required to remain inpatient until count recovery following induction intensification with supportive care that includes prophylactic antibiotics. This is the current practice for children with AML and we feel the intensity of therapy and risk for infectious complications are on par if not greater than the pediatric AML population. In comparison to the COG infant trial AALL0631 pre-amendment, which experienced significant infectious toxicities, our cumulative anthracyclines, steroids, and vincristine dosing are less, while PEG asparaginase is equal (Table 6). Additionally, AALL0631 did not require an inpatient stay or prophylactic antibiotics other than trimethoprim/sulfamethoxazole (TMP/SMZ). For infants less than 30 days of age or less than 3 months of age and significantly premature, anthracyclines, PEG-asparaginase and cyclophosphamide during induction intensification will be administered at a 50% dose reduction. Using these measures, in addition to strict adherence of supportive care guidelines, we are confident the regimen will be well tolerated based on the number of infants we have treated at St. Jude.

Table 6. Comparison of Infant Induction Regimens[1]

	Interfant 99		AALL0631 Pre-amend		TINI	
Drug	Details	Cum	Details	Cum	Details	Cum
Steroid[2]	Pred 60 mg/m^2 d1-7 Dex 6 mg/m^2 d8-29	1208 mg/m^2	Pred 40 mg/m^2 d1-21	840/m^2	Dex 10 mg/m^2 d1-4,8-11, 15-18	750 mg/m^2
VCR	1.5 mg/m^2 d8, 15, 22, 20	0.2 mg/kg	0.05 mg/kg d1, 15 0.03 mg/kg d8	0.13 mg/kg	NA	NA
Anthra[3]	Dauno 30 mg/m^2 d8,9	1.67 mg/kg	Dauno 2.6 mg/kg d1,2	4.33 mg/kg	Mito 0.27 mg/kg d8,9	2.16 mg/kg
PEG	Elspar 10,000 U/m^2 d15,18,22,25,29,33	60,000 U/m^2	PEG 2500 U/m^2 d4	2500 U/m^2	PEG 2500 U/m^2 d5	2500 U/m^2
AraC	75 mg/m^2 d8-21	1050 mg/m^2	NA	NA	NA	NA
Bortez	NA	NA	NA	NA	0.043 mg/kg d1,4,8,11,15,18	0.258 mg/kg
SAHA[4]	NA	NA	NA	NA	180 mg/m^2 d1-4, 8-11, 15-18	2160 mg/m^2

[1]Induction intensification is omitted from this table [2]5mg prednisone = 0.8mg dexamethasone conversion
[3]doxorubicin equivalents (daunorubicin=0.833, mitoxantrone=4); mg/m^2 dosing converted to mg/kg using the rule of 30.
[4]Dose level 3 is shown in this table. For dosing during the run in dose escalation phase, see section 4.2 and 14.1

2.2.2 Consolidation

Consolidation therapy is comprised of high dose methotrexate (HDMTX; average of 5g/m^2 over 24 hours) given every other week for 4 doses with leucovorin rescue. The feasibility and effectiveness of HDMTX at 5g/m^2 has been documented in large number of patients treated on TOTXV in addition to the BFM and AIEOP cooperative studies. Specifically, outcomes in infants treated on BFM and POG regimens improved with the addition of HDMTX.[31] Studies suggest that while HDMTX at 2.5g/m^2 is sufficient for most ALL cases, non-hyperdiploid B lineage blasts accumulate more methotrexate polyglutamates with high dose than low dose methotrexate.[32] The goal is to provide average therapeutic systemic exposure that is achieved with 5g/m^2 while minimizing toxicity. Therefore, we will target dosing to a range of 65uM ± 20%. Using this approach on TOTXV we observed only 5.1% of courses with grade 3-4 mucositis (compared to as high as 44% of courses with BFMs administration of 5g/m^2 without targeting). All patients will receive 5g/m^2 over 24 hours for the first course and methotrexate levels will be drawn at 6, 23, 42, and every 24 hours thereafter from the start of infusion until the level is <0.1 uM. Levels will be reported to the study chair following clearance and dosing recommendations will be given for the next course and each of the subsequent courses. This approach has been successful for the six infants treated on TOTXVI, with only one patient experiencing grade 3-4 mucositis during the first course and improvement with subsequent courses due to dosing modifications. It is recommended that patients achieving MRD negative status at the end of Consolidation not be referred to stem cell transplant in first remission. However, patients that are MRD positive during Consolidation but whom achieve MRD negative status at the end of Consolidation may be referred to stem cell transplant following Reinduction if the treating physician and the family agree that this is in the best interest of the patient.

2.2.3 Reinduction

The inclusion of a reinduction phase has been demonstrated to improve outcomes in both low and high risk ALL.

We have limited this regimen to one reinduction with the following rationale:

- Infants on Interfant99 did not see an improvement in outcome for patients that received a second intensification phase.
- Infants on Interfant99 that were MRD negative prior to the second intensification phase had a low rate of relapse (17% vs. 71% in MRD+ patients). Patients who remain MRD positive following reinduction on this protocol are eligible for transplant if a suitable donor is available. Thus, their therapy will be intensified accordingly.
- Due to induction intensification, patients receive 134 days (~4.5 months) of intensive chemotherapy prior to maintenance on this protocol. By limiting reinduction courses to one, we hope to avoid the additional toxicity a second course would cause. Close monitoring of MRD as indicated above will allow us to identify patients at higher risk for relapse and these patients will go on to transplant following Reintensification.

The majority of infants that relapse, do so within a year of diagnosis. Therefore, to intensify therapy early in an attempt to eradicate persistent clones we will reinduce patients immediately following consolidation. The reinduction course is identical to induction with three exceptions: induction intensification has been eliminated, mitoxantrone has been moved up to day 1, and we have intensified PEG-asparaginase to include two doses. During the run in dose escalation phase, vorinostat dosing will the same as that received during induction (see section 4.4 and 14.1).

2.2.4 Reintensification

Patients with MRD > 0.01% on day 1 of Reinduction, OR patients with increasing MRD during Consolidation represent a high risk population that cannot be cured with chemotherapy alone. Patients that achieve MRD negative status following Reinduction may proceed directly to stem cell transplant if a suitable donor is available. Patients that remain MRD positive following Reinduction are considered upfront refractory and therefore may either receive chimeric antigen receptor (CAR) T cells if available, or proceed to a Reintensification phase followed by hematopoietic stem cell transplant.[33] To introduce agents not yet seen during the treatment regimen, the BFM HR3 block, which includes high dose cytarabine and etoposide will be given[34]. All aspects of the hematopoietic stem cell transplant including but not limited to the conditioning regimen, donor selection, and GVHD prophylaxis will be at the discretion of the transplant physicians.

2.2.5 Maintenance

Maintenance chemotherapy is comprised of the standard antimetabolite based regimen and vincristine and steroid pulses used in many pediatric cooperative group studies. Weekly low dose intravenous methotrexate will be held on weeks when patients get intrathecal therapy due to the risk of increased neurotoxicity when administered concurrently.[35] 80% of infants with MLLr ALL relapse within the first few years after diagnosis while on treatment; in keeping with this Interfant 99 treated non-high risk patients for 2 years duration from the date of diagnosis and observed few events for patients achieving MRD negative status at either 5 or 9 weeks into therapy.[1,12] We have therefore limited the duration of maintenance chemotherapy to 20 cycles of 28 days each, for a total duration of therapy of 686 days = 1.9 years. Patients with CNS disease at diagnosis will receive more frequent intrathecal therapy than those who are CNS1.

2.2.6 Summary of TINI therapy

Total duration of therapy: 686 days = 1.9 years
Cumulative anthracyclines: 160 mg/m^2 doxorubicin equivalents
ITMHA: CNS1 – 17 doses, CNS2 – 20 doses, CNS3 – 23 doses
Study drugs:
- Bortezomib: 12 doses
- Vorinostat: 24 doses

2.3 Background and Rationale for Correlative Research Studies

2.3.1 Monitoring of MRD and Immune Repertoire

The current standard of care for MRD detection utilizes flow cytometry that carries a sensitivity of 10^{-4}. At St. Jude, we supplement this with quantitative RT-PCR detection of MLL fusion transcripts for cases that cannot be followed by flow cytometry or aspirates with hypocellular marrows where quantitation by flow is challenging due to limiting numbers of cells. Recently, next generation sequencing of T cell and B cell receptor variable regions has been used to monitor MRD with a sensitivity of up to 10-6.[36,37] The prognostic significance of MRD levels less than 10-4 have yet to be defined. We have established an in house assay to measure lymphocyte clonality by massive parallel next generation sequencing of rearranged immune receptor loci (both complete and partially rearranged alleles). =We will utilize this assay to evaluate MRD status at multiple time points, correlate with flow cytometry and RT-PCR assays, and determine if there is a prognostic significance in this subtype of leukemia. As this is an exploratory aim and our assay is not CLIA-certified, deep sequencing results will not be reported back to families nor treating physicians and will be used for research purposes only.

In addition to MRD detection, this technique allows the determination of immune diversity, providing a platform in which to monitor each patient's lymphocyte repertoire. A diverse repertoire provides an individual with the ability to mount an immune response against an infectious agent. Infectious complications are a major source of morbidity and

mortality in infants with ALL and are felt to be a result of an immature immune system combined with immunosuppression secondary to chemotherapy. It is unclear, however, why some patients exhibit mild to moderate symptoms during an infection while others experience severe and often life threatening symptoms. We hypothesize that the diversity of a patient's repertoire correlates with rate and severity of infectious complications. To evaluate this, we will obtain 2.5 ml of peripheral blood at multiple time points during therapy to measure and monitor lymphocyte repertoire over the course of treatment.

2.3.2 Next Generation Sequencing

Whole genome sequencing of MLLr infant ALL revealed a paucity of somatic mutations. None the less, activating mutations in tyrosine kinases/PI3K/RAS pathway genes were detected in 48% of cases.[7] Surprisingly, however, these mutations were often present in only a minor sub-clone and were frequently lost at the time of relapse. Furthermore, in our small sample size we failed to identify any prognostic significance of these cooperating mutations in contrast to a previous report that identified RAS mutations as an independent predictor for poor outcome with a hazard ratio of 3.194.[38] This may reflect our smaller sample size (48 vs. 109) or components of the treatment regimen received. To further define the genomic landscape and clarify the role of tyrosine kinase mutations we will comprehensively sequence leukemia cells from all patients enrolled by whole genome (WGS), whole exome (WES) and RNA sequencing (RNAseq). The use of WGS, which identifies all mutations in the genome (coding and noncoding regions) will provide us with sufficient mutant alleles to analyze persistent subclones in MRD positive patients by targeted capture with deep sequencing. Thus allowing us to correlate genetic composition with response to treatment at a higher sensitivity than standard overall outcome measures by identifying clones that demonstrate resistance to chemotherapeutic agents. Targeted capture and deep sequencing to identify persistent subclones using mutations identified at diagnosis will also be done. All next generation sequencing will be done in a research setting, and will not be used for clinical decision making as the significance and contribution to relapse remain unknown.

2.3.3 Measurement of Epigenetic Changes in Response to Bortezomib and Vorinostat

Our preclinical data demonstrates alterations in histone ubiquitination and methylation in response to treatment of cells with bortezomib. Additionally, vorinostat's primary mechanism of action is through the inhibition of histone deacetylases. Therefore, to verify target inhibition and monitor the response of leukemic blasts to these agents, we will measure levels of histone acetylation, ubiquitination, and methylation by flow cytometry. Consenting patients will have peripheral blood specimens drawn prior to therapy, at 24 hours following administration of bortezomib and vorinostat and on day four.

2.3.4 NFkB Activity and Proteasome Inhibition in Response to Bortezomib

Proteasome inhibition and NFkB activity will be assessed in peripheral blood before chemotherapy, 6h and 24h after bortezomib administration by ImageStream

cytometry.[39,40] This technique allows a more sensitive measurement of NFkB activity and will evaluate proteasome target inhibition.

2.3.5 In Vitro Drug Sensitivity

We have established *in vitro* and *in vivo* assays to evaluate drug sensitivity of primary infant ALL patient samples as described in section 2.1. As proof of principle, we utilized this system to evaluate 30 chemotherapeutic agents. Our preliminary data suggests this system can identify compounds with clinical activity. We are currently using this system to screen >10,000 compounds including FDA approved drugs, known bioactives/scaffolds, as well as rare scaffolds and natural products. We will select 50-100 compounds that have the greatest activity and test sensitivity of all patient samples received (including relapsed specimens). Agents with promise will then be selected for further study with a goal of identifying compounds that can be developed and brought to clinical trial in the relapsed setting.

3.0 ELIGIBILITY CRITERIA AND STUDY ENROLLMENT

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

3.1 Inclusion Criteria

3.1.1 Patient is ≤ 365 days of age at the time of diagnosis.
3.1.2 Patient has newly diagnosed acute lymphoblastic leukemia (ALL) or acute undifferentiated leukemia with ≥25% blasts in the bone marrow (M3), with or without extramedullary disease. Patients with T-cell ALL are eligible. Patients with bilineage or biphenotypic acute leukemia are eligible, provided the morphology and immunophenotype are predominantly lymphoid.
3.1.3 Limited prior therapy, including hydroxyurea for 72 hours or less, systemic glucocorticoids for one week or less, one dose of vincristine, and one dose of intrathecal chemotherapy.
3.1.4 Written informed consent following Institutional Review Board, NCI, FDA, and OHRP Guidelines.

3.2 Exclusion Criteria

3.2.1 Patients with prior therapy, other than therapy specified in 3.1.3.
3.2.2 Patients with mature B-cell ALL or acute myelogenous (AML).
3.2.3 Patients with Down syndrome.
3.2.4 Inability or unwillingness of legal guardian/representative to give written informed consent.

3.3 Research Participant Recruitment and Screening

19 institutions will collaborate in the proposed project: St. Jude Children's Research Hospital (SJCRH), Children's Hospital Los Angeles, Texas Children's Hospital (correlative research only), Children's Hospital of Minnesota, Rainbow Babies and Children's Hospital, Rady Children's Hospital San Diego, Children's Hospital of Michigan, Cincinnati Children's Hospital and Medical Center, Lucile Packard Children's Hospital, Doernbecher Children's Hospital, Children's Hospital of Orange County, Novant Health Hemby Children's Hospital, Children's Hospital-King's Daughters, BC Children's Hospital, CHU Sainte-Justine, McMaster Children's Hospital, Alberta Children's Hospital, Montreal Children's Hospital, Children's Hospital of Eastern Ontario, and Stollery Children's Hospital Edmonton, Alberta.

3.4 Enrollment on Study at St. Jude

Eligibility will be reviewed for completeness and corrected by the study team. A research participant-specific consent form will be released for participant/parent signature.

The study team will enter the Participant Eligibility checklist information into the central enrollment system and release the informed consent document. The signed consent form must be faxed to [redacted] or scanned and emailed the Clinical Trials Operations (CTO) office at [redacted] in order to complete enrolment.

To assist with enrollments and consent release, the CTO staff is available Monday through Friday. After hours, weekends, and holidays, the study team is referred to the CTO webpage for additional resources and instructions, Link: [redacted]

3.5 Enrollment on Study at Collaborating Sites

Before the collaborating or enrolling affiliate sites screen or enroll a study participant, the site completes a RIN request form and submits it to CTO by e-mail: [redacted] or fax [redacted] (follow fax by a phone call to [redacted] to ensure receipt). A RIN is an eight-digit automated number beginning with an "R". Once the form is received, St. Jude will r*egister* the research participant and then email the RIN to the email address provided on your registration form. This will register the participant only; it will not enroll the participant on the study. After hours, holidays, and weekends the site RIN request will be answered by the Patient Registration team.

To *enroll* the study participant, after the RIN is obtained, the site study team will complete an eligibility checklist and scan and email to the CTO at [redacted] or fax it to [redacted] (follow fax by a phone call to [redacted] to ensure receipt). Clinical Trials Operations (CTO) will enter the Eligibility Checklist information into the central enrollment system to officially enroll the participant on the trial. Collaborating sites are not required to forward a fully executed (signed) Informed Consent Form to St. Jude.

4.0 TREATMENT PLAN

4.1 Overview of Therapy

Phase	Treatment
Induction (~42 days)	ITMHA 1, 4*, 8, 11*,15, 22 (*CNS2/3) with leucovorin rescue
	Dexamethasone 10mg/m^2/day divided BID PO/NG/IV Days 1-4, 8-11, 15-18
	Vorinostat see dose escalation plan PO/NG Days 1-4, 8-11, 15-18
	Bortezomib 0.043mg/kg/dose IV Days 1, 4, 8, 11, 15, 18
	PEG-asparaginase 2500units/m^2/dose IV Day 5
	Mitoxantrone 0.27mg/kg/dose IV Days 8, 9
	Cyclophosphamide 300mg/m^2/dose IV Q12 hours x 4 doses Days 22-23
	Cytarabine 75mg/m^2 IV Days 23-26, 30-33
	6MP 50mg/m^2 PO/NG Days 22-35
Consolidation (~56 days)	ITMHA Days 1, 15, 29, 43
	HDMtx 5g/m^2 IV (targeted to 65uM) Days 1, 15, 29, 43
	6MP 25mg/m^2 PO/NG Days 1-56
Reinduction (~36 days)	IT MHA Day 1, 15* (*CNS3)
	Mitoxantrone 0.27mg/kg/dose IV Days 1, 2
	PEG-asparaginase 2500units/m^2/dose IV Days 3, 18
	Dexamethasone 10mg/m^2/day divided BID PO/NG/IV Days 1-4, 8-11, 15-18
	Bortezomib 0.043mg/kg/dose IV Days 1, 4, 8, 11, 15, 18
	Vorinostat see dose escalation plan PO/NG Days 1-4, 8-11, 15-18
Reintensification (~28 days) If MRD>0.01% on Reinduction Day 1 OR increasing MRD during Consolidation	ITMHA Day 5
	Dexamethasone 20mg/m^2/day divided BID PO/NG/IV Days 1-5
	Cytarabine 2g/m^2/dose Q12H x 4 doses Days 1, 2
	Etoposide 100mg/m^2 Days 3-5 (see dosing table 4.5.3)
	PEG-asparaginase 2500units/m^2/dose IV Day 6
Maintenance (20 cycles, 28 days each)	ITMHA Day 1* (*cycles 1-8 for CNS1; cycles 1-11 CNS2; cycles 1-13 for CNS3)
	Decadron 6mg/m^2/day divided BID PO/NG/IV Days 1-5
	VCR 0.05mg/kg/dose IV Day 1
	6MP 50mg/m^2 PO/NG Days 1-28
	Methotrexate 40mg/m^2/dose IV/IM/PO Days 1*, 8, 15, 22 (*cycles with no IT MHA only)

Weight and height measurements for dosing calculations should be done with each phase of therapy for the purpose of chemotherapy dosing calculations during that cycle of chemotherapy. Every effort should be made to record accurate measurements as small errors can result in significant dosing changes for patients less than 10kg. Within each phase, no dose adjustment is needed if changes in the patient's BSA/dosing weight results in a change of less than 15% in the

drug dose. For patients that have a significant change in weight during a chemotherapy cycle requiring new dosing calculations, the new BSA/dosing weight should be double checked by another individual to verify accuracy.

The timing and duration for administration for all agents are provided in the treatment phase sections as guidelines only. Variations in the timing and duration of chemotherapy infusions according to institutional practice or variations based on patient care needs are acceptable, as long as the treating investigator and/or PI determines that there was no impact on patient safety. These variations will not be considered protocol deviations, as long as the total dose is given within 10% of protocol specified dose calculated at the beginning of the treatment phase.

Complications during treatment may result in chemotherapy doses and/or procedures being held or delayed. These variations based on patient care needs are acceptable and will not be considered protocol deviations. Please contact the principal investigator for any questions regarding chemotherapy drug delays or omissions based on the clinical status of a patient.

Efforts should be made to perform all procedures on the days specified in the protocol. Modifications due to scheduling difficulties, weekends and holidays, or the clinical status of a patient are acceptable and not considered protocol deviations.

4.2 Remission Induction

4.2.1 Remission Induction Intrathecal Chemotherapy

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

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

- All patients will receive triple intrathecal treatment on **days 1, 8, 15 and 22**.
- Patients with any of the following features will receive additional triple intrathecal treatment on **days 4 and 11**:
    - CNS3 status (i.e., ≥5 WBC/μL of CSF with blasts or cranial nerve palsy)
    - CNS2 status (<5 WBC/μL of CSF with blasts)
    - Traumatic LP (≥10 RBC/μL of CSF with blasts)

Dose throughout the treatment protocol is based on age, as follows:

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

**Centers may use their institutional standard volumes for intrathecal chemotherapy provided the methotrexate, hydrocortisone, and cytarabine doses are as specified in the table.*

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

Intraventricular chemotherapy via Ommaya catheter may be used in place of intrathecal therapy delivered by LP. Intraventricular chemotherapy should be given according to the same schedule, but at **50% of the corresponding age-based doses for methotrexate and cytarabine** that would be given by LP. Hydrocortisone dosing does not require adjustment. We recommend using a total volume of 2 mL for patients less than 35 months and 3 mL for 36 months or greater for chemotherapy administered via Ommaya catheter.

4.2.1 Dose Escalation Cohort

Vorinostat, initially at 100mg/m^2/day will be administered on days 1-4, 8-11, and 15-18 and escalated based on tolerability. A classical 3-on-3 dose escalation will be done as a run-in phase. Three patients will be enrolled sequentially at the 100mg/m^2/day level. At any of the first two dose levels (100mg/m^2 or 150mg/m^2), if no dose limiting toxicity (DLT) is observed then we will move on to the next higher does level; if there is 1 DLT then we will enroll 3 more patients at the same dose level, if there is no more DLTs we will then move to the next higher dose level, otherwise deem the current dose level too toxic and use the dosage at one level lower as the maximum tolerated dose (MTD). If at any dose level 2 or more DLTs occur, we will deem the current dose level too toxic and use the dosage at one level lower as the MTD. If the 100mg/m^2 level is deemed intolerable (2/3 or 2/6 DLTs) then we move to dose level -1. If we escalate the dose to the highest dose level at 180mg/m^2 and it is tolerated (0/3 or 1/6 DLT), then this dosage will be used for the remaining patients as the MTD. Patients that are part of the dose escalation cohort will receive the same vorinostat dosing for induction and reinduction.

Dose Level	**Vorinostat Dosing**
-1	75mg/m^2/day
1*	100mg/m^2/day
2	150mg/m^2/day
3	180mg/m^2/day

*starting dose level

4.2.1.1 Definitions of Dose-Limiting Toxicities (DLT)

Toxicities will be graded according the CTEP Common Terminology Criteria for Adverse Events (CTCAE) version 4.0. DLT is based on non-hematologic toxicities that occur during induction (planned for 42 days). All three subjects in a cohort must complete the DLT monitoring phase before accrual may resume during the dose escalation phase. Please note elective intubation for procedures and as a precaution in infants with hyperleukocytosis will not be considered a DLT.

Non-Hematologic DLT

- Any Grade 5 event, unless the event is clearly and incontrovertibly due to extraneous causes or disease progression
- Any Grade 4 event , unless the event is clearly and incontrovertibly due to extraneous causes or disease progression with the exception of the following:
    - Grade 4 infection or fever
    - Grade 4 elevation in hepatic transaminases, alkaline phosphatase, GGT, bilirubin, amylase, lipase, and triglycerides
    - Grade 4 electrolyte disturbances that resolve to < grade 3 within 24 hours.
    - Grade 4 electrolyte disturbances due to tumor lysis
    - Grade 4 hyperglycemia or hypoglycemia that resolve to < grade 3 within 24 hours
    - Grade 4 intracranial hemorrhage secondary to hyperleukocytosis
    - Grade 4 pulmonary leukostasis syndrome
    - Grade 4 acute kidney injury due to tumor lysis
    - Grade 4 seizures secondary to intrathecal chemotherapy
    - Grade 4 leukoencephalopathy secondary to intrathecal chemotherapy

4.2.2 Remission Induction Chemotherapy

Agent	Dosage	Route	Days	# Doses
Mitoxantrone (MITO)	0.27 mg/kg/dose	IV	Days 8, 9	2
PEG-asparaginase (PEG-ASP)	2500 international units/m^2/dose	IV*	Day 5	1
Dexamethasone (DEX)	10 mg/m^2/day divided BID	PO/NG (may give IV)	Days 1-4,8-11,15-18	24
Bortezomib (BORT)	0.043 mg/kg/dose	IV (may give subQ)	Days 1,4,8,11,15,18	6
Vorinostat (VORINO)	see dose escalation plan	PO/NG	Days 1-4,8-11,15-18	12
Cyclophosphamide (CYCLO)	300 mg/m^2/dose	IV Q12H	Days 22-23	4
Cytarabine (ARAC)	75 mg/m^2/dose	IV	Days 23-26,30-33	8
Mercaptopurine (6MP)	50 mg/m^2	PO/NG	22-35	14

** Intravenous is the preferred method of PEG-asparaginase administration as anaphylactic reactions are extremely rare in infants and intramuscular injection can lead to hematomas. See Section 5.4 for intramuscular administration, if applicable.*

Bone marrow exam will be performed at diagnosis, Day 22 and at end of Remission Induction upon count recovery with day 1 Consolidation procedures. Patients with residual leukemia identified on Day 22 should receive cyclophosphamide, mercaptopurine, and cytarabine as scheduled if their clinical condition permits, regardless of their ANC. For other patients, the treatment may be delayed for 3 to 7 days to allow some degree of hematopoietic recovery if APC (ANC + monocyte) <300/mm^3. When possible, specimens should be shipped overnight Sunday-Thursday such that they are received by St. Jude Monday-Friday. If shipping on Friday or Saturday, please notify the study PI so that personnel can be called in to process the specimen on weekends and holidays. See section 8.2 for acceptable alternate timing of bone marrow examinations.

4.2.2.1 Dose modifications for infants < 30 days of age or < 3 months of age and significantly premature, as defined by born at less than 35 weeks gestation.

- Mitoxantrone and PEG-asparaginase will be at 50% dosing.
- Cyclophosphamide will be at 50% dosing for patients less than 30 days of age at the time cyclophosphamide is due, or for infants that are less than 3 months of age that are significantly premature.

4.2.2.2 Cyclophosphamide administration

Patients should receive D5 0.45% NaCl at 2250 ml/m^2/24 hours (=94ml/m^2/hr) on days 22-23 during cyclophosphamide administration. Hydration should be initiated 2 hours

prior to the first dose of cyclophosphamide and continued 12 hours after the final dose of cyclophosphamide. There is no need to measure urine specific gravity.

Cyclophosphamide doses should be administered over 30 minutes. For patients with microscopic hematuria (or a history of microscopic hematuria), mesna may be given at 20% of the cyclophosphamide dose immediately before (or mixed with) cyclophosphamide infusion, and again 4 and 8 hours after the start of the infusion. Alternatively, 60% of the cyclophosphamide dose may be given as an 8 hour continuous infusion beginning when cyclophosphamide infusion starts.

4.2.2.3 Vorinostat liquid administration

A suspension can be prepared locally by mixing 20 mL of OraPlus with the contents of twenty 100 mg vorinostat capsules in a 4 ounce glass bottle. After shaking for up to 3 minutes to disperse, add 20 mL of OraSweet. Shake the container to disperse, resulting in a final concentration of 50 mg/mL. The suspension should be stored at room temperature for a maximum of 4 weeks. Smaller volumes can be prepared to avoid wasting capsules. If the calculated dose can be accurately dispensed, then this dose should be administered. If the calculated dose cannot be accurately dispensed, then the dose may be rounded to the nearest 5 mg (0.1 mL) provided it is within 10% of the calculated dose. See also Appendix III.

4.2.2.4 Treatment modifications

Patients who have hyper leukocytosis, evidence of liver dysfunction at diagnosis secondary to leukemia infiltration, or are unable to initiate treatment for other clinical reasons may begin with steroid monotherapy until they are clinically stable to begin induction. The choice of steroid, dose, and duration of monotherapy are at the discretion of the treating physician and study team. For patients in which the treating physician is initiating steroid monotherapy, the principal and co-principal investigators should be notified of the reasons and rationale within 24 hours. Induction dexamethasone dosing will not be omitted in these patients, they will receive the full dosing schedule, e.g. this steroid administration will be in addition to induction chemotherapy.

See Section 6.0 for treatment modifications for mitoxantrone, bortezomib and PEG-asparaginase.

4.2.2.5 Supportive care requirements:

- Inpatient during Induction until signs of count recovery with a minimum ANC of 200. We recommend an ANC of at least 300 for two consecutive days for discharge criteria, however this is at the discretion of the treating physician and therefore small differences in ANC values at the time of discharge will not be considered protocol deviations or violations.
- IVIG for patients that have total immunoglobulin level of less than 400 mg/dL

- Micafungin at treatment dosing (4mg/kg IV Q24 hours) or caspofungin at treatment dosing (see section 10.10) and cefepime at prophylactic dosing (50mg/kg IV Q12 hours) for all patients until APC ≥500/mm^3 for two consecutive days
- Patients unable to receive cefepime due to drug shortage, allergy, or other cause should receive an alternative antibiotic with similar bacterial coverage. Patients who are discharged with an ANC of >200 but less than 500 may be sent out on oral levofloxacin without fungal coverage to facilitate a feasible outpatient regimen.
- Synagis prophylaxis during RSV season
- Patients with mucositis should be evaluated for herpes simplex infection and treated with acyclovir or famciclovir if work-up is positive
- PCP prophylaxis with TMP/SMZ should begin on day 15 of induction therapy and continue until the patient is 3 months off therapy. In patients who are allergic or who demonstrate significant cytopenias secondary to TMP/SMZ, intravenous pentamidine every 4 weeks may be substituted. Patients less than 2 months of age should receive pentamidine every 4 weeks as TMP/SMZ is contraindicated.
- GM-CSF and/or G-CSF should not be administered to aid in count recovery. Growth factor administration is reserved for patients with severe infections that are not predicted to recover in the near future. Please contact the PI for approval prior to initiating.

4.2.2.6 End of Induction Response:

A bone marrow aspirate will be performed at the end of remission induction upon count recovery with the day 1 consolidation LP. If the date falls on a Friday, week-end or holiday such that the bone marrow specimen will not be received by St. Jude Monday-Friday, the procedure may be performed on closest working day that allows the specimen to be received during the work week. MRD level will be determined on this bone marrow sample.

4.3 Consolidation Chemotherapy

Agent	Dosage	Route	Days	# Doses
High-dose Methotrexate (HDMTX)	5g/m^2 IV (targeted to 65uM)	IV	Days 1, 15, 29, 43	4
Mercaptopurine (6MP)	25 mg/m^2	PO/NG	1-56	56
ITMHA	See section 4.2.1	IT	Days 1, 15, 29, 43	4

4.3.1 Criteria for Starting Consolidation

When ANC ≥500/mm^3, WBC ≥1000/mm^3, and platelet count ≥50 x 10^9/L, consolidation treatment will be started, comprised of high dose methotrexate (every other week for 4 doses), daily mercaptopurine and IT chemotherapy on the same dates of high dose methotrexate (see section 5.3.4).

4.3.2 Mercaptopurine Administration

Mercaptopurine should be taken daily at approximately the same time. Due to recent data that commonly-practiced restrictions surrounding 6MP ingestion (i.e., taking medication only in the evening without food/dairy) might not influence outcome and the difficulty in withholding feeds in infants, these restrictive practices are not required.[41] In patients for whom high dose methotrexate treatment is delayed, mercaptopurine may be continued until 14 days after the last course of high dose methotrexate. Mercaptopurine may be held in the presence of ANC <500/mm^3, WBC <1,000/mm^3, platelet count <50 x 10^9/L or grade 3 or 4 mucositis. Mercaptopurine may be resumed once mucositis has resolved. Dosage of mercaptopurine in subsequent courses may be reduced to 12.5 mg/m^2/day in patients who have prolonged neutropenia after high dose methotrexate and mercaptopurine treatment.

4.3.3 High-Dose Methotrexate (HDMTX) Administration

High-dose methotrexate (HDMTX) administration: Patients will receive 5 gm/m^2, (or a dose targeted to achieve a steady-state plasma concentration of 65 uM) administered over 24 hr intravenously. The subsequent courses of high dose methotrexate and intrathecal treatment will be delayed if ANC <500/mm^3, WBC < 1000/mm^3, platelet count < 50 x 10^9/L, SGPT >500 U/L, total bilirubin >2 mg/dl and direct bilirubin >1.4 mg/dl, or mucositis is present. Sodium bicarbonate may be given orally at 1 gm/m^2 every 6 hours or intravenously with pre-hydration fluid starting the day before high dose methotrexate. Methotrexate levels will be drawn at 6, 23, 42, and every 24 hours thereafter from the start of infusion until the level is <0.1 uM. Following each course, methotrexate levels will be evaluated by a St. Jude clinical pharmacist and a recommended dose will be given for the subsequent course to achieve the targeted concentration. Methotrexate levels, associated lab values, leucovorin dosing and adverse events during consolidation will be maintained in a targeting database within the department of pharmaceutical sciences at St. Jude Children's Research Hospital for the purpose of exploratory objective 1.3.9.
Pre-hydration: At least two hours before high dose methotrexate, prehydration IV fluid (D5W + 40 mEq NaHCO3/L + 20 mEq KCl/L) will be administered at the rate of 200 ml/m^2/hr. At start of prehydration, one IV dose of NaHCO3 (unless otherwise clinically indicated, 25 mEq/m^2) will be given. Prehydration fluid may also be given overnight at a rate of at least 125 ml/m^2/hr. High dose methotrexate treatment will follow, provided that urinary pH is >/=6.5; exceptions must be cleared with the pharmacokinetics service and the attending physician.

High-dose methotrexate infusion: A 10% methotrexate loading dose will be given over 1 hour, followed immediately by maintenance infusion over 23 hours of the remaining 90%. During the methotrexate infusion, patients should receive hydration fluid with D5W + 40 mEq/L $NaHCO_3$ + 20 mEq KCl/L at 100-150 ml/m^2/hr. Urine pH will be monitored with each void during infusion. An IV bolus of 12 mEq/m^2 $NaHCO_3$ will be given if urine pH is 6.0; and 25 mEq/m^2 will be given if urine pH is <6.0. Acetazolamide 500 mg/m^2 orally every 6 to 8 hours may be used if systemic alkalosis limits the administration of bicarbonate for urinary alkalinization. Patients with evidence of renal

dysfunction or delayed clearance during the methotrexate infusion may receive less than a 24 hour methotrexate infusion.

Leucovorin rescue: Leucovorin, 15 mg/m^2 (IV or PO) will be started at 42 hours after the start of methotrexate and repeated every 6 hours for a total of three doses, as described Reiter, et al.[30] The dosage of leucovorin will be increased in patients with high plasma methotrexate concentrations (>1.0 μM at 42 hours) and continued until the methotrexate concentration is less than 0.10 μM. Additional measures, such as hydration, hemoperfusion, or glucarpidase will be considered in patients with 42-hour methotrexate levels > 10μM.

Patients with a history of delayed Grade 3 or 4 gastrointestinal toxicity with prior methotrexate or a history of typhlitis with any chemotherapy, should have leucovorin continue for a minimum of 5, rather than 3 doses; those with early toxicity should have leucovorin begin at 36 hours with subsequent methotrexate and receive a minimum of 6 rather than 3 doses; if toxicity recurs, the baseline leucovorin dosage should also be increased.

Leucovorin Dosing[1,2]

42 Hour MTX Level	Leucovorin Rescue
≤1 μM	15mg/m^2 q6h x 3 doses.
1.01-9.9 μM	15mg/m^2 q6h until MTX level <0.1μM.
10-39.9 μM	15mg/m^2 q3h until MTX level <0.1μM. Consider glucarpidase[2].
40-200 μM	100mg/m^2 q6h until MTX level <0.1μM. Consider glucarpidase[2].
>200 μM	1000mg/m^2 q6h until MTX level <0.1μM. Consider glucarpidase[3].

[1]If the 42 hour level is high, but then the patient "catches up" and the level falls to the expected value of <0.4 μM at 48 hours resume standard leucovorin and hydration as long as urine output remains satisfactory.
[2]Doses of LV may be increased outside of this table at the recommendation of the primary MD based on the clinical status of the patient.
[3]For patients who have markedly delayed MTX clearance secondary to renal dysfunction, consider using glucarpidase (carboxypeptidase G2, Voraxaze®). Glucarpidase is a commercially available agent designed to treat patients with excessive methotrexate levels and renal dysfunction. To obtain supplies of glucarpidase or for related questions, in the US, contact ASD Healthcare or Voraxaze® Customer Service at +1.855.7.VORAXAZE (+1.855.786.7292); available 24 hours/day, operating 365 days/year.

4.3.4 Intrathecal Chemotherapy

All patients will receive triple intrathecal therapy every other week for four doses on Days 1, 15, 29, and 43 (dosages are based upon age, according to Section 4.2.1). The intrathecal treatment should be given on the same day of the high dose methotrexate administration. Consult the PI or Pharmacokinetics if the IT and high dose methotrexate become separated by more than 12 hours.

4.3.5 Bone Marrow Examination

Patients that are MRD positive following induction will have bone marrow exams with each intrathecal/high dose methotrexate course. When possible, specimens should be shipped overnight Sunday-Thursday such that they are received by St. Jude Monday-Friday. If shipping on Friday or Saturday, please notify the study PI so that personnel can be called in to process the specimen on weekends and holidays. See section 8.2 for acceptable alternate timing of bone marrow examinations. For patients that do not have a decrease in MRD levels during consolidation, the phase may be aborted and patients may proceed to reinduction provided they are clinically stable (see section 7.0).

4.3.6 Interim Continuation Treatment

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

Interim treatment will consist of oral mercaptopurine 50 mg/m^2 per day (25 mg/m^2 per day in those with ANC<500/mm^3) and intravenous methotrexate 40 mg/m^2 per week; intrathecal therapy may be given every other week during this period of time and continued during the subsequent high dose methotrexate treatment for a total of 4 doses. High dose methotrexate will be started when the patient's physical condition allows. In the event that the interim therapy is longer than 4 weeks, an extra intrathecal therapy may be given with the last course of high dose methotrexate. Patients with a defective TPMT status may receive lower doses of mercaptopurine.

4.4 Re-Induction Therapy

Agent	Dosage	Route	Days	# Doses
Mitoxantrone (MITO)	0.27mg/kg/dose	IV	1, 2	2
PEG-asparaginase (PEG-ASP)	2500 international units/dose	IV	3, 18	2
Dexamethasone (DEX)	10 mg/m^2/day	PO/NG divided BID (may give IV)	1-4, 8-11, 15-18	24
Bortezomib (BORT)	0.043 mg/kg/dose	IV	1, 4, 8, 11, 15, 18	6
Vorinostat (VORINO)	see dose escalation plan	PO/NG	1-4, 8-11, 15-18	12
ITMHA	See section 4.2.1	IT	1, 15*	1 (*2 for CNS3 or traumatic tap at diagnosis)

4.4.1 Criteria for Starting Reinduction

Reinduction treatment begins after the completion of consolidation, provided that the ANC $\geq$500/mm^3, WBC $\geq$1000/mm^3, and platelet count $\geq$50 x 10^9/L as well as no evidence of grade 3 or 4 mucositis.

4.4.2 Bone Marrow Examination

A bone marrow will be done following count recovery prior to initiation of reinduction chemotherapy. When possible, specimens should be shipped overnight Sunday-Thursday such that they are received by St. Jude Monday-Friday. If shipping on Friday or Saturday, please notify the study PI so that personnel can be called in to process the specimen on weekends and holidays. See section 8.2 for acceptable alternate timing of bone marrow examinations.

4.4.3 Dose Modifications

Patients >1 year of age at the time of Re-Induction AND >10kg will receive mitoxantrone at 8mg/m^2/dose and bortezomib at 1.3mg/m^2/dose.

4.4.4 Treatment Modifications

See section 6.0 for treatment modifications for mitoxantrone, bortezomib, and PEG-asparaginase.

4.4.5 Intrathecal Treatment

Day 1 Intrathecal treatment will be followed by leucovorin rescue (5mg/m^2/dose PO or IV, max 5mg) at 24 and 30 hours only in patients with prior CNS toxicities or in patients with WBC <1500/mm^3 or ANC <500/mm^3. CNS3 patients will receive an additional dose of intrathecal chemotherapy on day 15. Day 15 intrathecal treatment **will** be followed by leucovorin rescue (5mg/m^2/dose PO or IV, max 5mg) at 24 and 30 hours as patients will be neutropenic at this time. See section 4.2.1 for dosing.

4.4.6 Supportive Care Requirements

- Inpatient during Reinduction until signs of count recovery with a minimum ANC of 200. We recommend an ANC of at least 300 for two consecutive days for discharge criteria, however this is at the discretion of the treating physician and therefore small differences in ANC values at the time of discharge will not be considered protocol deviations or violations.
- IVIG for patients that have total immunoglobulin level of less than 400 mg/dL
- Micafungin at treatment dosing (4mg/kg IV Q24 hours) or caspofungin at treatment dosing (see section 10.10) and cefepime at prophylactic dosing (50mg/kg IV Q12 hours) for all patients until APC $\geq$500/mm^3 for two consecutive days.

- Patients unable to receive cefepime due to drug shortage, allergy, or other cause should receive an alternative antibiotic with similar bacterial coverage. Patients who are discharged with an ANC of >200 but less than 500 may be sent out on oral levofloxacin without fungal coverage to facilitate a feasible outpatient regimen.
- Synagis prophylaxis during RSV season.
- Patients with mucositis should be evaluated for herpes simplex infection and treated with acyclovir or famciclovir if work-up is positive.
- GM-CSF and/or G-CSF should not be administered to aid in count recovery. Growth factor administration is reserved for patients with severe infections that are not predicted to recover in the near future. Please contact the PI for approval prior to initiating.

4.5 Re-Intensification Therapy

Agent	Dosage	Route	Days	# Doses
Cytarabine (ARAC)	2g/m^2/dose Q12H	IV	1, 2	4
Etoposide (VP16)	100 mg/m^2/dose*	IV	3-5	3
Dexamethasone (DEX)	20 mg/m^2/day	PO/NG divided BID (may give IV)	1-5	10
PEG-asparaginase (PEG-ASP)	2500 international units/dose	IV	6	1
ITMHA	See section 4.2.1	IT	5	1

*See dosing table for etoposide, Section 4.5.3.

4.5.1 Criteria for Starting Reintensification

Patients with MRD >0.01% on day 1 of Reinduction, OR patients with increasing MRD during Consolidation are considered upfront refractory and at high risk for disease progression. Patients that achieve MRD negative status following reinduction may proceed directly to stem cell transplant. Patients that remain MRD positive are eligible for CAR T cells if available, or they may undergo a Reintensification phase following Reinduction and then proceed to hematopoietic stem cell transplant in first remission due to their high risk of relapse.

Re-Intensification Treatment may begin after the completion of Reinduction, provided that the ANC ≥500/mm^3, WBC ≥1000/mm^3, and platelet count ≥50 x 10^9/L.

4.5.2 Bone Marrow Examination

A bone marrow will be done following count recovery prior to initiation of Reintensification chemotherapy. When possible, specimens should be shipped overnight Sunday-Thursday such that they are received by St. Jude Monday-Friday. If shipping on Friday or Saturday, please notify the study PI so that personnel can be called in to process

the specimen on weekends and holidays. See section 8.2 for acceptable alternate timing of bone marrow examinations.

4.5.3 Intrathecal Treatment

Intrathecal treatment will be followed by leucovorin rescue (5mg/m^2/dose PO or IV, max 5mg) at 24 and 30 hours only in patients with prior CNS toxicities or in patients with WBC <1500/mm^3 or ANC <500/mm^3. See section 4.2.1 for dosing.

4.5.3 Etoposide Administration

- All intravenous doses should be given over 1-2 hours (maximum rate 100 mg/sq.m/hour)
- If infusion-related reaction occurs, may prolong infusion to 2-4 hours or substitute etoposide phosphate (Etoposphos™) and infuse it over 1-2 hours.
- Patients with a body surface area < 0.6 m^2 should receive standardized dosing as follows:

Standardized etoposide dosing

BSA (m^2)	**Dose (mg)**
0.2-0.24	12
0.25-0.3	20
0.31-0.34	24
0.35-0.4	28
0.41-0.44	36
0.45-0.5	44
0.51-0.54	52
0.55-0.59	56
≥0.6	100/m^2

4.5.4 Treatment Modifications

See section 6.0 for treatment modifications for PEG-asparaginase, etoposide, and cytarabine.

4.5.5 Supportive Care Requirements

- Inpatient during Reintensification until signs of count recovery with a minimum ANC of 200. We recommend an ANC of at least 300 for two consecutive days for discharge criteria, however this is at the discretion of the treating physician and therefore small differences in ANC values at the time of discharge will not be considered protocol deviations or violations.
- IVIG for patients that have total immunoglobulin level of less than 400 mg/dL
- Micafungin at treatment dosing (4mg/kg IV Q24 hours) or caspofungin at treatment dosing (see section 10.10) and cefepime at prophylactic dosing (50mg/kg IV Q12 hours) for all patients until APC ≥ 500/mm^3 for two consecutive days.
- Patients unable to receive cefepime due to drug shortage, allergy, or other cause should receive an alternative antibiotic with similar bacterial coverage. Patients that

are discharged with an ANC of >200 but less than 500 may be sent out on oral levofloxacin without fungal coverage to facilitate a feasible outpatient regimen.
- Synagis prophylaxis during RSV season
- Patients with mucositis should be evaluated for herpes simplex infection and treated with acyclovir or famciclovir if work-up is positive.
- GM-CSF and/or G-CSF should not be administered to aid in count recovery. Growth factor administration is reserved for patients with severe infections that are not predicted to recover in the near future. Please contact the PI for approval prior to initiating.

4.6 Maintenance Chemotherapy

20 Cycles; One cycle = 28 days

Agent	Dosage	Route	Days	# Doses per cycle
Dexamethasone (DEX)	6 mg/m^2/day	PO/NG divided BID (may give IV)	1-5	10
VinCRIStine (VCR)	1.5 mg/m^2 (max 2 mg) for patients > 1 year and weighing ≥10 kg, **or** 0.05 mg/kg for patients < 1 year or weighing <10 kg	IV	1	1
Mercaptopurine (6MP)	50 mg/m^2	PO/NG	1-28	28
Methotrexate (MTX)	40 mg/m^2	IV/IM/PO	1*,8,15,22	3 (4*)
ITMHA	See section 4.6.2	IT	1	1

*Methotrexate will be given on day 1 of cycles that do not have an intrathecal treatment. For CNS1 patients this is cycles 9-20, CNS2 patients cycles 12-20, and CNS3 or traumatic LP cycles 14-20.

4.6.1 Criteria for Starting Maintenance

Treatment begins after the completion of Re-Induction, provided that the ANC ≥500/mm^3, WBC ≥ 1000/mm^3 and platelet count ≥ 50 x 10^9/L as well as no evidence of grade 3 or 4 mucositis.

4.6.2 Intrathecal Treatment

Patients will receive intrathecal treatments without leucovorin rescue during maintenance provided they are not neutropenic. Patients that are neutropenic should have the intrathecal treatment delayed until counts recover.

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

- Patients with CNS1 status at diagnosis will receive triple intrathecal treatment on day 1 of **cycles 1-8**.
- Patients with CNS2 status at diagnosis will receive triple intrathecal treatment on day 1 of **cycles 1-11**.
- Patients with any of the following features at diagnosis will receive triple intrathecal treatment on day 1 of **cycles 1-13**:
    - CNS3 status (i.e., ≥5 WBC/μL of CSF with blasts or cranial nerve palsy)
    - Traumatic LP (≥10 RBC/μL of CSF with blasts)

Dose is based on age, as follows:

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

**Centers may use their institutional standard volumes for intrathecal chemotherapy provided the methotrexate, hydrocortisone, and cytarabine doses are as specified in the table.*

4.6.3 Treatment Modifications

Patients > 1 year of age AND >10kg will receive vincristine at 1.5mg/m^2/dose

Dexamethasone and vincristine should be given regardless of blood counts, provided that the patient is not sick. Methotrexate and mercaptopurine will be held if ANC <300/mm^3, WBC <1000/mm^3, or platelet count <50 x 10^9/L (see section 6.0 for other dose modifications). Dosage of treatment during maintenance should be titrated to keep WBC between 1800 and 3000/mm^3, ANC between 500 and 1200/mm^3, and platelet count ≥ 50 x 10^9/L. Disproportionate dose reduction of one agent compared to another should be avoided unless clinically indicated. Frequent (e.g. weekly) changes of mercaptopurine doses should be avoided. Patients who receive mercaptopurine at a reduced dose for longer than 2 weeks should have a TGN level checked after at least 14 days of uninterrupted mercaptopurine if the plan is to continue at reduced doses to ensure therapeutic target is being achieved.

4.6.4 Dose Modifications for Inadequate Myelosuppression

Patients who miss less than 25% of therapy but have persistently (>50% of time; not counting the week after dexamethasone/vincristine) high WBC (≥3 x 10^9/L) and high ANC (≥1200 x 10^9/L) should be counseled on compliance, particularly if 6TGN levels are <100 pmol/8x10^8 RBCs. If the WBC remains high, mercaptopurine and methotrexate doses should be increased by 30% (e.g. to 65mg/m^2 and 50mg/m^2 respectively), using a stepwise approach if needed. If patients have a TPMT defect, mercaptopurine dosage should not be increased unless the TGN levels are <100 pmol/8x10^8 RBCs.

5.0 DRUG/DEVICE/BIOLOGIC AGENT INFORMATION

Dose rounding will be allowed. For chemotherapy the resultant dose must be within ±10% of the prescribed dose. For etoposide, please use standardized dosing table for patients with a body surface area of less than 0.6 m^2, for patients with a body surface area of greater than or equal to 0.6 m^2, dose rounding is allowed. For all other medications including but not limited to leucovorin and antibiotics, the resultant dose must be within ±15% of the prescribed dose.

5.1 Intrathecal Triples

(ITMHA, methotrexate/hydrocortisone/cytarabine)

Source and pharmacology**:** The intrathecal route of administration of a drug produces more consistent CSF drug concentrations at relatively smaller doses because of the volume difference between the CSF and blood compartments (140 mL vs. 3500 mL in an adult). (The CSF volume of children after the first 3 years is equivalent to that of an adult). Drug half-lives are longer as well because clearance is related to flow rather than metabolism or protein binding. Intrathecal methotrexate has a biphasic elimination curve from the CSF with a t½ of 4.5 and 14 hours respectively. Following IT injection of cytarabine the elimination of the drug from the CSF is biphasic with a t½ of 1 and 3.4 hours respectively which is 8-fold longer than the clearance from plasma. The elimination of hydrocortisone is similarly prolonged.

Formulation and stability: Methotrexate 25 mg/mL preservative free 2 mL vial or methotrexate 20 mg preservative free sterile powder for injection vial. Cytarabine 100 mg preservative free sterile powder for injection. Hydrocortisone sodium succinate100 mg vial sterile powder for injection.

Toxicity: Nausea, vomiting, fever, headache.

Guidelines for administration: Intrathecal, See Treatment Plan and Dosage Modification sections of the protocol.

Supplier: Commercially available. See individual drug package inserts for more detailed information

5.2 Dexamethasone (Decadron®, Hexadrol®, Dexone®, Dexameth®)

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.

Toxicity:

	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	Hyperuricemia
Prompt: *Within 2-3 weeks, prior to the next course*	Immunosuppression, personality changes (mood swings, euphoria, anxiety, depression), pituitary- adrenal axis suppression, acne (L)	Hyperglycemia, facial erythema, poor wound healing, infections (bacterial, fungal, parasitic, viral), edema	Pancreatitis (L), increased intraocular pressure (L), hypertension, psychosis, vertigo, headache
Delayed: *Any time later during therapy*	Cushing's syndrome (moon facies, truncal obesity)	Striae and thinning of the skin, easy bruising, muscle weakness, osteopenia	Spontaneous fractures (L), growth suppression, peptic ulcer and GI bleeding, pseudotumor cerebri (increased intracranial pressure with papilledema, headache), aseptic necrosis of the femoral and humeral heads (L), urolithiasis[1] (L)
Late: *Any time after completion of treatment*		Cataracts (may be reversible on discontinuation of dexamethasone in children)	
Unknown frequency and timing:	**Fetal and teratogenic toxicities**: dexamethasone crosses the placenta with 54% metabolized by enzymes in the placenta. In animal studies, large doses of cortisol administered early in pregnancy produced cleft palate, stillborn fetuses, and decreased fetal size. Chronic maternal ingestion during the first trimester has shown a 1% incidence of cleft palate in humans. There are no reports of dexamethasone excretion into breast milk in humans; however, it is expected due to its low molecular weight that it would partition into breast milk.		

[1] *Mainly reported in pediatric patients with ALL. Howard SC et al. Urolithiasis in pediatric patients with acute lymphoblastic leukemia. Leukemia 2003; 17: 541-6.*
(L) Toxicity may also occur later.

Formulation and stability: *Oral*: Available in 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 1 mg/1 mL concentrations. 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.

Injection: Dexamethasone Sodium Phosphate Solution for Injection is available as 4 mg/mL (1 mL, 5 mL, and 30 mL vials) and 10 mg/mL (1 mL and 10 mL vial sizes). Vials 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: Divided BID, PO/NG/IV; see Treatment and Dose Modifications section of the protocol.

Supplier: Commercially available; see package insert for more detailed information.

5.3 Mitoxantrone (Novantrone®, Mitozantrone)

Source and pharmacology: Mitoxantrone is a substituted alkylaminoanthraquinone and is a potent inhibitor of DNA and RNA synthesis *in vitro* and binds strongly to DNA. Mitoxantrone most likely acts through intercalation between base pairs of the DNA double helix causing crosslinks and strand breaks. In addition, it is a topoisomerase II inhibitor, an enzyme responsible for uncoiling and repairing damaged DNA. It has a cytocidal effect on both proliferating and non-proliferating cultured human cells, suggesting lack of cell cycle phase specificity. The drug disappears rapidly from plasma (drug found only in the 3-minute sample) and < 1% appears in the urine in 24 hours. The mean alpha half-life of mitoxantrone is 6 to 12 minutes, the mean beta half-life is 1.1 to 3.1 hours and the mean gamma (terminal or elimination) half-life is 23 to 215 hours (median approximately 75 hours). Primary excretion is biliary with 25% appearing in the feces; renal excretion accounting for only 11% of the total dose. Mitoxantrone clearance is reduced by hepatic impairment. Patients with severe hepatic dysfunction (bilirubin > 3.4 mg/dL) have an AUC more than three times greater than that of patients with normal hepatic function receiving the same dose. Mitoxantrone is approximately 95% protein bound.

Toxicity:

	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, diarrhea, fever, anorexia, green blue discoloration of the urine and/or sclera	Abdominal pain, back pain, headache, phlebitis, constipation	Anaphylaxis, angioedema, cardiac arrhythmias[1] (bradycardia), seizures, extravasation reactions rare but if occur can lead to: (erythema, swelling, pain, burning and/or blue

	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*
			discoloration of the skin and rarely tissue necrosis), tumor lysis
Prompt: *Within 2-3 weeks, prior to the next course*	Myelosuppression (L), mucositis /stomatitis, immunosuppression, alopecia, fatigue	Transient elevation of LFTs, pruritis with desquamation of the skin due to progressive dryness	Rash, conjunctivitis, (GI) hemorrhage, interstitial pneumonitis
Delayed: *Any time later during therapy*	Amenorrhea, menstrual disorders, temporary reduction in sperm count	Cardiotoxicity (decreased LVEF)[2] (L)	CHF, hepatotoxicity
Late: *Any time after completion of treatment*			Secondary malignancy
Unknown frequency and timing:	Fetal toxicities and teratogenic effects of mitoxantrone have been noted in animals. Toxicities include: low birth weight and prematurity. Mitoxantrone is excreted in human milk and significant concentrations (18 ng/mL) have been reported for 28 days after the last administration.		

[1] *Rarely clinically significant.*
[2] *Risk increases with chest radiation and prior anthracycline dosage.* ***(L) Toxicity may also occur later.***

Formulation and stability: The concentrate is a sterile, non-pyrogenic, non-preserved, dark blue aqueous solution containing mitoxantrone hydrochloride equivalent to 2 mg/mL mitoxantrone free base, with sodium chloride (0.80% w/v), sodium acetate (0.005% w/v), and acetic acid (0.046% w/v) as inactive ingredients with 0.14 mEq of sodium per mL. Mitoxantrone is provided as 20 mg (10 mL), 25 mg (12.5 mL) and 30 mg (15 mL) vials. Store intact vials at 15°-25°C (59°-77°F). Undiluted mitoxantrone injection should be stored not longer than 7 days between 15°-25°C (59°-77°F) or 14 days under refrigeration. Refrigeration of the concentrate may result in a precipitate, which redissolves on warming to room temperature. DO NOT FREEZE.

Guidelines for administration: See Treatment and Dose Modifications sections of the protocol.

Mitoxantrone must be diluted prior to injection. DO NOT GIVE IV PUSH. The dose of mitoxantrone should be diluted in at least 50 mL or to a concentration ≤ 0.5 mg/mL with either NS or D5W. The dilution is stable at room temperature for 48 hours with no loss of potency. Admixture with heparin may result in precipitation. Mitoxantrone is an irritant: Care should be taken to avoid extravasation; the use of a central line is suggested. If it is known or suspected that subcutaneous extravasation has occurred, it is recommended that intermittent ice packs be placed over the area of extravasation and that the affected extremity be elevated. Because of the progressive nature of extravasation reactions, the area of injection should be frequently examined and surgery consultation obtained early if there is any sign of a local reaction.

Supplier: Commercially available; see package insert for more detailed information.

5.4 Pegaspargase (PEG-asparaginase, Oncaspar®)

Source and pharmacology: Pegaspargase is a modified version of the enzyme L-asparaginase. L-asparaginase is modified by covalently conjugating units of monomethoxypolyethylene glycol (PEG), molecular weight of 5000, to the enzyme, forming the active ingredient PEG-L-asparaginase. The L-asparaginase (L-asparagine amidohydrolase, type EC-2, EC 3.5.1.1) used in the manufacture of Pegaspargase is derived from *Escherichia coli,* which is purchased in bulk from Merck, Sharp and Dohme. 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 (2500 IU/m^2), *E. coli* L-asparaginase (25000 IU/m^2), or *Erwinia* (25000 IU/m^2), 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.

Toxicity:

	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*	Allergic reactions (total likelihood of local, and or systemic reaction especially if previous hypersensitivity reaction to native asparaginase), pain at injection site, weakness, fatigue, diarrhea	Allergic reactions (total likelihood of local, and or systemic reaction if no previous hypersensitivity reaction to native asparaginase), rash	Anaphylaxis, hyper/hypotension, tachycardia, periorbital edema, chills, fever, dizziness, dyspnea, bronchospasm, lip edema, arthralgia, myalgia, urticaria, mild nausea/vomiting, abdominal pain, flatulence, somnolence, lethargy, headache, seizures (L), hyperuricemia
Prompt: *Within 2-3 weeks, prior to the next course*	Hyperammonemia (L), coagulation abnormalities with	Hyperglycemia, abnormal liver function tests,	Hemorrhage (L), DIC, thrombosis, anorexia, weight loss, CNS ischemic

	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*
	prolonged PTT, PT and bleeding times (secondary to decreased synthesis of fibrinogen, AT-III & other clotting factors) (L)	pancreatitis (L), increased serum lipase/amylase	attacks, edema, azotemia and decreased renal function, mild leukopenia, granulocytopenia, thrombocytopenia, pancytopenia, hemolytic anemia, infections (sepsis with/without septic shock, subacute bacterial endocarditis [SBE], URI), CNS changes including irritability, depression, confusion, EEG changes, hallucinations, coma and stupor, paresthesias, hypertriglyceridemia, hyperlipidemia, Parkinson-like syndrome with tremor and increase in muscular tone, hyperbilirubinemia, chest pain
Delayed: *Any time later during therapy*			Renal failure, urinary frequency, hemorrhagic cystitis, elevated creatinine and BUN, fatty liver deposits, hepatomegaly, liver failure
Unknown frequency and timing:	Animal reproduction studies have not been conducted with pegaspargase. It is not known whether pegaspargase can cause fetal harm when administered to a pregnant woman or can affect reproduction capacity. However, fetal toxicities and teratogenic effects of asparaginase have been noted in animals. It is unknown whether the drug is excreted in breast milk.		

(L)Toxicity may also occur later.

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 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°-8°C (36°-46°F). Do not use if stored at room temperature 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.

Guidelines for administration: See Treatment and Dose Modifications sections of the protocol. Intravenous is the preferred method of administration as anaphylactic reactions

are extremely rare in infants and intramuscular injection can lead to hematomas. For I.M. administration, limit the volume at a single injection site to 2 mL; if the volume to be administered is >2 mL, use multiple injection sites I.V. infusion in 100 mL of D_5W or NS through an infusion that is already running (can be TKO).

If the patient weighs ≥ 10 kg infuse PEG-asparaginase over 1 hour.
If the patient weighs < 10 kg infuse PEG-asparaginase over 2 hours.

Supplier: Commercially available; see package insert for more detailed information.

5.5 Asparaginase Erwinia Chrysanthemi (*Erwinia chrysanthemi*, Erwinase®, Erwinaze™, Crisantaspase)

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

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. Neoplastic cells associated with acute lymphoblastic leukemia, acute myeloid leukemia and lymphoblastic lymphosarcoma are asparagine-dependent but lack asparagine synthetase activity. The administration of L-asparaginase produces an anti-neoplastic effect by catalyzing asparagine into aspartic acid and ammonia. As a result, these cells lack the ability to produce the asparagine necessary for protein metabolism and survival. Deamination of glutamine may also play a role in the antineoplastic activity of asparaginase.

Asparaginase *Erwinia chrysanthemi* (Erwinaze™) is asparaginase derived from cultures of *Erwinia chrysanthemi*. L-asparaginase is a tetrameric enzyme; each of the four identical subunits has a molecular weight of approximately 35 kDa. Asparaginase *Erwinia chrysanthemi* is immunologically distinct from *E. coli* L-asparaginase and may allow continued asparaginase therapy when a hypersensitivity reaction occurs to *Escherichia coli*-derived asparaginase. The package labeling states that there is insufficient information to characterize the incidence of antibodies to asparaginase *Erwinia chrysanthemi*. Several factors are involved in immunogenicity assay results and the assessment of antibodies, including assay methodology, assay sensitivity and specificity, sample handling, timing of sample collection, concomitant medications, and the underlying disease state. The following data have been reported on each of the three preparations of asparaginase:

Clinical Pharmacology of Asparaginase Formulation	Elimination half-life (IM)	% Anti-Asparaginase Antibody positive patients
Native *Escherichia Coli*	26-30 hours	45-75
Pegylated-asparaginase	5.5-7 days	5-18
Erwinia Asparaginase	16 hours (7-13 hrs package insert)	30-50

From: Avramis, V; Panosyan, E; Pharmacokinetic/Pharmacodynamic Relationships of Asparaginase Formulations: The Past, the Present and Recommendations for the Future. Clin Pharmacokinet 2005; 44 (4): 367-393.

Effective asparaginase levels have been defined as activity of ≥ 0.1 International Units per mL. Clinical trials with asparaginase *Erwinia chrysanthemi* demonstrated that 100% of patients achieved effective asparaginase levels at 48 and 72 hours (n=35 and n=13, respectively) following the third total dose when given on a Monday, Wednesday, Friday schedule. No formal drug interaction studies have been performed with asparaginase *Erwinia chrysanthemi*.

Toxicity

	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*		Allergic reactions, anaphylaxis, urticaria	Local injection site reactions, fever
Prompt: *Within 2-3 weeks, prior to the next course*			Pancreatitis, glucose intolerance, thrombosis, hemorrhage, transient ischemic attack, disseminated intravascular coagulation, hyperbilirubinemia, alanine aminotransferase increased, aspartate aminotransferase increased, hyperglycemia, hyperammonemia, vomiting, nausea, abdominal pain, headache, diarrhea, seizure
Unknown frequency and timing:	Fetal toxicities and teratogenic effects of L-asparaginase have been noted in animals. It is unknown whether the drug is excreted in breast milk. Adequate, well-controlled studies of asparaginase *Erwinia chrysanthemi* have NOT been conducted. It is not known whether asparaginase *Erwinia chrysanthemi* will cause fetal harm or affect the ability to reproduce. It is not known if asparaginase *Erwinia chrysanthemi* is excreted into breast milk. The use of asparaginase *Erwinia chrysanthemi* should be avoided in pregnant or lactating patients.		

(L) Toxicity may also occur later.

Formulation and stability: Asparaginase *Erwinia chrysanthemi* is supplied as a sterile, white lyophilized powder for reconstitution in a clear glass vial with a 3 mL capacity. Each vial contains 10,000 International Units of asparaginase *Erwinia chrysanthemi* and the following inactive ingredients: glucose monohydrate (5.0 mg), sodium chloride (0.5 mg). Store intact vials between 2°C and 8°C (36° to 46ºF). Protect from light.

Guidelines for administration: See Treatment and Dose Modification sections of the protocol. Intravenous (preferred) or intramuscular.

Use appropriate precautions for preparation of a hazardous agent. Visually inspect the powder in vial for foreign particles or discoloration prior to reconstitution. The contents of each vial should be reconstituted by slowly adding 1 mL or 2 mL of sterile, preservative-free NS to the inner vial wall. The final concentration is 10,000 International Units per mL when using 1 mL for reconstitution or 5,000 International Units per mL when using 2 mL for reconstitution. Gently mix or swirl the contents to dissolve the contents of the vial. Do not shake or invert the vial. The resulting solution should be clear

and colorless. Discard if any particulate matter or protein aggregates are visible. Withdraw the appropriate dosing volume into a polypropylene syringe within 15 minutes of reconstitution. Polycarbonate luer-lok syringes from B-D (1 mL) are also acceptable (personal communication, EUSA Pharma). Discard any unused drug; do not save or use any unused drug remaining the in the vial.

Administer the dose within a 4 hour time period from reconstitution. If the dose is not used within this time period, discard the dose. Do not freeze or refrigerate the reconstituted solution. If administering the dose IM, no more than 2 mL should be given at any one injection site. Doses larger than 2 mL should be divided and given in separate administration sites.

Supplier: Commercially available; see package insert for more detailed information.

5.6 Bortezomib (Velcade®, PS-341, MLN341, LDP-341) IND# 127,270

Source and pharmacology: Bortezomib (PS-341) is a reversible inhibitor of the chymotrypsin-like activity of the 26S proteasome (a multicatalytic protease present in all eukaryotic cells). The 26S proteasome is a large protein complex that degrades proteins that have been conjugated to ubiquitin. The ubiquitin-proteasome pathway plays an essential role in regulating the intracellular concentration of specific proteins, and constitutes the major mechanism for intracellular protein degradation (80%). Those intracellular proteins that maintain homeostasis within cells include numerous regulatory proteins involved in cellular integrity, such as cell cycle control, cellular apoptosis, transcription factor activation, and tumor growth via ATP-dependent processes. Inhibition of the 26S proteasome prevents this targeted proteolysis, which can affect multiple signaling cascades within the cell. This disruption of normal homeostatic mechanisms can lead to cell death.

The binding of bortezomib to human plasma proteins averages 83% over a concentration range of 100 to 1000 ng/mL. The mean elimination half-life of bortezomib after multiple dosing ranged from 40 to 193 hours after the 1 mg/m^2 dose and 76 to 108 hours after the 1.3 mg/m^2 dose. In vitro studies with human liver microsomes and human cDNA-expressed cytochrome P450 isozymes indicate that bortezomib is primarily oxidatively metabolized via cytochrome P450 enzymes 3A4, 2C19, and 1A2. Bortezomib metabolism by CYP 2D6 and 2C9 enzymes is minor. The major metabolic pathway is deboronation to form 2 deboronated metabolites that subsequently undergo hydroxylation to several metabolites. Deboronated bortezomib metabolites are inactive as 26S proteasome inhibitors.

In vitro and *in vivo* studies showed that green tea compounds, ascorbic acid (vitamin C) and other antioxidants, have the potential to significantly inhibit the activity of bortezomib. Green tea constituents, in particular epigallocatechin gallate (EGCG) and other polyphenols with 1,2-benzenediol moieties, effectively prevented tumor cell death induced by bortezomib both *in vitro* and *in vivo*. In multiple myeloma cell lines or mouse xenografts, EGCG directly reacted with bortezomib and blocked its proteasome inhibitory function. As a result, bortezomib could not trigger endoplasmic reticulum

stress or caspase-7 activation and could not induce tumor cell death. A more recent study investigated whether clinically relevant levels of EGCG or ascorbic acid could inhibit the antitumor activity of bortezomib in murine xenograft tumors. The addition of EGCG to bortezomib demonstrated no effect on tumor growth inhibition at lower concentrations of EGCG that the investigators compare to human dietary intake. Similar results were found for ascorbic acid at normal daily doses. When bortezomib was given concurrently with much higher concentrations of EGCG, the investigators found that all antitumor activity was eliminated. The authors concluded that there is no interaction between EGCG and ascorbic acid when plasma concentrations are commensurate with dietary oral intake.

Vitamin C, at concentrations achieved during vitamin supplementation, has also been shown to inhibit the activity of bortezomib both *in vitro* and *in vivo*. Direct binding between the hydroxyl group of vitamin C and the boronic acid of bortezomib reduced the affinity of the proteasome inhibitor for the chymotrypsin-like subunit of the proteasome. In addition, it was noted that besides vitamin C, other natural agents carrying a hydroxyl group, such as flavonoid compounds (quercetin among others), bind and inhibit the activity of bortezomib *in vitro*.

To avoid the risk of any possible interaction it is recommended that green tea containing products and any supplemental products containing vitamin C, flavonoids or other antioxidants (e.g., vitamins, herbal supplements) be discontinued from at least 24 hours prior to the initiation of bortezomib through 72 hours after the last bortezomib dose. Injectable multivitamins used as a component of parenteral nutrition should also be avoided during this time period to minimize the risk of direct vitamin C inactivation of bortezomib. In addition, it is recommended that the total dietary intake of vitamin C not exceed the RDA for age. Normally balanced diets are acceptable; supplementation with high doses of vitamin C or injectable vitamin C should be avoided.

Toxicity:

Likely (>20%)	Less Likely (<20%)	Rare but Serious (<3%)
BLOOD AND LYMPHATIC SYSTEM DISORDERS		
Anemia		
CARDIAC DISORDERS		
		Heart failure
GASTROINTESTINAL DISORDERS		
Constipation Diarrhea Nausea Vomiting	Abdominal pain Dyspepsia Gastrointestinal hemorrhage Ileus	Gastrointestinal perforation
GENERAL DISORDERS AND ADMINISTRATION SITE CONDITIONS		
Fatigue Fever	Chills Edema limbs	
INFECTIONS AND INFESTATIONS		
Infections		
INVESTIGATIONS		
Thrombocytopenia	Neutropenia Weight loss	

Likely (>20%)	Less Likely (<20%)	Rare but Serious (<3%)
METABOLISM AND NUTRITION DISORDERS		
Anorexia	Dehydration	
MUSCULOSKELETAL AND CONNECTIVE TISSUE DISORDERS		
	Arthralgia Back pain Bone pain Muscle spasms Myalgia Pain in extremity	
NERVOUS SYSTEM DISORDERS		
Peripheral motor neuropathy Peripheral sensory neuropathy	Dizziness Headache Neuralgia Paresthesia	Leukoencephalopathy Reversible posterior leukoencephalopathy syndrome
PSYCHIATRIC DISORDERS		
	Anxiety Insomnia	
RENAL AND URINARY DISORDERS		
		Acute kidney injury
RESPIRATORY, THORACIC AND MEDIASTINAL DISORDERS		
	Cough Dyspnea Pharyngeal mucositis	Adult respiratory distress syndrome Pulmonary hypertension
SKIN AND SUBCUTANEOUS TISSUE DISORDERS		
	Maculo-papular rash	
VASULAR DISORDERS		
	Hypotension	

Formulation and stability: Bortezomib is supplied as a lyophilized powder in sterile vials containing 3.5 mg and 35 mg mannitol, USP. Unopened vials may be stored at controlled room temperature 25ºC (77ºF); excursions permitted from 15ºC to 30ºC (59ºF to 86ºF). Retain in original package to protect from light. Reconstitute bortezomib with 3.5 mL normal saline, USP. Each milliliter of solution will contain 1 mg of bortezomib at a pH of approximately 5 to 6. The drug solution is clear and colorless. Bortezomib contains no antimicrobial preservative. When reconstituted as directed, bortezomib may be stored at 25°C (77°F). Reconstituted bortezomib should be administered within 8 hours of preparation. The reconstituted material may be stored in the original vial and/or the syringe prior to administration. The product may be stored for up to 8 hours in a syringe; however, total storage time for the reconstituted material must not exceed 8 hours when exposed to normal indoor lighting.

Guidelines for administration: See Treatment and Dose Modification Sections of the protocol. Bortezomib is to be given without further dilution as an IV push over 3 to 5 seconds. Consecutive doses must be separated by at least 72 hours. Grapefruit and its juice should be avoided for the duration of treatment with bortezomib.

Special precautions: FOR INTRAVENOUS USE ONLY. The syringe containing bortezomib should be clearly labeled "For intravenous use only. Fatal if given by other routes." Additional wording may be considered at the institution's discretion. Three fatalities have been reported following accidental intrathecal administration of bortezomib. Special precautions should be employed to ensure that intravenous bortezomib and intrathecal medications are not inadvertently interchanged.

For patients with neuropathy, bortezomib doses may be administered subcutaneously (concentration of 2.5 mg/mL) into the thigh or abdomen, rotating the injection site with each dose; injections at the same site within a single cycle were avoided. Administer at least 1 inch from an old site and never administer to tender, bruised, erythematous, or indurated sites. If injection site reaction occurs, the more dilute 1 mg/mL concentration may be used SubQ (or IV administration of 1 mg/mL concentration may be considered).

Supplier: Commercially available; see package insert for more detailed information.

Agent Accountability: The Investigator, or a responsible party designated by the Investigator, must maintain a careful record of the inventory and disposition of bortezomib, as it is conducted under an IND.

5.7 Vorinostat (Zolinza®, Suberoylanidide Hydroxamic Acid, SAHA) IND# 127,270

Source and pharmacology: Vorinostat also known as suberoylanilide hydroxamic acid (SAHA), is a histone deacetylase (HDAC) inhibitor. Its chemical name is *N*-hydroxy-*N*'-phenyl-octane-1, 8-diotic acid diamide, *N*-hydroxy-*N*'-phenyl (9CI) octanediamide. The HDAC enzymes catalyze the removal of acetyl groups from the lysine residues of proteins, such as histones and transcription factors. In some cancer cells, there is an overexpression of HDACs or an abnormal recruitment of HDACs to oncogenic transcription factors causing hypoacetylation of core nucleosomal histones. Hypoacetylation of histones is associated with a condensed chromatin structure and repression of gene transcription.

Vorinostat inhibits HDAC by binding directly to the catalytic pocket of HDAC1, HDAC2, and HDAC3 (Class I) and HDAC6 (Class II) enzymes. Inhibition of HDAC activity allows for the accumulation of acetylated histones. This accumulation influences the regulation of gene expression. *In vitro*, exposure of cultured transformed cell to vorinostat led to G1 or G2 phase cell-cycle arrest, apoptosis, or differentiation and demonstrated synergistic and additive activity in combination with other cancer therapies (including radiation, kinase inhibitors, cytotoxic agents, and differentiating agents). The mechanism of the antineoplastic effect of vorinostat has not been fully characterized.

After oral administration, vorinostat is rapidly absorbed, however, administration with a high-fat meal resulted in a 33% increase in the extent of absorption and a 2.5-hour delay in the rate of absorption compared to the fasted state. Vorinostat is approximately 71% bound to human plasma protein. It is extensively metabolized to inactive metabolites, primarily by glucuronidation and hydrolysis followed by beta-oxidation. The two metabolites, *O*-glucuronide of vorinostat and 4-anilino-4-oxobutanoic acid are

pharmacologically inactive. *In vitro* studies indicate that vorinostat is not metabolized by and does not inhibit the activity of cytochrome P-450 enzymes. Less than 1% of an administered dose is excreted unchanged in the urine. Approximately 35-52% of an oral dose of vorinostat is excreted in the urine as the two major metabolites. The mean terminal half-life of vorinostat and the *O*-glucuronide metabolite is approximately 2 hours, while that of the 4-anilino-4-oxobutanoic acid metabolite it is 11 hours.

Prolongation of prothrombin time (PT) and International Normalized Ratio (INR) were observed in patients receiving vorinostat with coumarin-derivative anticoagulants (e.g., warfarin). Therefore, PT and INR should be monitored when coumarin-derivative anticoagulants are started or discontinued.

When vorinostat was administered with other HDAC inhibitors (e.g., valproic acid), severe thrombocytopenia and gastrointestinal bleeding have been reported.

Toxicity:

Likely (>20%)	Less Likely (<20%)	Rare but Serious (<3%)
BLOOD AND LYMPHATIC SYSTEM DISORDERS		
Anemia		
GASTROINTESTINAL DISORDERS		
Diarrhea Nausea Vomiting	Abdominal pain Constipation Dry mouth Dyspepsia	
GENERAL DISORDERS AND ADMINISTRATION SITE CONDITIONS		
Fatigue	Fever	
INFETIONS AND INFESTATIONS		
	Infection	
INVESTIGATIONS		
	Alanine aminotransferase increased Aspartate aminotransferase increased Bilirubin increased Creatinine increased Lymphopenia Neutropenia Weight loss White blood cell decreased	
METABOLISM AND NUTRITION DISORDERS		
Anorexia	Dehydration Hyperglycemia Hypocalcemia Hypokalemia Hypophosphatemia	
MUSCULOSKELETAL AND CONNECTIVE TISSUE DISORDERS		
	Muscle weakness	

Likely (>20%)	Less Likely (<20%)	Rare but Serious (<3%)
NERVOUS SYSTEM DISORDERS		
	Dizziness Dysgeusia	
RESPIRATORY, THORACIC AND MEDIASTINAL DISORDERS		
	Cough Dyspnea	
SKIN AND SUBCUTANEOUS TISSUE DISORDERS		
	Alopecia	Skin necrosis

Prolongation of prothrombin time and International Normalized Ratio have been observed in patients using vorinostat concomitantly with coumarin-derivative anticoagulants.

Formulation and stability: Vorinostat is supplied as a white, opaque gelatin, size 3 capsule, containing 100 mg of vorinostat. The inactive ingredients in each capsule include icrocrystalline cellulose, sodium croscarmellose, and magnesium stearate. Vorinostat 100 mg capsules are supplied in bottles containing 120 capsules.

Store vorinostat capsules at room temperature, 15 to 30ºC (59 to 86ºF). Do not store above 30ºC and avoid exposure to excessive moisture.

Guidelines for administration: See Treatment and Dose Modification sections of the protocol. Vorinostat should be taken with food/breastmilk. The capsules should not be opened or crushed. A suspension can be prepared by the pharmacy (to prepare accurate doses) for patients that cannot swallow pills. Please see Appendix III for instructions on suspension preparation. Antacids such as proton pump inhibitors and H2 blockers are not contraindicated. Vorinostat may be administered with dexamethasone.

Direct contact of the powder in vorinostat capsules with the skin or mucous membranes should be avoided. If such contact occurs, wash thoroughly. Clean powder spills from broken or damaged vorinostat capsules carefully minimizing inhalation. Wash spill area at least 3 times with ethyl alcohol, followed by water.

Supplier: Commercially available; see package insert for more detailed information.

Agent Accountability: The Investigator, or a responsible party designated by the Investigator, must maintain a careful record of the inventory and disposition of vorinostat, as it is conducted under an IND.

5.8 Cyclophosphamide (Cytoxan®)

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.

Toxicity

	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*	Anorexia, nausea & vomiting (acute and delayed)	Abdominal discomfort, diarrhea	Transient blurred vision, nasal stuffiness with rapid administration, arrhythmias (rapid infusion), skin rash, anaphylaxis, SIADH
Prompt: *Within 2-3 weeks, prior to the next course*	Leukopenia, alopecia, immune suppression	Thrombocytopenia, anemia, hemorrhagic cystitis (L)	Cardiac toxicity with high dose (acute – CHF hemorrhagic myocarditis, myocardial necrosis) (L), hyperpigmentation, nail changes, impaired wound healing, infection secondary to immune suppression
Delayed: *Any time later during therapy*	Gonadal dysfunction: azoospermia or oligospermia (prolonged or permanent)[1] (L)	Amenorrhea[1]	Gonadal dysfunction: ovarian failure[1] (L), interstitial pneumonitis, pulmonary fibrosis[2] (L)
Late: *Any time after completion of treatment*			Secondary malignancy (ALL, ANLL, AML), bladder carcinoma (long term use > 2 years), bladder fibrosis
Unknown Frequency and Timing:	Fetal toxicities and teratogenic effects of cyclophosphamide (alone or in combination with other antineoplastic agents) have been noted in humans. Toxicities include: chromosomal abnormalities, multiple anomalies, pancytopenia, and low birth weight. Cyclophosphamide is excreted into breast milk. Cyclophosphamide is contraindicated during breast feeding because of reported cases of neutropenia in breast fed infants and the potential for serious adverse effects.		

[1] *Dependent on dose, age, gender, and degree of pubertal development at time of treatment.*
[2] *Risk increased with pulmonary chest irradiation and higher doses. (L) Toxicity may also occur later.*

Formulation and stability: Cyclophosphamide for injection is available as powder for injection or lyophilized powder for injection in 500 mg, 1 g, and 2 g 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 25ºC (77ºF) is recommended. The product will withstand brief exposures to temperatures up to 30ºC (86ºF).

Guidelines for administration**:** See Treatment and Dose Modifications sections of the protocol.

Supplier: Commercially available; see package insert for more detailed information.

5.9 Mercaptopurine (6-MP; Purinethol®)

Source and pharmacology: Mercaptopurine is an analogue of the purine bases adenine and hypoxanthine. The main intracellular pathway for MP activation is catalyzed by the enzyme hypoxanthine-guanine phosphoribosyl transferase (HGPRT) which catalyzes the conversion of 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 TG nucleotides as well and thus produce toxicity. The absorption of an oral dose of MP is incomplete and variable, with only about 16%-50% of an administered dose reaching the systemic circulation secondary to a first pass metabolism in the liver. Co-administration with cotrimoxazole (TMP/SMX) significantly reduces absorption of MP. After IV administration, MP has a plasma half-life of 21 minutes in children. Approximately 19% is bound to protein. Mercaptopurine is well distributed into most body compartments except the CSF. (With high dose IV MP the CSF to plasma ratio is 0.15.) MP is metabolized by xanthine oxidase in the liver to 6-Thiouric acid an inactive metabolite. In patients receiving both MP and allopurinol (a xanthine oxidase inhibitor) the dose of MP must be reduced by 50-75%. Since TPMT, 6-thiopurine methyltransferase, is also one of the enzymes involved in the metabolism of MP, those individuals who have an inherited deficiency of the enzyme may be unusually sensitive to the myelosuppressive effects of MP and prone to develop rapid bone marrow suppression following the initiation of treatment. Mercaptopurine 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.

Toxicity:

	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*		Anorexia, nausea, vomiting, diarrhea, malaise	Urticaria, hyperuricemia
Prompt: *Within 2-3 weeks, prior to the next course*	Myelosuppression (L)	Erythematous rash (L)	Oral lesions resembling thrush, toxic hepatitis(L), increased AST/ALT hyperpigmentation (L), pancreatitis
Delayed: *Any time later during therapy, excluding the above conditions*		Oligospermia	Hepatic fibrosis(L), hyperbilirubinemia, alopecia

	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*
Late: *Any time after the completion of treatment*			Pulmonary fibrosis, secondary malignancies
Unknown frequency and timing:	Fetal toxicities and teratogenic effects of mercaptopurine have been noted in animals. Women receiving mercaptopurine in the first trimester of pregnancy have an increased incidence of abortion. It is unknown whether the drug is excreted in breast milk		

Formulation and stability: Mercaptopurine is available as a 50 mg tablet containing mercaptopurine and 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. Mercaptopurine is also available as an oral suspension in a concentration of 20 mg/mL (2000 mg/100 mL per bottle). The oral suspension is a pink to brown viscous liquid supplied in amber glass multiple-dose bottles with a child resistant closure. It should be stored at 15°-25°C (59°-77°F) in a dry place. NOTE: the concentration of the commercially available suspension (20 mg/mL) and the compounded suspension (50 mg/mL) are NOT the same; doses should be prescribed in the milligrams required, not mL.

Guidelines for administration: See Treatment and Dose Modifications sections of protocol.

Supplier: Commercially available; see package insert for more detailed information.

5.10 Methotrexate – IV/IM/PO

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. 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.

Toxicity:

	Common *Happens to 21-100 children out of every*	**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*	Transaminase elevations	Nausea, vomiting, anorexia	Anaphylaxis, chills, fever, dizziness, malaise, drowsiness, blurred vision, acral erythema, urticaria, pruritis, toxic epidermal necrolysis, Stevens-Johnson Syndrome, tumor lysis syndrome, seizures, photosensitivity
Prompt: *Within 2-3 weeks, prior to the next course*		Myelosuppression, stomatitis, gingivitis, photosensitivity, fatigue	Alopecia, folliculitis, acne, renal toxicity (ATN, increased creatinine/BUN, hematuria), enteritis, GI ulceration and bleeding, acute neurotoxicity (headache, drowsiness, aphasia, paresis, blurred vision, transient blindness, dysarthria, hemiparesis,
Delayed: *Any time later during therapy, excluding the above conditions*		Learning disability (L)	Pneumonitis, pulmonary fibrosis (L), hepatic fibrosis (L), osteonecrosis (L), leukoencephalopathy (L), pericarditis, pericardial effusions, hyperpigmentation of the nails
Late: *Any time after the completion of therapy*			Progressive CNS deterioration
Unknown Frequency and Timing:	Methotrexate crosses the placenta. Fetal toxicities and teratogenic effects of methotrexate have been noted in humans. The toxicities include: congenital defects, chromosomal abnormalities, severe newborn myelosuppression, low birth weight, abortion, and fetal death. Methotrexate is excreted into breast milk in low concentrations.		

[1]*May be enhanced by HDMTX and/or cranial irradiation.*
(L) Toxicity may also occur later.

Formulation and stability: Methotrexate for Injection is available as a lyophilized powder for injection in 1000 mg vials. The powder for injection contains approximately 7 mEq sodium in the 1000 mg vial. Methotrexate for Injection is also available as a 25 mg/mL solution in 2, 4, 8, 10, and 40 mL preservative free vials and 2 and 10 mL vials with preservative. The 2, 4, 8, 10, and 40 mL solutions contain approximately 0.43, 0.86, 1.72, 2.15, 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 20º-25ºC (68º-77ºF); excursions permitted to 15º-30ºC (59º- 86 Fº). Protect from light.

Guidelines for administration: See Treatment and Dose Modifications sections of protocol. Leucovorin rescue may be necessary with certain doses of methotrexate.

For IV use: Powder for injection: Dilute 1000 mg vial with 19.4 mL of preservative free SWFI, D5W or NS to a 50 mg/mL concentration. The powder for injection may be further diluted in NS or dextrose containing solutions to a concentration of ≤ 25mg/mL for IV use.

Do not use the preserved solution for high dose methotrexate administration due to 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, proton pump inhibitors, and NSAIDS as renal excretion of MTX is inhibited by these agents.

Supplier: Commercially available; see package insert for further information.

5.11 Leucovorin Calcium (Wellcovorin®, folinic acid)

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.

Toxicity:

	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*			Anaphylaxis, urticaria, seizure
Unknown frequency and timing:	Fetal toxicities and teratogenic effects of leucovorin in humans are unknown. It is unknown whether the drug is excreted in breast milk.		

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, and 350 mg vials. Store at room temperature 15°-25°C (59°-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 > 10 mg/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 SWFI, 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 protocol.

Supplier: Commercially available; see package insert for further information.

5.12 Cytarabine (Ara-C; Cytosar-U®)

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.

Toxicity:

	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, anorexia	Flu-like symptoms with fever, rash	Ara-C syndrome (fever, myalgia, bone pain, occasionally chest pain, maculopapular rash, malaise, conjunctivitis), anaphylaxis
Prompt: *Within 2-3 weeks, prior to the next course*	Myelosuppression (anemia, thrombocytopenia, leukopenia, megaloblastosis, reticulocytopenia), stomatitis, alopecia	Diarrhea, hypokalemia, hypocalcemia, hyperuricemia	Hepatotoxicity, sinusoidal obstruction syndrome (SOS, formerly VOD), urinary retention, renal dysfunction, pain and erythema of the palms and soles
Delayed: *Any time later during therapy, excluding the above conditions*			Asymptomatic nonoliguric rhabdomyolysis
Unknown frequency and timing:	Fetal toxicities and teratogenic effects of cytarabine have been noted in humans. It is unknown whether the drug is excreted in breast milk.		

Formulation and stability: Cytarabine for Injection 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 (25 mL per vial) or as a preservative free solution (5 mL, 50 mL per vial), and at a 100 mg/mL concentration with benzyl alcohol (20 mL vial) or as preservative free solution (20 mL vial). Hydrochloric acid and/or sodium hydroxide may be added to adjust the pH. Store at 25°C (77°F); excursions permitted to 15°-30°C (59°-86°F). Cytarabine solutions should be protected from light. 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 D5W or NS 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.

Supplier: Commercially available; see package insert for further information.

5.13 Etoposide (VP-16, Vepesid®) and Etoposide Phosphate (Etophos®)

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. Etoposide phosphate will be used if the patient has drug reaction with etoposide.

Toxicity:

	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	Anorexia	Transient hypotension during infusion; anaphylaxis (chills, fever, tachycardia, dyspnea, bronchospasm, hypotension)
Prompt: *Within 2-3 weeks, prior to next course*	Myelosuppression (anemia, leukopenia), alopecia	Thrombocytopenia, diarrhea, abdominal pain, asthenia, malaise, rashes and urticaria	Peripheral neuropathy, mucositis, hepatotoxicity, chest pain, thrombophlebitis, congestive heart failure, Stevens-Johnson Syndrome, exfoliative dermatitis

	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*
Delayed: *Any time later during therapy*			Dystonia, ovarian failure, amenorrhea, anovulatory cycles, hypomenorrhea, onycholysis of nails
Late: *Any time after completion of treatment*			Secondary malignancy (preleukemic or leukemic syndromes)
Unknown frequency and timing:	Fetal toxicities and teratogenic effects of etoposide have been noted in animals at 1/20th of the human dose. It is unknown whether the drug is excreted in breast milk.		

Formulation and stability: Etoposide for Injection is available as a 20 mg/mL solution in sterile multiple dose vials (5 mL, 25 mL, or 50 mL each). 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 D5W or NS. 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 the risk of precipitate formation. 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 0.4 mg/mL in NS. 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 to minimize exposure to DEHP.

Etoposide phosphate: Reconstitute the 100 mg vial with 5 or 10 mL of Sterile Water for Injection, D5W, NS, 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 equivalent (22.7 mg/mL or 11.4 mg/mL etoposide phosphate), respectively. **Use diluents 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 SWFI, D5W, or NS store at controlled room temperature for up to 24 hours.

Following reconstitution, etoposide phosphate may be further diluted to a concentration as low as 0.1 mg/mL of etoposide with D5W or NS. The diluted solution can be stored under refrigeration or at controlled room temperature for 24 hours.

Supplier: Commercially available; see package insert for more information.

5.14 VinCRIStine (Oncovin®)

Source and pharmacology: Vincristine is an alkaloid isolated from Vinca rosea Linn (periwinkle). It binds to tubulin, disrupting microtubules 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.

Toxicity:

	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 = local ulceration, shortness of breath, and bronchospasm

	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*
Prompt: *Within 2-3 weeks, prior to the next course*	Alopecia, constipation	Weakness, abdominal pain, mild brief myelosuppression (leukopenia, thrombocytopenia, anemia)	Paralytic ileus, ptosis, diplopia, night blindness, hoarseness, vocal cord paralysis, SIADH, seizure, defective sweating
Delayed: *Any time later during therapy*	Loss of deep tendon reflexes	Peripheral paresthesias including numbness, tingling and pain; clumsiness; wrist drop, foot drop, abnormal gait	Difficulty walking or inability to walk; sinusoidal obstruction syndrome (SOS, formerly VOD) (in combination); blindness, optic atrophy; urinary tract disorders (including bladder atony, dysuria, polyuria, nocturia, and urinary retention); autonomic neuropathy with postural hypotension; 8th cranial nerve damage with dizziness, nystagmus, vertigo and hearing loss
Unknown frequency and timing:	Fetal toxicities and teratogenic effects of vincristine (either alone or in combination with other antineoplastic agents) have been noted in humans. The toxicities include: chromosome abnormalities, malformation, pancytopenia, and low birth weight. It is unknown whether the drug is excreted in breast milk.		

Formulation and stability: Vincristine is supplied in 1 mL and 2 mL vials in which each mL contains vincristine sulfate 1 mg (1.08 µmol), mannitol 100 mg, SWFI; 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, preservative free 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: See Treatment and Dose Modifications sections of protocol.

The World Health Organization, the Institute of Safe Medicine Practices (United States) and the Safety and Quality Council (Australia) all support the use of minibag rather than syringe for the infusion of vincristine. The delivery of vincristine via either IV slow push or minibag is acceptable for this study. Vincristine should **NOT** be delivered to the patient at the same time with any medications intended for central nervous system administration. Vincristine is fatal if given intrathecally.

Injection of vincristine sulfate should be accomplished as per institutional policy. 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.

Special precautions: FOR INTRAVENOUS USE ONLY. The container or the syringe containing vinCRIStine must be enclosed in an overwrap bearing the statement: "Do not remove covering until moment of injection. For intravenous use only - Fatal if given by other routes."

Supplier: Commercially available; see package insert for more detailed information.

5.15 Drug Shortages and Unavailability

In the case of drug shortages and unavailability of any agent used in this protocol, treating investigators are urged to consult with the PI or co-PI and use their best clinical judgment in optimizing therapeutic intent and ensuring patient safety in managing the protocol-specified therapy.

Although these decisions may constitute "Protocol Violations," they are unavoidable and made in consideration of the best interest of an individual patient. These will NOT be considered monitoring/audit findings if appropriately documented. Most importantly, all protocol deviations must be noted in the research database and the alterations in therapy due to the agent shortage will be captured. This should be accomplished by entering "dose modified" and details noted in the comments field.

6.0 TREATMENT MODIFICATIONS

6.1 Renal Dysfunction

6.1.1 Methotrexate

Subclinical renal impairment (normal serum creatinine but decreased GFR) may be present in patients receiving concurrent nephrotoxic drugs (e.g. IV acyclovir) which, if possible, should be held during and for 20 hours after HDMTX infusions or until adequate MTX clearance has been documented. Consideration to delaying MTX should be given if a patient's serum creatinine indicates renal impairment.

6.1.2 Cytarabine doses $\geq$1000g/m^2

If serum creatinine is > 2 mg/dL or > 2 x normal for age, perform a CrCl. If the CrCl is <60 mL/min/1.73m^2, then high dose cytarabine should be reduced from twice to once daily dosing, (50% dose reduction).[42]

6.1.3 Etoposide

Dose modification for renal dysfunction

CCl or GFR (ml/min per 1.73m^2)	Etoposide Dose to Administer
> 60 ml/min per 1.73 m^2	Full Dose
30-60 ml/min per 1.73 m^2	50%
< 30 ml/min per 1.73 m^2	omit

6.2 Hepatic Dysfunction

Anthracyclines and vincristine dosages should be modified in patients with elevated direct bilirubin concentrations or other evidence of biliary obstruction.

- Direct bilirubin ≤3.0: Full dose
- Direct bilirubin 3.1-5 mg/dl: 50% dosage decrease
- Direct bilirubin 5.1-6 mg/dl: 75% dosage decrease
- Direct bilirubin >6 mg/dl: Withhold dose

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

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

Subclinical hypertransaminasemia (SGPT >500 IU/L) is an indication to delay only high-dose methotrexate but not other chemotherapy.

Patients with mild hepatic impairment do not require dose adjustment of bortezomib.

Patients with moderate or severe hepatic impairment should receive bortezomib at modified doses as outlined below:

	Direct bilirubin level	Bortezomib dose modification
Moderate	> 1.5x – 4x ULN	Reduce bortezomib in patients with moderate to severe elevated direct bilirubin levels to: • 0.7 mg/m^2 for patients ≥10kg and ≥12months of age or • 0.023 mg/kg for patients <10kg and/or <12months of age If direct bilirubin levels decrease to ≤1.5x ULN consider dose escalation for subsequent doses based on patient tolerability to: • 1 mg/m^2 for patients ≥10kg and ≥12months of age or • 0.033mg/kg for patients <10kg and/or <12months of age If direct bilirubin levels normalize consider dose escalation for subsequent doses based on patient tolerability to: • 1.3 mg/m2 for patients ≥10kg and ≥12months of age or
Severe	> 4x ULN	

	Direct bilirubin level	Bortezomib dose modification
		• 0.043mg/kg for patients <10kg and/or <12months of age or if direct bilirubin levels continue to rise consider <u>further dose reduction</u> to: • 0.5 mg/m^2 for patients ≥10kg and ≥12months of age or • 0.017mg/kg for patients <10kg and/or <12months of **age**

**No adjustment necessary for elevated SGOT (ALT).*

6.3 Testicular Leukemia at Diagnosis

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

6.4 Vincristine Neurotoxicity

The maximum single dose of vincristine must not exceed 2 mg. Mild vincristine toxicities (jaw pain, constipation, and decreased deep tendon reflexes) are anticipated. Loss of voice due to vocal cord paralysis may be a complication of vincristine toxicity but it must be differentiated from pharyngitis or Candida infection of the cord.

6.4.1 Dose Modifications for Toxicity: PLEASE USE ―BALIS SCALE FOR GRADING NEUROPATHY (See text box below)

- **Severe neuropathic pain (Grade 3 or greater):** Hold dose(s). When symptoms subside, resume at 50% previous calculated dose (maximum dose: 1 mg), then escalate to full dose as tolerated. NOTE: neuropathic pain can be not only severe but difficult to treat. However, because vincristine is an important component of curative therapy and the majority of neuropathies are ultimately reversible, vincristine therapy may be given at full dose at investigator discretion. Severe peripheral neuropathies, with or without a positive family history might suggest the need for a molecular

diagnostic evaluation to rule out Charcot Marie Tooth Disease (CMT), Type 1A or Hereditary neuropathy. Drugs such as gabapentin may be of value.

- **Vocal cord paralysis**: Hold dose(s). When symptoms subside, resume at 50% previous calculated dose (maximum dose: 1 mg), then escalate to full dose as tolerated. See above for comment on CMT.
- **Foot drop, paresis**: Should be Grade 3 to consider holding or decreasing dose. These toxicities are largely reversible but over months to years. Accordingly, holding doses of vincristine and/or lowering the dose may not result in rapid resolution of symptoms and may compromise cure. See above for comment on CMT. Physical therapy may be beneficial to maintain range of motion and provide AFO's and other forms of support. Drugs such as gabapentin may be of value.
- **Jaw pain**: Treat with analgesics; do not modify vincristine dose.
- **Hyperbilirubinemia**-see section 6.2
- **Constipation or ileus (≥ Grade 3) or typhlitis**: Hold dose(s); institute aggressive regimen to treat constipation if present. When symptoms abate resume at 50% of calculated dose (maximum dose: 1 mg) and escalate to full dose as tolerated.
- **Extravasation**: Discontinue the IV administration of the drug and institute appropriate measures to prevent further extravasation and damage according to institutional practice

6.4.2 Modified (Balis) Pediatric Scale of Peripheral Neuropathies

Peripheral Motor Neuropathy:

- Grade 1: Subjective weakness, but no deficits detected on neurological exam, other than abnormal deep tendon reflexes.
- Grade 2: Weakness that alters fine motor skills (buttoning shirt, coloring, writing or drawing, using eating utensils) or gait without abrogating ability to perform these tasks.
- Grade 3: Unable to perform fine motor tasks (buttoning shirt, coloring, writing or drawing, using eating utensils) or unable to ambulate without assistance.
- Grade 4: Paralysis.

Peripheral Sensory Neuropathy:

- Grade 1: Paresthesias, pain, or numbness that do not require treatment or interfere with extremity function.
- Grade 2: Paresthesias, pain, or numbness that are controlled by non-narcotic medications (without causing loss of function), or alteration of fine motor skills (buttoning shirt, writing or drawing, using eating utensils) or gait, without abrogating ability to perform these tasks.
- Grade 3: Paresthesias or pain that are controlled by narcotics, or interfere with extremity function (gait, fine motor skills as outlined above), or quality of life (loss of sleep, ability to perform normal activities severely impaired).
- Grade 4: Complete loss of sensation, or pain that is not controlled by narcotics.

Motor Neuropathy in Infants: Please see comments in section 6.7.

6.5 Cytarabine Neurotoxicity

Discontinue cytarabine for > Grade 3 CTCAE v 4 nervous system disorders. The most common nervous system disorder is an acute cerebellar syndrome that may manifest itself as ataxia, nystagmus, dysarthria, or dysmetria. However, seizures and encephalopathy have also occurred following therapy with high dose cytarabine.

6.6 Methotrexate Neurotoxicity

Infants that experience a seizure following IT MHA or high dose methotrexate should be started on levetiracetam for seizure control and continue on this medication until one month following the last dose of intrathecal. If the seizure occurred following IT MHA, all subsequent IT MHA should be followed by leucovorin rescue regardless of phase or ANC. If the patient has further seizures following IT MHA while on levetiracetam with leucovorin rescue, the methotrexate should be removed from the intrathecals and the patient should receive IT HA for all subsequent doses. Patients who experience a seizure secondary to high dose methotrexate should be started and maintained on levetiracetam as described above. The subsequent courses of high dose methotrexate should be given at $1 g/m^2$. If the patient experiences another seizure on the lower dose of methotrexate, then the remaining courses of high dose methotrexate may be omitted and the patient can proceed directly to reinduction once they meet WBC, ANC, and platelet criteria.

6.7 Mitoxantrone Cardiac Toxicity

Do not give MITOX if there is significant evidence of cardiac disease by echocardiogram or MUGA (shortening fraction <28% or ejection fraction <55%). Do not re-start if held for left ventricular shortening dysfunction that is not associated with a microbiologically proven bacteremia or sepsis. If the left ventricular shortening dysfunction occurred in the setting of microbiologically proven bacteremia or sepsis, then MITOX may be reinstituted at the treating clinician's discretion once the shortening fraction has returned to ≥28% or ejection fraction is ≥55%.

6.8 Bortezomib Neurotoxicity

Peripheral neuropathy will be closely monitored during each block of treatment and toxicities graded. A complete neurological exam should be documented prior to the start of therapy. Neuropathy is often difficult to diagnose in infants. Symptoms may include hypotonia, delayed motor development, areflexia, and/or foot deformities. Facial weakness and tongue fasciculations may be present.

As a reference, timing of developmental reflexes are indicated in the table below.

Reflex	Stimulation	Response	Duration
Babinski	Sole of foot stroked	Fans out toes and twists foot in	Disappears at nine months to a year
Blinking	Flash of light or puff of air	Closes eyes	Permanent
Grasping	Palms touched	Grasps tightly	Weakens at three months; disappears at a year
Moro	Sudden move; loud noise	Startles; throws out arms and legs and then pulls them toward body	Disappears at three to four months
Rooting	Cheek stroked or side of mouth touched	Turns toward source, opens mouth and sucks	Disappears at three to four months
Stepping	Infant held upright with feet touching ground	Moves feet as if to walk	Disappears at three to four months
Sucking	Mouth touched by object	Sucks on object	Disappears at three to four months
Swimming	Placed face down in water	Makes coordinated swimming movements	Disappears at six to seven months
Tonic neck	Placed on back	Makes fists and turns head to the right	Disappears at two months

Neuropathy grading should be based on the maximum toxicity occurring during the previous block. All dose modifications should be based on the worst preceding toxicity. Bortezomib dose will be decreased for sensory peripheral neuropathy per Section 6.7.1.

6.8.1 Dose Modifications for Bortezomib-Related Neuropathic Pain and/or Peripheral Sensory Neuropathy

Severity of sensory peripheral neuropathy	Bortezomib modification
Grades 1-3 with no pain	Same dose as previous
Grade 1 with pain or Grade 2	Initiate gabapentin at 15 mg/kg/day divided into three doses[43].
Grade 3 with pain	Hold bortezomib treatment until symptoms have resolved to ≤ Grade 1. Do not make up missed doses. When toxicity resolves, re-initiate bortezomib at: • 1 mg/m^2/dose subQ for patients ≥10kg and ≥12months of age and • 0.033mg/kg/dose subQ for patients <10kg and/or <12 mo of age.
Grade 4	Discontinue bortezomib

Patients experiencing any grade of peripheral neuropathy should receive all subsequent doses of bortezomib subcutaneously due to the lower incidence of neuropathic side effects with this alternative dosing route.[44]

If Grade 3 sensory peripheral neuropathy with pain persists for >2 weeks, bortezomib should be discontinued.

Patients experiencing Grade 4 sensory peripheral neuropathy will discontinue treatment. If Grade 2 or 3 sensory neuropathy with pain recurs despite bortezomib dose reduction to 1 mg/m^2, the patient should discontinue protocol treatment.

Patients whose sensory peripheral neuropathy resolves to ≤ Grade 1 or baseline within 2 weeks can receive bortezomib in subsequent at 1 mg/m^2/dose or 0.033mg/kg/dose subQ.

6.9 Venous Thromboembolism

Patients who develop cerebral or other venous thrombosis will receive low molecular weight heparin throughout treatment with asparaginase (during Reinduction). Acute management of thrombosis will be managed as per individual institutional guidelines; questions should be referred to the PI or co-PI. Also see section 6.10.

6.10 Avascular Necrosis of Bone

MRI exams will be interpreted by the radiologist. If there is evidence of epiphyseal or metaphyseal hip lesions, knee epiphyseal or metaphyseal lesions, or lesions of talus consistent with avascular necrosis of the bone, the patient will be referred to the orthopedic surgeon, who will evaluate symptoms, and will assess the severity and estimated risk of progression. Physical therapy, activity modifications, and surgical procedures will be recommended as needed. Patients with hip epiphyseal lesions or talus lesions affecting > 30% of weight-bearing area will have an X-ray of the affected area, and will be assessed at higher risk for progression. Symptomatic patients with such findings will likely have their dexamethasone stopped, especially if they are past reinduction in therapy. Asymptomatic patients with such findings will likely have their dexamethasone dose halved, especially if they are past reinduction in therapy. Any patients with X-ray findings of AVN are candidates for dexamethasone modification, regardless of symptoms. All modifications (or lack thereof) of dosage will be recorded in the research database. Patients with progression of any lesions or with worsening symptoms will be re-evaluated by imaging and, if appropriate, by additional orthopedic follow-up. If the dexamethasone is discontinued, the, first choice will be to replace each week's dosing with one dose of methotrexate (40 mg/m^2), if tolerated.

6.11 PEG-Asparaginase Dose Modifications

Patients with allergic reactions or intolerance to PEG-asparaginase subsequently will be given *Erwinia* L-asparaginase intramuscularly or intravenously over 30-60 minutes duration. Each dose of PEG will be replaced by Erwinia at 25,000 units/m^2/dose three times weekly for 6 doses for each planned PEGaspargase dose. Native E. coli

asparaginase can also be given when clinically indicated, but this should be discussed and approved by the PI or co-PI. Patients who are intolerant to all asparaginase formulations can have asparaginase held.

- Coagulopathy
  - If symptomatic, hold asparaginase until symptoms resolve, then resume with the next scheduled dose. Consider factor replacement (FFP, cryoprecipitate, factor VIIa). Do not withhold dose for abnormal laboratory findings without clinical symptoms.
  - *Thrombosis*: Withhold asparaginase until resolved, and treat with appropriate antithrombotic therapy, as indicated. Upon resolution of symptoms consider resuming asparaginase, while continuing LMWH or antithrombotic therapy. Do not withhold dose for abnormal laboratory findings without clinical correlate. For significant thrombosis, not line related, consider evaluation for inherited predisposition to thrombosis.
  - *CNS Events (bleed, thrombosis or infarction)*: Hold asparaginase. Treat with FFP, factors or anticoagulation as appropriate. Resume at full dose when all symptoms have resolved (and evidence of recanalization in case of thrombosis by CT/MRI). Consider evaluation for inherited predisposition to thrombosis
- Hyperbilirubinemia: see section 6.2.
- Hyperglycemia: Do not modify dose. Treat hyperglycemia as medically indicated.
- Hyperlipidemia: Do not modify dose.
- Ketoacidosis: Hold asparaginase until blood glucose can be regulated with insulin.

6.12 Dexamethasone Dose Modifications

- Hypertension: Dose should not be reduced. Sodium restriction and anti-hypertensives should be employed in an effort to control hypertension. Avoid calcium channel blockers due to their potential prohemorrhagic effect.
- Hyperglycemia: Dose should not be reduced for hyperglycemia. Rather, insulin therapy should be employed to control the blood glucose level.
- Pancreatitis: Do not modify dose for asymptomatic elevations of amylase and/or lipase. Contact the PI or co-PI to discuss the management if there is a possibility that the pancreatitis is due to Dexamethasone.
- Osteonecrosis (ON): Do not modify corticosteroid therapy for osteonecrosis (also referred to as avascular necrosis) during Reinduction and Reintensification. Omit Maintenance steroid for osteonecrosis Grade 2 or greater. Consider resuming steroid after 6 months if joint symptoms have resolved and if MRI findings have significantly improved or normalized.
- Varicella: Steroids should be held during active infection except during Induction, Reinduction and Reintensification. Do not hold during incubation period following exposure.
- Inability to use oral doses: Substitute the IV preparation mg for mg.
- Severe infection: Do not hold or discontinue steroids during Induction, Reinduction, and Reintensification without serious consideration, as this is a

critical period in the treatment of ALL. Later one may consider holding steroid until patient achieves cardiovascular stability, except for ―stress doses.

- Severe psychosis: Dexamethasone dose may be reduced by 50% for severe psychosis. If symptoms persist, switch to prednisone.

6.13 Management of Pancreatitis

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

7.0 CONTINGENCY PLANS FOR REFRACTORY DISEASE OR RELAPSE

7.1 Increasing MRD During Consolidation

Patients that achieve complete remission following induction (<5% leukemic blasts in bone marrow) but have detectable disease by MRD that fails to decrease during consolidation may abort methotrexate courses and proceed directly to reinduction provided they are clinically stable. These patients should be considered for referral to transplant in first remission.

7.2 Induction Failures

Patients who do not attain complete remission (≥5% leukemic blasts in bone marrow) after remission induction, consolidation, and reinduction treatment will be removed from the protocol. Those with >0.01% leukemic blasts after consolidation therapy are candidates for allogeneic hematopoietic stem cell transplantation.

7.3 Hematologic Relapse

Patients with ≥25% lymphoblasts in the marrow aspirate will be removed from protocol.

7.4 Extramedullary Relapse

Patients with any form of extramedullary relapse (testes, ovarian, etc.) except that of CNS will become eligible for relapse protocols. Patients with overt CNS relapse (i.e. ≥5 WBC/ul of CSF with blasts confirmed by TdT staining) will be removed from protocol. Patients with <5 WBC/ul of CSF with detectable blasts confirmed by TdT staining that remain in hematologic remission, will remain on study and receive twice weekly intrathecals until they have two negative taps or receive a total of 6 doses (whichever comes first) in addition to the systemic chemotherapy that is due as per the protocol. This will be followed by intrathecals every 4 weeks until the completion of chemotherapy.

8.0 REQUIRED EVALUATIONS, TESTS, AND OBSERVATIONS

8.1 Pre-Study Evaluations

All entry/eligibility studies must be performed within 1 week prior to entry onto the trial.

- Complete history and physical exam with careful notation and assessment of clinical signs relevant to leukemia
- CBC w/differential
- Chemistry profile: glucose, electrolytes, BUN, creatinine, LDH, uric acid, bilirubin, AST, ALT, calcium, phosphorous, magnesium, total protein and albumin
- Coagulation screen (PT, PTT)
- Urinalysis
- Thyroid function tests (free T4, T4, TSH)
- Cholesterol/lipid screen
- Total IgG level
- 25-OH Vitamin D level
- Chest X-ray
- Lumbar puncture with CSF examination (cell count with differential of cytospin preparation
- Bone marrow evaluation: morphology, cytochemistry, immunophenotyping, cytogenetics, molecular diagnosis, MRD studies
- Tumor: Next Generation Sequencing studies
- Cardiac echocardiogram or MUGA
- Baseline electrocardiogram (EKG)
- Peripheral blood and bone marrow for biological studies (see tables under Section 8.2)
- HLA typing – *recommended as per institutional practice*

8.2 Evaluations During Therapy

Test/Evaluation	Induction	Consolidation	Reinduction	Reintensification	Maintenance
Physical Exam	Q3-7 days	Weekly	Q3-7 days	Q3-7 days	Q28 days
CBC w/Diff[1]	Weekly, or as indicated	Weekly	As indicated	As indicated	Q28 days
Electrolytes, BUN, Cr[1]	As indicated	Weekly	As indicated	As indicated	Q28 days
Uric Acid, LDH, Phos, Ca, Mg[1]	As indicated	Before each HDMTX	As indicated	As indicated	As indicated
Serum glucose, urinalysis	As indicated	As indicated	As indicated	As indicated	As indicated
Bilirubin, AST, ALT, Total Protein, Albumin	As indicated	Weekly	As indicated	As indicated	Q28 days
Total IgG Level[2]	---	Day 1	Day 1	Day 1	Q28 days
25-OH vitamin D level[2]	---	Day 1	As indicated	As indicated	End of Therapy
Chest x-ray	As indicated	Day 1 in cases w/mediastinal mass at diagnosis	As indicated	As indicated	As indicated
CSF Studies (cell count with differential of cytospin preparation)	With ea IT	With ea IT	With ea IT	With ea IT	With ea IT
Bone marrow/MRD[3] (See table below)	Day 22	Day 1 (± 15, 29, 43)	Day 1	Day 1	End of Therapy
PB biology studies[3]	Day 1, 22	Day1 (± 15, 29, 43)			
Germline DNA[2]	---	PBL Day 1	---	---	---
MTX levels	As indicated	ea HDMTX	As indicated	As indicated	As indicated
ECHO or MUGA[4]	As indicated	---	Day 1	---	---
EKG[4]	As indicated	---	Day 1	---	---

[1]***During Induction, it is critical to monitor chemistries frequently due to tumor lysis***. *At a minimum, they should be checked daily until values are stable and within normal limits for 48-72 hours. Patients with high tumor burden may need tumor lysis labs as frequently as every 4-6 hours. Contact the study chair if there are questions or concerns.*
[2]*Level may be drawn following count recovery prior to day 1*
[3]*Please see tables on the next page for allowances of alternate time sample draws*
[4]*ECHO and EKG may be done anytime following completion of consolidation chemotherapy prior to administration of mitoxantrone*

Bone Marrow Samples required for clinical MRD studies (10ml heparin)

Phase	Induction		Consolidation[1]				Reinduct[1]	Reinten[1]	Mtn.
Time point	Day1[1,2]	Day22[3]	Day 1[1]	Day15[4]	Day29[4]	Day43[4]	Day1[1]	Day1[1]	End of Therapy
Patients	All	All	All	MRD>0.01% Consol Day1	MRD>0.01% Consol Day15	MRD>0.01% Consol Day29	All	MRD>0.01% Reinduct Day1	All

Bone Marrow Samples for Biologic Studies

Sample	Induction		Consolidation[1]				Reinduct[1]	Reinten[1]	Maint	Relapse
Time point	Day1[1,2]	Day22[3]	Day 1[1]	Day15[4]	Day29[4]	Day43[2]	Day1[1]	Day1[1]	End of Therapy	
Tumor NGS[5]	4 ml heparin	---	---	---	---	---	---	---	---	5 ml heparin
Drug Studies[5]	4 ml heparin	---	---	---	---	---	---	---	---	5 ml heparin
MRD NGS[5]	---	5 ml heparin	5 ml heparin	5 ml heparin	5 ml heparin	5 ml heparin	5 ml heparin	5 ml heparin	5 ml heparin	---

[1]Bone marrow specimens should be obtained prior to initiation of therapy and upon count recovery prior to chemotherapy at these time points and are not required to be drawn on the exact days specified in the table.
[2]If the patient is unable to undergo a bone marrow aspirate, the aspirate is unsuccessful, or insufficient bone marrow was obtained for all studies may substitute peripheral blood sample or tumor cells obtained by leukapheresis for Day1 specimens.
[3]If the patient is not clinically stable to undergo a procedure on day 22 or this day falls on a weekend/holiday, may delay obtaining this specimen so long as it is obtained prior to initiation of day 22 chemotherapy.
[4]Specimens should coincide with LPIT and HD-MTX courses, therefore if this is delayed for clinical or scheduling purposes the bone marrow will also be delayed.
[5]Only if patient is having a bone marrow done for clinical purposes (see above table).

Peripheral Blood Samples for Biologic Studies

	Induction					**Consol**	**Reind**	**Reinten**	**Maint**	**EOT**
Sample	PreTx	6hr	24hr	Day4	Day18	Day1[1]	Day1[1]	Day1[1]	Day 1[1,2]	
NFkB/ Proteasome[3]	2ml cell save 1ml heparin	2ml cell save 1ml heparin	2ml cell save 1ml heparin	---	---	---	---	---	---	---
Histone Analysis[4]	2.5ml	---	2.5 ml	2.5ml						
Immune Repertoire	1ml heparin	---	---	---	---	---	2ml heparin	2ml heparin	2ml heparin	2ml heparin
Germline DNA	---	---	---	---	---	5ml heparin	---	---	---	---

[1]Peripheral blood specimens should be obtained upon count recovery prior to chemotherapy at these time points and are not required to be drawn on the exact days specified in the table.
[2]Day 1 of maintenance cycles 1, 5, 10, and 15
[3]1ml may be drawn in either lithium heparin or sodium heparin. If no cell save tubes are available all 3 ml can be sent in the heparin tube.
[4]Blood should be drawn in EDTA and transferred to fixative tubes provided to sites – fill fixative tube with blood ONLY TO THE BLACK LINE The addition of blood from the EDTA to the fixative tube must be accurate; if the ratio is off the assay is no longer valid. The amount of blood added to the line is roughly 2ml, due to small volume inaccuracies when drawing blood, 2.5 ml should be drawn to prevent having insufficient amounts to transfer to the fixative.

Total peripheral blood samples during Induction:

- ≥10kg: 17.5 ml over 7 weeks = ~1.75 ml /kg over 7 weeks
- <10kg: 17.5 ml over 7 weeks = ~3 ml /kg over 7 weeks for the average infant (6kg)

For patients in which all induction samples cannot be drawn due to size or other clinical reason, peripheral blood research samples should be prioritized as follows:

1. Histone analysis
2. NFKB/Proteasome analysis - cell save tubes should be prioritized over heparin tubes
3. Immune repertoire

8.3 Long-Term Follow-up Evaluations

It is recommended that patients will be followed every 4 months for 1 year, every 6 months for 1 year, and then yearly until the patient is in remission for 10 years. Once a patient has been in remission 5 years or more, they may be referred for long term follow up per institutional guidelines. During the visit, CBC with differential and other laboratory studies as clinically indicated should be obtained. Patients will be considered off therapy 30 days after the last treatment is taken. Adverse events will not be reported while patient is off-therapy unless they are deemed related to therapy by the site PI.

9.0 EVALUATION CRITERIA

9.1 Response Criteria

- Complete remission: M1 marrow status with restoration of normal hematopoiesis as defined by Platelets >75,000/mm^3 and ANC>750/mm^3
- Induction failure: ≥5% leukemic blasts in marrow after 42 days of remission induction treatment
- Bone marrow relapse: ≥25% leukemic blasts in marrow
- CNS relapse: ≥5 WBC/ul of CSF with definite blasts confirmed by TdT staining on cytospin preparation.
- Testicular relapse: Isolated testicular relapse must be confirmed pathologically; in the event of bone marrow relapse, combined testicular relapse can be based on testicular enlargement (documented by sonogram) without biopsy.

9.2 Toxicity Evaluation Criteria

This study will utilize the CTCAE Version 4.0 for toxicity and performance reporting. A copy of the CTCAE version 4.0 can be downloaded from the CTEP home page (http://ctep.info.nih.gov). Additionally, the toxicities are to be reported on the appropriate data collection forms.

10.0 SUPPORTIVE CARE

10.1 Fever at Diagnosis

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

10.2 Metabolic Derangements

It is important to prevent or treat hyperuricemia and hyperphosphatemia with secondary hypocalcemia resulting from spontaneous or chemotherapy-induced leukemic cell lysis. Patients with large leukemic cell burden should receive hydration and oral phosphate binder.

Patients with large leukemic cell burden with or without hyperuricemia (e.g. WBC $\geq$100/mm^3, uric acid $\geq$6.5 mg/dl may be treated with rasburicase if they have no history of G6PD deficiency. For patients not at high risk of hyperuricemia, hydration, allopurinol, and judicious use of alkalinization (keeping urine pH between 6.5 and 7.4) may be sufficient.

10.3 Hyperleukocytosis

For patients with extreme hyperleukocytosis (e.g. WBC $\geq$ 300/mm^3), leukapheresis or exchange transfusion may be considered. Aggressive hydration and management of tumor lysis syndrome should be immediately implemented. Platelets should be transfused at counts below 20,000/mm^3 to prevent CNS bleed, or in the presence of any active mucosal or visceral bleeds. A platelet transfusion does not increase the blood viscosity significantly. Red blood cell transfusions are not indicated in a hemodynamically stable child, as it adversely affects the blood viscosity. In the case of severe anemia, with impending congestive cardiac failure, leukapheresis or exchange transfusion are better options. If coagulopathy is present, it should be corrected by transfusion of 10-15ml/kg of fresh frozen plasma.

In patients with hyperleukocytosis who are not stable for bone marrow procedures, all diagnostic tests should be submitted with peripheral blood specimens.

10.4 Skin Breakdown

Infants are at high risk for skin breakdown in the diaper region during times of neutropenia such as Induction and Reinduction, as well as during Consolidation following courses of high dose methotrexate. Additional factors that contribute include increased stooling secondary to infections, antibiotics, or chemotherapy. It is very important to protect the skin of the diaper area and minimize breakdown beginning at diagnosis and continuing throughout therapy. Consistency and frequent diaper changes are two key factors that help prevent skin breakdown. It is recommended that at diagnosis for patients with intact skin +/- erythema, a thick layer of butt balm is applied with each diaper change. To make butt balm, mix equal parts of Desitin®, Aquaphor®, and

Nystatin. When changing the diaper, stool and old paste should be removed gently. Aggressive scrubbing will increase the chance of breakdown. Mineral oil and a cotton ball can help gently remove paste that is mixed with stool. Allow skin to air dry before applying new layer of butt balm. For patients that develop skin breakdown, they have failed butt balm and we recommend trying a product such as Sensi-Care which is able to adhere to weepy, denuded skin and protect it from further breakdown.

10.5 Avascular Necrosis of Bone

Osteonecrosis of the bone, a known complication of treatment with corticosteroids, is extremely rare in infants. Any patient diagnosed with osteonecrosis will be referred to orthopedics. Any patient who develops symptoms of joint pain should have an MRI performed to rule out osteonecrosis or progression of this complication. For patients who require surgical intervention, treatment will vary based on degree of progression, e.g. observation, core decompression, bone grafting and resurfacing hemiarthroplasty.

10.6 Pancytopenias

Patients with prolonged (>3 weeks) unexplained anemia (hemoglobin <7g/dl) or neutropenia (ANC<300/mm^3) during remission should be evaluated for B19 parvovirus infection or hemolysis or toxicity from non-chemotherapeutic agents (e.g. TMP/SMZ). For patients with neutropenia secondary to TMP/SMZ, intravenous pentamidine every four weeks may be substituted for PCP prophylaxis.

10.7 Nutritional Supplementation

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

Evidence suggests that patients with hematologic malignancy and low 25-hydroxyvitamin D [25(OH)D] levels at diagnosis have worse outcomes.[45-47] The 25(OH)D level of infants is dependent on the 25(OH)D level of the mother during pregnancy. As vitamin D deficiency is epidemic in the US due to lifestyle and diet, all patients will have a 25(OH)D level measured at diagnosis. Patients with levels <30 ng/mL at diagnosis will be supplemented with 400 units D3 daily. All patients should have a repeat level drawn at the end of induction and those with normal levels at diagnosis but low levels following induction therapy should start supplementation. For all infants receiving supplementation, periodic repeat levels should be drawn as indicated to verify correction of the deficiency.

10.8 Drug Interactions

Because concurrent use of enzyme inducing anticonvulsants (e.g. phenytoin, phenobarbital, and carbamazepine) with antileukemic therapy has recently been associated with inferior EFS, every effort should be made to avoid these agents, as well as rifampin which also

induces many drug metabolizing enzymes. Keppra does not induce hepatic drug metabolizing enzymes and may be a suitable alternative anticonvulsant.

Azole antifungals (fluconazole, itraconazole, voriconazole, and ketoconazole) and the macrolide group of antibiotics (e.g. erythromycin, rifampin, and Zithromax) may have potent inhibitory effects on drug-metabolizing enzymes, and the doses of some antileukemic drugs (e.g. vincristine, anthracyclines, steroids, etoposide, bortezomib) may need to be reduced in some patients on chronic azole antifungals or antibiotics. During induction and reinduction, micafungin will be given at treatment doses due to the high risk of fungal infections. If a patient develops a fungal infection requiring an azole, monitor side effects of vincristine and bortezomib closely, and implement dose reductions as delineated in section 6.0. Consult the study PI if long-term use of these interacting drugs is unavoidable.

Penicillins interfere with tubular excretion of methotrexate, and it is recommended that an alternative non-penicillin antibiotic be used. Conversely, methotrexate clearance has been demonstrated not to be affected by TMP/SMZ and so this prophylactic medication does not need to be held during methotrexate administration.

10.9 RSV Prophylaxis

All infants less than 1 year of age should receive RSV prophylaxis between October and April.

Those patients greater than one year of age should prophylaxis until they have completed reinduction (or Reintensification if applicable) as patients are at high risk for RSV pneumonia during times of neutropenia. RSV prophylaxis is not required during maintenance for patients greater than one year of age.

10.10 Mandatory Prophylactic Antibiotics During Periods of Prolonged Neutropenia

Infants with ALL are severely immunocompromised due to their immature immune systems, the presence of leukemia, and exposure to chemotherapeutic agents. Patients may developed serious fungal infections while treated on this protocol including non-albicans Candida. It is mandatory that all patients receive anti-fungal coverage using either the echinocandin class (e.g. caspofungin or micafungin) or an amphotericin agent during Induction, Reinduction, and Reintensification (if applicable). The doses used should be in the treatment range for the particular agent more so than prophylactic as that is when colonization and infection may occur on this study with patients at high risk for fungal infection. Recommended treatment doses are as follows: micafungin 4mg/kg/dose daily; caspofungin 25mg/m^2/dose daily for infants less than 3 months of age; caspofungin 70mg/m^2/dose day 1 followed by 50mg/m^2/dose daily for infants 3 months of age or greater (may increase to 70mg/m^2/dose daily if necessary); Ambisome 5mg/kg/dose daily. Azole antifungal agents (i.e. fluconazole, itraconazole, voriconazole) given concurrently with bortezomib may increase risk of neurotoxicity. Caution is advised if azole antifungals are used.

In addition to fungal coverage, prophylactic bacterial coverage with cefepime during times of neutropenia is required. Patients unable to receive cefepime due to drug shortage, allergy, or other cause should receive an alternative antibiotic with similar bacterial coverage. Coverage should be broadened as indicated following episodes of fever or development of symptoms consistent with a localized infection. Examples include additional gram negative coverage for patients with evidence of colitis or hypotension and vancomycin in patients with mucositis. Patients that continue to have fever on broad spectrum antibiotics with no source should be evaluated for fungal infections. Please contact the study PI for any questions or concerns.

For patients discharged following induction, reinduction, or Reintensification courses that have ANC >200 but less than 500, fungal prophylaxis may be discontinued and monotherapy bacterial prophylaxis with oral levofloxacin is acceptable until ANC≥500. TMP/SMZ for PJP prophylaxis should continue.

11.0 CRITERIA FOR OFF STUDY AND OFF TREATMENT

11.1 Off-Study Criteria

- Death
- Lost to follow-up
- Request of the patient/parent
- Found to be ineligible (e.g. incorrect diagnosis)
- Discretion of the Study PI, such as the following
  - The researcher decides that continuing in the study would be harmful
  - A treatment is needed that is not allowed on this study
  - The participant misses so many appointments that the data cannot be used in the study
  - New information is learned that a better treatment is available, or that the study is not in the participant's best interest

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

11.2 Off-Therapy Criteria

- Research participants who fail to achieve morphological complete remission after treatment according to the contingency plan
- Any relapse as defined in section 7.0
- Second malignancy
- Development of unacceptable toxicity during treatment (with concurrence of the PI or co-PI)
- Refusal of therapy
- Investigator decides continued protocol treatment is no longer in patient's best interest

- Completion of all protocol-prescribed treatment
- Patients referred to transplant or CAR T cells will be considered off therapy the day they begin lymphodepleting chemotherapy in preparation of CAR T cell administration or are admitted for their stem cell transplant, prior to the initiation of the conditioning regimen.

12.0 SAFETY AND ADVERSE EVENT REPORTING REQUIREMENTS

12.1 Reporting Adverse Experiences and Deaths to the IRB

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

- Unexpected (in terms of nature, severity, or frequency) given (1) the research procedures that are described in the protocol-related documents, such as the IRB-approved research protocol and informed consent document, as well as other relevant information available about the research; (2) the observed rate of occurrence (compared to a credible baseline for comparison); and (3) the characteristics of the subject population being studied; and
- Related or possibly related to participation in the research; and
- Serious; or if not serious suggests that the research places subjects or others at a greater risk of harm (including physical, psychological, economic, or social harm) than was previously known or recognized.

Unrelated, expected deaths do not require reporting to the IRB if they occur > 30 days after last dose of protocol treatment. Though death is "serious", the event must meet the other two requirements of "related or possibly related" and "unexpected/unanticipated" to be considered reportable.

Deaths meeting reporting requirements are to be reported immediately to the IRB and FDA, but in no event later than 48 hours after the investigator first learns of the death. This includes all deaths on treatment, regardless of cause. Unexpected deaths related or possibly related to participation in the research will also be reported to the DSMB.

The following definitions apply with respect to reporting adverse experiences:

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

- results in death;

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

Unexpected Adverse Event (UAE):

- Any adverse event for which the specificity or severity is not consistent with the protocol-related documents, including the applicable investigator brochure, IRB approved consent form, Investigational New Drug (IND) or Investigational Device Exemption (IDE) application, or other relevant sources of information, such as product labeling and package inserts; or if it does appear in such documents, an event in which the specificity, severity or duration is not consistent with the risk information included therein; or
- The observed rate of occurrence is a clinically significant increase in the expected rate (based on a credible baseline rate for comparison); or
- The occurrence is not consistent with the expected natural progression of any underlying disease, disorder, or condition of the subject(s) experiencing the adverse event and the subject's predisposing risk factor profile for the adverse event.

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

External Events: Events experienced by participants enrolled at a site external to the jurisdiction of the St. Jude Institutional Review Board (IRB).

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

Such events, in general, meet all of the following criteria:

- unexpected;
- related or possibly related to participation in the research; and

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

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

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

Examples of unanticipated problems involving risks to subjects or others include:

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

This is an investigator-initiated study. The principal investigator, Tanja Gruber, and St. Jude are conducting the study and acting as the sponsor. Therefore, the legal/ethical obligations of the principal investigator include both those of a sponsor and those of an investigator.

12.2 Reporting to Regulatory Affairs Office and FDA

All treatment-related deaths and any unanticipated fatal or unanticipated life-threatening event judged by the PI to be at least possibly due to the study treatment, will be reported to the FDA by telephone or fax as soon as possible but no later than seven calendar days after notification of the event and followed by a written safety report as complete as possible within eight additional calendar days (i.e. full report 15 calendar days total after notification of event).

Unanticipated, non-fatal and non-life-threatening adverse events that occur in on-study patients and that are considered due to or possibly due to the investigational agent, will be reported to the FDA by written safety report as soon as possible but no later than 15 calendar days of the notification of the occurrence of the event. Expected SAEs, even

unexpected fatal SAEs, considered by the PI to be not related to the study, will be reported to the FDA in the Annual Review Report along with non-serious AEs. All FDA correspondence and reporting will be conducted through the St. Jude Office of Regulatory Affairs.

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

12.3 Recording Adverse Events and Serious Adverse Events

Adverse events (AEs) will be evaluated and documented by the clinical staff and investigators throughout inpatient hospitalizations and each outpatient visit. CRAs are responsible for reviewing documentation related to AEs and entering directly into CRIS protocol-specific database for all adverse events grade 3 or higher. Peripheral neuropathy and infectious complications will be captured if Grade 2 or higher. The data to be recorded are 1) the event description, 2) the NCI CTCAE v4.0 code and grade, 3) the onset date, 4) the resolution date (or ongoing if it has not resolved at time of off study), 4) action taken for event, 5) patient outcome 6) relationship of AE to protocol treatment/interventions, 7) if AE was expected or unexpected, and 8) comments, if applicable. AEs that are classified as serious, unexpected, and at least possibly related will be notated as such in the database as "SAEs". These events will be reported expeditiously to the St. Jude IRB within the timeframes as described above.

Cumulative summary of Grade 2 peripheral neuropathy, Grade 2 infectious complications and all Grades 3-5 events will be reported as part of the progress reports to IRB at the time of continuing review. Specific data entry instructions for AEs and other protocol-related data will be documented in protocol-specific data entry guidelines, which will be developed and maintained by study team and clinical research informatics.

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

12.4 Process for Reporting AEs Between St. Jude and Collaborating Sites

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

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

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

With the submission of the "Reportable Event" in St. Jude iRIS (Integrated Research Information System) application, the PI will indicate if all sites should be notified to report to their IRBs, and if the protocol and/or consent should be amended (consent will be amended if event is information that should be communicated to currently enrolled subjects). Generally, only events that warrant an amendment to the protocol and/or consent will be reported expeditiously to all sites. However, any event may be reported expeditiously to all sites at the discretion of the PI. A cumulative summary of Grade 2 peripheral neuropathy and infectious complications, all Grade 3-5 AEs, and expected/unrelated deaths that occur more than 30 days after protocol treatment will be reported to all sites with study progress report at the time of continuing review.

For collaborating sites: Serious AND unexpected events are to be reported to the St. Jude PI (Dr. Tanja Gruber) within 48-72 hours via fax or email.

All treatment-related deaths and unexpected deaths must be reported to the St. Jude PI at [redacted] or phone call to Dr. Gruber within 24 hours of the event. A written report must follow.

The study team should be copied on all correspondence regarding the event.

Sent report to:

Tanja A. Gruber, MD PhD
Pediatrics Hematology, Oncology, Stem Cell Transplant and Regenerative Medicine
Lucile Packard Children's Hospital, Stanford
Palo Alto, CA
Email: [redacted] or
Email: [redacted]

12.5 Data and Safety Monitoring Board

This study has been referred to the St. Jude Data and Safety Monitoring Board (DSMB) for regular monitoring and will be sent to the DSMB upon approval by the St. Jude CT-SRC and IRB. The DSMB is charged with advising the Director and other senior leaders of St. Jude Children's Research Hospital (SJCRH) on the safety of clinical protocols being conducted by SJCRH investigators and on their continuing scientific validity. Refer to the DSMB Charge and Criteria for Protocol Referral for more information regarding DSMB review.

13.0 DATA COLLECTION, STUDY MONITORING, AND CONFIDENTIALITY

13.1 Data Collection

Electronic case report forms (e-CRFs) will be completed by the clinical trials staff from the Cancer Center Comprehensive Center, Hematological Malignancies Program. Data will be entered from record directly into a secure CRIS database, developed and maintained by St. Jude Clinical Research Informatics.

Data Management will be supervised by the Director of Clinical Trials Management, and Manager of Clinical Research Operations for the Hematological Malignancies Program, working with Dr. Gruber or her designee. All protocol-specific data and all grade 2 peripheral neuropathy and infectious complications and grade 3-5 adverse events will be recorded by the clinical research associates into the CRIS database weekly during induction and reinduction as AE during these phases comprise DLTs. AEs will be recorded by the clinical research associates into the CRIS database within 2-4 weeks of completion of consolidation and Reintensification, and monthly during maintenance. All questions will be directed to the attending physician and/or PI and reviewed at regularly-scheduled working meetings. The attending physicians (or their designees) are responsible for keeping up-to-date roadmaps in the patient's primary SJCRH medical chart.

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

13.2 Data Collection Instructions for Collaborating Sites

Collaborating sites will collect data by using eCRFs via remote electronic data entry. All protocol-specific data and all grade 2 peripheral neuropathy and infectious complications and grade 3-5 adverse events will be recorded by the clinical research associates into the CRIS database weekly during induction and reinduction as AE during these phases comprise DLTs. AEs will be recorded by the clinical research associates into the CRIS database within 2-4 weeks of completion of Consolidation and Reintensification, and monthly during Maintenance.

13.3 Study Monitoring

Monitoring of this protocol is considered to be in the high-risk 3 category (HR-3). The study specific Monitoring Plan is a separate document from this protocol. The study team will meet at appropriate intervals to review case histories or quality summaries on participants. Highlights of the protocol monitoring plan are below:

The Clinical Research Monitor will assess protocol and regulatory compliance as well as the accuracy and completeness of all data points for the first two participants then 15% of study enrollees every six months. Accrual will be tracked continuously for studies that have strata. All SAE reports will be monitored for type, grade, attribution, duration, timeliness and appropriateness on all study participants *semi-annually.*

The monitor will also verify 100% of all data points on the first two participants and on 15% of cases thereafter. Protocol compliance monitoring will include participant status, eligibility, the informed consent process, demographics, staging, study objectives, subgroup assignment, treatments, evaluations, responses, participant protocol status, off-study, and off-therapy criteria. The Monitor will generate a formal report which is shared with the Principal Investigator (PI), study team and the Internal Monitoring Committee (IMC). Monitoring may be conducted more frequently if deemed necessary by administration, the Institutional Review Board (IRB), or the IMC.

Continuing reviews by the IRB and CT-SRC will occur at least annually. In addition, SAE reports in iRIS are reviewed in a timely manner by the IRB/OHSP.

Source document verification of eligibility for all SJCRH cases will be performed within two weeks of completion of enrollment. This will include verification of appropriate documentation of consent. Monitoring of timeliness of serious adverse event reporting will be done as events are reported in iRIS. St. Jude affiliates and collaborating study sites will be monitored on-site by a representative of St. Jude at intervals specified in the Data and Safety Monitoring Plan.

13.4 Confidentiality

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

14.0 STATISTICAL CONSIDERATIONS

14.1 Primary Objective

The primary objective is to determine the tolerability of incorporating bortezomib and Vorinostat into an ALL chemotherapy backbone for newly diagnosed infants with ALL.

Accrual: In this multi-institution collaborative trial, we expect to enroll 50 evaluable patients in 7 years for the tolerability study. To achieve 50 evaluable patients' global enrollment will increase from 50 patients to 60 patients with revision 5.1.

14.1.1 Dose Escalation Phase

Vorinostat, initially at 100mg/m^2/day will be administered on days 1-4, 8-11, and 15-18 and escalated based on tolerability. A classical 3-on-3 dose escalation will be done as a run-in phase. Three patients will be enrolled sequentially at the 100mg/m^2/day level. At any of the first two dose levels (100mg/m^2 or 150mg/m^2), if no dose limiting toxicity (DLT) is observed then we will move on to the next higher does level; if there is 1 DLT then we will enroll 3 more patients at the same dose level, if there is no more than 1/6

DLTs we will then move to the next higher dose level, otherwise deem the current dose level too toxic and use the dosage at one level lower as the maximum tolerated dose (MTD). If at any dose level 2 or more DLTs occur, we will deem the current dose level too toxic and use the dosage at one level lower as the MTD. If the 100mg/m^2 level is deemed intolerable (2/3 or 2/6 DLTs) then we move to dose level -1. If we escalate the dose to the highest dose level at 180mg/m^2 and it is tolerated (0/3 or 1/6 DLT), then this dosage will be used for the remaining patients as the MTD. Patients that are part of the dose escalation cohort will receive the same vorinostat dosing for induction and reinduction.

Dose Level	Vorinostat Dosing
-1	75mg/m^2/day
1*	100mg/m^2/day
2	150mg/m^2/day
3	180mg/m^2/day

*starting dose level

Definitions of Dose-Limiting Toxicities (DLT)

Toxicities will be graded according the CTEP Common Terminology Criteria for Adverse Events (CTCAE) version 4.0. DLT is based on non-hematologic toxicities that occur during induction (planned for 42 days). All three subjects in a cohort must complete the DLT monitoring phase before accrual may resume during the dose escalation phase. Please note elective intubation for procedures and as a precaution in infants with hyperleukocytosis will not be considered a DLT.

Non-Hematologic DLT

- Any Grade 5 event, unless the event is clearly and incontrovertibly due to extraneous causes or disease progression
- Any Grade 4 event , unless the event is clearly and incontrovertibly due to extraneous causes or disease progression with the exception of the following:
    - Grade 4 infection or fever
    - Grade 4 elevation in hepatic transaminases, alkaline phosphatase, GGT, bilirubin, amylase, lipase, and triglycerides
    - Grade 4 electrolyte disturbances that resolve to < grade 3 within 24 hours
    - Grade 4 electrolyte disturbances due to tumor lysis
    - Grade 4 hyperglycemia or hypoglycemia that resolve to < grade 3 within 24 hours
    - Grade 4 intracranial hemorrhage secondary to hyperleukocytosis
    - Grade 4 pulmonary leukostasis syndrome
    - Grade 4 acute kidney injury due to tumor lysis
    - Grade 4 seizures secondary to intrathecal chemotherapy
    - Grade 4 leukoencephalopathy secondary to intrathecal chemotherapy

14.1.2 Dose Expansion Phase

Based on the dose escalation phase, the MTD will be administered to the remaining patients for further evaluation of tolerability and response. Tolerability during the dose expansion phase will be defined as completing induction and reinduction blocks with no dose limiting toxicities as defined in section 14.1.1. A maximum of 50 evaluable patients will be enrolled. As a safety precaution, **enrollment to the study will be suspended intermittently to prevent more than 10 patients being treated simultaneously with induction and/or re-induction therapy.**

Prior experience suggests that the induction/re-induction TRM rate with the current treatment approach is approximately 5% for patients older than 90 days and 10% for younger patients. For this design, a proportion of 10% or less of patients experiencing a dose limiting toxicity will be considered as clinically tolerable and used as a monitoring benchmark.

Monitoring will be performed according to the following group-sequential rule. At each stage the null hypothesis H0: proportion (intolerable) ≤10% is tested against a one-sided alternative H1: proportion (intolerable)>10%. The boundary values are generated based on one-sample, one-sided Z test for a Binomial proportion at the 0.2 overall significance level. If the true proportion of intolerable patients is 20%, this monitoring rule has the power of 0.876 and expected sample size 30 to stop the trial.

Table 7. Monitoring of Intolerability

Interim Sample Size	Suspend if # Intolerable patients ≥	Z value
10	4	2.639
20	4	1.735
30	5	1.370
40	6	1.167
50	7	1.035

This design is generated by EAST6.0 with the following specific parameters: superiority experiment, total sample size 50, 5 equally spaced looks, alternative TRM (π_1) =20%, "spending functions" for boundary family, Lan-DeMets function with O'brien-Feleming parameters, overall type-I error probability=0.20.

This monitoring rule is not prescriptive -- the final decision about halting or continuing the study will rest with the Data and Safety Monitoring Board (DSMB).

The final analyses will also include calculating point estimates and 95% confidence intervals for the rates of TRM and other severe (CTCAE Grade 4) toxicities during Induction and Reinduction. The confidence interval for the TRM rate will be computed using the method adjusting for multiple interim looks[48].

In addition, we will separately monitor the frequency of grade 5 events, grade 4 sepsis, grade 4 hemorrhage, and grade 4 hepatic toxicity across all patients. The stopping boundaries for these specific toxicities are detailed in the table below and are based on

the frequency of these events in the TACL T2005-003 study which most closely resembles the treatment combination given to patients on TINI during induction and reinduction. Grade 4 seizures and leukoencephalopathy will also be monitored across all patients due to the use of methotrexate for CNS control in all phases of treatment. We consider a frequency of 10% or less (seizures and leukoencephalopathy combined) to be clinically tolerable. Therefore, the stopping boundaries included in Table 8 for these events are equivalent to the boundaries listed in Table 7 for DLTs. Grade 4 and 5 events that are clearly and incontrovertibly due to extraneous causes or disease progression will be excluded from these stopping boundaries.

Table 8. Monitoring of Specific Toxicities

Toxicity	#patients	Suspend if # of patients with monitored toxicities ≥
Grade 5 event (death)	5	1
	10	2
	15	3
	20	4
	30	5
	40	6
	50	7
Grade 4 sepsis	5	3
	10	6
	15	8
	20	10
	30	15
	40	20
	50	25
Grade 4 hemorrhage	5	2
	10	3
	15	5
	20	6
	30	9
	40	12
	50	15
Grade 4 hepatic toxicity	5	3
	10	6
	15	8
	20	10
	30	15
	40	20
	50	25
Grade 4 seizure or leukoencephalopathy	10	4
	20	4
	30	5
	40	6
	50	7

14.2 Statistical Analyses and Power for the Secondary Objectives

To estimate the event-free survival and overall survival of infants with ALL who are treated with bortezomib and vorinostat in combination with an ALL chemotherapy backbone.

Event-free survival (EFS) and overall survival (OS) functions will be estimated by the Kaplan-Meier estimator. For EFS, relapse and second malignancies will be considered as failures in addition to death in complete remission. The time to EFS will be set to 0 for patients who fail to achieve complete remission. EFS and OS probabilities at years 3, 5, 10 will be estimated with 95% confidence intervals.

We will monitor the possible worsened 4-year EFS starting at the 5th adverse event, and for every 5th adverse event as defined in the EFS calculation we shall calculate the 95% upper confidence bound of the 4-year EFS; if this bound is below 19% for MLLr younger than 3 month old, 44% for MLLr 3 month or older, or 75% for no MLL rearrangement patients, we will suspend enrollment and investigate.

The fourth amendment allows patients that are MRD positive at the end of consolidation to receive CART. Based on current enrollment and MRD data for patients on study, there will be likely less than 5 patients who receive CAR-T. If there are 10 or more patients that receive CAR-T, we will secondarily estimate the EFS and OS at years 3, 5 and 10, in the respective sub-cohorts of patients who did and/or did not receive CAR-T cell therapy, using point estimates and the 95% confidence interval.

To measure minimal residual disease (MRD) positivity using both flow cytometry and PCR.

MRD status and levels at the end of each therapy block will be summarized by descriptive statistics such as proportion (percentage) of MRD positive patients, mean and median levels of MRD and standard deviation.

To compare end of induction, end of consolidation, and end of reinduction MRD levels to Interfant99.

The preliminary data from TOTXVI indicate that 6 out of 6 infants achieved MRD negative status at the end of induction, giving the Blyth-Cassella 99% lower confidence bound of the probability of negative MRD status as 0.46 (46%). From Figures 1b and 3 of Van der Velden et al.[12] the point estimate of the MRD negative probability at the end of induction, consolidation and reinduction (corresponding to TP2, TP3 and TP4 therein respectively) is 0.43, 0.7, 0.89 respectively for the Interfant99 protocol, with respective sample size 81,60, 38. The following table shows the statistical power of the two-sample two-sided Z test at the 5% significance level in several scenarios. We are fully aware that the power is limited in several cases where the difference is small.

Table 8 Statistical power for the MRD comparison

	Num. pts n1 Interfant99	n2 TINI	π1 Interfant99	π2 TINI	Power
TP2 vs. end of Induction	81	50	0.43	0.9	1.00
TP2 vs. end of Induction	81	50	0.43	0.65	0.71
TP3 vs. end of Consolid.	60	50	0.7	0.95	0.96
TP3 vs. end of Consolid.	60	50	0.7	0.85	0.49
TP4 vs. end of Re-Induct.	38	50	0.89	0.99	0.48

The power was calculated using EAST6.0 with the following parameters: Asymptotic; nt/nc=n2/n1 (n1, n2 in the above table); Unpooled Estimate for variance.

14.3 Statistical Analyses for the Exploratory Objectives

To measure histone acetylation, ubiquitination, and methylation in leukemic blasts pre and post treatment with bortezomib and vorinostat

Assess NF-kB activity and proteasome inhibition pre and post treatment with bortezomib

These pre-post molecular changes will be summarized by descriptive statistics, and compared using the paired t test or rank-based procedures.

To assess the prognostic value of MRD by deep sequencing.

Association between MRD determined MRD levels and relapse and EFS will be modeled and tested by Fine-Gray and Cox hazard rate regression models.

Identify all genomic lesions by comprehensive whole genome, exome and transcriptome sequencing on all patients.

Population frequencies of the identified genomic lesions will be modeled as Binomial proportions and estimated by exact methods (e.g., Blyth-Cassela confidence intervals).

Evaluate the sensitivity of patient blasts in vitro to a panel of highly active agents identified by a high throughput drug screen on primary infant ALL specimens.

Responses to *in vitro* treatments (e.g., IC50) will be summarized by descriptive statistics, possibly after a proper transformation (e.g., $\log_{10}$) if needed.

Identify subclones in patients with detectable minimal residual disease by next generation deep sequencing and Determine clonal evolution of relapsed patients by next generation sequencing

These two objectives are in a fast-evolving area capitalizing on the maturation of the whole-genome sequencing technologies. We will utilize the latest and most effective approach to detecting subclones and modeling clonal evolution at the time of analysis.

Study immune repertoire diversity over the course of treatment by deep sequencing of lymphocyte variable regions.

The immune repertoire diversity will be estimated and described by the proportions of each observed variant in lymphocytes. Trajectory of each variant along time will be summarized and displayed graphically.

15.0 OBTAINING INFORMED CONSENT

15.1 Informed Consent Prior to Research Interventions

Initially, informed consent will be sought for obtaining extra bone marrow specimens for research tests, and for other procedures as necessary for standard medical care.

15.2 Consent at Enrollment

The process of informed consent for TINI will follow institutional policy. The informed consent process is an ongoing one that begins at the time of diagnosis and ends after the completion of therapy. Informed consent should be obtained by the attending physician or his/her designee, in the presence of at least one non-physician witness. Initially, informed consent will be sought for the acquisition of bone marrow specimens, blood transfusion and other procedures as necessary. After the diagnosis of leukemia is established, we will invite the patient to participate in the TINI protocol.

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

15.3 Consent When English is Not the Primary Language

When English is not the participant, parent, or legally authorized representative's primary language, the Social Work department will determine the need for an interpreter. This information will be documented in the participant's medical record. Either a certified interpreter or a telephone interpreter's service will be used to translate the consent information.

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APPENDIX I: TREATMENT/EVALUATION CALENDARS

REMISSION INDUCTION (approximately 42 days)

1 LP, **ITMHA** **LV rescue** **DEX** **VORINO** **BORTEZ** *See Section 8.1 for required pre-study tests and research tests (BMA and PB biology studies)*	2 **DEX** **VORINO**	3 **DEX** **VORINO**	4 LP, **ITMHA(*)** **LV rescue** **DEX** **VORINO** **BORTEZ** CSF studies PB – histone analysis	5 **PEG-ASP**	6	7
8 LP, **ITMHA,** **LV rescue** **DEX** **VORINO** **BORTEZ** **MITO** CSF studies	9 **DEX** **VORINO** **MITO**	10 **DEX** **VORINO**	11 LP, **ITMHA(*)** **LV rescue** **DEX** **VORINO** **BORTEZ** CSF studies	12	13	14
15 LP, **ITMHA** **LV rescue** **DEX** **VORINO** **BORTEZ** CSF studies	16 **DEX** **VORINO**	17 **DEX** **VORINO**	18 **DEX** **VORINO** **BORTEZ** PB – histone analysis	19	20	21
22 LP, **ITMHA** **LV rescue** **CYCLO** **CYCLO** **6MP** BMA and MRD MRD NGS CSF studies	23 **CYCLO** **CYCLO** **ARA-C** **6MP**	24 **ARA-C** **6MP**	25 **ARA-C** **6MP**	26 **ARA-C** **6MP**	27 **6MP**	28 **6MP**
29 **6MP**	30 **ARA-C** **6MP**	31 **ARA-C** **6MP**	32 **ARA-C** **6MP**	33 **ARA-C** **6MP**	34 **6MP**	35 **6MP**
36	37	38	39	40	41	42

[1]**During induction, it is critical to monitor chemistries frequently due to tumor lysis**. At a minimum, they should be checked daily until values are stable and within normal limits for 48-72 hours following Day 9 mitoxantrone. Patients with high tumor burden may need tumor lysis labs as frequently as every 4-6 hours. Once stable, obtain as clinically indicated. Contact the PI or co-PI if there are questions or concerns. Physical exam every 3-7 days; CBC w/diff weekly or as clinically indicated; Chemistries, UA as clinically indicated. *CSF studies with each IT treatment

Bone marrow/clinical MRD and MRD NGS Day 22. PB biology – NFKB pre-tx, 6 hr and 24 hour; histone analysis and immune repertoire (See section 8.2.). **See Section 8.1 for Pre-study evaluations – required within 1 week prior to study entry**.

CONSOLIDATION (approximately 56 days)

1 LP, **ITMHA LV** **rescue** **HDMTX#1** **6MP** PE, CBC, chemistries, IgG, vitD CXR (med mass at dx), CSF studies MRD and MRD NGS Germline DNA PB – histone analysis	2 **6MP**	3 **6MP**	4 **6MP**	5 **6MP**	6 **6MP**	7 **6MP**
8 **6MP** PE, CBC, diff Chemistries	9 **6MP**	10 **6MP**	11 **6MP**	12 **6MP**	13 **6MP**	14 **6MP**
15 LP, **ITMHA LV** **rescue** **HDMTX#2** **6MP** PE, CBC, diff chemistries, CSF studies, BMA/MRD and MRD NGS**	16 **6MP**	17 **6MP**	18 **6MP**	19 **6MP**	20 **6MP**	21 **6MP**
22 **6MP** PE, CBC, diff Chemistries	23 **6MP**	24 **6MP**	25 **6MP**	26 **6MP**	27 **6MP**	28 **6MP**
29 LP, **ITMHA LV** **rescue** **HDMTX#3** **6MP** PE, CBC, diff Chemistries, CSF studies, BMA MRD and MRD NGS**	30 **6MP**	31 **6MP**	32 **6MP**	33 **6MP**	34 **6MP**	35 **6MP**
36 **6MP** PE, CBC, diff Chemistries	37 **6MP**	38 **6MP**	39 **6MP**	40 **6MP**	41 **6MP**	42 **6MP**
43 LP, **ITMHA LV** **rescue** **HDMTX#4** **6MP** PE, CBC, diff Chemistries, CSF studies, BMA/MRD and MRD NGS**	44 **6MP**	45 **6MP**	46 **6MP**	47 **6MP**	48 **6MP**	49 **6MP**
50 **6MP** PE, CBC, diff Chemistries	51 **6MP**	52 **6MP**	53 **6MP**	54 **6MP**	55 **6MP**	56 **6MP**

Notes: CBC w/diff weekly; electrolytes/BUN/creatinine weekly; bilirubin/AST/ALT/TP/Albumin weekly; Uric Acid/LDH/Phos/Ca/Mg before each HDMTX; glucose/UA as indicated. PE weekly

*IT methotrexate + hydrocortisone + cytarabine, to be given on days 1, 15, 29, and 43; IT therapy should be given on same day as HDMTX administration (consult PI or PK if the IT and HDMTX become separated by more than 12 hours. **CSF studies with each IT.****Patients who are MRD positive on day 1 should have a repeat marrow on day 15 and with every HDMTX thereafter until they become MRD negative. Rising MRD is an indication for transplant.**

REINDUCTION

1 LP, **ITMHA LV rescue*** **MITO** **DEX** **BORTEZ** **VORINO** PE IgG CSF studies BMA/MRD and MRD ECHO OR MUGA NGS PB – immune repertoire	2 **MITO** **DEX** **VORINO**	3 **PEG-ASP** **DEX** **VORINO**	4 **DEX** **BORTEZ** **VORINO**	5	6	7
8 **DEX** **BORTEZ** **VORINO**	9 **DEX** **VORINO**	10 **DEX** **VORINO**	11 **DEX** **BORTEZ** **VORINO**	12	13	14
15 LP, **ITMHA(**)** **LV rescue** **DEX** **BORTEZ** **VORINO** CSF studies if CNS3	16 **DEX** **VORINO**	17 **DEX** **VORINO**	18 **PEG-ASP** **DEX** **BORTEZ** **VORINO**	19	20	21
22	23	24	25	26	27	28
29	30	31	32	33	34	35

PE weekly, clinical hematology and chemistry labs as indicated.
*Leucovorin rescue only in patients with prior CNS toxicities or in patients with WBC<1500/mm^3 or ANC<500/mm^3
**CNS3 patients only

RE-INTENSIFICATION - High-Risk Patients only*

1 **DEX** **ARA-C** **ARA-C** PE, IgG, BMA/MRD and MRD NGS PB – immune repertoire	2 **DEX** **ARA-C** **ARA-C**	3 **DEX** **VP16**	4 **DEX** **VP16**	5 LP, I**TMHA(*)** **LV rescue**** **DEX** **VP16** CSF studies	6 **PEG-ASP**	7
8	9	10	11	12	13	14
15	16	17	18	19	2-	21
22	23	24	25	26	27	28

*Patients with MRD >0.01% on day 1 of Reinduction, OR patients with increasing MRD during Consolidation phase.
** Leucovorin rescue only in patients with prior CNS toxicities or in patients with WBC <1500/mm^3 or ANC <500/mm^3

MAINTENANCE

Week	Cycle	Treatment	Schedule
1	1*	LP, ITMHA DEX + VCR + 6MP	Day 1: ITMHA CNS 1,2,3 Days 1-5: DEX Day 1: VCR Days 1-28: 6MP Days 8, 15, 22: MTX
2		6MP + MTX	
3		6MP + MTX	
4		6MP + MTX	
5	2	LP, ITMHA DEX + VCR + 6MP	Day 1: ITMHA CNS 1,2,3 Days 1-5: DEX Day 1: VCR Days 1-28: 6MP Days 8, 15, 22: MTX
6		6MP + MTX	
7		6MP + MTX	
8		6MP + MTX	
9	3	LP, ITMHA DEX + VCR + 6MP	Day 1: ITMHA CNS 1,2,3 Days 1-5: DEX Day 1: VCR Days 1-28: 6MP Days 8, 15, 22: MTX
10		6MP + MTX	
11		6MP + MTX	
12		6MP + MTX	
13	4	LP, ITMHA DEX + VCR + 6MP	Day 1: ITMHA CNS 1,2,3 Days 1-5: DEX Day 1: VCR Days 1-28: 6MP Days 8, 15, 22: MTX
14		6MP + MTX	
15		6MP + MTX	
16		6MP + MTX	
17	5*	LP, ITMHA DEX + VCR + 6MP	Day 1: ITMHA CNS 1,2,3 Days 1-5: DEX Day 1: VCR Days 1-28: 6MP Days 8, 15, 22: MTX
18		6MP + MTX	
19		6MP + MTX	
20		6MP + MTX	
21	6	LP, ITMHA DEX + VCR + 6MP	Day 1: ITMHA CNS 1,2,3 Days 1-5: DEX Day 1: VCR Days 1-28: 6MP Days 8, 15, 22: MTX
22		6MP + MTX	
23		6MP + MTX	
24		6MP + MTX	
25	7	LP, ITMHA DEX + VCR + 6MP + MTX	Day 1: ITMHA CNS 1,2,3 Days 1-5: DEX Day 1: VCR Days 1-28: 6MP Days 8, 15, 22: MTX
26		6MP + MTX	
27		6MP + MTX	
28		6MP + MTX	
29	8	LP, ITMHA DEX + VCR + 6MP	Day 1: ITMHA CNS 1,2,3 Days 1-5: DEX Day 1: VCR Days 1-28: 6MP Days 8, 15, 22: MTX
30		6MP + MTX	
31		6MP + MTX	
32		6MP + MTX	
33	9	LP, ITMHA(*) DEX + VCR + 6MP	Day 1: ITMHA CNS 2,3 Days 1-5: DEX Day 1: VCR Days 1-28: 6MP Days 1 (CNS1**), 8, 15, 22: MTX
34		6MP + MTX	
35		6MP + MTX	
36		6MP + MTX	

Leucovorin rescue following IT MHA only in patients with prior CNS toxicities or in patients with WBC<1500/mm^3 or ANC<500/mm^3. *PB for biology studies Cycles 1, 5, 10 and 15.

****<u>Methotrexate</u> will be given on day 1 of cycles that do not have an intrathecal treatment. For CNS1 patients this is cycles 9-20, CNS2 patients cycles 12-20, and CNS3 or traumatic LP cycles 14-20.**

MAINTENANCE - continued

Week	Cycle	Treatment	
37	10*	LP, ITMHA(*) DEX + VCR + 6MP	Day 1: ITMHA CNS 2,3 Days 1-5: DEX Day 1: VCR Days 1-28: 6MP Days 1 (CNS1**), 8, 15, 22: MTX
38		6MP + MTX	
39		6MP + MTX	
40		6MP + MTX	
41	11	LP, ITMHA(*) DEX + VCR + 6MP	Day 1: ITMHA CNS 2,3 Days 1-5: DEX Day 1: VCR Days 1-28: 6MP Days 1 (CNS1**) 8, 15, 22: MTX
42		6MP + MTX	
43		6MP + MTX	
44		6MP + MTX	
45	12	LP, ITMHA(*) DEX + VCR + 6MP	Day 1: ITMHA CNS 3 Days 1-5: DEX Day 1: VCR Days 1-28: 6MP Days 1 (CNS1,2**), 8, 15, 22: MTX
46		6MP + MTX	
47		6MP + MTX	
48		6MP + MTX	
49	13	LP, ITMHA(*) DEX + VCR + 6MP	Day 1: ITMHA CNS 3 Days 1-5: DEX Day 1: VCR Days 1-28: 6MP Days 1 (CNS1,2**),8, 15, 22: MTX
50		6MP + MTX	
51		6MP + MTX	
52		6MP + MTX	
53	14	DEX + VCR + 6MP	Days 1-5: DEX Day 1: VCR Days 1-28: 6MP Days 1, 8, 15, 22: MTX
54		6MP + MTX	
55		6MP + MTX	
56		6MP + MTX	
57	15*	DEX + VCR + 6MP	Days 1-5: DEX Day 1: VCR Days 1-28: 6MP Days 1, 8, 15, 22: MTX
58		6MP + MTX	
59		6MP + MTX	
60		6MP + MTX	
61	16	DEX + VCR + 6MP	Days 1-5: DEX Day 1: VCR Days 1-28: 6MP Days 1, 8, 15, 22: MTX
62		6MP + MTX	
63		6MP + MTX	
64		6MP + MTX	
65	17	DEX + VCR + 6MP	Days 1-5: DEX Day 1**: VCR Days 1-28: 6MP Days 1,8, 15, 22: MTX
66		6MP + MTX	
67		6MP + MTX	
68		6MP + MTX	
69	18	DEX + VCR + 6MP	Days 1-5: DEX Day 1: VCR Days 1-28: 6MP Days 1, 8, 15, 22: MTX
70		6MP + MTX	
71		6MP + MTX	
72		6MP + MTX	
73	19	DEX + VCR + 6MP	Days 1-5: DEX Day 1: VCR Days 1-28: 6MP Days 1, 8, 15, 22: MTX
74		6MP + MTX	
75		6MP + MTX	
76		6MP + MTX	
77	20	DEX + VCR + 6MP	Days 1-5: DEX Day 1: VCR Days 1-28: 6MP Days 1, 8, 15, 22: MTX
78		6MP + MTX	
79		6MP + MTX	
80		6MP + MTX	

Leucovorin rescue following IT MHA only in patients with prior CNS toxicities or in patients with WBC<1500/mm^3 or ANC<500/mm^3. BMA/MRD and MRD NGS at End of Therapy and at time of relapse. Tumor NGS and Drug Sensitivity studies at time of relapse. ***Methotrexate** will be given on day 1 of cycles that do not have an intrathecal treatment. For CNS1 patients this is cycles 9-20, CNS2 patients cycles 12-20, and CNS3 or traumatic LP cycles 14-20.**

APPENDIX II: TARGETED KINASE INHIBITORS AND INHIBITORS OF EPIGENETIC MARKS

Bendamustine
BEZ-235 (PI3K/MTOR dual inhibitor)
Bortezomib
CAL-101 (PI3Ki)
Clofarabine
Cytarabine
Daunorubicin
Decitabine
Dexamethasone
Doxorubicin
EPZ-5676 (DOT1Li)
Etoposide
JQ1 (BRDi)
L-Asparaginase
Mercaptopurine
Methotrexate
Methylprednisolone
Mitoxantrone
MLN4924
Panobinostat
PD-325901 (MEKi)
Prednisolone
Quizartinib (class III receptor TKI)
Sorafenib (TKI)
Thioguanine
Torin1 (mTORi)
Vincristine
Vinorelbine
Vorinostat

APPENDIX III: VORINOSTAT COMPOUNDING

Use compounding equipment designated for hazardous drugs and wear gloves and mask while preparing			
Product Description	**Recipe**		**References**
	Items Needed	**Preparation Instructions**	**References**
Name: Vorinostat **Strength**: 50 mg/ml **Dosage Form**: **Suspension Route**: PO **Stability**: expires 28 days after preparation **Packaging**: package in tight light-resistant *GLASS* bottles **Storage**: Room Temperature	#20 Vorinostat 100 mg capsules 20ml Ora-Plus 20ml Ora-Sweet	1. Place 20ml of Ora-Plus (Paddock Labs brand) in glass bottle. 2. Place contents of 20 vorinostat 100mg capsules in same bottle and shake to dispense. Note: shaking could take upwards of 3 minutes. 3. Add 20ml of Ora-Sweet (Paddock Labs brand) and shake to dispense.	REF: unpublished information from CTEP pediatric clinical trials provided by Merck. Ref: Reviewed date: 04/2011 Date Updated: 04/2009
Please use a Biological Safety Cabinet			

Date	Control number	Exp. Date	Total Qty Prepared	Manufacturer						Initials		
				Name	Manufacturer	NDC	Lot No.	Exp Date	Qty	Prep	Check	Final Check
				Vorinostat 100 mg capsules								
				Ora-Plus								
				Ora-Sweet								
				Vorinostat 100 mg capsules								
				Ora-Plus								
				Ora-Sweet								
				Vorinostat 100 mg capsules								
				Ora-Plus								
				Ora-Sweet								
				Vorinostat 100 mg capsules								
				Ora-Plus								
				Ora-Sweet								
				Vorinostat 100 mg capsules								
				Ora-Plus								
				Ora-Sweet								
				Vorinostat 100 mg capsules								
				Ora-Plus								
				Ora-Sweet								

Smaller volumes may be prepared to avoid wasting capsules.

APPENDIX IV: TESTS/EVALUATIONS FOR CLINICAL CARE AND FOR RESEARCH

Clinical care

- All drugs/agents (FDA approved or FDA-approved/off-label)
- History and physical exams
- CBC w/diff
- Chemistry profile
- Coagulation screen
- Urinalysis
- Lipid screen
- Thyroid function tests
- Total IgG level
- 25-OH Vitamin D level
- Chest X-ray
- Lumbar puncture with CSF examination
- Bone marrow: morphology cytochemistry, immunophenotyping, cytogenetics, DNA index molecular diagnosis and MRD studies
- Echocardiogram or MUGA
- Electrocardiogram (EKG)
- Methotrexate levels

Research

- Tumor next generation sequencing
- Drug sensitivity studies
- MRD next generation sequencing
- Peripheral blood for biologic studies (NFκB/proteasome, histone analysis, immune repertoire)
- Germline DNA