St. Jude
TSALV ID NCT02518750
Initial version, dated: 12-23-2014. Resubmitted to CT-SRC 03-31-2015. Resubmitted to IRB 05-08-2015. (IRB approved: 06-03-2015).
Revision 0.1, dated: 05-14-2015 (IRB approved: 06-04-2015)
Revision 0.2, dated: 07-15-2015 (IRB approved: 07-15-2015)
Activation date: 12-09-2015
Amendment 1.0, dated: 12-14-2015. Resubmitted to IRB 01-28-2016
(IRB approved: 02-01-2016)
Activation date: 03-30-2016

A PHASE II STUDY INCORPORATING PANOBINOSTAT, BORTEZOMIB, AND LIPOSOMAL VINCRISTINE INTO RE-INDUCTION THERAPY FOR RELAPSED PEDIATRIC T-CELL ACUTE LYMPHOBLASTIC LEUKEMIA OR LYMPHOMA

IND# 126,426

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PROTOCOL SUMMARY

TSALV, A PHASE II STUDY INCORPORATING PANOBINOSTAT, BORTEZOMIB, AND LIPOSOMAL VINCRISTINE INTO RE-INDUCTION THERAPY FOR RELAPSED PEDIATRIC T-CELL ACUTE LYMPHOBLASTIC LEUKEMIA OR LYMPHOMA

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Brief overview: This is a phase-II study to evaluate the efficacy of a salvage regimen consisting of liposomal vincristine, mitoxantrone, Peg-asparaginase, ITMHA, dexamethasone, panobinostat, and bortezomib in children with relapsed T-cell ALL or lymphoma.

Intervention: Systemic chemotherapy, intrathecal chemotherapy, panobinostat, bortezomib, and HSCT for children with relapsed T-cell ALL or lymphoma.

Brief outline of treatment plan: Three Block Induction**:**

Block A: approximately 5 weeks

- Dexamethasone 10 mg/m^2/day PO Days 1-8, 15-22 (Total 16 days)
- Panobinostat 24 mg/m^2/dose PO Day 2,4,6
- Liposomal vincristine (VSLI) 2.25 mg/m^2 no cap IV on Days 7, 14, 21, 28
- Mitoxantrone 10 mg/m^2 IV Day 7,14 (In the absence of peripheral blasts, Day 14 Mitoxantrone will be given if WBC $\geq$1000 and ANC $\geq$300)
- Peg-asparaginase 2500 units/m^2 on Days 9,23
- Bortezomib 1.3 mg/m^2 IV Days 16, 19, 23, 26
- ITMHA Days 1, 7, 14, 21, 28. Additional ITs on Days 10 and 17 for patients with CNS 2, 3 or traumatic tap with blasts

Block B: approximately 5 weeks

- High-dose methotrexate 8 g/m^2 IV over 24 hours (will not be given to patients with prior cranial irradiation) Day 1
- 6-mercaptopurine 50 mg/m^2 PO days 1-14
- ITMHA Day 1
- High-dose cytarabine 3 g/m^2 IV Q12H Days 15 and 16

Block C: approximately 3 weeks

- Nelarabine 650 mg/m^2/day IV Days 1-5
  (Clofarabine 40 mg/m^2/day IV Days 1-5 will be given instead of nelarabine for patients with B-lymphoblastic leukemia and lymphoma in stratum II)
- Cyclophosphamide 300 mg/m^2 IV Days 1-5
- Etoposide 100 mg/m^2/day IV Days 1-5

Response evaluation is performed after the end of each treatment block. All patients should proceed to HSCT after achieving negative MRD when a suitable donor is identified. Patients could continue on Block B and Block C if not ready for HSCT. If after completion of Block C MRD is persistently positive, the plan will be discussed with the PI/co PI and the transplant team. Enrollment on ongoing NK cell studies will be considered. For patients who require a second transplant, HAP3R may be an option. Donor will be selected according to institutional practices and transplant regimens will be used according to institutional HSCT protocols and guidelines.

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Study design:
Primary objective: To evaluate the complete remission (CR) rate for patients with T-cell lymphoblastic leukemia or lymphoma in first relapse: n=24. Point estimate and the Blyth-Casella exact 95% confidence interval of the CR rate will be computed. Secondarily a comparison to the historical CR rate (R16 and R17 combined) will be performed by Fisher's exact test.

Historical data:

Outcome in R16 and R17 T-cell patients

	R16 (n = 9)	R17 (n= 7)	Total
CR	7 (77.8%)	5 (71.4%)	12 (75.0%)
No CR	2 (22.2%)	2 (28.6%)	4 (25.0%)
Total	9	7	16

Stratum II: Patients with B-cell or T-cell lymphoblastic leukemia or lymphoma in second or third relapse, or refractory to 2 or 3 induction or re-induction attempts; n=10 patients for descriptive analyses only (exploratory objectives).

Sample size:
T-cell lymphoblastic leukemia or lymphoma in first relapse: n=24 evaluable patients
B-cell or T-cell lymphoblastic leukemia or lymphoma in second or greater relapse: n=10 evaluable patients. Total enrollment: n=40

Data management: Data management and statistical analysis will be provided locally by the Comprehensive Cancer Center Hematological Malignancies Program, and Biostatistics Department at St. Jude Children's Research Hospital.

Human subjects: The main risk to research participants will be the potential toxicities associated with the use of intensive salvage chemotherapy followed by HSCT. The research participants will be informed of the toxicities, which have been associated with the study drugs and potential side effects of procedures recommended in this study. Adverse events will be monitored, treated, and reported following institutional and federal guidelines and regulations.

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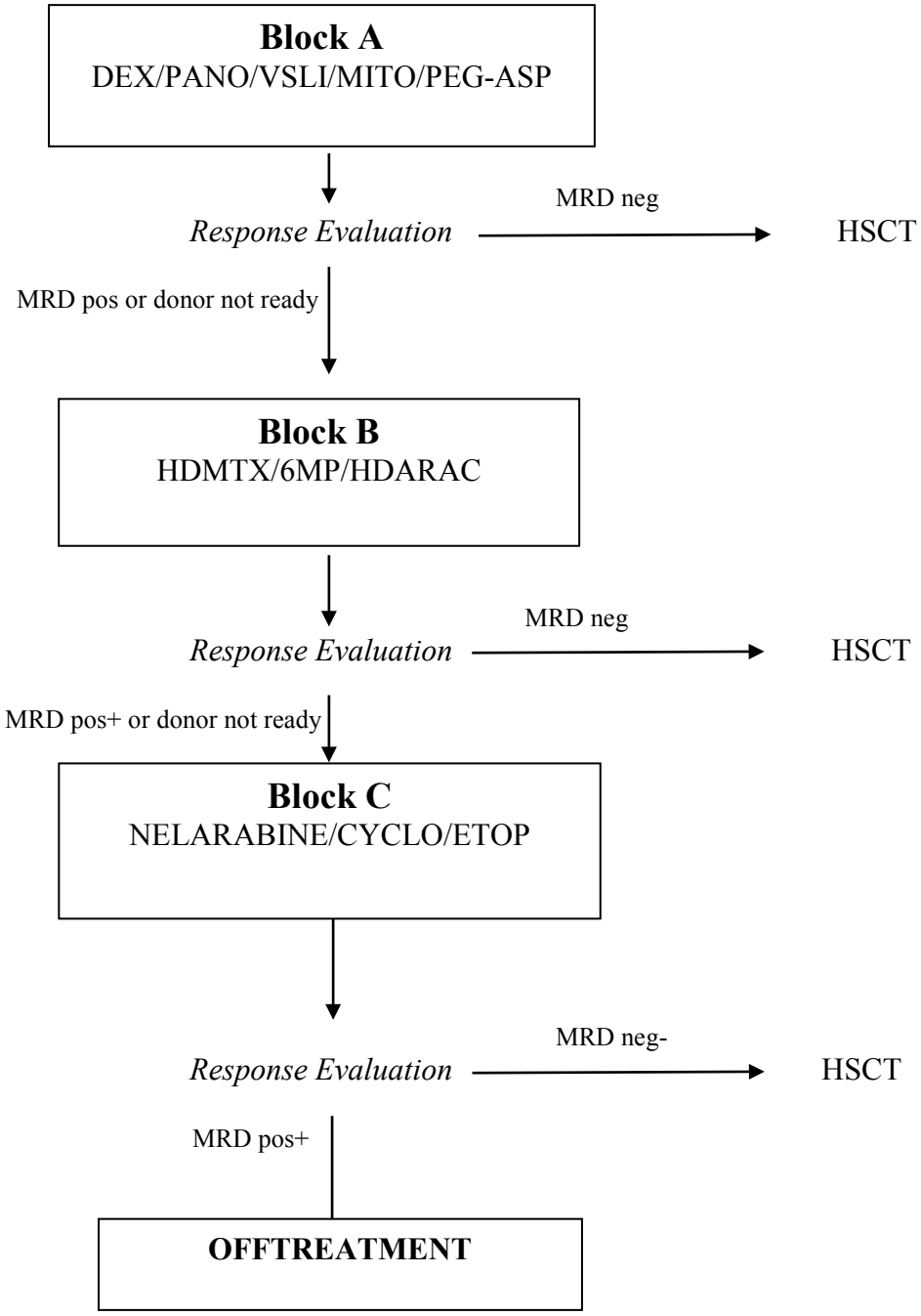
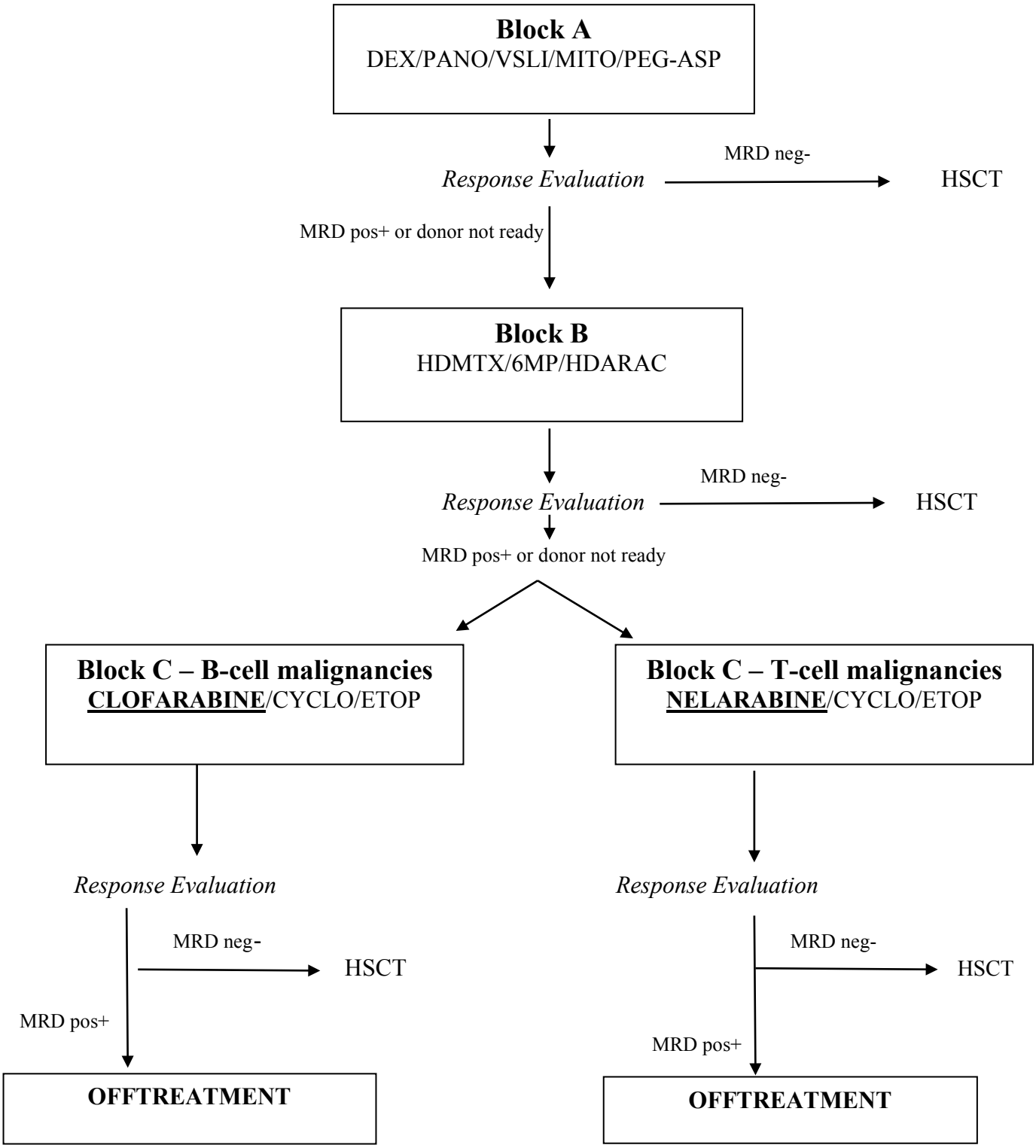
Stratum I: T-cell leukemia or lymphoma in 1st relapse



Stratum II: B-cell or T-cell lymphoblastic leukemia or lymphoma in 2ND or greater relapse



1.0 OBJECTIVES

The overall objective of this study is to improve the cure rate of relapsed T-cell lymphoblastic leukemia and lymphoma.

1.1 Primary Objective

To estimate the complete remission (CR) rate for patients with T-cell lymphoblastic leukemia and lymphoma in first relapse.

1.2 Secondary Objectives

1.2.1 To evaluate minimal residual disease (MRD) levels at end of each block of therapy.

1.2.2 To describe the toxicities of vincristine sulfate liposome injection (VSLI) when used in combination with chemotherapy and bortezomib.

1.3 Exploratory Objectives

1.3.1 To identify molecular determinants and biomarkers of histone deacetylase (HDAC) inhibitor response.

1.3.2 To study the pharmacokinetics of panobinostat.

1.3.3 To establish xenografts of relapsed T-cell lymphoblastic leukemia for downstream studies of cytotoxicity and therapy resistance.

1.3.4 To perform genomic studies of leukemic cells at relapse, diagnosis and MRD (where available) to investigate clonal evolution and identify targets for therapeutic intervention.

1.3.5 To validate new markers and methods for MRD detection.

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

1.3.7 To perform a comprehensive analysis of the intestinal microflora in patients with relapsed hematologic malignancies prior to and subsequent to each block of intensive salvage chemotherapy using next generation sequencing technologies as an exploratory approach.

1.3.8 To describe the frequency and severity of gastrointestinal illnesses during 3 blocks of intensive salvage chemotherapy in patients who did and those who did not have changes in intestinal microbiota.

2.0 BACKGROUND AND RATIONALE

2.1 Relapsed T-Cell ALL

Patients with newly diagnosed T-cell ALL treated on contemporary protocols have a 5-year EFS rates approaching 80%[1,2]. However, patients who relapse have a dismal outcome and only 5% to 16% survive their recurrence (Table 1) despite intensive salvage chemotherapy and hematopoietic stem cell transplantation (HSCT). In comparison to B-cell ALL, relapses in patients with T-cell ALL tend to occur early (within 3 years of initial diagnosis), and fewer patients achieve second remission and low levels MRD allowing them to proceed to HSCT. Thus, newer approaches to therapy are warranted.

Table 1: Outcomes for patients with T-cell ALL in first relapse

Study	N	CR2 rates	EFS
COG 1941[3]	17	82%	7% (3-year)
COG AALL01P2[4]	7	29%	0% (1-year)
*ALL REZ BFM 90[5]	73	NR	16.4% (10-year)
SJ ALLR16/17	16	75%	12.5% (3-year)
Italian ALL R-87[6]	15	60%	5% (7-year)
POG 8303[7]	27	81%	NR
Nelarabine phase II[8]	39	46%	NR

**composite of all T-cell ALL subtypes*
NR= not reported

2.2 Vincristine Sulfate Liposome Injection (VSLI, Marqibo®)

Vincristine is a very effective drug in hematologic malignancies and is an important component of therapeutic regimens in childhood ALL. However, to avoid toxicities (in particular neurological toxicities), the dose is capped at 2 mg which can compromise effectiveness especially in adolescents and adults. VSLI is a sphingomyelin and cholesterol based nanoparticle formulation of vincristine that was designed to improve on the pharmacokinetic properties of standard vincristine[9]. It circulates for a longer time in the plasma, accumulates in tumor tissue and slowly releases vincristine in the tumor instead of the systemic circulation. It is tolerated at a higher dose intensity compared to standard vincristine without increased toxicity[9,10]. In a phase II study of 65 adult patients with multiple relapsed ALL, single agent VSLI was administered at a dose of 2.25 mg/m^2 weekly without a dose cap[11]. Complete response was achieved in 13 patients (20%). Of the 10 patients with T-cell ALL treated in this study, 2 (20%) also achieved CR. Based on these results, the FDA granted accelerated approval for VSLI in August 2012 for the treatment of adults with Philadelphia chromosome-negative ALL in second or greater relapse. An ongoing phase III randomized study compares vincristine and VSLI on a standard chemotherapy backbone in adult patients with newly diagnosed ALL (NCT01439347). A pediatric phase I dose escalation study for this agent is nearing completion at the NCI (NCT1222780). At the current dose level (2.25 mg/m^2 weekly), it appears to be safe, tolerable and demonstrates preliminary activity in patients with refractory ALL and solid tumors[12]. In view of these encouraging findings and enhanced pharmacokinetic properties allowing dose intensification of vincristine without increased toxicity, we will incorporate VSLI weekly x 4 doses in Block A therapy of TSALV.

2.3 Panobinostat

2.3.1 Epigenetics and Cancer

In recent years, the importance of epigenetic regulatory mechanisms in normal and malignant cell development have become increasingly evident. The two best described epigenetic gene regulatory mechanisms leading to targeted therapeutic approaches are DNA promoter methylation and histone modification[13].

Abnormal DNA hypermethylation in promoter CpG islands leads to gene silencing. DNA methyltransferase inhibitors (e.g. azacytidine and decitabine) reverse promoter hypermethylation in tumor cells, lead to re-expression of aberrant silenced genes and induce tumor cell death. Both azacytidine and decitabine have demonstrated efficacy in myelodysplastic syndromes leading to FDA approval.

Histone proteins are responsible for the assembly of DNA in the nucleus and are modulated by post-translational modifications such as acetylation and/or phosphorylation. Acetylation of amino acid residues on the histone tails leads to an open configuration of DNA on the histones and increases accessibility of transcription factors to gene promoter regions[14]. Deacytelation has the opposite effect decreasing access of transcription factors to promoter regions. Histone acetylation is mediated by histone acetyl transferases (HATs) while acetyl groups are removed by histone deacetylases (HDACs). HDACs have a critical role in modulating balance between pro and anti-apoptotic proteins. High levels of HDAC enzymes and histone hypoacetylation is a common finding in multiple cancers[14]. HDAC-mediated deacetylation alters the transcriptional activity of nuclear transcription factors including p53, E2F, c-Myc, NF-kB and HIF-1α[15]. Several HDAC inhibitors are in clinical development[15,16]. Vorinostat was approved by the FDA for cutaneous T-cell lymphoma in October 2006.

2.3.2 HDAC Inhibitors in Hematologic Malignancies with a Focus on T-Cell ALL

By using gene expression profiling and small interfering RNA, it was shown by Pearl *et al* in 2005 that HDAC inhibitor exposure to T-lymphoblastic cell lines led to regulation of the expression of several genes (cyclins/cyclin dependent kinases, Bcl-2, caspases etc.), which favored induction of apoptosis and decreased cellular proliferation[17]. Multiple additional studies demonstrate modulation of the extrinsic and intrinsic apoptotic pathways as well as induction of non-apoptotic death (autophagy) by HDAC inhibitors in hematopoietic malignancies[14,18].

Resequencing efforts of matched diagnosis and relapsed ALL samples at St Jude have identified deleterious mutations of *CREBBP* in 20% of relapsed cases[19]. *CREBBP* encodes the CREB-binding protein which is a transcriptional co-activator that mediated glucocorticoid response and also plays a role in histone acetylation. Experiments in glucocorticoid-resistant ALL cell lines (including T-cell ALL) demonstrated sensitivity to vorinostat. Though all the mechanisms of action of HDAC inhibitors in ALL are not known, there is evidence to suggest that they lead to re-expression of pro-apoptotic *BIM* and restore sensitivity to glucocorticoids in resistant xenografts[20]. This finding has been validated in T-cell ALL cell lines at St Jude (Jun Yang, unpublished). In another study of

matched diagnosis and relapse blasts from patients with childhood ALL, a relapse specific gene-expression signature was identified. Using the Connectivity Map database that links disease to corresponding patterns produced by candidate drugs, vorinostat was the top candidate to reverse the relapse-specific signature[21]. Subsequent validation studies using primary patient samples and ALL cell lines demonstrated that vorinostat increased chemosensitivity to commonly used drugs such as prednisone, doxorubicin, cytarabine and etoposide.

Additional evidence of epigenetic deregulation in T-cell ALL is provided by a whole genome sequencing study of early T-cell precursor (ETP) ALL. Forty-five percent of cases were characterized by somatic alterations targeting histone modification (*EZH2, EED, SUZ12* and *EP300*)[22]. Twelve percent of non-ETP T-cell ALL cases also harbored alterations in these genes. *EZH2*, *EED* and *SUZ12* are components of the polycomb repressor complex and play a role in the methylation of histones while *EP300* is a HAT. Loss of function mutations in *EP300* alters the balance in favor of HDACs versus HATs suggesting that HDAC inhibitors may be beneficial.

Panobinostat is pan HDAC inhibitor with promising activity in solid tumors and hematologic malignancies[23]. It is more potent than vorinostat with a longer half-life. In a xenograft model of T-cell ALL, survival of the mice was significantly increased by treatment with panobinostat when compared to mice treated with conventional chemotherapy (vincristine and dexamethasone)[24]. The therapeutic effect of panobinostat was enhanced in combination with vincristine and dexamethasone without increased toxicity. *In vitro* studies at St Jude have demonstrated that panobinostat is highly cytotoxic across T-cell ALL cell lines, including ETP ALL cells at nanomolar concentrations. In addition, combination with steroids was highly synergistic in all T- cell ALL cell lines tested (Jun Yang, unpublished data, Figures 1 and 2). Panobinostat has a favorable clinical activity in phase I and II studies in leukemias and lymphomas, including peripheral T-cell lymphomas[16,25]. The pediatric phase I study in hematologic malignancies in ongoing (NCT 01321346). In TSALV, we will administer panobinostat along with dexamethasone during the first 6 days of therapy (window) to study the effects of panobinostat on dexamethasone sensitivity as well as its cytotoxic effects, pharmacokinetics and pharmacodynamics.

Figure 1: Cell cytotoxicity assays (MTT) of HDAC inhibitors at various concentrations in Loucy cell line (early T-cell precursor ALL). Both panobinostat and vorinostat were cytotoxic, but panobinostat was more potent than vorinostat.

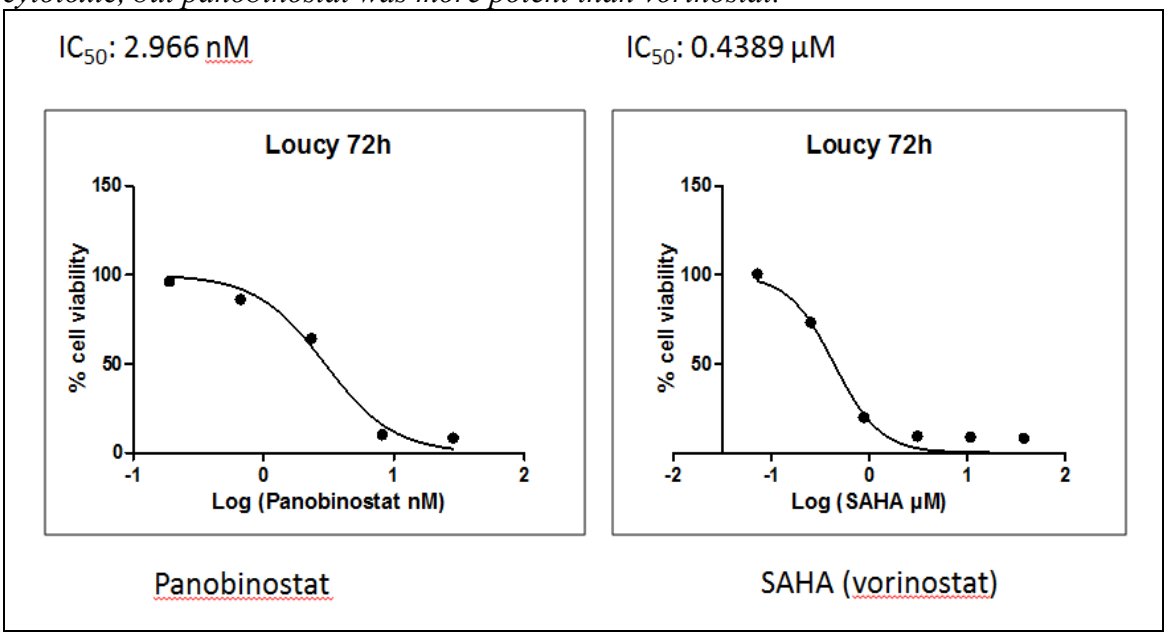
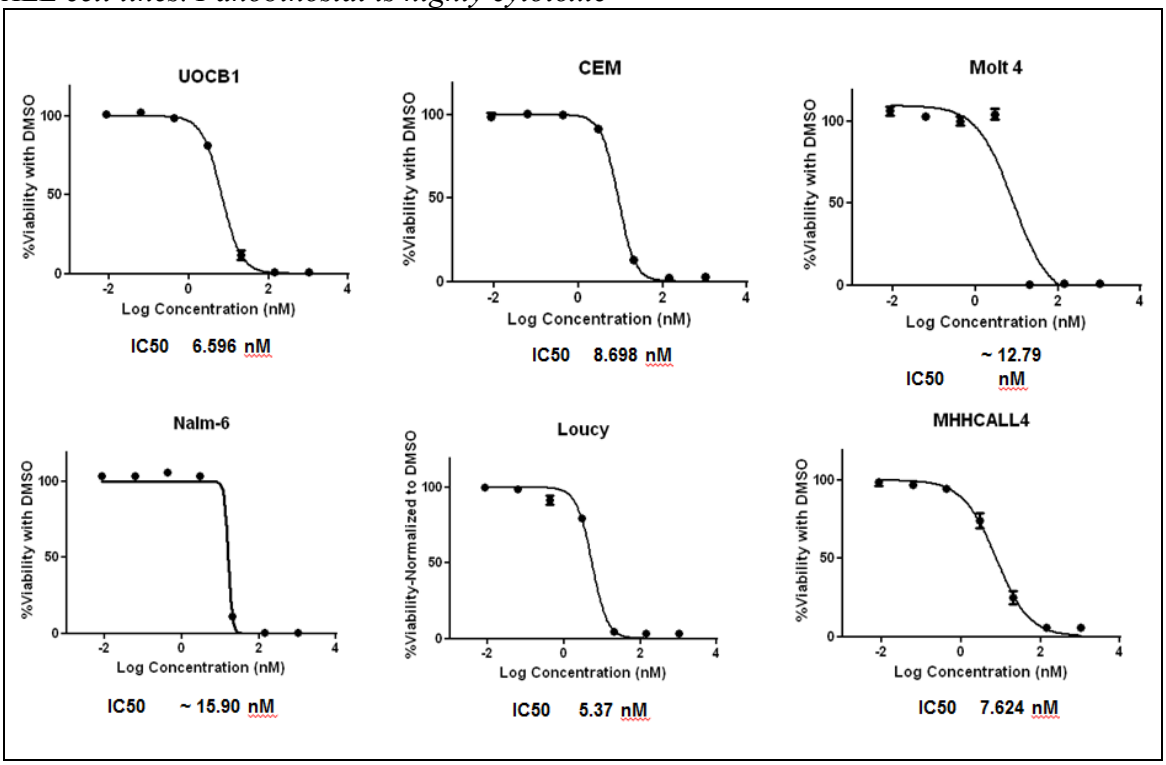


Figure 2: Cell cytotoxicity assays of panobinostat at various concentrations in multiple ALL cell lines. Panobinostat is highly cytotoxic



2.4 Bortezomib

Bortezomib, the first proteasome inhibitor approved by the Food and Drug Administration for multiple myeloma and relapsed non-Hodgkin lymphoma, showed preclinical activity against ALL and demonstrated synergy with dexamethasone[26]. Bortezomib was combined with a standard 4-drug regimen (vincristine, doxorubicin, prednisone, PEG-asparaginase) in the TACL2005-003 study which enrolled 22 patients with relapsed ALL that had failed 2 or 3 prior regimens[27]. The overall response rate was 73% and overall survival at 24 months was 41% ± 13%, which is a significant improvement from historical data[28].

2.5 Selection of Backbone Chemotherapy

2.5.1 Induction Block A

Block A is based on the TACL 2005-003 regimen[26] combining bortezomib and 4 drug induction with following modifications:

- One to two doses of mitoxantrone will be substituted for one dose of doxorubicin based on the UK ALL R3 trial demonstrating that a re-induction regimen incorporating mitoxantrone improved survival (3-year progression-free survival 64.6%) in patients with ALL in first relapse[28].
- Only 2 doses of PEG-asparaginase will be given, instead of 4.
- Dexamethasone will be given on a different schedule (8 days on, 6 days off, 8 days on, 6 days off) instead of on days 1-14.
- IT therapy is a different schedule and MHA is always given (not just Ara-C on Day 1).
- VSLI will be substituted for vincristine.
- A 6-day window of panobinostat and dexamethasone will explore the activity of this regimen in this patient population.

2.5.2 Induction Block B

Block B will comprise of high-dose methotrexate and high-dose cytarabine, which are established active drugs in T-cell leukemias and lymphomas and have excellent penetration into sanctuary sites like the CNS.

A 24-hour infusion of HDMTX was shown to have superior anti-leukemic effect than a 4-hour infusion of HDMTX in T-cell ALL[29]. Doses > 8 g/m^2 have been used in multiple protocols for short infusions; doses of ~ 8 g/m^2/24 hours (targeted to achieve steady-state plasma concentrations (Cpss) of 90 uM) were used in children with ALL in St. Jude Protocol Total XIV, although the number of patients receiving this dose was small[30]. However, we have experience targeting doses of ~ 5 g/m^2/24 hours to a Cpss of 65 uM in over 180 children enrolled on Total XV[31]. There are multiple studies to support use of a higher dose of HDMTX in T-cell ALL[29,32-38]. Thus, our plan is to use the 24 hour infusion and a higher dose of HDMTX, performing intra-infusion targeting of the dose of HDMTX to achieve a Cpss of 90 uM (average expected from 8 g/m^2 over 24 hours).

2.5.3 Induction Block C

Nelarabine is a purine analog with selective cytotoxicity for T-lineage hematologic malignancies. In the COG phase II study for relapsed T-cell ALL, the response rate at the dose of 650 mg/m^2 was 55% (11 CR, 2 partial response [PR]) for patients in first relapse and was 27% (7CR, 1PR) for those in second or greater relapse[8]. In a series of 7 patients with relapsed or refractory T-cell malignancies, nelarabine in combination with etoposide and cyclophosphamide induced complete remission in 5 patients and partial remission in 2 patients.[39] Thus, we will use this promising regimen for induction Block C. In patients with non-T-cell disease clofarabine will be substituted for nelarabine.

2.6 Background and Rationale for Secondary and Exploratory Objectives

2.6.1 Minimal Residual Disease (MRD)

MRD is an excellent surrogate marker for response and survival in childhood ALL at initial diagnosis[40]. At first relapse, studies at St Jude and other institutions have also demonstrated the prognostic implication of positive MRD ($\geq$ 0.01%) at the end of 4-6 weeks of therapy. In St. Jude ALLR11 and ALLR15 studies, the cumulative incidence of second relapse was 70.2% for MRD positive patients ($\geq$ 0.01%) versus 28% for MRD negative patients (p=0.008)[41]. In the COG AALL01P2 study, the 12 month EFS was 80% versus 48% in patients with positive versus negative MRD at the end of the first block of remission induction therapy[4]. The proportion of patients who are MRD positive at the end of re-induction therapy is relatively high: 68% in AALL01P2 and 71% in ALLR17. Thus, in TSALV, we will attempt to decrease the rate of MRD positivity by utilizing a novel induction regimen.

2.6.2 Toxicities of VSLI in Combination with Chemotherapy

Neurotoxicities (especially peripheral neuropathy) of VSLI in the adult single-agent study of patients with multiply relapsed ALL have been characterized and were not increased compared to those associated with standard vincristine despite increased dose intensity and prior exposure to standard vincristine[10]. The patient population in TSALV will be less heavily pre-treated (first relapse) with less baseline neurotoxicity. For detailed characterization of neuropathy, the pediatric-modified total neuropathy scale, balance, manual dexterity and gait testing will be done prior to the first dose of liposomal vincristine and repeated on day 15 (prior to initiation of bortezomib) and after completion of Induction Block A.

2.6.3 Molecular Determinants and Biomarkers of HDAC Inhibitor Response

Molecular analyses of the tumor tissue ideally would help discriminate which tumors will respond to HDAC inhibitor therapy. HR23B is a candidate cancer biomarker identified in a genome-wide loss-of-function screen which influences sensitivity to HDAC inhibitors[42]. High expression of HR23B in biopsies of cutaneous T-cell lymphoma (CTCL) was associated with response. HR23B plays a role in shuttling ubiquitinated proteins to the proteasome. Another retrospective analysis of pretreatment CTCL skin biopsies found that high nuclear STAT1 and phospho-STAT3 staining in lymphoma cells

correlated with lack of clinical response to vorinostat[43]. We will perform genomic profiling studies ex vivo (e.g., global gene expression analyses) of xenografted primary ALL samples before and after HDAC inhibitor treatment to identify additional biomarkers of HDAC inhibitor response.

2.6.4 Histone Acetylation

Pharmacodynamic (PD) studies are incorporated in clinical trials of molecularly targeted agents to determine the relationship between drug exposure and target inhibition in cancer cells. Hyperacetylation of histone H3and H4 has been associated with regulated gene expression and has been used for PD analysis in HDAC inhibitor clinical trials[44-46]. Changes in histone H3 and H4 acetylation in malignant and normal progenitor cells will be assessed pre- and post-treatment in bone marrow and/or blood samples by multi-parameter flow cytometry using cell lineage- and leukemic blast-specific markers.

Optional pharmacodynamic studies will be performed in bone marrow and peripheral blood. Samples will be collected pretreatment and on day 7 (course 1 only) to assess for histone H3 acetylation. Using multi-parameter flow cytometry and lineage and leukemic cell specific markers, H3 acetylation will be assessed in B cells (anti-CD19), T cells (anti-CD3), progenitors (anti-CD34), and leukemic blasts. Flow cytometry will be performed in Dr. Paul Mead's laboratory.

2.6.5 Pharmacokinetics of HDAC Inhibitors

Panobinostat will be administered in combination with dexamethasone, which has the potential to induce panobinostat clearance. Panobinostat pharmacokinetic (PK) studies will be performed on days 2 (first panobinostat dose) and 6 (third panobinostat dose) to characterize the PK profile and determine the effect of dexamethasone on panobinostat PK. The potential for drug-drug interactions will be explored by comparison of panobinostat PK data in the present study with historical PK data of single-agent panobinostat (TACL T2009-012 trial) and PK parameters on days 2 and 6 will be compared. Inter- and intra-patient variability in panobinostat systemic exposure will be characterized and correlated with pharmacodynamic effects.

2.6.6 Xenograft Models of ALL

Establishing xenografts of primary human ALL samples has proven to be a powerful tool to establish bio-repositories of human leukemic cells to examine the genetic and biologic basis of treatment failure, to examine clonal heterogeneity and to use as a platform for the testing of novel therapeutic agents *ex vivo* and *in vivo*. At St Jude, several investigators have established xenotransplants of multiple ALL subtypes, including *CRLF2*-rearranged, *MLL*-rearranged, *BCR-ABL1*-positive, *BCR-ABL1*-like, hypodiploid, and ETP ALL, and have achieved up to 80% engraftment rates with remarkable reproducibility between the tempo of engraftment between replicate mice transplanted with the same tumor. Moreover, for *CRLF2* rearranged and hypodiploid ALL downstream genomic assays demonstrated that the genomic alterations in the relapsed tumor recapitulate those present in the primary leukemia.

The use of xenograft models has also proven valuable in determining the genetic basis of the aggressiveness of disease, and the basis of relapse[47-49]. At St Jude, these studies have been performed with cryopreserved viable cells stored by the St Jude Biorepository. Cells harvested from patients in this study will be used for engraftment into murine models and will contribute to the ongoing efforts at St. Jude to establish a panel of xenografts of high risk acute leukemia samples to serve as a resource for a range of studies of the biologic basis of relapse in acute leukemia, as well as establishing a platform for the preclinical testing of novel therapeutics. Murine models will be used to test the effect of HDAC inhibition on dexamethasone sensitivity by ex vivo cytotoxicity assays of established and novel agents.

2.6.7 Genomic Studies on Leukemic Cells

Matched diagnosis and relapse leukemic samples from the same patient offer an excellent resource to study the mechanisms of relapse and to identify potential new therapeutic targets. Studies of DNA alterations, including DNA copy number alterations, loss-of-heterozygosity and sequence mutations at St Jude have provided insights into the clonal evolution from diagnosis to relapse[50] and have led to the identification of novel mutations including those in the *CREBBP* gene at relapse[19]. These studies had a limited number of samples from patients with T-cell ALL and thus it was not possible to investigate changes specific to this subtype at relapse and correlate to multiple clinical features and response. In one limited, low-resolution study of matched samples from patients with late relapsing T-cell ALL, TCR gene rearrangements and *NOTCH* mutations differed from diagnosis to relapse in a third of patients suggesting a second leukemia[51]. These findings require larger numbers for validation.

In this study, we will collect bone marrow samples at the time of relapse and retrieve cells from initial diagnosis from the St. Jude Biorepository (if available). MRD samples will also be studied where available. Genomic analyses will be performed on diagnosis and relapse samples, and where available, intermediated time points, including samples corrected at the time of measurement of MRD, samples obtained at remission, and samples collected during the course of therapy. Genomic analyses will be performed, where possible, on high blast purity samples, or samples flow sorted to at least 90% purity. If samples are limiting and/or flow sorting is not feasible, analyses will be performed in unsorted material. Assays will include SNP genotyping to asses DNA copy number alterations, and next generation sequencing including exome and/or mRNA-sequencing. Samples may also be studied using whole genome sequencing. DNA and RNA will be extracted using standard methods established in the laboratory of Dr Mullighan (e.g. phenol-chloroform extraction of DNA, TRIzol extraction of RNA). Genomic profiling, data analysis and verification of genomic alterations will be performed using the Hartwell Center for Bioinformatics and Biotechnology and established data analysis pipelines.

2.6.8 Clonal Evolution

MRD studies using deep sequencing provide an opportunity to follow clonal evolution using either IgH or TCR rearrangement as a biomarker of specific clones. Genomic DNA will be isolated from matched diagnosis (when available) and relapsed leukemic samples from the

same patient and submitted for deep sequencing. The clonal heterogeneity base on deep sequencing will be compared to the whole genomic sequencing studies to determine if there are concordances between IgH or TCR rearrangement changes with accumulation of new genomic mutations that may drive relapse. In selective cases, samples between diagnosis and relapse will be also studied to determine the temporal concordance between accumulation of the genomic mutations and deep sequencing. A good concordance would suggest IgH or TCR deep sequencing could predict the accumulation of new leukemic clones, acquisition of additional driver mutations, and relapse.

Recent genomic profiling studies from our group and others identified somatic mutation within the NT5C2 gene directly related to drug resistance in relapsed ALL (~15-20% in T and B-lineage ALL at relapse[52,53]. Patients with NT5C2 gain-of-function mutation are dramatically resistant to thiopurine therapy, and we expect a substantial proportion of patients in this trial would be NT5C2 mutant. Therefore, this protocol offers a unique opportunity to examine the dynamics of NT5C2 mutation during therapy (e.g., the change of % mutant cells at a function of time), especially as patients progress through various blocks of therapy. This can be combined with immunoglobulin or TCR-based clonal monitoring.

2.6.9 NK Cell Receptor Study

The normal physiological roles of NK cells are to control infection and prevent cancer. In fact, NK cells are the only immune cells that have been shown by prospective cohort study in healthy persons to have immunosurveillance capability against human cancer[54]. In a prospective study of more than 3000 healthy volunteers who were followed for 11 years, the risk of cancer was associated with decreased NK cell cytotoxicity against K562 leukemia cells, decreased expression of the NK receptor NKRP1, and decreased production of cytokines such as TNFα and IFNγ in NK cells.

Three studies of adult leukemia have demonstrated a direct link between leukemia and NK cell. In one study of patients with acute myeloid leukemia, the majority (16 of 18) of blood samples showed defective expression and function of NK cell–triggering receptors (NCRs) (dull)[55]. The expression of NK cell surface receptors was low, and the cytolytic activity against autologous leukemia cells, autologous B lymphoblasts, and NK cell–sensitive cell lines was weak. The abnormal NCR (dull) phenotype was confirmed in another study of 71 patients with acute myeloid leukemia and was found to be present in various morphologic and genotypic subtypes of leukemia[56]. In the third study of 25 AML and 14 ALL cases, the expression of HLA class I was frequently downregulated and that of the NK cell receptor ligands PVR and Nectin-2 were upregulated[57]. Together, these results suggest that NK receptor–ligand interaction may be crucial for the development of leukemia.

The normalization of NK cell receptor expression may be of prognostic value as NK cells may be important for the control of leukemia relapse and infection. These roles of NK cells have been shown by killer cell inhibitory receptor (KIR)-mismatched allogeneic stem cell transplantation[58]. Longitudinal study of adult AML patients showed that the NCR (dull) phenotype acquired during leukemia development was reversible in patients achieving complete remission after induction chemotherapy[56]. Reversibility of the NCR

(dull) phenotype after complete remission suggested that leukemia cells might be involved in NCR down-regulation. Alternatively, the recovery of normal NCRs may allow the recovery of normal NK cell function that contributes to the acquisition of remission status. Interestingly, a correlation was found between the NCR (dull) phenotype and poor survival in AML patients after chemotherapy[56], suggesting that NK-deficient activation caused by NCR down-regulation could play a role in treatment outcome. Therefore, we will study NK cell receptor expression at diagnosis and before reinduction to elucidate the role of NK cell in the development and treatment response of childhood ALL.

We are currently studying the prognostic significance of NK cell expression at diagnosis and following therapy in newly diagnosed ALL patients. This study will allow us to study the potential role of NK cells in first T-cell relapse.

2.6.10 Background and Rationale for Intestinal Microbiome Objectives

Increased susceptibility to a number of pathogens in children with hematologic malignancies can result in significant morbidity and treatment delays, thus compromising outcome[59]. Acute gastroenteritis is one of the most common diseases affecting children worldwide. Viruses are recognized as a major cause of this disease, particularly in children. Little is known about the prevalence of enteric viruses in immunocompromised children. Several studies from the 1980's have shown that children with HIV or T-cell deficiencies can develop chronic infections with several enteric viruses leading to persistent diarrhea, which can cause considerable problems of management. By evaluating the population dynamics of these enteric viruses prior to and after chemotherapy, we will obtain a greater understanding of the prevalence of these viruses in this patient population with the eventual goal of improved diagnostics and predictions of clinical complications.

The intestinal microbiome consists of all the intestinal micro-organisms and their associated genetic elements. Several lines of evidence suggest that the interactions between the host's enteric microbiota and the innate immune system are important in modulating the intestinal response to cancer therapy[60]. Alterations in the host microbiota can translate into alterations in the susceptibility of the host to infection[61]. *Clostridium difficile* is a common cause of antibiotic-associated diarrhea in this patient population. As perturbations of the normal gut microflora are thought to predispose patients to infections caused by *C. difficile*, these investigations will also address what alterations in the microflora correlate with the predominance of *C. difficile* in the gastrointestinal tract. The gut microbiota plays a crucial role in the development of an effective immune response. Alterations in the gut microbiota can have profound consequences not only for intestinal diseases but also for mounting an effective immune response against invading pathogens at various sites in the human body[62]. Recent studies have shown that certain bacterial species in the gut can be utilized to estimate the risk of antibiotic-associated diarrhea with an error rate of 2%, emphasizing the relevant application of these data for improved prediction of clinical outcomes[63]. We will study the impact of intensive salvage therapy on the intestinal microbiome, and the relationship between the intestinal microbiome and the increased susceptibility of children with relapsed hematologic malignancies to infections, including gastroenteritis.

In summary, existing literature suggests that the human microbiome plays a variety of important roles in immunology and infectious diseases. Alterations in the host microbiota can have profound effects on the type and magnitude of the immune response against invading pathogens and thus on the infectious complications. We are currently conducting an investigation on the effect of frontline chemotherapeutic regimens for pediatric ALL on the gut microbiome. The current study allows us to also assess the alterations of the microbiome in relapsed hematologic malignancies and the subsequent effects on infection-related clinical outcomes.

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 Participants must have relapsed or refractory acute lymphoblastic leukemia or lymphoma:

a. Stratum I: T-cell lymphoblastic leukemia or lymphoma in first relapse or refractory to one or two courses of frontline induction therapy.

b. Stratum II: B-cell or T-cell lymphoblastic leukemia or lymphoma in second or third relapse or refractory to 2 or 3 induction or re-induction attempts. Patients with Ph+ ALL must be refractory or relapsed after treatment with a regimen that included a tyrosine kinase inhibitor (TKI).

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

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

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

3.1.2 Age is ≤ 21 years (participant has not yet reached 22nd birthday).

3.1.3 Able to swallow capsules.

3.1.4 Karnofsky or Lansky performance score is ≥ 60%. The Lansky performance score should be used for participants < 16 years and the Karnofsky performance score for participants ≥ 16 years (see Appendix I).

3.1.5 Prior therapy:

a. There is no waiting period for participants who relapse while receiving therapy if they are free from side effects attributable to such therapy.

b. Emergent radiation therapy, one dose of intrathecal chemotherapy and up to 7 days of steroids or hydroxyurea are permitted before start of treatment in participants who relapse after completion of frontline therapy. Other circumstances must be cleared by PI or medical designee.

c. At least 90 days have elapsed since bone marrow transplant and participant is off immune suppression for $\geq$ 2 weeks, if applicable.

3.1.6 Adequate renal function defined as glomerular filtration rate $\geq$ 60 cc/min/1.73m^2 or serum creatinine based on age as follows:

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

3.1.7 Adequate hepatic function defined as:
a. Direct bilirubin $\leq$ 1.4 mg/dL (if total bilirubin > 1.4 mg/dL) and
b. AST and ALT $\leq$ 5 x ULN for age.

3.1.8 Adequate cardiac function defined as shortening fraction of $\geq$ 27% or ejection fraction $\geq$ 45%.

3.1.9 Lymphoma participants without bone marrow involvement must have:
a. Absolute neutrophil count (ANC) $\geq$1,000/mm^3, and
b. Platelet count $\geq$50,000/mm^3 (without transfusion support)

Note: these criteria are waived for participants with leukemia or lymphoma participants with bone marrow involvement.

3.1.10 Written, informed consent and assent following Institutional Review Board, NCI, FDA and OHRP guidelines.

3.2 Exclusion Criteria

3.2.1 Prior HDAC, DAC, HSP90 inhibitors or valproic acid for treatment of cancer.

3.2.2 Patients who will need valproic acid for any medical condition during the study or within 5 days prior to first panobinostat treatment.

3.2.3 Impaired cardiac function or clinically significant cardiac diseases, history of arrhythmia (including ventricular fibrillation or torsade de pointes), bradycardia <50 bpm, screening ECG with prolonged QTc or uncontrolled hypertension.

3.2.4 Impairment of GI function or GI disease that may significantly alter the absorption of panobinostat.

3.2.5 Patients with diarrhea > CTCAE grade 2.

3.2.6 Other concurrent severe and/or uncontrolled medical conditions (e.g., uncontrolled diabetes or active or uncontrolled infection) including abnormal laboratory values, that could cause unacceptable safety risks or compromise compliance with the protocol.

3.2.7 Patients using medications that have a relative risk of prolonging the QT interval or inducing torsade de pointes if treatment cannot be discontinued or switched to a different medication prior to starting treatment.

3.2.8 Patients who have received targeted agents within 2 weeks or within 5 half-lives of the agent and active metabolites (whichever is longer) and who have not recovered from side effects of those therapies.

3.2.9 Patients who have undergone major surgery ≤ 4 weeks prior to starting treatment or who have not recovered from side effects of such therapy.

3.2.10 Patients with known positivity for human immunodeficiency virus (HIV) or hepatitis B/C.

3.2.11 Inability to swallow capsules.

3.2.12 Active, uncontrolled infection or severe concurrent medical disease, including but not limited to congestive heart failure, cardiac arrhythmias, or psychiatric illness.

3.2.13 Isolated extramedullary relapse (leukemia) or isolated CNS lymphoma.

3.2.14 Pregnant or lactating (female participant of childbearing potential must have negative serum or urine pregnancy test required within 7 days prior to start of treatment). Male or female of reproductive potential has agreed to use effective contraception method for duration of study treatment.

3.2.15 Down syndrome.

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

3.3 Recruitment and Screening

Participants will be recruited by study investigators through their clinical practice.

3.4 Enrollment at St. Jude

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

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

3.5 Enrollment at Collaborating Sites

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

4.0 TREATMENT PLAN

This is a study of re-induction therapy that will comprise of three blocks of multi-agent chemotherapy. CR will be evaluated following each block of therapy. All patients will be candidates for HSCT once they achieve negative MRD. If patients cannot proceed to HSCT following Block A, they will continue therapy on Block B and Block C until ready for HSCT.

No dose modifications will be made for hematologic toxicity once a therapeutic block has been initiated with the exception of Block B where mercaptopurine dose can be adjusted and high-dose cytarabine can be delayed for WBC $\leq 1000/mm^3$, ANC $\leq 300/mm^3$ and platelet count $\leq 50{,}000/mm^3$). Dose calculations should be based on actual BSA.

4.1 Intrathecal Chemotherapy

age (months)	methotrexate (mg)	hydrocortisone (mg)	cytarabine (mg)	volume (ml)
< 12	6	12	18	6
12-23	8	16	24	8
24-35	10	20	30	10
≥ 36	12	24	36	12

Leucovorin rescue (5 mg/m^2/dose, max 5 mg) PO will be given at 24 and 30 hours after each triple intrathecal treatment. 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.

4.2 Block A: Approximately 5 Weeks

Day 1 DEX ITMHA	Day 2 DEX PANO	Day 3 DEX	Day 4 DEX PANO	Day 5 DEX	Day 6 DEX PANO	Day 7 DEX VSLI MITOX ITMHA
Day 8 DEX	Day 9 PEG-Asp	Day 10 (ITMHA)	Day 11	Day 12	Day 13	Day 14 VSLI MITOX ITMHA
Day 15 DEX	Day 16 DEX BORTEZ	Day 17 DEX (ITMHA)	Day 18 DEX	Day 19 DEX BORTEZ	Day 20 DEX	Day 21 DEX VSLI ITMHA
Day 22 DEX	Day 23 PEG-Asp BORTEZ	Day 24	Day 25	Day 26 BORTEZ	Day 27	Day 28 VSLI ITMHA

- Dexamethasone (DEX) 10 mg/m^2/day PO (may also give IV) divided TID, Days 1-8, 15-22 (total of 16 days)
- Panobinostat (PANO) 24 mg/m^2/dose PO Day 2, 4, 6 (3 doses). Round dose up to next 5 mg increment
- Liposomal vincristine (VSLI) 2.25 mg/m^2 no cap IV on Days 7, 14, 21, 28 (4 doses)
- Mitoxantrone (MITOX) 10 mg/m^2 IV over 1 hour on Days 7 and 14 (2 doses). *(Day 14 Mitoxantrone can be held in absence of peripheral blasts if WBC ≤ 1000 and ANC ≤ 300)*
- Peg-asparaginase (PEG-Asp) 2500 units/m^2 IV over 1 hour on Days 9 and 23 (2 doses)
- Bortezomib (BORTEZ) 1.3 mg/m^2 IV Days 16, 19, 23, 26 (4 doses)
- ITMHA Days 1, 7, 14, 21, 28. Additional ITs on Days 10 and 17 for patients with CNS 2, 3 or traumatic tap with blasts

4.2.1 Bone marrow aspirate and MRD will be performed on Day 7 (optional, see Section 8.2) and at completion of Block A after count recovery:

- WBC $\geq$1500/mm^3 and
- ANC $\geq$300/mm^3 and
- Platelet count $\geq$50,000/mm^3

In the absence of count recovery, a bone marrow aspiration should be performed to evaluate for persistent leukemia within 4-6 week period from initiation of Block depending on counts and clinical status.

4.2.2 Panobinostat administration: patients should be instructed to take their once-a-day oral dose of panobinostat at the same time each morning. Each dose of panobinostat should be taken with an 8 ounce/240 ml glass of water. Patients should be instructed to swallow the capsules whole and not chew them.

Patients must avoid grapefruit or grapefruit juice and Seville (sour) oranges during the entire study.

4.3 Block B: Approximately 5 Weeks

Block B will begin after count recovery from Block A (i.e., WBC $\geq$ 1500/mm^3, ANC $\geq$300/mm^3 and platelet count $\geq$50,000/mm^3)

Day 1 HDMTX ITMHA 6MP	Day 2 6MP	Day 3 6MP	Day 4 6MP	Day 5 6MP	Day 6 6MP	Day 7 6MP
Day 8 6MP	Day 9 6MP	Day 10 6MP	Day 11 6MP	Day 12 6MP	Day 13 6MP	Day 14 6MP
Day 15 HDAC HDAC	Day 16 HDAC HDAC	Day 17	Day 18	Day 19	Day 20	Day 21

- High-dose methotrexate (HDMTX) 8 g/m^2 IV over 24 hours (in patients with prior cranial irradiation HDMTX will be substituted with 2 doses of methotrexate 40 mg IV on Day 1 and Day 8)
- 6-mercaptopurine (6MP) 50 mg/m^2 PO days 1-14 (14 doses)
- ITMHA Day 1
- High-dose cytarabine (HDAC) 3 g/m^2 IV over 2 hours Q12H Days 15 and 16 (4 doses)

4.3.1 Bone marrow aspirate and MRD will be performed at end of Block B after count recovery:

- WBC $\geq$ 1500/mm^3, ANC $\geq$ 300/mm^3 and
- Platelet count $\geq$ 50,000/mm^3

In the absence of count recovery, a bone marrow aspiration should be performed to evaluate for persistent leukemia within 4-6 week period from initiation of Block B depending on counts and clinical status.

4.3.2 Mercaptopurine Administration

Mercaptopurine should be given daily at a consistent time that maximizes adherence with the prescribed treatment. Mercaptopurine may be held in the presence of ANC $\leq$300/mm^3, WBC $\leq$ 1,000/mm^3, platelet count $\leq$ 50,000/mm^3 or grade 3 or 4 mucositis.

4.3.3 High-Dose Methotrexate (HDMTX) Administration

The dosage of high dose methotrexate will be adjusted to achieve a targeted steady-state plasma concentration at the end of a 24 hour infusion of 90 uM, which is approximately the level expected from a dose of 8 g/m^2/24 hours, and one with which we have some experience.[30] Ten percent of the estimated total dose is administered as a loading dose over 1 hour and the remainder over 23 hours.

Clearance is estimated from the plasma concentrations up through 48 hours from methotrexate infusion using nonlinear curve fitting and a Bayesian estimation strategy.[31]

4.3.3.1 Pre-hydration

At least two hours before high dose methotrexate, pre-hydration IV fluid (D5W + 40 mEq NaHCO3/L + 20 mEq KCl/L) will be administered at the rate of 200 ml/m^2/hr. At start of pre-hydration, one IV dose of NaHCO3 (unless otherwise clinically indicated) 25 mEq/m2 diluted in 50 ml D5W will be given over 15 minutes. Pre-hydration fluid may also be given overnight at a rate of at least 150 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.

4.3.3.2 High dose methotrexate infusion

Methotrexate loading dose (generally 800 mg/m^2) will be given over 1 hour, followed immediately by a maintenance infusion over 23 hours. The maintenance infusion will begin at 7200 mg/m^2/23 hours; plasma methotrexate will be measured at least 2 time points within the first 6 hours of the infusion (e.g. at 1 and 3 hours), and the maintenance infusion will be adjusted to achieve a Cpss of 90 uM. During the methotrexate infusion, patients should receive hydration fluid with D5W + 40 mEq/L NaHCO3 + 20 mEq KCl/L at 125-150 ml/m^2/hr. Urine pH will be monitored with each void during infusion. An IV bolus of 12 mEq/m^2 NaHCO3 will be given if urine pH is 6.0; and 25 mEq/m^2 will be given if urine pH is <6.0. Acetazolamide 500 mg/m^2 orally every 6 to 8 hours may be

used if systemic alkalosis limits the administration of bicarbonate for urinary alkalinization. Patients with evidence of renal dysfunction or delayed clearance during the methotrexate infusion may receive less than a 24 hour methotrexate infusion.

Blood samples for methotrexate pharmacokinetics will be drawn; 2-3 ml of blood will be obtained in EDTA tubes (purple top) pre-dose, at the end of the loading dose (1 hour), and at 3 hours from start of loading dose (timing of samples is not critical, but at least samples should be available between 0.5 and 6 hour). Samples will also be drawn at 23 hours and 42 hours from the start of the infusion. Additional samples will be obtained in patients in whom there is clinical suspicion of poor clearance, or in those who have high plasma MTX concentration (e.g., >0.5 uM at 42 hours), to adjust leucovorin rescue.

4.3.3.3 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. The dosage of leucovorin will be increased in patients with high plasma methotrexate concentrations (>1.0 uM at 42 hours) and continued until the methotrexate concentration is less than 0.10 uM. Additional measures, such as hydration, hemoperfusion, or carboxypeptidase will be considered in patients with 42-hour methotrexate levels > 10 uM. Patients with a history of gastrointestinal toxicity or a history of typhlitis with any chemotherapy should have leucovorin continue for 5, rather than 3 doses.

4.3.4 Intrathecal Chemotherapy

All patients will receive triple intrathecal therapy 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 Conjunctivitis Prophylaxis

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

4.4 Block C: Approximately 3 Weeks

Block C will begin after count recovery from Block B (i.e., WBC ≥ 1500/mm^3, ANC ≥300/mm^3 and platelet count ≥ 50,000/mm^3)

Day 1 NELAR *(Clofar)** CYCLO ETOP	Day 2 NELAR *(Clofar)** CYCLO ETOP	Day 3 NELAR *(Clofar)** CYCLO ETOP	Day 4 NELAR *(Clofar)** CYCLO ETOP	Day 5 NELAR *(Clofar)** CYCLO ETOP

- Nelarabine (NELAR) 650 mg/m^2/day IV Days 1-5
  *[or c*lofarabine (CLOFAR) 40 mg/m^2/day IV Days 1-5 will be given instead of nelarabine for patients with B-lymphoblastic leukemia and lymphoma in stratum II*]
- Cyclophosphamide (CYCLO) IV 300 mg/m^2 Days 1-5
- Etoposide (ETOP) 100 mg/m^2/day IV Days 1-5

4.4.1 Bone marrow aspirate and MRD will be performed after count recovery: (WBC ≥ 1500/mm^3, ANC ≥ 300/mm^3 and platelet count ≥ 50,000/mm^3).

In the absence of count recovery, a bone marrow aspiration should be performed to evaluate for persistent leukemia within 4-6 week period from initiation of Block C depending on counts and clinical status.

4.5 Timing of Bone Marrow Aspirate and MRD Assessments

- Baseline, prior to initiation of Block A therapy
- Optional Day 7 Block A (pre VSLI and MITO; see Section 8.2)
- After count recovery from Block A
- After count recovery from Block B
- After count recovery from Block C

4.6 Recommendations for Post-Protocol Therapy

4.6.1 Hematopoietic Stem Cell Transplantation (HSCT)

All patients should proceed to HSCT after completion of Block C if a suitable donor has been found and the patient achieves negative MRD (<0.01%). If MRD is persistently positive, the plan will be discussed with the PI or co-PI and the transplant team. The donor will be selected according to institutional practices and transplant regimens will be used according to institutional HSCT protocols and guidelines.

4.7 CNS and Testicular Leukemia

4.7.1 Craniospinal Irradiation

Craniospinal irradiation is recommended for participants with CNS3 disease at relapse and whose first CR was less than 18 months; cranial irradiation only will be given for those whose CR was more than 18 months. The doses are 18 Gy cranial and 12 Gy spinal. Not all participants with CNS2 disease will require irradiation. These cases will be discussed individually with the radiation oncologist and PI/co-PI. Participants who will receive total body irradiation (TBI) for pre-transplant conditioning can receive a cranial irradiation boost (to a cumulative dose approximating 18 Gy in temporal proximity (typically prior) to TBI. Participants who will not receive TBI conditioning can receive cranial or craniospinal irradiation as above just prior to HSCT conditioning.

4.7.2 Treatment of Testicular Involvement

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

5.0 DRUG/DEVICE/BIOLOGIC AGENT INFORMATION

5.1 Dexamethasone (Decadron®)

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

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

Supplier: The drug is commercially available.

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

pituitary unresponsiveness particularly in times of stress as in trauma, surgery or illness, osteoporosis and muscle wasting.

Dosage and route of administration: 10 mg/m^2/day PO divided TID Days 1-8 and 15-22 (total of 16 days) of Block A see Section 4.2.

5.2 Panobinostat (LBH589)

Source and pharmacology: The oral formulation of panobinostat is 2-hydroxypropaonic acid compound with 2(E)-N-Hydroxy-3-(4-[[2-(2-methyl-1H-indol-3-yl)-ethylamino]-methyl]-phenyl)-2-propenamide, a histone deacetylase inhibitor being developed for the treatment of patients with leukemias and lymphomas. Panobinostat has also been shown to block tumor cell growth and to activate the p21 promoter. Tumor cells undergo apoptosis in the presence of low nanomolar concentrations of panobinostat for at least 16 hours. However, normal healthy cells do not experience cell death when exposed to the same concentrations of panobinostat for 72 hours. Furthermore, increased levels of acetylated histones were observed in tumor cell cultures from xenograft mice models treated with panobinostat, proving that the drug functions as a histone deacetylase inhibitor.

Formulation and stability: The oral formulation of panobinostat is an immediate-release solid hard gelatin capsule. Panobinostat lactate salt is the active ingredient in the capsule, provided in dosage strengths of 5 mg and 20 mg. The inactive ingredients of the capsule consist of mannitol, microcrystalline cellulose, starch, and magnesium stearate. The capsule formulation is created by an aqueous granulation and blending process.

Supplier: Supplied by Novartis Pharmaceuticals.

Toxicity: The most common adverse event from oral panobinostat is reversible thrombocytopenia; the degree of thrombocytopenia is dose-dependent. Other side effects include fatigue (dose-dependent), nausea, vomiting, and diarrhea, which can be controlled with loperamide. For orally administered panobinostat, no grade 4 QTc has been reported with “interrupted” dosing schedules. For oral dose of 20 mg or 60 mg given three times weekly, the incidence of grade 3 QTc prolongation ranges from <1% to 6%. The largest QTc change is seen approximately five days after the drug is given and does not correlate with drug serum levels. See Investigator’s Brochure for further details.

Dosage and route of administration: The oral panobinostat capsules will be provided in HDPE bottles with an induction seal and plastic (CR) closure. 24 mg/m^2/dose PO Days 2, 4, and 6 of Block A, see Section 4.2.

5.3 vinCRIStine sulfate LIPOSOME injection (VSLI, Marqibo®)

Source and pharmacology: Marqibo is vincristine encapsulated in sphingomyelin/cholesterol liposomes for intravenous injection. The active ingredient in Marqibo is vincristine sulfate. Vincristine sulfate is a vinca alkaloid isolated as a 1:1 sulfate salt from the periwinkle plan (*Catharanthus roseus*).

Formulation and stability: Marqibo is prepared on site from the components in the Marqibo Kit (see package insert for preparation instructions for pharmacy). Following the preparation procedure, each single-dose vial of Marqibo (vinCRIStine sulfate LIPOSOME injection) contains 5 mg/31 mL (0.16 mg/mL) vincristine sulfate.

Supplier: Supplied by Spectrum Pharmaceuticals.

Toxicity: The most commonly reported adverse reactions (incidence ≥ 30%) in clinical studies include constipation, nausea, pyrexia, fatigue, peripheral neuropathy, febrile neutropenia, diarrhea, anemia, decreased appetite, and insomnia.

Warnings:

- **Intrathecal administration is fatal**
- Extravasation causes tissue injury
- Neurologic toxicity: Monitor patients for peripheral motor and sensory, central and autonomic neuropathy, and reduce, interrupt, or discontinue dosing. Patients with preexisting severe neuropathy should be treated with Marqibo only after careful risk-benefit assessment
- Myelosuppression: Monitor blood counts prior to each dose of Marqibo. Neutropenia, thrombocytopenia, or anemia may occur; consider Marqibo dose reduction or interruption and supportive care measures
- Tumor lysis syndrome: Anticipate, monitor for, and manage
- Constipation, bowel obstruction, and/or paralytic ileus: Institute a prophylactic bowel regimen to prevent potential constipation, bowel obstruction, and/or paralytic ileus
- Fatigue: Severe fatigue can occur
- Hepatic toxicity: Monitor liver function and modify or interrupt dosing (Section 6.3)
- Embryo-fetal toxicity: Can cause fetal harm. Advise women of potential harm to the fetus.

See package insert for additional information.

Dosage and route of administration: FOR INTRAVENOUS USE ONLY. 2.25 mg/m^2 (no cap) IV on Days 7, 14, 21, and 28 of Block A, see Section 4.2.

5.4 Mitoxantrone (Novantrone®)

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

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

Supplier: commercially available.

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

Guidelines for administration: 10 mg/m^2/day IV on days 7 and 14 of Block A, see Section 4.2.

5.5 Peg-L-Asparaginase (Pegaspargase, Oncaspar®)

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

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

Supplier: commercially available.

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

Guidelines for administration: 2500 U/m^2 IV or IM on Days 9 and 23 of Block A, see Section 4.2.

5.6 Erwinia L-Asparaginase (Erwinase®)

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

Source and pharmacology: Erwinia asparaginase is an enzyme. It is derived from *Erwinia chrysanthemi* and may be useful in patients with an allergy to the *E. coli* derived product. Asparaginase hydrolyzes serum asparagine (an amino acid required to synthesize proteins) to aspartic acid and ammonia, and is therefore lethal to cells that cannot synthesize asparagine. Asparaginase is active during all phases of the cell cycle. Asparaginase is not absorbed from the GI tract and must be given parenterally. Asparaginase does not cross into the CSF. The plasma half-life of Erwinia asparaginase when given IM is approximately 16 hours. Only minimal urinary and biliary excretion occurs. Clearance is unaffected by age, renal function or hepatic function.

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

Supplier: Commercially available.

Toxicity: Acute toxicity includes anaphylactic reactions that occur most commonly when the drug is given IV. These can be characterized by laryngeal constriction, hypotension, diaphoresis, fever, chills, edema and loss of consciousness. Allergic reactions at the site of IM injection include pain, swelling and erythema. Other adverse effects include neutropenia and associated immunosuppression, mild nausea and vomiting, malaise, anorexia, elevated LFTs, pancreatitis and hyperglycemia. A decrease in protein synthesis including albumin, fibrinogen and other coagulation factors may occur, which can result

in hemorrhage. Thrombosis and/or pulmonary embolism can also occur. Less common side effects include renal dysfunction and CNS complications including somnolence, weakness, lethargy, coma and seizures.

Guidelines for administration: Intravenous or intramuscular injection. *For additional information about this drug, please see package insert.*

5.7 Bortezomib (Velcade, PS-341, MLN341, LDP-341)

Source and pharmacology: Bortezomib (PS-341) is a reversible inhibitor of the chymotrypsin-like activity of the 26S proteasome (a multi-catalytic 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/m2 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. In addition, it is recommended that the total dietary intake of vitamin C not exceed the RDA for age (i.e., normally balanced diets are acceptable).

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.

Supplier: Commercially available.

Toxicity: The most commonly reported adverse reactions (incidence ≥ 20%) in clinical studies include nausea, diarrhea, thrombocytopenia, neutropenia, peripheral neuropathy, fatigue, neuralgia, anemia, leukopenia, constipation, vomiting, lymphopenia, rash, pyrexia, and anorexia.

Warnings:

- Peripheral neuropathy: Manage with dose modification or discontinuation. Patients with pre-existing severe neuropathy should be treated with VELCADE only after careful risk-benefit assessment.
- Hypotension: Use caution when treating patients taking anti-hypertensives, with a history of syncope, or with dehydration.
- Cardiac toxicity: Worsening of and development of cardiac failure has occurred. Closely monitor patients with existing heart disease or risk factors for heart disease.
- Pulmonary toxicity: Acute respiratory syndromes have occurred. Monitor closely for new or worsening symptoms.
- Posterior reversible encephalopathy syndrome: Consider MRI imaging for onset of visual or neurological symptoms; discontinue VELCADE if suspected.

- Gastrointestinal toxicity: Nausea, diarrhea, constipation, and vomiting may require use of antiemetic and antidiarrheal medications or fluid replacement.
- Thrombocytopenia or neutropenia: Monitor complete blood counts regularly throughout treatment.
- Tumor lysis syndrome: Closely monitor patients with high tumor burden.
- Hepatic toxicity: Monitor hepatic enzymes during treatment.
- Embryo-fetal risk: Women should avoid becoming pregnant while being treated with VELCADE. Advise pregnant women of potential embryo-fetal harm.

See package insert for further details.

Dosage and route of administration: 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. 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.

Dose 1.3 mg/m^2 IV Days 16, 19, 23 and 26 of Block A, see Section 4.2.

5.8 Methotrexate (IV)

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

Formulation and stability: Methotrexate is supplied in single-dose vials containing 50mg, 100mg, 200mg, and 250 mg of methotrexate as a 25 mg/ml preservative-free solution and in vials containing 20mg, 50 mg, 100mg, 250 mg and 1000mg of lyophilized drug. It is also available in 2.5 mg tablets. Methotrexate preservative-free solution and lyophilized drug should be stored at room temperature and protected from light. Methotrexate tablets

can also be stored at room temperature. The vials containing 20, 50, 100 and 250 mg of lyophilized product can be reconstituted by adding sterile water, 0.9% NaCl or D5W to a final concentration not exceeding 25 mg/ml. The 1000mg vials containing lyophilized product are reconstituted to a final concentration of 50mg/ml.

Supplier: commercially available

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

Guidelines for administration: Intrathecal and intravenous. High dose methotrexate 8 g/m^2 IV over 24 hours Day 1 of Block B; see Section 4.2. Also given IT with ITMHA during Blocks A and B, see Sections 4.1 and 4.2.

5.9 Mercaptopurine (6-MP) (Purinethol®)

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

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

Supplier: Commercially available.

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

Guidelines for administration: oral tablets at consistent time each day to maximize adherence, 50 mg/m^2/day PO days 1-14 of Block B, see Section 4.3.

5.10 Cytarabine (Ara-C) (Cytosar-U®)

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

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

Supplier: Commercially available.

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

Guidelines for administration: Intrathecal and intravenous. High dose Ara-C 3g/m^2 every 12 hours Days 15 and 16 of Block B, see Section 4.2. Also given IT with ITMHA during Blocks A and B.

5.11 Nelarabine (Arranon®, Compound 506U78)

Source and pharmacology: Nelarabine (compound 506U78) is a water soluble pro-drug of 9-β-Darabinofuranosylguanine (ara-G), a deoxyguanosine analog being developed for the treatment of patients with T-cell and B-cell leukemias and lymphomas. Nelarabine is rapidly converted by adenosine deaminase in the peripheral blood to ara-G. In vitro studies and biochemical studies have demonstrated that intracellular ara-G is phosphorylated via deoxycytosine kinase (dCK) and deoxyguanosine kinase (dGK) to its active 5'-triphosphate (ara-GTP). Ara-GTP incorporation into DNA is a primary cause of cell death.

The pharmacokinetics of nelarabine in humans is characterized by a rapid and extensive conversion to ara-G. The average elimination half-lives for nelarabine and ara-G were approximately 20-25 minutes and 2.6-4.0 hours, respectively. Virtually all nelarabine was converted to ara-G. Peak plasma concentration and area under the curve for both nelarabine and ara-G was essentially proportional to the administered dose. The steady-state volume of distribution of ara-G was similar in adult and pediatric patients. Neither compound showed accumulation with the dosing studies used in the pre-clinical trials with this agent. The pharmacokinetics of both nelarabine and ara-G appeared to be independent of diagnosis and gender. Intracellular leukemic blast concentrations of ara-GTP were characterized by a long elimination half-life (median approximately 24 hours) and diagnosis dependent accumulation; with T-lymphoblasts generally demonstrating greater accumulation of ara-GTP than other cell types. Nelarabine and ara-G is < 25% bound to human plasma proteins.

Formulation and stability: Nelarabine (compound 506U78) is formulated to provide 5 mg of nelarabine per mL in 0.45% saline solution. Nelarabine Injection consists of a clear, colorless solution, contained in a clear 50 mL glass vial with a gray rubber closure and lacquered overseals made of polypropylene and aluminum. The drug is supplied as a 5 mg/mL liquid in 50 mL vials with a total of 250 mg nelarabine per vial. The pH of the solution is between 5 and 7. Intact 50 mL glass vials containing 250 mg of nelarabine for injection should be stored at or below 30 C (86 F). Do not store vials in the refrigerator as crystallization of the product may occur. Nelarabine as a clear colorless solution is stable in glass vials for at least 30 months at 30 C.

All vials should be visually inspected for any particulate matter prior to use. **The solution is intended to be used full strength directly from the vials with no further dilution.** Glass or plastic containers may be used. Nelarabine is stable in polyvinylchloride (PVC) infusion bags and glass containers for up to 8 hours at up to 30°C. Nelarabine is stable in normal saline for up to 8 hours. CAUTION: The single-use dosage form contains no antibacterial preservatives. Therefore, it is advised that the product be discarded 8 hours after initial entry.

Supplier: Commercially available.

Toxicity: Nelarabine contains a "black-box" warning regarding severe neurological events that occurred in clinical trials, including severe somnolence, CNS effects including convulsions, and peripheral neuropathies ranging from numbness and

paresthesias to motor weakness and paralysis. Over 60% of the patients who received nelarabine had some nervous system event (e.g., headache, somnolence, and peripheral neuropathy). Providers should closely monitor for signs and symptoms of neurological events. Other common adverse effects include fatigue, hematologic disorders (e.g., anemia, thrombocytopenia and neutropenia), gastrointestinal (GI) disorders (e.g., diarrhea, vomiting, and nausea), pulmonary disorders (e.g., cough and dyspnea) and fever. A small percentage of patients (4%) have complained of experiencing blurred vision while receiving nelarabine. Fatal opportunistic infections have also been reported periodically in patients receiving nelarabine.

Patients receiving nelarabine should receive intravenous hydration according to standard medical practice for the management of hyperuricemia in patients at risk for tumor lysis syndrome. Consideration should be given to the use of allopurinol in patients at risk of hyperuricemia. See package insert for further details.

Dosage and route of administration: 650 mg/m^2/day IV on Days 1-5 of Block B, see Section 4.3.

5.12 Clofarabine (Clolar™, Clofarex)

Clofarabine will be given instead of nelarabine for patients with B-lymphoblastic leukemia and lymphoma in Stratum II.

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

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

Supplier: The injection is commercially available.

Toxicity: The most common side effects are nausea, vomiting, diarrhea, headache, fever and pruritus. Also reported are pericardial effusion, tachycardia, hypotension, left ventricular systolic dysfunction, edema, flushing, hypertension, fatigue, anxiety, pain, dizziness, depression, irritability. Patients who receive clofarabine are at risk for tumor lysis syndrome and need to be monitored closely.

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

Dosage and route of administration: 40 mg/m^2/day IV, Days 1-5. *See Block C, section 4.4 for B-lymphoblastic leukemia and lymphoma in Stratum II only.*

5.13 Cyclophosphamide (Cytoxan®)

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

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

Supplier: Commercially available

Toxicity: Dose limiting toxicities of cyclophosphamide are bone marrow suppression and cardiac toxicity. Cardiac toxicity is typically manifested as congestive heart failure, cardiac necrosis or hemorrhagic myocarditis and can be fatal. Hemorrhagic cystitis may occur and necessitates withholding therapy. The incidence of hemorrhagic cystitis is related to cyclophosphamide dose and duration of therapy. Forced fluid intake and/or the administration of MESNA decrease the incidence and severity of hemorrhagic cystitis. Other toxicities reported commonly include nausea and vomiting (may be mild to severe depending on dosage), diarrhea, anorexia, alopecia, immunosuppression and sterility. Pulmonary fibrosis, SIADH, anaphylaxis and secondary neoplasms have been reported rarely.

Dosage and route of administration: 300 mg/m^2/day IV on Days 1-5 during Block C, see Section 4.4.

5.14 Etoposide (VP-16) (Vepesid®)

Source and pharmacology: Etoposide is an epipodophyllotoxin derived from *Podophyllum pelatatum.* It is thought to act mainly by inhibiting topoisomerase II, causing double and single strand DNA breaks. Etoposide is cell cycle, phase-specific, with activity in the G2 and S phases. Absorption of etoposide is approximately 30-40% and is highly variable and somewhat dose-dependent. It is extensively bound to serum proteins and is metabolized in the liver, including cytochrome P4503A metabolism to several moieties that include a reactive oxidized species. Etoposide and its metabolites are excreted mainly in the urine with a smaller amount excreted in the feces. Dosage adjustments should be considered in patients with liver dysfunction, kidney dysfunction or hypoalbuminemia.

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

Supplier: Commercially available.

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

Dosage and route of administration: 100 mg/m^2/day IV on Days 1-5 of Block C, see Section 4.4.

5.15 Etoposide Phosphate (Etopophos®)

To be used in case of etoposide reactions.

Source and pharmacology: Etoposide is an epipodophyllotoxin derived from *Podophyllum pelatatum.* It is thought to act mainly by inhibiting topoisomerase II, causing double and single strand DNA breaks. Etoposide is cell cycle, phase-specific, with activity in the G2 and S phases. Absorption of etoposide is approximately 30-40% and is highly variable and somewhat dose-dependent. It is extensively bound to serum proteins and is metabolized in the liver, including cytochrome P4503A metabolism to several moieties that include a reactive oxidized species. Etoposide and its metabolites are excreted mainly in the urine with a smaller amount excreted in the feces. Dosage adjustments should be considered in participants with liver dysfunction, kidney dysfunction or hypoalbuminemia.

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

Supplier: Commercially available.

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

Dosage and route of administration: Used in substitution for etoposide in participants that experience allergic reaction; IV administration.

5.16 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, as ITMHA during Blocks A and B.

5.17 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 PEG-Asparaginase Allergies/Hypersensitivity

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-asparaginase will be replaced by 6 doses of *Erwinia* L-asparaginase given at 25,000 units/m^2/dose thrice weekly (2 to 3 days apart, e.g., Monday, Wednesday and Friday) over 2 weeks.

Participants with possible allergic reactions should have measurements of anti-asparaginase antibodies and asparaginase at the time of the reaction and if re-challenged, after the next dose.

6.2 Participants with Renal Dysfunction

6.2.1 High Dose Methotrexate

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

6.2.2 High Dose Cytarabine

Patients who have received amphotericin B or nephrotoxic antibiotic regimens for at least 7 days, and patients in whom serum creatinine is greater than two times normal for age, should have their glomerular filtration rate (GFR) measured before they receive HDAC. In patients with a GFR ≤ 60 ml/min per 1.73m^2, we recommend decreasing the dosage of cytarabine from 3 g/m^2 to 2 g/m^2 every 12 hours.

6.3 Participants with Hepatic Dysfunction

Consider modifying the dosages for mitoxantrone, clofarabine, etoposide, vincristine, and bortezomib in participants with elevated direct bilirubin concentrations or other evidence of biliary obstruction.

Suggested modifications:

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

PEG-asparaginase may need to be withheld in participants 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 no other chemotherapy.

6.4 Cardiotoxicity

Mitoxantrone will be held for fractional shortening ≤ 28%. Consultation with cardiologist is suggested if clinically indicated.

All patients must have an assessment of serum potassium, magnesium, and calcium (total corrected for albumin, or ionized calcium) ≤ 72 hours prior to the administration of oral panobinostat on day 2 of cycle 1 and the results must all be ≥ LLN before the first dose of panobinostat is administered. Throughout Days 1-7, serum biochemistry values including serum potassium, calcium, phosphorous and magnesium will be monitored closely and corrected before administration of panobinostat.

6.5 Peripheral Neuropathy

Severity of Peripheral Neuropathy (PN)	VSLI modification	Bortezomib modification
Grade 3 or persistent Grade 2 at the time of the next dose	Interrupt VSLI for Grade 3 If PN remains Grade ≥ 3 until next dose then discontinue VSLI If PN recovers to Grade 1 or 2 from Grade 3, or persistent Grade 2, then reduce VSLI dose to 2 mg/m^2 No dose reduction if recovery to Grade < 2	Interrupt bortezomib for Grade 3 and resume at 1.3 mg/m^2 if PN recovers to Grade 1 or 2 by next dose
Stable Grade ≤ 2 after first VSLI dose reduction to 2 mg/m^2 or increase to Grade ≥ 3	No dose reduction for stable Grade 1 or 2 after first VSLI dose reduction to 2 mg/m^2 Reduce VSLI dose to 1.5 mg/m^2 if Grade 3 PN improves to Grade ≤ 2 at the time of the next dose Interrupt VSLI for Grade ≥ 3 PN	No dose reduction if stable Grade 1 or 2. Interrupt bortezomib for Grade 3 Resume at 1.3 mg/m^2 if PN recovers to Grade 1 and at 1 mg/m^2 if PN recovers to Grade 2 before next dose
Grade 4	Discontinue VSLI	Discontinue bortezomib

7.0 REQUIRED EVALUATIONS, TESTS, AND OBSERVATIONS

7.1 Pretreatment Evaluation

All participants should be invited to participate in the tissue banking protocol (TBANK) and PGEN5 at the time of study entry.

- Complete history
- Physical exam with vital signs and complete neurologic exam
- Height, weight, BSA
- Pulse oximetry
- Urinalysis
- Complete blood count with differential
- Chemistry profile: glucose, electrolytes, BUN, creatinine, LDH, uric acid, bilirubin, ALT, AST, calcium, phosphorous, magnesium, total protein and albumin
- Coagulation screen
- G6PD screen
- Lipid screen: Total cholesterol, triglycerides, free fatty acids and high-density lipoprotein cholesterol
- Thyroid function tests
- Hepatitis B and C Screen
- HIV
- HLA typing
- Chest x-ray (required for T-cell malignancies, as clinically indicated for B-cell)
- EKG
- Echocardiogram or MUGA
- Bone marrow evaluation: morphology, cytochemistry, immunophenotyping, cytogenetics, molecular diagnosis, MRD studies (*MRD leukemia patients only*)
- PET/CT *(lymphoma patients only)*
- Lumbar puncture with CSF cell count and cytology
- NK cell receptor studies, except in patients receiving steroids
- Pregnancy test of females of childbearing potential
- Other studies as clinically indicated, e.g., sickle cell prep, hemoglobin electrophoresis for black children; varicella titer; EBV, TOXO, CMV, HSV titers

7.2 Evaluations during Therapy

Studies to be Obtained	During Block A	During Block B	During Block C
Physical exam (includes vital signs, height, weight, BSA	Weekly	Weekly	Weekly
Comprehensive neurologic exam – ***(see note below)***	Prior to Day 6 and Day 15	Start of Block	Start of Block and EOT
CBC, differential, platelets	Weekly and as clinically indicated	Weekly and as clinically indicated	Weekly and as clinically indicated
GFR or creatinine clearance (in pts. with elevated serum creatinine, see Section 6.2.1 and 6.2.2)	---	Prior to HDMTX and HD Ara-C	---
Chemistries*	Weekly	Weekly	Weekly
Electrolytes#	Weekly	Weekly	Weekly
Lipase and amylase	Prior to each dose of asparaginase	---	---
Chest x-ray (T-cell patients)	Baseline	As clinically indicated	As clinically indicated
Pulse oximetry	Baseline	As clinically indicated	As clinically indicated
Urine or serum pregnancy test (if applicable)	Start of Block	Start of Block	Start of Block
EKG	Baseline	As clinically indicated	As clinically indicated
CSF cell count and differential	With each dose of ITMHA	With each dose of ITMHA	---
Bone marrow aspirate and/or biopsy for morphology for leukemia and lymphoma with marrow disease. MRD for leukemia patients.	Start of Block (and optional Day 7 pre VSLI and MITO; see Section 8.2)	Start of Block (after count recovery from Block A)	Start of Block (after count recovery from Block B) and EOT (after count recovery Block C)
PET/CT scan of involved areas for lymphoma patients	Start of Block	Start of Block	Start of Block (if not in CR after Block A) and EOT
Optional pharmacokinetics studies (**see Section 8.1**)	Day 2 and 6**	---	---
Optional pharmacodynamic, genomic and research MRD studies (**see Section 8.2**)	Start of Block, Day 2, 3 and 7		
Optional NK cell receptor studies (**see Section 8.3**)	Start of Block	---	Start of Block
Optional intestinal microbiome studies (**see Section 8.4**) *St. Jude participants only*	Start of Block	Start of Block	Start of Block and Completion of Block
Optional neuropathy & function (**see Section 8.5 and Appendix IV**)	Day 6 and 15	Start of Block	Start of Block and EOT

**BUN, creatinine, ALT, AST, LDH, alkaline phosphatase, glucose, total bilirubin, albumin, total protein, uric acid.*
#Sodium, potassium, calcium, magnesium and phosphorous. ***See Section 6.4 for daily monitoring serum potassium, magnesium, and calcium (total corrected for albumin, or ionized calcium) ≤ 72 hours prior to the administration of oral panobinostat on day 2 of cycle 1 of Block A, and the results must all be ≥ LLN before the first dose of panobinostat is administered.***
¥Post-menarchal females, per hospital policy.
***PK blood samples will be collected after the first dose (day 2) and third dose (day 6) of panobinostat at the following time points: pre-dose, 30 min, 1 h, 3 h, 6 h, 24 ± 3 h, and 48 ± 3h.*

- *Neurologic exam more often if clinically indicated. Comprehensive assessment of peripheral neuropathy and sensorimotor function prior to first dose of liposomal vincristine (VSLI) (day 6), prior to staring Bortezomib (day15), and on day of beginning of each block of therapy. Exam may be conducting within 4 days prior to the suggested day if scheduling necessitates (e.g., holidays etc.) If patients have Grade 4 neutropenia then CBCs should be checked at least every 3 to 4 days until recovery to Grade 3.*
- *Bone marrow procedures not required for lymphoma patients without marrow disease.*

Obtain other studies as needed for clinical care.

8.0 CORRELATIVE RESEARCH STUDIES

8.1 Pharmacokinetic Studies

Optional pharmacokinetic studies will be done on peripheral blood. Samples will be collected after the first dose (day 2) and third dose (day 6) of panobinostat at the following time points: pre-dose, 30 min, 1 h, 3 h, 6 h, 24 ± 3 h, and 48 ± 3h.

8.1.2 Sample Collection and Processing

Draw 4 mL of blood in a pre-cooled green top (heparinized) tube. Samples may be drawn from a central catheter using local hospital guidelines for flushing. Place samples on ice. Within 30 minutes of obtaining the sample, centrifuge in a refrigerated centrifuge for 10 minutes at 2000x g to separate blood cells and platelets from the plasma. The upper plasma layer will be split into 2 aliquots. Each aliquot should be transferred by pipette into a polypropylene screw-cap freezing vial. Each vial should be labeled with study ID number, and the DATE and TIME the specimen was drawn. Samples should be frozen immediately at -20 until shipping and analysis. Panobinostat will be measured in plasma using a validated LC-MS/MS assay in the St. Jude Children's Research Hospital Pharmacokinetics Shared Resource. Plasma samples will be batched for shipping.

8.2 Pharmacodynamic, Genomic and MRD Studies

Blood and bone marrow specimens will be routed through the St. Jude Biorepository for processing and distribution for these studies:

- PB and BM for MRD prior to each block
- Optional BM for PD pre treatment
- Optional BM for PD and MRD on day 7 of Block A
- Optional PB for PD pretreatment and on Day 2 (pre-panobinostat), Day 3 (approximately 24 hours post-panobinostat), and Day 7 of Block A

8.2.1 Sample collection and processing

In all patients:

Collect 5 mL of bone marrow aspirate in preservative-free heparin stored at ambient temperature. Samples will be collected for MRD:

- Pre-treatment
- Prior to each block

Collect 10 mL of peripheral blood in preservative-free heparin vacutainer stored at ambient temperature. Samples will be collected for MRD:

- Pre-treatment
- Day 7 of block A
- Prior to each block

In patients consenting for optional pharmacodynamic studies:

Collect 5 mL of bone marrow aspirate in purple top tubes (EDTA) stored at ambient temperature. Samples will be collected:

- Pre-treatment (5 mL)
- Day 7 of block A (10 mL: 5mL for PD and 5 mL for MRD)

Collect 15 mL of peripheral blood in purple top tubes (EDTA) stored at ambient temperature. Samples will be collected:

- Pre-treatment
- Days 2 (pre-panobinostat), 3 (approximately 24 hours post-panobinostat), and 7 of Block A

Note that flow cytometric studies are not performed on weekends. Hence, samples should be drawn Monday through Thursday and sent by overnight delivery. Samples that arrive on weekends are processed on Monday, but such delay may affect the quality of the assay.

Please send specimens Monday through Thursday only. Please do NOT ship Friday or before holidays. Send specimens via FedEx Priority Overnight. Notify the Biorepository of pending shipment [redacted] with tracking number.

8.3 NK Cell Receptor Studies

Peripheral blood (3 ml) will be obtained pre-treatment for immunophenotyping and (8.5 ml) before Block C for immunophenotyping and genotyping for NK cell receptors as described previously[58,64]. Blood samples will be collected in ACD (yellow-top) tubes and sent to [redacted] laboratory. We will assess NK cell receptors including KIRs, NCRs, NKG2D, DNAM-1, 2B4, and NTBA. The sample should be obtained before the administration of steroids.

8.4 Intestinal Microbiome Research

To participate in this research, participants cannot have a clinical or microbiologically-proven diarrheal illness.

Four stool specimens will be collected from research participants, one is during a time period before starting Block A (or until the first 72 hours after starting Block A), the second sample will be collected within 72 hours prior to starting Block B, the third within 72 hours prior to starting Block C, and the fourth within 3-4 weeks from starting Block C chemotherapy (post chemo). Paired stool samples will be batched to be analyzed simultaneously. Bacterial genomic DNA will be harvested from these samples and utilized for ribosomal DNA sequencing to determine the overall bacterial microflora prior to and following chemotherapy. These two methodologies will provide a preliminary investigation into any notable alterations in bacterial diversity resulting from therapeutic interventions. In tandem, we will also isolate RNA from these samples for sequencing in order to identify novel RNA viruses. Once notable differences in the microbiome before and after chemotherapy are determined, we will then attempt to describe the gastrointestinal complications during the 3 treatment blocks. Stool samples will be used to run the laboratory investigation in [redacted] research laboratories.

Medical records will be reviewed to collect data about infectious diseases, probiotics, antacids and antimicrobial use during the study period. Only descriptive statistics such as median, range and percentages will be used as appropriate. The proposed study is intended to be a pilot investigation to test feasibility of this approach and optimize experimental procedures. The data and information obtained in these investigations will be utilized as preliminary data for a larger scale study and future grant applications.

8.4.1 Laboratory Methods and Materials

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

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

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

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

8.5 Neuropathy and Function Assessments

8.5.1 Sensory, Motor and Autonomic Impairment

Peripheral sensory and motor integrity will be evaluated with the Pediatric Modified Total Neuropathy Scale (Ped-mTNS). This instrument includes a pediatric specific sensory, motor and autonomic symptoms inquiry and five-item neurologic exam (light touch, vibration, pin sensation, distal strength and deep tendon reflexes) designed to evaluate the integrity of the peripheral nervous system[65]. This assessment has been tested in children exposed to neurotoxic chemotherapy agents and has good internal consistency, inter-rater and test-retest reliability. Scores are associated with measures of balance and manual dexterity[66]. Scores range from 0-32; a score of zero indicates no impairment. This test requires a standard set of Semmes Weinstein monofilaments, a Medipin, a handheld Bioethesiometer, and a reflex hammer. Autonomic function will be evaluated using power spectral analysis of heart rate variability (PS/HRV) from time series during a thirty minute supine ECG and with heart rate and blood pressure responses from a tilt table test.

8.5.2 Balance and Manual Dexterity

The balance and manual dexterity subtests of the Bruininks-Oseretsky Test of Motor Proficiency, version 2 (BOT2) will be used to evaluate peripheral sensory and motor function[67]. This instrument has been evaluated for psychometric properties in 1,520 children with established inter-rater and test-retest reliability for each subtest, by sex and

age (4-21 years)[68]. Points are received for performance on each subtest and converted to sex- and age-standardized scores with a mean of 15 and a standard deviation of 5. Published standards will be used to administer the balance and manual dexterity sub-tests in our patient population[67]. This test requires a standard BOT2 Test Kit.

8.5.3 Energy Expenditure Requirements for Ambulation

The energy expenditure required for ambulation will be evaluated by calculating the physiologic cost of walking over a six minute time period. Children are asked to walk as fast as possible for six minutes on a level surface while pushing a measuring wheel that records the distance walked[69]. Heart rate (HR) and distance walked in meters will be recorded for each minute and a physiologic cost index determined [(HR during walking – HR at rest)/meters per minute]. The mean physiologic cost index over the last three minutes of walking will be used for analysis. This measure has been used to evaluate the ability of ankle foot orthoses to decrease the energy expenditure of walking in children with spastic diplegia,[70] to evaluate energy required for walking in persons with stroke,[71] and to compare different reciprocating gait orthoses in persons with paraplegia[72]. This test requires a stop watch, a corridor reasonably free of obstacles, rating of perceived exertion scale, measurement wheel, a heart rate monitor, and a lap counter.

9.0 EVALUATION CRITERIA

9.1 Response Criteria for Lymphoblastic Leukemia

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

- Complete response (CR): MRD-negative, < 0.01% blasts by flow cytometry
- Complete response (CRM): MRD-positive, 0.01% to < 5% blasts by flow cytometry
- Partial response (PR): A decrease of at least 50% in the percentage of blasts and 5% to 25% blasts by flow cytometry.
- No response (NR): No change in clinical or laboratory status.
- Progressive disease (PD): Deterioration of initial disease status
- Relapse:
    - Bone marrow relapse: Subsequent appearance, after achievement of CR, of ≥ 5% blasts in the bone marrow with confirmation by flow cytometry.
    - CNS relapse: ≥ 5 WBC/μL of CSF with definite blasts on cytospin.
    - Other extra-medullary relapse: Development of extra-medullary disease after achievement of CR.

9.2 Response Criteria for Lymphoblastic Lymphoma

- Complete response (CR): Disappearance of all evidence of disease from all sites as determined by physical exam and appropriate imaging studies. Bone marrow aspirate/biopsy as well as CSF examination should be normal. Any macroscopic nodules in any organs detectable on imaging studies should no longer be present. PET scan should be negative if initially positive.
- Complete response unconfirmed (CRu): Residual lymph node mass >1.5 cm in greatest transverse diameter (GTD) that has regressed by >75% in sum of the products of greatest perpendicular diameter or any residual lesions in organ have decreased by >75% with negative PET scan if initially positive.
- Partial response (PR): ≥50% decrease in SPD and no new lesions.
- Stable disease (SD): No new lesions and failure to qualify for partial response.
- Progressive disease (PD): 25% increase in lesions or any new lesions.

10.0 CONCOMITANT THERAPY

Concurrent anti-cancer therapy not defined within this protocol, including chemotherapy, radiation therapy, immunotherapy, or biologic therapy may NOT be administered to participants receiving treatment on this study. If these treatments are administered, the participant will be removed from protocol therapy. No other investigational agents may be given while the participant is on protocol therapy.

In general, the use of any concomitant medication/therapies deemed necessary for the care of the patient are allowed, including drugs given prophylactically (e.g., anti-emetics) with the following exceptions:

1) Any medications listed in Appendix II that may cause QTc prolongation or inducing torsade's de pointes should not be used unless clinically indicated and cleared by PI.
2) Any medications that have the potential to alter serum electrolytes (e.g., diuretics) should be monitored very closely for electrolyte abnormalities as these can contribute to the risk of QT prolongation and ventricular arrhythmias.
3) Hydroxyurea may be given per institutional guidelines prior to initiating protocol therapy and during the first course of therapy to help manage elevated white counts.
4) If the patient develops painful sites of disease that may benefit from localized radiation, the treating physician should contact the study chair to discuss the case. In certain circumstances, localized radiation will be allowed provided there are other sites of disease that can be evaluated for response.
5) Patients with diabetes may require close monitoring of blood glucose and adjustment of anti-diabetic medication.
6) Drugs that can inhibit CYP3A4/5: the concomitant use of strong CYP3A inhibitors should be avoided when possible (e.g., ketoconazole, itraconazole, voriconazole, posaconazole, clarithromycin, atazanavir, indinavir, nefazodone, nelfinavir, ritonavir, saquinavir, telithromycin).
7) Drugs that are potent CYP3A4/5 inducers: Similarly, the concomitant use of strong CYP3A inducers should be avoided (e.g., phenytoin, carbamazepine,

rifampin, rifabutin, rifapentine, phenobarbital, St. John's Wort). If given concomitantly, clinical judgment is to be exercised when medications that are metabolized by CYP3A are given with CYP3A4 inducers.

8) Patients with a prior seizure disorder requiring anti-convulsant therapy are not eligible to receive nelarabine. In addition, patients with Grade $\geq$ 2 peripheral neurotoxicity, as determined prior to Block C by the treating physician or a neurologist, are not eligible to receive nelarabine and will receive clofarabine instead. These restrictions in eligibility are designed to prevent excessive nelarabine-induced central and peripheral neurotoxicity in at-risk patients.

11.0 SUPPORTIVE CARE GUIDELINES

Best supportive care and treatment will be given as appropriate to each participant and according to current institutional practice (transfusions, oxygen therapy, nutritional support, palliative treatment for pain or cough, etc.). Participants may experience profound myelosuppression and immune suppression during this time. Steroids may mask fever as well as other components of the inflammatory response caused by sepsis during induction and may be associated with very mild and subtle symptoms. Caregivers must also be made aware that patients may experience very rapid clinical deterioration. This suggests the need for a supportive care network that can recognize and respond to sudden changes in a patient's condition. Aggressive supportive care improves outcome. The following guidelines are intended to give general heath direction for optimal patient care and to encourage uniformity in the treatment of this patient population. Notify the principal investigator of any unexpected or unusually severe complications.

11.1 Management of Hyperleukocytosis

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

11.2 Metabolic Derangements

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

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

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

11.3 Bacterial, Fungal and Viral Prophylaxis

Patients should receive prophylactic antibiotics (levofloxacin or ciprofloxacin; or vancomycin + ciprofloxacin or cefepime) and antifungals (micafungin during Block A and

during chemotherapy in Blocks B and C, or voriconazole if patient has completed all chemotherapy in the treatment phase). Antimicrobials may be given by the parents or other caregivers at home, according to institutional guidelines. All patients must receive prophylaxis for *pneumocystis jiroveci* pneumonia. Participants who have Herpes simplex virus (HSV) antibodies may receive prophylactic acyclovir or famcyclovir daily (PO or IV).

11.4 Management of Febrile Neutropenia

Episodes of fever and neutropenia should be managed according to institutional guidelines. Participants with fever (defined as a single temperature ≥38.3° C (101°F) or temperature of ≥38.0° C (100.4°F) sustained over a one hour period and neutropenia (defined as ANC ≤500 cells/μL or WBC ≤1,000 x 10^9/L) should be given IV broad-spectrum antibiotics immediately, after obtaining appropriate cultures.

11.5 Drug Interactions

Because concurrent use of enzyme inducing anticonvulsants (e.g., phenytoin, phenobarbital, and carbamazepine) with anti-leukemic therapy has recently been associated with inferior EFS, every effort should be made to avoid these agents, which induce many drug metabolizing enzymes. Keppra (levetiracetam) does not induce hepatic drug metabolizing enzymes and may be a suitable alternative anticonvulsant.

Azole antifungals (fluconazole, itraconazole, voriconazole, and ketoconazole) and the macrolide group of antibiotics (e.g., erythromycin, rifampin, and Zithromax) may have potent inhibitory effects on drug-metabolizing enzymes, and the doses of some anti-leukemic drugs (e.g., vincristine, anthracyclines, steroids, etoposide) may need to be reduced in some patients on chronic azole antifungals or antibiotics. Consult Pharmacokinetics if long-term use of these interacting drugs is unavoidable.

11.6 Growth Factors

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

11.7 Etoposide Reactions

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

resume infusion at ½ previous infusion rate until complete. Consider infusing NS at 1 – 1.5 x maintenance during remainder of infusion. For all subsequent doses, further dilute and infuse over 2 hours.

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

If an anaphylactoid reaction should occur:

1. Stop the infusion immediately and notify H/O Fellow or Attending MD
2. Administer the following as indicated:
    a) diphenhydramine 1mg/kg IV (max dose 50 mg)
    b) hydrocortisone 50 – 100 mg/m^2 IV
    c) epinephrine 0.01 mg/kg of a 1:1000 concentration for SQ administration (max. 0.5 mg)
    d) fluid bolus 10 mL/kg NS infused over 15 minutes
3. Once symptoms have resolved, resume infusion at ½ previous rate until infusion complete. Consider infusing NS at 1 – 1.5 x maintenance during remainder of infusion.
4. If anaphylaxis recurs, stop the infusion and re-treat as above. Do not administer remainder of dose. Consider substituting etoposide with etoposide phosphate (Etopophos®) for all subsequent doses.
5. If anaphylaxis does not recur, pre-medicate all subsequent doses with diphenhydramine 1mg/kg (max 50 mg) and hydrocortisone 50 – 100 mg/m^2. Consider slowing the loading dose to be administered over 1 hour.
6. Have at bedside all of the following for all subsequent infusions:
    a) Diphenhydramine 1mg/kg IV (max 50 mg)
    b) Hydrocortisone 50 – 100 mg/m^2 IV
    c) Epinephrine 0.01 mg/kg of a 1:1000 concentration for SQ administration (max 0.5 mg)

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

11.8 Nelarabine-Related Neurotoxicity

If neurologic toxicity develops prior to the completion of 5 days of therapy, nelarabine should be halted and the study chair should be called immediately. If Grade 4 nelarabine-related neurotoxicity develops, the patient will be taken off nelarabine indefinitely.

Investigators are cautioned to monitor patients carefully for the development of signs and symptoms of peripheral neuropathy. In the event that a patient develops initial signs or

symptoms of peripheral neuropathy attributed to the administration of nelarabine, the agent should be discontinued for the course.

Only resume nelarabine if peripheral neuropathy resolves to less than Grade 2 toxicity. Nelarabine should NOT be continued in patients who develop signs or symptoms suggestive of an ascending polyneuropathy, including a Guillain-Barré-like syndrome, even if these symptoms resolve. It is recommended that patients who develop neurotoxicity in association with nelarabine undergo a thorough neurologic evaluation to establish a diagnosis and to exclude other potential etiologies (e.g., disease progression, concomitant illness, etc.). If a Guillain-Barré-like syndrome is suspected, therapeutic measures considered appropriate for the individual patients (i.e., intravenous immunoglobulin, plasmapheresis, steroids, and supportive care) should be instituted as soon as possible. Neurology consult is recommended.

In patients who develop Grade 3 CNS events (e.g., somnolence, mood alteration, irritability, confusion, etc.) that return to < Grade 2 severity prior to the next planned course of nelarabine, the nelarabine dose is to be prescribed without treatment interruption. Nelarabine will not be made up if any doses are missed during a 5-day treatment course. Patients who are unable to receive a 5-day course of nelarabine because of toxicity should proceed to the next planned course of protocol therapy as soon as recovery allows.

If patient(s) develop myalgia or myoglobinuria, they should be evaluated for the potential of having rhabdomyolysis. The patient should receive a workup that includes AST, ALT, creatinine, and creatine kinase (CK)/ (CPK) at a minimum. Consideration should be given to consulting a nephrologist. The PI should be notified if the patient develops either of the above symptoms, and the nelarabine should be held. If the patient is stable, other protocol therapy may continue while the patient is undergoing evaluation. Following study chair notification and evaluation for rhabdomyolysis, a decision should be rendered regarding permanently discontinuing the nelarabine.

11.9 Bortezomib-Related Toxicities

Special criteria will be followed for peripheral neuropathy and pulmonary toxicities thought to be related to bortezomib. Bortezomib (and VSLI) will be decreased for sensory peripheral neuropathy as described in Section 6.5.

There have been rare reports of acute diffuse infiltrative pulmonary disease of unknown etiology such as pneumonitis, interstitial pneumonia, lung infiltration and acute respiratory distress syndrome (ARDS) in patients receiving bortezomib. For this reason, pulmonary toxicity will be monitored carefully during Block A treatment. Pulmonary symptoms such as increased respiratory rate may indicate toxicity related to bortezomib and pulse oximetry should be checked for persistent, unexplained hyperpnoea. Respiratory symptoms such as cough, hypoxia and dyspnea or chest infiltrates should be aggressively evaluated.

11.10 Special Considerations for Administration of Clofarabine

Patients with B-lymphoblastic leukemia and lymphoma in stratum II

Clofarabine is excreted primarily by the kidneys. Therefore, drugs with known renal toxicity should be avoided during the 5 days of clofarabine treatment in each cycle. Additionally, the liver is a known target organ for clofarabine toxicity. Therefore, concomitant use of medications known to induce hepatic toxicity should be avoided. Hepatic and renal function should be assessed prior to and during treatment with clofarabine and it is recommended that the patient's fluid status and hepatic and renal function be carefully monitored during the drug administration period. All patients should receive hydration each day of clofarabine treatment, giving careful consideration to the cardiac and renal function of the patient. To the extent possible, use of nephrotoxic (e.g., vancomycin, amphotericin B, etc.) and hepatotoxic (e.g., voriconazole, cyclosporine, etc.) agents is to be avoided during clofarabine administration.

Prophylactic steroid administration has been administered by some investigators and has been reported to minimize the occurrence and/or severity of the following potential clofarabine-related toxicities: nausea, vomiting, skin rash/desquamation, and capillary leak syndrome. Therefore, methylprednisolone, 0.5 to 1 mg/kg/dose IV, should be given prior to each dose of clofarabine.

12.0 OFF TREATMENT AND OFF STUDY CRITERIA

12.1 Off Treatment Criteria

- Research participants who fail to achieve complete remission after induction block C of treatment
- Progressive disease
- Second malignancy
- Development of unacceptable toxicity during treatment (with concurrence of the PI or medical designee)
- Participant/family decision to withdraw from protocol treatment at any time for any reason
- Discretion of the study PI or co-PI, such as the following:
    - The researcher decides that continuing in the study would be harmful
    - A treatment is needed that is not allowed on this study
    - The participant misses so many appointments that the data cannot be used in the study
    - The participant's condition gets worse
    - New information is learned that a better treatment is available, or that the study is not in the participant's best interest
- Completion of all protocol prescribed treatment

12.2 Off Study Criteria

- Death
- Lost to follow-up
- Withdrawal of consent

13.0 SAFETY AND ADVERSE EVENT REPORTING REQUIREMENTS

13.1 Reporting Adverse Experiences and Deaths to St. Jude IRB

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

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

Unrelated, expected deaths that occur after patient has completed protocol treatment do not require reporting to the IRB. Though death is "serious", the event must meet the other two requirements of "related or possibly related" and "unexpected/unanticipated" to be considered reportable. However, all deaths while on active protocol treatment (or within 30 days of last protocol treatment) will be require reporting to the IRB. Deaths meeting reporting requirements are to be reported immediately to the St. Jude IRB, but in no event later than 48 hours after the investigator first learns of the death.

The following definitions apply with respect to reporting adverse experiences:

Serious adverse event (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 that requires treatment to prevent any of the medical outcomes previously listed.

Unexpected adverse event:

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

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

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

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

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

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

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

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

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

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

The principal investigator has the obligation to report all serious adverse events to the FDA, IRB, and Novartis Pharmaceuticals Clinical Safety and Epidemiology Department (CS&E).

13.2 Reporting to Novartis Pharmaceuticals and Spectrum Pharmaceuticals

This is an investigator-initiated study. The principal investigator, Sima Jeha, 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.

Any unanticipated problem that is serious, at least possible related and unexpected occurring in a patient after providing informed consent, while receiving study drug, and until four weeks after stopping study drug will be reported by FAX [redacted] to Novartis Pharmaceuticals CS&E Department (panobinostat) and by FAX to Spectrum Pharmaceuticals (liposomal vinCRIStine, VSLI) within 24 hours of learning of its occurrence, even if it is not felt to be drug related.

Novartis instructions for rapid notification of unanticipated problems

The principal investigator has the obligation to report all unanticipated problems to the FDA, IRB, and Novartis Pharmaceuticals Integrated Medical Safety (IMS) Department. All events reported to the FDA by the investigator are to be filed utilizing the Form FDA 3500A (MedWatch Form).

All events must be reported, by FAX [redacted] to Novartis Pharmaceuticals IMS Department within 24 hours of learning of its occurrence. This includes serious, at least possibly related and unlabeled (unexpected) adverse experiences. **All deaths during treatment or within 30 days following completion of active protocol therapy must be reported within 24 hours**.

Any unanticipated problem occurring after the patient has provided informed consent and until 4 weeks after the patient has stopped study participation must be reported. This includes the period in which the study protocol interferes with the standard medical treatment given to a patient (e.g. treatment withdrawal during washout period, change in treatment to a fixed dose of concomitant medication).

Serious adverse events occurring more than 4 weeks after study discontinuation need only be reported if a relationship to the Novartis study drug (or therapy) is suspected. For Comparator Drugs/Secondary Suspects (Concomitant Medications), all serious adverse experiences will be forwarded to the product manufacturer by the investigator.

Pregnancies

To ensure patient safety, each pregnancy in a patient on study drug (or their partner) must be reported to Novartis within 24 hours of learning of its occurrence, provided the conception occurred between the time the patient started taking study drug, and 12 months after the discontinuation of study drug. The pregnancy should be followed up to determine outcome, including spontaneous or voluntary termination, details of birth, and the presence or absence of any birth defects, congenital abnormalities or maternal and newborn complications.

Pregnancy should be recorded on a Clinical Trial Pregnancy Form and reported by the investigator to the local Novartis Clinical Safety and Epidemiology Department. Pregnancy follow-up should be recorded on the same form and should include an assessment of the possible relationship to the Novartis study drug of any pregnancy outcome. Any SAE experienced during pregnancy must be reported on the SAE Report Form. Pregnancy outcomes must be collected for female patients who took study drug in this study.

Consent to report information regarding these pregnancy outcomes should be obtained from the patient.

Pregnancy outcomes should be collected for the female partners of any males who took study drug in this study. Consent to report information regarding these pregnancy outcomes should be obtained from the mother.

13.3 Reporting to Regulatory Affairs Office and FDA

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.

13.4 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 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 clinically relevant events 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.

Patients with abnormal blood counts due to bone marrow involvement by disease (i.e. all leukemia patients and lymphoma patients with bone marrow involvement) will be considered non-evaluable for hematological toxicities.

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.

13.5 Process for Reporting Adverse Events from and to Collaborating Sites

Adverse events from collaborating sites will also be reviewed by the PI and discussed in study team meetings as described above. SAE 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 TRACKS application, the PI will indicate if all sites should be notified to report to their IRBs, and if the protocol and/or consent should be amended (consent will be amended if event is information that should be communicated to currently enrolled subjects). Generally, only events that warrant an amendment to the protocol and/or consent will be reported expeditiously to all sites. However, any event may be reported expeditiously to all sites at the discretion of the PI. A cumulative summary of Grade 2-5 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. Sima Jeha) within 48-72 hours via fax or email.

Unexpected deaths must be reported to the St. Jude PI at [redacted] or phone call to Dr. Jeha within 24 hours of the event. A written report must follow. The study team should be copied on all correspondence regarding the event.

[redacted]

14.0 DATA COLLECTION, MONITORING AND CONFIDENTIALITY

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

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

14.2 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 TRACKS (Total Research and Knowledge System) 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 TRACKS.

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

14.3 Confidentiality

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

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

15.0 STATISTICAL CONSIDERATIONS

15.1 Analysis of Primary Objective

Primary objective: To estimate the complete remission (CR) rate for patients with T-cell lymphoblastic leukemia or lymphoma in first relapse.

CR is assessed at the end of each remission re-induction Block. CR is defined as CR or CRM in section 9.1 for ALL, and as CR in section 9.2 for lymphoma. All patients who start re-induction Block A therapy are considered evaluable.

Definition of Success and Failure: The same as in our previous practice (ALL-R16 and ALL-R17), CR is assessed at the end of each block; a patient in CR either goes to transplant if a matched donor is available and is consequently off the chemotherapy, or put on maintenance with the subsequent chemotherapy block while waiting for a donor. Therefore, **any patient who at any time point achieves CR and goes to transplant is considered as a success; or any patient who successfully reaches the end of block C and achieves/remains CR is considered a success; all other cases are considered as failure.** For example, a patient who achieves CR at the end of block A but put on block B while waiting for a donor, remains in CR, and then goes to transplant, is a success. An unlikely (and unfortunate) case of failure is that the patient achieves CR at the end of block A, but put on block B while waiting for a donor, and develops severe toxicities while on block B that the patient has be taken off treatment. A patient who could not find a matched donor but successfully reaches the end of block C with CR is a success.

Sample size: Logistically it is feasible to enroll 24 relapsed T cell ALL patients in 6 years. Thus the total sample size is set at 24.

Statistical Analysis: Analysis will be done by point and interval estimation of the Success (as defined above) rate/probability using the sample proportion and the Blyth-Casella exact 95% confidence interval.

Secondarily a comparison with historical Success (CR) rate from ALL-R16 and ALL-R17 (Table 1) will be done using Fisher's exact test.

Table 1: Induction response in R16 and R17 T-cell patients

	R16 (n = 9)	R17 (n= 7)	Total
CR	7 (77.8%)	5 (71.4%)	12 (75.0%)
No CR	2 (22.2%)	2 (28.6%)	4 (25.0%)
Total	9	7	16

Historical "control" is formed by combining ALL-R16 and ALL-R17, to give a sample (Sample1) of size n1=16 and number of success (CR) 12.

To safeguard against the possibility that the novel re-induction therapy is much inferior to the prior approaches, the following group-sequential design with interim analyses monitoring for early evidence of inferior re-induction will be applied. The historical data give an estimate of the CR probability 12/16=0.75 and the Blyth-Casella exact 95% confidence interval [0.5, 0.91]. Therefore we deem the novel re-induction therapy as unacceptable if there is evidence that the CR probability is less than 0.5. The following ad-hoc stopping rule is generated based on an analysis using the Binomial distribution, and has power of 0.79, 0.66, and 0.53 to stop the trial if the true Success rate of the novel treatment is 0.35, 0.4, and 0.45 respectively.

Table 2: Interim Analysis Plan

Interim Sample Size	**Stop the trial if the number *Failure* is ≥**
12	7
24	13

Monitoring of severe toxicities: Severe toxicities rendering patients' death or being taken off treatment will be monitored in a group-sequential manner the same as the interim analysis for CR, namely:

Table 3: Monitoring Plan for Severe Toxicities

Interim Sample S	**Stop the trial if the number of SAE is ≥**
12	7
24	13

15.2 Analysis of Secondary Objectives

To determine minimal residual disease (MRD) levels present at end of each block of therapy in patients with leukemia

Minimal residual disease (MRD) will be studies at each cycle of therapy. MRD is considered as positive (i.e., prevalent) if its level is ≥0.01% for ALL. The prevalence of MRD at end of each cycle is defined as proportion of MRD positives; we will estimate these proportions with point and interval estimates.

To characterize the toxicities of VSLI when used in combination with chemotherapy.

Proportions (probabilities) of relevant toxicities will be estimated with point and interval estimates.

15.3 Analysis of Exploratory Objectives

To identify molecular determinants and biomarkers of histone deacetylase (HDAC) inhibitor response

Global differential gene expression (and possibly genomic) profiling will be performed to identify genes differentially expressed and methylated (by H3K27 and H3K9 Acetylation markers) in xenograft cells treated with HDAC inhibitors vs. those untreated. Levels of gene expression will be generated by total-strand RNA-seq and measured by FPKM (or possibly the latest technology available at the time). Gene differential expression and methylation between treated and untreated cells will be detected by general linear models (upon proper transformation) and linear rank procedures such as the Wilcoxon test.

To study the pharmacokinetics of panobinostat

Panobinostat PK parameters (e.g. Cmax, Tmax, AUC, half-life, and clearance) will be estimated using population-based modeling techniques.

To establish xenografts of relapsed T-cell lymphoblastic leukemia for downstream studies of cytotoxicity and therapy resistance

This is a resource objective that does not require Statistical considerations.

To perform genomic studies of leukemic cells at relapse, diagnosis and MRD (where available) to investigate clonal evolution and identify targets for therapeutic intervention

This is a fast-evolving field capitalizing on the maturation of the whole-genome sequencing technologies. We will utilize the latest and most effective approach to modeling clonal evolution at the time of analysis.

To validate new markers and methods for MRD detection

Comparisons to the current gold standard by association (e.g., correlation) and agreement statistics (such as the kappa coefficient) will be applied to assess the new markers' ability in MRD detection.

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

Genotype-outcome and gene expression-outcome associations will be analyzed using proper statistical procedures such as the log-rank test and Cox regression modeling.

To perform a comprehensive analysis of the intestinal microflora in patients with relapsed hematologic malignancies prior to and subsequent to each block of intensive salvage chemotherapy using next generation sequencing technologies as an exploratory approach

Summary statistics (e.g. percentages) describing the intestinal microflora will be computed.

To describe the frequency and severity of gastrointestinal illnesses during 3 blocks of intensive salvage chemotherapy in patients who did and those who did not have changes in intestinal microbiota

Summary statistics describing the frequency and severity of gastrointestinal illnesses will be computed. Comparisons of gastrointestinal illnesses between patients who did and those who did not have changes in intestinal microbiota will be done by two-sample Binomial test or logistic regression.

15.4 Accrual and Study Duration

T-cell ALL or lymphoma in first relapse: n=24 *evaluable*
ALL or Lymphoblastic Lymphoma in second or third relapse: n=10 *evaluable*.
Total number or participants: n=40
Study duration: 6 years

16.0 OBTAINING INFORMED CONSENT

16.1 Consent Prior to Research Interventions

Initially, informed consent will be sought for the institutional banking protocol (TBANK research study), PGEN5, and for other procedures as necessary for standard medical care. During the screening process for eligibility, informed consent for SCREEN protocol OR for TSALV is required before any research tests are performed.

16.2 Consent at Enrollment

The process of informed consent for TSALV will follow institutional policy. The informed consent process is an ongoing one that begins at the time of diagnosis and ends

after the completion of therapy. Informed consent should be obtained by the attending physician or his/her designee, in the presence of at least one non-physician witness. Initially, informed consent will be sought for the institutional banking protocol (research study), blood transfusion and other procedures as necessary. After the diagnosis of relapsed or refractory leukemia/lymphoma is established, we will invite the patient to participate in the TSALV protocol.

Throughout the entire treatment period, participants and their parents receive constant education from health professionals at SJCRH and collaborating sites, and are encouraged to ask questions regarding alternatives and therapy. All families have ready access to chaplains, psychologists, social workers, and the St. Jude ombudsperson for support, in addition to that provided by the primary physician and other clinicians involved in their care. We will also obtain verbal assent from children 7 to 14 years old and written assent for all participants ≥14 years of age.

16.3 Consent at Age of Majority

Participants who reach the age of majority while on study will be re-consented for continued participation on TSALV at the time of their next clinic visit after turning 18 year according to Cancer Center and institutional policy.

16.4 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 the telephone interpreter's service will be used to translate the consent information. The process for obtaining an interpreter and for the appropriate use of an interpreter is outlined on the Interpreter Services, OHSP, and CPDMO websites.

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APPENDIX I: KARNOFSKY AND LANSKY PERFORMANCE STATUS SCALES/SCORES

PERFORMANCE STATUS CRITERIA *Karnofsky and Lansky performance scores are intended to be multiples of 10*					
ECOG (Zubrod)		**Karnofsky**		**Lansky**	
Score	**Description**	**Score**	**Description**	**Score**	**Description**
0	Fully active, able to carry on all pre-disease performance without restriction	100	Normal, no complaints, no evidence of disease	100	Fully active, normal
		90	Able to carry on normal activity, minor signs or symptoms of disease	90	Minor restrictions in physically strenuous activity
1	Restricted in physically strenuous activity by ambulatory and able to carry out work of a light or sedentary nature, e.g., light housework, office work	80	Normal activity with effort; some signs or symptoms of disease	80	Active, but tires more quickly
		70	Cares for self; unable to carry on normal activity or do active work	70	Both greater restriction of and less time spent in play activity
2	Ambulatory and capable of self-care but unable to carry out any work activities; up and about more than 50% of waking hours	60	Requires occasional assistance, but is able to care for most of his/her needs	60	Up and around, but minimal active play; keeps busy with quieter activities
		50	Requires considerable assistance and frequent medical care	50	Gets dressed, but lies around much of the day; no active play, able to participate in all quiet play and activities
3	Capable of only limited self-care, confined to bed or chair more than 50% of waking hours	40	Disabled, requires special care and assistance	40	Mostly in bed; participates in quiet activities
		30	Severely disabled, hospitalization indicated; death not imminent	30	In bed; needs assistance even for quiet play
4	Completely disabled; cannot carry on any self-care; totally confined to bed or chair	20	Very sick, hospitalization indicated. Death not imminent	20	Often sleeping; play entirely limited to very passive activities
		10	Moribund, fatal processes progressing rapidly	10	No play; does not get out of bed

APPENDIX II: TESTS PERFORMED FOR ROUTINE CARE AND FOR RESEARCH

Routine care

History & physical (including vital signs, height, weight, BSA)
CBC, differential, platelets
Chemistries
Serum Electrolytes
Pregnancy testing
Infectious disease screening
Echocardiogram/MUGA
EKG
Lumbar puncture for IT chemotherapy, CSF cell count and differential
Bone marrow procedure for morphology, response assessment, MRD
Peripheral blood for MRD, if done
Commercially available agents

Research

Investigational agents – panobinostat and liposomal vincristine (VSLI)
Pharmacokinetic studies
Pharmacodynamic studies
NK cell studies
Genomic studies
Intestinal microbiome studies
Neuropathy studies
Research MRD studies

APPENDIX III: MEDICATIONS TO AVOID OR BE USED WITH CAUTION WITH PANOBINOSTAT

I. Medications that have a risk of causing Torsade's de pointes ventricular arrhythmia should be avoided.

Patients who are currently receiving treatment of the medications in Table 1-1 and cannot either discontinue from this treatment or switch to an alternative medication prior to enrollment in a panobinostat clinical study, will be excluded from the study. Patients may not begin panobinostat treatment with any of the medications listed in Table 1-1 unless this is discussed with the PI and Novartis.

NOTE: It is of great importance to avoid combining drugs listed below in Table 1-1 and Table 2-1 (CYP3A inhibitors) especially in the presence of electrolyte abnormalities, notably decreased potassium or magnesium levels commonly associated with diuretic usage.

In generally, medications listed in Table 1-1 should be avoided while medications listed in Tables 2-1 and 3-1 are to be used with caution when co-administered with panobinostat. Please select the most stringent recommendation for concomitant medications (i.e., to be avoided), which are common among the tables (e.g., erythromycin, clarithromycin)

Table 0-1 Medications that have a risk of causing Torsade's de pointes to be avoided during treatment with panobinostat

All Class IA anti-arrythmics	
• quinidine • procainamide	• disopyramide • any other class IA antiarrhythmic drug
All Class III anti-arrythmics	
• amiodarone • sotalol • bretylium	• disopyramide • dofetilide • ibutilide • any other class III antiarrhythmic drug
Antibiotics **Macrolide antibiotics***	
• erythromycin • clarithromycin	• telithromycin • Quinolone antibiotics* • sparfloxacin
Antipsychotics	
• thioridazine • mesoridazine	• chlorpromazine • pimozide
Anti-malarials	
• halofantrine • chloroquine	

Medications that have a risk of causing Torsade's de pointes to be avoided during treatment with panobinostat

Miscellaneous drugs	
• arsenic trioxide	• levomethadyl
• astemizole	• methadone
• bepridil	• pentamidine
• domperidone	• droperidol
*Note: azithromycin, ciprofloxacin, levofloxacin, pefloxacin, ofloxacin, tosufloxacin, difloxacin, temafloxacin, fleroxacin, acrosoxacin, nalidixic acid and enoxacin are allowed.	

This is not a comprehensive list of medications which may prolong the QT interval or have a risk of causing Torsade's de pointes. This list of medications was developed in collaboration with an external cardiology consultant, and represents those medications which are deemed to have an unacceptable risk of co-administration with panobinostat.

The following website may be referenced as a supplemental guide for drugs which have been associated with Torsade's de pointes or prolonging the QT interval but at this point lack substantial evidence for causing Torsade's de pointes:

http://www.qtdrugs.org/medical-pros/drug-lists/drug-lists.htm#.

Medications listed on the website which do not appear in Table 1-1 above may be used with caution at the discretion of the investigators.

The serotonin ($5HT_3$) antagonists, often used as anti-emetics, such as ondansetron dolasetron, (also are known CYP2D6 substrates, see Table 3-1), or granisetron have been associated with Torsade's de points and QT prolongation but have not been shown to cause Torsade's de pointes. Therefore, $5HT_3$ antagonists are not per se prohibited but close monitoring for signs and symptoms of QT prolongation is recommended. Caution is to be exercised when using these or other agents that may prolong QT intervals in combination with panobinostat.

II. Medications that are known strong CyP3A4/5 Inhibitors to be used with caution

Medications that are known strong CYP3A4/5 inhibitors should be used with caution. Panobinostat is a substrate of CYP3A4 with minor involvement of CYP2D6, and CYP2C19 in in *vitro* evaluation of its metabolism. Thus, a clinical drug-drug interaction study was conducted using ketoconazole, a strong CYP3A inhibitor, in combination with panobinostat in Novartis study CLBH589B2110.

Multiple ketoconazole doses at 400 mg increased C_{max} and AUC of panobinostat by 1.6- and 1.8-fold, respectively, but with no change in T_{max} or half-lives in 14 cancer patients. The less than 2-fold increase in panobinostat AUC upon co-administration of a strong CYP3A inhibitor is considered a weak drug inhibition and not clinically relevant, as panobinostat doses at least 2-fold greater than the evaluated 20 mg dose (i.e., 40 mg and 60 mg) have been safely administered in patients. Thus, co-administration of panobinostat with a moderate or weak CYP3A inhibitor is allowed. However, clinical monitoring of signs and symptoms of panobinostat treatment related adverse events is recommended when long-term (≥ 1 week) concomitant administration of any

strong CYP3A inhibitors and panobinostat is medically indicated or investigated in a clinical study.

Patients with impaired liver function (as defined by NCI CTEP criteria)[1] are recommended not to receive panobinostat concomitantly with strong CYP3A inhibitors because potential interaction has not been established in this population.

Table 2-1: Medications that are known strong CYP3A4/5 inhibitors to be used with caution

Macrolide antibiotics*
• clarithromycin • telithromycin • troleandomycin • erythromycin
Antifungals (azoles)*
• ketoconazole • itraconazole • fluconazole
Antidepressants
• nefazodone
Calcium channel blockers*
• diltiazem • verapamil
HIV protease inhibitors
• indinavir • nelfinavir • ritonavir • saquinavir
Miscellaneous drugs or products
• aprepitant • grapefruit product or juice
**azithromycin, voriconazole, regular orange juice and dihydropyridine calcium channel blockers (e.g. amlodipine, felodipine, nicardipine and nifedipine) are allowed.*

This is not a comprehensive list of medications which may inhibit CYP3A4/5. Additional updated versions with moderate and weak CYP3A inhibitors, which are meant to be used as a guide, may be found at the following website: http://medicine.iupui.edu/clinpharm/DDIs

III. Medications that are known CYP2D6 substrates to be used with caution

Panobinostat was also shown to be a CYP2D6 inhibitor (K_i 0.17 μM) *in vitro*. Thus, clinical drug-drug interaction study with panobinostat as CYP2D6 inhibitor and dextromethorphan as CYP2D6 substrate was recently conducted in Novartis study CLBH589B2109.

Multiple panobinostat doses increased C_{max} and AUC of dextromethorphan by a mean of 1.8- and 1.6-fold respectively, but with no change in T_{max} in 17 cancer patients. An approximately 2-fold increase in dextromethorphan AUC upon co-administration with panobinostat indicated that *in vivo* CYP2D6 inhibition of panobinostat is weak.

As the study was conducted using a sensitive CYP2D6 substrate which resulted in a weak inhibition, drugs with a large therapeutic index such as anti-emetics, anti-hypertensives, and anti-depressants are generally safe to be co-administered with panobinostat.

Patients should be carefully monitored for potential signs and symptoms of toxicity and may require dose titration or dose reduction of a sensitive CYP2D6 substrate which also have a narrow therapeutic window (e.g., the ratio of toxicity exposure is ≤ 2-fold higher than the efficacious or therapeutic exposure).

Table 3-1: Medications that are known CYP2D6 substrates to be used with caution

Beta blockers (listed below):	Antipsychotics (listed below):
• carvedilol • metoprolol • propafenone • timolol • Antidepressants (listed below): • amitriptyline • chlormipramine • desipramine • imipramine • fluoxetine • paroxetine • venlafaxine • bupropion • duloxetine • Antiemetics (listed below): • dolasetron • ondansetron • metoclopramide	• aripiprazole • haloperidol • perphenazine • risperidone • thioridazine • zuclopenthixol • amphetamine • alprenolol • bufuralol • chloropheniramine • Anti-arrythmics (listed below): • quinidine • lidocaine • mexiletine • propafenone • Others: • oxycodone • codeine • hydrocodone • terbinafine • promethazine • tamoxifen • tramadol

This is not a comprehensive list of CYP2D6 substrates. Additional updated versions of this list, which are meant to be used as a guide, may be found at the following website: http://medicine.iupui.edu/clinpharm/DDIs

Synold TW, Takimoto CH, Doroshow JH, Gandara D, Mani S, Remick SC, Mulkerin DL, Hamilton A, Sharma S, Ramanathan RK, Lenz HJ, Graham M, Longmate J, Kaufman BM, Ivy SP. Dose-Escalating and Pharmacological Study of Oxaliplatin in Adult Cancer Patients with Impaired Hepatic Function: A National Cancer Institute Organ Dysfunction Working Group Study, Clin Cancer Res. 2007 13; *3660*

PEDIATRIC - MODIFIED TOTAL NEUROPATHY SCALE

DATE OF EXAM: _____ PATIENT NAME**:** _____ MRN**:** _____

Evaluation time point:

☐ Block A Day 6 ☐ Prior to Block B ☐ Prior to Block C
☐ Block A Day 15 ☐ Completion of Block C

1. **Sensory Symptoms**: _____ (record worst score for the three sensations)

"*Do you have any parts of your body that are tingly, numb (can hardly feel), or hurt*?"

_____ Tingly _____ Numb _____ Hurts (record number for each)

If yes, "Where you have those feelings?"

0 None
1 Symptoms limited to fingers or toes
2 Symptoms extend to ankles or wrists
3 Symptoms extend to knee or elbow
4 Symptoms above knee or elbow

2. **Functional Symptoms**: _____ (record worst score of the three questions)

"*Do you have trouble buttoning shirts or tying shoes (zipping zippers)*?" _____
"*Do you have trouble walking such as tripping frequently?*" _____
"*Do you have trouble going up or down stairs?*" _____

If yes to any, "Is it….. (read choices)" and record after each question:

0 Not difficult
1 A little difficult
2 Somewhat difficulty
3 I need help
4 I can't do that at all

3. **Autonomic Symptoms**: _____ (record worst score of the three questions)

"*Do you feel dizzy or light-headed when you get up out of bed?*" _____
"*Do your hands or feet feel hotter or colder than normal?*" _____

0 Never
1 A little bit
2 Sometimes
3 Very much
4 Almost always

DATE OF EXAM: _____ PATIENT NAME: _____ MRN: _____

4. Clinical Testing:

Light Touch Sensation: _____

0 Normal
1 Reduced in fingers/toes
2 Reduced up to wrist/ankle
3 Reduced up to elbow/knee
4 Reduce to above elbow/knee

Semmes			Semmes	
Toes	R		Finger	R
	L			L
Med Mal	R		Wrist	R
	L			L
Knee	R		Elb	R
	L			L

Pin Sensibility: _____

0 Normal
1 Reduced in fingers/toes
2 Reduced up to wrist/ankle
3 Reduced up to elbow/knee
4 Reduce to above elbow/knee

Vibration Sensibility: _____ (worst score)

0 Normal
1 Reduced in fingers/toes
2 Reduced up to wrist/ankle
3 Reduced up to elbow/knee
4 Reduce to above elbow/knee

Bioesth			Bioesth	
Toes	R	/	Finger	R /
	L	/		L /
Med Mal	R	/	Wrist	R /
	L	/		L /
Knee	R	/	Elb	R /
	L	/		L /

Strength: _____ (worst score) (MRC Score R / L)

MRC level: Great toe _____/_____ Ankle DF _____/_____

Finger abd. _____/_____ Wrist ext. _____/_____

0 Normal
1 Mild weakness (MRC 4)
2 Moderate weakness (MRC 3)
3 Severe weakness (MRC 2)
4 Paralysis (MRC 1-0)

DATE OF EXAM: _____ PATIENT NAME**:** _____ MRN**:** _____

DTR: _____ (Achilles, patellar)

0 Normal
1 Ankle reflex reduced (Achilles +1)
2 Ankle reflex absent (Achilles 0, others +2)
3 Ankle reflex absent, others reduced (Achilles 0, others +1)
4 All reflexes absent (all 0)

TOTAL SCORE: ___ / 32

DATE OF EXAM: _____ PATIENT NAME**:** _____ MRN**:** _____

TSALV SIX MINUTE WALK CRF

Time	Heart rate	Distance (meters)	Rating of Perceived exertion (6-20)
Rest			
1 minute			
2 minutes			
3 minutes			
4 minutes			
5 minutes			
6 minutes			
1 minute recovery			
2 minutes recovery			