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RAVEN: A PHASE I/II TRIAL TREATING RELAPSED ACUTE LYMPHOBLASTIC LEUKEMIA WITH VENETOCLAX AND NAVITOCLAX

IND# 157865 (St. Jude Children's Research Hospital)

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Protocol Summary

RAVEN: A PHASE I/II TRIAL TREATING RELAPSED ACUTE LYMPHOBLASTIC LEUKEMIA WITH VENETOCLAX AND NAVITOCLAX
Principal Investigator: Seth E. Karol, MD
Institution/Sponsor-IND holder: St. Jude Children's Research Hospital. IND# 157865
Brief overview: This is a Phase I/II clinical trial. In Block I, all patients receiving common therapy evaluating the activity of combination chemotherapy with venetoclax and navitoclax in children with relapsed or refractory acute lymphoblastic leukemia or lymphoma (rALL). In Block 2, a phase 1 rolling six design and phase 2 extension will be used to assess the combination dose of venetoclax combinations with either blinatumomab for CD19-postive patients or navitoclax and high-dose cytarabine for CD19-negative patients.

St. Jude Children's Research Hospital
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RAVEN: A PHASE I/II TRIAL TREATING RELAPSED ACUTE LYMPHOBLASTIC LEUKEMIA WITH VENETOCLAX AND NAVITOCLAX

Intervention:

Block 1:
Venetoclax 120mg/m^2 (max 200mg) PO D1; 240mg/m^2 (max 400mg) PO D2-22
Navitoclax 25mg (20-44.9kg) OR 50mg (>=45kg) D3-22
Dexamethasone 5mg/m^2/dose PO BID D1-7, 15-22
Vincristine 1.5mg/m^2/dose (max 2mg) IV D1, 8, 15, 22
Calaspargase pegol 2500units/m^2 IV/ D2
Intrathecal chemotherapy: LPIT D -7 to 1, then weekly until clear if CNS+
Dasatinib 80mg/m^2/day (max 140mg) QD/PO D1-28 for ABL-fusion-positive and T-cell

Block 2a (CD19 negative):
Venetoclax 240mg/m^2 (max 400mg) PO D1-7
Navitoclax 25mg (20-44.9kg) OR 50mg (>=45kg) PO D1-7
Dexamethasone 3mg/m^2/dose PO BID D1-5
Calaspargase pegol 1000units/m^2 IV/ D3
Dasatinib 80mg/m^2/day (max 140mg) QD/PO D1-28 for ABL-fusion-positive and T-cell

Cytarabine dosing during phase 1 portion for Block 2a

Dose level	Dose (with maximum daily dose if applicable) and Route	Number of doses	Schedule
1	3000mg/m^2/dose q12h IV infusion over 3 hours	4	Days 1-2
0	1500mg/m^2/dose q12h IV infusion over 3 hours	4	Days 1-2
-1	1000mg/m^2/dose q12h IV infusion over 3 hours	2	Day 1

Block 2b (CD19 positive):
Blinatumomab 5mcg/m^2/day D1-7 (max 9mcg/day) IV (if Block 1 MRD>5%)
Blinatumomab 15mcg/m^2/day (max 28mcg/day) IV D8-28 (if Block 1 MRD >5%)
Blinatumomab 15mcg/m^2/day (max 28mcg/day) IV D1-28 (if Block 1 MRD ≤5%)
Dexamethasone 10mg/m^2 (max 20mg) PO/IV 6-12 hrs. and 5mg/m^2 30 min prior to blinatumomab Day 1
Dasatinib 80mg/m^2/day (max 140mg) QD/PO D1-28 for ABL-fusion-positive

Venetoclax dosing during phase 1 portion for Block 2b

Dose level	Dose (with maximum daily dose if applicable) and Route	Number of doses	Schedule
1	240mg/m^2 (max 400mg) PO	21	Days 8-28
0	240mg/m^2 (max 400mg) PO	14	Days 8-21
-1	240mg/m^2 (max 400mg) PO	7	Days 8-14

Brief outline of treatment plan: Patients will receive therapy as described in the intervention. Response evaluation will be performed on Day 29 of Block 1. MRD-negative (<0.01%) patients will await ANC recovery to 500/µL or 14 days, whichever is shorter, before proceeding to Block 2

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therapy. Patients who are MRD positive proceed to Block 2 therapy without awaiting hematological recovery. Patients receiving Block 2b therapy will begin venetoclax according to the current dose level. Venetoclax may be held if the peripheral absolute lymphocyte count is <300/microL or the ANC is <500/microL. Response evaluation following Block 2 therapy will occur with count recovery. Following Block 2 of therapy, late (≥36 months from diagnosis) first relapse B-ALL who are MRD negative after Block 1 will continue chemotherapy using adapted R3 intensification, interim, and continuation therapies. Other patients are off therapy for alternative therapy including cellular therapy/ transplant.
Study design: Multi-center non-randomized phase I/II trial with a common Block 1 therapy and biologically-determined Block 2 therapy utilizing a rolling six dose finding phase I followed by a phase II extension.
Sample size: We anticipate 78 enrollees to yield 70 research participants evaluable for Block 1 response. We also anticipate that additional patients may enroll on exploratory cohorts to assess therapy in specific populations or with specific therapy modifications. This will not exceed 20 patients in total with no more than 8 patients >21.99 years old.
Data management: Data management and statistical analysis will be provided by the Comprehensive Cancer Center Hematological Malignancies Program and the Biostatistics Department at St. Jude Children's Research Hospital.
Human subjects: The risks to subject will be related to the toxicity of therapy for relapsed ALL and the investigational agents venetoclax and navitoclax. The research participants will be informed of the toxicities that 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.

TABLE OF CONTENTS

APPENDICES

1.0 OBJECTIVES

1.1 Primary Objective

1.1.1 To compare MRD-negative CR/CRi rate in children with relapsed or refractory acute lymphoblastic leukemia or lymphoma (rALL) following Block 1 therapy with venetoclax and navitoclax based reinduction to historical controls.
1.1.2 To identify the recommended phase 2 combination dose (RP2D) of venetoclax based consolidation in novel combinations with a) high-dose cytarabine and navitoclax or b) blinatumomab.

1.2 Secondary Objectives

1.2.1 To estimate the tolerability and activity of venetoclax based consolidation in novel combinations with a) high-dose cytarabine and navitoclax or b) blinatumomab.
1.2.2 To describe event-free and overall survival in patients treated with this regimen.

1.3 Exploratory Objectives

1.3.1 To evaluate MRD-negative CR/CRi rates in each prespecified groups: late first relapse B-ALL; early first relapse and second or greater relapse B-ALL; and relapsed T-ALL.
1.3.2 To identify drug sensitivity patterns in patient samples prior to and after receiving combination therapy and evaluate mechanisms of disease resistance/ escape.
1.3.3 To explore immune subsets during and after this regimen.
1.3.4 Evaluate response to therapy in rare relapse patient subsets.
1.3.5 Explore breakthrough infections in children and young adults with relapsed or refractory ALL

2.0 BACKGROUND AND RATIONALE

2.1 Background

2.1.1 Relapsed Acute Lymphoblastic Leukemia/Lymphoma

Acute lymphoblastic leukemia (ALL) is the most common malignancy of childhood.[1] While frontline therapy for children with newly diagnosed ALL will cure the majority of patients, 10-20% of children with ALL will relapse.[2-6] The outcome for children with relapsed ALL (rALL) is generally poor and varies by site and timing of relapse as well as immunophenotype, with very early relapse (<18 months from diagnosis) and early (18-36 months from diagnosis) relapses, medullary relapse (compared to isolated extramedullary relapse), and T-ALL relapse faring even more poorly.[7,8] These poor outcomes are reflected in both early disease clearance as determined by end of induction minimal residual disease (MRD) as well as survival. Survival rates for 1) late (≥36 months from diagnosis) or isolated extramedullary, 2) early, and 3) very early or T-cell marrow relapse approximate 60, 30, and 25%, respectively.[9] Patients with relapsed ALL have chemotherapeutic

resistance which results in both initial and retrieval treatment failure and have outcomes inferior to those of patients with newly diagnosed ALL.[10-12]

Intensification of chemotherapy accompanied by hematopoietic cell transplantation (for patients with either early relapse or residual disease after induction) is the current standard of care, with combination chemotherapy in first reinduction including a glucocorticoid (dexamethasone or prednisone), vincristine, asparaginase, and an anthracycline (doxorubicin or mitoxantrone).[8,13-15] For patients with B-cell precursor ALL (B-ALL), early (or very early) marrow relapse, or detectable residual disease (positive-MRD) after induction, transplantation after achieving an MRD-negative remission is the standard of care.

Two standard of care regimens are most frequently employed in the United States. The UKALL R3 regimen, which was also utilized in the recently completed AALL1331 trial,[16] utilizes high-dose dexamethasone (20mg/m^2), vincristine, pegaspargase, and mitoxantrone.[8] The Children's Oncology Group (COG) AALL01P2 trial replaced dexamethasone with prednisone and mitoxantrone with doxorubicin in efforts to reduce toxicity.[14] Typical therapy involves a 4-drug induction (as described above) followed by 2 consolidation blocks including intermediate- or high-dose methotrexate, cyclophosphamide, etoposide (Block 2) and high-dose cytarabine and asparaginase (Block 3). For patients with B-ALL who experience a late relapse and are MRD-negative after Block 1, continued chemotherapy with continuation and maintenance therapy after the completion of the induction and 2 consolidation blocks are standard. Total therapy for these patients lasts 2 years from the beginning of reinduction for relapsed disease.

Despite therapy intensifications, many patients still experience reinduction failure and few achieve MRD-negative remissions after induction Block 1 (Table 1). MRD-negative remission, in which fewer than 0.01% bone marrow mononuclear cells are leukemia blasts, is strongly associated with a favorable prognosis in relapsed ALL. Patients without MRD-negative remissions are more likely to experience both therapy toxicity and refractory or recurrent disease, resulting in lower event-free and overall survival (EFS and OS). In the Children's Oncology Group AALL01P2 trial, failure to achieve MRD-negative CR after Block 1 resulted in a 58% 1-year event-free survival compared to 80% in those who achieved MRD-negativity.[14] On the AALL07P1 trial, patients with early relapse (18-36 months from diagnosis) who were MRD-negative after Block 1 had a 3-year EFS of 58% vs. 10% in those MRD-positive, while those with very early relapse (<18 months from diagnosis) who were MRD-negative had 3-year EFS of 70% vs. 3% in those MRD-positive.[15] Overall survival was also lower in MRD-positive patients, with 3-year OS of 0% and 19% for very early and early relapse vs. 70% and 65% in those same populations who were MRD-negative.

Despite the need to achieve an MRD-negative remission, current therapy fails to do this for most patients. Patients treated with the R3 backbone who experienced a late relapse achieved MRD-negative remission after 1 block of therapy in 82/193 cases (42.5%).[17] AALL01P2 achieved an MRD-negative remission among patients in CR in 49% (21/43) of late relapse patients and 25% (9/36) of early relapse patients.[14] However, data was incomplete among patients in CR and CR was not obtained in an additional 32% of early

and 4% of late relapse. For patients with T-ALL in first relapse, MRD-negative remission was achieved in 4/20 reported (of 22 evaluable) patients treated with bortezomib based therapy on AALL07P1.[15] For early relapse B-ALL treated on the same trial, 29% of patients in CR were MRD-negative, and only 68% of patients (70/103) patients achieved a CR. Thus, only 19.7% of evaluable patients achieved a CR.[15] In the AALL1331 trial, end of induction 1 bone marrow results for early relapse patients have been reported for 189 of 206 patients who began induction, with other patients off therapy prior to assessment. Only 52/206 (25%) achieved MRD-negative CR using the protocol defined recovery threshold of ANC ≥500/microL and platelets of ≥50,000/microL.(Brown et al)[18]

Table 1: Proportion of patients achieving MRD-negative remission according to timing of relapse and immunophenotype

Study	**Early and Very Early (< 36 months from diagnosis)**		**Late (≥ 36 months from diagnosis)**	
	B	T	B	T
R3[8,17#]	Not reported	Not reported	42.5% [82/193]	Not reported
AALL1331^	25% [52/206]	Not included	Not reported	Not included
AALL07P1[15]	27% [27/100]	18.2% [8/22]	No included	Not included
AALL01P2[14]*	25% [9/36]	0%	47% [21/43]	0
#B/T not reported separately **of available samples, with samples not available for the entire cohort* *^Brown et al.[18]; early and very early relapse combined*				

For patients with multiply relapsed ALL, response rates are even lower. In a retrospective cohort from the Therapeutic Advances in Childhood Leukemia (TACL) consortium, less than half of patients in >1st relapse B-ALL achieved a complete response or complete response with incomplete hematological recovery (CR/CRi: <5% bone marrow disease without extramedullary disease but without peripheral blood count criteria).[19] Specifically, 51% of second salvage attempts (84/165), 37% of third salvage attempts (27/73), and 31% of subsequent relapses (16/52) achieved a CR/CRi. Combining these treatment attempts, patients in >1st relapse achieved a CR/CRi in 43.8% (127/290) of courses. Data are relatively similar from a historical Berlin-Frankfurt-Münster cohort of 74 second relapse patients.[20] In this cohort, 30/74 were able to achieve a 3rd CR (40.5%), including 30/55 (55.6%) for whom therapy details were available and who did not receive palliative care. For patients in 3rd relapse (18 of the 30 who achieved a CR after 2nd relapse), only 6 (33.3%) achieved a 4th CR. Combining 2nd and 3rd salvage attempts, CR/CRi was achieved in 39% of treated patients. MRD responses in these populations are not available.

2.1.2 Venetoclax and Navitoclax Background

Venetoclax (ABT-199) is a first-in-class orally available selective inhibitor of B cell CLL/ lymphoma 2 (BCL-2).[21] Overexpression of BCL-2 and BCL-2 family member proteins result in reduced sensitivity to therapy-induced apoptosis, resulting in treatment failure. These proteins sequester pro-apoptotic initiator and effector proteins needed for mitochondrial outer membrane permeabilization (MOMP). Venetoclax binds to BCL-2, displacing the pro-apoptotic proteins BIM and BAX and allowing initiation of MOMP.

This in turn results in outer membrane depolarization, cytochrome c release, and the activation in intracellular caspases resulting in apoptosis.[22]

Venetoclax has demonstrated in vitro cytotoxicity to ALL cell lines including RS4;11, a KMT2A-rearranged line.[21] Early clinical data in adults suggested venetoclax had wide-ranging activity against B-cell malignancies, including CLL, myeloma, and lymphoma (summarized by Roberts and Huang).[23] Preclinical data suggested that sensitivity extended to ALL, with venetoclax sensitivity observed in B-ALL from multiple genetic backgrounds.[24] In a separate analysis, 8/11 B-ALL primary patient samples tested and 1/6 T-ALL samples were responsive to venetoclax.[25] In vivo studies of xenografted patient samples demonstrated the efficacy of venetoclax in prolonging leukemia free survival in these animals and mirrored the responsiveness seen in vitro. Furthermore, synergy was noted between venetoclax and the classical chemotherapeutic agents dexamethasone, vincristine, and asparaginase). Data also suggests venetoclax sensitivity among other high risk genetic subgroups of ALL, including hypodiploid ALL,[26] *KMT2A* rearranged ALL,[24,27,28] Ph+ ALL,[29,30] and the highly chemotherapy resistant (and rare) *TCF3-HLF* fusion ALL. In addition to specific genetic subtypes, venetoclax is also active against pediatric leukemias with diverse genetic lesions which dysregulate *CELSR2*, with venetoclax showing synergism with prednisone in this common mechanism of glucocorticoid resistance.[31]

Preclinical data suggests that common mechanisms of venetoclax resistance involve dependence on alternative BCL-2 family members for apoptosis inhibition. While many cell lines and primary samples are sensitive to venetoclax, some show resistance while remaining sensitive to navitoclax (ABT-263), a combined BCL-2/ BCL-X_L/ BCL-W inhibitor.[32] Notably, many T-ALL samples appear to be more dependent on BCL-X_L with corresponding relative sensitivity to navitoclax compared to venetoclax.[25,33] Additional work has shown that T-cell maturation stage may play a significant role in the susceptibility of T-ALL to venetoclax and navitoclax, with early T-cell precursor (ETP) ALL demonstrating venetoclax sensitivity and more mature T-ALL having navitoclax (BCL-X_L) or MCL-1 inhibitor sensitivity.[34,35]

2.1.3 Clinical Experience with Venetoclax and Navitoclax in Pediatric Leukemia

While data suggests that many high-risk B-cell precursor (BCP) ALL subtypes are susceptible to the BCL-2 inhibitor venetoclax, a common mechanism of resistance to venetoclax is dependence on alternative BCL family members including BCL–X_L. Additionally, some BCP ALL and most T–ALL (with the notable exception of ETP) have primary dependence on BCL–X_L, rendering venetoclax therapy less effective. This suggests that combination therapy with navitoclax, an inhibitor of BCL-2, BCL–X_L, and BCL-W, is likely to have broader activity. Results from the M13–833 (Place et al., 2020, ASH annual meeting) and M16–106[36] studies support these preclinical data. In the M13-833 trial which combined venetoclax with one of two chemotherapy backbones, children treated with a vincristine/ asparaginase/ high-dose dexamethasone (VLD) backbone achieved a complete response/ complete response with incomplete hematological recovery/ complete response with incomplete platelet recovery (CR/CRi/CRp) in 9/16 cases, including 1/3 patients with T-ALL. Minimal residual disease (MRD) negativity <0.01%

was achieved in 6/16 cases. In the M16-106 trial which utilized venetoclax and navitoclax in combination with VLD, a CR/CRi/CRp was achieved in 9/12 pediatric patients including 6/12 who were MRD negative. Both trials allowed the addition of a tyrosine kinase inhibitor (including dasatinib) in patients with ABL1 fusions. These patients did not experience excessive toxicity with the combination therapy.

Interestingly, the predicted BCL-2 dependency pattern was not observed, with 3/4 T-ALL showing primary BCL-2 dependence and 4/8 B-ALL showing primary BCL–X_L dependence. However, despite these encouraging initial responses (60% CR/CRp/CRi in all patients including adults and children, 75% in children), 18-month overall survival was ~20% in the combined adult and pediatric cohort. Responses in T-ALL were encouraging but limited, with 10/19 patients (including adults) achieving a CR/CRi of which 6 were MRD-negative and 3 of these patients proceeding to transplantation. Together, these data suggest that initial response rates are high (given the heavily pretreated nature of the population) but better consolidative strategies are needed for patients treated with these agents to provide long-term cure.

2.1.4 Rationale for Dasatinib in T-ALL

Primary treatment failure and relapse are more common in T-ALL than in B-ALL. In many studies, response to a 7-day prednisone window is used to identify poor response. In studies of T-ALL, hyperactivation of lymphocyte cell-specific protein-tyrosine kinase (LCK), a SRC family kinase potentially targetable by dasatinib, was associated with a poor prednisone response.[37] Separately, in silico analysis also identified LCK overexpression in T-ALL.[38] In vitro evaluation of 22 primary T-ALL samples demonstrated nanomolar sensitivity to dasatinib in 6/22 cases (27%). Separately, dasatinib sensitivity was identified in 12/40 (30%) of patients with T-ALL patients not harboring ABL-class fusions.[24] Notably, ETP ALL does not appear to be sensitive to dasatinib.

To further understand these findings, pharmacotyping to assess in vitro drug sensitivity was performed on 45 T-ALL samples including 18 adult and 27 pediatric cases.[39] In this cohort, 12/27 (44%) of pediatric cases were exquisitely dasatinib sensitive. This sensitivity was confirmed in vivo using patient derived xenografts. Analysis of gene interactome networks confirmed LCK as a driver of this sensitivity. Using phosphoproteomics, introduction of a dasatinib resistance mutation in LCK, and specific inhibitors of LCK, this kinase was confirmed as being the target and driver of dasatinib sensitivity in T-ALL. Interestingly, there appeared to be a direct, correlative relationship between dasatinib and navitoclax sensitivity in available cases while there was an inverse correlation between dasatinib and venetoclax sensitivity [i.e. venetoclax resistant cases were sensitive to dasatinib (via LCK) and navitoclax (due to upregulation of BCL–X_L), while venetoclax sensitive cases (primarily ETP ALL) were resistant to dasatinib]. These changes in sensitivity appear to be related to the maturational stage of the T-ALL; T-cell receptor signaling; and LCK, BCL-2, and BCL–X_L activity. These patterns observed in bulk leukemia samples were also observed in clonal subpopulations evaluated with single-cell RNA sequencing. Preliminary studies have also evaluated synergy between dasatinib and navitoclax in T-ALL samples and have shown promising results, suggesting a novel mechanism for targeting this challenging population.

While work is underway to identify optimal biomarkers to select T-ALL patients for dasatinib therapy, there is not currently a CLIA approved test to identify such patients. Given the extremely poor prognosis of patients with relapsed T-ALL (<50% 3-year OS for first-relapse receiving bortezomib based reinduction and <15% in non-bortezomib combinations),[14,40] the fact that dasatinib was well tolerated when combined with M16-106 therapy,[36] and preclinical data demonstrating synergy between navitoclax and dasatinib in T-ALL, we will combine these agents for all patients with T-ALL.

2.1.5 Rationale for Combining Venetoclax and Blinatumomab

Blinatumomab, a bispecific T-cell engager targeting CD19, is rapidly becoming the standard of care for consolidation therapy for patients with relapsed CD19-positive B-ALL. It is FDA approved for adults and children with relapsed or refractory ALL. In adults with relapsed ALL, blinatumomab provided better CR and MRD-negative CR rates than chemotherapy and improved survival.[41] Blinatumomab has also been shown to be active in children with bulk disease with ~20% of patients achieving an MRD-negative CR within 2 cycles.[42] The Children's Oncology Group AALL1331 trial compared blinatumomab vs. standard of care consolidation chemotherapy based on the UKALL R3 backbone (intermediate-dose methotrexate combined with cyclophosphamide and etoposide in Block 2 and high-dose cytarabine in Block 3) for children with CD19-positive B-ALL in first relapse. Results for patients at intermediate and high risk have now been published.[16,43] In these patients, blinatumomab was superior to conventional therapy in achieving CR and MRD-negative CR and was associated with lower toxicity, resulting in more patients reaching transplant. This translated into an improvement in 2-year overall survival (71.3% for blinatumomab vs. 58.4% for chemotherapy, p=0.02). Separately, a single cycle of blinatumomab consolidation improved EFS compared to a third chemotherapy based consolidation block in children with high-risk B-cell relapsed ALL.[44] Blinatumomab has also been shown to be effective when used as a bridge to transplantation in children who achieved a CR with conventional therapy.[45] Blinatumomab has been successfully combined with dasatinib in large clinical trials of adults with BCR-ABL1 ALL with excellent results.[46]

There have been case reports of combining blinatumomab with venetoclax in relapsed B-ALL.[47] In that report, 2 adult patients received venetoclax 400mg/day after a ramp-up phase and blinatumomab 9mcg/day on days 1-7 and 28mcg/day on Days 8-28. This therapy was well tolerated without significant cytopenias. Both patients tolerated the therapy well and achieved an MRD-negative remission after 1 cycle before proceeding to transplantation. Another case report by a different group describes a patient who received blinatumomab after a reinduction failure (33% disease) and had persistent disease (45%) after 1 week of therapy with blinatumomab alone. Venetoclax was added at that time and disease responded with undetectable disease by MRD on Day 21, following 14 days of venetoclax and blinatumomab combination (Herrera Rojas et al., 2017. Rev Hematol Mex). This strategy has also been employed in several adult patients at MD Anderson with relapsed ALL with similar efficacy and tolerability observed (Elias Jabbour, personal communication).

These clinical responses are supported by preclinical data on the potential combinatorial efficacy of blinatumomab and venetoclax. Venetoclax enhanced the cellular cytotoxicity of antigen specific T-cells directed against CMV in both an antigen specific and an antigen non-specific manner.[48] Separately, chimeric antigen receptor T-cells (CAR T-cells) had enhanced cytotoxicity in the presence of ABT-737, a preclinical compound with similar activity to navitoclax (BCL-2 and BCL-X_L inhibition).[49] This synergy appeared to be mediated by increased sensitivity of blasts to CAR T-cells and could be achieved either by pre-sensitizing with ABT-737 or by co-exposure of blasts to CAR T-cells and ABT-737. Venetoclax has also be successfully combined with PD-1 blockade in pre-clinical models of breast cancer with demonstration of improved intra-tumoral cellular cytotoxicity from tumor infiltrating T-cells.[50] This study demonstrated the preservation of effector memory and activated T-cells with a loss of naïve T-cells during venetoclax therapy. Regulatory T-cells were also affected by the addition of venetoclax, with sensitivity intermediate to CD-4 and CD-8 positive T-cells. Surviving T-cells had unimpaired function in the presence of venetoclax. The preservation of these effector populations is relevant to combinations with blinatumomab. Effector memory T-cells appear to be the cells most responsible for blinatumomab activity in preclinical models of blinatumomab.[51] Indeed, loss of regulatory T-cells from venetoclax may actually be beneficial, as non-responders to blinatumomab had higher levels of peripheral blood T-regs than responders.[52] Thus, combining venetoclax may selectively eliminate immune cell subsets which reduce blinatumomab efficacy while retaining effector cells. Published reports describe the use of venetoclax with blinatumomab for all 28 days of the blinatumomab cycle. Because patients on RAVEN will have just completed Block 1 when proceeding to Block 2b, we will hold venetoclax for the first 7 days of the blinatumomab infusion to support further hematological recovery.

2.1.6 Rationale for Combining Venetoclax with Navitoclax and High-Dose Cytarabine

Venetoclax has previously been successfully combined with high-dose cytarabine and idarubicin in children with relapsed and refractory acute myeloid leukemia (AML). In the St. Jude VENAML trial,[53] all evaluated dose levels of venetoclax with high-dose cytarabine were well tolerated. The recommended phase 2 dose combination was venetoclax 360mg/m^2 (equivalent to an adult dose of 600mg) combined with cytarabine 1000mg/m^2 x 8 doses and idarubicin 12mg/m^2 x1 dose. Hematological and infectious toxicities were consistent with the backbone chemotherapy without excess toxicity attributable to venetoclax. These results have since been extended in adults with both newly diagnosed and relapsed/ refractory AML at MD Anderson, where FLAG-Ida has shown both tolerability and impressive activity.[54]

Acute lymphoblastic leukemia resistant to chemotherapy is frequently characterized by immature/ stem cell characteristics.[35,55] Moreover, within the bulk leukemic population, leukemic stem cells act as a reservoir which are more resistant to chemotherapy and drive the development of relapse.[55] Cytarabine has been demonstrated to target leukemic stem cells in AML[56], and the addition of cytarabine to condition regimens prior to transplant improve outcomes in ALL.[57] One proposed mechanism of this interaction is a downregulation of MCL1 through cellular stress from cytarabine administration.[56]

Based on these data, we will combine high-dose cytarabine (which may downregulate MCL1) with the BCL2 inhibitor venetoclax and the BCL-X_L inhibitor navitoclax to try to eliminate leukemic stem cells which persist following Block 1 therapy.

For combination therapy as described in Block 2a, we believe the experience of the VENAML trial (NCT03194932[53]) provides an appropriate signal to indicate the planned therapy is likely to be safe. In that trial for children with relapsed AML, all dose levels were deemed to be tolerable with excellent activity. To increase the safety of the planned therapy in RAVEN, we have pre-emptively modified the planned treatment to attempt to reduce toxicity. Specifically, we have reduced the starting dose of venetoclax (400mg vs. 600mg), utilize fewer doses of cytarabine (4 doses vs. 8 doses), and are not using an anthracycline (omitting 12mg/m^2 of idarubicin). We have further reduced therapy intensity to minimize myelotoxicity compared to the historical R3 Block 3 treatment by stopping chemotherapy after day 7 rather than repeating high-dose cytarabine on days 8 and 9.

2.2 Study Plan Rationale

As described in the background, outcomes for children with relapsed acute lymphoblastic leukemia and lymphoma who fail to achieve MRD-negative remissions are extremely poor, with most MRD-positive patients progressing to frank relapse. Current standard of care options (AALL01P2 and AALL1331/ R3) fail to achieve an MRD-negative remission in most patients resulting in adverse outcomes. Thus, achieving this level of remission is a meaningful therapeutic outcome for children with relapsed ALL.

While data from the phase I and expansion cohorts of M16-106 suggest that the combination therapy of Block 1 is active and able to achieve MRD-negative responses, the pediatric experience with this combination is limited to 12 patients. Additional assessment of this combination in children is needed to refine estimates of response and further characterize toxicities. Additionally, overall survival rates following this combination remain lower than response rates, suggesting that persistent residual disease below the level of MRD detection may persist in heavily pretreated populations such as those who received therapy on M16-106. Thus, additional consolidative strategies prior to cellular therapy or transplantation are needed to deepen remissions and allow for long-term survival. As described above, combining venetoclax with blinatumomab (for CD19-positive ALL) or navitoclax and high-dose cytarabine (for other patients) provide rational approaches to such consolidation therapies which build on existing standards of care while providing needed assessments of these combinations. As these combinations have not previously been explored in pediatrics, assessments of both tolerability and response to these combinations are needed.

Dosing for this study is based on the M16-106 study.[36] Here, dosing of venetoclax was capped at 400mg daily and was combined with navitoclax (25mg for those 20-44.9kg and 50mg for those 45kg and heavier). With this dosing, the selective BCL-2 inhibition of venetoclax combines with the combination BCL-2, BCL-X_L, and BCL-w inhibition of navitoclax to achieve higher levels of combined BCL-2 family inhibition. Dasatinib dosing is identical to that used in two recent trials of children with newly diagnosed ALL.[2,58]

2.2.1 Rationale for intensification of CNS directed therapy compared to R3 backbone for late first relapse who continue therapy past Block 2

As described in the study plan, patients with B-cell precursor ALL who experience a late relapse (≥ 36 months from diagnosis) and achieve an MRD-negative remission after Block 1 of therapy will continue protocol therapy after Block 2. Therapy after Block 2 has been modeled after the UKALL R3 backbone.[8,17] Post-induction therapy using this backbone includes intermediate dose methotrexate, an intensification block utilizing high-dose cytarabine (similar to RAVEN Block 2A), and continuation therapy including daily 6-mercaptopurine, weekly oral methotrexate, and every 4 week dexamethasone and vincristine "pulses". This backbone was utilized as the standard of care/ control arm of the recently completed Children's Oncology Group relapse trial AALL1331, which compared this control arm to an arm including blinatumomab. However, unpublished data suggests that the isolated extramedullary relapse rate in both the control and experimental arms of AALL1331 for patients treated with this therapy was unexpectedly high (Mignon Loh and Patrick Brown, personal communication; AALL1821 investigator memo dated March 23, 2021). As a result of these findings, intensification of post-induction CNS directed therapy is being planned for the current COG B-ALL relapse trial AALL1821.

The implications of the findings from AALL1331 for the RAVEN trial are unknown. Venetoclax does penetrate the cerebrospinal fluid, albeit at low levels, and thus may contribute to the antileukemic activity of chemotherapy in the CNS. However, to ensure effective extramedullary control for patients treated on RAVEN, additional modifications to the R3 backbone were felt to be needed. These include: the addition of an additional dose of intrathecal chemotherapy for CNS1 patients during Block 1, the exclusion of all patients with isolated extramedullary relapse from Block 2B (to enable them to receive a block of high-dose cytarabine for extramedullary disease), the addition of an intensification block utilizing two cycles of high-dose methotrexate (HD-MTX) immediately after Block 2, changing intermediate dose methotrexate to high-dose methotrexate during interim continuation therapy, and adding additional intrathecal therapy to high-risk populations during the first year of continuation.

In addition to HD-MTX being the standard of care for patients with NCI-high risk B-ALL during initial therapy,[2,3] both St. Jude and the COG have utilized HD-MTX in patients with relapsed ALL. The recently completed ALLR18 protocol (NCT01700946) used 2 cycles of HD-MTX spaced 1 week apart without exclusion for patients with prior CNS irradiation and did not note unexpected neurological toxicity. The ALLR15 trial also used HD-MTX and none of the 4 patients who had received 18 Gy cranial irradiation in frontline therapy experienced grade 3 or higher neurotoxicity during this therapy.[59] A retrospective evaluation of 11 patients treated on relapse ALL trials at St. Jude who had received radiation therapy during frontline treatment identified 1 patient who experienced a seizure and no cases of leukoencephalopathy or necrosis (Matthew Krasin, personal communication). The COG AALL0433 trial used 9 cycles of HD-MTX in patients with intermediate risk relapse and observed a rate of leukoencephalopathy of <1%.[60] Notably, no patients died during either induction 2 or intensification 1, both blocks which contained HD-MTX.[60] Data from newly diagnosed patients treated on St. Jude Total V-X protocols compared the frequency of seizures in patients receiving ongoing methotrexate therapy

after CNS directed radiation. These data demonstrated that the rate of seizure was higher in patients receiving intrathecal or intravenous methotrexate earlier than 12 months after CNS directed radiation therapy but not after 12 months. Together, these data suggest that HD-MTX will be tolerable in relapse patients on this trial including those who received CNS directed radiation therapy during initial treatment, as all of these patients will be >18 months from initial radiation at the time of study entry.

2.3 Background and Rationale for Ancillary and Exploratory Studies

In vitro drug sensitivity is associated with clinical response in children with newly diagnosed ALL.[12,31,61] The drug responsiveness of large cohorts of relapsed or refractory ALL patients has not been systematically characterized, making it more challenging to identify appropriate combination therapies in this population. Similarly, the BH3 (BCL-2 Homology 3) dependence of relapsed ALL has not been previously systematically characterized.

As described in the background, immunotherapy is rapidly emerging as a standard part of therapy for relapsed ALL. Challenges with immunotherapy include understanding reasons for treatment failure and the optimal timing of immunotherapy within combination therapy platforms. Mechanisms of leukemic escape from immunotherapy include loss of the target antigen (CD19 loss or CD22 downregulation)[41,42,62-67] as well as failure to respond to therapy despite antigen preservation.[41,42,62,63,65,67] Treatment failure without antigen loss is frequently mediated by T-cell exhaustion. This exhaustion may be mediated by cytokine secretion within the leukemic microenvironment as well as immune checkpoint signaling mediated by PD-1/ PD-L1.[68,69] These mechanisms also impair the function of tumor neoantigen-specific T-cells which may be generated during both immunotherapy and conventional chemotherapy and can be present in significant numbers of patients with ALL.[70] These signals impair T-cell function and can impair the activity of blinatumomab and CAR-T therapy. This signature can be detected using single-cell genomics.[71] Evaluation of changes in T-cell potential across therapy is needed to optimize use of these therapeutics.

Mixed phenotype acute leukemia (MPAL), also sometimes termed acute leukemia of ambiguous lineage (ALAL), is a heterogenous disease with characteristics of multiple lineages, including, variably, B-ALL, T-ALL, and acute myeloid leukemia (AML).[72,73] Data on newly diagnosed patients suggests that ALL-based therapy is more likely to be beneficial in these patients than AML therapy.[72] These patients are now being included on frontline ALL trials. However, the optimal treatment strategy for these patients in relapse remains unknown. Venetoclax and cytarabine have activity in relapsed AML.[53] Thus, the proposed therapy offers therapy likely to be beneficial in this population which has characteristics of both ALL and AML.

Similarly, there are limited data available on therapies for patients who are intolerant to asparaginase due to toxicity. Data from frontline trials clearly demonstrate a decrement in therapeutic efficacy when asparaginase is inactivated (as in immune mediated inactivation or accelerated drug clearance) or omitted due to shortage or toxicity.[74-76] There are, however, limited trial data suggesting optimal therapy for these patients without

asparaginase. Venetoclax and navitoclax have synergy with multiple chemotherapeutic agents including asparaginase.[77,78] Thus, although patients may benefit more from therapy including both asparaginase and these agents, synergy between non-asparaginase chemotherapy and these agents may still provide therapeutic benefit to such patients who have limited other therapeutic options.

Young adults also comprise a group who may experience differential toxicity to combination therapy compared to the general pediatric population.[6,79,80] Although data are limited, experts in the treatment of young adults with ALL have suggested prolonging time between asparaginase exposure as a mechanism of reducing asparaginase-induced hepatotoxicity.[81] Small case-series data also suggest the use of a reduced dose of asparaginase (i.e. 1000u/m^2 compared to 2500u/m^2) to reduce toxicity without an obvious decrement in overall efficacy.[82] To evaluate such therapy modifications for this population, they will be considered separately from pediatric patients in this cohort.

Patients with isolated extramedullary relapse are frequently excluded from clinical trials due to their different therapeutic needs. The planned therapy includes multiple agents which are active against central nervous system (CNS) leukemia including dexamethasone, asparaginase, vincristine, and, for patients who will receive it, dasatinib. Venetoclax also enters the cerebrospinal fluid at levels ~1000-fold lower than in the plasma[83] and thus may also contribute to extramedullary disease clearance.

Infectious toxicities in children receiving reinduction therapy for relapsed ALL are extremely common. In AALL1331 therapy for 206 patients with relapse before 36 months from diagnosis (early relapses), grade 4 or 5 toxicity occurred in 33% of patients, including a 5% toxic death rate.[18,43] In a retrospective review of real-world data evaluating 59 patients including 16 in >1st relapse, the TACL consortium indicated 97% of patients experienced a grade 3 or higher non-hematological toxicity and 90% experienced a grade 3 or higher infection.[84] Additionally, 15.5% of patients required intensive care unit admission for toxicities (primarily infectious). Further characterization of infections experienced by these patients is needed to identify strategies which mitigate such toxicities

3.0 ELIGIBILITY CRITERIA AND STUDY ENROLLMENT

3.1 Inclusion Criteria

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

3.1.1 Diagnosis

3.1.1.1 Relapsed or refractory acute lymphoblastic leukemia or lymphoma with ≥1% bone marrow disease as measured by flow cytometry, PCR, or next generation sequencing. However, if an adequate bone marrow sample cannot be obtained, patients may be enrolled if there is unequivocal evidence of leukemia with ≥ 5% blasts in the peripheral blood.

- Patients with 1-4.99% bone marrow involvement must have disease confirmed in one of the following ways: an alternative minimal residual disease assay (e.g. flow cytometry and PCR or NGS), cytogenetic abnormality consistent with patient's leukemia, FISH abnormality, or a second bone marrow with MRD ≥1% separated by 1-4 weeks.
- Patients with ≥5% bone marrow disease by a single measurement as measured by flow cytometry, PCR, or next generation sequencing do not require a second confirmatory test.
- Refractory disease is defined as residual leukemia ≥1% after at least 2 prior lines of frontline therapy with curative intent.
- Patients in exploratory cohort I must have measurable extramedullary disease but may have <1% bone marrow disease.
- Patients in exploratory cohort M must have ≥1% bone marrow disease as measured by flow cytometry of mixed phenotype acute leukemia (MPAL)/ acute leukemia of ambiguous lineage (ALAL).

3.1.2 Age ≥ 4 to < 30 years. Patients ≥ 22 years old are only eligible for exploratory cohort O. Sites may have different (lower) maximum ages based on institutional guidelines but may not exceed 30 years.

3.1.3 Patient weighs ≥ 20 kg.

3.1.4 Patient is able to swallow pills.

3.1.5 Lansky/Karnofsky 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).

3.1.6 Participant has adequate organ function as defined by the following:

- Direct bilirubin ≤ 1.5x the institutional upper limit of normal (ULN) unless attributable to leukemic involvement. At institutions which do not obtain a direct bilirubin in patients with a normal total bilirubin, a normal total bilirubin may be used as evidence that the direct bilirubin is not > 1.5 x the ULN. Patients with a direct bilirubin ≥ 2 mg/dl may not enroll regardless of attribution.
- Aspartate transaminase (AST) and alanine transaminase (ALT) < 3.0 x the ULN unless increase is attributable to leukemic involvement.
- Normal creatinine for age or a calculated creatinine clearance ≥ 60 mL/min/1.73 m^2.
- Left ventricular ejection fraction (LVEF) ≥ 40% or shortening fraction ≥ 25%.
    - Patients with a history of reduced LVEF which subsequently improved with medical management are eligible if they meet the criteria above.

3.1.7 Patients must have fully recovered from the acute effects of all prior therapy (defined as resolution of all such toxicities to ≤ Grade 2).

3.1.8 For patients with prior hematopoietic stem cell transplant (HSCT), at least 90 days must have elapsed since transplant, the patient cannot have evidence of active graft-versus-host disease (GVHD), and they must be off calcineurin inhibitors for ≥4 weeks, and off other immunosuppression for ≥2 weeks.

3.1.9 Patients with Down Syndrome/ germline Trisomy 21 are eligible for Block 1 and Block 2b therapies but are ineligible for Block 2a therapy. Patients with Down Syndrome and CD19-negative disease are off therapy after the response evaluation to Block 1.

3.1.10 Prior therapy

- ≥14 days must have elapsed since the completion of cytotoxic therapy, with the exception of standard maintenance therapy (glucocorticoids, vincristine, methotrexate, 6-mercaptopurine), tyrosine kinase inhibitors, and steroids.
- Cytoreduction with prednisone, methylprednisolone, or hydroxyurea for ≤ 120 hours (5 days) in patients with hyperleukocytosis or extramedullary disease compromising organ function can be initiated and continued until up to 24 hours prior to the start of protocol therapy.
- At least 21 days must have elapsed since completion of therapy with a biologic agent excluding blinatumomab. For agents that have known adverse events occurring beyond 21 days after administration, this period prior to enrollment must be extended beyond the time during which adverse events are known to occur. At least 7 days must have elapsed since blinatumomab infusion and patients must have recovered from all toxicities as described above.
- Intrathecal cytotoxic therapy: No waiting period is required for patients having received intrathecal cytarabine, methotrexate, and/or hydrocortisone. Intrathecal chemotherapy given at the time of diagnostic LP to evaluate for relapse prior to study enrollment is allowed.
- Patient has not had prior exposure to navitoclax

3.1.11 Male or female participant of reproductive potential must agree to use appropriate methods of contraception for the duration of study treatment and for at least 30 days after last dose of protocol treatment.

3.1.12 Additional criteria for exploratory cohorts

- **Cohort I**: Diagnosis of isolated extramedullary relapse as defined by bone marrow blasts of <1% AND 1) central nervous system white blood cell count (WBC) of ≥ 5WBC/mL with blasts or 2) biopsy confirmed extramedullary leukemia.

- **Cohort M**: Diagnosis of relapsed or refractory mixed phenotype acute leukemia (MPAL)/ acute leukemia of ambiguous lineage (ALAL).

- **Cohort N**: Patients with relapsed or refractory ALL who, in the view of the provider, are unable to tolerate further asparaginase therapy due to prior toxicities.

- **Cohort O**: Patients with relapsed or refractory ALL who are ages 22-29.9 years. This cohort may not enroll patients at all sites based on institutional guidelines or capacity.

3.2 Exclusion Criteria

3.2.1 Known HIV infection or active hepatitis B (defined as hepatitis B surface antigen–positive) or C (defined as hepatitis C antibody–positive).

3.2.2 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).

3.2.4 Concomitant medications and food

- Treatment with moderate or strong cytochrome P450 3A (CYP3A) inhibitors within 3 days of starting protocol therapy (see Appendix III for examples).
- Treatment with moderate or strong CYP3A inducers within 7 days of starting protocol therapy (see Appendix III for examples).
- Administration or consumption within 3 days prior to the first dose of study drug or grapefruit or grapefruit products, Seville oranges (including marmalade containing Seville oranges), or star fruit.

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

3.3 Definitions

3.3.1 Bone marrow status

- M1: <5% leukemic blasts
- M2: 5-25% leukemic blasts
- M3: >25% leukemic blasts

3.3.2 Minimal residual disease status (MRD)

- Positive: ≥ 0.01% detectable leukemia cells
- Negative: < 0.01% detectable leukemia cells within a marrow with adequate cellular composition to enable evaluation of at least 100,0000 viable mononuclear cells.
- Equivocal: no detectable leukemia but with inadequate cellular composition to enable evaluation of at least 100,000 viable mononuclear cells.

3.3.3 CNS Status

- CNS1: Absence of blasts in the cerebral spinal fluid (CSF), regardless of the number of white blood (WBC) cells present
- CNS2: <5 WBC/ microL CSF and cytospin positive for blasts
- Traumatic LP with blasts: ≥ 10 red blood cells (RBC)/ microL CSF with morphologically identifiable blasts on cytospin
- CNS3: ≥5 WBC/ microL CSF and cytospin positive for blasts OR cranial nerve palsy

3.3.4 Definitions of Relapse

Relapse: Re-emergence of measurable leukemic burden after obtaining a CR.

Marrow Relapse:

- Rel-M3: A single bone marrow sample with M3 morphology
- Rel-M2: A single bone marrow sample with M2 morphology and confirmatory testing showing ≥ 5% leukemic blasts by flow cytometry, FISH testing or other molecular method
- Rel-M1: A single bone marrow sample with M1/M2 morphology and at least two tests showing ≥1% leukemic blasts (Rel-M1); examples of tests include:
  - flow cytometry showing leukemia ≥1% of all cells
  - karyotypic abnormality (must display at least 1 metaphase similar/identical to diagnosis)
  - FISH abnormality identical to one present at diagnosis/ prior relapse
  - PCR or NGS-based demonstration of Ig or TCR rearrangement that matches diagnosis and is quantifiable as ≥1% in a CLIA approved laboratory.
  - PCR or NGS-based demonstration of validated leukemogenic lesion (e.g., fusion, mutation) that matches diagnosis and is quantifiable as ≥ 1% in a CLIA approved laboratory.

Equivocal Marrow (Equiv-M): A single bone marrow sample with M1 morphology, but also with at least one evaluation that suggests recurrence of leukemia (e.g., flow cytometry, karyotype, FISH, PCR, NGS) but does not meet the definition of a definitive relapse.

In the case of Equiv-M, bone marrow evaluation should be repeated at least 1 week and at most, 4 weeks later.

- To convert to definitive relapse, the repeat marrow must either:
  a. meet criteria for definitive relapse (Rel-M3, Rel-M2 or Rel-M1), or
  b. demonstrate ≥1% MRD

CNS Relapse: Occurrence, after remission, of either a single LP with CNS3 or two-consecutive LP with CNS2 status and supportive testing demonstrating lymphoblasts. Supportive testing can include positive TdT staining, flow cytometry, FISH, or PCR positivity.

Extramedullary Relapse: Biopsy proven recurrence of disease after first remission.

Timing of relapse:

- Early: less than 36 months from initial diagnosis
- Late: ≥ 36 months from initial diagnosis

Response criteria: Disease burden within the bone marrow will be determined by flow cytometry when available for the patient. Peripheral blood count criteria will utilize a CBC obtained within one day before and 2 weeks after the corresponding bone marrow evaluation.

Complete remission (CR)

- Bone marrow with < 5% blasts, no evidence of circulating blasts or extramedullary disease and with peripheral count recovery.
- Peripheral count recovery defined as absolute neutrophil count higher than or equal to 500/μL and platelet count higher than or equal to 50,000/μL.
- Patients who begin therapy with <5% blasts must achieve an MRD-negative (<0.01%) to be deemed a CR or CRi.

Complete remission with incomplete blood count recovery (CRi)

- Bone marrow with < 5% blasts, no evidence of circulating blasts or extramedullary disease but without peripheral count recovery (ANC<500/μL or platelets <50,000/μL).
- Peripheral count recovery defined as absolute neutrophil count higher than or equal to 500/μL and platelet count higher than or equal to 50,000/μL.
- Patients who begin therapy with <5% blasts must achieve an MRD-negative (<0.01%) response to be deemed a CR or CRi.

Partial response (PR)

- A decrease of at least 50% in the percentage of blasts and 5% to 25% blasts by flow cytometry.
- Patients who began therapy with <5% blasts, have at least a 50% decrease in the percentage of blasts, and do not achieve and MRD-negative CR/CRi will be deemed PR.
- At least a 50% reduction in the sum of the product of the diameters of up to the six largest nodes or nodal masses without new lesions.

No response (NR)

- Leukemia/ lymphoma reduction less than required to meet the definition of PR or CR/CRi, or increase in leukemia/ lymphoma after treatment.

- If a patient also meets the definition of treatment failure (TF), the patient will be recorded as a TF in the research database.

Treatment Failure (TF)

- Failure to achieve CR/CRi/PR criteria after cycle 2.

3.4 Research Participant Recruitment and Screening

RAVEN is be conducted on two continents. Fourteen (14) institutions in North America will collaborate in the proposed project: St. Jude Children's Research Hospital; University of North Carolina School of Medicine; Nationwide Children's Hospital Columbus; Children's Hospital of Philadelphia; Vanderbilt University Medical Center; Memorial Sloan Kettering Cancer Center; Rady Children's Hospital-San Diego; Cook Children's Medical Center; Children's Hospital of Michigan; Children's Healthcare of Atlanta; Texas Children's Hospital/ Baylor; University of California San Francisco; Sanford Children's Hospital Sioux Falls; Sanford Roger Maris Cancer Center.

Ten (10) institutions in Australia and New Zealand as part of the ANZCHOG group: John Hunter Children's Hospital, Queensland Children's Hospital, Monash Children's Hospital, Perth Children's Hospital, Royal Children's Hospital, Sydney Children's Hospital, Women's and Children's Hospital Adelaide, the Children's Hospital at Westmead, Starship Children's Hospital, and Christ Church Hospital.

3.5 Enrollment on Study at St. Jude

A member of the study team will confirm potential participant eligibility as defined in Sections 3.1-3.2, and complete the 'Participant Eligibility Checklist' in OnCore. A research participant-specific consent form and assent document (where applicable) will be generated. The entire signed consent/assent form(s) must be scanned into the Electronic Health Record (EHR) by the study team designee.

3.6 Enrollment on Study for Affiliate Sites Sharing St. Jude Participants

For Affiliate sites sharing patients, eligibility will already have been determined at St. Jude and the participant will already have been enrolled via the St. Jude participant enrollment process. The CTO Clinical Research Monitor (CRM) will monitor the completed consent forms of the shared participants during scheduled monitoring vests with the affiliate sites.

3.7 Enrollment on Study at Collaborating Sites

Collaborating sites and enrolling affiliate sites will screen potential study participants. Determine eligibility status through the OnCore system. Enroll participants meeting eligibility criteria into the OnCore system.

OnCore will generate a unique patient ID number. The OnCore system will also generate a patient research record in the TrialMaster database.

4.0 TREATMENT PLAN

4.1 Design and Study Overview

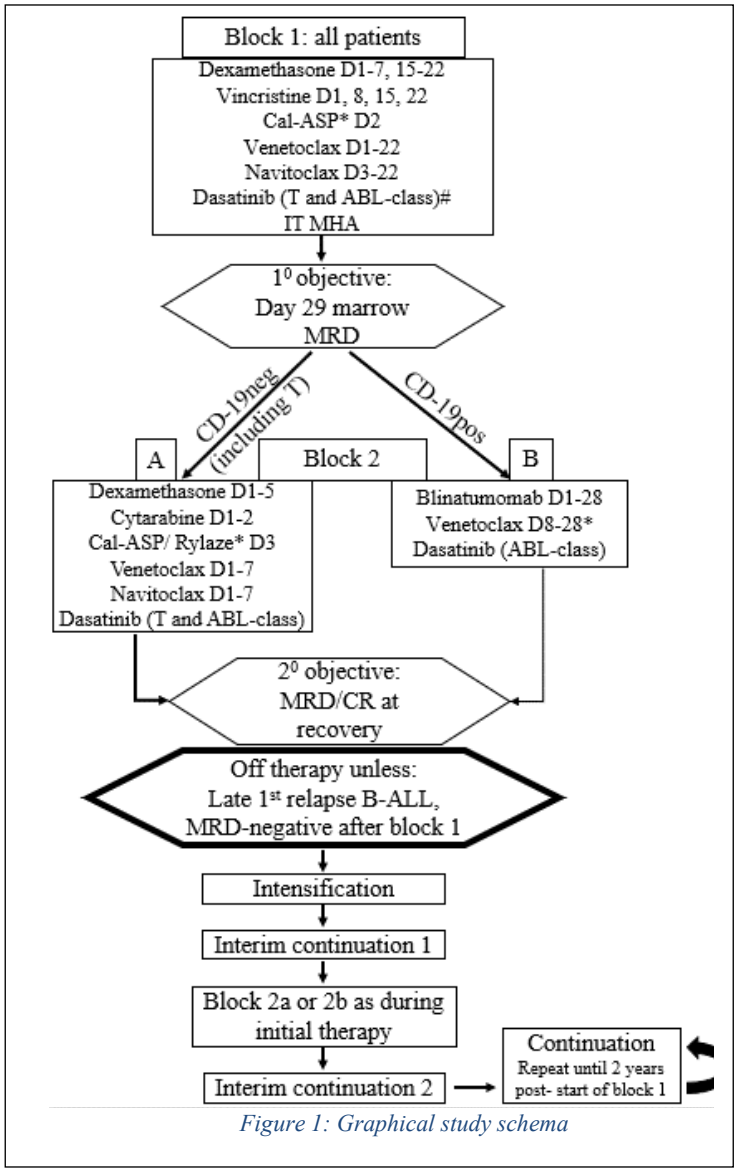

Figure 1: Graphical study schema

Enrolled patients will receive protocol therapy as described. A graphical schema is shown in Figure 1. The primary outcome assessment will be a bone marrow with MRD testing on Day 29 of Block 1 therapy. Following that assessment, patients should continue with protocol therapy including either Block 2A (which includes high-dose cytarabine with venetoclax and navitoclax) for patients with CD19 negative leukemia, those with isolated extramedullary relapse, or whose treating physician determines that blinatumomab therapy is not in the patient's best interest; or Block 2B for patients with leukemia which is CD19 positive. Following recovery from Block 2 therapy, all patients except those with late first relapse of B-ALL (≥ 36 months from diagnosis) who are MRD-negative at <0.01% after Block 1 therapy are off therapy. Further therapy for these patients is at the treating physician's discretion. Patients with late first relapse B-ALL relapse and who are MRD negative will proceed with intensification, interim continuation, and continuation therapy as described. Patients with Down's Syndrome with CD19 negative leukemia will be off therapy after Block 1 as they are ineligible for Block 2A. Patients in exploratory cohorts I and N who are late first relapse B-ALL and who are MRD-negative after Block 1 therapy may continue on protocol therapy after Block 2 or receive alternative therapy at their treating physician's discretion. Patients in exploratory cohort M and O are off therapy after Block 2.

4.2 Treatment Administration and Schedule

4.2.1 Block 1 Therapy

Agent	Dose (with maximum daily dose if applicable) and Route	Number of doses	Schedule
Dexamethasone	5mg/m^2/dose BID PO/IV	30	Days 1-7, 15-22
Vincristine	1.5mg/m^2/dose IV (Max 2mg)	4	Days 1, 8, 15, 22
Calaspargase*	2500units/m^2/dose IV	1	Day 2
Dasatinib#	80mg/m^2/day QD/PO (Max 140mg)	28	Days 1-28
Venetoclax	120mg/m^2 (max 200mg) QD/PO	1	Day 1
Venetoclax	240mg/m^2/dose (max 400mg) QD/PO	21	Days 2-22
Navitoclax	50mg QD/PO (for patients ≥45kg) 25mg (for patients 20 - <45kg)	20	3-22
IT MHA	Methotrexate 12mg IT Hydrocortisone 24mg IT Cytarabine 36mg IT	1-4	See table below
Leucovorin	5mg/m^2 (max 5mg) PO or IV	2+	24 and 30 hours after each lumbar puncture

Calaspargase may be replaced by alternative forms of asparaginase due to local practice or prior allergy.* ***If pegaspargase is used, two doses (on days 2 and 15) of 2500units/m^2 replace the single day 2 dose of calaspargase doses**. Asparaginase formulation must be documented in the research database. Other substitutions should be discussed with PI and documented in the research database. For patients in exploratory cohort N, asparaginase will be omitted. For patients in exploratory cohort O, the Day 15 dose of pegaspargase may be omitted and the Day 2 dose may be capped at 2000units/m^2 with a maximum dose of 3750 units. These modifications should be documented in the research database.*

 # Dasatinib will be given to patients with ABL-class fusions (in-frame fusions of ABL1, ABL2, CSF1R, PDGFRB, PDGFRA, or LYN that include the tyrosine kinase domain) and those with non-ETP T-ALL/ALLy. For patients who are discovered to have ABL-class fusions while on therapy, dasatinib will be begun upon identification of that fusion.

Intrathecal schedule

CNS status	Relapse	# Doses	Days
1 (no blasts)	Untreated first relapse	3	-7 to 1, 8, 22
1 (no blasts)	>1st including refractory after relapse	1	-7 to 1
2, 3, traumatic with blasts (blasts present)	Any	3-4	-7 to 1, 8, 15, *22

**Give Day 22 intrathecal therapy to patients with persistent CSF blasts on Day 8 of Induction. Day 1 LPIT can be delayed if clinical condition requires.*

Continue intrathecal chemotherapy weekly until negative x 2. Patients with persistent CSF blasts on day 15 should have a diagnostic LP performed with end of Block 1 therapy evaluations (days 29-32). Patients with persistent CSF blasts at the end of Block 1 are treatment failures and off therapy.

4.2.2 Block 2a and 2b Therapy

Criteria to begin Block 2 therapy: Patients who achieve an MRD-negative (MRD<0.01%) CR/CRi after Block 1 are recommended to wait up to 2 weeks for count recovery, defined as a WBC ≥1500/microL, ANC ≥500/microL and platelets ≥50,000/microL. Patients without count recovery at 2 weeks should have a repeat bone marrow assessment at 2 weeks to confirm continued MRD-negative remission but should then proceed with Block 2 therapy. Patients who are MRD-positive after Block 1 should begin Block 2 when the MRD result is known and they have recovered from toxicities to ≤ grade 2. Variations needed for patient care are allowable but should be discussed with the PI.

4.2.2.1 Block 2a Therapy

For patients with ≥ 5% of leukemic blasts which do not have detectable CD19 on their surface (i.e., at least 5 of every 100 blasts do not express CD19), those with isolated extramedullary relapse, or whose physician determines this therapy arm is in their patient's best interest. **Patients with Down Syndrome are not eligible for Block 2a therapy**. Patients in exploratory cohort O are not eligible for the phase I/ dose de-escalation phase of the study but may enroll on block 2 therapy after the dose has been established.

Agent	**Dose (with maximum daily dose if applicable) and Route**	**Number of doses**	**Schedule**
Dexamethasone	3mg/m^2/dose BID PO/IV	10	Days 1-5
Calaspargase*	1000units/m^2/dose IV	1	Day 3
Cytarabine	According to current dose level: see table below		

Agent	Dose (with maximum daily dose if applicable) and Route	Number of doses	Schedule
Dasatinib#	80mg/m^2/day QD/PO (Max 140mg)	28	Days 1-28
Venetoclax&	240mg/m^2 (max 400mg) QD/PO	7	Days 1-7
Navitoclax&	50mg QD/PO (for patients ≥45kg) 25mg (for patients 20 - <45kg)	7	Days 1-7
IT MHA	Methotrexate 12mg IT Hydrocortisone 24mg IT Cytarabine 36mg IT	1	With end of cycle bone marrow assessment

**Calaspargase may be replaced by alternative forms of asparaginase due to local practice or prior allergy. Asparaginase formulation must be documented in the research database. For patients in exploratory cohort N, asparaginase will be omitted. This omission should be documented in the research database.*
 # Dasatinib will be given to patients with ABL-class fusions (in-frame fusions of ABL1, ABL2, CSF1R, PDGFRB, PDGFRA, or LYN that include the tyrosine kinase domain) and those with non-ETP T-ALL/LLy.
&Patients with late relapse and MRD-negative response to Block 1 who are receiving a repeat of Block 2 following interim continuation therapy will not receive venetoclax or navitoclax during the repeated Block 2 therapy.

Cytarabine dosing during phase 1 portion for Block 2a

Dose level	Dose (with maximum daily dose if applicable) and Route	Number of doses	Schedule
1	3000mg/m^2/dose q12h IV infusion over 3 hours	4	Days 1-2
~~0~~	~~1500mg/m^2/dose q12h IV infusion over 3 hours~~	~~4~~	~~Days 1-2~~
~~-1~~	~~1000mg/m^2/dose q12h IV infusion over 3 hours~~	~~2~~	~~Day 1~~

Rules for dose selection are described in section 12.2.
All of the first 5 patients enrolled at dose level 1 tolerated therapy without a dose limiting toxicity as defined in section 12.2.2.2. With 5/5 patients tolerating this dose, dose level 1 was declared the recommended phase two dose and future patients will be enrolled at this RP2D.

4.2.2.2 Block 2b Therapy

For patients with >95% of leukemic blasts with detectable CD19 on their surface. **Patients with isolated extramedullary relapse are ineligible for Block 2b therapy**. Patients in exploratory cohort O are not eligible for the phase I/ dose de-escalation phase of the study but may enroll on block 2 therapy after the dose has been established.

Agent	Dose (with maximum daily dose if applicable) and Route	Number of doses	Schedule
Blinatumomab; for patients with end of Block 1 MRD >5%	5mcg/m^2/day (max 9mcg/day; patients weighing ≥45kg receive 9mcg/day fixed dose) IV	7	Days 1-7

Agent	Dose (with maximum daily dose if applicable) and Route	Number of doses	Schedule
Blinatumomab; for patients with end of Block 1 MRD >5%	15mcg/m^2/day (max 28mcg/day; patients weighing ≥45kg receive 28mcg/day fixed dose) IV	21	Days 8-28
Blinatumomab; for patients with end of Block 1 MRD ≤5%	15mcg/m^2/day (max 28mcg/day; patients weighing ≥45kg receive 28mcg/day fixed dose) IV	28	Days 1-28
Dexamethasone	10mg/m^2 (max 20mg) 6-12 hours and 5mg/m^2 (max 20mg) 30-60 minutes prior to day 1 blinatumomab* PO/IV	2*	Day 1, 8*
Dasatinib#	80mg/m^2/day QD/PO (Max 140mg)	28	Days 1-28
Venetoclax&	According to current dose level: see table below^		
IT MHA	Methotrexate 12mg IT Hydrocortisone 24mg IT Cytarabine 36mg IT	1	Day 29 With end of cycle bone marrow

Dasatinib will be given to patients with ABL-class fusions (in-frame fusions of ABL1, ABL2, CSF1R, PDGFRB, PDGFRA, or LYN that include the tyrosine kinase domain).
^ Venetoclax will be held for patient with a peripheral lymphocyte count (WBC x percent lymphocytes) <300/mm^3 or if ANC <500/microL, or in the presence of grade 3 or higher cytokine release syndrome.
* For patients who start blinatumomab at 5mcg/m^2/day, an additional dose of dexamethasone will be given on day 8 30-60 minutes prior to dose increase. For patients whose blinatumomab is interrupted for more than 4 hours for any reason, an additional dose of dexamethasone will be given prior to restarting blinatumomab.
&Patients with late relapse and MRD-negative response to Block 1 who are receiving a repeat of Block 2 following interim continuation therapy will not receive venetoclax during the repeated Block 2 therapy.
Patients with Down syndrome should receive leucovorin rescue after every IT (5 mg/m^2 [max 5mg] PO q6h x 2 doses starting 24 hours after IT).

Venetoclax dosing during phase 1 portion for Block 2b

Dose level	Dose (with maximum daily dose if applicable) and Route	Number of doses	Schedule
1	240mg/m^2 (max 400mg) QD/PO	21	Days 8-28
~~0~~	~~240mg/m^2 (max 400mg) QD/PO~~	~~14~~	~~Days 8-21~~
~~-1~~	~~240mg/m^2 (max 400mg) QD/PO~~	~~7~~	~~Days 8-14~~

Rules for dose selection are described in section 12.2.
All of the first 5 patients enrolled at dose level 1 tolerated therapy without a dose limiting toxicity as defined in section 12.2.2.2. With 5/5 patients tolerating this dose, dose level 1 was declared the recommended phase two dose and future patients will be enrolled at this RP2D.

4.2.2.3 End of Block 2 Evaluations

Patients on Block 2A will undergo a bone marrow with count recovery ANC ≥ 300/microL and platelets ≥50,000/microL or on Day 35, whichever is sooner.

Patients on Block 2b will undergo a bone marrow on Day 28-30.

Patients who are MRD-negative without count recovery by Day 42 should undergo repeat bone marrow evaluation weekly until count recovery or Day 70. Patients without count recovery by Day 70 will be removed from protocol therapy.

St. Jude Children's Research Hospital
IRB NUMBER: 21-0719
IRB APPROVAL DATE: 02/19/2024

4.2.3 Intensification Therapy

For late first relapse B-ALL and MRD<0.01% after Block 1 only. Patients in exploratory cohort O are excluded.

Patients should begin therapy when ANC ≥ 300/microL and platelets ≥50,000/microL.

Agent	Dose (with maximum daily dose if applicable) and Route	Number of doses	Schedule
HD-Methotrexate	5000mg/m^2/dose IV: 500mg/m^2 over 60 minutes then 4500mg/m^2 over 23 hours; or targeted 65 microM	2	Day 1, 15#
Mercaptopurine	50mg/m^2/dose PO daily*	28	Days 1-28
IT MHA	Methotrexate 12mg IT Hydrocortisone 24mg IT Cytarabine 36mg IT	1	Day 15
Leucovorin	15mg/m^2/dose PO/IV every 6 hours: Start 42 hours after HD-MTX begins ^	At least 3	Days 2, 16

#The subsequent dose of high dose methotrexate and mercaptopurine will be delayed if ANC <300/mm^3, WBC < 1500/mm^3, platelet count < 50 x 10^9/L, SGPT >500 U/L, total bilirubin >2 mg/dl and direct bilirubin >1.4 mg/dl, or mucositis is present.
** Mercaptopurine may be held in the presence of ANC < 300/microL, WBC < 1,500/microL, platelet count < 50x 10^9/L or grade 3 or 4 mucositis. Dosage of mercaptopurine may be reduced to 25 mg/m^2/day in participants who have developed neutropenia after the first high dose methotrexate and mercaptopurine treatment. See section 5.2.7 for modifications of mercaptopurine based on TPMT status.*
^ Patients with a history of Grade 3 or 4 gastrointestinal toxicities with prior methotrexate or a history of typhlitis with any chemotherapy should have leucovorin continue for 5, rather than 3 doses; those with early toxicity should have leucovorin begin at 36 hours with subsequent methotrexate. Leucovorin may be extended in the presence of evidence of mucositis or other methotrexate toxicity. Leucovorin dose should be increased if 42 hour MTX level is >1 microM and continued until the methotrexate concentration is less than the threshold level for toxicity (historically 0.1 microM by TDx assay; ~0.15 microM by ARK assay). See section 5.2.2 for additional information

4.2.4 Interim Continuation Therapy 1 and 2 (8 weeks each)

For late first relapse B-ALL (see Section 3.3 for definitions) and MRD<0.01% after Block 1 only. Patients should begin therapy when ANC ≥ 500/microL and platelets ≥50,000/microL.

Agent	Dose (with maximum daily dose if applicable) and Route	Number of doses	Schedule
Dexamethasone	3mg/m^2/dose BID PO/IV	10	Days 1-5
Vincristine	1.5mg/m^2 (max 2mg) IV	1	Day 1
Mercaptopurine	75mg/m^2/dose PO daily*	49	Days 1-49
Methotrexate	20mg/m^2/dose PO/IV@	4	Days 8, 15, 29, 36
HD-Methotrexate	5000mg/m^2/dose IV: 500mg/m^2 over 60 minutes then 4500mg/m^2	1	Day 22$

Agent	Dose (with maximum daily dose if applicable) and Route	Number of doses	Schedule
	over 23 hours; or targeted 65 microM		
Leucovorin	15mg/m^2/dose PO/IV every 6 hours: Start 42 hours after HD-MTX^	At least 3	Days 24, 25^
Cyclophosphamide	300mg/m^2/dose IV over 15-30 minutes	2	Days 43$, 50
Etoposide	150mg/m^2/dose IV over 1-2 hours	2	Days 43, 50$
Cytarabine	50mg/m^2/dose IV over 1-30 minutes	8	Days 44-47, 51-54
Dasatinib#	80mg/m^2/day QD/PO (Max 140mg)	56	Days 1-56
IT MHA	Methotrexate 12mg IT Hydrocortisone 24mg IT Cytarabine 36mg IT	2	Day 22, 43

*For patients with known heterozygous inactivating mutations of TPMT or NUDT15, reduce dose to 60mg/m^2/day. For patients with known homozygous/ biallelic inactivating mutations, consult PI. Mercaptopurine dose may be decreased in the presence of neutropenia <500/microL or platelets <50,000/microL.

@IV delivery is preferred. PO is acceptable for logistical reasons, intolerance of IV delivery, and is preferred in patients who have previously received cranial radiation. Day 29 low-dose methotrexate may be delayed or omitted if a patient is still receiving leucovorin following HD-MTX administration.

^Patients with a history of Grade 3 or 4 gastrointestinal toxicities with prior methotrexate or a history of typhlitis with any chemotherapy should have leucovorin continue for 5, rather than 3 doses; those with early toxicity should have leucovorin begin at 36 hours with subsequent methotrexate. Leucovorin may be extended in the presence of evidence of mucositis or other methotrexate toxicity. Leucovorin dose should be increased if 42 hour MTX level is >1 microM and continued until the methotrexate concentration is less than the threshold level for toxicity (historically 0.1 microM by TDx assay; ~0.15 microM by ARK assay). See section 5.2.2 for additional information.

$ANC must be ≥500/microL and platelets must be ≥50,000/microL prior to Day 22 and 43/44 therapies. Day 22 therapy may be delayed up to 1 week due to cytopenias. If counts have not recovered by day 29, omit HD-methotrexate and proceed with Day 29 low-dose methotrexate. Consider mercaptopurine dose modification if cytopenias precluded HD-methotrexate administration. Once Day 43 therapy begins, Day 44 therapy begins 1 day later and Day 50 therapy proceeds 1 week from Day 43 therapy unless non-hematological toxicity precludes administration.

#Dasatinib will be given to patients with ABL-class fusions (in-frame fusions of ABL1, ABL2, CSF1R, PDGFRB, PDGFRA, or LYN that include the tyrosine kinase domain). Dasatinib should be held beginning 48 hours prior to HD-methotrexate administration and until methotrexate levels are <0.5microM without evidence of residual methotrexate toxicity (e.g. mucositis). Day 22 intrathecal treatment should be given on the same day as HD-MTX administration. Consult the PI or clinical pharmacist if the IT and HD-MTX are separated by more than 12 hours.

Following recovery from Interim Continuation therapy 1 (ANC must be ≥500/microL and platelets must be ≥50,000/microL), patients will repeat Block 2 therapy according to their initial Block assignment (2A for CD19-negative, 2B for CD19-positive) and receive a second cycle of interim continuation. Patients will not receive venetoclax or navitoclax during this repeated Block 2 cycle. Following recovery from Interim Continuation 2, patients will proceed to Continuation therapy.

4.2.5 Continuation Therapy

For late first relapse B-ALL and MRD <0.01% after Block 1 only.

Continuation cycles last 8 weeks (56 days) each. Continuation therapy continues until 2 years from the start of protocol therapy.

Agent	Dose (with maximum daily dose if applicable) and Route	Number of doses	Schedule
Dexamethasone	6mg/m^2/dose BID PO/IV	20	Days 1-5, 29-33
Vincristine	1.5mg/m^2 (max 2mg) IV	2	Day 1, 29
Mercaptopurine	75mg/m^2/dose PO daily*	56	Days 1-56
Methotrexate	20mg/m^2/dose PO/IV@	6	Days 8, 15, 22, 36, 43, 50
Dasatinib#	80mg/m^2/day QD/PO (Max 140mg)	56	Days 1-56
IT MHA^	Methotrexate 12mg IT Hydrocortisone 24mg IT Cytarabine 36mg IT	1	Day 1, 29%

#Dasatinib will be given to patients with ABL-class fusions (in-frame fusions of ABL1, ABL2, CSF1R, PDGFRB, PDGFRA, or LYN that include the tyrosine kinase domain).
*For patients with known heterozygous inactivating mutations of TPMT or NUDT15, reduce dose to 60mg/m^2/day. For patients with known homozygous/ biallelic inactivating mutations, consult PI.
@ IV delivery is preferred. PO is acceptable for logistical reasons, intolerance of IV delivery, and is preferred in patients who have previously received cranial radiation.
^For patients who are CNS3 at the time of relapse and receive radiation therapy after cycle 2 of continuation, IT MHA will be replaced by oral methotrexate 20mg/m^2 on Day 1 of subsequent cycles. Lumbar puncture for monitoring of CNS remission is indicated every 2 cycles.
%Patients with CNS2 or traumatic lumbar puncture with blasts present at the time of relapse will receive day 1 and 29 IT MHA for the first year of continuation therapy. Following this, patients will receive IT MHA on day 1 of each cycle until the completion of therapy. Patients with CNS3 disease at relapse will receive day 1 and 29 IT MHA during cycles 1 and 2 of continuation and then proceed to chemoradiation as described.

St. Jude Children's Research Hospital
IRB NUMBER: 21-0719
IRB APPROVAL DATE: 02/19/2024

4.2.6 Chemoradiation for those with CNS3 disease at relapse (late first relapse [≥36 months from diagnosis] B-ALL and MRD<0.01% after Block 1 only)

Patients with CNS3 disease at relapse will receive 18Gy cranial irradiation after Cycle 2 of Continuation Therapy. Patients will not receive additional intrathecal chemotherapy following irradiation. Chemoradiation lasts 21 days.

Agent	Dose (with maximum daily dose if applicable) and Route	Number of doses	Schedule
Dexamethasone	6mg/m^2/dose BID PO/IV	20	Days 1-5, 15-19
Vincristine	1.5mg/m^2 (max 2mg) IV	3	Day 1, 8, 15
Calaspargase*	2500units/m^2/dose IV	1	Day 2
Dasatinib#	80mg/m^2/day QD/PO (Max 140mg)	21	Days 1-21

*Calaspargase may be replaced by alternative forms of asparaginase due to local practice or prior allergy. Asparaginase formulation must be documented in the research database. If pegaspargase is used, two doses of 2500units/m^2 may be given on Day 2 and 15 to replace the single Day 2 dose of calaspargase. For patients in exploratory cohort N, asparaginase will be omitted. This omission should be documented in the research database.
#Dasatinib will be given to patients with ABL-class fusions (in-frame fusions of ABL1, ABL2, CSF1R, PDGFRB, PDGFRA, or LYN that include the tyrosine kinase domain).

4.2.7 Testicular Radiation

Patients with persistent testicular involvement by leukemia at the end of Block 1 may continue on study and may receive testicular radiation during Block 2 therapy. Suggested radiation for patients with persistent testicular involvement is 24Gy, although this can be adjusted due to prior therapy, plans for transplant, or other patient specific factors. Testicular radiation should be documented in the research database. Radiation should not be given concurrently with venetoclax or navitoclax.

Radiation for scenarios not described in sections 4.2.6 and 4.2.7 should be discussed with the PI. Radiation therapy techniques are at the treating physician's discretion.

5.0 DOSE MODIFICATIONS AND SUPPORTIVE CARE

5.1 Dose Modifications during Continuation Treatment

Dexamethasone and vincristine can be given regardless of counts. For all other drugs, doses are recommended to be reduced as follows:

- WBC >1,500/mm^3 and ANC >300 /mm^3; give full dose
- WBC > 1,000/mm^3 but <1,500/mm^3 and ANC > 300 /mm^3; reduce the dose by 50%.
- WBC < 1,000/mm^3 or ANC <300/mm^3; hold chemotherapy.
- Participants with an inactivating TPMT allele should receive no more than 60 mg/m^2 of 6MP.

- Patients with recurrent myelosuppression should have doses of methotrexate and mercaptopurine reduced 50% with slow increases back to protocol dose as tolerated.
- Patients receiving dasatinib who experience recurrent myelosuppression may decrease dasatinib to 60mg/m^2/day or 100mg/day.

5.2 Dose Modifications for Agent-Specific Toxicity

5.2.1 Asparaginase Toxicity

Asparaginase may be dosed based on ideal body weight or the dose may be capped at 3,750u/ dose based on local practice.

Allergic reactions

Patients with allergic reactions to Pegaspargase or calaspargase pegol may receive desensitization (prolonged administration with anti-allergic premedication), Erwinia asparaginase at 25,000 units/m^2/dose IM thrice weekly (2 to 3 days apart, e.g., Monday, Wednesday and Friday), or (if FDA approved for use in pediatric patients with ALL), Recombinant Crisantaspase Pseudomonas fluorescens (RC-P) at the FDA approved dose. Patients who undergo desensitization for allergic reactions should be monitored for rapid drug clearance resulting in asparaginase activity < 0.1 IU/mL on Day 15. Patients receiving Erwinia or RC-P during Block 2A either due to allergy or local practice will receive only a single dose (25,000 units/m^2 Erwinia) on day 3, while dosing during Block 1 will be thrice weekly as described. Premedication prior to asparaginase of all forms may be given at the investigator's discretion.

Pancreatitis

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

5.2.2 High Dose Methotrexate

Participants with Down Syndrome

Dosage of high dose methotrexate in patients with Down Syndrome receiving interim continuation therapy will be modified because participants with Down syndrome have altered MTX pharmacokinetics and enhanced tissue sensitivity to the effects of MTX. The hydration and alkalinization regimen should be the same as outlined in Section 5.2.13. However, the dose of HD-MTX should be 500 mg/m^2 (50 mg/m^2 over 1 h and 450 mg/m^2 given over 23 h). Baseline leucovorin rescue will begin early (at hour 30 at 30 mg/m^2 IV q6h × 2 doses, followed by 10 mg/m^2 IV q6h × 6 doses). If MTX plasma levels are elevated, increased leucovorin rescue is recommended. Vigorous hydration should be ensured until the 42-hr MTX level is known.

Hepatic dysfunction

High-dose methotrexate 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.

Renal dysfunction

Subclinical renal impairment (normal serum creatinine but decreased GFR) may be present in patients receiving concurrent nephrotoxic drugs (e.g. IV acyclovir) which, if possible, should be held during and for 20 hours after HD-MTX infusions or until adequate MTX clearance has been documented. Consideration to delaying MTX administration should be given if a patient's serum creatinine indicates renal impairment, and/or GFR/CrCl is < 50 ml/min/1.73m^2.

SLCO1B1 inhibitors

Coadministration of high dose methotrexate with SLCO1B1 inhibitors such as proton pump inhibitors, aminopenicillins, or dasatinib may result in delayed methotrexate clearance and increased toxicity. These agents should be held for at least 48 hours prior to high-dose methotrexate and until methotrexate clearance is complete.

5.2.3 Etoposide Toxicity

Hepatic dysfunction

Etoposide should be modified in participants with elevated direct bilirubin concentrations or other evidence of biliary obstruction:

- 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

Renal dysfunction

Both renal and liver functions affect drug clearance. Each route of clearance takes on added significance with higher vs lower doses and short vs long IV infusions. The following dose adjustment should be performed.

- GFR/CrCl 10-50 mL/minute/1.73m^2: Administer 75% of dose.
- GFR/CrCl <10 mL/minute/1.73m^2: Administer 50% of dose.
- Hemodialysis/peritoneal dialysis (PD) (after dialysis on dialysis days): administer 50% of dose.
- Continuous renal replacement therapy (CRRT): Administer 75% of dose and reduce for hyperbilirubinemia.

Hypotension

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, lacrimation, 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
2. Administer the following as indicated:
   a. diphenhydramine 1mg/kg IV (max dose 50 mg) or similar anti-histamine according to institutional practice.
   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)

5.2.4 Venetoclax Toxicity

Venetoclax should be reduced 50% in patients with a direct bilirubin of 4-6 mg/dl and held in patients with a direct bilirubin >6 mg/dl.[85]

Venetoclax must be dose adjusted if given with moderate-strong CYP3A inhibitors. See Appendix III for a list of prohibited CYP3A inducers and a list of CYP3A inhibitors requiring dose adjustment. See appendix IV for appropriate dose adjustments in the presence of strong-moderate CYP3A inhibitors. These dose adjustments and the reason for adjustment should be included in the research database.

5.2.5 Vincristine Toxicity

Neurotoxicity

Mild vinca alkaloid toxicities are anticipated. These include jaw pain, constipation, decreased deep tendon reflexes. If there are persistent severe abdominal cramps, gait impairment, severe pain that requires narcotics or SIADH develops, the dose may be reduced to 50%. Motor paralysis or typhlitis will warrant temporary discontinuation of vincristine.

Hepatic dysfunction

Vincristine should be modified in participants with elevated direct bilirubin concentrations or other evidence of biliary obstruction.

- 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

5.2.6 Blinatumomab Toxicity

Adverse Reaction	Grade	Patients Weighing 45 kg or More	Patients Weighing Less than 45 kg
Cytokine Release Syndrome (CRS)	Grade 3	• Interrupt Blinatumomab • Administer dexamethasone 8 mg every 8 hours IV or orally for up to 3 days and taper thereafter over 4 days • When CRS is resolved, restart blinatumomab at 9 mcg/day, and escalate to 28 mcg/day after 7 days if the adverse reaction does not recur.	• Interrupt blinatumomab • Administer dexamethasone 5 mg/m^2 (max 8 mg) every 8 hours intravenously or orally for up to 3 days and taper thereafter over 4 days. • When CRS is resolved, restart blinatumomab at 5 mcg/m^2/day, and escalate to 15 mcg/m^2/day after 7 days if the adverse reaction does not recur.
	Grade 4	Discontinue blinatumomab permanently. Administer dexamethasone as instructed for Grade 3 CRS	
Neurological Toxicity	Seizure	Discontinue blinatumomab permanently if more than one seizure occurs	
	Grade 3	Withhold blinatumomab until no more than Grade 1 (mild) and for at least 3 days, then restart blinatumomab at 9 mcg/day. Escalate to 28 mcg/day after 7 days if the adverse reaction does not recur. If the adverse reaction occurred at 9 mcg/day, or if the adverse reaction takes more than 7 days to resolve, discontinue blinatumomab permanently	Withhold blinatumomab until no more than Grade 1 (mild) and for at least 3 days, then restart blinatumomab at 5 mcg/m^2/day. Escalate to 15 mcg/m^2/day after 7 days if the adverse reaction does not recur. If the adverse reaction occurred at 5 mcg/m^2/day, or if the adverse reaction takes more than 7 days to resolve, discontinue blinatumomab permanently.
	Grade 4	Discontinue blinatumomab permanently	
Other Clinically Relevant Adverse Reactions	Grade 3	Withhold blinatumomab until no more than Grade 1 (mild), then restart blinatumomab at 9 mcg/day. Escalate to 28 mcg/day after 7 days if the adverse reaction does not recur. If the adverse reaction takes more than 14 days to resolve, discontinue blinatumomab permanently	Withhold blinatumomab until no more than Grade 1 (mild), then restart blinatumomab at 5 mcg/m^2/day. Escalate to 15 mcg/m^2/day after 7 days if the adverse reaction does not recur. If the adverse reaction takes more than 14 days to resolve, discontinue blinatumomab permanently.
	Grade 4	Consider discontinuing blinatumomab permanently.	

These are strong recommendations. Variations from these recommendations should be discussed with the PI and documented

5.2.7 TPMT and NUDT15 Status and Thiopurine Dosage

Participants who have at least one mutant TPMT allele often require dosage decreases of 6MP to avoid severe myelosuppression.[86,87] Thus, we recommend that participants with phenotype or genotype consistent with at least one variant allele start mercaptopurine at 60 mg/m^2 and adjust the subsequent doses according to blood count and red blood cell 6 thioguanine nucleotide level. Patients with two copies of non-functional TPMT alleles should have mercaptopurine dose adjusted in discussion with the PI. For the participants who did not have TPMT/NUDT15 testing in the frontline protocol and will receive mercaptopurine, a blood sample (5-10 ml) will be drawn along with routine lab work at the start of Block 2 to allow for timely TPMT genotyping and/or phenotyping. In participants in whom TPMT genotype and phenotype are discordant, or in whom suspected noncompliance, problems with toxicities or high blood counts are present, samples for red blood 6TGN and/or repeat TPMT activity may be measured. The dose of 6MP may be adjusted based on participants' TPMT status, 6TGN levels and WBC.

Similarly, patients with inherited non-functional alleles in NUDT15 also experience excessive myelosuppression and can tolerate significantly lower mercaptopurine dosages during maintenance therapy.[86,88-90] NUDT15 encodes nucleotide diphosphatase which directly inactive thiopurine metabolites. *In vitro* and *in vivo* data indicate that loss of NUDT15 (e.g., patients inheriting no functional alleles) leads to increased levels of thiopurine active metabolite (TGTP) and higher incorporation of thioguanine into DNA (DNA-TG) and thus toxicity. Because ALL blasts with non-functional NUDT15 alleles are also more sensitive to thiopurine,[91] reduction of mercaptopurine dose in at-risk patients is likely to mitigate toxicity without compromising anti-leukemic efficacy. Following the same principle of TPMT-driven thiopurine dosing, the starting dose of mercaptopurine should be adjusted to 60 mg/m^2/day for patients with only one copy of non-functional NUDT15 alleles. Patients with two copies of non-functional NUDT15 alleles should have mercaptopurine dose adjusted in discussion with the PI.

5.2.8 Obesity

Actual body weight will be used to calculate body surface area in all participants and used for dosage calculations.

5.2.9 Minor Modifications

Timing of protocol therapy administration, response assessment studies, and surgical interventions are based on schedules derived from the experimental design or on established standards of care. Minor unavoidable departures (up to 72 hours) from protocol directed therapy and/or disease evaluations for valid clinical, patient and family logistical, or facility, procedure and/or anesthesia scheduling issues are acceptable.

The term "every" is an approximate term meaning that these medications noted will be administered approximately "every" 12 hours. The drug administration timing in the case of "every 12 hours" may be modified by approximately +/- 4 hours or as clinically indicated

such as in the case of surgical procedures or to accommodate other necessary medication, blood product delivery or procedures (such as a needed CT scan).

The term "day" does not refer to an absolute calendar day. It refers to a general 24-hour period.

Medication doses may be rounded to the nearest integer or to the nearest appropriate quantity when clinically or pharmaceutically indicated as per the MD and PharmD.

5.2.10 Dose Modifications for Collaborating Sites

Doses of intrathecal therapy may be modified to match institutional standard practice. Single agent intrathecal cytarabine or methotrexate may be administered if standard practice at collaborating institutions. Mercaptopurine doses may be modified such that the weekly total dose is equal with differing doses given on differing days of the week to support the use of pills/ tablets. There are no other dose modifications anticipated for collaborating sites.

5.2.11 Participation of St. Jude Affiliates in the Treatment Plan

Patients with late 1st relapse B-ALL who are MRD-negative at the end of Block 1 may receive treatment after Block 2a or 2b (including the repeat of block 2a or 2b without venetoclax or navitoclax) at St. Jude affiliates.

5.2.12 Concomitant Therapy

Additional, non-protocol specified, leukemia directed therapy while receiving protocol therapy is prohibited. Use of steroids for other reasons (e.g. as premedication) is allowed.

5.2.13 Supportive Care

Prophylaxis and Treatment of Tumor Lysis Syndrome

Although not reported among patients with ALL, significant tumor lysis syndrome (TLS) has been observed in patients with CLL who were treated with venetoclax. Therefore, during Block 1, all patients should receive IV hydration at 1500-3000 mL/m^2/day, starting prior to the first dose of venetoclax and continuing until 24 hours after the administration of the first dose of navitoclax. Patients will receive allopurinol unless there is a specific contraindication and should also receive oral phosphate binders or recombinant urate oxidase as needed. Tumor lysis labs, including potassium, calcium, phosphate, uric acid, and creatinine, will be monitored prior to the first dose and every 6-8 hours until 24 hours after the first dose of navitoclax is given. A physician or designee should review TLS labs prior to each ramp-up dose.

Venetoclax and navitoclax should be held in patients who develop significant TLS, including, but not limited to grade 3 or greater hyperkalemia, grade 3 or greater acute

kidney injury, and grade 3 TLS. Venetoclax and navitoclax should be resumed at the planned dose when electrolyte abnormalities related to TLS resolve or return to baseline.

Hydration for High Dose Methotrexate

The following guidelines for hydration during and around high dose methotrexate are **strongly recommended**. Minor deviations to match institutional practice are allowed.

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 (25 mEq/m^2) 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 125 ml/m^2/hr. High-dose methotrexate treatment will follow, provided that urinary pH is $\geq$ 6.5.

During the methotrexate infusion, participants 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. Participants with evidence of renal dysfunction or delayed clearance during the methotrexate infusion may receive less than a 24 hour methotrexate infusion. Hydration fluids should continue until the last dose of leucovorin rescue is given.

Leucovorin Rescue for High Dose Methotrexate

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 to five doses, as determined by primary physician and Pharm D based on patient's prior treatment history or present condition. The dosage of leucovorin will be increased in participants with high plasma methotrexate concentrations (>1.0 microM at 42 hours) and continued until the methotrexate concentration is less than the threshold level for toxicity (historically 0.1 microM by TDx assay; ~0.15 microM by ARK assay). Additional measures, such as hydration, hemoperfusion, or carboxypeptidase will be considered in participants with 42-hour methotrexate levels > 10 microM. Participants with a history of delayed Grade 3 or 4 gastrointestinal toxicity with prior methotrexate or a history of typhlitis with any chemotherapy should have leucovorin continue for 5 doses; those with early toxicity should have leucovorin begin at 36 hours with subsequent methotrexate; if toxicity recurs, the baseline leucovorin dosage should also be increased.

Prophylactic Antibiotics during Periods of Immunosuppression

All patients should receive prophylactic antibiotics (levofloxacin preferred) and antifungals (micafungin or caspofungin preferred during concomitant venetoclax therapy, voriconazole or posaconazole during periods without venetoclax) from the start of therapy (if neutropenic at therapy start) or onset of neutropenia (ANC <500/microL) until neutrophil recovery. Azoles, if used, should be held for 24 hours before and after

vincristine. Use of an azole with venetoclax requires venetoclax dose adjustment as described in Appendix IV . If azoles are used in a patient receiving dasatinib, the dasatinib dose should be reduced by 75% (i.e. to 20mg/m^2/day or a max of 40mg/day).

Prophylaxis for *Pneumocystis jiroveci* Pneumonia

All participants should receive TMP/SMZ (trimethoprim 150 mg/m^2/day in 2 divided doses on Monday, Tuesday, and Wednesday of each week) or according to institutional guidelines. For those who cannot tolerate TMP/SMZ, monthly pentamidine may be substituted. Other options include atovaquone or dapsone.

Immunoglobulin prophylaxis

Low levels of gamma globulin are common in children with relapsed ALL. Monitoring of IgG levels should be considered throughout treatment. If the IgG level falls below age-determined normal levels (recommended threshold 400mg/dl), administration of IVIG at 400 mg/kg is recommended.

Vomited Doses

If doses of venetoclax or navitoclax are vomited within one hour of ingestion, it will be replaced. If vomiting occurs more than 1 hour after dosing, it will be considered a complete dose.

Delayed or Missed Doses

Both venetoclax and navitoclax will be administered as an oral tablet. The tablets are intended to be swallowed intact and are not to be crushed or otherwise altered for administration.

If a dose of venetoclax and/or navitoclax is missed by less than 8 hours, the dose should be taken right away. The next dose should be taken at the usual time the next day. If a dose of venetoclax and/or navitoclax is missed by more than 8 hours, the dose should not be taken that day. The next dose should be taken at the usual time the next day.

Growth Factors

Prophylactic use of hematopoietic growth factors (GM-CSF or G-CSF) is not recommended. However, GM-CSF (250 mcg/m^2/day) or G-CSF (5-10 mcg/kg/day) may be considered for participants who have life threatening fungal infections or bacterial sepsis after discussion with PI. Use of growth factors must be documented in the research database. Biosimilar versions of G-CSF are allowed. Long acting G-CSF such as Pegfilgrastim is not allowed.

Management of Febrile Neutropenia

All patients with fever ≥ 38.3° C on a single occasion or ≥ 38.0°C that persists for one hour should be hospitalized and treated immediately with broad spectrum antibiotics according to institutional guidelines.

6.0 DRUG INFORMATION

Dose rounding at St. Jude will be allowed according to institutional policy (See St. Jude Policy # 20.109, Institutional Policy and Procedure Manual).

Dose rounding for standard of care medications may follow each institution's policy.

6.1 Venetoclax (ABT-199)

Source and pharmacology: Venetoclax is a selective and orally bioavailable small-molecule inhibitor of BCL2, an antiapoptotic protein. Overexpression of BCL2 has been demonstrated in CLL cells where it mediates tumor cell survival and has been associated with resistance to chemotherapeutics. Venetoclax helps restore the process of apoptosis by binding directly to the BCL2 protein, displacing pro-apoptotic proteins like BIM, triggering mitochondrial outer membrane permeabilization and the activation of caspases. In nonclinical studies, venetoclax has demonstrated cytotoxic activity in tumor cells that overexpress BCL2.

Formulation and stability: Venetoclax tablets for oral administration are supplied in bottles or blister cards that contain 10, 50, or 100 mg venetoclax as the active ingredient. Venetoclax dispersible tablets for oral suspension are supplied in blister cards that contain 25 mg tablets. Venetoclax powder sachets contain 3, 10, 25, or 100 mg of venetoclax and are packaged in bottles or cartons or in blisters in cartons. See clinical label for appropriate storage condition requirements.

Supplier: AbbVie, Inc.

Toxicity: The safety of single agent venetoclax at the 400 mg recommended daily dose following a dose ramp-up schedule is based on pooled data of 240 patients with previously treated CLL from two phase 2 trials and one phase 1 trial. In the pooled dataset, the median age was 66 years (range: 29 to 85 years), 95% were white, and 69% were male. The median number of prior therapies was 3 (range: 1 to 12). The median duration of treatment with venetoclax at the time of data analysis was approximately 10.3 months (range: 0 to 34.1 months). Approximately 46% of patients received venetoclax for more than 48 weeks. The most common adverse reactions (≥20%) of any grade were neutropenia, diarrhea, nausea, anemia, upper respiratory tract infection, thrombocytopenia, and fatigue. Serious adverse reactions were reported in 43.8% of patients. The most frequent serious adverse reactions (≥2%) were pneumonia, febrile neutropenia, pyrexia, autoimmune hemolytic anemia (AIHA), anemia, and TLS. Discontinuations due to adverse reactions occurred in 8.3% of patients. The most frequent adverse reactions leading to drug discontinuation were thrombocytopenia and AIHA. Dosage adjustments due to adverse reactions occurred in

9.6% of patients. The most frequent adverse reactions leading to dose adjustments were neutropenia, febrile neutropenia, and thrombocytopenia. See Investigational Brochure for additional information.

Dosage and route of administration: Oral. See Treatment and Dose Modifications sections of the protocol.

Accountability: Because this study is being conducted under an IND, participating pharmacies will be required to submit Drug Accountability Logs at the time of monitoring documenting receipt and shipment of drug supply, dispensing/ordering of supply, and destruction of unused study medication and/or damaged or expired drug.

6.2 Navitoclax

Source and pharmacology: Navitoclax is an oncolytic agent designed to bind and inhibit antiapoptotic Bcl-2 family proteins with high affinity. Thus, it selectively induces program death in cells that have a Bcl-2 protein ratio shifted towards survival as observed in human cell lines derived from small cell lung carcinomas and lymphoid malignancies (concentration required for 50% effect [EC50] ≤ 1 μM). Additionally, navitoclax potently enhances the cytotoxicity of both chemotherapy and radiation in cells derived from multiple, major tumor types, regardless of whether single-agent efficacy is achieved.

Formulation and stability: Navitoclax is available in 25 mg tablets in 30 count HDPE bottles, which should be stored at 15º to 25ºC (59º to 77ºF).

Supplier: AbbVie, Inc.

Toxicity: The most common toxicities include thrombocytopenia, lymphopenia and neutropenia. See Investigator's brochure for additional information.

Dosage and route of administration: Oral, see Treatment and Dose Modifications sections of the protocol.

Accountability: Because this study is being conducted under an IND, participating pharmacies will be required to submit Drug Accountability Logs at the time of monitoring documenting receipt and shipment of drug supply, dispensing/ordering of supply, and destruction of unused study medication and/or damaged or expired drug.

6.3 Dexamethasone

Source and pharmacology: Dexamethasone is a synthetic fluorinated glucocorticoid devoid of mineralocorticoid effects. Dexamethasone, 0.75 mg, has potent anti-inflammatory activity equivalent to approximately 5 mg of prednisone. Glucocorticoids produce widespread and diverse physiologic effects on carbohydrate, protein, and lipid metabolism, electrolyte and water balance, functions of the cardiovascular system, kidney, skeletal muscle, and the nervous systems. Glucocorticoids reduce the concentration of thymus-dependent lymphocytes (T-lymphocytes), monocytes, and eosinophils. Glucocorticoids

selectively bind to the cortisol receptors on human lymphoid cells which are found in larger numbers on leukemic lymphoblasts. They also decrease binding of immunoglobulin to cell surface receptors and inhibit the synthesis and/or release of interleukins, thereby decreasing T-lymphocyte blastogenesis and reducing expansion of the primary immune response. The specific cellular mechanisms that act to halt DNA synthesis are thought to be related to inhibition of glucose transport or phosphorylation, retardation of mitosis, and inhibition of protein synthesis. Elimination half-lives for the following age groups have been reported to be: infants and children under 2 years of age: 2.3 to 9.5 hours, 8 to 16 years: 2.82 to 7.5 hours, and adults (age not specified): 3 to 6 hours. The biologic half-life is 36-72 hours. It is primarily metabolized in the liver and excreted by the kidneys.

Toxicities (>20%): Insomnia, hyperphagia, immunosuppression, personality changes (mood swings, euphoria, anxiety, and depression), pituitary-adrenal axis suppression, acne (L) Cushing's syndrome (moon facies, truncal obesity).

Formulation and stability: Oral: Available in 0.5 mg, 0.75 mg, 1 mg, 1.5 mg, 2 mg, 4 mg, and 6 mg tablets; liquid formulations are available in 0.5 mg/5 mL and 1 mg/1 mL concentrations. Liquid formulations may include: 5%-30% alcohol, benzoic acid, sorbitol, sodium saccharin, glycerin, purified water, and various dyes. Injection: Dexamethasone Sodium Phosphate Solution for Injection is available as 4 mg/mL (1 mL, 5 mL, and 30 mL vials) and 10 mg/mL (1 mL and 10 mL vial sizes). Vials are available in multi-dose vials as well as unit of use vials and syringes.

Guidelines for administration: See Treatment and Dose Modifications sections of the protocol. Tablets and oral solution are preferred; intravenous preparations may be used if needed.

Supplier: Commercially available. See package insert for further information.

6.4 Vincristine

Source and pharmacology: Vincristine is an alkaloid isolated from Vinca rosea Linn (periwinkle). It binds to tubulin, disrupting microtubules and inducing metaphase arrest. Its serum decay pattern is triphasic. The initial, middle, and terminal half-lives are 5 minutes, 2.3 hours, and 85 hours respectively; however, the range of the terminal half-life in humans is from 19 to 155 hours. The liver is the major excretory organ in humans and animals; about 80% of an injected dose of vincristine sulfate appears in the feces and 10% to 20% can be found in the urine. The p450 cytochrome involved with vincristine metabolism is CYP3A4. Within 15 to 30 minutes after injection, over 90% of the drug is distributed from the blood into tissue, where it remains tightly, but not irreversibly bound. It is excreted in the bile and feces. There is poor CSF penetration.

Toxicities (>20%): Alopecia, constipation, loss of deep tendon reflexes.

Formulation and stability: vincristine is supplied in 1 mL and 2 mL vials in which each mL contains vincristine sulfate 1 mg (1.08 μmol), mannitol 100 mg, SWFI; acetic acid and sodium acetate are added for pH control. The pH of vincristine sulfate injection, *USP*

ranges from 3.5 to 5.5. This product is a sterile, preservative free solution. Store refrigerated at 2˚-8˚C or 36˚-46˚F. Protect from light and retain in carton until time of use. Do not mix with any IV solutions other than those containing dextrose or saline.

Guidelines for administration: See Treatment and Dose Modifications sections of protocol. The World Health Organization, the Institute of Safe Medicine Practices (United States) and the Safety and Quality Council (Australia) all support the use of minibag rather than syringe for the infusion of vincristine. Vincristine should **NOT** be delivered to the patient at the same time with any medications intended for central nervous system administration. Vincristine is fatal if given intrathecally.

Injection of vincristine sulfate will be as per institutional policy. Vincristine sulfate must be administered via an intact, free-flowing intravenous needle or catheter. Care should be taken to ensure that the needle or catheter is securely within the vein to avoid extravasation during administration. The solution may be injected either directly into a vein or into the tubing of a running intravenous infusion.

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

Supplier: Commercially available. See package insert for further information.

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

6.5 Pegaspargase

Source and pharmacology: Pegaspargase is a modified version of the enzyme L-asparaginase. L-asparaginase is modified by covalently conjugating units of monomethoxypolyethylene glycol (PEG), molecular weight of 5000, to the enzyme, forming the active ingredient PEG-L-asparaginase. The L-asparaginase (L-asparagine amidohydrolase, type EC-2, EC 3.5.1.1) used in the manufacture of Pegaspargase is derived from *Escherichia coli* which is purchased in bulk from Merck, Sharp and Dohme. L-asparagine is a nonessential amino acid synthesized by the transamination of L-aspartic acid by a reaction catalyzed by the enzyme L-asparagine synthetase. The ability to synthesize asparagine is notably lacking in malignancies of lymphoid origin. Asparaginase depletes L-asparagine from leukemic cells (especially lymphoblasts) by catalyzing the conversion of L-asparagine to aspartic acid and ammonia. In predominately L-asparaginase naive adult patients with leukemia and lymphoma, initial plasma levels of L-asparaginase following intravenous administration of pegaspargase were determined. Apparent volume of distribution was equal to estimated plasma volume. L-asparaginase was measurable for at least 15 days following the initial treatment with Pegaspargase. The approximate t½ in adult patients is 5.73 days. The enzyme could not be detected in the urine. The half-life is independent of the dose administered, disease status, renal or hepatic function, age, or gender. In a study of newly diagnosed pediatric patients with ALL who received either a

single intramuscular injection of pegaspargase (2500 units/m^2), *E. coli* L-asparaginase (25000 units/m^2), or *Erwinia* (25000 units/m^2), the plasma half-lives for the three forms of L-asparaginase were: 5.73 ± 3.24 days, 1.24 ± 0.17 days, and 0.65 ± 0.13 days respectively. The plasma half-life of pegaspargase is shortened in patients who are previously hypersensitive to native L-asparaginase as compared to non-hypersensitive patients. L-asparaginase is cleared by the reticuloendothelial system and very little is excreted in the urine or bile. Cerebrospinal fluid levels are <1% of plasma levels.

Toxicities (>20%): Allergic reactions (total likelihood of local, and or systemic reaction especially if previous hypersensitivity reaction to native asparaginase), pain at injection site, weakness, fatigue, diarrhea, hyperammonemia (late), coagulation abnormalities with prolonged PTT, PT and bleeding times (secondary to decreased synthesis of fibrinogen, AT-III & other clotting factors) (late).

Formulation and stability: Each milliliter of pegaspargase contains: PEG-L-asparaginase 750 IU ± 20%, monobasic sodium phosphate, *USP* 1.20 mg ± 5% dibasic sodium phosphate, *USP* 5.58 mg ± 5%, sodium chloride, *USP* 8.50 mg ± 5% , Water for Injection, *USP* qs to 1 mL. The specific activity of pegaspargase is at least 85 IU per milligram protein. Available in 5 mL vials as Sterile Solution for Injection in ready to use single-use vials, preservative free. Keep refrigerated at 2°-8°C (36°-46°F). Do not use if stored at room temperature for more than 48 hours. DO NOT FREEZE. Do not use product if it is known to have been frozen. Freezing destroys activity, which cannot be detected visually.

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

For IV administration: dilute pegaspargase in 100 mL of NS or D5W and infuse over 1 to 2 hours through a NS or D5W running infusion line. Pegaspargase admixed in 100 mL of NS or D5W is stable for 48 hours at room temperature. Pegaspargase diluted in 100 mL of NS is stable for up to 72 hours refrigerated (4ºC [39°F]) (refrigerated stability data on file with Sigma-Tau). Avoid excessive agitation. DO NOT SHAKE. Do not use if cloudy or if precipitate is present. If necessary (e.g., shortage of intravenous fluid), pegaspargase can be given intramuscularly in this protocol.

Supplier: Commercially available. See package insert for further information

6.6 Erwinia asparaginase

To be used in case of hypersensitivity or intolerance to Pegaspargase or calaspargase pegol.

Source and pharmacology: L-asparagine is a nonessential amino acid synthesized by the transamination of L-aspartic acid by a reaction catalyzed by the enzyme L-asparagine synthetase. Neoplastic cells associated with acute lymphoblastic leukemia, acute myeloid leukemia and lymphoblastic lymphosarcoma are asparagine-dependent but lack asparagine synthetase activity. The administration of L-asparaginase produces an anti-neoplastic effect by catalyzing asparagine into aspartic acid and ammonia. As a result, these cells lack

the ability to produce the asparagine necessary for protein metabolism and survival. Deamination of glutamine may also play a role in the antineoplastic activity of asparaginase.

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

The following data have been reported on each of the three preparations of asparaginase:

Asparaginase Preparations

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

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

Effective asparaginase levels have been defined as activity of ≥0.1 International Units per mL. No formal drug interaction studies have been performed with asparaginase *Erwinia chrysanthemi*.

Toxicities (5-20%): Allergic reactions, anaphylaxis, urticaria.

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

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

Use appropriate precautions for preparation of a hazardous agent. Visually inspect the powder in vial for foreign particles or discoloration prior to reconstitution. The contents of each vial should be reconstituted by slowly adding 1 mL or 2 mL of sterile, preservative-

free NS to the inner vial wall. The final concentration is 10,000 International Units per mL when using 1 mL for reconstitution or 5,000 International Units per mL when using 2 mL for reconstitution. Gently mix or swirl the contents to dissolve the contents of the vial. Do not shake or invert the vial. The resulting solution should be clear and colorless. Discard if any particulate matter or protein aggregates are visible. **Withdraw the appropriate dosing volume into a polypropylene syringe within 15 minutes of reconstitution.** Polycarbonate luer-lok syringes from B-D (1 mL) are also acceptable (personal communication, EUSA Pharma). Discard any unused drug; do not save or use any unused drug remaining in the vial.

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

Supplier: Commercially available. See package insert for further information.

6.7 Calaspargase Pegol

Source and pharmacology: L-asparaginase is an enzyme which catalyzes the deamidation of asparagine to aspartic acid and ammonia, reducing circulating levels of asparagine. Leukemic cells with low asparagine synthetase expression have a reduced ability to synthesize L-asparagine. L-asparaginase reduces the exogenous asparagine source for the leukemic cells, resulting in cytotoxicity specific to leukemic cells. Calaspargase pegol contains an E. coli-derived asparagine-specific enzyme, as a conjugate of L-asparaginase and monomethoxypolyethylene glycol (mPEG) with a succinimidyl carbonate (SC) linker, which produces a chemically stable carbamate bond between the mPEG component and the L-asparaginase lysine groups. L-asparaginase is a tetrameric enzyme that is produced endogenously by E. coli and consists of identical 34.5 kDa subunits. Approximately 31 to 39 molecules of SC-PEG are linked to L-asparaginase; the molecular weight of each SC-PEG molecule is about 5 kDa. The activity of calaspargase pegol is expressed in units (U).

Calaspargase pegol is FDA-approved as a component of a multi-agent chemotherapeutic regimen for the treatment of acute lymphoblastic leukemia in pediatric and young adult patients age 1 month to 21 years.

Asparagine concentrations in plasma were maintained below the assay limit of quantification for more than 18 days following a single dose of calaspargase pegol 2,500 U/m2 during the induction phase. Mean CSF asparagine concentrations decreased from a pretreatment concentration of 0.8 μg/mL to 0.2 μg/mL on Day 4 and remained decreased at 0.2 μg/mL 25 days after the administration of a single dose of calaspargase pegol 2,500 U/m2 in the induction phase. Plasma asparaginase activity pharmacokinetics are nonlinear following calaspargase pegol administration. The time to peak concentration (Tmax) = 1.17 hours and Cmax = 1.62 U/mL. Area under the curve (AUC0-∞) is 25.5 day x U/mL. Calaspargase pegol has a long elimination half-life (T1/2) of 16.1 days, volume of distribution (Vss) of 2.96 L, and clearance of 0.147 L/day.

Toxicities: Grade 3 and 4 hypersensitivity reactions including anaphylaxis have been reported in clinical trials with calaspargase pegol and include angioedema, lip swelling, eye swelling, erythema, blood pressure decreased, bronchospasm, dyspnea, pruritis, and rash. Other toxicities include pancreatitis, thrombosis, hemorrhage, transaminitis, and increased bilirubin.

Formulation and stability: Calaspargase pegol is supplied as a clear, colorless, preservative-free, isotonic sterile solution in phosphaste-buffered saline, pH 7.3 that requires dilution prior to intravenous infusion. Each vial of calaspargase pegol-mknl contains 3,750 units in 5 mL of solution. Each milliliter contains 750 units of calaspargase pegol-mknl; dibasic sodium phosphate, USP (5.58 mg); monobasic sodium phosphate, USP (1.20 mg); and sodium chloride, USP (8.5 mg) in water for injection, USP. Store calaspargase pegol refrigerated at 2°C to 8°C (36°F to 46°F) in the original carton to protect from light. Do not shake or freeze product. Unopened vials may be stored at room temperature (15°C to 25°C [59°F to 77°F]) for no more than 48 hours.

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

For IV administration: dilute in 100 mL of 0.9% Sodium Chloride Injection, USP or 5% Dextrose Injection, USP using sterile/aseptic technique. After dilution, administer immediately into a running infusion of either 0.9% sodium chloride or 5% dextrose, respectively. Administer over one hour. The diluted solution may be stored for up to 4 hours at room temperature (15°C to 25°C [59°F to 77°F]) for up to 24 hours. Protect from light. Do not shake or freeze.

Supplier: Commercially available. See package insert for further information.

6.8 Methotrexate

Source and pharmacology: A folate analogue which reversibly inhibits dihydrofolate reductase, the enzyme that reduces folic acid to tetrahydrofolic acid. Inhibition of tetrahydrofolate formation limits the availability of one carbon fragments necessary for the synthesis of purines and the conversion of deoxyuridylate to thymidylate in the synthesis of DNA and cell reproduction. The polyglutamated metabolites of MTX also contribute to the cytotoxic effect of MTX on DNA repair and/or strand breaks. MTX cytotoxicity is highly dependent on the absolute drug concentration and the duration of drug exposure. MTX is actively transported across cell membranes. At serum methotrexate concentrations exceeding 0.1 μmol/mL, passive diffusion becomes a major means of intracellular transport of MTX. The drug is widely distributed throughout the body with the highest concentration in the kidney, liver, spleen, gallbladder and skin. Plasma concentrations following high dose IV MTX decline in a biphasic manner with an initial half-life of 1.5-3.5 hours, and a terminal half-life of 8-15 hours. About 50% is bound to protein. After oral administration, approximately 60% of a 30 mg/m^2 dose is rapidly absorbed from the GI tract, with peak blood levels at 1 hour. At doses >30 mg/m^2 absorption decreases significantly. Even at low doses absorption may be very erratic, varying between 23% and 95%. The elimination of

MTX from the CSF after an intrathecal dose is characterized by a biphasic curve with half-lives of 4.5 and 14 hours. After intrathecal administration of 12 mg/m², the lumbar concentration of MTX is ~100 times higher than in plasma (ventricular concentration is ~ 10% of lumbar concentration). MTX is excreted primarily by the kidneys via glomerular filtration and active secretion into the proximal tubules. Renal clearance usually equals or exceeds creatinine clearance. Small amounts are excreted in the feces. There is significant entero-hepatic circulation of MTX. The distribution of MTX into third-space fluid collections, such as pleural effusions and ascitic fluid, can substantially alter MTX pharmacokinetics. The slow release of accumulated MTX from these third spaces over time prolongs the terminal half-life of the drug, leading to potentially increased clinical toxicity.

Toxicities (>20%): Transaminase elevations. Intrathecal administration: nausea, headache.

Formulation and stability: Methotrexate for Injection is available as a lyophilized powder for injection in 1000 mg vials. The powder for injection contains approximately 7 mEq sodium in the 1000 mg vial. Methotrexate for Injection is also available as a 25 mg/mL solution in 2, 4, 8, 10, and 40 mL preservative free vials and 2 and 10 mL vials with preservative. The 2, 4, 8, 10, and 40 mL solutions contain approximately 0.43, 0.86, 1.72, 2.15, and 8.6 mEq sodium per vial, respectively. The preserved vials contain 0.9% benzyl alcohol as a preservative. Sterile methotrexate powder or solution is stable at 20º-25ºC (68º-77ºF); excursions permitted to 15º-30ºC (59º- 86 Fº). Protect from light.

Methotrexate tablets are available as 2.5 mg, 5 mg, 7.5 mg, 10 mg and 15 mg. Inactive ingredients vary depending on manufacturer but tablet formulations may include: anhydrous lactose, crospovidone, hydroxypropyl methylcellulose, magnesium stearate, microcrystalline cellulose, polyethylene glycol, polysorbate 80, pregelatinized starch, sodium carbonate monohydrate, talc and titanium dioxide and various dyes. Store at controlled room temperature 15°-30°C (59°-86°F) and protect from light.

Australia/New Zealand – only 2.5 mg and 10 mg tablets available.

Methotrexate is also available as a clear yellow to orange oral solution (Xatmep®) that contains 2.5 mg of methotrexate per milliliter (equivalent to 2.74 mg of methotrexate sodium/mL) in a 120 mL bottle. Inactive ingredients include purified water, sodium citrate, citric acid, methylparaben sodium, propylparaben sodium, and sucralose. It may also contain sodium hydroxide or hydrochloric acid for pH adjustment. It is packaged in a high-density polyethylene (HDPE) bottle with a child-resistant cap and tamper-evident seal. Store oral solution under refrigeration (2°C to 8°C/36°F to 46°F) prior to dispensing. Avoid freezing and excessive heat. After dispensing, patients may store methotrexate oral solution at room temperature (20°C to 25°C/68°F to 77°F) for up to 60 days; excursions permitted to 15°C to 30°C (59°F to 86°F).

Guidelines for administration: IV, IM, PO, and Intrathecal. See Treatment and Dose Modifications sections of protocol.

Supplier: Commercially available. See package insert for further information.

6.9 Dasatinib

Source and pharmacology: Dasatinib (BMS-354825) (an aminothiazole analogue) is indicated for treatment of chronic, accelerated, myeloid or lymphoid blast phase chronic myeloid leukemia in adults who are resistant or intolerant to prior therapy including imatinib. It is also indicated for the treatment of Philadelphia chromosome- positive acute lymphoblastic leukemia in adults who are resistant or intolerant to prior therapy. Dasatinib is a potent, broad-spectrum ATP-competitive inhibitor of 5 critical oncogenic tyrosine kinase families: BCR-ABL, SRC family kinases, c-KIT, ephrin (EP) receptor kinases, and PDGFβ receptor. Each of these protein kinases has been strongly linked to multiple forms of human malignancies. Dasatinib is rapidly absorbed following oral administration. At the adult therapeutic dose of 70 mg BID, neither disease status nor study day had a marked influence on the Tmax of dasatinib in subjects with leukemia. The overall mean half-life was 4 to 6 hours. Dasatinib is likely to reach steady state conditions by the second day of treatment at 70 mg BID. Dasatinib is highly bound to serum proteins, has extensive extravascular distribution and has a moderate rate of blood clearance. Elimination is predominantly in the feces, mostly as metabolites. Following a single oral dose of [14C]-labeled dasatinib, approximately 85% of the dose was recovered in the feces within 10 days, and approximately 4% of the administered radioactivity was recovered in the urine. Unchanged dasatinib accounted for 19% and 0.1% of the administered dose in feces and urine, respectively, with the remainder of the dose being metabolites. Dasatinib is primarily metabolized in the liver by the human CYP3A4 enzyme and is a significant inhibitor of CYP3A4. It may decrease the metabolic clearance of drugs that are significantly metabolized by the CYP3A4 enzyme. CYP3A4 substrates known to have a narrow therapeutic index should be administered with caution in patients receiving dasatinib. Concomitant use of dasatinib and drugs that inhibit CYP3A4 may increase exposure to dasatinib and should be avoided. Drugs that induce CYP3A4 activity may reduce exposure to dasatinib and concomitant use of potent CYP3A4 inducers with dasatinib should be avoided.

Due to the potential of dasatinib to prolong the QT/QTc, use caution when administering dasatinib with other potential QTc-prolonging medications. Due to the possibility of gastrointestinal, cardiac, and cutaneous hemorrhage, avoid using medications that inhibit platelet function or anticoagulants with dasatinib. Dasatinib is not a p-glycoprotein inhibitor.

Toxicity (>20%): anemia, diarrhea, nausea, fatigue, neutrophil count decreased, platelet count decreased, myalgia, headache, dyspnea, pleural effusion, and maculo-papular rash.

Formulation and stability: Dasatinib is commercially available as a 20 mg, 50 mg, 70 mg, 80 mg, 100 mg, or 140 mg oral tablet. The intact bottles should be stored at controlled room temperature 15°-25°C (59°-77°F) and protected from light. Excursions are permitted up to 30 °C. Stability studies are ongoing. Additional formulations may be provided as available from the manufacturer.

Guidelines for administration: See the Treatment and Dose Modifications sections of the protocol. Administer orally with or without food, per institutional guidelines.

Supplier: Commercially available. See package insert for further information.

6.10 Blinatumomab

Source and pharmacology: Blinatumomab is a fusion protein composed of two single-chain antibodies (scFv), murine anti-CD19 scFv and murine anti-CD3 scFv. Through CD3 binding, blinatumomab recruits and engages T cells for redirected lysis of CD19-positive B cells, including those expressed with B-cell malignancies. T cells are bound by its anti-CD3 moiety, whereas B cells are bound by the anti-CD19 moiety. The subsequent serial lysis of multiple malignant cells by a single blinatumomab-activated T cell closely resembles a natural cytotoxic T cell reaction. Treatment with blinatumomab is associated with a rapid depletion of peripheral B cells, accompanied by T cell activation and a transient increase in cytokines.

Blinatumomab consists of a single chain of 504 amino acids with a molecular weight of approximately 54 kDa. The pharmacokinetics of blinatumomab was assessed over a dose range from 5 to 90 mcg/m^2/day (approximately equivalent to 9-162 mcg/day). Following continuous intravenous infusion, the steady state serum concentration (Css) was achieved within a day and remained stable over time. The estimated mean (SD) volume of distribution based on terminal phase (Vz) was 4.52 (2.89) L. The estimated mean (SD) systemic clearance was 2.92 (2.83) L/hour and the estimated mean (SD) half-life was 2.11(1.42) hours. Negligible amounts of blinatumomab were excreted in the urine at the tested clinical doses. Like other protein therapeutics, blinatumomab is expected to be degraded into small peptides and amino acids via catabolic pathways. At the clinical doses of 9 mcg/day and 28 mcg/day for the treatment of adult relapsed/refractory ALL, the mean (SD) Css was 211 (258) pg/mL and 621 (502) pg/mL, respectively.

Formulation and stability: Intravenous powder for solution 35 mcg/vial mcg single-use vial. See package insert for more information.

Toxicities (>20%): Diarrhea, nausea, fatigue, fever, lymphocyte count decreased, white blood cell decreased, hypokalemia, headache, tremor.

Guidelines for administration: See Treatment and Dose Modifications sections of protocol. Preparation and administration of blinatumomab may follow institutional guidelines.

Supplier: Commercially available. See package insert for further information.

6.11 Cyclophosphamide

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

produced by mechlorethamine. Approximately 70% of a dose of cyclophosphamide is excreted in the urine as the inactive carboxyphosphamide and 5-25% as unchanged drug. The plasma half-life ranges from 4.1 to 16 hours after IV administration.

Toxicities (>20%): Anorexia, nausea and vomiting (acute and delayed), leukopenia, alopecia, immune suppression, gonadal dysfunction: azoospermia or oligospermia (prolonged or permanent) (late).

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

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

Supplier: Commercially available. See package insert for further information.

6.12 Etoposide

Source and pharmacology: A semisynthetic derivative of podophyllotoxin that forms a complex with topoisomerase II and DNA, which results in single and double strand DNA breaks. Its main effect appears to be in the S and G2 phase of the cell cycle. The initial t½ is 1.5 hours and the mean terminal half-life is 4 to 11 hours. It is primarily excreted in the urine. In children, approximately 55% of the dose is excreted in the urine as etoposide in 24 hours. The mean renal clearance of etoposide is 7 to 10 mL/min/m² or about 35% of the total body clearance over a dose range of 80 to 600 mg/m². Etoposide, therefore, is cleared by both renal and non-renal processes (i.e., metabolism and biliary excretion). The effect of renal disease on plasma etoposide clearance is not known. Biliary excretion appears to be a minor route of etoposide elimination. Only 6% or less of an intravenous dose is recovered in the bile as etoposide. Metabolism accounts for most of the non-renal clearance of etoposide.

The maximum plasma concentration and area under the concentration time curve (AUC) exhibit a high degree of patient variability. Etoposide is highly bound to plasma proteins (~94%), primarily serum albumin. Pharmacodynamic studies have shown that etoposide systemic exposure is related to toxicity. Preliminary data suggests that systemic exposure for unbound etoposide correlates better than total (bound and unbound) etoposide. There is poor diffusion into the CSF < 5%.

Etoposide phosphate is a water-soluble ester of etoposide which is rapidly and completely converted to etoposide in plasma. Pharmacokinetic and pharmacodynamic data indicate that etoposide phosphate is bioequivalent to etoposide when it is administered in molar equivalent doses.

Toxicities (>20%): Nausea, vomiting Myelosuppression (anemia, leukopenia), alopecia.

Formulation and stability: *Etoposide for Injection* is available as a 20 mg/mL solution in sterile multiple dose vials (5 mL, 25 mL, or 50 mL each). The pH of the clear, nearly colorless to yellow liquid is 3 to 4. Each mL contains 20 mg etoposide, 2 mg citric acid, 30 mg benzyl alcohol, 80 mg modified polysorbate 80/tween 80, 650 mg polyethylene glycol 300, and 30.5 percent (v/v) alcohol. Vial headspace contains nitrogen. Unopened vials of etoposide are stable until expiration date on package at controlled room temperature (20˚-25˚C or 68˚-77˚F).

Etoposide phosphate for injection is available for intravenous infusion as a sterile lyophilized powder in single-dose vials containing etoposide phosphate equivalent to 100 mg etoposide, 32.7 mg sodium citrate *USP*, and 300 mg dextran 40. Etoposide phosphate must be stored under refrigeration (2°-8°C or 36°-46°F). Unopened vials of etoposide phosphate are stable until the expiration date on the package

Supplier: Commercially available. See package insert for further information.

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

6.13 Leucovorin

Source and pharmacology: Leucovorin is a mixture of the diastereoisomers of the 5-formyl derivative of tetrahydrofolic acid (THF). The biologically active compound of the mixture is the (-)- l-isomer, known as Citrovorum factor or (-)-folinic acid. Leucovorin does not require reduction by the enzyme dihydrofolate reductase in order to participate in reactions utilizing folates as a source of "one-carbon" moieties. Administration of leucovorin can counteract the therapeutic and toxic effects of folic acid antagonists such as methotrexate, which act by inhibiting dihydrofolate reductase. In contrast, leucovorin can enhance the therapeutic and toxic effects of fluoropyrimidines used in cancer therapy, such as 5-fluorouracil. Leucovorin is readily converted to another reduced folate, 5,10-methylenetetrahydrofolate, which acts to stabilize the binding of fluorodeoxyuridylic acid (an active metabolite of 5-FU) to thymidylate synthase and thereby enhances the inhibition of this enzyme. Peak serum levels of 5-methyl THF (an active metabolite) were reached at approximately 1.3-1.5 hours (IV/IM) and 2.3 hours for the oral form. The terminal half-life of total reduced folates was approximately 6.2 hours. Following oral administration, leucovorin is rapidly absorbed and expands the serum pool of reduced folates. At a dose of 25 mg, almost 100% of the *l*-isomer (the biologically active form) but only 20% of the *d*-isomer is absorbed. Oral absorption of leucovorin is saturable at doses above 25 mg. The apparent bioavailability of leucovorin was 97% for 25 mg, 75% for 50 mg, and 37% for 100 mg doses. Both oral and parenteral leucovorin raise the CSF folate levels.

Toxicities (rare): anaphylaxis, urticaria, seizure.

Formulation and stability: Leucovorin calcium for injection is supplied as a sterile ready to use liquid and a sterile powder for injection. The 10 mg/mL preservative free liquid is

available in 50 mL vials containing sodium chloride 400 mg/vial. Store preservative free liquid in the refrigerator at 2°-8°C (36°-46°F) protected from light. The powder for injection is available in 50 mg, 100 mg, 200 mg, and 350 mg vials. Store at room temperature 15°-25°C (59°-77°F) protected from light. Reconstitute the sterile powder with sterile water for injection or bacteriostatic water for injection to a concentration of 10 mg/mL leucovorin calcium. **Do not use diluents containing benzyl alcohol for doses > 10 mg/m² or in infants < 2 years of age or patients with allergy to benzyl alcohol.** When Bacteriostatic Water is used, the reconstituted solution is good for 7 days. If reconstituted with SWFI, use solution immediately as it contains no preservative. One milligram of leucovorin calcium contains 0.004 mEq of leucovorin and 0.004 mEq of calcium.

The oral form of leucovorin is available as 5 mg, 10 mg, 15 mg, and 25 mg tablets. Inactive ingredients vary depending on manufacturer but tablet formulations may include corn starch, dibasic calcium phosphate, magnesium stearate, pregelatinized starch, lactose, microcrystalline cellulose, and sodium starch glycolate.

Supplier: Commercially available. See package insert for further information.

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

6.14 Mercaptopurine

Source and pharmacology: Mercaptopurine is an analogue of the purine bases adenine and hypoxanthine. The main intracellular pathway for MP activation is catalyzed by the enzyme hypoxanthine-guanine phosphoribosyl transferase (HGPRT) which catalyzes the conversion of MP to several active nucleotide metabolites including thioinosinic acid, a ribonucleotide which can interfere with various metabolic reactions necessary for nucleic acid (RNA and DNA) biosynthesis. It can also cause pseudofeedback inhibition of the first step in de novo purine biosynthesis or convert to another ribonucleotide which can cause feedback inhibition. Mercaptopurine can be incorporated into DNA in the form of TG nucleotides as well and thus produce toxicity. The absorption of an oral dose of MP is incomplete and variable, with only about 16%-50% of an administered dose reaching the systemic circulation secondary to a first pass metabolism in the liver. Co-administration with cotrimoxazole (TMP/SMX) significantly reduces absorption of MP. After IV administration, MP has a plasma half-life of 21 minutes in children and 47 minutes in adults. Approximately 19% is bound to protein. Mercaptopurine is well distributed into most body compartments except the CSF. (With high dose IV MP the CSF to plasma ratio is 0.15.) MP is metabolized by xanthine oxidase in the liver to 6-Thiouric acid an inactive metabolite. In patients receiving both MP and allopurinol (a xanthine oxidase inhibitor) the dose of MP must be reduced by 50-75%. Since TPMT, thiopurine methyltransferase, is also one of the enzymes involved in the metabolism of MP, those individuals who have an inherited deficiency of the enzyme may be unusually sensitive to the myelosuppressive effects of MP and prone to develop rapid bone marrow suppression following the initiation of treatment. Mercaptopurine is excreted in urine as metabolites and some unchanged drug; about half an oral dose has been recovered in 24 hours. A small proportion is excreted over several weeks.

Toxicities (>20%): Anemia, neutrophil count decreased, white blood cell decreased, platelet count decreased.

Formulation and stability: Mercaptopurine is available as a 50 mg tablet containing mercaptopurine and the inactive ingredients corn and potato starch, lactose, magnesium stearate, and stearic acid. Store at 15°-25°C (59°-77°F) in a dry place. Mercaptopurine is also available as an oral suspension in a concentration of 20 mg/mL (2000 mg/100 mL per bottle). The oral suspension is a pink to brown viscous liquid supplied in amber glass multiple-dose bottles with a child resistant closure. It should be stored at 15°-25°C (59°-77°F) in a dry place.

NOTE: the concentration of the commercially available suspension (20 mg/mL) and the compounded suspension (50 mg/mL) are NOT the same; doses should be prescribed in the milligrams required, not mL. Please contact PI for compounding instructions if needed.

Supplier: Commercially available. See package insert for further information.

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

6.15 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: See treatment plan.

Supplier: Commercially available. Refer to the package insert for complete information.

6.16 Cytarabine (Cytosine arabinoside, Ara-C, Cytosar®)

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

Toxicities: Most common (>20%) nausea, vomiting, anorexia, conjunctivitis (with high dose), myelosuppression, stomatitis, alopecia.

Formulation and stability: Cytarabine for Injection is available in vials of 100 mg, 500 mg, 1 g, and 2 g containing a sterile powder for reconstitution. It is also available at a 20 mg/mL concentration with benzyl alcohol (25 mL per vial) or as a preservative free solution (5 mL, 50 mL per vial), and at a 100 mg/mL concentration with benzyl alcohol (20 mL vial) or as preservative free solution (20 mL vial). Hydrochloric acid and/or sodium hydroxide may be added to adjust the pH. Store at 25°C (77°F); excursions permitted to 15°-30°C (59°-86°F). Cytarabine solutions should be protected from light. When reconstituted with Bacteriostatic Water for Injection, cytarabine is stable for 48 hours at room temperature. Solutions reconstituted without a preservative should be used immediately. Discard if solution appears hazy. Diluted solutions in D5W or NS are stable for 8 days at room temperature; however, the diluted cytarabine should be used within 24 hours for sterility concerns.

Guidelines for administration: See Treatment Plan.

Supplier: Commercially available. Refer to the package insert for complete information.

7.0 EVALUATIONS, TESTS, OBSERVATIONS

7.1 Pre-Treatment Clinical Evaluations

- History and physical exam with height (cm), weight (kg), and BSA
- Performance status
- Diagnosis of relapsed/ refractory ALL/ALLy/ALAL confirmed by bone marrow or peripheral blood as described in inclusion criteria

- Imaging of extramedullary disease (excluding CNS disease identified by cerebrospinal fluid analysis)
- Laboratory studies as needed to confirm eligibility (direct bilirubin [may be omitted if total bilirubin is within normal limits], AST, ALT, creatinine)
- Echocardiogram

Obtain other studies as needed for good patient care.

7.2 Evaluations during Therapy – all Participants

STUDIES TO BE OBTAINED	Day -7 to 1	Block 1	Block 2	End of Treatment@
History and Physical	X	Weekly	Start of Block	X
CBC, differential, platelets	X	Weekly®	Weekly	X
Creatinine, ALT, Bilirubin	X	Weekly	Weekly	X
Tumor lysis monitoring: potassium, creatinine, calcium, phosphorus, uric acid		Prior to Day 1 dose of venetoclax and every 6-8 hours until 24 hours after the first dose of navitoclax. See section 5.2.13.		
*Pregnancy Test	X			
CSF cell count and cytospin	X	With each IT	With each IT	X
Bone marrow (BM) with minimal residual disease evaluation	X	Day 29-32	With count recovery (Block 2A); Day 28-30 (Block 2B)	X
Bone marrow for pharmacotyping	X			
Bone marrow for comprehensive leukemia genomics#	X			
Skin biopsy for germline comparator sample#	X			
Bone marrow for BH3 profiling	X			
Bone marrow immune profiling	X	End of Block	End of Block	X
Peripheral blood immune profiling%	X	Day 15, end of Block	Day 1 (if >7 days from end of Block 1), Days 8, 15, end of block	X
TPMT/NUDT15 genotyping^			Once during block	

® Platelet count required on Day 4

@ End of treatment studies should be obtained at the end of Block 2 for all patients except late (≥36 months from diagnosis) first relapse B-cell ALL who are MRD-negative at <0.01% after Block 1. For these patients, they will be obtained at the end of continuation.

** Required as a condition of enrollment for all females of childbearing potential.*

 # To be obtained only in patients consenting to comprehensive leukemia sequencing without prior germline sequencing at SJCRH and collaborating sites. For patients who wish to consent to these studies at sites with pending agreements, consider collection of appropriate samples and banking as feasible.

^ For patients with late first relapse of B-ALL who are MRD-negative at <0.01% after Block 1 (and thus will continue chemotherapy after Block 2) and who do not have prior TPMT/NUDT15 genotyping.

% Sample collection may be +/- 2 days if needed to accommodate patient scheduling.

Patients with peripheral blasts and resulting inability to obtain bone marrow at diagnosis may submit peripheral blood for pharmacotyping, leukemia genomics, and BH3 profiling. Patients with isolated extramedullary leukemia/ lymphoma may submit relevant samples as available for these studies.
Patients will be offered enrollment in TBANK so that samples and data may be used for future research to better understand the biology of relapsed ALL and to identify potential treatments for this population.

7.3 Evaluations for Late Relapsing B-ALL Participants Receiving Therapy After Protocol Block 2

STUDIES TO BE OBTAINED	Intensification, Interim continuation 1 & 2	Block 2 cycle 2	Continuation	End of Treatment
History and physical	Weekly	Start of Block	Every 2 weeks	X
CBC, differential, platelets	Weekly	Weekly	Every 2 weeks	X
Creatinine, ALT, bilirubin	Weekly	Weekly	Every 2 weeks	X
*Pregnancy test				
CSF cell count and cytospin	With each IT	With each IT	With each IT	X
Bone marrow (BM) with minimal residual disease evaluation			Start of cycle 1	X

Obtain other studies as needed for good patient care.
** Pregnancy tests in females of childbearing potential should be repeated during treatment according to institutional guidelines.*

7.4 Evaluations after Completion of Therapy

A peripheral blood or bone marrow sample should be submitted for pharmacotyping, genomics, and BH3 profiling at the time of relapse if possible. If this cannot be done, it will not be a deviation.

It is recommended that participants completing continuation therapy be followed at least every 4 months for 1 year and then every 6 months for 1 additional year after completion of therapy.

Participants will be followed for 30 days after last treatment taken. Adverse events will not be recorded off-therapy unless they are unanticipated and deemed related to study treatment.

All patients will be followed for relapse and survival for 5 years from enrollment.

Evaluations will be performed as described for consenting patients. Minor modifications for good patient care or to align with institutional practice are allowed.

8.0 EVALUATION CRITERIA

8.1 Response Criteria

See section 3.3 for definitions of response. Response will be assessed after Blocks 1 and 2 and at the completion of therapy.

8.2 Toxicity Evaluation Criteria

Common Terminology Criteria for Adverse Events v5 (CTCAE): This study will utilize the CTCAE of the National Cancer Institute (NCI) for toxicity and performance reporting. A copy of the current version of the CTCAE can be downloaded from the Cancer Therapy Evaluation Program home page (http://ctep.info.nih.gov). Additionally, toxicities will be reported on the appropriate data collection screens.

8.3 Breakthrough Infections during Treatment

All infection adverse events will be prospectively assessed, categorized, and recorded. Identified organisms and their sensitivities will be obtained.

8.4 Acceptable Percentage of Missed Doses for Drugs

It is understood that deviations from the protocol should be avoided, except when necessary to eliminate an immediate hazard to a research participant. In such case, the deviation must be reported to the IRB. However, it is expected that participants will occasionally miss some doses or receive the wrong dose of oral chemotherapy. Appropriately documented doses of missed or wrong doses of chemotherapy will not constitute a deviation unless the amount in question is over *10%* of the expected total dose due in the respective protocol cycles. Missed doses do not include doses held or reduced for medical reasons (toxicity, illness) and will not be considered protocol deviations or violations.

9.0 OFF-TREATMENT AND OFF-STUDY CRITERIA

9.1 Off-Treatment Criteria

- Research participants who fail to achieve MRD-negative complete remission after Block 2 of therapy
- 30 days after completion of protocol directed therapy, or if a participant proceeds to alternative therapy (including cellular therapy/ transplant), off therapy day is defined as the first date of receipt of non-protocol therapy (including conditioning therapy)
- Any relapse
- Second malignancy
- Development of unacceptable toxicity during treatment which precludes continuing therapy
- Refusal of therapy

- Physician determination that protocol therapy is no longer appropriate

9.2 Off-Study Criteria

- Death
- Lost to follow up
- Withdrawal of consent
- 5 years from enrollment

10.0 SAFETY AND ADVERSE EVENT REPORTING REQUIREMENTS

10.1 Reporting Adverse Experiences and Deaths

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

As further described in the definition of unanticipated problem, this includes any event that in the PI's opinion was:

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

Unrelated, expected deaths do not require reporting to the IRB. Though death is "serious", the event must meet the other two requirements of "related or possibly related" and "unexpected/unanticipated" to be considered reportable.

Deaths meeting reporting requirements are to be reported immediately to the St. Jude IRB, but in no event later than 48 hours after the investigator first learns of the death.

The following definitions apply with respect to reporting adverse experiences:

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

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

Unexpected Adverse Event: Any adverse event for which the specificity or severity is not consistent with the protocol-related documents, including the applicable investigator brochure, IRB approved consent form, Investigational New Drug (IND) or Investigational Device Exemption (IDE) application, or other relevant sources of information, such as product labeling and package inserts; or if it does appear in such documents, an event in which the specificity, severity or duration is not consistent with the risk information included therein; or the observed rate of occurrence is a clinically significant increase in the expected rate (based on a credible baseline rate for comparison); or

The occurrence is not consistent with the expected natural progression of any underlying disease, disorder, or condition of the subject(s) experiencing the adverse event and the subject's predisposing risk factor profile for the adverse event.

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

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

Unanticipated Problem Involving Risks to Subjects or Others: An 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:

- 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 and IRB.

The following adverse events that are commonly observed in this patient population will not be reported as individual expedited reports, but with annual continuing review and progress reports:

- Grade 3 or 4 infection or fever
- Grade 3 or 4 fever and neutropenia with or without infection
- Grade 3 or 4 elevation in hepatic transaminases, alkaline phosphatase, GGT, bilirubin, and triglycerides that resolve to less than grade 3 within 5 days
- Grade 3 or 4 elevation of amylase or lipase
- Grade 3 electrolyte disturbances and Grade 4 electrolyte disturbances that resolve to < grade 3 within 24 hours allowing for replacement as needed
- Grade 3 and 4 electrolyte disturbances due to tumor lysis
- Grade 3 hyperglycemia or hypoglycemia and Grade 4 hyperglycemia or hypoglycemia that resolve to < grade 3 within 24 hours, allowing for replacement or insulin as needed
- Grade 3 fatigue and nausea
- Grade 3 vomiting or diarrhea that resolves to ≤ Grade 2 within 7 days with supportive care as needed (antiemetics, antidiarrheal medications)
- Grade 3 or 4 hypotension explained by sepsis, pancreatitis, or infusion reaction

- Grade 3 mucositis that resolves to ≤ Grade 2 within 14 days
- Grade 3 anorexia
- Grade 3 hypertension
- Grade 3 enterocolitis, typhlitis, or colitis
- Grade 3 gastric hemorrhage, gastric ulcer, gastritis, and/or upper GI bleed
- Grade 3 glucose intolerance
- Grade 3 leukoencephalopathy and/or seizures secondary to intrathecal chemotherapy
- Any Grade 3 event not specified above as an exception which resolves within 48 hours
- Any Grade 3 event secondary to an infectious process or thrombocytopenia

Collaborating and affiliating sites: On treatment deaths must be reported to the St. Jude PI, Dr. Seth Karol, within 24 hours of the event. A written report must follow. The study team should be copied on all correspondence regarding the event. Unanticipated problems should be reported promptly, but in no event later than 10 working days after the investigator first learns of the unanticipated problem. Send report to:

Seth E. Karol, MD
Department of Oncology
St. Jude Children's Research Hospital
262 Danny Thomas Place
Memphis, TN 38105
Phone: [redacted]
FAX: [redacted]
Email: [redacted]

This is an investigator-initiated study. The principal investigator, Seth Karol 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.

10.2 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.

10.3 Reporting to Drug Manufacturer (AbbVie, Inc.)

In the event of a serious adverse event except as described above which are excluded from expedited reported, whether associated with study drug or not, the Investigator will notify AbbVie Clinical Pharmacovigilance within 24 hours of the site being made aware of the serious adverse event. Pregnancy in a study subject or study subject's partner must be reported to AbbVie within 15 days, and subjects who become pregnant must be discontinued from the study. Product complaints will be reported within 1 calendar day of site becoming aware of the event.

E-mail: [redacted]
FAX to: [redacted]

10.4 Recording Adverse Events and Serious 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 the protocol-specific database for all clinically significant non-hematologic grade 2 adverse events, and all non-hematological grade 3 or higher. The data to be recorded are 1) the event description, 2) the NCI CTCAE v5.0 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) attribution (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.

Attribution is the relationship between an adverse event or serious adverse event and the study treatment. Attribution will be assigned as follows:

- Definite – The adverse event is clearly related to the study treatment.
- Probable – The adverse event is likely related to the study treatment.
- Possible – The adverse event may be related to the study treatment.
- Unlikely – The adverse event is doubtfully related to the study treatment.
- Unrelated – The adverse event is clearly NOT related to the study treatment.

Cumulative summary of all clinically significant non-hematologic grade 2 adverse events, and all non-hematological grade 3 or higher will be reported as part of the progress reports to IRB at the time of continuing review. Specific data entry instructions for AEs and other protocol-related data will be documented in protocol-specific data entry guidelines, which will be developed and maintained by study team and clinical research informatics. The

study team will meet regularly to discuss AEs. The PI will review AE reports generated from the research database, and corrections will be made if applicable. Once the information is final the PI will sign and date reports, to acknowledge his review and approval of the AE as entered in the research database.

All adverse events reported from the time of study drug administration until 30 days following discontinuation of study drug administration have elapsed will be collected, whether solicited or spontaneously reported by the subject. In addition, protocol-related serious adverse events and protocol-related nonserious adverse events will be collected from the time the subject signed the study-specific informed consent/assent.

10.5 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 iRIS 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 all clinically significant non-hematologic grade 2adverse events, and all non-hematological grade 3 or higher 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.

11.0 DATA COLLECTION, MONITORING AND CONFIDENTIALITY

11.1 Data Collection

Data will be entered directly into a secure 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. Karol or his designee. All protocol-specific data and all clinically significant non-hematologic grade 2 adverse events, and all non-hematological grade 3 or higher adverse events will be recorded by the clinical research associates into a protocol-specific 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 St. Jude medical chart.

Regular summaries of toxicity and protocol events will be generated for the PI and the Department of Biostatistics to review.

11.2 Study Monitoring

This study is considered high risk (HR-3) for monitoring purposes. Protocol and regulatory compliance, including essential regulatory documentation, will be assessed as well as the accuracy and completeness of all data points relating to the primary and secondary objectives semi-annually. If the study design has strata, accrual will be tracked continuously. The first two enrollees will be monitored and 15 % of the study enrollees thereafter, semi-annually.

The PI and study team are responsible for protocol and regulatory compliance, and for data accuracy and completeness. The study team will meet frequently to review active case histories or quality summaries on all participants and retain copies of the minutes which are signed by the PI.

Clinical Trials Operations (CTO) will verify informed consent documentation and eligibility status on 100% of St. Jude participants within 5 working days of enrollment completion.

The Clinical Research Monitor (CRM) will verify the informed consent and eligibility processes of all non-St. Jude participants and perform a quality verification of all St. Jude enrollments during routine monitoring intervals (every 6 months). Overall study conduct, compliance with primary and secondary objectives, age of majority consenting, safety assessments and reporting, and the timeliness and accuracy of database entries are monitored routinely.

Study documents routinely monitored on selected participants include medical records, database entries, study worksheets, and case report forms. Study documents are monitored for participant status, demographics, staging, subgroup assignment, treatments,

investigational drug accountability, evaluations, responses, participant protocol status, off-study and off-therapy criteria, and for other specifics as detailed in a separate study-specific monitoring plan. The study-specific monitoring plan may be revised over time, to adapt monitoring frequency and/ or intensity to a changing environment when appropriate (for example: new safety signals; positive history of compliance; all participants are in long term follow-up; or the enrollment period has ended).

The recording and reporting of Adverse Events, Serious Adverse Events (SAEs), and Unanticipated Problems (UPs) to include type, grade, attribution, duration, timeliness and appropriateness will be reviewed by the Monitor/ CRM. The CRM will generate a formal report which is shared with the Principal Investigator (PI), study team and the Internal Monitoring Committee (IMC).

Continuing reviews by the Institutional Review Board (IRB) and Clinical Trials- Scientific Review Committee (CT-SRC) will occur at least annually. In addition, unanticipated problems are reviewed in a timely manner by the IRB.

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

12.0 STATISTICAL CONSIDERATIONS

12.1 Total Sample Size and Accrual

We anticipate it is logistically possible to enroll 78 patients, yielding 70 evaluable patients for the primary objective. The most recent SJCRH relapsed ALL trial ALL-R18 accrued 42 patients in 6 years. Notably, this study was primarily performed at a single center and limited to 1st relapse of B-ALL only. With expanded eligibility and multicenter participation, we expect to obtain 70 evaluable patients in 2.5 years. Anticipated numbers in distinct strata are shown in Table 2 below. Estimates are based on the proportion of early and late first relapse B-ALL on recent trials. We anticipate a relatively higher proportion B-ALL beyond first relapse and T-ALL relapse due to the potential presence of competing trials at collaborating institutions. As described in the summary, we also anticipate enrolling up to 20 patients total into the exploratory cohorts (including no more than 8 age 22 years or older) in addition to the 70 patients in the primary cohort (i.e. up to 90 patients total on trial).

By enrolling 70 patients we project that at least 56 will enroll in a combined cohort comprised of early first relapse B-ALL, relapsed T-ALL, and ALL beyond first relapse (Table 2). These groups comprise the highest risk relapse groups with a historical MRD-negativity rate of ≤25%. A sample size of 56 in this cohort assures sound statistical behavior for the group-sequential design for futility monitoring and declaration of efficacy as shown below in Table 3.

Table 2: Anticipated accrual by stratum with 70 total patients

Stratum	Number and percentage of enrolled patients for 70 patients total	Historical response (MRD-neg CR after Block 1)	Source of historical/literature
B-ALL, 1st relapse, late (≥36 months from diagnosis)	14, 21%	42.5% [82/193]	UKALL R3[17]
B-ALL, 1st relapse, early (<36 months from diagnosis)	12, 17%	25% [52/206]	AALL1331
>1st relapse or primary refractory	32, 45%	20%	BFM[20] and AALL01P2[14]
T-ALL, 1st relapse	12, 17%	18.2% [4/22]	AALL07P1[15]

12.2 Primary Objectives

To compare MRD-negative CR/CRi rate in children following Block 1 therapy with venetoclax and navitoclax based reinduction to historical controls.

Responsible investigators: Seth Karol, MD
Responsible statistician: Cheng Cheng, PhD

Historical MRD negativity rates according to stratum are shown above in Table 2. For patients in first relapse, these patients include patients who achieved a CR following 1 block of therapy. For patients treated on the UKALL R3 protocol, no specified count recovery was required, with patients having to achieve a "less than 5% blasts in the marrow or no blasts in the CSF."[8,17] Patients treated on AALL1331 needed to achieve a CR with count recovery as defined in section 3.3.4, while patients treated on AALL07P1 required count recovery with ANC ≥750/microL and platelets of ≥75,000/microL.[15] To enable an effective comparison to historical data while simultaneously ensuring results of the present study inform future use, we will use the current definitions of CR utilized in ongoing Children's Oncology Group protocols (ANC ≥500/microL and platelets of ≥50,000/microL) for all patients in first relapse. We regard this comparison as reasonable despite the difference in bone marrow recovery parameters as the MRD-negative response to induction for patients with early relapse B-ALL is highly similar in AALL01P2 (25%), AALL07P1 (27%), and AALL1331 (25%) despite the differing count threshold used in AALL1331. This suggests that the difference in count recovery parameters between prior generation and current generation trials do not significantly alter the observed MRD-negativity rate.

For patients beyond first relapse, there are not MRD response data available for patients with either B- or T-ALL, and the published data indicates that hematological recovery was not required to determine remission in these patients. "Complete remission (CR) was characterized by a non-aplastic BM with <5% blasts, absence of blasts in peripheral blood and no evidence of any extra-medullary leukemic disease spread."[20] Both historically and in current practice, hematological recovery is not required as these patients frequently

proceed to transplant prior to full hematological recovery. Thus, we will evaluate these patients in CR or CRi as long as bone marrow cellularity is sufficient to assess MRD. In the absence of historical MRD data for this population, we have conservatively estimated that 50% of patients in CR/CRi will be MRD positive, a rate consistent with the best-responding group in our historical control (i.e. the late first relapse of B-ALL). Given the high rate of MRD detected in patients with marrows without morphologic leukemia,[14,15,17,18] we believe that the estimated rate of MRD-negativity in the historical population is conservative (i.e. the true rate is likely higher than the estimate provided).

The primary endpoint is a binary (success/failure) MRD response at the end of remission Reinduction Block 1. Patients in first relapse must achieve a CR according to definitions in section 3.3.4, while patients beyond first relapse must achieve a CR or CRi. A patient achieving an MRD level <0.01% at the end of Block 1 is a success; MRD level 0.01% or higher, treatment related death during Block 1, as well as severe toxicity resulting in incomplete Block 1 treatment (i.e. unable to continue therapy or undergo disease assessments) are failures; other scenarios rendering a patient not being able to finish Block 1 are considered unevaluable. Patients in the exploratory cohorts will not be counted toward the primary objective, and their responses will be provided descriptively.

The MRD-negative CR/CRi rate will be estimated separately for patients in late first relapse and other patients by the sample proportion (projected denominators n=14 and 56, respectively) and the 95% Binomial exact confidence interval and compared to the historical/literature controls. Patients with late first relapse in the historical control (Table 2) have an MRD-negative CR rate of 42.5%. With a 14-patient sample, there is an 80% power to detect an increase to 71% with a one-sided alpha of 0.1. Given the small size of this cohort, we regard this elevated alpha as reasonable for this phase 2 study and note that identification of a greater MRD-negativity rate without anthracycline therapy would be clinically meaningful.

From Table 2 above, the overall MRD-negative rate in historical/literature control for other patients (early first B-ALL, first T-ALL, beyond first relapse) is approximately 20.7% = [(12/56)*0.25+(32/56)*0.20+(12/56)*0.182]. To plan the sample size, we have conservatively estimated that all patients in this cohort would have a historical response as good as those observed in early first relapse (i.e. 25%). The projected cohort of 56 patients assures sound statistical behavior for group-sequential analysis with futility monitoring shown in Table 3 below.

The group-sequential design tables below are produced by an in-house R program using an algorithm based on exact binomial probabilities. It assumes that individual patients develop AEs (or respond to treatment) independently to each other. With a projected total number of patients, the stopping boundaries and postulated true success rates specified to the program, it compute the probabilities of stopping at each stage for each postulated true success rate.

12.2.1. Efficacy monitoring using group sequential analysis for Block 1

In light of the historical differences in MRD-negative rate between late first relapse B-ALL (42.5%) and all other patients (25% for early first relapse B-ALL, 18.2% for first relapse T-ALL, 20% for patients beyond first relapse), we will monitor futility in these 2 groups separately.

For patients in late first relapse B-ALL, we will consider the treatment sufficiently active if the response rate is at least 50% (null 49.9%). We will monitor for futility in an ad hoc fashion. If at any time the number of failures reaches 7, we will suspend enrollment in the trial for late first relapse B-ALL and investigate.

For other patients in the primary analysis cohort (early first relapse B-ALL, first relapse T-ALL, and patients beyond first relapse), the therapy will be considered sufficiently effective if the overall response rate is 25% (null 24.9%). We will monitor for futility against this rate. The decision boundary and operating characteristics outlined in Table 3 below are derived from a group sequential design. By this rule, we will watch the number of failures sequentially in the first 28 patients, if at any point this number reaches 21, the trial will be suspended. The probability of suspension in the first 28 patients is 0.82, 0.37, and 0.18 if the true success probability is 0.2, 0.3, and 0.35 respectively. Likewise, we will watch the cumulative number of failures in the second stage and suspend the trial if this number reaches 41. The cumulative probability to suspend the trial in any stage is 0.94, 0.48, and 0.23 if the true success probability is 0.2, 0.3, and 0.35 respectively. We regard these operating characteristics reasonable. Conversely, if the true response rate is 0.35 or 0.4, we have a 0.77 or 0.92 probability to declare efficacy.

Table 3: Stopping boundary and probabilities for several scenarios of true MRD-negative CR/CRi rate (Monitor against <25% MRD-negative responses)

	True MRD-negative rate (probability)				
	0.20	**0.25**	**0.30**	**0.35**	**0.40**
Stop if ≤7/28 successes	0.82	0.60	0.37	0.18	0.07
Stop if ≤ 15/56 successes	0.12	0.16	0.11	0.05	0.01
Obtain ≥ 16/56 successes Declare sufficient efficacy	0.06	0.24	0.52	0.77	0.92

12.2.2 Safety Monitoring

Patients in exploratory cohort I (extramedullary disease only), M (MPAL/ALAL), and N (without asparaginase) will be included in the safety (not efficacy) stopping rules with patients from the primary cohort. Efficacy/ futility will be monitored separately for these patients either because MRD is unlikely to be informative (cohort I), there is no historical control available to determine response (cohort M), or therapy modifications make comparison to historical controls and other patients treated on the study uninformative (cohort N). Patients is cohort O will be monitored separately based on published data that young adult patients experience greater toxicity with identical therapy compared to pediatric patients. Specifically, induction death and high-grade toxicity requiring therapy

discontinuation was more common on CALGB10403 when compared to AALL0232.[3,79] There was also a higher incidence of heart failure and thrombosis in young adults treated on NOPHO ALL2008. Thus, we will monitor patients in cohort O separately.

12.2.2.1 Safety monitoring in Block 1

Patients in the primary analysis cohort as well as exploratory cohorts I (extramedullary disease), M (MPAL/ALAL), and N (without asparaginase) will be included in the safety cohort. Thus, we project the sample size for toxicity monitoring to be 82 patients (70 from the primary analysis and 12 from the 3 exploratory cohorts).

- Death: By literature (Raetz et al., 2008[14]; von Stackelberg et al., 2016[42]) we believe a toxic death rate (i.e. not related to disease progression) of ≤5% is consistent with background toxicity for this population. We will use a group sequential design with 3 stages to monitor against a >5% toxic death rate. The decision boundary and operating characteristics are outlined in Table 4.

  Table 4: Stopping boundary and probabilities for several scenarios of true rate of toxic death (monitor against death rate of >5%)

	True death rate (probability)		
	0.08	**0.05**	**0.02**
Suspend if 1/8 deaths	0.49	0.34	0.15
Suspend if 2/16 deaths	0.07	0.04	0.01
Suspend if 3/24 deaths	0.03	0.01	0.00
Suspend if 3/32 deaths	0.07	0.04	0.01
Suspend if 4/40 deaths	0.02	0.01	0.00
Suspend if 4/48 deaths	0.05	0.03	0.00
Suspend if 4/56 deaths	0.06	0.04	0.01
Suspend if 5/64 deaths	0.01	0.01	0.00
Suspend if 5/72 deaths	0.03	0.02	0.00
Suspend if 5/82 deaths	0.05	0.04	0.00
Obtain ≤ 4/82 deaths	0.12	0.42	0.82

- Grade 4 or higher non-hematologic, non-infection AEs not attributable to underlying disease excluding asymptomatic laboratory abnormalities which resolve to grade 1 or less within 72 hours with or without supplementation, AST/ ALT elevations which resolve to grade 1 or less within 7 days, fever, or nausea/ vomiting/ or diarrhea adequately managed with supportive care. We will use a group sequential design to monitor against a >20% rate of these toxicities. The decision boundary and operating characteristics are outlined in Table 5.

Table 5: Stopping boundary and probabilities for several scenarios of true rate of monitored toxicities (monitor against rate >20%)

	True monitored toxicity rate (probability)		
	0.25	**0.20**	**0.15**
Suspend if 3/8 AEs	0.32	0.20	0.11
Suspend if 5/16 AEs	0.13	0.08	0.03
Suspend if 7/24 AEs	0.08	0.04	0.02
Suspend if 9/32 AEs	0.05	0.03	0.01
Suspend if 10/40 AEs	0.09	0.06	0.02
Suspend if 12/48 AEs	0.04	0.02	0.01
Suspend if 14/56 AEs	0.03	0.02	0.00
Suspend if 16/64 AEs	0.02	0.01	0.00
Suspend if 17/72 AEs	0.04	0.03	0.01
Suspend if 18/82 AEs	0.06	0.05	0.01
Obtain if ≤ 17/82 AEs	0.15	0.45	0.79

- Uncontrolled Grade 4 or Grade 5 infections: Grade 4 infections which are uncontrolled after 7 days, or any Grade 5 infection, will be included as a targeted toxicity. Uncontrolled infections are defined as exhibiting ongoing signs/ symptoms related to the infection and without improvement, despite appropriate antibiotics or other treatment. We will use a group sequential design monitor against a >20% rate of such infections. The decision boundary and operating characteristics are outlined in Table 5.
- Hematological toxicity: Patients will be termed prolonged aplasia if they have not recovered to an ANC ≥500/microL after day 42 in the absence of evidence of leukemia. We will use a group sequential design to monitor against a >20% rate of these toxicities. The decision boundary and operating characteristics are outlined in Table 5.

Monitoring of patients who receive dasatinib: These patients are included in the above monitoring. Additionally, patients receiving dasatinib will be monitored for toxicities as described above to ensure a rate of monitored toxicity of no more than 20% using the characteristics in Table 6 below, with hematological toxicity, uncontrolled infectious toxicities, and high-grade non-hematological/ non-infectious toxicities monitored separately.

Table 6: Stopping boundary and probabilities for several scenarios of true rate of monitored toxicities in patients receiving dasatinib (monitor against rate >20%)

	True grade 4+ toxicity rate (probability)		
	0.25	**0.20**	**0.15**
Suspend if 3/8 AEs	0.32	0.20	0.11
Suspend if 4/16 AEs	0.29	0.22	0.13
Suspend if 7/24 AEs	0.03	0.02	0.00
Obtain if ≤ 6/24 AEs	0.44	0.56	0.76

Monitoring of patients in cohort O: Patients is cohort O will be monitored separately based on published data that young adult patients experience greater toxicity with identical therapy compared to pediatric patients. Specifically, induction death and high-grade toxicity requiring therapy discontinuation was more common on CALGB10403 when compared to AALL0232.[3,79] There was also a higher incidence of heart failure and thrombosis in young adults treated on NOPHO ALL2008. Due to the small size of this cohort, patients in this cohort will be monitored ad hoc. We will pause enrollment in this cohort and investigate if we observe either 1 death or 3 other monitored toxicities (see definitions above for grade 4 or higher infections, non-hematological toxicities, and hematological toxicity).

To identify the recommended phase 2 combination of venetoclax based consolidation in novel combinations with a) high-dose cytarabine and navitoclax or b) blinatumomab.

Responsible investigators: Seth Karol, MD
Responsible statistician: Cheng Cheng, PhD

12.2.2.2 Dose finding stage: For Blocks 2a and 2b we will first run a dose de-escalation study (for cytarabine and venetoclax, respectively) to identify the safe dose level for the subsequent phase-II component. In each block we will start at the highest dose and de-escalate (or stay) according to the rolling-6 design. Patients in exploratory cohort O are not eligible for the phase I dose de-escalation part of the study.

Cytarabine dosing during phase 1 portion for Block 2a

Dose level	Dose (with maximum daily dose if applicable) and Route	Number of doses	Schedule
1	3000mg/m^2/dose q12h IV infusion over 3 hours	4	Days 1-2
0	1500mg/m^2/dose q12h IV infusion over 3 hours	4	Days 1-2
-1	1000mg/m^2/dose q12h IV infusion over 3 hours	2	Day 1

Venetoclax dosing during phase 1 portion for Block 2b

Dose level	Dose (with maximum daily dose if applicable) and Route	Number of doses	Schedule
1	240mg/m^2 (max 400mg) PO	21	Days 8-28
0	240mg/m^2 (max 400mg) PO	14	Days 8-21
-1	240mg/m^2 (max 400mg) PO	7	Days 8-14

The rolling-6 design will be used for the conduct of this portion of the study. Dose/ combination limiting toxicities (DLTs) are defined separately for Blocks 2a and 2b.

- For block 2a, dose limiting toxicities are defined as:
    - Toxic death due to any cause.
    - Grade 4 or higher non-hematologic, non-infection AEs not attributable to underlying disease excluding asymptomatic laboratory abnormalities which

resolve to grade 1 or less within 72 hours with or without supplementation, AST/ ALT elevations which resolve to grade 1 or less within 7 days, fever, or nausea/ vomiting/ or diarrhea adequately managed with supportive care.
    - Grade 4 infections which are uncontrolled after 7 days.
    - Prolonged aplasia, defined as failure to recover to an ANC ≥500/microL by day 42 in the absence of leukemia.
- For block 2b, dose limiting toxicities are defined as:
    - Toxic death due to any cause.
    - Grade 3 or higher non-hematologic, non-infection AEs not attributable to underlying disease excluding asymptomatic laboratory abnormalities which resolve to grade 1 or less within 72 hours with or without supplementation, AST/ ALT elevations which resolve to grade 1 or less within 7 days, fever, nausea/ vomiting/ or diarrhea adequately managed with supportive care, or grade 3 cytokine release syndrome which does not resolve to grade 2 or less within 48 hours with supportive care.
    - Grade 3 or 4 infections which are uncontrolled after 7 days.
    - Prolonged aplasia, defined as failure to recover to an ANC ≥500/microL past day 28 in the absence of evidence of leukemia.

Following completion of Block 1 therapy, patients will enroll on the appropriate arm (2a or 2b, based on the criteria in 4.2.2). One to six participants can be concurrently enrolled onto a dose level, depending on the number of participants enrolled at the current dose level, the number of participants who have experienced a dose/ combination limiting toxicity (DLT) at the current dose level, and the number of participants entered but with tolerability data pending. Dose de-escalation will occur if 2 patients at a dose level experience a targeted toxicity. If 6 patients complete a therapy block and experience 0-1 DLT, that dose will be considered the recommended phase 2 dose (RP2D). New enrollment on an arm (2a/2b) will be paused if further enrollment would result in more than 6 patients being treated on that arm before the RP2D is identified.

If dose level 1 is not tolerable, patients will be enrolled on level 0.
If dose level 0 is not tolerable, patients will be enrolled on level -1.
If dose level -1 is not tolerable, the block will be suspended for evaluation.

12.3 Secondary Objectives

12.3.1 *To estimate the tolerability and activity of venetoclax based consolidation in novel combinations with a) high-dose cytarabine and navitoclax or b) blinatumomab.*

Responsible investigators: Seth Karol, MD
Responsible statistician: Cheng Cheng, PhD

The (secondary) endpoint for tolerability of Block 2a / 2b (consolidation) is the development of grade 3 or higher non-hematologic adverse effects (AE) according to CTCAE criteria and death due to any cause other than disease progression. Tolerability must be assessed separately in Block 2a and 2b. The rate of AEs will be estimated by

sample proportions along with the 95% exact Binomial confidence interval in Block 2a and 2b respectively.

Safety in Block 2a and 2b will be monitored separately as well. We anticipate that the cytarabine-containing treatment (Block 2a) is more toxic than the blinatumomab (Block 2b) regimen. Monitoring will be carried out in a group-sequential fashion.

12.3.1.1 Safety monitoring in Block 2a:

Phase 1/ dose finding monitoring and de-escalation schema are shown above. The below monitoring describes safety monitoring rules for patients being treated on the recommended phase 2 dose (RP2D). The projected estimates below assume that 6 patients will be treated during the phase 1 portion of the study (all at the RP2D). In the event that additional patients are treated during the phase 1 portion of the study, the tables below will be used. For the rules below, patients treated at the RP2D during the phase 1 portion will be counted toward the rules for study suspension.

- Death: By literature (Raetz et al., 2008[14]; von Stackelberg et al., 2016[42]) we believe a toxic death rate (i.e. not related to disease progression) of ≤5% is consistent with background toxicity for this population. We will use a group sequential design for patients treated at the recommended phase 2 dose (RP2D), including those treated during the dose finding phase. The design will monitor against a >5% toxic death rate. The decision boundary and operating characteristics are outlined in Table 7.

  Table 7: Stopping boundary and probabilities for several scenarios of true rate of toxic death (monitor against death rate of >5%)

	True death rate (probability)		
	0.08	**0.05**	**0.02**
Suspend if 1/8 deaths	0.49	0.34	0.15
Suspend if 2/16 deaths	0.07	0.04	0.01
Suspend if 3/24 deaths	0.03	0.01	0.00
Suspend if 3/32 deaths	0.07	0.04	0.00
Suspend if 4/40 deaths	0.02	0.01	0.00
Suspend if 4/48 deaths	0.05	0.03	0.00
Suspend if 4/52 deaths	0.03	0.02	0.00
Obtain if ≤ 3/52 deaths	0.24	0.51	0.83

- Grade 4 or higher non-hematologic, non-infection AEs not attributable to underlying disease excluding asymptomatic laboratory abnormalities which resolve to grade 1 or less within 72 hours with or without supplementation, AST/ ALT elevations which resolve to grade 1 or less within 7 days, fever, or nausea/ vomiting/ or diarrhea adequately managed with supportive care. We will use a group sequential to monitor against a >20% rate of these toxicities. The decision boundary and operating characteristics are outlined in Table 8.

Table 8: Stopping boundary and probabilities for several scenarios of true rate of monitored toxicities (monitor against rate >20%)

	True monitored toxicity rate (probability)		
	0.25	**0.20**	**0.15**
Suspend if 3/8 AEs	0.32	0.20	0.10
Suspend if 5/16 AEs	0.13	0.09	0.03
Suspend if 7/24 AEs	0.08	0.04	0.01
Suspend if 9/32 AEs	0.05	0.03	0.01
Suspend if 10/40 AEs	0.09	0.06	0.02
Suspend if 12/48 AEs	0.04	0.02	0.01
Suspend if 12/52AEs	0.06	0.05	0.02
Obtain if ≤ 11/52 AEs	0.23	0.51	0.80

- Uncontrolled Grade 4 or Grade 5 infections: Grade 4 infections which are uncontrolled after 7 days, or any Grade 5 infection, will be included as a targeted toxicity. Uncontrolled infections are defined as exhibiting ongoing signs/symptoms related to the infection and without improvement, despite appropriate antibiotics or other treatment. We will use a group sequential design to monitor against a >20% rate of such infections. The decision boundary and operating characteristics are outlined in Table 8.

Hematological toxicity: Patients will be termed prolonged aplasia if they have not recovered to an ANC ≥500/microL after day 42 in the absence of evidence of leukemia. We will use a group sequential design to monitor against a >20% rate of such aplasia. The decision boundary and operating characteristics are outlined in Table 8.

12.3.1.2 Safety monitoring in Block 2b:

Phase 1/ dose finding monitoring and de-escalation schema are shown above. The below describes safety monitoring rules for patients being treated on the recommended phase 2 dose (RP2D). The projected estimates below assume that 6 patients will be treated during the phase 1 portion of the study (all at the RP2D). In the event that additional patients are treated during the phase 1 portion of the study, the tables below will be used. For the rules below, patients treated at the RP2D during the phase 1 portion will be counted toward the block suspension rules.

- Death: By literature (Raetz et al., 2008[14]; von Stackelberg et al., 2016[42]) we believe a toxic death rate (i.e. not related to disease progression) of ≤5% is consistent with background toxicity for this population. We will use a group sequential design for patients treated at the recommended phase 2 dose (RP2D), including those treated during the dose finding phase. The design will monitor against a >5% toxic death rate. The decision boundary and operating characteristics are outlined in Table 9.

Table 9: Stopping boundary and probabilities for several scenarios of true rate of toxic death (monitor against death rate of >5%)

	True death rate (probability)		
	0.08	**0.05**	**0.02**
Suspend if 1/8 deaths	0.49	0.34	0.15
Suspend if 1/16 deaths	0.25	0.22	0.13
Suspend if 2/24 deaths	0.03	0.03	0.01
Suspend if 3/30 deaths	0.01	0.00	0.00
Obtain ≤ 2/30 deaths	0.22	0.41	0.71

- Grade 3 or higher non-hematologic AEs excluding infection, asymptomatic laboratory abnormalities which resolve to grade 1 or less within 72 hours with or without supplementation, AST/ ALT elevations which resolve to grade 1 or less within 7 days, fever, or nausea/ vomiting/ or diarrhea adequately managed with supportive care. Grade 3 cytokine release syndrome which resolves to grade 2 or less within 2 days with supportive care is also excluded. During the phase 2 portion, we will use a group sequential design to monitor against a >20% rate of these toxicities. The decision boundary and operating characteristics are outlined in Table 10.

Table 10: Stopping boundary and probabilities for several scenarios of true rate of monitored toxicities (monitor against toxicity rate of >20%)

	True monitored toxicity rate (probability)		
	0.25	**0.20**	**0.15**
Suspend if 3/8 AEs	0.32	0.20	0.11
Suspend if 4/16 AEs	0.29	0.22	0.13
Suspend if 7/24 AEs	0.03	0.02	0.00
Suspend if 7/30 AEs	0.11	0.08	0.04
Obtain ≤ 6/30 AEs	0.25	0.47	0.72

- Grade 3-4 infections which are uncontrolled after 7 days, or any grade 5 infection, will be included as a targeted toxicity. Uncontrolled infections are defined as exhibiting ongoing signs/symptoms related to the infection and without improvement, despite appropriate antibiotics or other treatment. During the phase 2 portion, we will use a group sequential design to monitor against a >20% rate of these toxicities. The decision boundary and operating characteristics are outlined in Table 10 (above).

- Hematological toxicity: Patients will be termed prolonged aplasia if they have not recovered to an ANC ≥500/microL after day 28 in the absence of evidence of leukemia. During the phase 2 portion, we will use a group sequential design with 2 stages to monitor against a >20% rate of these toxicities. The decision boundary and operating characteristics are outlined in Table 10 (above).

Activity of the consolidation blocks (2a, 2b) will be analyzed descriptively. We will estimate among the patients who are MRD-positive after Block 1 the proportion of patients who attain negative MRD after Block 2a/ 2b, along with an exact 95% confidence interval. We will also estimate the overall proportion of MRD-negative patients after Block 2a/ 2b using the sample proportion and the denominator of all evaluable patients after Block 2a/ 2b.

12.3.1.3 Safety monitoring for cohort O during Block 2

Patients is cohort O will be monitored separately based on published data that young adult patients experience greater toxicity with identical therapy compared to pediatric patients. Specifically, induction death and high-grade toxicity requiring therapy discontinuation was more common on CALGB10403 when compared to AALL0232.[3,79] There was also a higher incidence of heart failure and thrombosis in young adults treated on NOPHO ALL2008.[80] Due to the small size of this cohort, patients in this cohort will be monitored ad hoc. We will pause enrollment in block 2 for this cohort and investigate if we observe either 1 death or 3 other monitored toxicities (as defined above for blocks 2a and 2b in sections 12.3.1.1 and 12.3.1.2, respectively). As indicated above, patients in cohort O are not eligible for the phase I/ dose de-escalation phase.

12.3.1.4 Safety monitoring beyond Block 2

Because only last first relapse B-ALL who are MRD-negative after Block 1 are eligible to continue therapy after Block 2, we anticipate no more than 14 patients will receive this therapy. Due to the small number of patients, we will monitor safety in this cohort ad hoc to ensure a rate of death of <5% and <20% of other monitored AEs (grade 4 or higher non-hematologic, non-infectious AEs excluding asymptomatic laboratory abnormalities which resolve to grade 1 or less within 72 hours with or without supplementation, AST/ ALT elevations which resolve to grade 1 or less within 7 days, fever, or nausea/ vomiting/ or diarrhea adequately managed with supportive care; grade 4 or higher infections not controlled within 7 days). We will suspend post-Block 2 therapy for the trial and investigate if 1 death or 3 other monitored AEs occur.

12.3.2 To describe event-free and overall survival in patients treated with this regimen.

Responsible investigators: Seth Karol, MD
Responsible statistician: Cheng Cheng, PhD

Patients will be followed for 5 years from enrollment. Failure in overall survival (OS) is death of any cause. Patients staying alive at the last follow up are censored. Failures in event-free survival (EFS) is death of any cause, relapse, or refractory disease (≥5% MRD at the end of Block 2). Patients remaining failure-free are censored at the last follow up date. The time to event is the time between on-treatment date and the date of failure or last follow up. Kaplan-Meier estimates of the OS and EFS functions will be calculated, along with the standard error at any given time point (1 year, 3 year, etc.) computed by Peto's method.

12.4 Exploratory Objectives

12.4.1 To evaluate MRD-negative CR/CRi rates in each prespecified groups: late first relapse B-ALL; early first relapse and second or greater relapse B-ALL; and relapsed T-ALL.

Responsible investigators: Seth Karol, MD
Responsible statistician: Cheng Cheng, PhD

Sample proportions of MRD-negative CR/CRi rate will be computed in each stratum along with standard error.

12.4.2 To identify drug sensitivity patterns in patient samples prior to and after receiving combination therapy and evaluate mechanisms of disease resistance/ escape.

Responsible investigators: Charles Mullighan, MBBS, Jun Yang, PhD, Joseph Opferman, PhD
Responsible statistician: Cheng Cheng, PhD

Statistical approaches as described previously will be applied.[31,92] Specifically, for each anti-leukemic agent, we will treat the in vitro drug sensitivities and BH3 protein sensitivities as continuous phenotypes in data analyses. Associations between various genomic features/ factors and the drug sensitivity phenotypes will be analyzed/ tested by regression modeling or rank correlations. For each type of genomic feature, to assess the top hits' (genomic features') ability to different sensitive and resistant leukemic cells, we will separate patients into top 25%, middle 50%, and bottom 25% groups based on their drug sensitivities, ranging from the most resistant to the most sensitive, perform a model based clustering (MCLUST) using the top k genomic features, and pick the value for k as the number of features for which the generated clusters agree the most with the quartile grouping by the Chi-square/ Fisher's test (minimum P value). Approaches based on false discovery rate will also be applied to determine a threshold to define the set of top genomic features highly associated with drug sensitivity. Given anticipated limited sample availability, exploratory analyses will be performed on residual leukemia samples after treatment to identify mechanisms of resistance. Xenografting to further evaluate these findings may be utilized for samples from consenting patients.

12.4.3 To explore immune subsets during and after this regimen.

Responsible investigators: Paul Thomas, PhD, Benjamin Youngblood, PhD, Caitlyn Zebley, MD, PhD

In the Thomas lab, high resolution flow cytometry will be used to identify infiltrating immune cell subsets and their phenotypes, including, but not limited to CD4+ T cells, CD4+ Treg cells, CD8+ cells, γδ T cells, NK cells, neutrophils, macrophages, monocytes, eosinophils, and B cells. T cell populations will be analyzed for activation status, transcriptional profile, and T cell receptor sequence using a single cell sequencing platform such as the 10X Genomics 5' Kit or smartSeq3. Responses to tumor-specific and associated

antigens will be probed with functional assays such as intracellular cytokine staining. T cell receptors of interest will be cloned and characterized for specificity. Patient HLA information will be acquired from the clinical chart or from dedicated RNA-sequencing to construct immune multimers where possible. We will explore associations between changes in immune cell phenotypes, transcriptional profiles, activation states, frequencies, with objective responses as well as with PFS/OS using logistic regression models and Cox proportional hazards models, respectively.

For T cell repertoire studies, we will analyze the clonal distribution and composition of tumor-associated T cells at different time points relative to therapy. Specific receptor profiles will be characterized using clustering methods developed by the Thomas lab (TCRdist and related algorithms)[93] and by using approaches that relate TCR specificity to gene expression profiles (clonotype neighbor graph analysis, CoNGA). These will be associated with objective responses and PFS/OS. TCR specificity will also be inferred, where possible, by matching to known receptor specificities in public and private databases.

In parallel, DNA will be extracted from T cells and bisulfite treated to convert all unmethylated cytosines to uracils. Bisulfite-modified DNA will be submitted for whole genome sequencing to quantify the methylation status of CpG sites. The T cell Multipotency Index[71,94] will be determined to score of patients' T cells and correlate this multipotency score to clinical response in patients. A multivariate linear regression model will be used to model the relationship between clinical response in patients and the multipotency score with clinical response as the dependent variable as methods previously described.[71,94-101]

12.4.4 Evaluate response to therapy in rare relapsed acute leukemias to this regimen.
Responsible investigators: Seth Karol, MD
Responsible statistician: Cheng Cheng, PhD

MRD levels at the end of Block 1 and Block 2 will be summarized with mean, standard deviation, and the 5-number summary. Proportion of MRD-negative response will be estimated by sample proportion.

12.4.5 Explore breakthrough infections in children and young adults with relapsed or refractory ALL.

Responsible investigators: Joshua Wolf, MBBS, PhD
Responsible statistician: Li Tang, PhD

All enrolled participants will be eligible for this component. Descriptive statistics, such as frequency and proportion, will be summarized for breakthrough infections, antibiotic-resistant infections, febrile neutropenia episodes and adverse events. Cumulative incidence of breakthrough infection, febrile neutropenia and adverse events will also be explored, with competing risks and/or recurrent event appropriately adjusted. Death not due to the toxicity of interest, relapse, and other reasons unrelated to the toxicity of interest rendering off treatment are considered as competing events. For cumulative incidence, time to the

first episode will be considered as the time to failure. Recurrence rate will be estimated by Poisson process modeling.

12.5 Anticipated Completion Dates

Anticipated primary completion date: 2.5 years from study opening.
Anticipated study completion date: 4.5 years from study opening.

13.0 OBTAINING INFORMED CONSENT

13.1 Consent Prior to Research Interventions

Initially, informed consent will be sought for the institutional banking protocol (TBANK or local banking protocol as applicable) and for other procedures as necessary. During the screening process for eligibility, informed consent is required before any research tests are performed.

The process of informed consent and assent for RAVEN 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. Throughout the entire treatment period, participants and their parents receive constant education from health professionals at St. Jude and collaborating sites and are encouraged to ask questions regarding alternatives and therapy. All families have ready access to chaplains, psychologists, social workers, and child life specialists, in addition to that provided by the primary clinicians involved in their care.

13.2 Consent at Age of Majority

The age of majority in the state of Tennessee is 18 years old. Research participants must be consented at the next clinic visit after their 18th birthday. If an affiliate or collaborating site is located in a country or state where a different age of majority applies, that location must consent the participants according to their local laws.

13.3 Consent When English is Not the Primary Language

When English is not the patient, parent, or legally authorized representative's primary language, the Social Work department will determine the need for an interpreter. This information 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 CTO websites. Collaborating sites will follow institutional policy.

14.0 REFERENCES

1. Howlader N NA, Krapcho M, Miller D, Brest A, Yu M, Ruhl J, Tatalovich Z, Mariotto A, Lewis DR, Chen HS, Feuer EJ, Cronin KA: SEER Cancer Statistics Review, 1975-2016, in Institute NC (ed): (ed Based on November 2018 SEER data submission, posted to the SEER web site, April 2019). Bethesda, MD, 2019
2. Jeha S, Pei D, Choi J, et al: Improved CNS Control of Childhood Acute Lymphoblastic Leukemia Without Cranial Irradiation: St Jude Total Therapy Study 16. J Clin Oncol 37:3377-3391, 2019
3. Larsen EC, Devidas M, Chen S, et al: Dexamethasone and High-Dose Methotrexate Improve Outcome for Children and Young Adults With High-Risk B-Acute Lymphoblastic Leukemia: A Report From Children's Oncology Group Study AALL0232. J Clin Oncol 34:2380-8, 2016
4. Dunsmore KP, Winter SS, Devidas M, et al: Children's Oncology Group AALL0434: A Phase III Randomized Clinical Trial Testing Nelarabine in Newly Diagnosed T-Cell Acute Lymphoblastic Leukemia. J Clin Oncol 38:3282-3293, 2020
5. Moorman AV, Robinson H, Schwab C, et al: Risk-directed treatment intensification significantly reduces the risk of relapse among children and adolescents with acute lymphoblastic leukemia and intrachromosomal amplification of chromosome 21: a comparison of the MRC ALL97/99 and UKALL2003 trials. J Clin Oncol 31:3389-96, 2013
6. Toft N, Birgens H, Abrahamsson J, et al: Results of NOPHO ALL2008 treatment for patients aged 1-45 years with acute lymphoblastic leukemia. Leukemia 32:606-615, 2018
7. Oskarsson T, Soderhall S, Arvidson J, et al: Relapsed childhood acute lymphoblastic leukemia in the Nordic countries: prognostic factors, treatment and outcome. Haematologica 101:68-76, 2016
8. Parker C, Waters R, Leighton C, et al: Effect of mitoxantrone on outcome of children with first relapse of acute lymphoblastic leukaemia (ALL R3): an open-label randomised trial. Lancet 376:2009-17, 2010
9. Locatelli F, Schrappe M, Bernardo ME, et al: How I treat relapsed childhood acute lymphoblastic leukemia. Blood 120:2807-16, 2012
10. Kaspers GJ, Veerman AJ, Pieters R, et al: In vitro cellular drug resistance and prognosis in newly diagnosed childhood acute lymphoblastic leukemia. Blood 90:2723-9, 1997
11. Klumper E, Pieters R, Veerman AJ, et al: In vitro cellular drug resistance in children with relapsed/refractory acute lymphoblastic leukemia. Blood 86:3861-8, 1995
12. Holleman A, Cheok MH, den Boer ML, et al: Gene-expression patterns in drug-resistant acute lymphoblastic leukemia cells and response to treatment. N Engl J Med 351:533-42, 2004
13. August KJ, Guest EM, Lewing K, et al: Treatment of children with relapsed and refractory acute lymphoblastic leukemia with mitoxantrone, vincristine, pegaspargase, dexamethasone, and bortezomib. Pediatr Blood Cancer 67:e28062, 2020
14. Raetz EA, Borowitz MJ, Devidas M, et al: Reinduction platform for children with first marrow relapse of acute lymphoblastic Leukemia: A Children's Oncology Group Study[corrected]. J Clin Oncol 26:3971-8, 2008
15. Horton TM, Whitlock JA, Lu X, et al: Bortezomib reinduction chemotherapy in high-risk ALL in first relapse: a report from the Children's Oncology Group. Br J Haematol 186:274-285, 2019
16. Brown P, Ji L, Xu X, et al: A Randomized Phase 3 Trial of Blinatumomab Vs. Chemotherapy As Post-Reinduction Therapy in High and Intermediate Risk (HR/IR) First Relapse of B-Acute Lymphoblastic Leukemia (B-ALL) in Children and Adolescents/Young Adults (AYAs)

Demonstrates Superior Efficacy and Tolerability of Blinatumomab: A Report from Children's Oncology Group Study AALL1331, American Society of Hematology Annual Meeting. Orlando, FL, 2019
17. Parker C, Krishnan S, Hamadeh L, et al: Outcomes of patients with childhood B-cell precursor acute lymphoblastic leukaemia with late bone marrow relapses: long-term follow-up of the ALLR3 open-label randomised trial. Lancet Haematol 6:e204-e216, 2019
18. Abstracts from the 52th Congress of the International Society of Paediatric Oncology (SIOP) Virtual Congress, October 14-17, 2020. Pediatr Blood Cancer 67 Suppl 4:e28742, 2020
19. Sun W, Malvar J, Sposto R, et al: Outcome of children with multiply relapsed B-cell acute lymphoblastic leukemia: a therapeutic advances in childhood leukemia & lymphoma study. Leukemia 32:2316-2325, 2018
20. Reismuller B, Peters C, Dworzak MN, et al: Outcome of children and adolescents with a second or third relapse of acute lymphoblastic leukemia (ALL): a population-based analysis of the Austrian ALL-BFM (Berlin-Frankfurt-Munster) study group. J Pediatr Hematol Oncol 35:e200-4, 2013
21. Souers AJ, Leverson JD, Boghaert ER, et al: ABT-199, a potent and selective BCL-2 inhibitor, achieves antitumor activity while sparing platelets. Nature Medicine 19:202-208, 2013
22. Place AE, Goldsmith K, Bourquin JP, et al: Accelerating drug development in pediatric cancer: a novel Phase I study design of venetoclax in relapsed/refractory malignancies. Future Oncol 14:2115-2129, 2018
23. Roberts AW, Huang D: Targeting BCL2 With BH3 Mimetics: Basic Science and Clinical Application of Venetoclax in Chronic Lymphocytic Leukemia and Related B Cell Malignancies. Clin Pharmacol Ther 101:89-98, 2017
24. Frismantas V, Dobay MP, Rinaldi A, et al: Ex vivo drug response profiling detects recurrent sensitivity patterns in drug-resistant acute lymphoblastic leukemia. Blood 129:e26-e37, 2017
25. Diamanti P, Ede BC, Dace PE, et al: Investigating the response of paediatric leukaemia-propagating cells to BCL-2 inhibitors. Br J Haematol 192:577-588, 2021
26. Diaz-Flores E, Comeaux EQ, Kim KL, et al: Bcl-2 Is a Therapeutic Target for Hypodiploid B-Lineage Acute Lymphoblastic Leukemia. Cancer Res 79:2339-2351, 2019
27. Benito JM, Godfrey L, Kojima K, et al: MLL-Rearranged Acute Lymphoblastic Leukemias Activate BCL-2 through H3K79 Methylation and Are Sensitive to the BCL-2-Specific Antagonist ABT-199. Cell Reports 13:2715-2727, 2015
28. Khaw SL, Suryani S, Evans K, et al: Venetoclax responses of pediatric ALL xenografts reveal sensitivity of MLL-rearranged leukemia. Blood 128:1382-95, 2016
29. Leonard JT, Rowley JS, Eide CA, et al: Targeting BCL-2 and ABL/LYN in Philadelphia chromosome-positive acute lymphoblastic leukemia. Sci Transl Med 8:354ra114, 2016
30. Budhraja A, Turnis ME, Churchman ML, et al: Modulation of Navitoclax Sensitivity by Dihydroartemisinin-Mediated MCL-1 Repression in BCR-ABL(+) B-Lineage Acute Lymphoblastic Leukemia. Clin Cancer Res 23:7558-7568, 2017
31. Autry RJ, Paugh SW, Carter R, et al: Integrative genomic analyses reveal mechanisms of glucocorticoid resistance in acute lymphoblastic leukemia. Nat Cancer 1:329-344, 2020
32. Alford SE, Kothari A, Loeff FC, et al: BH3 Inhibitor Sensitivity and Bcl-2 Dependence in Primary Acute Lymphoblastic Leukemia Cells. Cancer Res 75:1366-75, 2015
33. Daver N, Konopleva M: Cotargeting BCL-2 and BCL-XL for maximal efficacy in ALL. Blood 128:1316-7, 2016
34. Peirs S, Matthijssens F, Goossens S, et al: ABT-199 mediated inhibition of BCL-2 as a novel therapeutic strategy in T-cell acute lymphoblastic leukemia. Blood 124:3738-3747, 2014

35. Chonghaile TN, Roderick JE, Glenfield C, et al: Maturation stage of T-cell acute lymphoblastic leukemia determines BCL-2 versus BCL-XL dependence and sensitivity to ABT-199. Cancer Discov 4:1074-87, 2014
36. Pullarkat VA, Lacayo NJ, Jabbour E, et al: Venetoclax and Navitoclax in Combination with Chemotherapy in Patients with Relapsed or Refractory Acute Lymphoblastic Leukemia and Lymphoblastic Lymphoma. Cancer Discov 11:1440-1453, 2021
37. Serafin V, Capuzzo G, Milani G, et al: Glucocorticoid resistance is reverted by LCK inhibition in pediatric T-cell acute lymphoblastic leukemia. Blood 130:2750-2761, 2017
38. Laukkanen S, Gronroos T, Polonen P, et al: In silico and preclinical drug screening identifies dasatinib as a targeted therapy for T-ALL. Blood Cancer Journal 7, 2017
39. Gocho Y, Liu J, Hu J, et al: Network-based systems pharmacology reveals heterogeneity in LCK and BCL2 signaling and therapeutic sensitivity of T-cell acute lymphoblastic leukemia. Nature Cancer 2:284-299, 2021
40. Einsiedel HG, von Stackelberg A, Hartmann R, et al: Long-term outcome in children with relapsed ALL by risk-stratified salvage therapy: Results of trial acute lymphoblastic leukemia-relapse study of the Berlin-Frankfurt-Miinster Group 87. Journal of Clinical Oncology 23:7942-7950, 2005
41. Kantarjian H, Stein A, Gokbuget N, et al: Blinatumomab versus Chemotherapy for Advanced Acute Lymphoblastic Leukemia. N Engl J Med 376:836-847, 2017
42. von Stackelberg A, Locatelli F, Zugmaier G, et al: Phase I/Phase II Study of Blinatumomab in Pediatric Patients With Relapsed/Refractory Acute Lymphoblastic Leukemia. J Clin Oncol 34:4381-4389, 2016
43. Brown PA, Ji L, Xu X, et al: Effect of Postreinduction Therapy Consolidation With Blinatumomab vs Chemotherapy on Disease-Free Survival in Children, Adolescents, and Young Adults With First Relapse of B-Cell Acute Lymphoblastic Leukemia: A Randomized Clinical Trial. JAMA 325:833-842, 2021
44. Locatelli F, Zugmaier G, Rizzari C, et al: Effect of Blinatumomab vs Chemotherapy on Event-Free Survival Among Children With High-risk First-Relapse B-Cell Acute Lymphoblastic Leukemia: A Randomized Clinical Trial. JAMA 325:843-854, 2021
45. Keating AK, Gossai N, Phillips CL, et al: Reducing minimal residual disease with blinatumomab prior to HCT for pediatric patients with acute lymphoblastic leukemia. Blood Adv 3:1926-1929, 2019
46. Foa R, Bassan R, Vitale A, et al: Dasatinib-Blinatumomab for Ph-Positive Acute Lymphoblastic Leukemia in Adults. N Engl J Med 383:1613-1623, 2020
47. Campos-Cabrera G, Vega-Tapia NB, Campos-Cabrera V, et al: Blinatumomab and Venetoclax for Minimal Residual Disease Relapse in Acute Lymphoblastic Leukemia. Blood 134, 2019
48. Murakami S, Suzuki S, Hanamura I, et al: Combining T-cell-based immunotherapy with venetoclax elicits synergistic cytotoxicity to B-cell lines in vitro. Hematol Oncol 38:705-714, 2020
49. Karlsson H, Lindqvist AC, Fransson M, et al: Combining CAR T cells and the Bcl-2 family apoptosis inhibitor ABT-737 for treating B-cell malignancy. Cancer Gene Ther 20:386-93, 2013
50. Haikala HM, Anttila JM, Marques E, et al: Pharmacological reactivation of MYC-dependent apoptosis induces susceptibility to anti-PD-1 immunotherapy. Nat Commun 10:620, 2019
51. Huo Y, Sheng Z, Lu DR, et al: Blinatumomab-induced T cell activation at single cell transcriptome resolution. BMC Genomics 22:145, 2021

52. Duell J, Dittrich M, Bedke T, et al: Frequency of regulatory T cells determines the outcome of the T-cell-engaging antibody blinatumomab in patients with B-precursor ALL. Leukemia 31:2181-2190, 2017
53. Karol SE, Alexander TB, Budhraja A, et al: Venetoclax in combination with cytarabine with or without idarubicin in children with relapsed or refractory acute myeloid leukaemia: a phase 1, dose-escalation study. Lancet Oncology 21:551-560, 2020
54. DiNardo CD, Lachowiez CA, Takahashi K, et al: Venetoclax Combined With FLAG-IDA Induction and Consolidation in Newly Diagnosed and Relapsed or Refractory Acute Myeloid Leukemia. J Clin Oncol 39:2768-2778, 2021
55. Senft D, Jeremias I: A rare subgroup of leukemia stem cells harbors relapse-inducing potential in acute lymphoblastic leukemia. Exp Hematol 69:1-10, 2019
56. Teh TC, Nguyen NY, Moujalled DM, et al: Enhancing venetoclax activity in acute myeloid leukemia by co-targeting MCL1. Leukemia 32:303-312, 2018
57. Arai Y, Kondo T, Shigematsu A, et al: High-dose cytarabine added to CY/TBI improves the prognosis of cord blood transplantation for acute lymphoblastic leukemia in adults: a retrospective cohort study. Bone Marrow Transplant 51:1636-1639, 2016
58. Shen S, Chen X, Cai J, et al: Effect of Dasatinib vs Imatinib in the Treatment of Pediatric Philadelphia Chromosome-Positive Acute Lymphoblastic Leukemia: A Randomized Clinical Trial. JAMA Oncol 6:358-366, 2020
59. Hijiya N, Gajjar A, Zhang Z, et al: Low-dose oral etoposide-based induction regimen for children with acute lymphoblastic leukemia in first bone marrow relapse. Leukemia 18:1581-6, 2004
60. Lew G, Chen Y, Lu X, et al: Outcomes after late bone marrow and very early central nervous system relapse of childhood B-acute lymphoblastic leukemia: a report from the Children's Oncology Group phase III study AALL0433. Haematologica 106:46-55, 2021
61. Escherich G, Troger A, Gobel U, et al: The long-term impact of in vitro drug sensitivity on risk stratification and treatment outcome in acute lymphoblastic leukemia of childhood (CoALL 06-97). Haematologica 96:854-62, 2011
62. Maude SL, Laetsch TW, Buechner J, et al: Tisagenlecleucel in Children and Young Adults with B-Cell Lymphoblastic Leukemia. N Engl J Med 378:439-448, 2018
63. Park JH, Riviere I, Gonen M, et al: Long-Term Follow-up of CD19 CAR Therapy in Acute Lymphoblastic Leukemia. N Engl J Med 378:449-459, 2018
64. Gardner R, Wu D, Cherian S, et al: Acquisition of a CD19-negative myeloid phenotype allows immune escape of MLL-rearranged B-ALL from CD19 CAR-T-cell therapy. Blood 127:2406-10, 2016
65. Bhojwani D, Sposto R, Shah NN, et al: Inotuzumab ozogamicin in pediatric patients with relapsed/refractory acute lymphoblastic leukemia. Leukemia 33:884-892, 2019
66. Fry TJ, Shah NN, Orentas RJ, et al: CD22-targeted CAR T cells induce remission in B-ALL that is naive or resistant to CD19-targeted CAR immunotherapy. Nat Med 24:20-28, 2018
67. Zhao Y, Aldoss I, Qu C, et al: Tumor-intrinsic and -extrinsic determinants of response to blinatumomab in adults with B-ALL. Blood 137:471-484, 2021
68. Deng Q, Han G, Puebla-Osorio N, et al: Characteristics of anti-CD19 CAR T cell infusion products associated with efficacy and toxicity in patients with large B cell lymphomas. Nat Med 26:1878-1887, 2020
69. Finney OC, Brakke HM, Rawlings-Rhea S, et al: CD19 CAR T cell product and disease attributes predict leukemia remission durability. J Clin Invest 129:2123-2132, 2019

70. Zamora AE, Crawford JC, Allen EK, et al: Pediatric patients with acute lymphoblastic leukemia generate abundant and functional neoantigen-specific CD8(+) T cell responses. Sci Transl Med 11, 2019
71. Abdelsamed HA, Zebley CC, Nguyen H, et al: Beta cell-specific CD8(+) T cells maintain stem cell memory-associated epigenetic programs during type 1 diabetes. Nat Immunol 21:578-587, 2020
72. Orgel E, Alexander TB, Wood BL, et al: Mixed-phenotype acute leukemia: A cohort and consensus research strategy from the Children's Oncology Group Acute Leukemia of Ambiguous Lineage Task Force. Cancer 126:593-601, 2020
73. Alexander TB, Gu Z, Iacobucci I, et al: The genetic basis and cell of origin of mixed phenotype acute leukaemia. Nature 562:373-379, 2018
74. Kawedia JD, Liu C, Pei D, et al: Dexamethasone exposure and asparaginase antibodies affect relapse risk in acute lymphoblastic leukemia. Blood 119:1658-64, 2012
75. Gupta S, Wang C, Raetz EA, et al: Impact of Asparaginase Discontinuation on Outcome in Childhood Acute Lymphoblastic Leukemia: A Report From the Children's Oncology Group. J Clin Oncol 38:1897-1905, 2020
76. Vrooman LM, Stevenson KE, Supko JG, et al: Postinduction dexamethasone and individualized dosing of Escherichia Coli L-asparaginase each improve outcome of children and adolescents with newly diagnosed acute lymphoblastic leukemia: results from a randomized study--Dana-Farber Cancer Institute ALL Consortium Protocol 00-01. J Clin Oncol 31:1202-10, 2013
77. Richard-Carpentier G, Jabbour E, Short NJ, et al: Clinical Experience With Venetoclax Combined With Chemotherapy for Relapsed or Refractory T-Cell Acute Lymphoblastic Leukemia. Clin Lymphoma Myeloma Leuk 20:212-218, 2020
78. El-Cheikh J, Moukalled NM, El Darsa H, et al: Feasibility of the Combination of Venetoclax and Asparaginase-based Chemotherapy for Adult Patients With Relapsed/Refractory Acute Lymphoblastic Leukemia. Clin Lymphoma Myeloma Leuk 18:e441-e444, 2018
79. Stock W, Luger SM, Advani AS, et al: A pediatric regimen for older adolescents and young adults with acute lymphoblastic leukemia: results of CALGB 10403. Blood 133:1548-1559, 2019
80. Toft N, Birgens H, Abrahamsson J, et al: Toxicity profile and treatment delays in NOPHO ALL2008-comparing adults and children with Philadelphia chromosome-negative acute lymphoblastic leukemia. Eur J Haematol, 2015
81. Stock W, Douer D, DeAngelo DJ, et al: Prevention and management of asparaginase/pegasparaginase-associated toxicities in adults and older adolescents: recommendations of an expert panel. Leukemia & Lymphoma 52:2237-2253, 2011
82. Derman BA, Streck M, Wynne J, et al: Efficacy and toxicity of reduced vs. standard dose pegylated asparaginase in adults with Philadelphia chromosome-negative acute lymphoblastic leukemia. Leuk Lymphoma 61:614-622, 2020
83. Reda G, Cassin R, Dovrtelova G, et al: Venetoclax penetrates in cerebrospinal fluid and may be effective in chronic lymphocytic leukemia with central nervous system involvement. Haematologica 104:e222-e223, 2019
84. Sun W, Orgel E, Malvar J, et al: Treatment-related adverse events associated with a modified UK ALLR3 induction chemotherapy backbone for childhood relapsed/refractory acute lymphoblastic leukemia. Pediatr Blood Cancer 63:1943-8, 2016
85. Salem AH, Dave N, Marbury T, et al: Pharmacokinetics of the BCL-2 Inhibitor Venetoclax in Subjects with Hepatic Impairment. Clin Pharmacokinet 58:1091-1100, 2019

86. Relling MV, Schwab M, Whirl-Carrillo M, et al: Clinical Pharmacogenetics Implementation Consortium Guideline for Thiopurine Dosing Based on TPMT and NUDT15 Genotypes: 2018 Update. Clin Pharmacol Ther 105:1095-1105, 2019
87. Relling MV, Hancock ML, Rivera GK, et al: Mercaptopurine therapy intolerance and heterozygosity at the thiopurine S-methyltransferase gene locus. J Natl Cancer Inst 91:2001-8, 1999
88. Moriyama T, Yang YL, Nishii R, et al: Novel variants in NUDT15 and thiopurine intolerance in children with acute lymphoblastic leukemia from diverse ancestry. Blood 130:1209-1212, 2017
89. Moriyama T, Nishii R, Perez-Andreu V, et al: NUDT15 polymorphisms alter thiopurine metabolism and hematopoietic toxicity. Nat Genet 48:367-73, 2016
90. Yang JJ, Landier W, Yang W, et al: Inherited NUDT15 variant is a genetic determinant of mercaptopurine intolerance in children with acute lymphoblastic leukemia. J Clin Oncol 33:1235-42, 2015
91. Nishii R, Moriyama T, Janke LJ, et al: Preclinical evaluation of NUDT15-guided thiopurine therapy and its effects on toxicity and antileukemic efficacy. Blood 131:2466-2474, 2018
92. Paugh SW, Bonten EJ, Savic D, et al: NALP3 inflammasome upregulation and CASP1 cleavage of the glucocorticoid receptor cause glucocorticoid resistance in leukemia cells. Nat Genet 47:607-14, 2015
93. Dash P, Fiore-Gartland AJ, Hertz T, et al: Quantifiable predictive features define epitope-specific T cell receptor repertoires. Nature 547:89-93, 2017
94. Abdelsamed HA, Moustaki A, Fan YP, et al: Human memory CD8 T cell effector potential is epigenetically preserved during in vivo homeostasis. Journal of Experimental Medicine 214:1593-1606, 2017
95. Ghoneim HE, Fan Y, Moustaki A, et al: De Novo Epigenetic Programs Inhibit PD-1 Blockade-Mediated T Cell Rejuvenation. Cell 170:142-157 e19, 2017
96. Hale JS, Youngblood B, Latner DR, et al: Distinct memory CD4+ T cells with commitment to T follicular helper- and T helper 1-cell lineages are generated after acute viral infection. Immunity 38:805-17, 2013
97. Scharer CD, Barwick BG, Youngblood BA, et al: Global DNA Methylation Remodeling Accompanies CD8 T Cell Effector Function. J Immunol, 2013
98. Youngblood B, Hale JS, Akondy R: Using epigenetics to define vaccine-induced memory T cells. Curr Opin Virol 3:371-376, 2013
99. Youngblood B, Noto A, Porichis F, et al: Cutting edge: Prolonged exposure to HIV reinforces a poised epigenetic program for PD-1 expression in virus-specific CD8 T cells. J Immunol 191:540-4, 2013
100. Youngblood B, Oestreich KJ, Ha SJ, et al: Chronic virus infection enforces demethylation of the locus that encodes PD-1 in antigen-specific CD8+ T cells Immunity 35:13, 2011
101. Youngblood B, Reich NO: The early expressed HIV-1 genes regulate DNMT1 expression. Epigenetics 3:149-56, 2008

APPENDIX I: Performance Status Criteria

PERFORMANCE STATUS CRITERIA *Karnofsky and Lansky performance scores are intended to be multiples of 10*					
ECOG (Zubrod)		**Karnofsky (P**articipants ≥16 years)		**Lansky (Participants <16 years)**	
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: SAMPLE PROCESSING

Refer to the RAVEN Procedures Manual for specimen submission guidelines and shipping information.

Specimen descriptions

Immune profiling: 3mL of blood or bone marrow as appropriate in ACD.

Pharmacotyping/ genomics/ BH3 profiling: 10mL of bone marrow in preservative free sodium heparin tube (dark green top tube).
Clinical genomics specimens must be labeled with name and date of birth.

Skin biopsy (for patients consenting to comprehensive genomic sequencing) should be done at the site of port/line placement or BMA. A minimum of 3mm punch biopsy is needed, preferably 4mm. The biopsy should be placed in a sterile container with RPMI with the lid wrapped with parafilm. Seal in a bag and ship ambient overnight. This specimen must be labeled with name and date of birth.

MRD (if St. Jude will serve as reference lab): Specimens should be collected in a preservative free sodium heparin tube (dark green top tube) and shipped ambient. 5mL of sample is required. The MRD assay is performed in a CLIA lab, and specimens for MRD must be labelled with patient name and date of birth.

Note that biomarker studies are not performed on weekends. Hence, samples should be drawn Monday through Thursday and sent by overnight delivery.

APPENDIX III: CYP3A INDUCERS AND INHIBITORS

Excluded during protocol therapy:
Strong CYP3A inducers - avasimibe, carbamazepine, enzalutamine, mitotane, phenytoin, rifampin, St. John's wort **Moderate CYP3A inducers** - bosentan, efavirenz, etravirine, modafinil, nafcillin
Excluded during Block 1; caution during Block 2; if used during Block 2, dose adjustments of venetoclax may be needed: contact PI
Strong CYP3A inhibitors - boceprevir, clarithromycin, cobicistat, conivaptan, danoprevir/ritonavir, elvitegravir/ritonavir, idelalisib,* indinavir, itraconazole, ketoconazole, mibefradil, lopinavir/ritonavir, nefazodone, nelfinavir, ritonavir, paritaprevir/ritonavir combinations, posaconazole, saquinavir, telaprevir, telithromycin, tipranavir/ritonavir, voriconazole **Moderate CYP3A inhibitors -** amprenavir, aprepitant, atazanavir, cimetidine, ciprofloxacin, clotrimazole, crizotinib*, cyclosporine*, darunavir/ritonavir, diltiazem[1], erythromycin, fluconazole, fosamprenavir, imatinib*, isavuconazole, tofisopam, verapamil
Cautionary
Warfarin** **P-gp substrates** Aliskiren, ambrisentan, colchicine, dabigatran etexilate, digoxin, everolimus*, fexofenadine, lapatinib*, loperamide, maraviroc, nilotinib*, ranolazine, saxagliptin, sirolimus*, sitagliptin, talinolol, tolvaptan, topotecan* **BCRP substrates** Methotrexate*, mitoxantrone*, irinotecan*, lapatinib*, rosuvastatin, sulfasalazine, topotecan* **OATP1B1/1B3 substrates** Atrasentan, atorvastatin, ezetimibe, fluvastatin, glyburide, rosuvastatin, simvastatin acid, pitavastatin, pravastatin, repaglinide, telmisartan, valsartan, olmesartan **P-gp inhibitors** Amiodarone, azithromycin, captopril, carvedilol, dronedarone, felodipine, quercetin, quinidine, ronalzine, ticagrelor **BCRP inhibitors** Geftinib*

APPENDIX IV: DOSE ADJUSTMENTS WITH CYP3A INHIBITORS

Patient size	Standard venetoclax (Ven) dosing	Ven dose when also using moderate CYP3A4 inhibitors	Ven dose when also using strong CYP3A4 inhibitors	Ven dose when also using Posaconazole
BSA adjusted	240mg/m^2	120mg/m^2	60mg/m^2	45mg/m^2
Maximum	400mg	200mg	100mg	70mg

APPENDIX V: LIST OF PROTOCOL CHANGES IN AMENDMENTS

AMENDMENT 1:

- Expanded screening window from 4 to 7 days.
    - Reduce need for repeat assessments to avoid out of window screening data.
- Increased eligibility for Block 2b to patients with >95% blasts expressing CD19.
    - The threshold for CD19+ needed to respond to blinatumomab has not been previously well defined.
- Clarified that blinatumomab dosing for patients ≥45kg is a fixed dose rather than BSA based to align with FDA label.
    - To align with FDA label.
- Removed requirement that PI be notified for coadministration of venetoclax and azole antifungals provided appropriate dose adjustment is made.
    - Improve ease of therapy delivery. The dose adjustments are explicit within the protocol so PI input for these adjustments are not needed.
- Added information on the venetoclax powder sachet formulation.
    - Change in available venetoclax formulation.
- Indicated that patients with non-ETP T-cell acute lymphoblastic lymphoma (ALLy) can receive dasatinib.
    - Similar genomics present in ALL and ALLy suggest agent may be active. These patients do poorly with existing therapy.
- Added the option of asparaginase dose capping or dosing based on ideal body weight.
    - Alignment with other ALL trials and practice across sites.
- Changed listed asparaginase from pegaspargase to calaspargase and removed requirement to notify PI of alternative asparaginase formulations.
    - Address change in available formulation in the US but variable availability of asparaginase across sites. Research database continues to document formulation.
- Clarified that prophylactic antibiotics did not need to be started at protocol entry if the patient was not neutropenic.
    - Reduce unneeded antibiotic exposure for patients without neutropenia.
- Removed leukemia sample collection at end of therapy and added sample submission at time of relapse.
    - Align sample collection timepoints with periods most likely to be informative for mechanisms of resistance.
- Added option for peripheral blood or extramedullary leukemia/ lymphoma sample submission for correlative biology sample at diagnosis in patients with leukocytosis precluding initial bone marrow sampling.
    - Align with standard practice and with ability of patients with high peripheral blast burden to go on protocol therapy without a bone marrow, and allow analysis of samples without isolated extramedullary disease.
- Indicated that the day 1 LPIT could be delayed if required by the patient's clinical condition.
- Indicated that samples and data may be used for future research to better understand the biology of relapsed ALL and to identify potential treatments for this population.
    - To increase generalizable knowledge gained for future patients in this population with a high unmet need.

- Increased flexibility of peripheral blood immune profile sample collection.
    - To minimize the need for visits just for blood sample collection and align with blinatumomab bag changes or clinical visits.
- Increased availability for post-block 2 therapy to be given at affiliates.
    - To improve access to local therapy for these patients.
- Minor clarifications and corrections of typographical errors.

AMENDMENT 2:

- Updated consents to further clarify infectious risks.

AMENDMENT 3:

- Consents and protocol updated to include RP2D defined during phase I of blocks 2A and 2B.
- Added definition of No Response (NR) to describe patients not achieving CR or PR after block 1. The definition of treatment failure continues to describe these patients after block 2.
- Minor clarifications and corrections of typographical errors.