CHILDREN'S ONCOLOGY GROUP

The world's childhood cancer experts

Group Chair
Peter C. Adamson, M.D.
adamson@email.chop.edu

Group Statistician
Todd Alonzo, Ph.D.
talonzo@childrensoncologygroup.org

Group Vice Chair
Susan Blaney, M.D.
smblaney@txch.org

Chief Operating Officer
Elizabeth O'Connor, M.P.H.
econnor@childrensoncologygroup.org

Executive Director of Administration
Deborah L. Crabtree, M.S.
crabtreed@email.chop.edu

Group Chair's Office
The Children's Hospital of Philadelphia
3501 Civic Center Blvd
CTRB 10060
Philadelphia, PA 19104

P 215 590 6359
F 215 590 7544

Group Operations Center
222 E. Huntington Drive
Suite 100
Monrovia, CA 91016

P 626 447 0064
F 626 445 4334

Statistics & Data Center Headquarters
222 E. Huntington Drive
Suite 100
Monrovia, CA 91016

P 626 447 0064
F 626 445 4334

Gainesville Office
6011 NW 1st Place
Gainesville, FL 32607

P 352 273 0556
F 352 392 8162

A National Cancer Institute-supported member group of the National Clinical Trials Network

May 29, 2019

Martha Kruhm, MS, RAC
Head, Protocol and Information Office
Operations and Informatics Branch
Cancer Therapy Evaluation Program
Division of Cancer Treatment and Diagnosis
National Cancer Institute
Executive Plaza North Room 730
Bethesda, MD 20892

Dear Ms. Kruhm,

Enclosed please find Amendment #6 to protocol **AALL1231**, *A Phase III Randomized Trial Investigating Bortezomib (NSC# 681239; IND# [redacted]) on a Modified Augmented BFM (ABFM) Backbone in Newly Diagnosed T- Lymphoblastic Leukemia (T-ALL) and T- Lymphoblastic Lymphoma (T-LLy)*, for CTEP review.

This amendment is being submitted in response to a Request for Amendment (RRA) from Dr. John Wright, dated April 16th 2019. In this amendment, the revised CAEPR for Bortezomib, (Version 2.7, March 25, 2019) has been inserted in the protocol and the associated risk information in the informed consent document has been revised accordingly. In addition an update has been made to reflect CTCAE V5 for CTEP-AERS reporting. Language has also been added regarding permanent closure to accrual as of 5/24/19. Revisions to the protocol and consent are detailed in the pages below.

The AALL1231 study team looks forward to approval of this amendment. Please let me know if we can offer further information.

Sincerely,

Arshi Reyaz, MS, Protocol Coordinator (on behalf of)

David Teachey, M.D., Study Chair, AALL1231
Elizabeth Ann Raetz, M.D., Study Vice Chair, AALL1231
Meenakshi Devidas, Ph.D., Study Statistician, AALL1231
Mignon Loh, M.D., ALL Committee Chair
Peter Adamson, Group Chair, Children's Oncology Group

AALL1231 SUMMARY OF CHANGES
SUMMARY OF CHANGES: PROTOCOL DOCUMENT

In accordance with the above discussion, the following specific revisions have been made to the protocol. Additions are in **boldfaced** font and deletions in ~~strikethrough~~ font.

Section	Page	Comments
Title Page	1	Protocol version date and amendment number have been updated and the closure date was added.
Table of Contents	2-6	Table of contents has been updated to account for repagination.
Study Committee	7	The address for Meenakshi Devidas has been updated.
6.1	93-98	The revised CAEPR for Bortezomib (Version 2.7, March 25, 2019) has been inserted. This CAEPR, which was previously in CTCAE 4.0 language (version 2.6), has been migrated to CTCAE 5.0 language (version 2.7). There is no new or modified risk information for Bortezomib.
9.3.1.1	137	Language added regarding permanent closure to accrual as of 5/24/19.
11.0	146-150	Language was updated to reflect the use of CTCAE V5 throughout.

SUMMARY OF CHANGES: INFORMED CONSENT DOCUMENTS (CHANGES BELOW APPLY TO BOTH STUDY CONSENTS)

In accordance with the above discussion, the following specific revisions have been made to the protocol. Additions are in **boldfaced** font and deletions in ~~strikethrough~~ font.

Section	Page	Comments
Throughout	Throughout	The version date has been updated.
What side effects or risks can I expect from being in the study?	11-12	The term “muscle spasms” was removed. This risk is captured as part of “pain” under Occasional. “Velcade” has been added. Dehydration and weight loss have been combined. Additional minor administrative updates have been added.

Activated: 09/29/14
Closed: 05/24/19
Version Date: 05/29/19
Amendment #6

CHILDREN'S ONCOLOGY GROUP

AALL1231

A Phase III Randomized Trial Investigating Bortezomib (NSC# 681239; IND# [redacted]) on a Modified Augmented BFM (ABFM) Backbone in Newly Diagnosed T- Lymphoblastic Leukemia (T-ALL) and T- Lymphoblastic Lymphoma (T-LLy)

A Groupwide Phase III Study

NCI Supplied Agent: Bortezomib (NSC# 681239; IND# [redacted])

Sponsor: CTEP, NCI, DCTD
Protocol IND: [redacted]

STUDY CHAIR

Name: David T. Teachey
Address: The Children's Hospital of Philadelphia
3501 Civic Center Boulevard, CTRB 3008
Philadelphia, PA 19104
Phone: (267) 426-5802
Fax: (215) 590-3770
E-mail: teacheyd@email.chop.edu

TABLE OF CONTENTS

STUDY COMMITTEE

STUDY CHAIR
David T. Teachey, MD
Hematology/Oncology
The Children's Hospital of Philadelphia
3615 Civic Center Boulevard, ARC902
Philadelphia, PA 19104
Phone: (267) 426-5802
Fax: (215) 590-3770
E-mail: teacheyd@email.chop.edu

STUDY VICE CHAIR
Elizabeth Ann Raetz, MD
Hematology/Oncology
Primary Children's Hospital
100 N Mario Capecchi Drive
Salt Lake City, UT
Phone: (801) 662-4700
Fax: (801) 662-4707
E-mail: elizabeth.raetz@hci.utah.edu

STUDY STATISTICIAN
Meenakshi Devidas, PhD
Director, Global Analytics Unit
St. Jude Children's Research Hospital
262 Danny Thomas Place, Mail Stop 721
Memphis, TN 38105-3678
Phone: (901) 595-1764
Fax: (901) 595-5319
E-mail: mini.devidas@stjude.org

STUDY COMMITTEE MEMBERS
Robert D. Annett, PhD
Behavioral Science
University of Mississippi Medical Center
2500 N. State Street
Jackson, MS 39216
Phone: (601) 984-5863
Fax: (601) 984-5857
E-mail: rdannett@umc.edu

Barbara L. Asselin, MD
Hematology/Oncology
University of Rochester
601 Elmwood Avenue, Box 777
Rochester, NY 14642
Phone: (585) 275-2981
Fax: (585) 275-1039
E-mail: barbara_asselin@urmc.rochester.edu

STUDY COMMITTEE MEMBERS
Keith Jason August, MD MSc
Hematology/Oncology
Children's Mercy Hospitals and Clinics
2401 Gillham Road
Kansas City, MO 64108
Phone: (816) 302-6808
Fax: (816) 302-9894
E-mail: kjaugust@cmh.edu

Steve Yoon-Ho Cho, MD
Diagnostic
Wisconsin Institute for Medical Research
1111 Highland Ave
Madison, WI 53705
Phone: (608) 263-5084
Fax: (608) 265-7390
E-mail: scho@uwhealth.org

Robert J. Hayashi, MD
Hematology/Oncology
Washington University School of Medicine
One Children's Place
St. Louis, MO 63110
Phone: (314) 454-2041
Fax: (314) 454-2780
E-mail: hayashi_r@kids.wustl.edu

Stephen P. Hunger, MD
Hematology/Oncology
Children's Hospital Philadelphia
Colket Translational Research Building
3501 Civic Center Blvd
Philadelphia, PA 19104
Phone: 267 425-0197
E-mail: hungers@email.chop.edu

Brent Lee Wood, MD PhD
Pathology
Seattle Children's Hospital
Seattle Cancer Care Alliance G7-800
Seattle, WA 98109-1028
Phone: (206) 288-7060
Fax: (206) 288-7127
E-mail: woodbl@u.washington.edu

STUDY COMMITTEE MEMBERS

Alok I. Jaju, MD
Diagnostic Imaging
225 E Chicago Avenue, Box #9
Chicago, IL 60614
Phone: (312) 227-3513
Fax : (312) 227-9786
E-mail: ajaju@luriechildrens.org

Allison Lam, PharmD, BCPS
Pharmacy
Miller Children's and Women's Hospital Long Beach
2801 Atlantic Avenue
Long Beach. CA 90806
Phone: (562) 833-8161
Fax: (562) 933-8337
E-mail: alam@memorialcare.org

Mignon Lee-Cheun Loh, MD
Hematology/Oncology
Helen Diller Family Comprehensive Cancer Center
1450 3rd St HD284
San Francisco, CA 94158-3112
Phone: (415) 514-9389
Fax: (415) 353-2657
E-mail: Mignon.Loh@ucsf.edu

Yoav H. Messinger, MD
Hematology/Oncology
Children's Hospitals and Clinics of Minnesota - Minneapolis
2525 Chicago Ave S CSC-175
Minneapolis, MN 55404
Phone: (612) 813-5940
Fax: (612) 813-6325
E-mail: Yoav.Messinger@childrensmn.org

Samir I Patel, MD
Radiation Oncology
University of Alberta- Stollery Children's Hospital
11560 University Avenue
Edmonton, AB T6G1Z2
Phone: (780) 432-8518
Fax: (780) 432-8380
E-mail: samir.patel2@albertahealthservices.ca

Kathleen W. Rao, PhD
Cytogenetics
University of North Carolina at Chapel Hill
Room 1071, 1st Floor Memorial Hospital
Chapel Hill, NC 27599-7220
Phone: (919) 966-1595
Fax: (919) 966-1411
E-mail: krao@med.unc.edu

STUDY COMMITTEE MEMBERS

Eric Stephen Schafer, MD
Hematology/Oncology
Baylor College of Medicine
1102 Bates Avenue, Suite 1590.
Houston, TX 77030
Phone: (832) 824-4241
Fax: (832) 825-4170
E-mail: esschafe@txch.org

Tal Tova Schechter-Finkelstein, MD
Stem Cell Transplantation
Hospital for Sick Children
555 University Avenue
Toronto, ON M5G1X8
Phone: (416) 813-4505
Fax: (416) 813-5372
E-mail: tal.schechter-finkelstein@sickkids.ca

Kristin Ammon Shimano, MD
Hematology/Oncology
UCSF Medical Center-Mission Bay
550 16th Street, Box 0434
San Francisco, CA 94158
Phone: (415) 476-3831
Fax: (415) 353-2657
E-mail: ShimanoK@peds.ucsf.edu

Amii Corbisiero Steele, PhD
Behavioral Science
Carolinas Medical Center/Levine Cancer Institute
1001 Blythe Blvd, MCP Suite 601
Charlotte, NC 28232
Phone: (704) 381-9966
E-mail: amii.steele@carolinashealthcare.org

Stuart Sheldon Winter, MD
Hematology/Oncology
University of New Mexico
1 University of New Mexico, MSC 10-5590
Albuquerque, NM 87131-0001
Phone: (505) 272-4461
Fax: (505) 272-8699
E-mail: swinter@salud.unm.edu

Patrick A. Zweidler-McKay, MD PhD
Hematology/Oncology
M D Anderson Cancer Center
1515 Holcombe Blvd. Box 853
Houston, TX 77030
Phone: (713) 563-5395
Fax: (713) 563-5407
E-mail: pzweidler@mdanderson.org

STUDY COMMITTEE MEMBERS

Michelle Hermiston, MD PhD
Hematology/Oncology
UCSF Medical Center – Mission Bay
550 16th Street, Box 0434
San Francisco, CA 94158-3112
Phone: (415) 476-3831
Fax: (415) 353-2657
E-mail: Michelle.Hermiston@ucsf.edu

Terzah M. Horton, MD PhD
Hematology/Oncology
Baylor College of Medicine
1102 Bates Avenue, Suite 750
Houston TX, 77030
Phone: (832) 824-4269
Fax: (832) 824-1206
E-mail: tmhorton@txch.org

Kimberly Panter Dunsmore, MD
Hematology/Oncology
University of Virginia Cancer Center
Box 800386
Charlottesville, VA 22908
Phone: (434) 924-5105
Fax: (434) 982-1927
E-mail: kpd6u@virginia.edu

Brian Thomas Fisher, DO MSCE
Pediatrics, Division of Infectious Diseases
The Children's Hospital of Philadelphia
34th Street and Civic Center Boulevard
Philadelphia, PA 19104
Phone: (267) 426-7110
Fax: (215) 590-2017
E-mail: fisherbria@email.chop.edu

Paul John Galardy, MD
Hematology/Oncology
Mayo Clinic
200 First Street SW
Rochester, MN 55906
Phone: (507) 284-2695
galardy.paul@mayo.edu

Rodney R. Miles, MD PhD
Pathology
Primary Children's Hospital
15 N. Medical Dr. East JMRB Rm 2100
Salt Lake City, UT 84112-0565
Phone: (801) 213-3448
Email: rodney.miles@path.utah.edu

Maki Okada, RN, CPNP
Nursing
Miller Children's & Women's Hospital Long Beach
2801 Atlantic Ave
Long Beach, CA 90806
Phone: (562) 728-5000
Fax: (562) 933-1815
E-mail: mokada@memorialcare.org

Neelam Singh, PhD
Clinical Research Associate
Michigan State University Clinical Center
Sparrow Professional Building Suite 145
1200 East Michigan Avenue
Lansing, MI 48912
Phone: (517) 364-5591
Email: neelam.singh@hc.msu.edu

COG RESEARCH COORDINATOR

Charlotte Wood, CCRP
Children's Oncology Group - Data Center (Gaines)
6011 NW 1st Place
Gainesville, FL 32607
Phone: (352) 273-0573
Fax: (352) 392-8162
E-mail: cwood@cog.ufl.edu

Jason Lawrence Freedman, MD
Hematology/Oncology
The Children's Hospital of Philadelphia
3501 Civic Center Boulevard, CTRB 10405
Philadelphia, PA 19104
Phone: (215) 906-7885
E-mail: feedmanj@email.chop.edu

COG PROTOCOL COORDINATOR

Sarah L. Vargas, Ph.D.
Children's Oncology Group - Operations Center
222 E Huntington Drive Suite 100
Monrovia, CA 91016
Phone: (626) 241-5116
Fax: (626) 445-4334
Email: svargas@childrensoncologygroup.org

Maria Luisa Sulis, MD
Hematology/Oncology
Columbia University Medical Center
161 Fort Washington Ave -IP7
New York, NY 10032
Phone: (212) 305-5808
Fax: (212) 305-5848
Email: mls95@columbia.edu

CTEP Supplied Agent: Bortezomib, NSC# 681239
IND# [redacted]
(IND Sponsor: CTEP)
Cyclophosphamide, NSC# 026271, Commercial
Cytarabine, NSC# 063878, Commercial
Daunorubicin, NSC# 082151, Commercial
Dexamethasone, NSC# 034521, Commercial
Doxorubicin, NSC# 123127, Commercial
Etoposide, NSC# 141540, Commercial
Filgrastim, NSC# 614629, Commercial
Hydrocortisone, NSC# 010483, Commercial
Ifosfamide, NSC# 109724, Commercial
Leucovorin, NSC# 003590, Commercial
6-Mercaptopurine, NSC# 000755, Commercial
Mesna, NSC# 113891, Commercial
Methotrexate, NSC# 000740, Commercial
Pegaspargase, NSC# 624239, Commercial
6-Thioguanine, NSC# 000752, Commercial
Vincristine, NSC# 675574, Commercial

The Children's Oncology Group has received a Certificate of Confidentiality from the federal government, which will help us protect the privacy of our research subjects. The Certificate protects against the involuntary release of information about your subjects collected during the course of our covered studies. The researchers involved in the studies cannot be forced to disclose the identity or any information collected in the study in any legal proceedings at the federal, state, or local level, regardless of whether they are criminal, administrative, or legislative proceedings. However, the subject or the researcher may choose to voluntarily disclose the protected information under certain circumstances. For example, if the subject or his/her guardian requests the release of information in writing, the Certificate does not protect against that voluntary disclosure. Furthermore, federal agencies may review our records under limited circumstances, such as a DHHS request for information for an audit or program evaluation or an FDA request under the Food, Drug and Cosmetics Act. The Certificate of Confidentiality will not protect against mandatory disclosure by the researchers of information on suspected child abuse, reportable communicable diseases, and/or possible threat of harm to self or others.

ABSTRACT

AALL1231 is a COG group-wide Phase III study for patients between 1-30 years of age with newly diagnosed T- lymphoblastic leukemia (T-ALL) or T-Lymphoblastic Lymphoma (T-LLy) that will assess whether the addition of bortezomib (PS-341) to a modified augmented BFM backbone will decrease relapse risk and improve event-free survival (EFS) and overall survival (OS). Although EFS and OS continue to increase for children and young adults with T-ALL and T-LLy, patients who relapse have a dismal prognosis. These patients are extremely difficult to salvage as they tend to relapse early and with chemotherapy-refractory disease. There is strong biological rationale for introducing bortezomib early in treatment to prevent relapse. Bortezomib has been shown to have a favorable synergistic interaction with backbone chemotherapy drugs and the potential to reverse both innate and acquired steroid resistance. In addition, early phase clinical trials suggest bortezomib is a therapeutically active agent in T-ALL and T-LLy.

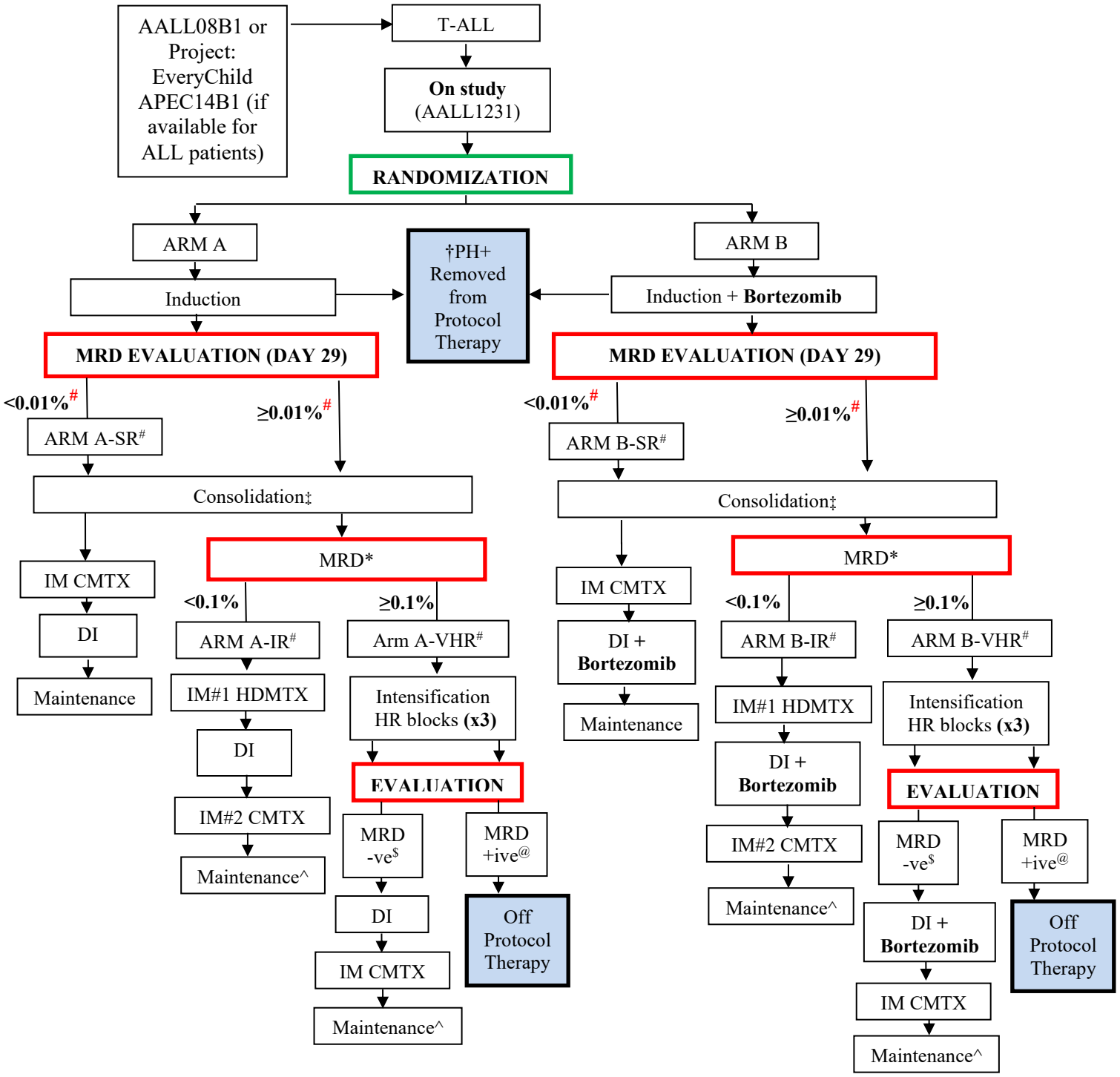
Patients will be randomized to receive backbone therapy with or without bortezomib during Induction and Delayed Intensification. T-ALL patients will be stratified into standard risk (SR), intermediate risk (IR), or very high risk (VHR) groups based on assessments minimal residual disease (MRD) at Day 29 and End of Consolidation (EOC). T-LLy patients will be risk stratified into the same groups, based on percentage disease in marrow at diagnosis and radiographic disease response at end of Induction.

The augmented BFM backbone will be modified in this trial to include dexamethasone as the sole corticosteroid throughout therapy and to increase the exposure to pegaspargase, based on the success of this approach in the recently completed UKALL 2003 trial. This trial also aims to establish whether prophylactic cranial radiation therapy (CRT) can be safely eliminated from the treatment in the vast majority (~90%) of children with T-ALL and to determine if intensification of chemotherapy in VHR T-ALL and T-LLy patients will prevent relapse. Finally, this trial aims to determine whether a response assessment after Intensification therapy in VHR patients (T-ALL and T-LLy) can identify patients who are chemotherapy refractory and who may potentially benefit from novel agents and/or stem cell transplant.

Amendment 3 includes the correction to an error in the date of administration of PEG-Aspargase during Delayed Intensification, and emphasizes supportive care guidelines to remind clinicians of the potential and expected risk of Invasive Fungal Infections, especially during Induction and periods of prolonged neutropenia.

Amendment 4 revises the eligibility criteria for T-cell Lymphoblastic Lymphoma (T-LLy) patients, and clarifies classification study enrollment for T-cell Acute Lymphoblastic Leukemia (T-ALL) patients.

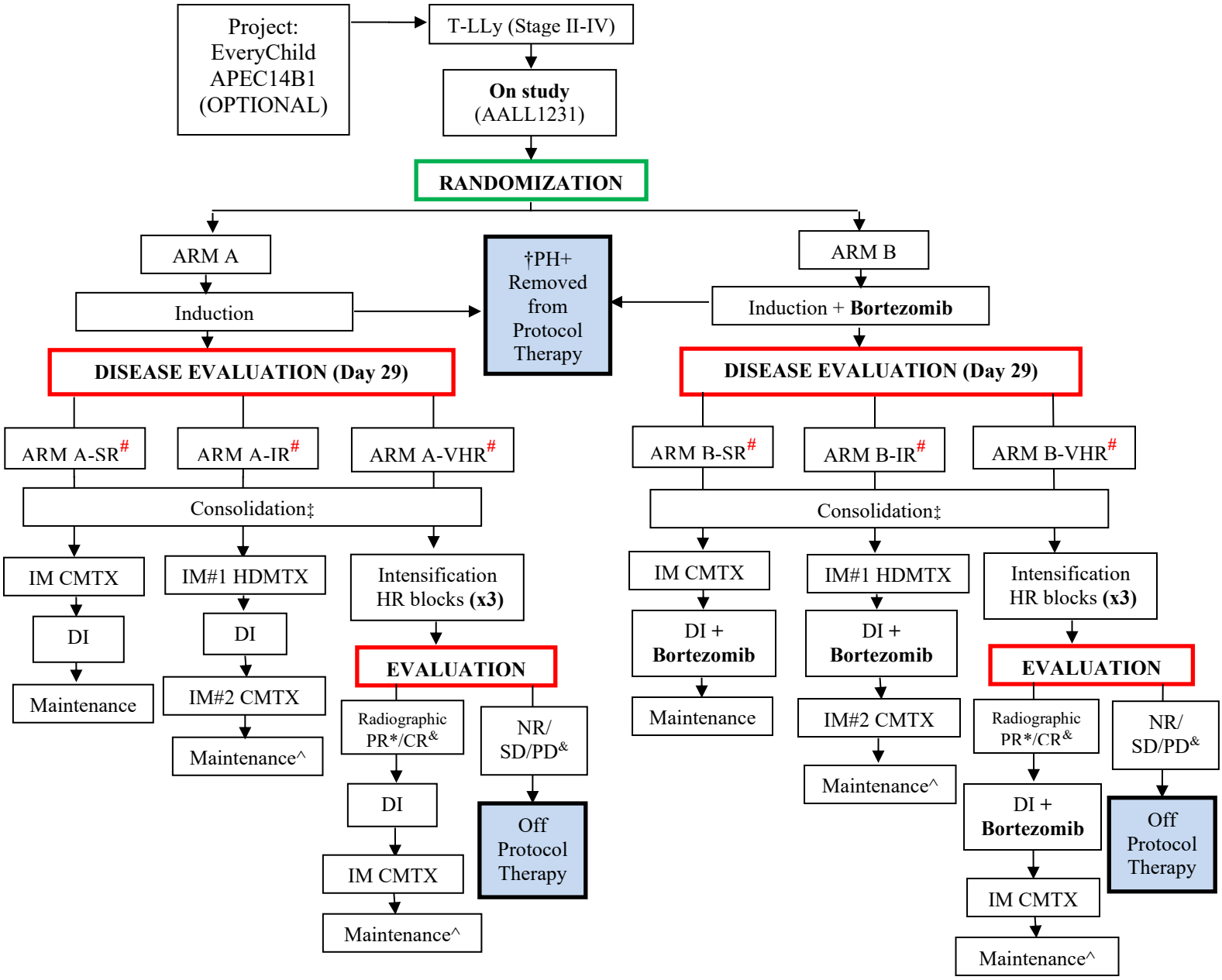
EXPERIMENTAL DESIGN SCHEMA: T-ALL



T-ALL: T-Lymphoblastic Leukemia **MRD:** Minimal Residual Disease
HR: High Risk **SR:** Standard Risk **IR:** Intermediate Risk **VHR:** Very High Risk
IM: Interim Maintenance **DI:** Delayed Intensification **CMTX:** Capizzi Methotrexate
HDMTX: High Dose Methotrexate $Undetectable MRD @Detectible MRD

#See Section 3.4 for definitions. **OF NOTE**, subjects who are CNS2, CNS3, or have testicular disease, or were steroid pre-treated CANNOT be SR.
†See Section 3.5 for details ^ T-ALL: all VHR subjects regardless of CNS status, and IR subjects with CNS3 disease will receive cranial radiation therapy (CRT). See Section 16.1 for details.
‡ Patients with persistent testicular disease at end-Induction should receive radiation to the testes during Consolidation. A testicular biopsy should be performed if the clinical findings are equivocal. See Section 16.2 for details.
*Induction failures are treated as VHR regardless of MRD status

EXPERIMENTAL DESIGN SCHEMA: T-LLy (Stages II-IV)



T-LLy: T-Lymphoblastic Lymphoma **MRD:** Minimal Residual Disease
HR: High Risk **SR:** Standard Risk **IR:** Intermediate Risk **VHR:** Very High Risk
IM: Interim Maintenance **DI:** Delayed Intensification
CMTX: Capizzi Methotrexate **HDMTX:** High Dose Methotrexate
CR: complete response **PR:** Partial Response

#See Section 3.4 for definitions. **OF NOTE**, subjects who are CNS2, CNS3, have testicular disease, or were steroid pre-treated, CANNOT be SR.
†See Section 3.5 for details
^ T-LLy subjects with CNS3 disease receive cranial radiation therapy (CRT). See Section 16.1 for details
*Patients who are in a radiographic PR at the end of the 3 Intensification HR blocks should be re-biopsied. If the biopsy finds residual disease, the patient comes off protocol therapy. If the biopsy does not demonstrate active disease, the patient continues on therapy at DI
‡ Patients with persistent testicular disease at end-Induction should receive radiation to the testes during Consolidation. A testicular biopsy should be performed if the clinical findings are equivocal. See Section 16.2 for details. &See Section 10.4 for details

1.0 GOALS AND OBJECTIVES (SCIENTIFIC AIMS)

1.1 Primary Aims

1.1.1 To compare EFS in patients with newly diagnosed T-ALL and T-LLy who are randomized to a modified ABFM backbone versus bortezomib plus the modified ABFM backbone.

1.2 Secondary Aims

1.2.1 To determine the safety and feasibility of modifying standard therapy for T-ALL and T-LLy based on the results of UKALL 2003, which includes a dexamethasone-based Induction, additional doses of pegaspargase (PEG-ASP) during Induction and Delayed Intensification (DI), and dexamethasone pulses during Maintenance therapy

1.2.2 To determine if prophylactic (presymptomatic) cranial radiation therapy (CRT) can be safely and effectively eliminated in the 85-90% of T-ALL patients classified as standard or intermediate risk.

1.2.3 To determine the proportion of EOC MRD ≥ 0.1% T-ALL patients who become MRD negative (undetectable by flow cytometry) after intensification of chemotherapy, using three high risk (HR) BFM blocks, and to compare EFS between the patients who become MRD negative after the three HR BFM blocks and continue on chemotherapy with those who continue to have detectable MRD and are eligible for other treatment strategies, including hematopoietic stem cell transplant (HSCT). Similarly, to compare the EFS between very high risk (Induction failure) T-LLy patients treated with HR BFM intensification blocks who have partial or complete response (PR or CR) with those who do not respond (NR).

1.3 Correlative Aims

1.3.1 To investigate the prognostic significance of Day 29 BM MRD in T-LLy patients.

1.3.2 To determine if protein expression patterns can predict bortezomib response and drug resistance in T-ALL

1.3.3 To analyze and target relevant signaling pathways in T-ALL blasts, focusing on Early T cell Precursor (ETP) ALL

2.0 BACKGROUND

2.1 Trial Rationale

T-cell lymphoid malignancies have distinct biochemical, immunologic and clinical features which set them apart from non-T lymphoid malignancies.[1,2] Historically, the diagnosis of T-Lymphoblastic Leukemia (T-ALL) portended a worse prognosis than other forms of non-T childhood ALL.[3,4] Over the past three decades, the introduction of intensive, high-dose, multi-agent pulse chemotherapy has improved the EFS for patients with T-ALL from 15%-20% to 50%-85%. The outcomes for T-ALL are now comparable to those of children with high risk B Lymphoblastic Leukemia (B-ALL).[5-8] Lymphoblastic Lymphoma (LL) accounts for approximately 25% of all pediatric non-Hodgkin lymphoma (NHL) cases. The history of the treatment for LL over the past 3 decades reflects the evolution of treatment from conventional lymphoma-based treatment to ALL-based treatment. With current therapies for T-LLy (~90% of pediatric LL), the majority of patients, including those with advanced stage disease, can expect a likelihood of 80%-85% EFS utilizing regimens modified from those used to treat ALL.[9-11]

Despite these significant advances, relapsed T-ALL and T-LLy have a dismal prognosis with reported 3-year EFS rates of <15%.[12,13,14] These patients are extremely difficult to salvage as most have chemotherapy-refractory disease. Accordingly, the primary goal in this trial is to prevent relapse. AALL1231 will test two major strategies to meet this goal: (1) to modify the existing chemotherapy platform to mirror the most efficacious backbone that has been tested in Phase 3 trials; and, (2) to randomize patients to receive/not receive a novel agent (bortezomib), with strong biologic rationale, introduced early into therapy in a safe and feasible manner to determine if this will improve EFS and OS.

2.1.1 Rationale for Incorporating Bortezomib

AALL1231 is a Phase 3 randomized trial with the primary objective of testing the safety and efficacy of adding bortezomib (PS-341) to a modified ABFM backbone in patients with newly diagnosed T-ALL and T-LLy. Bortezomib has been studied extensively in preclinical models of T-ALL. The pediatric preclinical testing program (PPTP) tested bortezomib *in vitro* in T-ALL cell lines finding single agent activity and *in vivo* using xenografted NOD-SCID mice and found a 100% response rate in T-ALL (2 of 2 samples) compared with a 40% (2 of 5 samples) response rate in pre-B ALL.[15] These data are not surprising as T-ALL blasts often have considerable activation of the NF-kappa B pathway, frequently as a consequence of activated Notch1.[16]

In order to move a new agent into a complex chemotherapy backbone, it is important to investigate the activity of the novel agent with conventional cytotoxics in preclinical models. Bortezomib has a favorable additive or synergistic interaction against T-ALL when combined with a number of cytotoxic agents, including dexamethasone (DEX), cytarabine, doxorubicin, asparaginase, vincristine, and etoposide *in vitro* (Table 1).[17] One benefit of targeting the proteasome with bortezomib is the potential to overcome chemotherapy resistance. Bortezomib has been shown to overcome chemotherapy resistance to anthracyclines, alkylators, and corticosteroids in a number of malignancies.[18-22] Preclinical data suggest that bortezomib may sensitize steroid resistant T-ALL cells to corticosteroids.[23] As corticosteroid resistance is correlated with a particularly poor prognosis in T-ALL, the possibility of overcoming steroid resistance is quite attractive. The plan to treat subjects with bortezomib during Induction and Delayed Intensification (DI) gives the potential to reverse both innate and acquired steroid resistance as well as take advantage of the synergistic interaction between DEX and bortezomib. Finally, preclinical data suggest that proteasome inhibition may also reverse anthracycline resistance in T-ALL.[24]

Bortezomib has been studied in early phase trials in adults and children and has an acceptable toxicity profile. Dose limiting toxicities (DLTs) in early phase trials in adults with hematologic malignancies included thrombocytopenia, hyponatremia, fluid retention, diarrhea, hypokalemia, fatigue, and malaise.[25,26] A Phase 1 study in children with refractory leukemia (ADVL0317) demonstrated a maximum tolerated dose (MTD) of 1.3 mg/m^2 given twice weekly for 2 weeks as a single agent, followed by a 10-day rest.[27] DLTs were febrile neutropenia with hypotension and azotemia. Early data also suggest that bortezomib may be active against T-cell lymphomas in adults when given as a single agent or in combination with chemotherapy.[28-32]

Bortezomib was safely incorporated into an intensive ALL re-induction backbone (TACL 2005-003) and is currently under investigation in the COG AALL07P1 first relapse ALL trial.[33] AALL07P1 incorporates bortezomib into the AALL01P2 backbone. AALL07P1 recently successfully completed the second stage of the Simon 2-stage design. Sixty-one eligible evaluable B-ALL patients were accrued, including 28 with early (<18 months) and 33 with intermediate (18-36 months) relapse. Forty-two CRs were observed in this phase, resulting in exceeding the efficacy boundary, and bortezomib was deemed worthy of further investigation in pediatric ALL.[34] AALL07P1 also enrolled T-ALL and T-LLy patients with descriptive statistics regarding response. Thus far, 17 eligible, evaluable T-ALL patients have been treated on AALL07P1 and 11 of 17 (65%) have obtained CR2 at the end of Block 1. This is in contrast to AALL01P2, in which only 1 of 7 (17%) T-ALL patients attained CR2 at the end of the first block of re-induction therapy (Block 1). While patient numbers are small, the response to chemotherapy plus bortezomib is extremely encouraging, as the CR2 rate is very similar to that of B-ALL patients with early relapse (64%). Relapsed B-ALL patients have also been treated with bortezomib in combination with re-induction chemotherapy on the TACL study (2005-003) with a highly promising 80% objective response rate (ORR) (CR +CRp).[34] The preclinical data strongly suggest T-ALL/T-LLy should be at least as sensitive to bortezomib as B-ALL. Together the response rates of relapsed B- and T-ALL on AALL07P1 in conjunction with published TACL data in B-ALL support testing bortezomib in patients with newly diagnosed ALL. Bortezomib has also been shown to be active as a single agent and in combination with chemotherapy in multiple studies in adults with advanced T-cell lymphomas.[28,29,35] More detailed data describing the use of bortezomib in clinical trials is included in the relevant data (Section 2.4) below.

Based on these data, all patients with T-ALL and T-LLy will be randomized to receive or not receive 8 doses of bortezomib 1.3 mg/m^2/dose (4 doses in Induction on Days 1, 4, 8, and 11 and 4 doses in Delayed Intensification (DI) on Days 1, 4, 15, and 18). These schedules of bortezomib are designed to optimize the co-administration of bortezomib and DEX.

2.1.2 Rationale for Updating Risk Stratification

MRD is the single most powerful prognostic factor in childhood ALL.[36-38] A number of large studies have investigated the sensitivity and specificity of MRD at different time points on a variety of treatment protocols, using either flow- or PCR-based technologies.[39] While the majority of data suggest that end Induction MRD assessment is the best predictor of outcome in B-ALL, recent data from AIEOP-BFM ALL 2000 strongly suggest that EOC MRD is a better predictor of adverse outcome in T-ALL.[38] These data are based on European studies that measure MRD using molecular techniques. In COG T-ALL studies, MRD is measured by flow cytometry. While the MRD cutoffs that predict outcome using flow-based MRD are not as clearly defined, several studies have shown that flow cytometry

and PCR amplification of antigen-receptor genes yield remarkably concordant measurements if MRD is present at a ≥ 0.01% level.[40-42] On AIEOP-BFM ALL 2000, patients were stratified into 3 risk groups based on MRD and early prednisone response. Patients with a prednisone good response who were MRD <0.01% at end Induction and EOC were standard risk. Patients who had an EOC MRD ≥ 0.1% or were prednisone poor responders were considered high risk. All others were classified as intermediate risk. The same MRD cutoffs will be used on AALL1231.

A recent review of data from children enrolled on AALL0434 (the current open COG trial for newly diagnosed T-ALL and T-LLy) validated the prognostic value of these MRD cutoffs for patients with T-ALL (unpublished and DMSC protected confidential data).

On AALL1231, T-ALL patients will be stratified into 3 risk groups based on MRD: Standard Risk (SR) - Day 29 M1 marrow, Day 29 BM MRD < 0.01%, CNS 1, no testicular disease, no pre-treatment with corticosteroids; Intermediate Risk (IR) - Day 29 M1 or M2 marrow, Day 29 marrow MRD ≥ 0.01%, EOC MRD < 0.1%, any CNS and testicular disease status; and, Very High Risk (VHR) - EOC MRD ≥ 0.1% or Induction failure (M3 marrow), any CNS or testicular disease status. This stratification is estimated to allocate subjects as follows: SR T-ALL: 40%-50% of T-ALL patients; IR T-ALL: 35%-50% of patients; VHR T-ALL: 10%-15% of patients.

T-ALL subjects enrolled on AALL1231 will receive a modified ABFM backbone based on the data from UKALL 2003 (described below) and will be randomized to receive or not receive bortezomib during Induction and the re-Induction portion of DI. Modifications from the COG ABFM backbone include the use of DEX 6 mg/m^2/day x 28 days during Induction, administration of 2 rather than 1 dose of pegaspargase during Induction and the re-Induction portion of Delayed Intensification, and the use of DEX rather than prednisone pulses during Maintenance therapy.

After Induction, the three risk groups will be treated differently. SR subjects will receive 1 Interim Maintenance (IM) phase with Capizzi methotrexate (CMTX) and PEG-ASP. They will not receive prophylactic cranial radiation therapy (CRT). IR subjects will receive 2 IM phases: the first with high dose methotrexate (HD MTX) and the second with CMTX and PEG-ASP. They will not receive prophylactic CRT. Patients with CNS disease (CNS3) will receive 1800 cGy CRT during the first Maintenance cycle. VHR subjects will receive 3 HR BFM blocks in lieu of IM #1. If they become MRD negative (undetectable) at end of HR3, then they will remain on study and receive DI (± bortezomib as randomized) and IM #2 with CMTX and PEG-ASP. During the first cycle of maintenance therapy they will either receive 1200 cGy CRT (CNS1 or CNS2) or 1800 cGy CRT (CNS3). Subjects who remain MRD positive (any level of detectable MRD) at the end of HR3 will go off study and presumably most will undergo HSCT.

All other components of therapy will remain the same for all three risk groups. The rationale for these differences is described in the Section 2.1.3 below describing the backbone modifications and plans to eliminate prophylactic CRT in most patients.

On AALL0434, T-LLy patients were risk stratified into SR, HR, or Induction failure based on the disease burden in their marrow at diagnosis and by disease response, measured radiographically and by bone marrow morphology at the end of Induction. T-LLy patients with < 1% disease in the marrow at diagnosis will continue to be classified as SR on this study and those with ≥ 1% marrow disease at diagnosis or pre-treated with corticosteroids will be classified as IR. Patients who fail to achieve at least a radiographic partial response

(PR) or who do not achieve morphological marrow remission at the end of Induction will be designated VHR (Induction failures). In addition, similar to patients with T-ALL, Day 29 marrow MRD may be obtained. Patients who have identifiable marrow disease at diagnosis by morphology will need a repeat bone marrow at Day 29. Patients who do not have identifiable marrow disease by morphology at diagnosis have the option of a Day 29 marrow for correlative studies. MRD at diagnosis will be used in T-LLy risk stratification. Day 29 MRD will not be used for risk stratification but will be important for correlative endpoints. Response definitions for T-LLy patients are provided in Section 9.0. SR T-LLy subjects will receive 1 IM phase with CMTX and PEG-ASP, IR subjects will receive 2 IM phases (the first with HD MTX and second with CMTX and PEG-ASP), and VHR subjects will receive 3 HR BFM blocks; the same as the treatment strategy for T-ALL. If VHR patients achieve a CR/PR at end of HR3, then they will remain on study and receive DI (± bortezomib as randomized) and IM #2 with CMTX and PEG-ASP. They will either receive 1800 cGy CRT if CNS3. No T-LLy patient will receive prophylactic CRT. The rationale for these differences is described below.

2.1.3 Rationale for Modifying Backbone.

Rationale for steroid modifications

UKALL 2003 is a recently completed Phase 3 clinical trial that tested whether treatment can be safely and effectively stratified using MRD in patients with B- and T-ALL.[43] UKALL 2003 utilized a modified COG ABFM regimen that included Capizzi MTX with PEG-ASP, rather than HD MTX during IM. Even though only CNS3 patients received CRT, T-ALL patients treated on this trial had an 84% 5yr EFS, a rate superior to any published results from a multi-center trial in newly diagnosed T-ALL.[44-46] In addition, T-ALL patients had markedly improved survival when compared to the preceding trial, UKALL 97/99 (86% vs. 73% 3yr EFS; 90% vs. 78% 3 yr. OS).[45,46] The most significant protocol changes were a transition to PEG-ASP from native *E. Coli* asparaginase and the use of DEX as the only corticosteroid for all patients. The UKALL 2003 Induction included DEX 6 mg/m^2/day x 28 days with the same dose of DEX given for 5 days every 4 weeks during Maintenance therapy. They also found that rapid responder (RER) T-ALL subjects with NCI HR features had a 5yr EFS and OS of 86.7% and 91.2%, respectively.[46] This was in contrast to RER T-ALL subjects with NCI SR features who had a worse outcome (80.1% EFS and 85.6% 5 year OS). One major difference between the two groups was that the RER NCI SR subjects received a three-drug DEX based Induction and a 4-week oral 6-MP based Consolidation, while the RER NCI HR subjects received a four-drug DEX-based Induction and a BFM style Consolidation, confirming the importance of a 4-drug Induction and early intensive therapy in T-ALL. All T-ALL subjects on the recently opened successor trial, UKALL 2011, will receive a 4-drug Induction with DEX and the intensified PEG-ASP with either a standard BFM consolidation or an ABFM style consolidation based on Day 29 MRD, as given in UKALL 2003.

The UKALL 2003 regimen was well tolerated with acceptable rates of toxicity, including Induction mortality and osteonecrosis.[43,46] The Induction mortality rate on UKALL 2003 was 2% for ALL patients treated with the anthracycline containing Induction, DEX at 6mg/m^2/day, and an extra dose of PEG-ASP on day 18. [43] This TRM rate is quite similar to that seen in other recent studies using a 4-drug Induction regimen, including AIEOP-BFM ALL 2000 and COG AALL0232 (2.16% for AYA patients vs. 1.67% for younger patients).[47] The vast majority of studies that encountered excess toxicity with DEX-based 4-drug Inductions used higher doses of 10 mg/m^2/day, as opposed to the 6 mg/m^2/day schedule proposed in AALL1231. The rate of symptomatic osteonecrosis in patients over 10 years of age treated on UKALL 2003 was lower than the rate found in the prednisone arm of AALL0232 (12% vs 15%).[43,46,48] Two older studies did include DEX in Induction

at 6 mg/m^2/day for 28 days. DFCI 91-01 found a higher rate of infectious toxicity, using DEX at 6mg/m^2/day in Induction as compared with prednisone; however, this trial also included high dose MTX (4gm/m^2) on day 2-3 of Induction.[49] POG 9905/6 initially included DEX at 6mg/m^2/day for 28 days in a four-drug Induction regimen.[50] DEX was changed to prednisone after 2 infectious deaths occurred among the first 32 treated subjects. Nevertheless, on the UKALL2003 trial over 1000 patients were treated with a 4-drug Induction regimen, including DEX at 6mg/m^2/day, and it was a safe and well-tolerated regimen with modern supportive care guidelines.

The current AIEOP-BFM ALL 2009 trial uses 7 days of prednisone at 60 mg/m^2/day followed by 21 days of DEX at 10 mg/m^2/day (plus taper) for prednisone good responder T-ALL patients. The AIEOP-BFM ALL 2000 trial compared this DEX-based Induction with a prednisone-based Induction. On that trial the Induction treatment-related mortality (TRM) was higher in the DEX arm (2% vs 0.9%); however, the EFS/OS for T-ALL patients was superior in the DEX arm with a threefold reduction in the cumulative incidence of relapse (6% vs. 20%, p =0.003), leading to the continued use of DEX for T-ALL patients with a good prednisone response in the successor AIEOP-BFM ALL 2009 trial.[46,51] No difference was seen in the overall rate of symptomatic osteonecrosis, comparing the two arms.[52]

The DFCI ALL Protocol 00-01 randomized patients to receive DEX or prednisone pulses every 3 weeks for 104 weeks of Intensification and Maintenance.[53] T-ALL patients and all patients 10+ years old were classified as high-risk patients, and they were treated with 120 mg/m^2/day of prednisone vs. 18 mg/m^2/day of DEX, during a 30-week intensification phase and with 40 mg/m^2/day of prednisone vs. 6 mg/m^2/day of DEX, during a 72-week consolidation phase. They found a higher rate of symptomatic ON in children over 10 years of age treated with DEX. They also found a significant 5-year EFS advantage for DEX among all patients (90% vs. 81%; p = 0.01). While the number of T-ALL patients was relatively small (n=39), the advantage for DEX was particularly striking in this group with 5-year EFS 96% vs. 65%. Accordingly, the Dana Farber Cancer Institute (DFCI) is also continuing to use DEX in all adolescents based on the improved survival. Of note, the Maintenance pulses of DEX proposed on AALL1231 are every 4 weeks, not every 3 weeks, as used in the DFCI, and the DFCI regimen contained 10 pulses of DEX at doses 3-fold higher than those used by the COG.

The MRC and BFM recently opened Phase 3 ALL trials (AIEOP-BFM ALL 2009 and UKALL 2011) and in both of these trials T-ALL patients are treated with a DEX, regardless of age, based on its superior efficacy in T-ALL. SJCRH also uses DEX during maintenance/continuation for teenagers.

The 6mg/m^2/day DEX schedule has the potential to provide the enhanced efficacy of DEX over prednisone without the toxicity found in the 10 mg/m^2/day regimens. In addition, infectious toxicity on AALL07P1 (bortezomib plus backbone) during Block 1 therapy is similar to AALL01P2 (backbone alone) and ADVL04P2 (backbone plus epratuzumab). Each of these relapsed ALL trials have a 45% Grade 3-5 infection rate in Block 1. Accordingly, it is not anticipated that bortezomib will add significant infectious risk (Horton, Rheingold, and Raetz, personal communications).

Rationale for additional Asparaginase therapy
Based on these promising results, all patients enrolled in the current trial will be treated with the same DEX-based Induction and DEX will be used as the sole steroid in other blocks, mirroring UKALL 2003. In addition, a number of studies suggest additional ASP

may improve outcome in T-ALL (UKALL 2003, POG 8704, DFCI (85-01, 87-01, 91-01), and St. Jude Total Therapy XV).[6,8,54] Accordingly, this trial will non-randomly include a modest increase in the number of doses of PEG-ASP, as compared to AALL0434, with one additional dose during Induction and during DI. UKALL 2003 only included one additional dose during Induction. Additionally, two doses will be included in the second Capizzi IM Phase. The vast majority of subjects on this trial therefore will receive 9 doses of PEG-ASP as compared with 5 or 7 doses on AALL0434. Subjects on the high-risk stratum of UKALL 2003 received 12 well-tolerated doses of PEG-ASP delivered during the first 6 courses of therapy. NCI HR patients on the Capizzi MTX arm of AALL0232 received 2 Capizzi IM and 2 DI phases with a total of 11 doses of PEG ASP with acceptable toxicity.

2.1.4 Rationale for Eliminating Prophylactic CRT in the Majority of Patients

Regardless of treatment modality, between 3%-8% of children treated for *de novo* ALL ultimately have a CNS relapse.[55] A significant majority of patients with T-ALL treated on COG and POG trials have traditionally received prophylactic CRT in order to reduce CNS relapse, though RER non-CNS3 T-ALL patients did not receive CRT on CCG 1961.[56] RER non-CNS T-ALL patients (who did not receive CRT) had a higher incidence (7.9%) of CNS relapse than slow early responder (SER) non-CNS T-ALL patients (0.8%, who did receive 1200cGy CRT) on CCG 1961, leading to increased use of CRT in T-ALL in subsequent COG trials.[57] AALL0434 is non-randomly investigating whether CRT can be safely eliminated from low-risk T-ALL patients, but this population includes only ~10% of patients. While effective, the usefulness of CRT may be offset by substantial long-term adverse effects, including second cancers, irreversible endocrinopathies, neurocognitive, and neurotoxic effects. Pui *et al*. showed that previous irradiation in 10-year event-free survivors of childhood ALL was associated with a 20.9% cumulative risk of second neoplasms at 20 years (30 years from remission Induction), a higher mortality rate than the general population, and an increased unemployment rate.[58]

Partly, in an effort to reduce these events, between 1983-1989, the European Organization for the Research and Treatment of Cancer (EORTC) treated medium and high risk ALL patients on studies 58831 and 58832 with early, frequent IT MTX, systemic HD MTX, increased doses of ASNase and a DI phase while randomizing patients to receive or not to receive standard prophylactic CRT. While patients with overt CNS disease at diagnosis were not eligible for 58831 or 58832, there was no statistically significant difference in 6-year DFS (66%-68%), isolated CNS relapse rate (7%) or total CNS relapse rate (9%-15%) in the two study arms.[59] T-ALL patients were enrolled and randomized to receive or not receive prophylactic radiation on 58831 or 58832; however, immunophenotypic data was not systematically collected, precluding analysis of T-ALL specific outcomes. Based on these results, several subsequent studies have continued to intensify systemic and intrathecal therapy and non-randomly omit CRT for a majority of ALL patients. EORTC 58881 and 58951, the follow-up studies to 58831 and 58832, eliminated CRT for all *de novo* ALL patients, including those with CNS manifestations at diagnosis (CNS3), while further intensifying systemic and intrathecal therapies. Both studies included multiple courses of HD MTX and triple (MTX, hydrocortisone and ARAC) intrathecal chemotherapy (ITT), and 58951 included an intensified Induction. In 58881, the 8 year isolated and overall CNS relapse incidences in T-ALL patients decreased to 6.8% and 10.9% respectively, and these decreased further in T-ALL patients to 5.3% and 8.5% respectively in 58951.[59,60]

St. Jude Total Therapy trials have also eliminated CRT for all patients with *de novo* ALL. In Total Therapy XV, patients received systemic chemotherapy with DEX as the Induction

and post-Induction steroid, HD MTX, intensified ASNase and an intensified regimen of ITT and with no prophylactic CRT. Isolated CNS relapse occurred in 7.7% of T-ALL patients.[54] This showed relative equivalence to the study's predecessor, Total Therapy XIIIB which showed a 7% isolated CNS relapse rate despite utilizing prophylactic CRT in patients with either CNS3 disease at diagnosis or T-ALL presenting with a WBC count of ≥50,000/μL.[61] In addition, all Total Therapy XV patients with isolated CNS relapse were successfully salvaged after additional chemotherapy plus CRT.[54] The Dutch Childhood Oncology Group (DCOG) eliminated CRT from all ALL patients in protocol ALL-9. While T-ALL specific outcomes have not been published, the 5-year isolated CNS relapse rate was reported to be only 2.6% and providing evidence of decreased toxicities with elimination of CRT, only 2 second malignancies, neither of them in the brain, were noted during follow-up.[62] The Israeli National Studies 89 and 98 (INS89/98) used BFM-90 based therapy with intensified ITT to eliminate CRT from 76% of T-ALL patients; the remaining 24% of patients received prophylactic cCRT secondary to high risk disease, defined as patients presenting with CNS3 disease or WBC ≥100 000/μL and/or a poor response to the prednisone prophase. With this strategy, INS 89 had a cumulative incidence of any CNS relapse of 7.1% while INS 98 reduced the incidence to 1.7%.[63]

Finally, the United Kingdom Medical Research Council (UK MRC) eliminated CRT in all T-ALL patients except those with CNS3 disease at presentation in UKALL 97, 97/99, and 2003. The UKALL 97 and 97/99 trials randomized subjects to prednisone at 40 mg/m^2 vs DEX at 6.5 mg/m^2. The results of the trial showed an overall improvement in EFS in the DEX arm across all risk groups; in particular, patients with T-ALL had only a 3.8% risk of isolated CNS relapse. Notably, there was no difference in Induction deaths or deaths during complete remission between the two arms. In UKALL 2003, using DEX as the Induction and Maintenance steroid for all patients, extra doses of PEG-ASP, no HD MTX, and intrathecal methotrexate rather than ITT, the current actuarial incidence of isolated CNS and overall CNS relapse is 3% and 4%, respectively.[45]

Together, these studies support the elimination of CRT from the majority of T-ALL patients without significantly increasing CNS relapse rates or compromising survival.[54,62-64] In these trials, a number of interventions were incorporated into existing backbones to compensate for the removal of CRT, including HD MTX, intensified ASP, DEX as the corticosteroid, and triple intrathecal chemotherapy (ITT). Not all of these interventions were used in each of the above trials, none of which were designed to prove if any single intervention could reduce the rate of CNS relapse. AALL1231 will eliminate CRT from the majority of patients with T-ALL (all SR and IR) by incorporating the interventions that were used successfully to eliminate CRT in UKALL 2003 (and are retained in UKALL 2011) in to a modified ABFM backbone. AIEOP-BFM ALL 2000 recently demonstrated that T-ALL patients with EOC MRD >0.1% had an isolated CNS relapse rate of 7.2% and a cumulative extramedullary relapse rate of 18.6% despite the use of DEX, intensified ASNase, IT MTX, systemic HD MTX and prophylactic CRT.[38] Accordingly, VHR patients on AALL1231 will continue to receive 1200 cGy prophylactic CRT.

No IR patients with CNS1 or 2 disease or SR T-ALL or T-LLy patients will receive prophylactic radiation therapy. HR T-LLy patients who are CNS1 or 2 will not receive prophylactic radiation therapy. IR and VHR T-ALL and T-LLy patients who are CNS3 will receive CRT during first cycle of Maintenance (1800 cGy). VHR T-ALL patients who are CNS1 or 2 disease will receive 1200 cGy CRT during the first cycle of Maintenance.

Additional backbone modifications based on UKALL 2003 and the elimination of CRT in most patients

The AALL1231 backbone is based on the UKALL 2003 backbone. On UKALL2003, 231 patients with more favorable disease characteristics were treated with 1 IM phase and 100 patients with less favorable disease characteristics were treated with 2 IM phases (both Capizzi MTX with PEG-ASP).

SR patients on AALL1231 will receive a single IM phase using Capizzi MTX with PEG-ASP. This will mirror the standard arm of AALL0434 and the practice at COG institutions for over 10 years. If AALL0434 finds HD MTX is superior to Capizzi MTX, AALL1231 may be amended to change to HD MTX. A subset of T-ALL patients in this arm would have received prophylactic CRT on AALL0434. Nevertheless, this is a favorable risk group and favorable risk patients on UKALL 2003 who did not receive prophylactic CRT, received 1 IM, and had excellent outcomes.

IR patients on AALL1231 will receive 2 IM phases, the first with HD MTX and the second with Capizzi. These patients would have received prophylactic CRT on AALL0434. On UKALL 2003 less favorable patients received 2 IM phases. The rationale to use HD MTX as one of the IM phases instead of two Capizzi IM phases is there are numerous other studies demonstrating a reduced risk of CNS relapse with HD MTX.[54,59,60] The combination will intensify both ASNase and MTX in an effort to reduce the risk of recurrence and improve outcomes. This is identical to the approach used for VHR B-cell precursor ALL patients on COG AALL1131.

2.1.5 Rationale for Intensification of Therapy for Patients with EOC BM MRD ≥ 0.1%

T-ALL patients with EOC MRD ≥ 0.1% and T-ALL patients who fail Induction (M3 marrow at day 29) have a poor prognosis.[38,65] Intensification of therapy in order to achieve an MRD-negative remission may improve outcome. AALL1231 will non-randomly assign T-ALL patients who fail Induction or have persistent MRD ≥ 0.1% at the EOC to receive 3 additional intensive multi-agent blocks of therapy in lieu of the HD MTX IM phase. The blocks that will be used in this study are the BFM High Risk (HR) Blocks 1-3, which have been used for over 15 years in Europe in AIEOP and BFM cooperative group trials in patients with high risk B-LL and T-ALL.[38,66,67] These HR blocks were non-randomly incorporated into treatment in ALL-BFM 95 and AIEOP-ALL95.[66,67] In both studies, the HR blocks were well tolerated and survival was improved when compared with predecessor trials. The blocks subsequently became standard of care for high-risk patients in AIEOP and BFM trials. These blocks are also included in the backbone therapy for patients in the COG AALL1122 Ph^+ ALL trial.

It is hypothesized that most patients with T-ALL who have EOC MRD ≥ 0.1% will become MRD negative (undetectable by flow cytometry) after these blocks of relatively intensive therapy. It is anticipated that the patients who become MRD negative will have improved survival as compared to those that have detectable MRD present at end HR Block 3; and, therefore, they will continue on study at DI with or without bortezomib (as randomized) followed by an IM phase with Capizzi MTX with PEG-ASP. The patients who remain MRD positive at end HR Block 3 are unlikely to be cured with chemotherapy alone and will be removed from protocol therapy and eligible to participate in COG HSCT trials. This approach, which is analogous to that used in AIEOP-BFM ALL 2009 and COG AALL1122, is designed to capture T-ALL subjects who are very high risk, yet may benefit from intensification, using established drugs. This approach also establishes a possible framework for future collaborative international trials, where a novel agent or novel combination may be studied in these high-risk EOC MRD ≥ 0.1% T-ALL patients. Similarly, HR T-LLy patients have a poor prognosis and will be treated by these HR BFM blocks.[64] T-LLy patients who are in a PR or better at the end of these blocks can resume

protocol therapy at DI with or without bortezomib (as randomized) followed by an IM phase with Capizzi MTX with PEG-ASP. T-LLy patients who are not in a PR will be removed from protocol therapy. HD MTX is included in the 3 HR blocks. VHR T-ALL and T-LLy patients who resume protocol therapy after the 3 HR blocks will receive CRT in the 1st cycle (1st four weeks) of maintenance (1200cGy non-CNS3; 1800cGy CNS3).

2.1.6 Rationale for Supportive Care Guidelines for Invasive Fungal Infections.

AALL1231 amendment #3 incorporates the use of dexamethasone throughout the Induction phase of chemotherapy for all patients based on promising results from the UKALL2003 and AIEOP BFM ALL 2000 studies. These studies showed improvements in event-free survival (EFS) with the use of dexamethasone vs. prednisone during Induction in patients with T-ALL. For example, the results of the AIEOP BMF 2000 trial were recently published and demonstrated a significant improvement in overall survival (OS) from 83% to 91% in children with T-ALL and a prednisone good response when treated with dexamethasone in Induction instead of prednisone).[68]

IFIs are an expected complication on any ALL protocol, and other studies demonstrate the use of dexamethasone may increase that risk of IFIs over prednisone-based backbones. In the UKALL2003 trial the incidence of Invasive Fungal Infection was 2%, 6% and 3% in the standard, intermediate and high risk groups, respectively.[69] In the AIEOP BFM ALL 2000, the reported incidence of IFI on the dexamethasone arm was 1.6% during Induction. Direct comparisons between trials is challenging based on the level of details (e.g. pathogen) provided and different monitoring and reporting requirements. While IFIs have been seen on AALL1231, the current IFI rate for AALL1231 has not exceeded overall reported rates of IFIs with other dexamethasone–based induction regimens and the induction mortality (from all causes) on the trial has been low and within expected rates (<1.5%).

2.2 Early T cell Precursor ALL (ETP ALL)

Early T cell Precursor (ETP) ALL is a recently identified type of T-ALL that may carry a poor prognosis.[70] ETP ALL blasts are defined by a unique immunophenotype, expressing T-lineage markers and myeloid/early progenitor markers; however, ETP ALL is not ambiguous-lineage leukemia. Despite their T cell origin, very recent work suggests ETP ALL blasts have a gene expression profile more similar to myeloid/early progenitor leukemia than lymphoid leukemia.[70,71] ETP ALL blasts also frequently have mutations more commonly found in myeloid leukemia, including mutations in histone-modifying proteins and in genes that regulate Ras and cytokine receptor signaling.[71]

Data from St. Jude Children's Research Hospital (SJCRH) and older AIEOP trials suggested ETP ALL patients respond poorly to current chemotherapeutic approaches, but this report included only 31 ETP patients.[70,72] More recent data from AIEOP-BFM ALL 2000 and UK ALL 2003 suggest not all of these patients do poorly. 387 T-ALL patients were treated on UKALL 2003. Of these, 239 had immunophenotypic data allowing for classification as non-ETP, definitive/probable ETP, or possible ETP with the definitive, probable, and possible having similar outcome.[73] ETP ALL patients were less likely to be MRD low risk at the end of induction (4% vs 27%; p = 0.008). They also found the ETP ALL patients had a non-statistically significant inferior 5-year EFS (76% vs 84.6%; p =0.2) and 5-year OS (84.1% vs 90.9%); p = 0.08). The data from UKALL 2003 thus argue against stem cell transplant in CR1 for ETP ALL. AIEOP-BFM ALL 2000 demonstrated that ETP ALL patients can be classified based on response to chemotherapy, e.g., the majority of patients who do poorly are already designated high risk based on prednisone response, end Induction morphologic response, and end Induction and EOC MRD[38] This study had data on ETP status for

only 19 patients, with 9/19 remaining in CR1. Fourteen were classified as high risk based on MRD alone (10), Induction failure (2), or prednisone poor response (2) with 7/14 relapsing and 2 deaths in CR after transplantation in first remission. Five ETP patients were classified as either standard (1) or intermediate (4) risk, with 4/5 remaining in CR with chemotherapy alone.

Over 100 patients with ETP ALL have been identified to date on AALL0434, and we project that approximately 150 ETP ALL patients will eventually be identified on AALL0434. Once AALL0434 data mature, this experience will allow us to assess whether or not ETP ALL is an independent predictor of poor risk regardless of MRD response. Based on the conflicting results between UKALL 2003, AIEOP-BFM ALL 2000, and SJCRH and the lack of mature outcome data from COG AALL0434, it is currently not justified to use ETP as an independent risk factor on AALL1231. UKALL 2003 is the largest of these studies and provides the most data in ETP ALL and is the backbone of therapy for AALL1231.

2.3 Impact

Relapsed T-ALL and T-LLy have a dismal prognosis with the majority of patients relapsing early in therapy with chemotherapy refractory disease. This study will test whether the addition of bortezomib, which is a very promising agent based on responses seen in relapsed B-ALL and T-ALL, to an ABFM backbone will decrease relapse risk, improving EFS and OS in children, adolescents and young adults with T-ALL and T-LLy. It is anticipated the modifications in the chemotherapy backbone will also decrease relapse risk. This trial will also establish if prophylactic CRT can be safely eliminated from the treatment in the vast majority (~90%) of pediatric patients with T-ALL. We also expect to determine if intensification of chemotherapy in VHR T-ALL and T-LLy will prevent relapse in these patients and further identify patients who are chemotherapy refractory and require novel agents and/or HSCT for cure. This model could potentially be used for future international collaborative trials in VHR patients. Finally, it is anticipated that this trial will answer a number of important correlative biologic aims, improving the understanding of the biology of T-ALL and T-LLy, which may translate into better therapies in the future.

2.4 Relevant Pre-clinical Data on Bortezomib

2.4.1 Biologic Rationale for Bortezomib

Bortezomib, a dipeptidyl boronic acid, is a selective inhibitor of the ubiquitin proteasome pathway (UPP), which is essential for the degradation of most short-lived and many long lived intracellular proteins in eukaryotic cells.[74] Bortezomib specifically inhibits the 26S proteasome, an ATP-dependent multi-subunit protein that degrades ubiquitinated proteins involved in multiple cellular processes, including cell cycle regulation, transcription factor activation, apoptosis, and cell trafficking.[75,76] Proteasome inhibition stabilizes many cell cycle-regulatory proteins that are overexpressed in leukemia cells.[77] Previous studies have shown that proteasome inhibition may sensitize malignant hematologic cells to apoptosis induced by both radiation and chemotherapy.[78-80] Apoptosis following proteasome inhibition is seen in leukemia cells lines[17,81,82] as well as in primary ALL lymphoblasts[17] but not in normal hematopoietic progenitors.[83,84] Important regulatory proteins affected by inhibition of the UPP system include NF-κB, p53, Bax and other cell cycle regulatory proteins such as the cyclin-dependent kinase inhibitors p27 and p21.[85] It is believed that proteasome inhibition alters the ratio of pro-apoptotic and anti-apoptotic proteins within a cell, resulting in an increased sensitivity to apoptosis.[86]

2.4.2 NF-κB Alterations in Hematopoietic Malignancies

In non-proliferative cells, the inhibitor protein IκB sequesters NF-κB in the cytoplasm.[87] Cellular stress results in ubiquitination and the subsequent proteasomal degradation of IκB.[87] NF-κB dimerizes and rapidly translocates into the nucleus, is phosphorylated, and activates the promoter regions of numerous genes, including genes encoding several anti-

apoptotic proteins such as Bcl-2, X-linked inhibitor of apoptosis protein (XIAP), and c-Jun N-terminal kinase (JNK).[88] Inhibiting proteasomal degradation of IκB inhibits NF-κB nuclear translocation and binding of NF-κB to NF-κB response elements; this may prevent anti-apoptotic responses induced by chemotherapy.[87]

Studies have shown constitutive activation of NF-κB in many hematologic malignancies,[80] including primary ALL,[89] acute myeloid leukemia (AML),[81] and numerous leukemia cell lines.[90,91] Constitutive activation of NF-κB occurs through a variety of mechanisms, including NF-κB gene amplification, NF-κB chromosomal rearrangements, IκB mutations, induction of IκB kinase (IKK), and the induction of upstream regulators of NF-κB.[88] Constitutive NF-κB activation has been observed in Philadelphia chromosome positive (Ph+) ALL[91] and in primary T-cell leukemia.[92] Amplification and chromosomal rearrangements of NF-κB subunits have also been found in adult T-ALL.[93] Abnormal signaling through Notch1 and PI3K/Akt/mTOR is very common in pediatric T-ALL, and one or both of these pathways are dysregulated in the majority of T-ALL blasts.[94] NF-κB was recently demonstrated to be one of the major mediators of Notch1-induced transformation in T-ALL.[16] Constitutively activated Notch1 activates NF-κB through IKK, and inhibiting the proteasome is very effective in preclinical models of Notch1-driven cancers.[16] NF-κB can also be activated as a consequence of activated Akt in T cells.[95] Accordingly, proteasome inhibition with bortezomib has the potential to target two of the major dysregulated signaling pathways in T-ALL.

Confounding the understanding of the mechanism of action of proteasome inhibition in malignancies are recent reports suggesting proteasome inhibitors, including bortezomib, may induce NF-κB despite degrading IKK *in vivo*.[96-98] Similar to drugs that inhibit other biologic and signaling pathways, *in vivo* assessment of biomarkers can be difficult to interpret. Targeting pathways with biologic agents can lead to compensatory feedback inhibition, making it difficult to determine the best biomarkers to measure and the appropriate time to measure them *in vivo,* in order to assess pathway activation and response to target inhibition. Nevertheless, multiple studies suggest proteasome inhibitors are very active in tumors with activated NF-κB, including T-ALL.[15,99-102]

2.4.3 Proteasome Inhibition Results in Increased Sensitivity to Chemotherapeutic Agents and May Overcome Chemotherapy Resistance

Several studies have shown that inhibition of NF-κB can enhance leukemia cell sensitivity to chemotherapeutic agents.[82,103,104] Both lymphoid and myeloid malignancies show enhanced sensitivity to doxorubicin after transient over-expression of mutant IκB or proteasome inhibition.[24,87] Leukemia cells are more sensitive to anthracyclines, etoposide and cytarabine in the presence of proteasome inhibitors such as bortezomib.[17,83,105-107] Bortezomib potentiation of anthracyclines and cytarabine in leukemia cells appears to be independent of p53 status and multi-drug resistance mechanisms.[107,108] Bortezomib also enhances doxorubicin and cytarabine apoptosis in vitro in T-cell lymphomas and human T-lymphotropic virus type I (HTLV-I) associated adult T-cell leukemia.[109,110] Bortezomib has been shown to overcome chemotherapy resistance to anthracyclines, alkylators, and corticosteroids in a number of malignancies.[18-22] Preclinical data suggest that bortezomib may sensitize steroid resistant T-ALL cells to corticosteroids.[23] Preclinical data also suggest that proteasome inhibition may reverse anthracycline resistance in T-ALL.[24] Finally, recent work suggests bortezomib may help overcome chemotherapy resistance in leukemic cells by down-regulation of mdr1 and reduction of p-glycoprotein (p-gp) expression.[98]

2.4.4 Proteasome Inhibition Alters Expression of P53 and Other Regulatory Cell Cycle Proteins

Many short-lived regulatory proteins are degraded by the UPP system in addition to IκB, and apoptosis following proteasome inhibition may not be exclusively dependent on NF-κB activation.[85] Evidence for this has been provided by a murine AML xenograft model[111] and a phase 1 clinical trial.[27] Several other regulatory cell cycle proteins are ubiquitinated and degraded by the 26S proteasome and could contribute to proteasome-induced apoptosis. p53, an ubiquitin-regulated cell cycle checkpoint protein and tumor suppressor, accumulates after proteasome inhibition.[90] The cyclin-dependent kinase inhibitors p21 and p27 are also ubiquitinated and alterations in p21 accumulate in many relapsed hematopoietic malignancies.[112]

2.4.5 Preclinical Studies Using Bortezomib in T-ALL

The anti-leukemia activity of bortezomib has been tested in both cell culture and xenotransplantation systems. Houghton et al. tested bortezomib as part of the NIH-sponsored CTEP pediatric preclinical testing program (PPTP).[15] Potent bortezomib activity was noted in vitro and bortezomib demonstrated significant in vivo activity in murine leukemia ALL xenografts, finding a 100% response rate against T-ALL (2 of 2 samples) as compared with a 40% (2 of 5 samples) response rate in pre-B ALL.[15] Work in the Horton lab has shown that bortezomib and DEX have synergistic anti-leukemia interactions in vitro.[17] Bortezomib also had additive anti-leukemia effects when combined with asparaginase, vincristine, doxorubicin or cytarabine.[17] Preliminary data indicate that bortezomib and etoposide also appear to have at least additive interactions in vitro when drug-drug interactions are analyzed using the stringent universal response surface approach (URSA)[17,113] (Table 1)

Table 1: Drug Interaction Summary: Strength of in vitro bortezomib drug interactions with chemotherapy agents using the URSA modeling method.

Drug	Drug interaction parameter*	Confidence interval	Interaction
Dexamethasone	3.2	1.9 to 5.5	Synergistic
Cytarabine	0.19	-0.03 to 0.5	Additive
Doxorubicin	0.007	-0.09 to 0.09	Additive
Asparaginase	0.26	-0.04 to 0.57	Additive
Vincristine	0.001	-0.33 to 0.33	Additive
Etoposide	0.0002	-0.28 to 0.28	Additive

*Drug interactions were considered synergistic if the combination interaction parameter, α, was significantly greater than 0 (i.e., 95% confidence interval of α did not include zero).[113]

2.5 Clinical Experience with Bortezomib

2.5.1 Bortezomib in Adult Clinical Trials: Toxicities in Phase 1 and 2 Clinical Trials

Bortezomib has been evaluated as a single agent and in combination with chemotherapeutics in adults with leukemia, multiple myeloma and non-Hodgkin lymphoma (NHL), leading to its FDA approval for use in adults with multiple myeloma and relapsed NHL. [25,26,114,115] Adult early phase trials have demonstrated that the maximum tolerated dose (MTD) of bortezomib is schedule dependent. When administered twice weekly for 2 weeks in a 21-day cycle, the MTD, alone or in combination with chemotherapy, is 1.3-1.5 mg/m^2.[32,116,117] In a compilation of results from early phase studies in multiple myeloma, the most commonly reported adverse events were nausea (62%), fatigue (54%), diarrhea (48%), constipation (41%), thrombocytopenia (41%), pyrexia

(36%), peripheral neuropathy (35%), vomiting (34%), and anorexia (30%).[118] Similar toxicities were also found in early phase trials using bortezomib in adults with other hematologic malignancies.[25,26,119-121] Additional reported toxicities of bortezomib from early phase trials in adults include mild elevation of liver transaminases, hyponatremia, hypokalemia, fluid retention, orthostatic hypotension, neutropenia, lymphopenia, infection, coagulopathy, and anemia.[25,26,119-121]

Bortezomib has been safely combined with chemotherapeutics in a number of adult trials in hematologic malignancies, including: 1) bortezomib (1.3 mg/m^2) combined with etoposide/prednisone/vincristine/cyclophosphamide and doxorubicin (EPOCH) in adults with diffuse large-cell B lymphoma (DLCBL);[122] 2) bortezomib (1.5 mg/m^2) combined with pegylated doxorubicin in adults with refractory hematologic malignancies;[32] 3) bortezomib (1.3 mg/m^2) combined with rituximab in NHL;[123] 4) bortezomib (1.3 mg/m^2) combined with CHOP-R (cyclophosphamide, doxorubicin, vincristine, prednisone plus rituximab) in adults with lymphoma;[124] 5) bortezomib (1.5 mg/m^2) in combination with idarubicin/cytarabine for adults with AML;[125] 6) bortezomib (1.6 mg/m^2) in combination with bendamustine and rituximab in adults with follicular lymphoma;[126] 7) bortezomib (1.3 mg/m^2) combined with fludarabine and rituximab in mantle cell lymphoma;[117] and 8) bortezomib (1.6 mg/m^2) combined with CHOP in adults with T-cell lymphoma.[127] In all of these studies, bortezomib-related toxicities were similar to those found in single agent studies, and primarily consisted of electrolyte abnormalities (hyponatremia, hypokalemia), cytopenias (neutropenia, lymphopenia, and thrombocytopenia), neuropathy, fatigue, malaise, nausea, vomiting, and diarrhea.

2.5.2 Bortezomib in Adults with T-cell Lymphomas

Kim and colleagues performed a Phase 2 trial evaluating bortezomib (Days 1 and 8 at 1.6 mg/m^2) in addition to CHOP chemotherapy every 3 weeks for six cycles in adults (median age 51 years) with newly diagnosed stage III/IV peripheral T-cell lymphoma.[127] Forty-six patients were treated and 65% had CR. The ORR was 76%, and 3 year OS and PFS were 47% and 35%, respectively. The only Grade 3 or higher toxicities reported in over 10% of patients were hematologic cytopenias. This same group had previously reported a Phase 1 trial of bortezomib and CHOP in 13 patients with aggressive T-cell lymphoma, finding an MTD of 1.6mg/m^2/dose of bortezomib and similar rates of response (CR rate 62%) and toxicity.[28] Zinzani and colleagues reported a Phase 2 trial of bortezomib as a single agent (1.3mg/m^2, Days 1, 4, 8 and 11) in 15 adults with relapsed or refractory cutaneous T-cell lymphoma.[29] The ORR rate was 67% with a 17% CR and 50% PR rate. Grade 3 toxicities included neutropenia, thrombocytopenia, and sensory neuropathy, each seen in 2 patients. No patient had a Grade 4 toxicity. Falchook and colleagues reported a Phase 1 trial of bortezomib (Days 1 and 8; escalating dose from 0.7 mg/m^2 to 1.3 mg/m^2) in combination with gemcitabine and liposomal doxorubicin in adults with advanced refractory malignancies. Sixty five patients were treated and the best responses were found in patients with cutaneous T-cell lymphoma (6 of 7 PR).[30]

2.5.3 Single Agent Bortezomib in Pediatric Clinical Trials: DLT and Pharmacokinetics

Blaney et al. conducted a bortezomib phase 1 study in pediatric patients with relapsed/refractory solid tumors.[128] Bortezomib was administered twice weekly for 2 consecutive weeks at either 1.2 mg/m^2 or 1.6 mg/m^2 followed by a 10-day rest period. In the 12 patients evaluable for toxicity, thrombocytopenia was dose-limiting and prevented administration of a complete treatment cycle. The average proteasome inhibition 1 hour after drug administration on Day 1 was 67% ± 7% at 1.2 mg/m^2 and 76% ± 3% at 1.6 mg/m^2. Grade 3 thrombocytopenia was seen in 2 of 5 evaluable patients receiving bortezomib at 1.6 mg/m^2. A Phase 1 study of bortezomib in patients with

relapsed/refractory leukemia (ADVL0317) demonstrated a bortezomib MTD of 1.3 mg/m^2.[27] DLTs at the 1.7 mg/m^2 dose level were altered mental status (n=1) and Grade 4 hypotension followed by Grade 5 hypoxia in one patient with febrile neutropenia. Hypotension and pulmonary toxicity, although rare, have been previously reported in adults.[116] Pharmacokinetic profiles were similar to those observed in adults. At the 1.3 mg/m^2 dose, bortezomib had an estimated Cmax of 63 ± 16 ng/mL, clearance of 49 ± 13 L/h with a rapid distribution half life (3.5 ± 1.7 min) and a mean terminal half life of 12 ± 1.3 h.[27] The dose of bortezomib in this study will be 1.3 mg/m^2, the MTD determined in ADVL0317 and currently being studied in AALL07P1.

2.5.4 Pediatric Bortezomib Combination Studies in Hematologic Malignancies

The Therapeutic Advances in Childhood Leukemia (TACL) consortium recently completed a Phase 1-2 study of bortezomib (1.3 mg/m^2) combined with a 4-drug Re-Induction chemotherapy regimen (vincristine, DEX, pegylated L-asparaginase, and doxorubicin) (TACL 2005-03) in patients with second or greater relapse. In the Phase 1 portion of the study, 10 patients were treated with 4-drug Re-Induction combined with bortezomib.[33] Four patients were treated at 1 mg/m^2 with no DLT, and 6 were treated at the 1.3 mg/m^2 dose level and this combination was adequately tolerated. One patient with a prior history of hypophosphatemia had dose-limiting hypophosphatemia and rhabdomyolysis (secondary to hypophosphatemia) after one dose of bortezomib at 1.3 mg/m^2. This patient died from diffuse zygomycosis on Day 17. Five other patients treated at 1.3 mg/m^2 did not experience a DLT, and 1.3 mg/m^2 was the recommended Phase 2 dose. Of the 9 patients evaluable for bone marrow response, 6 achieved a CR and one had a bone marrow CR with persistent CNS leukemia. There was no overall increase in peripheral neuropathy. Only 2 patients had mild peripheral neuropathy (Grades 1 and 2). Twenty-two patients were treated on the phase 2 portion of the study which closed early secondary to reaching a favorable efficacy end-point.[129] The CR rate was 64%, the ORR was 73% (CR + CRp), and 2 yr. OS was 41%. Only 2 patients with T-ALL were enrolled. Both, who were in second relapse, did not respond. All 22 patients completed the 4 doses of bortezomib. Ten of 22 subjects had Grade 3 or higher infection and 3 subjects had infectious death, leading to the use of prophylactic antimicrobials. Of note, infectious toxicities are commonly far greater in relapsed ALL trials (as described below) than in the trials in newly diagnosed patients. Two patients developed Grade 3 peripheral neuropathy. Other Grade 3 and 4 toxicities were no more common than expected on backbone alone.

AALL07P1 is a Phase 2 trial currently open for patients with ALL in first relapse that incorporates bortezomib into the AALL01P2 backbone. Bortezomib (1.3 mg/m^2) is given on Days 1, 4, 8, and 11 of Block 1 and on Days 1, 4 and 8 of Block 2. AALL07P1 recently completed the second stage of the Simon 2-stage design. As described above (Section 2.1.1), forty-two CRs were observed amongst patients with early and intermediate risk relapses; the efficacy boundary was crossed, and bortezomib was deemed worthy of further investigation in B-lineage ALL. More patients who achieved CR2 on AALL07P1 were MRD <0.1% when compared to similar patients treated on AALL01P2 (backbone alone). Sixteen of 39 (41%) B-lineage patients who attained CR2 on AALL01P2 were MRD <0.1%, as compared with 25 of 40 (63%) B-lineage patients who attained CR2 on AALL07P1 (p = 0.07) AALL07P1 also enrolled T-ALL and T-LLy patients with descriptive statistics regarding response. In AALL01P2, only 1 of 7 T-ALL (17%) patients attained CR at the end of Block 1. Thus far, 17 evaluable T-ALL patients have been treated on AALL07P1 and 11 of 17 (65%) have attained CR at the end of Block 1. While the number of T-ALL patients treated is rather small, the observed CR2 rate is very promising in comparison to historical control data from patients with T-ALL and BM relapse and the CR2 rate observed is similar to that seen in early relapse B-ALL patients (64%).

In addition, infectious toxicity on AALL07P1 during Block 1 therapy is similar to AALL01P2 (backbone alone) and ADVL04P2 (backbone plus epratuzumab). Each of these relapsed ALL trials have a 45% Grade 3-5 infection rate with 2-4 infectious deaths in Block 1. Accordingly, these data suggest that the addition of bortezomib is unlikely to increase infectious toxicity significantly over that observed with the chemotherapy platform. As of January 2013, a total of 93 eligible patients have been enrolled on the trial. Other than infection, the only Grade 3 or higher non-hematologic toxicities that occurred in over 10% of subjects during Block 1 include hypokalemia (12.3%), hyponatremia (13.8%), and hypotension (12.3%). One subject developed reversible, Grade 3 sensory peripheral neuropathy.

2.5.5 Other Pediatric Studies

Two pediatric COG clinical trials testing bortezomib in combination with Re-Induction chemotherapy in children with relapsed hematologic malignancies were recently completed. AHOD0521 investigated bortezomib (1.2 mg/m^2, Days 1, 4, and 8) combined with ifosfamide and vinorelbine in pediatric patients and young adults with relapsed Hodgkin lymphoma. This study enrolled 25 subjects and closed early for not meeting an efficacy end-point based on CT imaging despite a favorable efficacy signal using PET imaging.[130] There were no Grade 3 or 4 non-hematologic toxicities that occurred in over 10% of patients. Rare Grade 3 or 4 events that were possibly related to bortezomib included hypokalemia, hypophosphatemia, anorexia/vomiting, limb pain, and neuralgia. One subject developed Grade 3 peripheral neuropathy early in therapy that did not recur with subsequent doses of bortezomib. AAML07P1 was a Phase 1/2 pilot study combining bortezomib with either idarubicin/cytarabine (patients with low prior anthracycline exposure) or etoposide/cytarabine (patients with high prior anthracycline exposure) in pediatric patients with relapsed AML. The study enrolled a total of 52 subjects between dose-finding phase and efficacy phases. The study closed early as it did not meet a pre-determined efficacy threshold, using a Simon 2-stage design. Bortezomib was tolerated at 1.3 mg/m^2 given on Days 1, 4, and 8. There were 4 deaths. This rate of death is comparable to the rate found on AALL01P2. Non-hematologic Grade 3 or higher toxicities were no more prevalent than expected on the chemotherapy backbone, and included infection, hypotension, hypokalemia, transaminitis, esophagitis, mucositis, nausea, vomiting, and abdominal pain (AAML07P1, study report March 31, 2012). AAML1031 is an on-going Phase 3 trial for children with de novo AML. A primary objective of the trial is to determine if bortezomib with cytarabine, daunomycin and etoposide (ADE) increases EFS when compared to ADE alone. All patients are randomized to receive or not receive bortezomib for 4 cycles of chemotherapy. This study opened to accrual on June 20, 2011. As of January 15, 2013, 249 patients have enrolled. Grade 3-4 toxicities are comparable between arms (Table 2). Grade 3 or higher toxicities and infectious toxicities by treatment blocks are shown in Table 3. There have been 2 deaths on the non-bortezomib arm and 4 deaths on the bortezomib arm during Induction 1.

Table 2a: Grade 3 or higher toxicities on AAML1031*

Treatment block	Bortezomib-containing arm**	Non-bortezomib containing arm**
Induction 1	93 of 112 (83%)	84 of 110 (76%)
Induction 2	57 of 93 (61%)	40 of 75 (53%)
Intensification 1	45 of 63 (71%)	40 of 58 (69%)
Intensification 2	32 of 40 (80%)	30 of 38 (79%)

Table 2b: Grade 3 or higher infections or infestations on AAML1031*

Treatment block	Bortezomib-containing arm**	Non-bortezomib containing arm**
Induction 1	49 of 112 (44%)	51 of 110 (46%)
Induction 2	39 of 93 (42%)	29 of 75 (39%)
Intensification 1	45 of 63 (71%)	25 of 58 (43%)
Intensification 2	30 of 40 (75%)	26 of 38 (68%)

*Based on data as of September 30, 2012
**n = total number of Grade 3 or higher infections and total number treated. If a subject has more than 1 infection it is considered more than one event.

2.5.6 Bortezomib and Bone Health

Unlike many agents used to treat cancer, bortezomib promotes bone formation and may be protective against ON.[131,132] At least eight different clinical trials (reviewed in PMID: 22226939) have found bortezomib to have positive effects on bone health, including lower rates of pathologic fractures, increased bone volume and bone density, and prevention of development of steroid-induced bone disease. For example, on the Phase 3 VISTA trial, previously untreated multiple myeloma patients were randomized to receive up to 54 weeks' therapy with bortezomib, melphalan, and prednisone or melphalan and prednisone alone.[133] Patients who developed osteopenia or evidence of lytic destruction of bone or osteopenia were to initiate bisphosphonates, following standardized ASCO guidelines.[134] On the bortezomib arm there were lower rates of progression because of worsening bone disease and rates of skeletal AEs, (3% vs. 11% and 4% vs. 5%, respectively), as well as need for bisphosphonates (73% vs. 82%). There have been over 30 clinical trials that have assessed biomarkers of bone formation in patients treated with bortezomib (also reviewed in PMID: 22226939). On many of these trials, patients were treated with corticosteroids (prednisone or DEX) with or without bortezomib. Consistently across all of these trials bortezomib was found to have a positive effect on biomarkers of bone health and to be protective against steroid-induced negative effects. No study has found a negative biomarker profile consistent with deleterious bone health in bortezomib treated patients.

2.6 Developmental Plans

AALL1231 is the successor study to AALL0434, which is the current COG Phase 3 trial for patients with newly diagnosed T-ALL and T-LLy, and will open to enrollment when AALL0434 meets accrual goals in late 2014.

AALL0434 is a COG group-wide Phase 3 clinical trial that uses a 2 x 2 factorial design to randomize patients with T-ALL and T-LLy to receive or not receive six 5-day courses of nelarabine and to randomize T-ALL patients to receive Capizzi MTX with PEG-ASP or HD MTX during a single IM phase. Nelarabine is given during Consolidation, the second half of DI, and the first 3 Cycles of Maintenance. AALL1231 has been designed such that the treatment backbone can be modified easily to incorporate potential changes to the treatment backbone that might be indicated based on results of the AALL0434 randomized questions. As nelarabine and bortezomib have potentially overlapping toxicities, and the combination of these two agents has not been studied to date, bortezomib will not be given during the blocks of therapy that include nelarabine in AALL0434. Accordingly, if the nelarabine arm on AALL0434 is found to be superior after AALL1231 opens, this trial will be amended to incorporate nelarabine during the same therapeutic blocks. The proposed changes to the backbone of this study will also not affect the ability to incorporate nelarabine, as these changes do not occur during consolidation, the second half of DI, or during maintenance while receiving nelarabine. Nevertheless, if the trial is amended to include nelarabine, additional toxicity monitoring will be added to the amended trial. Patients with IR and VHR T-ALL and T-LLy on this study will receive 2 IM phases as described above. SR T-ALL

patients and T-LLy patients will receive a single IM, using Capizzi MTX with PEG-ASP. If AALL0434 determines HD MTX is superior to Capizzi MTX with PEG-ASP in T-ALL, this study will be amended to change the SR T-ALL IM to HD-MTX. Because HD MTX is given in AALL1231 to IR (during IM#1) and VHR (during HR BFM Blocks), no modification will be needed for these patients if HD MTX is found to be superior to Capizzi MTX with PEG-ASP on AALL0434. If Capizzi MTX is found to be superior, no changes will need to be made to any risk group. SR, IR, and VHR T-ALL and T-LL patients are all receiving Capizzi MTX.

2.7 Correlative Biology Studies

2.7.1 Minimal Residual Disease Determination at Serial Time Points During Therapy

MRD is the single most powerful prognostic factor in childhood ALL.[36-38] A number of large studies have investigated the sensitivity and specificity of MRD at different time points on a variety of treatment protocols, using either flow- or PCR-based technologies.[39] While the majority of data suggest that end Induction MRD assessment is the best predictor of outcome in B-ALL, recent data from AIEOP-BFM ALL 2000 strongly suggest that EOC MRD is a better predictor of adverse outcome in T-ALL, though response at early time points is essential to identify the patients with the best prognosis.[38] Currently, therapeutic response in T-LLy is limited to bone marrow morphology and radiographic imaging. While MRD may be useful in determining prognosis, MRD has not been formally or extensively studied in patients with T-LLy.

AALL1231 will test the following hypotheses: (1) the persistence of positive MRD at both end Induction and end Consolidation in the bone marrow will correlate with inferior EFS in patients with T-ALL, and persistence of positive MRD at end of induction in bone marrow will correlate with inferior EFS in patients with T-LLy; (2) rates of MRD positivity will be decreased in the bortezomib-containing arm as compared with the control (non-bortezomib) containing arm; (3) the 3 HR BFM blocks will reduce MRD+ rates in VHR T-ALL and that the reduction in MRD+ rates will correlate with improved EFS and OS; and, (4) a higher percentage of ETP-ALL patients will be MRD >0.1% at the end of consolidation as compared with non-ETP ALL patients. Additional details regarding MRD testing are included in Appendix XI.

2.7.2 Evaluating Mechanisms of Bortezomib Response and Mechanisms of Bortezomib Resistance in T-cell ALL

AALL1231 is designed to test the hypothesis that the proteasome inhibitor bortezomib can be safely and effectively incorporated into a modified BFM backbone in patients with T-ALL and T-LLy. The goal of these correlative studies is to determine if changes in proteasome function or cell stress protein expression patterns can predict bortezomib response and drug resistance in T-ALL. The primary Aims of these correlative studies are: (1) to delineate the mechanisms of bortezomib action and resistance in T-ALL to determine if proteasome alterations correlate with clinical response, as measured by MRD and EFS; and (2) to determine if reverse phase protein lysate arrays (RPPA) predict chemotherapy response or resistance. Both arms are eligible for these optional studies.

The central hypothesis is that UPR activation, or induction of other protein cell stress pathways in ALL-lymphoblasts, can predict chemotherapy response, and ultimately clinical outcome. These studies are optional and performed on a research basis. No results will be given back to patients. Additional details describing the rationale, feasibility, preliminary data, experimental methods, and statistical analysis are included in Appendix XII.

2.7.3 Identifying Biomarkers and Mechanisms of Chemotherapy Resistance and Response in T-ALL, Focusing on ETP ALL.

T-ALL is a heterogeneous disease with variable responses to therapy. To date, the ability to predict outcome based upon biologic features in T-ALL has been limited as the majority of identified genetic alterations are largely not prognostic in therapy stratified by disease response. ETP ALL is a newly identified phenotype of T-ALL with a poor prognosis in some reports. ETP ALL blasts have a heterogeneous mix of genetic mutations; however, these aberrations manifest biochemically as changes in a limited set of signal transduction pathways that preliminary data suggest may be important in the majority of ETP ALL. Accordingly, T-ALL patients may be better risk allocated based on biochemical pathway alterations. These correlative studies will identify biomarkers and mechanisms of chemotherapy resistance and response in T-ALL, focusing on ETP ALL.

The central hypothesis is that the signaling network differences in ETP ALL with be distinct from signaling networks in non-ETP ALL and these differences will predict whether the leukemia will respond to chemotherapy. These studies are optional and performed on a research basis. No results will be given back to patients. Additional details describing the rationale, feasibility, preliminary data, experimental methods and statistical analysis are included in Appendix XII.

3.0 STUDY ENROLLMENT PROCEDURES AND PATIENT ELIGIBILITY

3.1 Study Enrollment

3.1.1 Patient Registration

Prior to enrollment on this study, patients must be assigned a COG patient ID number. This number is obtained via the COG Registry system once authorization for the release of protected health information (PHI) has been obtained. The COG patient ID number is used to identify the patient in all future interactions with COG. If you have problems with the registration, please refer to the online help.

In order for an institution to maintain COG membership requirements, every patient with a known or suspected neoplasm needs to be offered participation in APEC14B1, *Project:EveryChild A Registry, Eligibility, Screening, Biology and Outcome Study*.

A Biopathology Center (BPC) number will be assigned as part of the registration process. Each patient will be assigned only one BPC number per COG Patient ID. For additional information about the labeling of specimens please refer to the Pathology and/or Biology Guidelines in this protocol.

Please see Appendix XIII for detailed CTEP Registration Procedures for Investigators and Associates, and Cancer Trials Support Unit (CTSU) Registration Procedures including: how to download site registration documents; requirement s for site registration, submission of regulatory documents and how to check your site's registration status.

3.1.2 IRB Approval

Local IRB/REB approval of this study must be obtained by a site prior to enrolling patients. Sites must submit IRB/REB approvals to the NCI's Cancer Trials Support Unit (CTSU) Regulatory Office and allow 3 business days for processing. The submission must include a fax coversheet (or optional CTSU IRB Transmittal Sheet) and the IRB approval document(s). The CTSU IRB Certification Form may be submitted in lieu of the signed IRB approval letter. All CTSU forms can be located on the CTSU web page (https://www.ctsu.org). Any other regulatory documents needed for access to the study

enrollment screens will be listed for the study on the CTSU Member's Website under the RSS Tab.

IRB/REB approval documents may be submitted via the online portal via www.ctsu.org in the member's section, under the Regulatory Submission Portal submitted via the online portal via www.ctsu.org in the member's section, under the Regulatory Submission Portal where they will be entered and tracked in the CTSU RSS.

Regulatory Submission Portal: www.ctsu.org (members' area) → Regulatory Tab →Regulatory Submission.

Institutions with patients waiting that are unable to use the Portal should alert the CTSU Regulatory Office immediately at 1-866-651-2878 in order to receive further instruction and support. For general (non-regulatory) questions call the CTSU General Helpdesk at: 1-888-823-5923.

3.1.3 Study Enrollment

Patient enrollment will be facilitated using the Oncology Patient Enrollment Network (OPEN). OPEN is a web-based registration system available on a 24/7 basis. To access OPEN, the site user must have an active CTEP-IAM account (check at < https://eapps-ctep.nci.nih.gov/iam/index.jsp >) and a 'Registrar' role on either the lead protocol organization (LPO) or participating organization roster.

All site staff will use OPEN to enroll patients to this study. It is integrated with the CTSU Enterprise System for regulatory and roster data and, upon enrollment, initializes the patient position in the Rave database. OPEN can be accessed at https://open.ctsu.org or from the OPEN tab on the CTSU members' side of the website at https://www.ctsu.org.

Prior to accessing OPEN, site staff should verify the following:

- All eligibility criteria have been met within the protocol stated timeframes.
- All patients have signed an appropriate consent form and HIPAA authorization form (if applicable).

Note: The OPEN system will provide the site with a printable confirmation of registration and treatment information. Please print this confirmation for your records.

Further instructional information is provided on the CTSU members' web site OPEN tab or within the OPEN URL (https://open.ctsu.org). For any additional questions contact the CTSU Help Desk at 1-888-823-5923 or ctsucontact@westat.com

3.1.4 Timing

a. **T-ALL PATIENTS MUST BE ENROLLED ON AALL08B1 OR PROJECT:EVERYCHILD (APEC14B1, IF OPEN FOR CLASSIFICATION OF NEWLY DIAGNOSED ALL PATIENTS) BEFORE ENROLLING ON AALL1231**.

T-LLY: PATIENTS WITH T-LLY ARE INELIGIBLE FOR AALL08B1.

EVERY EFFORT SHOULD BE MADE TO ACQUIRE AS MUCH TISSUE AS POSSIBLE. SPECIFIC INSTRUCTIONS REGARDING TISSUE SUBMISSION ARE OUTLINED IN SECTION 13.6. OF NOTE, T-LLY SPECIMENS, INCLUDING DIAGNOSTIC MRD, ARE SUBMITTED AS PER THE INSTRUCTIONS IN AALL1231 AS OUTLINED IN SECTIONS 13 AND 14.

b. Informed consent: Except for administration of intrathecal cytarabine or allowable steroid pretreatment (defined below), *informed consent/parental permission* MUST be signed before protocol therapy begins.

c. Study enrollment: Patients are randomized to receive or not receive bortezomib before protocol therapy begins. Accordingly**, Patients must be enrolled on AALL1231 before protocol therapy begins.** The only exceptions are the first dose of intrathecal chemotherapy may be given before enrollment when administered as part of the initial diagnostic lumber puncture, and in select circumstances, corticosteroids or emergent radiation may be given before enrollment as defined in Section 3.2.2 and Section 3.3.

The date protocol therapy is projected to start must be no later than ***five (5)*** calendar days after the date of study enrollment.

d. Eligibility studies: Patients must meet all eligibility criteria prior to enrollment. Unless otherwise indicated in the eligibility section below, all clinical and laboratory studies to determine eligibility must be performed within 7 days prior to enrollment.

e. Initiation of systemic protocol therapy: Systemic Induction therapy, with the exception of steroid pretreatment as outlined below (Section 3.2.2), must begin within 72 hours of the first dose of intrathecal chemotherapy.

3.1.5 Inclusion of Women and Minorities

Both men and women of all races and ethnic groups are eligible for this study.

3.1.6 Randomization

Randomization will take place at the time a patient is enrolled via OPEN. Randomization will occur prior to Induction therapy for all patients (T-ALL and T-LLy).

3.2 Patient Eligibility Criteria

Important note: **The eligibility criteria listed below are interpreted literally and cannot be waived (per COG policy posted 5/11/01). All clinical and laboratory data required for determining eligibility of a patient enrolled on this trial must be available in the patient's medical/research record which will serve as the source document for verification at the time of audit.**

All clinical and laboratory studies to determine eligibility must be performed within 7 days prior to enrollment unless otherwise indicated. Imaging studies, if applicable, must be obtained within 2 weeks prior to start of protocol therapy (repeat the tumor imaging if necessary).

See Section 7.1 for required studies to be obtained prior to starting protocol therapy.

3.2.1 INCLUSION CRITERIA

a. Classification Study:

- T-ALL: T-ALL patients must be enrolled on AALL08B1 or Project:EveryChild (APEC14B1, if open for the classification of ALL patients) prior to treatment and enrollment on AALL1231.

b. Age at Diagnosis: All patients must be > 1 and < 31 years of age.

c. Diagnosis: Patients must have newly diagnosed T-Lymphoblastic Leukemia (T-ALL) or T-Lymphoblastic Lymphoma (T-LLy) Stages II-IV (see Appendix VIII).

Note: A diagnosis of T-ALL is established when leukemic blasts lack myeloperoxidase or evidence of B-lineage derivation (CD19/CD22/CD20), and express either surface or cytoplasmic CD3 or two or more of the antigens CD8, CD7, CD5, CD4, CD2 or CD1a, and are present either in peripheral blood or >25% in the bone marrow. If surface CD3 is expressed on all leukemic cells, additional markers of immaturity, including TdT, CD34 or CD99 will be assessed for expression. Cases with uncertain expression will receive additional review within the appropriate COG reference laboratory.

For T-LLy patients with tissue available for flow cytometry, the criterion for diagnosis should be analogous to T-ALL. For tissue processed by other means (i.e. paraffin blocks), the methodology and criteria for immunophenotypic analysis to establish the diagnosis of T-LLy defined by the submitting institution will be accepted. See pathologic diagnosis recommendation in Section 13.4 and required studies in Table 7.2, including diagnostic bone marrow MRD.

d. Informed consent: All patients and/or their parents or legal guardians must sign a written informed consent. Assent, when appropriate, will be obtained according to institutional guidelines.

3.2.2 EXCLUSION CRITERIA

a. Prior Therapy: Patients must not have received any cytotoxic chemotherapy for either the current diagnosis of T-ALL, T-LLy or for any cancer diagnosis prior to the initiation of protocol therapy on AALL1231, with the exception of:

- Steroid pretreatment: Prednisone or methylprednisolone for ≤ 120 hours (5 days) in the 7 days prior to initiating Induction chemotherapy or for ≤ 336 hours (14 days) in the 28 days prior to initiating Induction chemotherapy. Prior exposure to ANY steroids that occurred > 28 days before the initiation of protocol therapy does not affect eligibility. The dose of prednisone or methylprednisolone does not affect eligibility.
- Intrathecal cytarabine (The CNS status must be determined based on a sample obtained prior to administration of any systemic or intrathecal chemotherapy, except for steroid pretreatment as discussed in Section 3.3) Systemic chemotherapy must begin within 72 hours of this IT therapy; or
- Pretreatment with hydroxyurea; or
- 600 cGy of chest irradiation, if medically necessary.

Pre-treatment with dexamethasone in the 28 days prior to initiation of protocol therapy is not allowed with the exception of a single dose of dexamethasone used during sedation to prevent or treat airway edema. Inhalation steroids and topical steroids are not considered pretreatment.

b. Peripheral neurotoxicity: Pre-existing ≥ grade 2 sensory or motor peripheral neurotoxicity.

c. Seizures disorder: Uncontrolled seizure disorder

d. Diagnosis of Down syndrome (Trisomy 21)

e. Patients who are pregnant since fetal toxicities and teratogenic effects have been noted for several of the study drugs. A pregnancy test is required for female patients of childbearing potential.

f. Lactating females who plan to breastfeed.

g. Sexually active patients of reproductive potential who have not agreed to use an effective contraceptive method for the duration of their study participation.

h Patient has hypersensitivity to bortezomib, boron, or mannitol.

i Serious medical or psychiatric illness likely to interfere with participation in this clinical study.

j Participation in clinical trials with other investigational agents not included in this trial, within 14 days of the start of this trial and within 30 days of any dose of bortezomib.

3.2.3 Regulatory Requirement

a. All institutional, FDA, and NCI requirements for human studies must be met.

3.3 DEFINITIONS

3.3.1 INITIAL WBC

The first WBC at the treating COG institution. If prior therapy (i.e. steroids) or IV hydration has been administered then the initial WBC prior to therapy and/or hydration should be used.

3.3.2 CNS STAGING AT DIAGNOSIS (for both T-ALL and T-LLy patients)

CNS 1: In cerebral spinal fluid (CSF), absence of blasts on cytospin preparation, regardless of the number of white blood cells (WBCs).

CNS 2: In CSF, presence of < 5/ µL WBCs and cytospin positive for blasts or ≥ 5/µL WBCs with negative Steinherz Bleyer algorithm (see below).

CNS 2a: < 10/ µL RBCs; < 5/ µL WBCs and cytospin positive for blasts;
CNS 2b: ≥ 10/ µL RBCs; < 5/ µL WBCs and cytospin positive for blasts; and
CNS 2c: ≥ 10/ µL RBCs; ≥ 5/ µL WBCs and cytospin positive for blasts but negative by Steinherz/Bleyer algorithm (see below).

CNS3: In CSF, presence of ≥ 5/ µL WBCs and cytospin positive for blasts and/or clinical signs of CNS Leukemia.

CNS 3a: < 10/ µL RBCs; ≥ 5/µL WBCs and cytospin positive for blasts;
CNS 3b: ≥ 10/µL RBCs, ≥ 5/µL WBCs and positive by Steinherz/Bleyer algorithm (see below); and
CNS 3c: Clinical signs of CNS leukemia (such as facial nerve palsy, brain/eye involvement or hypothalamic syndrome).

3.3.3 TESTICULAR INVOLVEMENT AT DIAGNOSIS

Unilateral or bilateral testicular disease based on clinical exam or imaging. Biopsy is required if clinical findings are equivocal or suggestive of hydrocele or a non-leukemic mass.

3.3.4 BONE MARROW STATUS for T-ALL

M1: < 5% lymphoblasts
M2: 5 - 25% lymphoblasts
M3: > 25% lymphoblasts.

3.3.5 BONE MARROW STATUS FOR T-LLy PATIENTS

The MRD status of T-LLy patients is required at diagnosis in the MRD central reference laboratory and patients will be risk-stratified as described below. Marrow must have <25% morphologic blasts to be classified as T-LLy.

3.3.6 METHOD OF EVALUATING INITIAL TRAUMATIC LUMBAR PUNCTURES:

If the patient has leukemic cells in the peripheral blood and the lumbar puncture is traumatic and contains ≥ 5 WBC/μL and blasts, the following Steinherz/Bleyer algorithm should be used to distinguished between CNS2 and CNS3 disease:

$$\frac{\text{CSF WBC}}{\text{CSF RBC}} > 2X \quad \frac{\text{Blood WBC}}{\text{Blood RBC}}$$

A patient with CSF WBC ≥ 5/μL blasts, whose CSF WBC/RBC is 2X greater than the blood WBC/RBC ratio, has CNS disease at diagnosis. Example: CSF WBC = 60/μL; CSF RBC = 1500/μL; blood WBC = 46000/μL; blood RBC = 3.0 X 10^6/μL:

$$\frac{60}{1500} = 0.04 > 2X \quad \frac{46000}{3.0 \times 10^6} = 0.015$$

3.4 RISK STRATIFICATION

Criteria for risk stratification as Standard Risk (SR), Intermediate Risk (IR) or Very High Risk (VHR) for patients with T-ALL or T-LLy enrolled on this trial are outlined in the table below.

Note: Chemotherapy should not be delayed awaiting formal risk stratification by the study team in iMEDIDATA Rave. Please contact the study chair if there are questions regarding risk stratification

		T-ALL				T-LLy%		
	SR#	**IR**	**IR**	**VHR**	**SR**	**IR**	**IR**	**VHR**
Bone Marrow Results								
MRD at diagnosis*					<1%	<1%	≥1%	Any
Day 29 Status	M1	M1	M1 or M2	M3^				
MRD at Day 29	<0.01%	<0.01%	≥0.01%	Any				
MRD at EOC+			<0.1%	≥0.1%^				
CNS Status**	CNS1	Any	Any	Any	CNS1	Any	Any	Any
LP prior to steroids	Yes	Yes or No	Yes or No	Yes or No	Yes	Yes or No	Yes or No	Yes or No
Other Considerations								
Testicular Involvement$	None	Any	Any	Any	None	Any	Any	Any
Steroid Pretreatment@	None	Any	Any	Any	None	Any	Any	Any
End Induction Response					PR or CR	PR or CR	PR or CR	SD or NR

*MRD result from central reference lab ^M3 at day 29 or MRD≥0.1% at EOC = VHR

#SR=standard risk; IR=intermediate risk; VHR=very high risk

**CNS status: Any patient who is CNS2 or CNS3 cannot be Standard Risk and will be assigned to IR or VHR based on MRD response (T-ALL) or end Induction radiographic response (T-LLy)

$Testicular disease: Any patient who has testicular involvement at diagnosis cannot be Standard Risk and will be assigned to IR or VHR based on MRD response (T-ALL) or end Induction radiographic response (T-LLy)

@Any patient receiving corticosteroids within 4 weeks prior to the diagnostic lumbar puncture cannot be standard risk and will be assigned to IR or VHR based on MRD response.

% T-LLy: See Section 10.4 for additional information regarding the type of imaging and subsequent evaluations used to determine response. See also, evaluation tables in Sections 7.2, 7.3, and 7.4.
+ Patients with Day 29 MRD <0.01% do not need EOC MRD, regardless of features used in risk stratification, including steroid pretreatment, CNS or testicular disease status

3.4.1 CORTICOSTEROID PRETREATMENT AND RISK STRATIFICATION

It is recognized that T-ALL and T-LLy patients often present with hyperleukocytosis and/or mediastinal mass that requires emergent therapy. In addition, many patients present, having already recently received corticosteroids prior to the diagnosis of T-ALL or T-LLy. Corticosteroid exposures that may affect eligibility for the trial are listed in Section 3.2.2. Note: Inhalation steroids and topical steroids are not considered pre-treatment.

Patients with T-ALL receiving steroids within one month (Day -28 to Day -1) prior to the diagnostic lumbar puncture can not be SR and will be assigned to IR or VHR category depending on MRD response

Patients with T-LLy receiving steroids within one month (Day -28 to Day -1) prior to the diagnostic lumbar puncture or bone marrow can not be SR and will be assigned to the IR or VHR category depending on end Induction response

Patients with T-ALL or T-LLy who received steroids > 28 days preceding diagnosis but did not receive corticosteroids within the 28 days (Day -28 to Day -1) preceding diagnosis will not have risk allocation changed and can be SR.

Pre-treatment with prednisone or equivalent does not change the classification of CNS status, e.g. if a patient is pre-treated with corticosteroids and diagnostic LP demonstrates CNS 1 or CNS2, then that patient is NOT treated as CNS3 because of the prior steroid exposure.

Pre-treatment with dexamethasone in the 28 days prior to initiation of protocol therapy is not allowed with the exception of a single dose of dexamethasone used during sedation to prevent or treat airway edema. Patients who receive a single dose of dexamethasone to prevent or treat airway edema in the 28 days preceding diagnosis as described in section 3.2.2 are eligible for study; however, if this dose was given prior to the diagnostic lumbar puncture they can not be SR and will be assigned IR or VHR category based on MRD response. Pre-treatment with a single dose of dexamethasone does not change the classification of CNS status.

3.5 PHILADELPHIA CHROMOSOME POSITIVE (Ph+)

BCR-ABL1 (formerly known as BCR-ABL) fusion determined by FISH or RT-PCR

T-ALL patients entered onto AALL1231 who are later found to meet eligibility criteria for the AALL1122 Ph+ ALL study (or successor) are not eligible for post-Induction therapy on AALL1231 and should be taken off protocol therapy prior to Day 15 of Induction therapy.

T-LLy patients entered onto AALL1231 who are later found to meet the criteria for Ph+ T-LLy are not eligible for post-Induction therapy on AALL1231 and should be removed from protocol therapy prior to or at the end of Induction.

4.0 TREATMENT PLAN

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 (and up to 1 week for surgery) for valid clinical, patient and family logistical, or facility, procedure and/or anesthesia scheduling issues are acceptable (except where explicitly prohibited within the protocol).

4.1 Overview of Treatment Plan

See Experimental Design Schema T-ALL and Experimental Design Schema:T-LLy

4.1.1 Randomization

All T-ALL and T-LLy patients will be randomized upon enrollment to receive Induction treatment ± bortezomib. **Arm A is treatment without bortezomib and Arm B is treatment with bortezomib**. After Induction, all patients will receive the same 8 weeks of Consolidation therapy. Subsequent therapy will be dependent on risk assignment, as detailed below.

4.1.2 Treatment based on Risk Assignment

(see also Experimental Design Schema T-ALL and Experimental Design Schema: T-LLy)

a. Standard Risk T-ALL and T-LLy patients will be assigned to backbone therapy with one Interim Maintenance (IM) phase with Capizzi Methotrexate (CMTX) ± bortezomib. (See Section 3.3 for definition of SR-T-ALL and SR-T-LLy)

Standard Risk T-ALL and T-LLy patients will NOT receive CRT. Pretreatment with steroids may preclude Standard Risk status (see Section 3.3).

b. Intermediate Risk T-ALL and T-LLy will be assigned to backbone therapy with 2 IM phases: the first IM phase with High Dose Methotrexate (HDMTX) and the second occurring after Delayed Intensification (DI) with Capizzi Methotrexate (CMTX) ± bortezomib. (See Section 3.3 for definition of IR-T-ALL and IR T-LLy)

Only Intermediate Risk T-ALL and T-LLy patients who are CNS3 will receive CRT (1800 cGy) during 1st cycle (first 4 weeks) of Maintenance.

c. Very High Risk T-ALL patients will be assigned to backbone therapy that includes 3 HR Intensification Blocks. These patients will be risk assessed again after the 3 HR Blocks: T-ALL patients who are MRD positive (detectable by flow cytometry at any level) will be taken off protocol therapy and T-ALL patients who are MRD negative (undetectable at any level by flow cytometry) will remain on protocol therapy and will receive one DI (with or without bortezomib as randomized) and one CMTX IM phase. (See Section 3.3 for definition of VHR-T-ALL)

All VHR T-ALL patients receive CRT during the first cycle of Maintenance. CNS1 or CNS2 will receive 1200 cGy prophylactic CRT. CNS3 will receive 1800 cGy therapeutic CRT.

d. Very High Risk T-LLy patients will initially receive backbone therapy for Induction and Consolidation and then receive 3 HR Intensification Blocks. Disease evaluation will again be performed after the 3 HR Blocks.

1. T-LLy patients with no response or progressive disease as defined in Section 10.4 will be removed from protocol therapy.

2. Patients with a radiographic partial response as defined in Section 10.4 should be re-biopsied. T-LLy patients with biopsy proven persistent disease and/or morphologically positive bone marrow will be taken off protocol therapy. If re-biopsy is not feasible because of surgical morbidity concerns, the study chair should be contacted and the subject may be eligible to continue on therapy.
3. T-LLy patients who are radiographic partial responders with negative biopsies or complete responders will remain on protocol therapy and be treated with backbone therapy with 1 DI and 1 CMTX IM phase in treatment Arm A (without bortezomib)-VHR or Arm B (with bortezomib)-VHR. (See Section 3.3 for definition of VHR-T-LLy)

VHR T-LLy patients who are CNS3 will receive 1800 cGy therapeutic CRT.

4.1.3 Treatment Arms (T-ALL and T-LLy)

Risk Category- Arm A (Without Bortezomib)
(See Appendix I for Therapy Delivery Maps)

Standard Risk (A-SR)	Intermediate Risk (A-IR)	Very High Risk (A-VHR)
Induction (no bortezomib)	Induction (no bortezomib)	Induction (no bortezomib)
Consolidation	Consolidation	Consolidation
IM (CMTX)	IM#1 (HDMTX)	3 HR Intensification blocks
DI (no bortezomib)	DI (no bortezomib)	DI (no bortezomib)
Maintenance	IM#2 (CMTX)	IM (CMTX)
	Maintenance	Maintenance

Risk Category- Arm B (With Bortezomib)
(See Appendix II for Therapy Delivery Maps)

Standard Risk (B-SR)	Intermediate Risk (B-IR)	Very High Risk (B-VHR)
Induction (with bortezomib)	Induction (with bortezomib)	Induction (with bortezomib)
Consolidation	Consolidation	Consolidation
IM (CMTX)	IM#1 (HDMTX)	3 HR Intensification blocks
DI (with bortezomib)	DI (with bortezomib)	DI (with bortezomib)
Maintenance	IM#2 (CMTX)	IM (CMTX)
	Maintenance	Maintenance

4.2 Concomitant Therapy Restrictions

4.2.1 Drug Interactions with Conventional Chemotherapy

Since concurrent use of enzyme inducing anticonvulsants (e.g., phenytoin, phenobarbital, and carbamazepine) with anti-leukemic therapy has recently been associated with inferior EFS, every effort should be made to avoid these agents, as well as rifampin, which also induces many drug metabolizing enzymes.[62] Neither gabapentin nor levetiracetam induce hepatic drug metabolizing enzymes and may be suitable alternative anticonvulsant. Azole antifungals (listed in the table below) and the macrolide group of antibiotics (listed in the

table below) may have potent inhibitory effects on drug-metabolizing enzymes, and the doses of some anti-leukemic drugs (e.g., vincristine, anthracyclines, etoposide) may need to be reduced in some patients on chronic azole antifungals or antibiotics (see table below).

DRUG or DRUG CLASS^	POTENTIAL INTERACTION	ACTION TO BE TAKEN
Anticonvulsants	Induction of drug metabolizing enzymes Lowered EFS	AVOID phenytoin, phenobarbital, carbamazepine Consider gabapentin or levetiracetam (Keppra) as alternative
Rifampin	Induction of drug metabolizing enzymes	DO NOT USE
Azole Antifungals fluconazole itraconazole* posaconazole voriconazole ketoconazole	Inhibition of drug metabolizing enzymes	CONSIDER ALTERNATIVE MEDICATIONS May need dose reductions of vincristine*, anthracyclines, etoposide, steroids
Macrolide Antibiotics erythromycin clarithromycin azithromycin roxithromycin telithromycin	Inhibition of drug metabolizing enzymes	CONSIDER ALTERNATIVE MEDICATIONS May need dose reductions of vincristine, anthracyclines, etoposide, steroids

* Itraconazole should NOT be used in patients who are receiving vincristine due to a serious drug-drug interaction leading to severe neurotoxicity.[63,64]

^ For a more complete list of CYP 3A 4/5 Inhibitors and Inducers, go to: http://medicine.iupui.edu/flockhart/

4.2.2 Drug Interactions Specific to Bortezomib

In vitro and *in vivo* studies showed that green tea compounds, ascorbic acid (vitamin C) and other antioxidants, have the potential to significantly inhibit the activity of bortezomib.[135,136] One study concluded that there is not an interaction when plasma concentrations are commensurate with dietary oral intake.[137] To avoid the risk of possible interaction it is recommended that green tea containing products, and supplemental products containing vitamin C, flavonoids or other antioxidants (e.g., vitamins, herbal supplements), be discontinued for at least 24 hours prior to the initiation of bortezomib through 72 hours after the last bortezomib dose. In addition, it is recommended that the total dietary intake of vitamin C not exceed the recommended daily allowance (RDA) for age (i.e., normally balanced diets are acceptable).

4.2.3 Drug Interactions Specific to High-Dose Methotrexate

Possible Drug Interactions with Methotrexate:
Avoid non-steroidal anti-inflammatory drugs (NSAIDs), trimethoprim/sulfamethoxazole (TMP/SMX), penicillins, probenecid, IV contrast media, proton pump inhibitors, phenytoin and fosphenytoin. Urinary acidifiers can cause methotrexate to precipitate in the urinary tract.

Hold such agents on the days of a high dose methotrexate infusion and for at least 72 hours after the start of the infusion, until the methotrexate level is < 0.4 μM. If there is delayed methotrexate clearance, continue to hold such medications until the methotrexate level is <

0.1 µM.

4.3 General Guidelines

See Section 6.0, DRUG INFORMATION, for detailed information about drug administration.

4.3.2 Parenteral Chemotherapy Administration Guidelines

See Parenteral Chemotherapy Administration Guidelines (CAG) on the COG website at: https://members.childrensoncologygroup.org/_files/disc/Pharmacy/ChemoAdminGuidelines.pdf for special precautions and suggestions for patient monitoring during the infusion. As applicable, also see the CAG for suggestions on hydration, or hydrate according to institutional guidelines.

4.3.3 Supportive Care

4.3.3.1 Supportive Care Guidelines Regarding Management of Invasive Fungal Infections.

All treating physicians should have a low index of suspicion for invasive fungal infection (IFI), especially during Induction and during periods of prolonged neutropenia, including consolidation, delayed intensification, and the 3 intensification blocks. In patients with a new or unusual mucocutaneous lesion(s), prompt biopsy of suspicious lesions in an effort to confirm the diagnosis and guide further therapy is recommended as well as targeted imaging and sampling/culture of other clinically suspected areas of infection.

Children with prolonged fever in the setting of neutropenia that does not resolve with the use of antibiotics should receive empiric anti-mold antifungal therapy according to the COG endorsed fever and neutropenia guidelines: (https://childrensoncologygroup.org/downloads/COG_SC_FN_Guideline_Document.pdf). Please follow the guidelines for patients at high risk for Invasive Fungal Infections.

For patients with documented or suspected invasive fungal infection and neutropenia, consider growth factor support with GM-CSF (sargramostim) and/or G-CSF (filgrastim or pegfilgrastim).

While receiving corticosteroids, elevated blood glucose levels may be an additional risk factor. Thus, in patients with sustained hyperglycemia consider endocrinology evaluation. Treating oncologists are encouraged to work in close collaboration with their dermatology, infectious disease, otolaryngology, and surgical colleagues to aggressively and timely treat patients affected by IFIs with medical and surgical interventions, as indicated.

If an IFI is identified, please notify the study chair immediately.

For COG Supportive Care Guidelines see: https://members.childrensoncologygroup.org/prot/reference_materials.asp under Standard Sections for Protocols.

4.4 INDUCTION ARM A (without bortezomib)

This Induction is for all T-ALL and T-LLy patients randomized to treatment on Arm A (without bortezomib). See Section 4.1 for details. The therapy delivery map (TDM) for INDUCTION-Arm A (without bortezomib) is in APPENDIX I-A.

CRITERIA TO BEGIN INDUCTION

Patients must complete and sign the appropriate informed consent and undergo bortezomib randomization before starting Induction therapy. Treatment on Arm A is for patients who are randomized to treatment WITHOUT BORTEZOMIB.

Intrathecal Cytarabine: IT
Given at time of diagnostic lumbar puncture (if within 72 hours from the start of protocol therapy) OR Day 1.

Age-based dosing:

Age (yrs)	Dose
1 – 1.99	30 mg
2 – 2.99	50 mg
≥ 3	70 mg

Use preservative free formulation. The volume to be given IT should be in the range of 5-10 mL. The volume of CSF removed should be equal to at least half the volume delivered (see drug monograph).

Note: Larger volumes approximating at least 10% of the CSF volume, isovolumetric delivery, with the patient remaining lying down after the procedure may facilitate drug distribution. These procedures have not been validated in clinical trials. They are allowed but not mandated for patients on COG studies.

VinCRIStine: Administer IV push over 1 minute or infusion via minibag per institutional policy
Days: 1, 8, 15 and 22
Dose: 1.5 mg/m^2/dose (max dose 2 mg)

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."

VinCRIStine is available in a liposomal formulation (vinCRIStine sulfate liposomal injection, VSLI, Marqibo®). Use conventional vincristine only; the conventional and liposomal formulations are NOT interchangeable.

Dexamethasone: PO (may be given IV)
Days 1-28 (do not taper)
Dose: 3 mg/m^2/dose BID (i.e. total daily dose: 6 mg/m^2/day, divided BID)

If using tablets, adjust dose upward to the nearest 0.25 mg. Oral solutions are acceptable and intravenous preparations may be used on a temporary basis, if needed.

Of note, corticosteroid pretreatment using prednisone or methylprednisolone DOES NOT CHANGE the number of doses of dexamethasone.

DAUNOrubicin: IV push/infusion (over 1-15 minutes)
Days 1, 8, 15 and 22
Dose: 25 mg/m^2/dose

The reconstituted solution or the commercially available solution (5mg/ml) can be administered diluted or undiluted by slow IV push or infusion over 1-15 minutes. Short infusion times may be lengthened slightly (and up to 60 minutes) if institutional policies mandate. It is suggested that DAUNOrubicin be administered through the tubing of rapidly infusing solution of D_5W or 0.9% NaCl and that it is infused into a large vein. Protect from sunlight.

Special precautions: **Medication errors have occurred due to confusion between DAUNOrubicin and DOXOrubicin. DAUNOrubicin is available in a liposomal formulation (DAUNOrubicin citrate). The conventional and liposomal formulations are NOT interchangeable. The liposomal formulation is not allowed.**

Pegaspargase: IV over 1-2 hours through the tubing of a freely infusing solution of D_5W or 0.9% NaCl
Days 4 and 18
Dose: 2500 International units/m^2/dose

Intrathecal Methotrexate: IT
Days 8 and 29 (CNS3 patients ONLY also receive IT MTX on Days 15 & 22).

Aged-based dosing:

Age (yrs)	Dose
1 – 1.99	8 mg
2 – 2.99	10 mg
3 – 8.99	12 mg
≥ 9	15 mg

SEE PROTOCOL SECTION 5.0 FOR DOSE MODIFICATIONS. SEE THE COG MEMBER WEBSITE FOR SUPPORTIVE CARE GUIDELINES.

CRITERIA TO BEGIN CONSOLIDATION
Following completion of Induction Arm A, the next course (Consolidation, Section 4.6) starts on Day 36 (7 days following day 29 LP) or when peripheral counts recover, whichever occurs later. If the Day 29 marrow is M2 or M3 or has MRD >5%, the patient should begin Consolidation therapy as soon as possible and should not wait until Day 36 or for count recovery to occur. See below for additional details regarding peripheral count parameters. All patients receive common Consolidation therapy.

TESTICULAR BIOPSY
A testicular biopsy should be performed in patients with persistent testicular disease at the end of Induction if the clinical findings are equivocal.

DAY 29 ± 1 BONE MARROW MRD SAMPLES
THESE SAMPLES MUST BE OBTAINED AND SHIPPED TO THE COG ALL FLOW CYTOMETRY REFERENCE LABORATORY SO THAT RESULTS ARE AVAILABLE FOR RISK STRATIFICATION AT THE END OF CONSOLIDATION. ANY DEVIATION THAT EXCEEDS 1 DAY FROM DAY 29 MUST BE DISCUSSED WITH THE STUDY CHAIR. IF MRD IS NOT SENT THEN THE PATIENT WILL NOT BE ELIGIBLE TO CONTINUE ON A COG ALL TRIAL FOLLOWING COMPLETION OF INDUCTION THERAPY.

4.5 INDUCTION ARM B (with bortezomib)

CRITERIA TO BEGIN INDUCTION

Patients must complete and sign the appropriate informed consent and undergo bortezomib randomization before starting Induction therapy. Treatment on Arm B is for patients who are randomized to treatment WITH BORTEZOMIB. The therapy delivery map (TDM) for INDUCTION ARM B (with bortezomib) is in APPENDIX II-A.

Bortezomib: IV push over 3-5 seconds
Days 1, 4, 8, and 11
Dose: 1.3 mg/m^2/dose

Special precautions: FOR INTRAVENOUS USE ONLY.
The container or the syringe containing bortezomib must be clearly labeled "For intravenous use only -Fatal if given by other routes."

Note: Consecutive doses of bortezomib must be separated by at least 72 hours. Grapefruit and its juice should be avoided for the duration of treatment with bortezomib during each course. To avoid the risk of any possible interaction it is recommended that green tea containing products and any supplemental products containing vitamin C, flavonoids or other antioxidants (e.g., vitamins, herbal supplements) be discontinued from at least 24 hours prior to the initiation of bortezomib through 72 hours after the last bortezomib dose. In addition, it is recommended that the total dietary intake of vitamin C not exceed the RDA for age (i.e., normally balanced diets are acceptable).

Bortezomib is supplied by Millennium Pharmaceuticals and distributed by the NCI DTCD. **Do not use commercially available drug.**

If bortezomib is not available on Day 1 of Induction, then administer the first bortezomib dose as soon as possible and do not delay the start of other Induction chemotherapy. Subsequent bortezomib doses should be given after 72 and 144 hours. All doses must be at least 72 hours apart.

Intrathecal Cytarabine: IT
Given at time of diagnostic lumbar puncture (if within 72 hours from the start of protocol therapy) OR Day 1.

Age-based dosing:

Age (yrs)	Dose
1 – 1.99	30 mg
2 – 2.99	50 mg
≥ 3	70 mg

For IT administration use preservative free formulation. The volume to be given IT should be in the range of 5-10 mL. The volume of CSF removed should be equal to at least half the volume delivered (see drug monograph).

Note: Larger volumes approximating at least 10% of the CSF volume, isovolumetric delivery, with the patient remaining lying down after the procedure may facilitate drug distribution. These procedures have not been validated in clinical trials. They are allowed but not mandated for patients on COG studies.

VinCRIStine: Administer IV push over 1 minute or infusion via minibag per institutional policy
Days 1, 8, 15 and 22
Dose: 1.5 mg/m^2/dose (max dose 2 mg)

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."

VinCRIStine is available in a liposomal formulation (vinCRIStine sulfate liposomal injection, VSLI, Marqibo®). Use conventional vincristine only; the conventional and liposomal formulations are NOT interchangeable.

Dexamethasone: PO (maybe given IV)
Days 1-28 (do not taper)
Dose: 3 mg/m^2/dose BID (i.e. total daily dose: 6 mg/m^2/day, divided BID)

If using tablets, adjust dose upward to the nearest 0.25 mg. Oral solutions are acceptable and intravenous preparations may be used on a temporary basis, if needed.

DAUNOrubicin: IV push/infusion over 1-15 minutes
Days 1, 8, 15 and 22
Dose: 25 mg/m^2/dose

The reconstituted solution or the commercially available solution (5mg/ml) can be administered diluted or undiluted by slow IV push or infusion over 1-15 minutes. Short infusion times may be lengthened slightly (and up to 60 minutes) if institutional policies mandate. It is suggested that DAUNOrubicin be administered through the tubing of rapidly infusing solution of D_5W or 0.9% NaCl and that it is infused into a large vein or central venous access device. Protect from sunlight.

Special precautions: **Medication errors have occurred due to confusion between DAUNOrubicin and DOXOrubicin. DAUNOrubicin is available in a liposomal formulation (DAUNOrubicin citrate). The conventional and liposomal formulations are NOT interchangeable. The liposomal formulation is not allowed.**

Pegaspargase: IV over 1-2 hours through the tubing of a freely infusing solution of D_5W or 0.9% NaCl.
Day 4 and 18
Dose: 2500 International units/m^2/dose

Intrathecal Methotrexate: IT
Administer on Days 8 and 29 (CNS3 patients also receive IT MTX on Days 15 & 22).

Aged-based dosing:

Age (yrs)	Dose
1 – 1.99	8 mg
2 – 2.99	10 mg
3 – 8.99	12 mg
≥ 9	15 mg

Use preservative free formulation. The volume to be given IT should be in the range of 5-10 mL. The volume of CSF removed should be equal to at least half the volume delivered (see drug monograph).

SEE PROTOCOL SECTION 5.0 FOR DOSE MODIFICATIONS. SEE THE COG MEMBER WEBSITE FOR SUPPORTIVE CARE GUIDELINES.

CRITERIA TO BEGIN CONSOLIDATION

Following completion of INDUCTION ARM B (with bortezomib), the next course (Consolidation, Section 4.6) starts on Day 36 (7 days following day 29 LP) or when peripheral counts recover (whichever occurs later). If the Day 29 marrow is M2 or M3 or has MRD >5%, the patient should begin Consolidation therapy as soon as possible and should not wait until Day 36 or for count recovery to occur. See below for additional details regarding peripheral count parameters. All patients receive common Consolidation therapy.

TESTICULAR BIOPSY

A testicular biopsy should be performed in patients with persistent testicular disease at the end of Induction if the clinical findings are equivocal.

DAY 29 ± 1 BONE MARROW MRD SAMPLES

THESE SAMPLES MUST BE OBTAINED AND SHIPPED TO THE COG ALL FLOW CYTOMETRY REFERENCE LABORATORY SO THAT RESULTS ARE AVAILABLE FOR RISK STRATIFICATION AT THE END OF CONSOLIDATION. ANY DEVIATION THAT EXCEEDS 1 DAY FROM DAY 29 MUST BE DISCUSSED WITH THE STUDY CHAIR. IF MRD IS NOT SENT THEN THE PATIENT WILL NOT BE ELIGIBLE TO CONTINUE ON A COG ALL TRIAL FOLLOWING COMPLETION OF INDUCTION THERAPY.

4.6 CONSOLIDATION-All Patients

This Consolidation course is for all Arm A and Arm B T-ALL and T-LLy patients. See Section 4.1 for details. The therapy delivery map (TDM) for CONSOLIDATION is in APPENDIX I-B (for subjects on Arm A) and APPENDIX II-B (for subjects on Arm B).

CRITERIA TO BEGIN CONSOLIDATION

Start Consolidation on Day 36 from Induction (7 days following Day 29 LP) or when peripheral counts recover with ANC ≥ 750/µL and platelets ≥ 75,000/µL (whichever occurs later.) T-ALL patients who are M2 or M3 on Day 29 or have MRD >5% should proceed directly to Consolidation without waiting for count recovery, but there should be a minimum of 3 days between Day 29 Induction IT therapy and Day 1 Consolidation IT therapy).

Once Consolidation therapy has begun, interruptions for myelosuppression (ANC ≤ 750/µL and platelets ≤ 75,000/µL) should occur only at Day 29. Once the Day 1 or Day 29 cyclophosphamide has been given, the remainder of the therapy should not be held solely for myelosuppression, which is expected to occur. Therapy must be interrupted for patients with serious proven or presumed infection and resumed when the signs of infection have abated. Therapy should not be interrupted for fever, if there are no signs of serious infection. Hold Day 29 chemotherapy until ANC ≥ 750/µL and platelets ≥ 75,000/µL.

Intrathecal Methotrexate: IT
Days 1, 8, 15[#] and 22[#]
[#] if CNS3 T-ALL or CNS3 T-LLy: omit Days 15 & 22 and administer on days 1 and 8 only

Aged-based dosing:

Age (yrs)	Dose
1 – 1.99	8 mg
2 – 2.99	10 mg
3 – 8.99	12 mg
≥ 9	15 mg

Use preservative free formulation. The volume to be given IT should be in the range of 5-10 mL. The volume of CSF removed should be equal to at least half the volume delivered (see drug monograph).

Cyclophosphamide: IV over 30-60 minutes
Days 1 and 29
Dose: 1000 mg/m^2/dose

May be administered as undiluted drug (20 mg/mL, reconstitute with 0.9% NaCl only to avoid hypotonic solution) or further diluted. Mesna is not required for this dose of cyclophosphamide, but may be administered at institutional discretion.

Cytarabine: IV (administer over 1-30 minutes) or SubQ
Days 1-4, 8-11, 29-32 and 36-39
Dose: 75 mg/m^2/dose

Subcutaneous Administration: Reconstitute to a concentration not to exceed 100 mg/mL. Rotate injection sites to thigh, abdomen, and flank regions. Avoid repeated administration to a single site. Aspirate prior to injection to avoid injection into a blood vessel.

Mercaptopurine: PO
Days 1-14 and 29-42
Dose: 60 mg/m^2/dose once daily*

*See Section 5.11 for suggested starting dose based on TPMT and NUDT15 status (if status is known)

It is strongly recommended that mercaptopurine be taken at the same time each day.

The liquid or tablet formulation may be used. If using tablets, adjust daily dose using the dosing nomogram in Appendix VI to attain a weekly cumulative dose as close to 420 mg/m^2/week as possible. Do not escalate or reduce dose based on blood counts during this cycle.

Pegaspargase: IV over 1-2 hours through the tubing of a freely infusing solution of D_5W or 0.9% NaCl
Days 15 and 43
Dose: 2500 International units/m^2/dose

VinCRIStine: Administer IV push over 1 minute or infusion via minibag per institutional policy
Days 15, 22, 43 and 50
Dose: 1.5 mg/m^2/dose (max dose 2 mg)

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."

VinCRIStine is available in a liposomal formulation (vinCRIStine sulfate liposomal injection, VSLI, Marqibo®). Use conventional vincristine only; the conventional and liposomal formulations are NOT interchangeable.

See the Parenteral Chemotherapy Administration Guidelines (CAGs) on the COG website at: https://members.childrensoncologygroup.org/_files/disc/Pharmacy/ChemoAdminGuidelines.pdf for special precautions and suggestions for patient monitoring during the infusions. As applicable, also see the CAGs for suggestions on hydration, or hydrate according to institutional guidelines.

SEE PROTOCOL SECTION 5.0 FOR DOSE MODIFICATIONS. SEE THE COG MEMBER WEBSITE FOR SUPPORTIVE CARE GUIDELINES.

TESTICULAR RADIATION THERAPY
T-ALL and T-LLy patients with persistent testicular disease at end-Induction should receive radiation to the testes during Consolidation. A testicular biopsy should be performed if the clinical findings are equivocal. During the first two weeks of Consolidation, testicular radiation therapy will be given at 2400 cGy in 12 once-daily fractions of 200 cGy (see Section 16.2). Testicular radiation must be started during Consolidation and should be completed before the end of this phase of therapy. **Patients with testicular leukemia at diagnosis that clinically resolves completely by end-Induction, and those that have a negative testicular biopsy at end-Induction will NOT receive testicular irradiation.**

BONE MARROW AT END CONSOLIDATION
a. T-ALL SR only:
SR T-ALL patients DO NOT require a bone marrow at the end of Consolidation (see Section 4.1 for definitions of risk assignment). These patients should start the next course of therapy (Interim Maintenance with CMTX) following the completion of Consolidation as soon as counts have recovered with an ANC ≥750/μL and platelets ≥75,000/μl.

BONE MARROW AT END CONSOLIDATION (CONT'D)

b. T-ALL patients who are not SR and with end Induction BM MRD ≥ 0.01% **:**

- Following completion of Consolidation, end of consolidation marrow MRD will determine risk assignment (see Section 4.1 for details) and the next course of therapy.
- The end of Consolidation marrow should occur as close to Day 57 as possible, but should be delayed until counts have recovered with an ANC ≥ 500/µL and platelets ≥ 50,000/µL. The end of Consolidation marrow should not be performed prior to Day 57 even if counts have recovered.
- If on Day 57, the counts have recovered with an ANC ≥ 500/µL and platelets ≥ 50,000/µL, the bone marrow for end of Consolidation MRD should be performed on that day. A one day deviation is allowed, but any deviation that is greater than 1 day after count recovery must be discussed with the study chair.
- If on Day 57, the counts have not recovered (e.g., ANC< 500/µL or platelets < 50,000/µL), the bone marrow for end of Consolidation MRD should be delayed until ANC ≥ 500/µL and platelets ≥ 50,000/µL. The bone marrow for end of consolidation MRD should be performed within 2 days of count recovery (ANC ≥ 500/µL and platelets ≥ 50,000/µL). Any deviation that is greater than 3 days after count recovery must be discussed with the study chair. If counts have not recovered on Day 57, patients should have a CBC checked every 2-3 days (three times a week) at minimum until count recovery to minimize delay in obtaining the bone marrow.
- Patients who have not had count recovery (e.g. ANC ≥ 500/µL and platelets ≥ 50,000/µL) by Day 72 should undergo bone marrow to ensure they are M1 and not M2 or M3.

CRITERIA TO BEGIN NEXT COURSE OF THERAPY FOR T-ALL

a. If the End of Consolidation marrow is M2 or M3, the patient should immediately proceed to Intensification Block 1 and not wait for MRD results to proceed.

b. Patients should begin the next course of therapy (HD MTX if IR, HR BFM Block 1 if VHR) as soon as the results of EOC MRD are available and they have an ANC ≥750/µL and platelets ≥75,000/µl.

c. As there will likely be a few day delay waiting for MRD results between the end of Consolidation marrow and the start of the next course of therapy, the next block of therapy lumbar puncture with intrathecal chemotherapy should not be given early (e.g. at the same time as the end of consolidation bone marrow) and should be given with the next block of therapy.

DAY 57 END OF CONSOLIDATION BONE MARROW FOR MRD

THE END OF CONSOLIDATION BM MRD SAMPLES MUST BE OBTAINED AND SHIPPED TO COG ALL FLOW CYTOMETRY REFERENCE LABORATORY TO HAVE RESULTS AVAILABLE FOR END OF CONSOLIDATION (EOC) RISK STRATIFICATION. THE BONE MARROW FOR MRD MUST BE OBTAINED AS CLOSE TO DAY 57 AS POSSIBLE. DELAYS FOR COUNT RECOVERY AS DESCRIBED ABOVE ARE ALLOWED BUT OTHER DEVIATIONS MUST BE DISCUSSED WITH THE STUDY CHAIR. If MRD IS NOT SENT THEN THE PATIENT WILL NOT BE ELIGIBLE TO CONTINUE ON A COG ALL TRIAL FOLLOWING COMPLETION OF CONSOLIDATION THERAPY.

c. T-LLy only:

Following completion of Consolidation, the next course (based on risk assignment, see Section 4.1 for details) will start as soon as counts have recovered as described in the relevant block of

therapy. Bone marrow is only required for patients who had morphologic evidence of disease at the end of induction.

4.7 INTERIM MAINTENANCE with CMTX- – All Patients

This IM phase is administered to all patients. SR T-ALL and T-LLy patients receive it after Consolidation. IR T-ALL and T-LLy patients receive it after DI as IM#2. VHR T-ALL and T-LLy patients receive it after DI. See Section 4.1 for details.

The therapy delivery map (TDM) for INTERIM MAINTENANCE with CMTX is in APPENDIX I-C (Arm A-SR) APPENDIX I-G (Arm A-IR), APPENDIX I-L (Arm A-VHR), APPENDIX II-C (Arm B-SR), APPENDIX II-G (Arm B-IR), APPENDIX II-L (Arm B-VHR).

CRITERIA TO BEGIN INTERIM MAINTENANCE WITH CMTX: IR AND VHR PATIENTS

Start when ANC ≥750/µL and platelets ≥75,000/µl and need to meet renal and hepatic function requirements to initiate methotrexate as described in Section 5.9.

CRITERIA TO BEGIN INTERIM MAINTENANCE WITH CMTX: SR PATIENTS

SR T-ALL and T-LLy patients receive this block immediately after consolidation and **must meet all of following criteria.**

1. Post-Consolidation risk assignment has been completed as described in Section 4.1
2. ANC ≥750/µL and platelets ≥75,000/µl.
3. Need to meet renal and hepatic function requirements to initiate methotrexate as described in Section 5.9.1, Section 5.9.2, and Section 5.9.3

VinCRIStine: Administer IV push over 1 minute or infusion via minibag as per institutional policy
Days 1, 11, 21, 31 and 41
Dose: 1.5 mg/m^2/dose (max dose 2 mg)

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."

VinCRIStine is available in a liposomal formulation (vinCRIStine sulfate liposomal injection, VSLI, Marqibo®). Use conventional vincristine only; the conventional and liposomal formulations are NOT interchangeable.

Methotrexate: IV over 2-5 minutes (undiluted) or over 10-15 minutes (diluted).
Days 1, 11, 21, 31 and 41.
Dose: Start dose at 100 mg/m^2/dose and escalate by **50 mg/m^2/dose** on Days 1, 11, 21, 31 and 41 based on blood count requirements as described in Section 5.10.5. Discontinue escalation and resume at 80% of last dose if delay is necessary as described in Section 5.10.5.

Pegaspargase: IV over 1-2 hours through the tubing of a freely infusing solution of D_5W or 0.9% NaCl.
Days 2 and 22
Dose: 2500 International units/m^2/dose

Intrathecal Methotrexate: IT
Days 1 and 31

Aged-based dosing:

Age (yrs)	Dose
1 – 1.99	8 mg
2 – 2.99	10 mg
3 – 8.99	12 mg
≥ 9	15 mg

Use preservative free formulation. The volume to be given IT should be in the range of 5-10 mL. The volume of CSF removed should be equal to at least half the volume delivered (see drug monograph).

SEE PROTOCOL SECTION 5.0 FOR DOSE MODIFICATIONS. SEE THE COG MEMBER WEBSITE FOR SUPPORTIVE CARE GUIDELINES.

The therapy delivery map (TDM) for INTERIM MAINTENANCE with CMTX is in APPENDIX I-C (Arm A-SR) APPENDIX I-G (Arm A-IR), APPENDIX I-L (Arm A-VHR), APPENDIX II-C (Arm B-SR), APPENDIX II-G (Arm B-IR), APPENDIX II-L (Arm B-VHR).

Following completion of INTERIM MAINTENANCE with CMTX the next course (based on risk assignment, see Section 4.1 for details) starts on Day 57 or when blood count parameters are met (whichever occurs later).

4.8 DELAYED INTENSIFICATION- Arm A (without bortezomib)

This Delayed Intensification (DI) course is for all T-ALL and T-LLy patients randomized to treatment on Arm A (without bortezomib). See Section 4.1 for details.

The therapy delivery maps (TDMs) for DELAYED INTENSIFICATION- ARM A are in APPENDIX I-D (Arm A-SR), APPENDIX I-F (Arm A-IR), and APPENDIX I-K (Arm A-VHR)

CRITERIA TO BEGIN DELAYED INTENSIFICATION SR AND IR T-ALL AND T-LLY:

1. ANC ≥ 750/µL
2. Platelets ≥ 75,000/µL prior to starting therapy.

CRITERIA TO BEGIN DELAYED INTENSIFICATION VHR T-ALL:

VHR T-ALL patients receive this block immediately after HR Intensification 3 and **must meet all of the following criteria**

1. Post-HR Intensification Block 3 MRD is undetectable as described in Section 4.13
2. ANC ≥750/µL
3. Platelets ≥75,000/µl

CRITERIA TO BEGIN DELAYED INTENSIFICATION VHR T-LLY:

VHR T-LLy patients receive this block immediately after HR Intensification 3 and **must meet all of the following criteria**

1. Post-HR Intensification Block 3 disease evaluation demonstrates a radiographic CR OR a radiographic PR with a biopsy showing no residual disease as described in Section 10.4
2. ANC ≥750/µL
3. Platelets ≥75,000/µl

ADDITIONAL GUIDELINES DURING DI FOR ALL RISK GROUPS.

Patients should have ANC ≥ 750/µL and platelets ≥ 75,000/µL prior to starting therapy. Once Delayed Intensification has begun, it may be interrupted for myelosuppression (ANC ≤ 750/µL and platelets ≤ 75,000/µL) on Day 29 ONLY. Once the Day 1 therapy or Day 29 cyclophosphamide has been given, the remainder of the therapy should not be held solely for myelosuppression, which is expected to occur. Therapy must be interrupted for patients with serious proven or presumed infection and resumed when the signs of infection have abated.

VinCRIStine: Administer IV push over 1 minute or infusion via minibag as per institutional policy.
Days 1, 8, 15, 43 and 50
Dose: 1.5 mg/m^2/dose (max dose 2 mg)

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."

VinCRIStine is available in a liposomal formulation (vinCRIStine sulfate liposomal injection, VSLI, Marqibo®). Use conventional vincristine only; the conventional and liposomal formulations are NOT interchangeable.

Dexamethasone: PO (maybe given IV)
Days 1-7 and 15-21
Dose: All patients, regardless of age, receive discontinuous dexamethasone at 5 mg/m^2/dose BID

(i.e. total daily dose: 10 mg/m^2/day, divided BID)

If using tablets, adjust dose upward to the nearest 0.25 mg. Oral solutions are acceptable and intravenous preparations may be used on a temporary basis, if needed.

DOXOrubicin: IV push (over 15 minutes)
Days 1, 8 and 15
Dose: 25 mg/m^2/dose

Administer at a concentration not to exceed 2mg/ml by slow IV push or infusion over 1-15 minutes. Short infusion times may be lengthened slightly (and up to 60 minutes) if institutional policies mandate. It is suggested that DOXOrubicin be administered through the tubing of rapidly infusing solution of D_5W or 0.9% NaCl and that it is infused into a large vein.

Special precautions: **Medication errors have occurred due to confusion between DOXOrubicin and DAUNOrubicin. DOXOrubicin is available in a liposomal formulation. The conventional and liposomal formulations are NOT interchangeable.**

Pegaspargase: IV over 1-2 hours through the tubing of a freely infusing solution of D_5W or 0.9% NaCl
Days 4, 18 and 43
Dose: 2500 International units/m^2/dose

Intrathecal Methotrexate: IT
Administer on Days 1, 29 and 36.

Aged-based dosing:

Age (yrs)	Dose
1 – 1.99	8 mg
2 – 2.99	10 mg
3 – 8.99	12 mg
≥ 9	15 mg

Use preservative free formulation. The volume to be given IT should be in the range of 5-10 mL. The volume of CSF removed should be equal to at least half the volume delivered (see drug monograph).

Cyclophosphamide: IV over 30-60 minutes
Day 29
Dose: 1000 mg/m^2/dose

May be administered as undiluted drug (20 mg/mL, reconstitute with 0.9% NaCl only to avoid hypotonic solution) or further diluted. Mesna is not required for this dose of cyclophosphamide, but may be administered at institutional discretion.

Cytarabine: IV (Administer over 1-30 minutes) or SubQ
Days 29-32 and 36-39
Dose: 75 mg/m^2/dose

Subcutaneous Administration: Reconstitute to a concentration not to exceed 100 mg/mL. Rotate injection sites to thigh, abdomen, and flank regions. Avoid repeated administration to a single site. Aspirate prior to injection to avoid injection into a blood vessel.

Thioguanine: PO
Days 29-42
Dose: 60 mg/m^2/dose

Administer in the **evening** preferably on an empty stomach (at least 1 hour before or 2 hours after food or drink except water). Tablets are scored and doses can be rounded to half tablet. Adjust dose using ½ tablets and different doses on alternating days in order to attain a weekly cumulative dose as close to 420 mg/m^2/week as possible. See Appendix VII for details.

SEE PROTOCOL SECTION 5.0 FOR DOSE MODIFICATIONS. SEE THE COG MEMBER WEBSITE FOR SUPPORTIVE CARE GUIDELINES.

Following completion of DELAYED INTENSIFICATION - Arm A, the next course (based on risk assignment, see Section 4.1 for details) starts on Day 64 or when blood count parameters are met (whichever occurs later).

4.9 DELAYED INTENSIFICATION Arm B (with bortezomib)

This Delayed Intensification (DI) course is for all T-ALL and T-LLy patients randomized to treatment on Arm B (with bortezomib). See Section 4.1 for details.

The therapy delivery maps (TDMs) for DELAYED INTENSIFICATION- Arm B are in APPENDIX II-D (Arm B-SR), APPENDIX II-F (Arm B-IR) and APPENDIX II-K (Arm B-VHR).

CRITERIA TO BEGIN DELAYED INTENSIFICATION SR AND IR T-ALL AND T-LLY:

1. ANC ≥ 750/µL
2. Platelets ≥ 75,000/µL

CRITERIA TO BEGIN DELAYED INTENSIFICATION VHR T-ALL:

VHR T-ALL patients receive this block immediately after HR Intensification 3 and **must meet all of the following criteria**

1. Post-HR Intensification Block 3 MRD was completed and demonstrated undetectable MRD as described in Section 4.13
2. ANC ≥750/µL
3. Platelets ≥75,000/µl

CRITERIA TO BEGIN DELAYED INTENSIFICATION VHR T-LLY:

VHR T-LLy patients receive this block immediately after HR Intensification 3 and **must meet all of the following criteria**

1. Post-HR Intensification Block 3 disease evaluation was completed and demonstrated a radiographic CR OR a radiographic PR and a biopsy showing no residual disease as described in Section 10.4
2. ANC ≥750/µL
3. Platelets ≥75,000/µl

ADDITIONAL GUIDELINES DURING DI FOR ALL RISK GROUPS.

Patients should have ANC ≥ 750/µL and platelets ≥ 75,000/µL prior to starting therapy. Once Delayed Intensification has begun, it may be interrupted for myelosuppression (ANC ≤ 750/µL and platelets ≤ 75,000/µL) on Day 29 ONLY. Once the Day 1 therapy or Day 29 cyclophosphamide has been given, the remainder of the therapy should not be held solely for myelosuppression, which is expected to occur. Therapy must be interrupted for patients with serious proven or presumed infection and resumed when the signs of infection have abated. Hold Day 29 chemotherapy until ANC ≥ 750/µL and platelets ≥ 75,000/µL.

Bortezomib: IV push over 3-5 seconds
Days 1, 4, 15, and 18
Dose: 1.3 mg/m^2/dose

Special precautions: FOR INTRAVENOUS USE ONLY.
The container or the syringe containing Bortezomib must be clearly labeled "For intravenous use only - Fatal if given by other routes."

Note: Consecutive doses must be separated by at least 72 hours. Grapefruit and its juice should be avoided for the duration of treatment with bortezomib during each course. To avoid the risk of any possible interaction it is recommended that green tea containing products and any supplemental products containing vitamin C, flavonoids or other antioxidants (e.g., vitamins, herbal supplements) be discontinued from at least 24 hours prior to the initiation of bortezomib through 72 hours after the last bortezomib dose. In addition, it is recommended that the total dietary intake of vitamin C not exceed the RDA for age (i.e., normally balanced diets are acceptable).

Bortezomib is supplied by Millennium Pharmaceuticals and distributed by the NCI DTCD. **Do not use commercially available drug.**

VinCRIStine: Administer IV push over 1 minute or infusion via minibag as per institutional policy.
Days 1, 8, 15, 43 and 50
Dose: 1.5 mg/m^2/dose (max dose 2 mg)

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."

VinCRIStine is available in a liposomal formulation (vinCRIStine sulfate liposomal injection, VSLI, Marqibo®). Use conventional vincristine only; the conventional and liposomal formulations are NOT interchangeable.

Dexamethasone: PO (maybe given IV)
Days 1-7 and 15-21
Dose: All patients, regardless of age, receive discontinuous dexamethasone: 5 mg/m^2/dose BID (i.e. total daily dose 10 mg/m^2/day, divided BID)

If using tablets, adjust dose upward to the nearest 0.25 mg. Oral solutions are acceptable and intravenous preparations may be used on a temporary basis, if needed.

DOXOrubicin: IV push (over 15 minutes)
25 mg/m^2/dose on Days 1, 8 and 15

Administer at a concentration not to exceed 2mg/ml by slow IV push or infusion over 1-15 minutes. Short infusion times may be lengthened slightly (and up to 60 minutes) if institutional policies mandate. It is suggested that DOXOrubicin be administered through the tubing of rapidly infusing solution of D_5W or 0.9% NaCl and that it is infused into a large vein.

Special precautions: **Medication errors have occurred due to confusion between DOXOrubicin and DAUNOrubicin. DOXOrubicin is available in a liposomal formulation. The conventional and liposomal formulations are NOT interchangeable.**

Pegaspargase: IV over 1-2 hours through the tubing of a freely infusing solution of D_5W or 0.9% NaCl
Day 4, 18 and 43
Dose: 2500 International units/m^2/dose

Intrathecal Methotrexate: IT
Administer on Days 1, 29 and 36.

Aged-based dosing:

Age (yrs)	Dose
1 – 1.99	8 mg
2 – 2.99	10 mg
3 – 8.99	12 mg
≥ 9	15 mg

Use preservative free formulation. The volume to be given IT should be in the range of 5-10 mL. The volume of CSF removed should be equal to at least half the volume delivered (see drug monograph).

Cyclophosphamide: IV over 30-60 minutes
Day 29
Dose: 1000 mg/m^2/dose

May be administered as undiluted drug (20 mg/mL, reconstitute with 0.9% NaCl only to avoid hypotonic solution) or further diluted. Mesna is not required for this dose of cyclophosphamide, but may be administered at institutional discretion.

Cytarabine: IV (Administer over 1-30 minutes) or SubQ
Days 29-32 and 36-39
Dose: 75 mg/m^2/dose

Subcutaneous Administration: Reconstitute to a concentration not to exceed 100 mg/mL. Rotate injection sites to thigh, abdomen, and flank regions. Avoid repeated administration to a single site. Aspirate prior to injection to avoid injection into a blood vessel.

Thioguanine: PO
Days 29-42
Dose: 60 mg/m^2/dose

Administer in the evening preferably on an empty stomach (at least 1 hour before or 2 hours after food or drink except water). Tablets are scored and doses can be rounded to half tablet. Adjust dose using ½ tablets and different doses on alternating days in order to attain a weekly cumulative dose as close to 420 mg/m^2/week as possible. See Appendix X for details.

SEE PROTOCOL SECTION 5.0 FOR DOSE MODIFICATIONS. SEE THE COG MEMBER WEBSITE FOR SUPPORTIVE CARE GUIDELINES.

Following completion of DELAYED INTENSIFICATION- Arm B (with bortezomib), the next course (based on risk assignment, see Section 4.1 for details) starts on Day 64 or when blood count parameters are met (whichever occurs later).

4.10 INTERIM MAINTENANCE #1 with HD MTX- ALL INTERMEDIATE RISK SUBJECTS

This is IM#1 for all IR T-ALL and T-LLy patients randomized to either treatment Arm. SR and VHR T-ALL and T-LLy patients do not receive this block. See Section 4.1 for details.

The therapy delivery maps (TDMs) for Interim Maintenance #1 with HD MTX are in APPENDIX I-E (Arm A-IR) and APPENDIX II-E (Arm B-IR).

CRITERIA TO BEGIN INTERIM MAINTENANCE WITH HD MTX: IR PATIENTS ONLY

IR T-ALL and T-LLy patients receive this block immediately after consolidation and **must meet all of the following criteria**

1. Post-consolidation risk assignment completed as described in Section 4.1
2. ANC ≥750/µL and platelets ≥75,000/µl.
3. Need to meet renal and hepatic function to receive HD MTX as defined in Section 5.9.1, Section 5.9.2, and Section 5.9.3

High Dose Methotrexate: **IV over 24 hours**
Days 1, 15, 29, and 43
Dose: 5000 mg/m^2/dose (no maximum dose)

For HD MTX Infusion Guidelines and liver and kidney requirements to administer drug see Section 5.9 and Appendix IV.

Leucovorin: PO/IV
Days 3-4, 17-18, 31-32, and 45-46
Dose: 15mg/m^2/dose x minimum of 3 doses given at 42, 48, and 54 hours after the start of the HD MTX infusion. See Section 5.9 and Appendix IV for HD MTX Infusion and Leucovorin rescue guidelines.

VinCRIStine: Administer IV push over 1 minute or infusion via minibag as per institutional policy.
Days 1, 15, 29, 43
Dose: 1.5 mg/m^2/dose (max dose 2 mg)

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."

VinCRIStine is available in a liposomal formulation (vinCRIStine sulfate liposomal injection, VSLI, Marqibo®). Use conventional vincristine only; the conventional and liposomal formulations are NOT interchangeable.

Mercaptopurine: PO
Days 1-56
Dose: 25 mg/m^2/dose once daily*

*Other Considerations:
*See Section 5.11 for suggested starting dose based on TPMT and NUDT15 status (if status is known)

It is strongly recommended that mercaptopurine be taken at the same time each day.

The liquid or tablet formulation may be used. If using tablets, adjust daily dose using the dosing nomogram in Appendix VI to attain a weekly cumulative dose as close to 175 mg/m2/week as possible. Do not escalate or reduce dose based on blood counts during this cycle. Mercaptopurine should be held for ANC < 750/µL or platelets < 75 000/µL. Do not make up missed doses (see Section 5.11.1).

Intrathecal Methotrexate: IT
Days 1 and 29

When IT therapy and HD MTX are scheduled for the same day, deliver the IT therapy within 6 hours of the beginning of the IV MTX infusion (hour -6 to +6, with 0 being the start of the MTX bolus).

Age-based dosing:

Age (yrs)	Dose
1 – 1.99	8 mg
2 – 2.99	10 mg
3 – 8.99	12 mg
≥ 9	15 mg

SEE PROTOCOL SECTION 5.0 FOR DOSE MODIFICATIONS. SEE THE COG MEMBER WEBSITE FOR SUPPORTIVE CARE GUIDELINES.

Following completion of Interim Maintenance #1 the next course (based on randomization assignment, see Section 4.1 for details) starts on Day 57 or when blood count parameters are met (whichever occurs later).

4.11 INTENSIFICATION BLOCK 1 (HR1)- ALL VERY HIGH RISK SUBJECTS

This phase of treatment is for all VHR T-ALL and T-LLy patients who are randomized to either treatment Arm. SR and IR T-ALL and T-LLy patients do not receive this block.

The therapy delivery maps (TDMs) for INTENSIFICATION BLOCK 1 are in APPENDIX I-H (Arm A-VHR) and APPENDIX II-H (Arm B-VHR).

CRITERIA TO BEGIN INTENSIFICATION BLOCK 1

T-ALL patients who are M2 or M3 at the end of Consolidation should proceed directly to Intensification Block 1 without waiting for count recovery or MRD results to proceed.

VHR T-ALL and T-LLy patients receive this block immediately after consolidation and **must meet all of the following criteria**

1. Post-consolidation risk assignment must have been completed as described in Section 4.1
2. ANC≥750/µL
3. Platelets ≥75,000/µl
4. Need to meet renal and hepatic function requirements to receive HD MTX as defined in Section 5.9.2, Section 5.9.3, and Section 5.9.4.
5. Need to meet renal function requirements to receive HD ARAC as defined in Section 5.4.4

The 3 High Risk Intensification blocks are profoundly myelosuppressive and since they also include 5 days of high dose dexamethasone, clinical signs of infection may be blunted. Therefore, the following precautions are required for all subjects during each high risk block:

- CBC with differential every 2 days after completion of chemotherapy until there is evidence of marrow recovery. Recovery is defined as ANC >0.2 x 10^9/L (200µ/L) and platelet transfusion independence.
- G-CSF 5 µg/kg/day SC or IV starting anytime from 7 days to 11 days from the start of the block (e.g., at least 24 hours after completion of chemotherapy in each HR block) until the WBC count is >3.0 x 109/L (>3000/mm^3). [Pegfilgrastim 100 µg/kg (max 6 mg/dose) s.c. given once during the 7-11th day from the start of the block may be considered as an alternative.]

Additionally the following precautions are STRONGLY recommended for all subjects during each high risk block:

- Hospitalization of patients for close observation after each intensive block of chemotherapy, particularly during the period of profound myelosuppression, until there is evidence of blood count recovery because this is the intervention most likely to reduce the death rate.
- A very low threshold should be used for institution of empiric antimicrobial therapy since dexamethasone may blunt the signs of a life threatening infection.

Dexamethasone: PO (maybe given IV)
Days 1-5
Dose: 10 mg/m^2/dose (i.e. total daily dose: 20 mg/m^2/day, divided BID)

If using tablets, adjust dose upward to the nearest 0.25 mg. Oral solutions are acceptable and intravenous preparations may be used on a temporary basis, if needed.

High Dose Methotrexate: IV over 24 hours
Day 1
Dose: 5000 mg/m^2/dose (no maximum dose)

For HD MTX Infusion Guidelines and renal and hepatic function requirements see Section 5.9 and Appendix IV):

Leucovorin: PO/IV
Days 3-4
Dose: 15 mg/m^2/dose x minimum of 3 doses given at 42, 48, and 54 hours after the start of the HD MTX infusion. See Section 5.9 and Appendix IV for HD MTX Infusion and Leucovorin rescue guidelines.

VinCRIStine: Administer IV push over 1 minute or infusion via minibag as per institutional policy.
Days 1 and 6
Dose: 1.5 mg/m^2/dose (max dose 2 mg)

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."

VinCRIStine is available in a liposomal formulation (vinCRIStine sulfate liposomal injection, VSLI, Marqibo®). Use conventional vincristine only; the conventional and liposomal formulations are NOT interchangeable.

Cyclophosphamide: IV over 1-6 hours
Day 2-4
Dose: 200 mg/m^2/dose Q12 hours x5doses

May be administered as undiluted drug (20 mg/mL, reconstitute with 0.9% NaCl only to avoid hypotonic solution) or further diluted.

High Dose Cytarabine: IV (infuse over 3 hours)
Day 5
Dose: 2000 mg/m^2/dose Q12 hours x 2 doses

Suggested premedications and supportive care: Administer steroid eye drops (0.1% dexamethasone or 1% prednisolone ophthalmic solution), 2 drops to each eye every 6 hours, beginning immediately before the first dose and continuing for 24 hours after the last dose. If patient does not tolerate steroid eye drops, may administer artificial tears on an every 2-4 hour schedule.

Pegaspargase: IV over 1-2 hours
Day 6*
Dose: 2500 International units/m^2/dose

Administer over 1-2 hours through the tubing of a freely infusing solution of D_5W or 0.9% NaCl.
*Administer 3 hours after completion of the second High Dose Cytarabine infusion.

Triple Intrathecal Therapy: IT
Day 1*

Age-based dosing:
1 to < 2 yrs: MTX: 8 mg, HC: 8 mg, ARAC: 16 mg
2 to < 3 yrs: MTX: 10 mg, HC: 10 mg, ARAC: 20 mg
3 to < 9 yrs: MTX: 12 mg, HC: 12 mg, ARAC: 24 mg
≥ 9 yrs: MTX 15 mg, HC: 15 mg, ARAC: 30 mg

*Should be given 2 hours after start of High Dose MTX infusion (window of -6 to +6 hours in relation to start of HD-MTX is acceptable)

Filgrastim: SubQ/IV
5 mcg/kg/dose daily beginning on Day 7 and until WBC > 3000/µL.
Administer undiluted by subcutaneous injection (preferred). May also administer diluted in D5W by IV infusion over 15-30 minutes or by continuous infusion.

*Pegfilgrastim 100 mcg/kg (max 6 mg/dose) s.c. once during the 7-11th day from the start of the block may be considered as an alternative

SEE PROTOCOL SECTION 5.0 FOR DOSE MODIFICATIONS. SEE THE COG MEMBER WEBSITE FOR SUPPORTIVE CARE GUIDELINES.

Following completion of INTENSIFICATION BLOCK 1 Arm, the next course (INTENSIFICATION BLOCK 2 Section 4.12) starts on Day 22 or when blood count parameters are met (whichever occurs later).

4.12 INTENSIFICATION BLOCK 2- ALL VERY HIGH RISK SUBJECTS

This phase of treatment is for all VHR T-ALL and T-LLy patients who are randomized to either treatment Arm. SR and IR T-ALL and T-LLy patients do not receive this block.

The therapy delivery maps (TDMs) for INTENSIFICATION BLOCK 2 are in APPENDIX I-I (Arm A-VHR) and APPENDIX II-I (Arm B-VHR).

Start Day 1 when ANC ≥750/µL and platelets ≥75,000/µL. Need to meet renal and hepatic function requirements described in Section 5.9.2, Section 5.9.3, and Section 5.9.4. in order to receive HD MTX. HR2 lasts 21 days.

The 3 High Risk Intensification blocks are profoundly myelosuppressive and since they also include 5 days of high dose dexamethasone, clinical signs of infection may be blunted. Therefore, the following precautions are required for all subjects during each high risk block:

- CBC with differential every 2 days after completion of chemotherapy until there is evidence of marrow recovery. Recovery is defined as ANC >0.2 x 10^9/L (200µ/L) and platelet transfusion independence.
- G-CSF 5 µg/kg/day SC or IV starting anytime from 7 days to 11 days from the start of the block (e.g., at least 24 hours after completion of chemotherapy in each HR block) until the WBC count is >3.0 x 109/L (>3000/mm^3). [Pegfilgrastim 100 µg/kg (max 6 mg/dose) SC. given once during the 7-11^{th} day from the start of the block may be considered as an alternative.]

Additionally, the following precautions are STRONGLY recommended for all subjects during each high risk block:

- Hospitalization of patients for close observation after each intensive block of chemotherapy, particularly during the period of profound myelosuppression, until there is evidence of blood count recovery because this is the intervention most likely to reduce the death rate.
- A very low threshold should be used for institution of empiric antimicrobial therapy since dexamethasone may blunt the signs of a life threatening infection.

Dexamethasone: PO (maybe given IV)
Days 1-5
Dose: 10 mg/m^2/dose BID (i.e. total daily dose: 20 mg/m^2/day)

If using tablets, adjust dose upward to the nearest 0.25 mg. Oral solutions are acceptable and intravenous preparations may be used on a temporary basis, if needed.

High Dose Methotrexate: IV over 24 hours
Day 1
Dose: 5000 mg/m^2/dose (no maximum dose)

For HD MTX Infusion Guidelines and renal and hepatic requirements to administer see Section 5.9 and Appendix IV

Leucovorin: PO/IV
Days 3-4
Dose: 15mg/m^2/dose x minimum of 3 doses given at 42, 48, and 54 hours after the start of the HD MTX infusion. See Section 5.9 and Appendix IV for HD MTX Infusion and Leucovorin rescue guidelines.

VinCRIStine: Administer IV push over 1 minute or infusion via minibag as per institutional policy.
Days 1 and 6
Dose: 1.5 mg/m^2/dose (max dose 2 mg)

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."

VinCRIStine is available in a liposomal formulation (vinCRIStine sulfate liposomal injection, VSLI, Marqibo®). Use conventional vincristine only; the conventional and liposomal formulations are NOT interchangeable.

Ifosfamide: IV over 1 hour
Days 2-4*
Dose: 800 mg/m^2/dose Q12hours x5 doses

*Start immediately after completion of HD-MTX infusion

Suggested hydration: Administer 3,000 mL/m²/day (125 mL/m²/hour) using fluid containing D_5W/0.45% NaCl or 0.9% NaCl. Achieve urine specific gravity ≤ 1.010 prior to start of ifosfamide. Monitor for adequate urine output as per institution guidelines. May use diuretics (e.g., furosemide) to increase urine output. Consider adding potassium and magnesium to prevent electrolyte deficiencies.

Mesna: IV
Days: 2-4
Dose: 300 mg/m^2/dose Hour 0, 4, and 8 from start of each ifosfamide infusion

Hydration following ifosfamide administration: If patient stops methotrexate hydration for whatever reason, need to continue until 12 hours post ifosfamide infusion.

DAUNOrubicin: IV push/infusion over 1-15 minutes
Day 5
Dose: 30 mg/m^2/dose

The reconstituted solution or the commercially available solution (5mg/ml) can be administered diluted or undiluted by slow IV push or infusion over 1-15 minutes. Short infusion times may be lengthened slightly (and up to 60 minutes) if institutional policies mandate. It is suggested that DAUNOrubicin be administered through the tubing of rapidly infusing solution of D_5W or 0.9% NaCl and that it is infused into a large vein or central venous access device. Protect from sunlight.

Special precautions: **Medication errors have occurred due to confusion between DAUNOrubicin and DOXOrubicin. DAUNOrubicin is available in a liposomal formulation (DAUNOrubicin citrate). The conventional and liposomal formulations are NOT interchangeable. The liposomal formulation is not allowed.**

Pegaspargase: IV over 1-2 hours through the tubing of a freely infusing solution of D_5W or 0.9% NaCl
Day 6
Dose: 2500 International units/m^2/dose IV

Triple Intrathecal Therapy: Day 1*
Age-based dosing:
1 to < 2 yrs: MTX: 8 mg, HC: 8 mg, ARAC: 16 mg
2 to < 3 yrs: MTX: 10 mg, HC: 10 mg, ARAC: 20 mg
3 to < 9 yrs: MTX: 12 mg, HC: 12 mg, ARAC: 24 mg
≥ 9 yrs: MTX 15 mg, HC: 15 mg, ARAC: 30 mg

*Should be given 2 hours after start of High Dose MTX infusion (window of -6 to +6 hours in relation to start of HD-MTX is acceptable)

Filgrastim*: SubQ/IV
Day 7
Dose 5 mcg/kg/dose daily until WBC > 3000/µl

*Pegfilgrastim 100 mcg/kg (max 6 mg/dose) s.c. once during the 7-11th day from the start of the block may be considered as an alternative.

SEE PROTOCOL SECTION 5.0 FOR DOSE MODIFICATIONS. SEE THE COG MEMBER WEBSITE FOR SUPPORTIVE CARE GUIDELINES.

CRITERIA FOR INITIATION OF INTENSIFICATION BLOCK 3
Following completion of INTENSIFICATION BLOCK 2 Arm, the next course (INTENSIFICATION BLOCK 3, Section 4.13) starts on Day 22 or when blood count parameters are met (whichever occurs later).

4.13 INTENSIFICATION BLOCK 3 (HR3) ALL VERY HIGH RISK SUBJECTS

This phase of treatment is for all VHR T-ALL and T-LLy patients who are randomized to either treatment Arm. SR and IR T-ALL and T-LLy patients do not receive this block.

The therapy delivery maps (TDMs) for INTENSIFICATION BLOCK 3 are in APPENDIX I-J (Arm A-VHR) and APPENDIX II-J (Arm B-VHR).

CRITERIA FOR INITIATION OF INTENSIFICATION BLOCK 3

Start Day 1 when ANC ≥ 750/µL and platelets ≥ 75,000/µL. HR3 lasts 21 days. Patients must meet renal function requirements described in Section 5.4.4.

The 3 High Risk Intensification blocks are profoundly myelosuppressive and since they also include 5 days of high dose dexamethasone, clinical signs of infection may be blunted. Therefore, the following precautions are required for all subjects during each high risk block:

- CBC with differential every 2 days after completion of chemotherapy until there is evidence of marrow recovery. Recovery is defined as ANC >0.2 x 10^9/L (200µ/L) and platelet transfusion independence.
- G-CSF 5 µg/kg/day SC or IV starting anytime from 7 days to 11 days from the start of the block (e.g., at least 24 hours after completion of chemotherapy in each HR block) until the WBC count is >3.0 x 109/L (>3000/mm^3). [Pegfilgrastim 100 µg/kg (max 6 mg/dose) s.c. given once during the 7-11th day from the start of the block may be considered as an alternative.]

Additionally the following precautions are STRONGLY recommended for all subjects during each high risk block:

- Hospitalization of patients for close observation after each intensive block of chemotherapy, particularly during the period of profound myelosuppression, until there is evidence of blood count recovery because this is the intervention most likely to reduce the death rate.
- A very low threshold should be used for institution of empiric antimicrobial therapy since dexamethasone may blunt the signs of a life threatening infection.

Dexamethasone: PO (maybe given IV)
Days 1-5
Dose: 10 mg/m^2/dose BID (i.e. total daily dose: 20 mg/m^2/day, divided BID)

If using tablets, adjust dose upward to the nearest 0.25 mg. Oral solutions are acceptable and intravenous preparations may be used on a temporary basis, if needed.

High Dose Cytarabine: IV (over 3 hours)
Days 1-2
Dose: 2 gm/m^2/dose Q12 hours x 4 doses

Suggested premedications and supportive care: Administer steroid eye drops (0.1% dexamethasone or 1% prednisolone ophthalmic solution), 2 drops to each eye every 6 hours, beginning immediately before the first dose and continuing for 24 hours after the last dose. If patient does not tolerate steroid eye drops, may administer artificial tears on an every 2-4 hour schedule.

Etoposide: IV over 1-2 hours
100 mg/m^2/dose Q12 hours x 5 doses on Days 3-5
The first dose of etoposide should be given approximately 12 hours after the start of the 4th dose of high dose cytarabine on Day 2. Etoposide will be administered every 12 hours thereafter for a total of 5 doses.

Infuse diluted solution (concentration ≤ 0.4 mg/mL) over at least 60-120 minutes; slow rate of administration if hypotension occurs. Rate should not exceed 300 mg/m^2/hour (10 mg/kg/hour) (hypotension risk). The use of an in-line filter during the infusion is suggested.

Special precautions: Etoposide can be mixed in 0.9% NaCl or D_5W. Avoid use of large volumes of D_5W due to potential development of hyponatremia.

Pegaspargase: IV, administer over 1-2 hours through the tubing of a freely infusing solution of D_5W or 0.9% NaCl
Day 6
Dose: 2500 International units/m^2/dose

Triple Intrathecal Therapy: IT Methotrexate (MTX), Hydrocortisone (HC), Cytarabine (ARAC)
Day 5

Age-based dosing:
1 to < 2 yrs.: MTX: 8 mg, HC: 8 mg, ARAC: 16 mg
2 to < 3 yrs.: MTX: 10 mg, HC: 10 mg, ARAC: 20 mg
3 to < 9 yrs.: MTX: 12 mg, HC: 12 mg, ARAC: 24 mg
≥ 9 yrs.: MTX 15 mg, HC: 15 mg, ARAC: 30 mg

Filgrastim: SubQ/IV*
5 mcg/kg/dose daily beginning on Day 7 and until WBC > 3000/µl.

Administer undiluted by subcutaneous injection (preferred). May also administer diluted in D5W by IV infusion over 15-30 minutes or by continuous infusion.

*Pegfilgrastim 100 mcg/kg (max 6 mg/dose) SC. once during the 7-11th day from the start of the block may be considered as an alternative.

T-ALL: After completing the 3 HR Intensification Blocks, T-ALL patients continue on protocol therapy resuming at DI with or without bortezomib as randomized if MRD is undetectable. If MRD is detectable, patients are no longer eligible to continue on protocol therapy.

T-LLy: After completing the 3 HR Intensification Blocks, T-LLy patients continue on protocol therapy resuming at DI with or without bortezomib as randomized if they have a complete response as defined in Section 10.4 and a morphologically negative marrow. Patients with persistent disease by imaging should be re-biopsied. If there is active disease by pathologic examination, patients are no longer eligible to continue on protocol therapy.

TIMING OF BONE MARROW MRD FOR PATIENTS WITH VHR T-ALL.
The marrow for MRD that is obtained after Intensification HR3 should occur as close to Day 21 as possible, but should be delayed until counts have recovered with an ANC ≥ 500/µL and platelets ≥ 50,000/µL. OF NOTE, this is different from Day 29 MRD which must be sent on Day 29 regardless of counts. The end of HR3 marrow should not be performed before Day 21 even if the counts have recovered.

If on Day 21, the counts have recovered with an ANC ≥ 500/µL and platelets ≥ 50,000/µL, the bone marrow for end of HR3 MRD should be performed that day. A 1 day deviation is allowed, but any deviation that is greater than 1 day after count recovery must be discussed with the study chair.

If on Day 21, the counts have not recovered (e.g., ANC< 500/µL or platelets < 50,000/µL), the bone marrow for end of HR3 MRD should be delayed until ANC ≥ 500/µL and platelets ≥ 50,000/µL. The bone marrow for end of HR3 MRD should be performed within 2 days of count recovery (ANC ≥ 500/µL and platelets ≥ 50,000/µL). Any deviation that is greater than 2 days after count recovery must be discussed with the study chair. If counts have not recovered on Day 21, patients should have a CBC checked every 2-3 days (three times a week) at minimum until count recovery to minimize delay in obtaining the bone marrow.

As there will likely be a delay of a few days waiting for MRD results between the end of HR3 marrow and the start of the next course of therapy (DI), the DI lumbar puncture with intrathecal chemotherapy should not be given early (e.g. at the same time as the end of HR3 bone marrow) and should be given with the next block of therapy.

SEE PROTOCOL SECTION 5.0 FOR DOSE MODIFICATIONS. SEE THE COG MEMBER WEBSITE FOR SUPPORTIVE CARE GUIDELINES.

Following completion of INTENSIFICATION BLOCK 3 Arm A-VHR, subjects will either stop treatment due to lack of response or continue on to next course. The next course (based on randomization, see Section 4.1 for details) starts on Day 22 or when blood count parameters are met (whichever occurs later).

4.14 MAINTENANCE - All Patients

This Maintenance course is for all patients irrespective of study phase or treatment randomization assignment.

CRITERIA FOR STARTING MAINTENANCE

1. ANC ≥ 750/µL
2. Platelets ≥ 75,000/µL.

VinCRIStine: Administer IV push over 1 minute or infusion via minibag as per institutional policy.
Days 1, 29 and 57
Dose: 1.5 mg/m^2/dose (max dose 2 mg)

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."

VinCRIStine is available in a liposomal formulation (vinCRIStine sulfate liposomal injection, VSLI, Marqibo®). Use conventional vincristine only; the conventional and liposomal formulations are NOT interchangeable.

Dexamethasone: PO (may be given IV)
Days 1-5, 29-33, 57-61
Dose: 3 mg/m^2/dose BID (i.e. total daily dose: 6 mg/m^2/day, divided BID)

If using tablets, adjust dose upward to the nearest 0.25 mg. Oral solutions are acceptable and intravenous preparations may be used on a temporary basis, if needed.

Mercaptopurine: PO
Days 1-84
Dose: 75 mg/m^2/dose*

Other Considerations:
*See Section 5.11 for suggested starting dose based on TPMT and NUDT15 status (if status is known)

It is strongly recommended that mercaptopurine be taken at the same time each day.

If using tablets, adjust daily dose using the dosing nomogram in Appendix VI to attain a weekly cumulative dose as close to 525 mg/m^2/week as possible. See Section 5.11.2 for dose modifications during Maintenance.

Methotrexate: PO
Days 8, 15, 22, 29*, 36, 43, 50, 57, 64, 71 and 78
Dose: 20 mg/m^2/dose weekly
* Omit Day 29 of first 4 cycles for SR T-ALL and T-LLy patients and for first 2 cycles of Maintenance for IR T-ALL and T-LLy patients.

Administer on an empty stomach (at least 1 hour before or 2 hours after food or drink except water).

Intrathecal Methotrexate: IT
Administer on Day 1 **(also on Day 29 of the first 4 cycles of Maintenance for Standard Risk T-ALL and T-LLy patients ONLY and on Day 29 of the first 2 cycles of Maintenance for Intermediate Risk T-ALL and T-LLy patients ONLY)**.

Aged-based dosing:

Age (yrs)	Dose
1 – 1.99	8 mg
2 – 2.99	10 mg
3 – 8.99	12 mg
≥ 9	15 mg

Use preservative free formulation. The volume to be given IT should be in the range of 5-10 mL. The volume of CSF removed should be equal to at least half the volume delivered (see drug monograph).

Cranial Radiation Therapy

All CRT will be given during the 1st cycle (first 4 weeks) of Maintenance. See the tables below to determine which subjects will receive CRT. See Section 16.1 for details.

T-ALL- Cranial Radiation Therapy

	SR	IR	VHR
CNS 1	none	none	CRT (1200 cGy)
CNS 2	none	none	CRT (1200 cGy)
CNS 3	n/a	CRT (1800 cGy)	CRT (1800 cGy)

T-LLy- Cranial Radiation Therapy

	SR	IR	VHR
CNS 1	none	none	none
CNS 2	none	none	none
CNS 3	n/a	CRT (1800 cGy)	CRT (1800 cGy)

SEE PROTOCOL SECTION 5.0 FOR DOSE MODIFICATIONS. SEE THE COG MEMBER WEBSITE FOR SUPPORTIVE CARE GUIDELINES.

SEE SECTION 6.0, DRUG INFORMATION, FOR DETAILED INFORMATION ABOUT DRUG ADMINISTRATION.

The therapy delivery maps (TDMs) for MAINTENANCE are in APPENDIX I-M (Arm A) and APPENDIX II-M (Arm B).

CRITERIA TO BEGIN MAINTENANCE CYCLES

Begin subsequent Maintenance cycles regardless of counts. Only mercaptopurine and PO methotrexate will be interrupted for myelosuppression as outlined in Section 5.10.

DOSE MODIFICATIONS FOR MYELOSUPPRESSION DURING MAINTENANCE

Only mercaptopurine and PO methotrexate will be interrupted for myelosuppression as outlined in Section 5.10.

DURATION OF THERAPY

1. GIRLS SR and IR T-ALL: Continue to repeat 12-week cycles of Maintenance therapy until the total duration of therapy is **two** years from the start of Interim Maintenance (~ Week 119).

2. GIRLS VHR T-ALL: Continue to repeat 12-week cycles of Maintenance therapy until the total duration of therapy is **two** years from the start of Intensification Block #1 (~ Week 119).

3. BOYS SR and IR T-ALL: Continue to repeat 12-week cycles of Maintenance therapy until the total duration of therapy is **three** years from the start of Interim Maintenance (~ Week 171).

4. BOYS VHR T-ALL: Continue to repeat 12-week cycles of Maintenance therapy until the total duration of therapy is **three** years from the start of Intensification Block #1 (~ Week 171).

5. T-LLy patients (regardless of gender): Continue to repeat 12-week cycles of Maintenance therapy until the total duration of therapy is **two** years from the start of Interim Maintenance (~Week 119)

6. May stop therapy on anniversary date if dexamethasone is completed for the 5-day dexamethasone pulse. Anniversary date is defined as the date marking two (2) years (for T-ALL girls and all T-LLy patients, regardless of gender) and three (3) years (for T-ALL boys) from the start of Interim Maintenance #1 for SR and IR patients, and from the start of Intensification Block #1 for VHR patients.

5.0 DOSE MODIFICATION FOR TOXICITIES

Notify the Study Chair at the time of removing a patient from protocol therapy for toxicity. The drugs are listed in alphabetical order.

5.1 Asparaginase [E.coli, Pegaspargase (PEG-Asparaginase) or Erwinia]

5.1.1 Allergy

Local Allergic Reactions (inflammation at injection site, swelling): Note these recommendations only apply when the asparaginase product is administered intramuscularly. Continue asparaginase administration in the presence of Grade 1 allergy as defined by CTCAE v4.0 (transient flushing or rash; drug fever < 38ºC).

Systemic Allergic Reactions: In the event of Grade 1 reactions, characterized by transient flushing or rash and drug fever < 38ºC, without the need for treatment with antihistamines or steroids, the dose of asparaginase being administered intravenously may be continued with close observation.

Discontinuation is recommended for Grade 2 or higher allergic reactions, as defined by CTCAE v4.0, that require medical intervention.

Note: Premedication with antihistamines to decrease the risk of overt allergy symptoms is strongly discouraged since anti-histamine use may mask the appearance of systemic allergy. Systemic allergy is frequently associated with the presence of asparaginase neutralizing antibodies, which render asparaginase therapy ineffective. In the event of Grade 2 or higher systemic or recurrent local allergic reaction, Erwinia asparaginase should be substituted.

Arm A ONLY: Patients with allergy/anaphylaxis to pegaspargase may be eligible to receive IV pegcrisantaspase, a pegylated form of Erwinia asparaginase, on COG AALL1421 at doses prescribed on that protocol. Patients who have previously received *Erwinia* asparaginase or are receiving other investigational agents (including patients on Arm B of AALL1231) are **not** eligible for AALL1421.

5.1.2 Anaphylaxis

Discontinue pegaspargase or *E. coli* if the patient develops Grade 3 anaphylaxis as defined by CTCAE v4.0 (symptomatic bronchospasm, with or without urticaria, parenteral intervention indicated; allergy-related edema/angioedema; hypotension). If this occurs, Erwinia asparaginase should be substituted.

Erwinia asparaginase has a shorter half-life and is associated with a shorter duration of asparagine depletion than native *E. coli* asparaginase, with "head-to-head" comparisons of Erwinia and *E. coli* asparaginase, using the same dose and schedule for both preparations, demonstrating a superior outcome, favoring *E. coli* asparaginase.[138,139] Pegaspargase has a longer half-life and is associated with more prolonged asparagine depletion than native *E. coli* asparaginase, but the largest randomized trial comparing weekly native to bi-weekly pegaspargase wasn't powered to detect a difference in outcome.[140] Current COG trials have adopted pegaspargase as the preparation of choice, based on the results of CCG 1962.[141] COG AALL07P2 showed that Erwinia asparaginase was well tolerated and achieved nadir serum asparaginase activity at both 48 and 72 hours after dosing that was similar to that achieved with pegaspargase. Based on these and other data, the FDA approved Erwinia asparaginase for use following allergy to pegaspargase, with a dose of Erwinia 25,000 IU/m^2 x 6 doses IM on a Monday/Wednesday/Friday schedule substituted for a single dose of pegaspargase.

PATIENTS ENROLLED IN AALL0932, AALL1131 OR AALL1231 MAY BE ELIGIBLE TO ENROLL IN AALL1421 IF THEY DEVELOP AN ALLERGIC REACTION TO PEGASPARGASE THAT PRECLUDES FURTHER ADMINISTRATION OF THAT DRUG. The new AALL1421 trial was developed to study the pharmacokinetics and tolerability of pegcrisantaspase in patients with hypersensitivity to PEG-asparaginase. On AALL1421 patients will receive pegcrisantaspase at the assigned dose via 1 hour IV infusion as a replacement for each scheduled dose of PEG-asparaginase remaining on the original treatment protocol. Please see the AALL1421 protocol web page for more information.

The dose modification guidelines for ALL trials recommend the substitution for replacement of Erwinia asparaginase for either native or pegaspargase utilizing the following schedule:

Phase(s) of Treatment	**Drug(s)**	**Replacement Schedule for Erwinia asparaginase**[#]	**Important Notes**
Induction, Consolidation, Interim Maintenance (CMTX), Delayed Intensification, Intensification Blocks 1, 2, and 3	One or more doses of pegaspargase (2,500 IU/m^2)	25,000 IU/m^2/dose IM or IV# M/W/F x 6 doses for each dose of pegaspargase.	

[#]If a patient develops a Grade 3 or higher anaphylaxis to Erwinia, discontinue future asparaginase therapy. Consider discontinuation for severe Grade 2 or higher allergic reactions

To replace a dose of intravenous pegaspargase that was discontinued during the infusion due to an allergic reaction, the following recommendations may be used to guide patient care.

In the event that a pegaspargase infusion is discontinued for an allergic reaction, regardless of amount received, substitution with *Erwinia* asparaginase should begin approximately 48 hours after pegaspargase has been discontinued and preferably to coincide with the recommended Monday/Wednesday/Friday administration schedule detailed above in patients who are clinically stable. Up to 6 doses of *Erwinia* asparaginase may be administered, as tolerated, to replace the incomplete intravenous pegaspargase dose. Of note, *Erwinia* asparaginase is recommended only for pegaspargase hypersensitivity reactions, and not for pancreatitis, hepatitis, coagulation abnormalities, or other non-hypersensitivity toxicities associated with pegaspargase. To best suit the needs of each individual patient, additional modifications to these recommendations may be made at the discretion of the treating physician.

5.1.3 Coagulopathy

If symptomatic, hold asparaginase until symptoms resolve, then resume with the next scheduled dose. Consider factor replacement (FFP, cryoprecipitate, factor VIIa). Do not withhold dose for abnormal laboratory findings without clinical symptoms.

5.1.4 Hyperbilirubinemia
Asparaginase may need to be withheld in patients with an elevated direct bilirubin, since asparaginase has been associated with hepatic toxicity. No specific guidelines are available.

5.1.5 Hyperglycemia
Do not modify dose. Treat hyperglycemia as medically indicated.

5.1.6 Hyperlipidemia
Do not modify dose

5.1.7 Ketoacidosis
Hold asparaginase until blood glucose can be regulated with insulin.

5.1.8 Pancreatitis
Discontinue asparaginase in the presence of Grade 3 or 4 pancreatitis. In the case of asymptomatic Grade 2 pancreatitis (enzyme elevation or radiologic findings only), asparaginase should be held until symptoms and signs subside, and amylase/lipase levels return to normal and then resumed. Grade 3 or 4 pancreatitis is a contraindication to additional asparaginase administration.

5.1.9 Thrombosis
Withhold asparaginase until symptoms have resolved, and treat with appropriate antithrombotic therapy, as indicated. Upon resolution of symptoms consider resuming asparaginase, while continuing LMWH or antithrombotic therapy. Do not withhold dose for abnormal laboratory findings without clinical correlate. For significant thrombosis, which is not catheter-related, consider evaluation for inherited predisposition to thrombosis.

CNS Events (bleed, thrombosis or infarction): Hold asparaginase. Treat with FFP, factors or anticoagulation as appropriate. Consider resuming at full dose when all symptoms have resolved (and evidence of recanalization in case of thrombosis by CT/MRI). Consider evaluation for inherited predisposition to thrombosis.

Centers may elect to discontinue pegasparaginase and switch to erwinia asparaginase based upon laboratory evidence of silent inactivation of asparaginase activity in the absence of clinical symptoms of hypersensitivity at their discretion.

5.2 Bortezomib Related Toxicities

Special criteria will be followed for peripheral neuropathy and pulmonary toxicities thought to be possibly, probably or definitely related to bortezomib

5.2.1 Bortezomib-related peripheral neuropathy
Peripheral neuropathy will be closely monitored during each block of treatment that includes bortezomib and toxicities graded.

Neuropathy grading should be based on the maximum toxicity occurring during the previous block. Neuropathy should be graded using the "BALIS" scale (see Section 5.13). All dose modifications should be based on the worst preceding toxicity. Bortezomib dose will be decreased for sensory peripheral neuropathy as follows:

Table 2: Dose Modifications for Bortezomib-Related Neuropathic Pain and / or Peripheral Sensory Neuropathy

Severity of peripheral sensory neuropathy	Bortezomib modification
Grades 1-2 without pain	None
Grade 1 with pain	Decrease bortezomib to 1 mg/m^2/dose
Grade 2 with pain or any Grade 3	Hold bortezomib treatment until symptoms ≤ Grade 1. Do not make up missed doses. When toxicity resolves, re-initiate bortezomib at 1 mg/m^2/dose
Grade 2 with or any Grade 3 > 2 weeks duration or recurrence after prior dose reduction	Discontinue bortezomib
Grade 4	Discontinue bortezomib

5.2.2 Bortezomib-associated pulmonary toxicity

There have been rare reports of acute diffuse infiltrative pulmonary disease of unknown etiology such as pneumonitis, interstitial pneumonia, lung infiltration and acute respiratory distress syndrome (ARDS) in patients receiving bortezomib. For this reason, pulmonary toxicity will be monitored carefully during the study. See below for management of new-onset dyspnea, hypoxia or chest infiltrates not explained by infection or other known cause.

5.2.2.1 Identification and Management of Bortezomib-Related Pulmonary Toxicity

Pulmonary toxicities are more commonly seen when bortezomib is given to elderly adults. Pulmonary toxicity due to bortezomib may be delayed and can occur up to 3 weeks after the final dose of bortezomib is administered. Bortezomib-related pulmonary toxicities are rare in children and were not seen during AALL0P1. Nevertheless, as pulmonary toxicities secondary to bortezomib can be serious, a low threshold to evaluate for pulmonary toxicity is recommended. Patients with abnormal respiratory symptoms, including dyspnea, cough, or pleuritic chest pain or abnormal respiratory findings on exam, including tachypnea, increased work of breathing, or ausculatory abnormalities should have a chest radiograph and pulse oximetry performed.

If the patient develops pulmonary toxicity, including a decrease in oxygen saturation to < 90%, and/or bilateral infiltrates on chest x-ray, and/or pulmonary opacities on CT scan, in the absence of evidence of pneumonia or congestive heart failure, the following steroid therapy is recommended. Therapy can be tailored based on the severity of the pulmonary toxicity:

- Methylprednisolone 200-400 mg/m^2 IV X 1, followed by 250 mg/m^2 IV daily for 3 days (max dose 1000 mg/day), or
- Dexamethasone 6 mg/m^2 IV BID hrs. or
- Prednisone 40 mg/m^2 PO daily divided BID.

Other similar steroid regimens suitable for treatment of non-infectious pneumonitis may be used. In addition, it is suggested that a formal consultation from a pulmonologist be obtained. Bronchoscopy is encouraged if clinically indicated. The steroid dosage should be tapered as clinically appropriate once the clinical condition improves.

5.2.2.2 Dose De-escalation or Discontinuation of Bortezomib for Pulmonary Toxicity and

Other Bortezomib-Related Toxicities (Excluding Peripheral Neuropathy)
Bortezomib will be dose de-escalated to 1 mg/m^2 for any resolving Grade 3 pulmonary toxicity (excluding voice changes/laryngitis), including hypoxia (oxygen saturation below the threshold listed in 5.2.2.1) that is possibly, probably or definitely related to bortezomib. Patients that experience a Grade 4 pulmonary toxicity possibly, probably or definitely related to bortezomib (excluding voice changes/laryngitis) will discontinue bortezomib.

5.2.2.3 Patients re-experiencing the same bortezomib-related qualifying Grade 3 pulmonary toxicity following a single bortezomib dose reduction will discontinue bortezomib.

5.2.3 Bortezomib-Related Electrolyte Abnormalities
Bortezomib has been associated with hyponatremia, hypophosphatemia, and hypokalemia in some studies. Serum sodium, potassium, and phosphorus should be checked at the beginning of Induction and DI. Consider correcting low levels as clinically appropriate, balancing the risk of correction of these abnormalities with the risks of developing hyperkalemia or hyperphosphatemia from tumor lysis during induction chemotherapy. Also, consider monitoring electrolyte levels periodically during induction and DI and correcting if clinically indicated. Bortezomib will not be dose de-escalated for bortezomib-related electrolyte abnormalities

5.2.4 Non-Hematologic and non-Electrolyte Grade 3 or 4 Bortezomib-Related Toxicity
The bortezomib dose should be de-escalated to 1 mg/m^2 for non-hematologic and non-electrolyte Grade 3 and 4 toxicities that are possibly, probably or definitely related to bortezomib (see below).

Dose de-escalation within a block (Induction or DI):
If a patient experiences a Grade 3 or 4 bortezomib-related toxicity that does not require cessation of bortezomib (see above), and once this Grade 3 or 4 toxicity resolves to ≤ Grade 1, bortezomib can be restarted at a decreased dose of 1 mg/m^2 for **subsequent** doses. Missed doses of bortezomib will not be made up. Patients who have a qualifying Grade 3 pulmonary toxicity that worsens (i.e., > Grade 3) by the next scheduled dose of bortezomib should discontinue bortezomib.

Dose de-escalation between Induction and DI:
The bortezomib dose will be decreased to 1 mg/m^2 for qualifying Grade 3 or 4 toxicities (see above) that resolve to ≤ Grade 1 prior to the beginning of DI. Doses reduced for an adverse event will not be re-escalated, even if there is minimal or no toxicity at the reduced dose.

5.2.5 Bortezomib Dose Reductions for Hyperbilirubinemia:
Patients with mild hepatic impairment do not require dose adjustment of bortezomib. Patients with moderate or severe hepatic impairment should receive bortezomib at modified doses as outlined below:

	Direct Bilirubin Level	Bortezomib Dose Modification
Moderate	> 1.5x – 3x upper limit of normal	Reduce bortezomib to 0.7 mg/m^2 for all doses in the cycle in which hepatotoxicity is present.

Severe	> 3x upper limit of normal	Consider dose escalation to 1 mg/m^2 in subsequent cycles if transaminitis and hyperbilirubinemia resolves and hepatic toxicity was not possibly, probably, or definitively related to bortezomib.

* No adjustment necessary for elevated SGOT (ALT).

5.3 Cyclophosphamide

5.3.1 Hematuria

5.3.1.1 Omit in the presence of macroscopic hematuria.

5.3.1.2 If there is a history of previous significant hematuria:

- Hydrate before cyclophosphamide until specific gravity is <1.010
- Hydrate at 125 mL/m^2/hr for 24 hours after dose
- Monitor for adequate urine output as per institutional guidelines.
- Give IV mesna at a total dose that is 60% of the cyclophosphamide dose divided to 3 doses (e.g., if the cyclophosphamide dose is 1000 mg/m^2, the total mesna dose is 600 mg/m^2 or 200 mg/m^2/dose). Give the first mesna dose 15 minutes before or at the same time as the cyclophosphamide dose and repeat 4 and 8 hours after the start of cyclophosphamide. This total daily dose of mesna can also be administered as IV continuous infusion. The continuous infusion should be started 15-30 minutes before or at the same time as cyclophosphamide and finished no sooner than 8 hours after the end of cyclophosphamide infusion.

5.3.2 Renal Dysfunction

If creatinine clearance or radioisotope GFR is < 10 mL/min/1.73 m^2, reduce dose of cyclophosphamide by 50%. Prior to dose adjustment of cyclophosphamide, the creatinine clearance should be repeated with good hydration.

5.4 Cytarabine (ARAC)

5.4.1 Fever

- Do not withhold ARAC for fever if it is likely to have been caused by the ARAC (ARAC Syndrome).
- Obtain blood cultures if a central line is present.

5.4.2 Rash or Conjunctivitis

For rash or conjunctivitis, withhold for Grade 3-4 toxicity until resolved. Make up missed doses and consider concurrent treatment with hydrocortisone or dexamethasone, and/or with dexamethasone ophthalmic drops for conjunctivitis.

5.4.3 Myelosuppression

Once Consolidation (C) or Delayed Intensification (DI) has started do not interrupt for uncomplicated myelosuppression; do hold for proven or presumed serious infection. Do make up missed doses.

5.4.4 Renal Function

Adequate renal function (defined as creatinine within normal range) is required for the administration of high dose ARAC. Creatinine Clearance should be measured for patients with elevated creatinine or suspected renal insufficiency. For CrCl < 60 mL/min/1.73 m^2, hold pending recovery and omit if recovery requires > 3 weeks.

5.5 Daunorubicin and Doxorubicin

5.5.1 Cardiac Toxicity

Discontinue for clinical or echocardiographic evidence of cardiomyopathy (SF < 27% or EF < 50%) or Grade 3-4 left ventricular systolic dysfunction (LVSD) per CTCAE version 4.0.

5.5.2 Myelosuppression (beyond Induction)

If patient has severe infection or severe mucositis (Grade 3-4) and an ANC < 500/µL delay anthracycline during phases other than Induction. During Induction, continue with anthracycline administration. Subsequent doses should be given at full dose.

5.5.3 Hyperbilirubinemia [142]

Direct Bili	% Dose Reduction
< 1.2 mg/dL	Full dose
1.2 – 3.0 mg/dL	50%
3.1 – 5.0 mg/dL	75%
> 5.0 mg/dL	Withhold dose and administer next scheduled dose if toxicity has resolved.

Do not make up missed doses.

5.5.7 Extravasation

In the event of an extravasation, discontinue the IV administration of the drug and institute appropriate measures to prevent further extravasation and damage according to institutional guidelines. Also see https://members.childrensoncologygroup.org/_files/disc/Nursing/extravasationguidelines.pdf for COG guidelines.

5.6 Etoposide

5.6.1 Allergic Reaction

Premedicate with diphenhydramine (1-2 mg/kg slow IV push, maximum dose -50 mg). If symptoms persist, add hydrocortisone 100-300 mg/m^2. Continue to use premedication before etoposide in future. Also consider substituting an equimolar amount of etoposide phosphate, in the face of significant allergy and/or hypotension.

5.6.2 Hypotension

If diastolic or systolic blood pressure (BP) falls 20 mm Hg during infusion, reduce infusion rate by 50%. Start a simultaneous infusion of NS 10 mL/kg if BP fails to recover or falls further. Stop infusion if BP does not recover, continue NS. If the patient has had any episode of hypotension, prehydrate with 0.9% NaCl at 10 mL/kg/hr for 2 hours prior to any subsequent infusion.

5.6.3 Renal Insufficiency

If renal function decreases, adjust etoposide as follows: CrCl 10-50 mL/min/1.73 m^2, decrease dose by 25%; if CrCl < 10 mL/min/1.73 m^2, decrease dose by 50%.

5.6.4 Hyperbilirubinemia

If direct bilirubin is > 2 mg/dL, decrease dose by 50%. If direct bilirubin is > 5 mg/dL, hold etoposide.

5.7 Ifosfamide

5.7.1 Hematuria

Grade 2 – 3: Administer Mesna as under Section 4.12 with ifosfamide. Grade 4 notify Study Chair.

5.8 Intrathecal Methotrexate/Triple Intrathecal Therapy

5.8.1 Systemic toxicity

The dosage for IT methotrexate will not be reduced for systemic toxicity (myelosuppression, mucositis, etc.). Instead, leucovorin may be used at a dose of 5 mg/m^2/dose every 12 hours x 2 doses, beginning 48 hours after the IT therapy has been delivered. This may reduce the risk of worsening already existent myelosuppression (ANC < 500/µL) or mucositis. Do not administer leucovorin solely to prevent myelosuppression.

5.8.2 Dose modifications following an episode of acute neurotoxicity

Neurotoxicity has extremely protean manifestations, ranging from transient events, seizures or episodes of acute hemiparesis, to severe necrotizing encephalopathies.[143-145] These toxicities are poorly understood and currently it is impossible to predict who will suffer these complications. In addition, there are no data clearly linking the occurrence of an acute neurotoxic event with an increased risk of long-term neurocognitive dysfunction, nor do changes present on MRI at the time of an acute event clearly correlate with or predict outcome.[145-150] It is clear however, that CNS prophylaxis is a mandatory component of curative therapy for children with ALL. Effective prophylaxis generally takes 2 forms; cranial, or less commonly, craniospinal radiation, with a limited number of doses of IT therapy or prolonged IT therapy with either IT MTX or triple IT therapy (MTX, ARAC and hydrocortisone). Certain protocols, for example BFM 2000,[151] include fewer doses of IT MTX, with an acceptably low frequency of CNS relapse, but the backbone of the BFM therapies is not the same as those currently used by the Children's Oncology Group. The exclusive use of IT ARAC has not been studied or described in the context of ALL therapy nor can one demonstrate the safety of omitting multiple doses of IT therapy without concomitant use of cranial radiation therapy or high dose methotrexate.

The following guidelines are offered for consideration following an acute event, but it must be recognized that there are little data to support these approaches or any others. Thus the treating physician must evaluate the patient and, with the family, make the best possible decision with respect to the relative risk and benefit of continued therapy.

Following an acute neurotoxic event, a history and physical exam should guide the differential diagnosis. A neurology consult may be of value and should be considered. Seizures and other transient events may be linked to fever, infection, encephalitis, meningitis, hypertension, electrolyte disturbance, hypoglycemia, trauma, intracranial hemorrhage or thrombosis, narcotic withdrawal, illicit drug use, or other causes in addition to the direct side effects of chemotherapy. Appropriate laboratory studies may include, but are not limited to, blood cultures, a CBC, electrolytes, including glucose, calcium, magnesium and phosphorus, renal and liver function studies and/or an examination of the CSF. Imaging studies may include a CT scan and/or an MRI. The CT is commonly normal, in the absence of stroke, but if calcifications are present, this finding may be indicative of a more severe mineralizing leukoencephalopathy.[152] MRI abnormalities may be pronounced, but transient. Posterior reversible encephalopathy may be present on MR with extensive diffusion abnormalities, but these do not appear to correlate with subsequent demyelination or gliosis.[153-155] Additional studies, including MR angiography and/or venogram should be considered, if clinically indicated (e.g., focal deficits).

Many acute events, seizures or episodes of transient hemiparesis, are temporally related to the administration of intrathecal therapy, commonly 9 to 11 days after the IT administration.[156] For patients who return to their "pre-event" status, without residual deficits of physical or neurologic exam, there are few data to support or guide therapeutic interventions. It is reasonable to hold the next dose of IT therapy, or, substitute IT Ara-C for 1 dose of IT MTX, or triple IT therapy. It is also reasonable to include leucovorin rescue at a dose of 5 mg/m^2 q 12 hrs x 2 doses beginning 48 hours after the LP. This pattern of rescue was associated with a clear diminution in the incidence of acute neurotoxicity in one case series.[156] There have been questions about potential interference of leucovorin with the efficacy of the IT MTX, but there are little data to support or refute this position. Moreover, the administration 48 hours later would minimize any potential interference. If the event does not recur, resumption of standard therapy should be considered, following 1 modified or omitted IT dose. In the face of multiply recurrent events, or evidence of progressive encephalopathy, another evaluation is warranted and the treating physician may consider a more prolonged or definitive change in therapy. These decisions are extremely difficult and may hinge on an individual's view of the importance of quality of life versus an increase in the risk of relapse. Since the greatest impact of CNS prophylaxis occurs early in therapy, the timing of these events may also influence clinical decisions. Cranial radiation has been suggested as an alternative to continued IT therapy though much of the literature on long-term neurocognitive dysfunction supports a more deleterious effect from CRT than IT therapy.[157-160] Dramatic deviations from protocol recommended therapy might result in the child being taken off protocol therapy.

The use of dextromethorphan (DM) has been suggested as a neuroprotectant, capable of preventing NMDA mediated neurotoxicity without prohibitive toxicity. Low dose therapy has been recommended, in part, based on data suggesting that DM is concentrated in brain relative to serum. However, the literature on the use of DM supports a tight dose response relationship, with the likelihood of sparing an initially unaffected area, following ischemic damage, linked to dose, in both clinical trials and animal models of CNS ischemia.[161-164] At doses thought to be therapeutic, side effects have included nystagmus, nausea and vomiting, distorted vision, ataxia, and dizziness. In addition, Hollander et al[165] have raised concerns about the potential deleterious effects of long-term NMDA receptor blockade on memory because hippocampal long-term potentiation is dependent on the activation of the NMDA receptor. Thus in the absence of a clinical trial there are few data to support the addition of DM.

5.8.3 Hydrocephalus, microcephaly or known abnormality of CSF flow precluding intrathecal chemotherapy via lumbar puncture

Intraventricular chemotherapy via Ommaya catheter may be used in place of intrathecal therapy delivered by LP. Intraventricular chemotherapy should be given according to the same schedule, but at **50% of the corresponding age-based doses** that would be given by LP. NOTE: Obstruction to CSF flow may be a contraindication to intrathecal and/or intraventricular therapy.

5.8.4 Viral, bacterial, or fungal meningitis

Omit until resolved.

5.9 High-Dose Methotrexate (HDMTX) and Leucovorin Rescue

[Please note that **HDMTX** refers to IV MTX **5g/m^2 given over 24 hrs**]

Note: Review of methotrexate dosing on BFM-based protocols indicated that excessive methotrexate toxicity has not been encountered in patients larger than 2 m^2 who receive more than

10 grams of methotrexate. The investigator should base the methotrexate on the patient's meter-squared dosing and not cap at 10 grams of methotrexate.

5.9.1 HD MTX Infusion Guidelines

See Appendix IV for a flowchart of the HDMTX / LCV guidelines.

When IT therapy and HDMTX are scheduled for the same day, deliver the IT therapy within 6 hours of the beginning of the IV MTX infusion (hour -6 to +6, with 0 being the start of the MTX bolus).

Hold TMP-SMX on the days of HDMTX infusion and for at least 72 hours after the start of the HDMTX infusion and until the MTX level is less than 0.4 µM. *In the presence of delayed clearance continue to hold these medications until MTX level is less than 0.1 µM.*

Hold any nonsteroidal anti-inflammatory medications, penicillins, proton pump inhibitors or aspirin-containing medications on the day of HDMTX infusion and for at least 72 hours after the start of the HD MTX infusion and until the MTX level is less than 0.4 µM. *In the presence of delayed clearance continue to hold these medications until MTX level is less than 0.1 µM.*

Recommended Prehydration with D5 ¼ NS with 30 mEq $NaHCO_3$/L at 125 mL/m^2/hour until urine specific gravity is ≤ 1.010 and pH is ≥ 7.0 and ≤ 8.0. Ringers Lactate may be used as the initial fluid if a bicarbonate containing solution is unavailable. Adjust fluid volume and sodium bicarbonate to maintain urine specific gravity and pH at above parameters. An acetate or bicarbonate bolus (0.5-1 mEq/kg over 15 min) may be given to raise the urine pH relatively quickly; a normal saline bolus may also be helpful in facilitating hydration. Continue hydration and alkalinization throughout HDMTX infusion, and for a minimum of 54 hours after the MTX bolus started for patients who meet expected clearance parameters. In patients with delayed MTX clearance, continue hydration and leucovorin as instructed (Appendix IV) until the plasma MTX concentration is below 0.1uM.

Hour 0: MTX 500 mg/m^2 IV infused over 30 minutes. This is followed, immediately, by MTX 4500 mg/m^2 given by continuous IV infusion over 23.5 hours. Be certain that the HDMTX infusion is completed in the 24 hour period. Unintentional prolongation to as long as 26 hours though not encouraged is acceptable.

Hours 24, (36), 42 and 48: Draw MTX level and serum creatinine. NOTE: 36 hour level is only drawn if needed (see below).

For MTX levels that exceed these expected values modify the rescue regimen as noted below and increase hydration to 200 mL/m^2/hr, monitor urine pH to assure a value ≥ 7.0 and monitor urine output to determine if volume is ≥ 80% of the fluid intake, measured every 4 hours. If serum creatinine rises significantly (serum CR >125% of baseline), at any time point, assure appropriate urine pH and urine volume as above and draw a 42 hour level. If urine output fails to continue at 80% of the fluid intake, consider furosemide. Regardless of urine output, also consider glucarpidase (carboxypeptidase G_2) (see below). For patients with delayed clearance during a previous course, begin the following course with the increased hydration (200 mL/m^2/hr). If subsequent course is not associated with delayed clearance, attempt to use standard hydration.

If the 24 hour level is < 150 µM draw the next level at hour 42 and refer to table below.

If the 24 hour level is ≥ 150 µM and/or creatinine > 125% baseline, repeat level if MTX contamination is possible. If the value is "real" refer to the changes in hydration, etc., described above and repeat the level with a serum Cr at hour 36. Then refer to the table below.

If the 42 and 48 hour levels are ≤ 1 and 0.4 µM, respectively, give Leucovorin at 15 mg/m^2 IV/PO at 42, 48 and 54 hours post the start of methotrexate loading dose. No additional levels are needed, nor is additional leucovorin.

36 hr MTX level	42 hr MTX level	48 hr MTX level	Leucovorin Rescue++
Only required if 24 hr level is ≥ 150 µM.**	1.01 to 9.9 µM	0.41 to 5.9 µM	Continue 15 mg/m^2 q 6h until MTX level < 0.1 µM (draw q12-24 h).
	10 to 19.9 µM	6 to 9.9 µM	Increase to 15 mg/m^2 q 3h until MTX level < 0.1 µM (draw q 6-24 h). Consider glucarpidase.
	20 to 200 µM	10 to 100 µM	Increase to 100 mg/m^2 q 6h until MTX level < 0.1 µM (draw q 6-24 h). Consider glucarpidase.
	> 200 µM	> 100 µM	Increase to 1000 mg/m^2 q 6h until MTX level < 0.1 µM (draw q 6-24 h). Consider glucarpidase.

** **If the 36 hour level exceeds 3** µM, increase hydration to 200 mL/m^2/hr, monitor urine pH to assure a value ≥ 7.0 and monitor urine output to determine if volume is ≥ 80% of the fluid intake, measured every 4 hours. If urine output fails to continue at 80% of the fluid intake, consider furosemide. Regardless of urine output, also **consider glucarpidase if 36 hour MTX level exceeds 10** µM (see below).

++ If the level is high at hour 36 or 42, but then the patient "catches up" and the level falls to the expected values of ≤ 1 and/or ≤ 0.4 µM at hours 42 and 48, respectively, resume standard leucovorin and hydration as long as urine output remains satisfactory.

5.9.2 Nephrotoxicity

Postpone course if pre-treatment (MTX) serum creatinine is > 1.5x baseline or GFR creatinine clearance < 65 mL/minute/1.73m^2. If renal function does not recover, omit MTX. Do not give HDMTX to a patient with this degree or renal impairment, assuming that prolonged excretion can be managed with glucarpidase.

NOTE: For patients who have markedly delayed MTX clearance secondary to renal dysfunction, consider using glucarpidase (carboxypeptidase G_2, Voraxaze™).[166,167] To obtain supplies of glucarpidase in the US contact the Voraxaze 24-hour Customer Service line at 855-786-7292. Additional information can be found at http://www.btgplc.com/products/specialty-pharmaceuticals/voraxaze regarding product availability through ASD Healthcare, Cardinal, and McKesson. Canadian sites should contact McKesson at (877) 384-7425 for further information. Sites in Australia and New Zealand should contact Hospira at 1300-046-774 (local) or medicalinformationAUS@hospira.com. Patients requiring glucarpidase rescue will remain on study.

5.9.3 Elevated Transaminases

Samples for the determination of ALT value must be drawn within 72 hours, PRIOR to a course of intravenous MTX. Blood samples for ALT should not be drawn following the start of MTX infusions as MTX causes significant short term elevation in ALT levels.

ALT	IV MTX
< 10 X ULN	Continue with therapy as scheduled
10 – 20 X ULN	Continue with therapy as scheduled for 1 cycle
10 – 20 X ULN for 2 consecutive cycles	Discontinue TMP/SMX* Hold therapy until ALT < 10 X ULN, then resume at full dose at point of interruption. Do not skip doses.
> 20 X ULN	Hold therapy until ALT < 10 X ULN, then resume at full dose at point of interruption. Do not skip doses.
> 20 X ULN for > 2 weeks	Evaluate with AST, bili, alkaline phosphatase, PT, albumin, total protein, and hepatitis A, B, C, CMV, and EBV serologies. Consider liver biopsy before additional therapy given. Notify Study Chair.

* *Please see COG Supportive care Guidelines at: https://members.childrensoncologygroup.org/prot/reference_materials.asp for TMP/SMX substitutions.

5.9.4 Hyperbilirubinemia

Hold IV MTX for direct hyperbilirubinemia of > 2.0 mg/dL.

5.9.5 Mucositis

For Grade 3-4 mucositis, withhold IV MTX until resolved. Increase leucovorin rescue following the next course from 3 to 5 doses on a q6 hr schedule. If subsequent course is not associated with Grade 3-4 mucositis, attempt to decrease the leucovorin. If mucositis recurs despite the extended leucovorin, decrease the dose of MTX by 25%, increase hydration to 200 mL/m^2/hr and continue increased leucovorin as above. Should subsequent courses be well tolerated, use a stepwise approach to resuming a standard approach to drug delivery. Consider culturing lesions for herpes simplex if mucositis persists or recurs.

5.9.6 Myelosuppression

All chemotherapy should be held for ANC < 750/µL and platelets < 75 000/µL.

5.10 Capizzi Methotrexate Regimens

5.10.1 Liver Dysfunction

Samples for the determination of ALT value must be drawn within 72 hours, PRIOR to a course of intravenous MTX. Blood samples for ALT should not be drawn following the start of MTX infusions as MTX causes significant short term elevation in ALT levels.

ALT	IV MTX
< 10 X ULN	Continue with therapy as scheduled
10 – 20 X ULN	Continue with therapy as scheduled for 1 cycle
10 – 20 X ULN for 2 consecutive cycles	Discontinue TMP/SMX* Hold therapy until ALT < 10 X ULN, then resume at full doses at point of interruption. Do not skip doses.
> 20 X ULN	Discontinue TMP/SMX* Hold therapy until ALT < 10 X ULN, then resume at full doses at point of interruption. Do not skip doses.
> 20 X ULN for > 2 weeks	Evaluate with AST, bili, alkaline phosphatase, PT, albumin, total protein, and hepatitis A, B, C, CMV, and EBV serologies. Consider liver biopsy before additional therapy given.

* Please see COG Supportive care Guidelines at: https://members.childrensoncologygroup.org/prot/reference_materials.asp for TMP/SMX substitutions.

5.10.2 Nephrotoxicity

Postpone course if serum creatinine is >1.5 x baseline or GFR creatinine clearance < 65 mL/1.73m²/minute.

5.10.3 Hyperbilirubinia

Hold IV MTX for direct hyperbilirubinemia of > 2.0 mg/dL.

5.10.4 Mucositis

For Grade 3-4 mucositis, withhold IV MTX until resolved. Discontinue MTX dose escalation and resume at 80% of last dose if therapy is delayed for myelosuppression or Grade 3 or greater mucositis. If mucositis persist or recurs, consider culturing lesions for herpes simplex.

5.10.5 Myelosuppression

A) If ANC is < 500/µL or platelets < 50 000/µL, hold all chemotherapy and repeat blood counts in 4 days.
   1. In 4 days, if ANC ≥ 500/µL and platelets ≥ 50 000/µL, give same dose of methotrexate as previous cycle.
   2. In 4 days, if ANC is still < 500/µL or platelets < 50 000/µL, give VCR (and IT MTX if Day 31) and pegaspargase (if due) (omitting IV MTX) and repeat counts in 7 days to begin next dose of VCR and IV MTX if counts are adequate.
      a. If after 7 days ANC ≥ 500/µL or platelets ≥ 50 000/µL, reduce dose of IV MTX by 20% (Do not make up missed dose of MTX). For subsequent doses, resume escalation as per A-C.

b. If after 7 days ANC is still < 500/µL or platelets < 50 000/µL, hold therapy until counts recover to ANC > 500/µL and platelets > 50,000/µL. When ANC ≥ 500/µL and platelets ≥ 50 000/µL, resume at 80% of last dose of MTX. For subsequent doses, resume escalation as per A-C.

B) If ANC ≥ 500 but < 750/µL and platelets ≥ 50 000 but < 75 000/µL, give same dose of MTX as previously (i.e., no escalation).

C) If ANC ≥ 750 and platelets ≥ 75 000 escalate MTX by 50 mg/m^2.

D) Do not escalate MTX dose and resume at 80% of last dose if it had been delayed secondary to myelosuppression and/or Grade 3 mucositis. For subsequent doses, resume escalation as per A-C.

5.11 PO Methotrexate (MTX) and 6-Mercaptopurine (MP)

5.11.1 Interim Maintenance #1 with HDMTX

If ANC is < 750/µL and/or platelets < 75 000/µL, hold mercaptopurine. Restart mercaptopurine at full dose with next cycle of HD MTX when ANC is ≥ 750/µL and platelets are ≥ 75 000/µL. Do not make up missed doses. Consider a marrow evaluation in the face of persistent or prolonged cytopenias. If patient develops severe or unexpected myelosuppression, see section below on thiopurine pharmacology testing.

If patient develops severe or unexpected myelosuppression, see section below on thiopurine pharmacology testing.

5.11.2 Maintenance:

a) Dose reduction for myelosuppression:

If neutrophil count falls below 500/µL or if platelet count falls below 50,000/µL during Maintenance, MP and MTX will be held until recovery above these levels. For the first drop in ANC or platelets, resume chemotherapy (both MP and MTX) at the same dose the patient was taking prior to the episode of myelosuppression. If neutrophil count falls below 500/µL or if platelet count falls below 50 000/µL for a second (or greater) time, discontinue doses of MP and MTX until ANC is ≥ 750/µL and platelets are ≥ 75 000/µL. Restart both MP and MTX at 50% of the dose prescribed at the time the medication was stopped. Then continue to increase to 75% and then 100% of the dose prescribed prior to stopping the medication at 2-4 week intervals provided ANC remains ≥ 750/µL and platelets remain ≥ 75 000/µL. May increase both MP and MTX simultaneously. Consider discontinuing TMP/SMX as per COG Supportive care Guidelines at: https://members.childrensoncologygroup.org/prot/reference_materials.asp. If neutrophil count falls below 500/µL or if platelet count falls below 50 000/µL on > 2 occasions during Maintenance, perform thiopurine pharmacology testing as described below. Should therapy be withheld for myelosuppression or elevated transaminase, do not "make up" that week. Resume therapy at the correct point, chronologically.

b) Dose escalation:

For ANC ≥ 1500/µL on 3 CBC(s) done over 6 weeks or 2 successive monthly CBC(s) alternately increase doses of MTX or MP by 25%. As a general rule, do not increase doses more often than every 4 weeks. If both MTX and MP are increased once without a fall in ANC, consider noncompliance as a possibility. Noncompliance can be assessed by obtaining a sample for RBC thioguanine nucleotides (TGNs). Consider observing the administration of an oral dose of MTX and checking plasma MTX concentration 2-4

hours later. This will document whether or not poor absorption contributes to lack of response and may facilitate discussions about noncompliance.

c) Mucositis Grade 3-4:
MTX should be reduced to 50% if Grade 3 toxicity develops; withhold in the presence of Grade 4 toxicity until there is a resolution, then resume at 50% of original dose with gradual dose escalation. If mucositis persists or recurs, consider culturing for herpes simplex.

d) Liver Dysfunction:
For increase in hepatic transaminases (SGPT/ALT or SGOT/AST) to greater than 5x ULN consistent with Grade 3 toxicity, obtain total bilirubin. Monitor SGPT/ALT or SGOT/AST and total bilirubin every 2 weeks during Consolidation and every 4 weeks during Maintenance as long as transaminases remain over 5x ULN.

Continue full dose therapy unless either of the following occurs:
1) Direct bilirubin > 2.0 mg/dL
2) SGPT/ALT or SGOT/AST > 20x ULN (consistent with Grade 4 toxicity) on 2 determinations at least 1 week apart.

If either of these occurs, hold MTX and monitor labs as above, weekly. Restart at full dose therapy when the transaminase is less than 5x ULN, if bilirubin is normal. If liver dysfunction persists, consider a trial period with MTX but without MP, especially if red cell MP methylated derivatives are elevated. Also consider liver biopsy.

Exclude infectious hepatitis (A, B, C) for persistent (> 1 month) elevations in SGPT/ALT or SGOT/AST above 5x ULN.

5.11.3 Thiopurine Pharmacology Testing and Dosage Adjustments in All Blocks that Contain Thiopurines

MP and 6-TG are methylated directly by thiopurine methyltransferase (TPMT) to an inactive metabolite. TPMT activity varies tremendously among patients, because of a common inherited genetic defect in TPMT. One in 300 patients is completely deficient (homozygous defective) and 10% of the population is moderately deficient in TPMT activity because they have inherited one variant (non-functional) TPMT allele (i.e., heterozygotes).[168-171] Patients with low TPMT form higher concentrations of the 6-thioguanine nucleotides (6-TGN) and are more susceptible to acute thiopurine toxicity (primarily myelosuppression, involving neutropenia, thrombocytopenia, and anemia). Patients with the complete deficiency of TPMT tolerate less than 10% of protocol doses of MP (10 to 30 mg/m^2/day 3 days per week). About 35% of heterozygotes require a lower dose of MP to avoid dose-limiting myelosuppression.[172]

Recently, germline variants in the gene encoding the nucleoside diphosphate–linked moiety X-type motif 15 (NUDT15) were reported in approximately 4% of Hispanic/Native American and nearly 10% of East Asian children with ALL; these polymorphisms are strongly associated with 6-MP intolerance. [173]

There are now CLIA certified tests for TPMT genotype and phenotype, for thiopurine metabolites (6-methyl mercaptopurine [6-MMP] and 6-TGN) measurements, and for *NUDT15* polymorphisms. Only 3 SNPs constitute well over 90% of the inactivating mutations in the gene, based on studies in numerous racial and ethnic groups worldwide.[168,174-177] Thus, the genotyping test has a low false negative rate, and may be preferable to TPMT phenotype testing in cases where a history of red cell transfusions

would potentially confound assessments of RBC TPMT activity. When the genotyping result is coupled with a phenotyping test for TPMT or with thiopurine metabolite concentrations in erythrocytes, the reliability of the tests will be even greater. Moreover, metabolite levels can provide an index of patient compliance with thiopurine therapy.

Recommendations for Thiopurine Monitoring and Dosage Adjustments:
When myelosuppression has led to significant delays in therapy (> 2 weeks) or is disproportionate to the therapy, thiopurine testing should be performed:

- For subjects who have received full dose thiopurine therapy during the 2 weeks immediately preceding the test, RBC thiopurine metabolites will likely predict TPMT status and actual thiopurine exposure.
- In the absence of RBC transfusions for 3 months prior, TPMT activity will accurately reflect TPMT status
- TPMT genotyping will be informative in all subjects, if at least 1 mutant allele is identified. If not, and myelosuppression continues, send samples for TPMT activity and/or metabolites since TPMT genotyping will miss 5%-10% of mutants. NOTE: *Genotyping can be done despite recent transfusions.*

5.11.4 Suggested Dose Adjustments in Patients With Unacceptable Myelosuppression

- If the subject is *homozygous deficient* for TPMT or *NUDT15*, the thiopurine dose should be *reduced to* 10-20 mg/m^2/day 3 days per week. If the subject is *heterozygous for TPMT and* has experienced significant myelosuppression, the thiopurine dose should be reduced by 30%-50%. It is not yet clear how the dose of thiopurine should be adjusted for patients who are heterozygous for *NUDT15* but such patients should be monitored carefully while on thiopurines. If a patient has two polymorphisms in *NUDT15* (i.e. heterozygous for both the R139C and R139H), they should be treated as if they were homozygous deficient. Gradual dose escalations should be attempted as outlined below.
- Do not increase the dose in response to a high ANC for 4 weeks to allow for achievement of steady state. All other myelosuppressive medications should be delivered at full dose, and the thiopurine dose should be titrated based on blood counts. Further thiopurine pharmacologic measures are not often necessary.
- If the subject is homozygous wild-type (high activity) for TPMT or *NUDT15*, then discontinue TMP/SMX and use pentamidine or dapsone. For modifications of the oral MP and MTX see the beginning of this Section.

5.12 Steroids (Dexamethasone and Prednisone)

5.12.1 Hypertension

Dose should not be reduced. Sodium restriction and anti-hypertensives should be employed in an effort to control hypertension. Avoid calcium channel blockers due to their potential prohemorrhagic effect.

5.12.2 Hyperglycemia

Dose should not be reduced for hyperglycemia. Rather, insulin therapy should be employed to control the blood glucose level.

5.12.3 Pancreatitis
Do not modify dose for asymptomatic elevations of amylase and/or lipase. Discontinue steroids, except for stress doses, in the presence of hemorrhagic pancreatitis or severe pancreatitis.

5.12.4 Osteonecrosis (ON)
Do not modify corticosteroid therapy for osteonecrosis (also referred to as avascular necrosis) during Induction or Delayed Intensification. Consider omitting Maintenance steroid for osteonecrosis Grade 1 (clinically asymptomatic, radiographic finding only). Omit Maintenance steroid for osteonecrosis Grade 2 or greater, and notify study chair. Consider resuming Maintenance steroid after 6 months if joint symptoms have resolved and if MRI findings have significantly improved or normalized.

5.12.5 Varicella
Steroids should be held during active infection except during Induction. Do not hold during incubation period following exposure.

5.12.6 Inability to use oral doses
For dexamethasone, substitute the IV preparation mg for mg. For prednisone, substitute IV methylprednisolone at 80% of the oral prednisone dose. Note that if substituting oral prednisolone for prednisone, the doses are the same; prednisone is converted in the liver to prednisolone.

5.12.7 Severe infection
Do not hold or discontinue steroids during Induction without serious consideration, as this is a critical period in the treatment of ALL. Later in therapy, one may consider holding steroid until patient achieves cardiovascular stability, except for "stress doses."

5.12.8 Severe psychosis
Dexamethasone dose may be reduced by 50% for severe psychosis. If symptoms persist, consider switching to an equivalent dose of prednisone.

5.13 PO 6-Thioguanine (TG)

Delayed Intensification
Oral TG will be held for suspected or proven serious infection.

For severe and/or unexpected myelosuppression, evaluate for TPMT activity as described in Section 5.10.

5.14 Vincristine

PLEASE USE "BALIS" SCALE FOR GRADING NEUROPATHY (See text box below)

5.14.1 Severe neuropathic pain (Grade 3 or greater)
Hold dose(s). When symptoms subside, resume at 50% previous calculated dose (maximum dose: 1 mg), then escalate to full dose as tolerated. NOTE: neuropathic pain can be not only severe but difficult to treat. However, because vincristine is an important component of curative therapy and the majority of neuropathies are ultimately reversible, vincristine therapy may be given at full dose at investigator discretion. Severe peripheral neuropathies, with or without a positive family history might suggest the need for a molecular diagnostic evaluation to rule out Charcot Marie Tooth Disease (CMT), Type 1A or Hereditary neuropathy with liability to pressure palsies. Drugs such as gabapentin may be of value.

5.14.2 Vocal Cord paralysis
Hold dose(s). When symptoms subside, resume at 50% previous calculated dose (maximum dose: 1 mg), then escalate to full dose as tolerated. See above for comment on CMT.

5.14.3 Foot Drop, paresis
Should be Grade 3 to consider holding or decreasing dose. These toxicities are largely reversible but over months to years. Accordingly, holding doses of vincristine and/or lowering the dose may not result in rapid resolution of symptoms and may compromise cure. See above for comment on CMT. Physical therapy may be beneficial to maintain range of motion and provide AFO's and other forms of support. Drugs such as gabapentin may be of value.

5.14.3 Jaw pain
Treat with analgesics; do not modify vincristine dose.

5.14.4 Hyperbilirubinemia[178,179]

Direct Bili	Dose reduction
< 3.1 mg/dL	Full dose (maximum dose: 2 mg),
3.1- 5.0 mg/dL	50% of calculated dose (maximum dose: 1 mg),
5.1-6.0 mg/dL	75% of calculated dose (maximum dose: 0.5 mg),
> 6.0 mg/dL	Withhold dose and administer next scheduled dose if toxicity has resolved. Do not make up missed doses.

5.14.5 Constipation or ileus (≥ Grade 3) or typhlitis
Hold dose(s); institute aggressive regimen to treat constipation if present. When symptoms abate resume at 50% of calculated dose (maximum dose: 1 mg) and escalate to full dose as tolerated.

5.14.6 Extravasation
In the event of an extravasation, discontinue the IV administration of the drug and institute appropriate measures to prevent further extravasation and damage according to institutional guidelines. Also see https://members.childrensoncologygroup.org/_files/disc/Nursing/extravasationguidelines.pdf for COG guidelines.

Modified ("Balis") Pediatric Scale of Peripheral Neuropathies

Peripheral Motor Neuropathy:

- Grade 1: Subjective weakness, but no deficits detected on neurological exam, other than abnormal deep tendon reflexes.

- Grade 2: Weakness that alters fine motor skills (buttoning shirt, coloring, writing or drawing, using eating utensils) or gait without abrogating ability to perform these tasks.

- Grade 3: Unable to perform fine motor tasks (buttoning shirt, coloring, writing or drawing, using eating utensils) or unable to ambulate without assistance.

- Grade 4: Paralysis.

Peripheral Sensory Neuropathy:

- Grade 1: Paresthesias, pain, or numbness that do not require treatment or interfere with extremity function.
- Grade 2: Paresthesias, pain, or numbness that are controlled by non-narcotic medications (without causing loss of function), or alteration of fine motor skills (buttoning shirt, writing or drawing, using eating utensils) or gait, without abrogating ability to perform these tasks.

- Grade 3: Paresthesias or pain that are controlled by narcotics, or interfere with extremity function (gait, fine motor skills as outlined above), or quality of life (loss of sleep, ability to perform normal activities severely impaired).

- Grade 4: Complete loss of sensation, or pain that is not controlled by narcotics.

6.0 DRUG INFORMATION

6.1 BORTEZOMIB

(Velcade™, N-Pyrazinecarbonyl-L-phenylalanine-L-leucine boronic acid, PS-341, MLN341, LDP-341) NSC# 681239, IND# [redacted] (05/21/19)

Source and Pharmacology:

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

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

In vitro and *in vivo* studies showed that green tea compounds, ascorbic acid (vitamin C) and other antioxidants, have the potential to significantly inhibit the activity of bortezomib. Green tea constituents, in particular epigallocatechin gallate (EGCG) and other polyphenols with 1,2-benzenediol moieties, effectively prevented tumor cell death induced by bortezomib both *in vitro* and *in vivo*. In multiple myeloma cell lines or mouse xenografts, EGCG directly reacted with bortezomib and blocked its proteasome inhibitory function. As a result, bortezomib could not trigger endoplasmic reticulum stress or caspase-7 activation and could not induce tumor cell death. A more recent study investigated whether clinically relevant levels of EGCG or ascorbic acid could inhibit the antitumor activity of bortezomib in murine xenograft tumors. The addition of EGCG to bortezomib demonstrated no effect on tumor growth inhibition at lower concentrations of EGCG that the investigators compare to human dietary intake. Similar results were found for ascorbic acid at normal daily doses. When bortezomib was given concurrently with much higher concentrations of EGCG, the investigators found that all antitumor activity was eliminated. The authors concluded that there is no interaction between EGCG and ascorbic acid when plasma concentrations are commensurate with dietary oral intake.

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

To avoid the risk of any possible interaction it is recommended that green tea containing products and any supplemental products containing vitamin C, flavonoids or other antioxidants (e.g.,

vitamins, herbal supplements) be discontinued from at least 24 hours prior to the initiation of bortezomib through 72 hours after the last bortezomib dose. Injectable multivitamins used as a component of parenteral nutrition should also be avoided during this time period to minimize the risk of direct vitamin C inactivation of bortezomib. In addition, it is recommended that the total dietary intake of vitamin C not exceed the RDA for age. Normally balanced diets are acceptable; supplementation with high doses of vitamin C or injectable vitamin C should be avoided. See Section 4.2.2 for additional information.

Toxicity:

Comprehensive Adverse Events and Potential Risks list (CAEPR)
for
Bortezomib (Velcade, NSC 681239)

The Comprehensive Adverse Event and Potential Risks list (CAEPR) provides a single list of reported and/or potential adverse events (AE) associated with an agent using a uniform presentation of events by body system. In addition to the comprehensive list, a subset, the Specific Protocol Exceptions to Expedited Reporting (SPEER), appears in a separate column and is identified with ***bold*** and ***italicized*** text. This subset of AEs (SPEER) is a list of events that are protocol specific exceptions to expedited reporting to NCI (except as noted below). Refer to the 'CTEP, NCI Guidelines: Adverse Event Reporting Requirements' http://ctep.cancer.gov/protocolDevelopment/electronic_applications/docs/aeguidelines.pdf for further clarification. *Frequency is provided based on 2084 patients.* Below is the CAEPR for bortezomib (Velcade).

NOTE: Report AEs on the SPEER **ONLY IF** they exceed the grade noted in parentheses next to the AE in the SPEER. If this CAEPR is part of a combination protocol using multiple investigational agents and has an AE listed on different SPEERs, use the lower of the grades to determine if expedited reporting is required.

Version 2.7, March 25, 2019[1]

Adverse Events with Possible Relationship to Bortezomib (Velcade) (CTCAE 5.0 Term) [n= 2084]			**Specific Protocol Exceptions to Expedited Reporting (SPEER)**
Likely (>20%)	**Less Likely (<=20%)**	**Rare but Serious (<3%)**	
BLOOD AND LYMPHATIC SYSTEM DISORDERS			
Anemia			***Anemia (Gr 3)***
CARDIAC DISORDERS			
		Heart failure	
GASTROINTESTINAL DISORDERS			
	Abdominal pain		***Abdominal pain (Gr 3)***
Constipation			***Constipation (Gr 3)***
Diarrhea			***Diarrhea (Gr 3)***
	Dyspepsia		***Dyspepsia (Gr 2)***
	Gastrointestinal hemorrhage[2]		
		Gastrointestinal perforation[3]	
	Ileus		***Ileus (Gr 3)***
Nausea			***Nausea (Gr 3)***
Vomiting			***Vomiting (Gr 3)***
GENERAL DISORDERS AND ADMINISTRATION SITE CONDITIONS			
	Chills		***Chills (Gr 2)***
	Edema limbs		***Edema limbs (Gr 3)***
Fatigue			***Fatigue (Gr 3)***
Fever			***Fever (Gr 3)***

Adverse Events with Possible Relationship to Bortezomib (Velcade) (CTCAE 5.0 Term) [n= 2084]			Specific Protocol Exceptions to Expedited Reporting (SPEER)
Likely (>20%)	**Less Likely (<=20%)**	**Rare but Serious (<3%)**	
HEPATOBILIARY DISORDERS			
		Hepatic failure[4]	
		Hepatobiliary disorders - Other (hepatitis)[4]	
INFECTIONS AND INFESTATIONS			
Infection[5]			***Infection[5](Gr 4)***
INVESTIGATIONS			
		Alanine aminotransferase increased[4]	
		Alkaline phosphatase increased[4]	
		Aspartate aminotransferase increased[4]	
		Blood bilirubin increased[4]	
		GGT increased[4]	
		INR increased[4]	
		Investigations - Other (albumin)[4]	
	Neutrophil count decreased		***Neutrophil count decreased (Gr 4)***
Platelet count decreased			***Platelet count decreased (Gr 4)***
	Weight loss		
METABOLISM AND NUTRITION DISORDERS			
Anorexia			***Anorexia (Gr 3)***
	Dehydration		
		Tumor lysis syndrome	
MUSCULOSKELETAL AND CONNECTIVE TISSUE DISORDERS			
	Arthralgia		***Arthralgia (Gr 3)***
	Back pain		***Back pain (Gr 3)***
	Bone pain		***Bone pain (Gr 2)***
	Muscle cramp		
	Myalgia		***Myalgia (Gr 3)***
	Pain in extremity		***Pain in extremity (Gr 3)***
NERVOUS SYSTEM DISORDERS			
	Dizziness		***Dizziness (Gr 3)***
	Headache		***Headache (Gr 3)***
		Leukoencephalopathy	
	Neuralgia		***Neuralgia (Gr 3)***
	Paresthesia		
Peripheral motor neuropathy			***Peripheral motor neuropathy (Gr 3)***
Peripheral sensory neuropathy			***Peripheral sensory neuropathy (Gr 3)***
		Reversible posterior leukoencephalopathy syndrome	
PSYCHIATRIC DISORDERS			
	Anxiety		
	Insomnia		***Insomnia (Gr 2)***
RENAL AND URINARY DISORDERS			

Adverse Events with Possible Relationship to Bortezomib (Velcade) (CTCAE 5.0 Term) [n= 2084]			Specific Protocol Exceptions to Expedited Reporting (SPEER)
Likely (>20%)	**Less Likely (<=20%)**	**Rare but Serious (<3%)**	
		Acute kidney injury	
RESPIRATORY, THORACIC AND MEDIASTINAL DISORDERS			
		Adult respiratory distress syndrome	
	Cough		***Cough (Gr 2)***
	Dyspnea		***Dyspnea (Gr 3)***
	Pharyngeal mucositis		***Pharyngeal mucositis (Gr 2)***
		Pulmonary hypertension	
SKIN AND SUBCUTANEOUS TISSUE DISORDERS			
	Rash maculo-papular		***Rash maculo-papular (Gr 3)***
VASCULAR DISORDERS			
	Hypotension		***Hypotension (Gr 4)***

[1]This table will be updated as the toxicity profile of the agent is revised. Updates will be distributed to all Principal Investigators at the time of revision. The current version can be obtained by contacting PIO@CTEP.NCI.NIH.GOV. Your name, the name of the investigator, the protocol and the agent should be included in the e-mail.

[2]Gastrointestinal hemorrhage includes Anal hemorrhage, Cecal hemorrhage, Colonic hemorrhage, Duodenal hemorrhage, Esophageal hemorrhage, Esophageal varices hemorrhage, Gastric hemorrhage, Hemorrhoidal hemorrhage, Ileal hemorrhage, Intra-abdominal hemorrhage, Jejunal hemorrhage, Lower gastrointestinal hemorrhage, Oral hemorrhage, Pancreatic hemorrhage, Rectal hemorrhage, Retroperitoneal hemorrhage, and Upper gastrointestinal hemorrhage under the GASTROINTESTINAL DISORDERS SOC.

[3]Gastrointestinal perforation includes Colonic perforation, Duodenal perforation, Esophageal perforation, Gastric perforation, Ileal perforation, Jejunal perforation, Rectal perforation, and Small intestinal perforation under the GASTROINTESTINAL DISORDERS SOC.

[4]Cases of acute liver failure have been reported in patients receiving multiple concomitant medications and with serious underlying medical conditions. Other reported hepatic reactions include hepatitis, increases in liver enzymes, and hyperbilirubinemia.

[5]Infection includes all 75 sites of infection under the INFECTIONS AND INFESTATIONS SOC.

Adverse events reported on bortezomib (Velcade) trials, but for which there is insufficient evidence to suggest that there was a reasonable possibility that bortezomib (Velcade) caused the adverse event:

BLOOD AND LYMPHATIC SYSTEM DISORDERS - Blood and lymphatic system disorders - Other (hematocrit low, hematocrit); Blood and lymphatic system disorders - Other (lymphadenopathy); Blood and lymphatic system disorders - Other (pancytopenia); Disseminated intravascular coagulation; Febrile neutropenia; Hemolytic uremic syndrome; Leukocytosis
CARDIAC DISORDERS - Asystole; Atrial fibrillation; Atrial flutter; Atrioventricular block complete; Cardiac arrest; Cardiac disorders - Other (cardiac amyloidosis); Cardiac disorders - Other (cardiomegaly); Chest pain - cardiac; Left ventricular systolic dysfunction; Mobitz type I; Myocardial infarction; Palpitations;

Pericardial effusion; Pericardial tamponade; Pericarditis; Right ventricular dysfunction; Sinus bradycardia; Sinus tachycardia; Supraventricular tachycardia; Ventricular arrhythmia; Ventricular fibrillation; Ventricular tachycardia

EAR AND LABYRINTH DISORDERS - Hearing impaired; Tinnitus

ENDOCRINE DISORDERS - Hypothyroidism

EYE DISORDERS - Blurred vision; Dry eye; Extraocular muscle paresis; Eye disorders - Other (chalazion); Eye disorders - Other (choroidal effusion); Eye disorders - Other (conjunctival hemorrhage); Eye disorders - Other (retinal hemorrhage with bilateral vision impairment); Keratitis; Watering eyes

GASTROINTESTINAL DISORDERS - Abdominal distension; Ascites; Belching; Bloating; Colitis; Dry mouth; Duodenal ulcer; Dysphagia; Enterocolitis; Esophagitis; Flatulence; Gastritis; Gastroesophageal reflux disease; Gastrointestinal disorders - Other (colonic wall thickening); Gastrointestinal disorders - Other (early satiety); Gastrointestinal disorders - Other (ileitis); Gastrointestinal disorders - Other (ischemic bowel); Gastrointestinal disorders - Other (mouth/tongue ulceration); Gastrointestinal disorders - Other (retching); Gastrointestinal pain; Gingival pain; Hemorrhoids; Mucositis oral; Oral pain; Pancreatitis; Small intestinal obstruction; Typhlitis

GENERAL DISORDERS AND ADMINISTRATION SITE CONDITIONS - Edema face; Flu like symptoms; Gait disturbance; General disorders and administration site conditions - Other (catheter related complication); General disorders and administration site conditions - Other (hepato-renal syndrome); Hypothermia; Injection site reaction; Malaise; Multi-organ failure; Non-cardiac chest pain; Pain; Sudden death NOS

HEPATOBILIARY DISORDERS - Portal vein thrombosis; Sinusoidal obstruction syndrome

IMMUNE SYSTEM DISORDERS - Allergic reaction; Anaphylaxis; Cytokine release syndrome

INJURY, POISONING AND PROCEDURAL COMPLICATIONS - Bruising; Fall; Fracture; Vascular access complication

INVESTIGATIONS - Activated partial thromboplastin time prolonged; CD4 lymphocytes decreased; CPK increased; Carbon monoxide diffusing capacity decreased; Cardiac troponin I increased; Cardiac troponin T increased; Cholesterol high; Creatinine increased; Ejection fraction decreased; Investigations - Other (BUN); Investigations - Other (low chloride); Lipase increased; Lymphocyte count decreased; Serum amylase increased; Weight gain; White blood cell decreased

METABOLISM AND NUTRITION DISORDERS - Acidosis; Hypercalcemia; Hyperglycemia; Hyperkalemia; Hyperuricemia; Hypoalbuminemia; Hypocalcemia; Hypoglycemia; Hypokalemia; Hypomagnesemia; Hyponatremia; Hypophosphatemia; Metabolism and nutrition disorders - Other (failure to thrive); Metabolism and nutrition disorders - Other (hypoproteinemia)

MUSCULOSKELETAL AND CONNECTIVE TISSUE DISORDERS - Arthritis; Avascular necrosis; Buttock pain; Chest wall pain; Generalized muscle weakness; Joint range of motion decreased; Muscle weakness lower limb; Osteonecrosis of jaw

NEOPLASMS BENIGN, MALIGNANT AND UNSPECIFIED (INCL CYSTS AND POLYPS) - Tumor pain

NERVOUS SYSTEM DISORDERS - Acoustic nerve disorder NOS; Akathisia; Ataxia; Cognitive disturbance; Depressed level of consciousness; Dysesthesia; Dysgeusia; Dysphasia; Edema cerebral; Encephalopathy; Facial muscle weakness; Facial nerve disorder; Hypersomnia; Intracranial hemorrhage; Ischemia cerebrovascular; Lethargy; Memory impairment; Nervous system disorders - Other (autonomic neuropathy, autonomic dysfunction); Nervous system disorders - Other (cranial palsy); Nervous system disorders - Other (dysautonomia); Nervous system disorders - Other (L sided facial droop); Nervous system disorders - Other (paralysis); Nervous system disorders - Other (polyneuropathy); Nervous system disorders - Other (tongue paralysis); Presyncope; Seizure; Somnolence; Spinal cord compression; Stroke; Syncope; Tremor; Vasovagal reaction

PSYCHIATRIC DISORDERS - Agitation; Confusion; Delirium; Depression; Personality change; Psychosis

RENAL AND URINARY DISORDERS - Bladder spasm; Chronic kidney disease; Cystitis noninfective; Hematuria; Proteinuria; Renal and urinary disorders - Other (bilateral hydronephrosis); Renal and urinary disorders - Other (glomerular nephritis proliferative); Renal calculi; Urinary frequency; Urinary incontinence; Urinary retention; Urinary tract pain

RESPIRATORY, THORACIC AND MEDIASTINAL DISORDERS - Allergic rhinitis; Aspiration; Atelectasis; Bronchopulmonary hemorrhage; Bronchospasm; Epistaxis; Hiccups; Hypoxia; Laryngeal edema; Mediastinal hemorrhage; Pharyngolaryngeal pain; Pleural effusion; Pleuritic pain; Pneumonitis; Postnasal drip; Pulmonary edema; Respiratory failure; Respiratory, thoracic and mediastinal disorders - Other (obstructive airways disease); Respiratory, thoracic and mediastinal disorders - Other (respiratory distress); Respiratory, thoracic and mediastinal disorders - Other (tachypnea); Tracheal mucositis; Tracheal stenosis; Voice alteration
SKIN AND SUBCUTANEOUS TISSUE DISORDERS - Alopecia; Bullous dermatitis; Dry skin; Erythema multiforme; Erythroderma; Hyperhidrosis; Pain of skin; Palmar-plantar erythrodysesthesia syndrome; Pruritus; Purpura; Rash acneiform; Skin and subcutaneous tissue disorders - Other (angioedema); Skin and subcutaneous tissue disorders - Other (leukoclastic vasculitis); Skin and subcutaneous tissue disorders - Other (skin lesion NOS); Urticaria
VASCULAR DISORDERS - Capillary leak syndrome; Flushing; Hematoma; Hypertension; Thromboembolic event; Vascular disorders - Other (trach site); Vasculitis

Note: Bortezomib (Velcade) in combination with other agents could cause an exacerbation of any adverse event currently known to be caused by the other agent, or the combination may result in events never previously associated with either agent.

Effect in Pregnancy and Lactation:
At ½ the clinical dose **bortezomib** was not teratogenic in non-clinical developmental toxicity studies in rats and rabbits. However, pregnant rabbits given **bortezomib** during organogenesis at a dose of 0.05 mg/kg (0.6 mg/m^2) experienced significant post-implantation loss and decreased number of live fetuses. Live fetuses from these litters also showed significant decreases in fetal weight. It is not known whether **bortezomib** is excreted in human milk.

Formulation and Stability:
Bortezomib is supplied as a lyophilized powder in sterile vials containing 3.5 mg and 35 mg mannitol, USP. Unopened vials may be stored at controlled room temperature 25ºC (77ºF); excursions permitted from 15ºC to 30ºC (59ºF to 86ºF). Retain in original package to protect from light.

Reconstitute bortezomib with 3.5 mL normal saline, USP. Each milliliter of solution will contain 1 mg of bortezomib at a pH of approximately 5 to 6. The drug solution is clear and colorless. Bortezomib contains no antimicrobial preservative. When reconstituted as directed, bortezomib may be stored at 25°C (77°F). Reconstituted bortezomib should be administered within 8 hours of preparation. The reconstituted material may be stored in the original vial and/or the syringe prior to administration. The product may be stored for up to 8 hours in a syringe; however total storage time for the reconstituted material must not exceed 8 hours when exposed to normal indoor lighting.

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

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

Supplier: Supplied by Millennium Pharmaceuticals and distributed by the NCI DTCD. Do not use commercially available drug.

Obtaining the Agent

Agent Ordering:

NCI supplied agent may be requested by the Principal Investigator (or their authorized designee) at each participating institution. Pharmaceutical Management Branch (PMB) policy requires that agent be shipped directly to the institution where the patient is to be treated. PMB does not permit the transfer of agents between institutions (unless prior approval from PMB is obtained). The CTEP assigned protocol number must be used for ordering all CTEP supplied investigational agents. The responsible investigator at each participating institution must be registered with CTEP, DCTD through an annual submission of FDA form 1572 (Statement of Investigator), Curriculum Vitae, Supplemental Investigator Data Form (IDF), and Financial Disclosure Form (FDF). If there are several participating investigators at one institution, CTEP supplied investigational agents for the study should be ordered under the name of one lead investigator at that institution.

Active CTEP-registered investigators and investigator-designated shipping designees and ordering designees must submit agent requests through the PMB Online Agent Order Processing (OAOP) application < https://eapps-ctep.nci.nih.gov/OAOP/pages/login.jspx >. Access to OAOP requires the establishment of a CTEP Identity and Access Management (IAM) account < https://eapps-ctep.nci.nih.gov/iam/ > and the maintenance of an "active" account status and a "current" password. For questions about drug orders, transfers, returns, or accountability, call (240) 276-6575 Monday through Friday between 8:30 am and 4:30 pm (ET) or email PMBAfterHours@mail.nih.gov anytime.

Agent Accountability:

Agent Inventory Records – The investigator, or a responsible party designated by the investigator, must maintain a careful record of the inventory and disposition of all agents received from DCTD using the NCI Drug Accountability Record Form (DARF). (See the CTEP home page at http://ctep.cancer.gov/protocolDevelopment/default.htm#agents_drugs for the Procedures for Drug Accountability and Storage and http://ctep.cancer.gov/forms/default.htm to obtain a copy of the DARF and Clinical Drug Request form.)

Agent Returns:

Investigators/Designees must return unused DCTD supplied investigational agent to the NCI clinical repository as soon as possible when: the agent is no longer required because the study is completed or discontinued and the agent cannot be transferred to another DCTD sponsored protocol; the agent is outdated or the agent is damaged or unfit for use. Regulations require that all agents received from the DCTD, NCI be returned to the DCTD, NCI for accountability and disposition. Return only unused vials/bottles. Do NOT return opened or partially used vials/bottles unless specifically requested otherwise in the protocol. See the CTEP web site for Policy and Guidelines for Investigational agent Returns at: http://ctep.cancer.gov/protocolDevelopment/default.htm#agents_drugs. The appropriate forms may be obtained at: http://ctep.cancer.gov/forms/docs/return_form.pdf.

6.2 CYCLOPHOSPHAMIDE - INJECTION (03/13/13)

(Cytoxan) NSC #26271

Source and Pharmacology:

Cyclophosphamide is an alkylating agent related to nitrogen mustard. Cyclophosphamide is inactive until it is metabolized by P450 isoenzymes (CYP2B6, CYP2C9, and CYP3A4) in the liver to active compounds. The initial product is 4-hydroxycyclophosphamide (4-HC), which is in equilibrium with aldophosphamide, which spontaneously releases acrolein to produce phosphoramide mustard.

Phosphoramide mustard, which is an active bifunctional alkylating species, is 10 times more potent *in vitro* than is 4-HC and has been shown to produce interstrand DNA cross-link analogous to those produced by mechlorethamine. Approximately 70% of a dose of cyclophosphamide is excreted in the urine as the inactive carboxyphosphamide and 5-25% as unchanged drug. Cyclophosphamide is well absorbed orally with a bioavailability greater than 75%. The plasma half-life ranges from 4.1 to 16 hours after IV administration and 1.3 to 6.8 hours after oral administration.

Toxicity:

	Common Happens to 21-100 children out of every 100	**Occasional** Happens to 5-20 children out of every 100	**Rare** Happens to < 5 children out of every 100
Immediate: Within 1-2 days of receiving drug	Anorexia, nausea & vomiting (acute and delayed)	Abdominal discomfort, diarrhea	Transient blurred vision, nasal stuffiness with rapid administration, arrhythmias (rapid infusion), skin rash, anaphylaxis, SIADH
Prompt: Within 2-3 weeks, prior to the next course	Leukopenia, alopecia, immune suppression	Thrombocytopenia, anemia, hemorrhagic cystitis (L)	Cardiac toxicity with high dose (acute – CHF hemorrhagic myocarditis, myocardial necrosis) (L), hyperpigmentation, nail changes, impaired wound healing, infection secondary to immune suppression
Delayed: Any time later during therapy, excluding the above conditions	Gonadal dysfunction: azoospermia or oligospermia (prolonged or permanent)[1] (L)	amenorrhea[1]	Gonadal dysfunction: ovarian failure[1] (L), interstitial pneumonitis, pulmonary fibrosis[2] (L)
Late: Any time after completion of treatment			Secondary malignancy (ALL, ANLL, AML), bladder carcinoma (long term use > 2 years), bladder fibrosis
Unknown Frequency and Timing:	Fetal toxicities and teratogenic effects of cyclophosphamide (alone or in combination with other antineoplastic agents) have been noted in humans. Toxicities include: chromosomal abnormalities, multiple anomalies, pancytopenia, and low birth weight. Cyclophosphamide is excreted into breast milk. Cyclophosphamide is contraindicated during breast feeding because of reported cases of neutropenia in breast fed infants and the potential for serious adverse effects.		

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

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

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

Cyclophosphamide for Injection:
If the drug will be administered as undiluted drug at the 20 mg/mL concentration, then reconstitute to 20 mg/mL with NS ONLY to avoid a hypotonic solution. If the drug will be further diluted prior to administration, then first reconstitute with NS, SWFI, or Bacteriostatic Water for Injection

(paraben preserved only) to a concentration of 20 mg/mL. Following reconstitution further dilute in dextrose or saline containing solutions for IV use.

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

6.3 CYTARABINE - ALL ROUTES (07/13/15)

(Cytosine arabinoside, Ara-C, Cytosar®) NSC #63878

Source and Pharmacology:

Cytarabine appears to act through the inhibition of DNA polymerase. A limited, but significant, incorporation of cytarabine into both DNA and RNA has also been reported. It exhibits cell phase specificity, primarily killing cells undergoing DNA synthesis (S-phase) and under certain conditions blocking the progression of cells from the G1 phase to the S-phase. Cytarabine is metabolized by deoxycytidine kinase and other nucleotide kinases to the nucleotide triphosphate (Ara-CTP), an effective inhibitor of DNA polymerase. Ara-CTP is inactivated by a pyrimidine nucleoside deaminase, which converts it to the nontoxic uracil derivative (Ara-U). It appears that the balance of kinase and deaminase levels may be an important factor in determining sensitivity or resistance of the cell to cytarabine. It has an initial distributive phase $t_{½}$ of about 10 minutes, with a secondary elimination phase $t_{½}$ 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 $t_{½}$ of about 2 hours.

Toxicity: (Intravenous, SubQ, IM)

	Common Happens to 21-100 children out of every 100	**Occasional** Happens to 5-20 children out of every 100	**Rare** Happens to < 5 children out of every 100
Immediate: Within 1-2 days of receiving drug	Nausea, vomiting, anorexia *With High Dose*: conjunctivitis	Flu-like symptoms with fever, rash	Ara-C syndrome (fever, myalgia, bone pain, occasionally chest pain, maculopapular rash, malaise, conjunctivitis), anaphylaxis, swelling, pain and redness at the site of the medication injection (SubQ or IM injection) *With High Dose:* cardiomyopathies (vasculitis, and pericarditis), cerebral and cerebellar dysfunction including: encephalopathy, aseptic meningitis, ataxia, dysphasia, nystagmus, a decreased level of consciousness, personality changes, somnolence, seizures
Prompt: Within 2-3 weeks, prior to the next course	Myelosuppression (anemia, thrombocytopenia, leukopenia, megaloblastosis, reticulocytopenia), stomatitis, alopecia	Diarrhea, hypokalemia, hypocalcemia, hyperuricemia *With High D*ose: capillary pulmonary leak syndrome (RDS, pulmonary edema)	Hepatotoxicity, sinusoidal obstruction syndrome (SOS, formerly VOD), urinary retention, renal dysfunction, pain and erythema of the palms and soles
Delayed: Any time later during therapy, excluding the above conditions			Asymptomatic nonoliguric rhabdomyolysis
Unknown Frequency and Timing:	Fetal toxicities and teratogenic effects of cytarabine have been noted in humans. It is unknown whether the drug is excreted in breast milk.		

Toxicity: (Intrathecal)

	Common Happens to 21-100 children out of every 100	**Occasional** Happens to 5-20 children out of every 100	**Rare** Happens to < 5 children out of every 100
Immediate: Within 1-2 days of receiving drug	Nausea, vomiting, fever, headache	Arachnoiditis	Rash, somnolence, meningismus, convulsions, paresis
Prompt: Within 2-3 weeks, prior to the next course			Myelosuppression, ataxia
Delayed: Any time later during therapy, excluding the above condition			Necrotizing leukoencephalopathy, paraplegia, blindness (in combination with XRT & systemic therapy)

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

IV Infusion:
Reconstitute the lyophilized powder with Bacteriostatic Water for Injection or NS injection. Solution containing bacteriostatic agent should not be used for the preparation of doses > 200 mg/m^2. May be further diluted with dextrose or sodium chloride containing solutions. May give by IV push injection, by IV infusion, or by continuous infusion.

Low Dose (≤ 200 mg/m²/dose): For administration by IV push, reconstitute to a concentration of 20-100 mg/mL.

High Dose (≥ 1000 mg/m²/dose): Administer steroid eye drops (dexamethasone or prednisolone), 2 drops each eye q6h beginning immediately before the first dose and continuing 24 hours after the last dose. If patient does not tolerate steroid eye drops, administer artificial tears on a q2-4 hour schedule.

Stability: When reconstituted with Bacteriostatic Water for Injection, cytarabine is stable for 48 hours at room temperature. Solutions reconstituted without a preservative should be used immediately. Discard if solution appears hazy. Diluted solutions in D5W or NS are stable for 8 days at room temperature; however, the diluted cytarabine should be used within 24 hours for sterility concerns.

Subcutaneous or IM:
Dilute with Bacteriostatic Water for Injection or NS to a concentration not to exceed 100 mg/mL. Rotate injection sites for subcutaneous/IM administration.

6.4 DAUNORUBICIN (05/09/11)

(Daunomycin, rubidomycin, Cerubidine®) NSC #82151

Source and Pharmacology:

Daunorubicin is an anthracycline antibiotic isolated from cultures of *Streptomyces coeruleorubidus*. Daunorubicin is closely related structurally to doxorubicin only differing in that the side chain of daunorubicin terminates in a methyl group rather than an alcohol. The cytotoxic effect of daunorubicin on malignant cells and its toxic effects on various organs are similar to those of doxorubicin and are thought to be related to nucleotide base intercalation and cell membrane lipid binding activities. Intercalation inhibits nucleotide replication and action of DNA and RNA polymerases. The interaction of daunorubicin with topoisomerase II to form DNA-cleavable complexes appears to be an important mechanism of cytocidal activity. Daunorubicin cellular membrane binding may affect a variety of cellular functions. Enzymatic electron reduction of daunorubicin by a variety of oxidases, reductases, and dehydrogenases generate highly reactive species including the hydroxyl free radical (OH•), which may lead to DNA damage or lipid peroxidation. Daunorubicin is metabolized more rapidly by aldoketoreductases to the active metabolite, daunorubicinol, than is doxorubicin. Daunorubicin hydrochloride is rapidly and widely distributed in tissues, with the highest levels in the spleen, kidneys, liver, lungs, and heart. Daunorubicin serum decay pattern is multiphasic. The initial $t_{½}$ is approximately 45 minutes followed by a terminal $t_{½}$ of 18.5 hours. By 1 hour after drug administration, the predominant plasma species is daunorubicinol, which disappears with a half-life of 26.7 hours. Twenty five percent of an administered dose of daunorubicin is eliminated in an active form by urinary excretion and an estimated 40% by biliary excretion.

Toxicity:

	Common Happens to 21-100 children out of every 100	**Occasional** Happens to 5-20 children out of every 100	**Rare** Happens to < 5 children out of every 100
Immediate: Within 1-2 days of receiving drug	Nausea, vomiting, pink or red color to urine, sweat, tears, and saliva	Hyperuricemia, sclerosis of the vein	Diarrhea, anorexia, abdominal pain, extravasation (rare) but if occurs = local ulceration, anaphylaxis, fever, chills, rash, urticaria, acute arrhythmias
Prompt: Within 2-3 weeks, prior to the next course	Myelosuppression (leukopenia, thrombocytopenia, anemia), alopecia	Mucositis (stomatitis and esophagitis), hepatotoxicity	Radiation recall reactions, myocarditis-pericarditis syndrome, conjunctivitis and lacrimation
Delayed: Any time later during therapy			Cardiomyopathy[1] (uncommon at cumulative doses ≤ 550 mg/m², 400 mg/m² with mediastinal radiation, 300 mg/m² in children, or 10 mg/kg in children < 2 yrs or 0.5 m²) (L), hyper-pigmentation of nail beds
Late: Any time after completion of treatment		Subclinical cardiac dysfunction	CHF (on long term follow up in pediatric patients), secondary malignancy (in combination regimens)
Unknown Frequency and Timing:	Fetal toxicities and teratogenic effects of daunorubicin have been noted in animals. It is unknown whether the drug is excreted in breast milk.		

[1] *Risk increases with cardiac irradiation, exposure at a young or advanced age.*

(L) Toxicity may also occur later

Formulation and Stability:
Daunorubicin is available as red-orange lyophilized powder[1] for injection in 20 mg single dose vials and a preservative free 5 mg/mL solution[2] in 20 mg (4 mL) and 50 mg (10 mL) vials.

[1] Each vial contains 21.4 mg of daunorubicin hydrochloride (equivalent to 20 mg of daunorubicin) and 100 mg mannitol.

[2] Each mL contains 5.3 mg daunorubicin hydrochloride (equivalent to 5 mg of daunorubicin), 9 mg of sodium chloride, sodium hydroxide or hydrochloric acid to adjust pH, and Sterile Water for Injection.

Powder for Injection:
Store intact, unreconstituted vials at room temperature, 15°-30°C (59°-86°F). Protect from light. Retain in carton until contents are used. Reconstitute a 20 mg vial with 4 mL SWFI to a final concentration of 5 mg/mL. After adding the diluent, the vial should be shaken gently and the contents allowed to dissolve. The reconstituted solution is stable for 24 hours at room temperature and 48 hours refrigerated. Protect from exposure to sunlight.

Aqueous Solution:
Store refrigerated 2°-8°C, (36°-46°F). Protect from light. Retain in carton until contents are used.

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

Administer by IV side arm into a rapidly flowing infusion solution. Alternately, daunorubicin may be further diluted in saline or dextrose containing solutions and administered by infusion. Protect final preparation from light. To avoid extravasation, the use of a central line is suggested.

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

6.5 DEXAMETHASONE (05/09/11)

(Decadron®, Hexadrol®, Dexone®, Dexameth®) NSC #34521

Source and Pharmacology:
Dexamethasone is a synthetic fluorinated glucocorticoid devoid of mineralocorticoid effects. Dexamethasone, 0.75 mg, has potent anti-inflammatory activity equivalent to approximately 5 mg of prednisone. Glucocorticoids produce widespread and diverse physiologic effects on carbohydrate, protein, and lipid metabolism, electrolyte and water balance, functions of the cardiovascular system, kidney, skeletal muscle, and the nervous systems. Glucocorticoids reduce the concentration of thymus-dependent lymphocytes (T-lymphocytes), monocytes, and eosinophils. Glucocorticoids selectively bind to the cortisol receptors on human lymphoid cells that are found in larger numbers on leukemic lymphoblasts. They also decrease binding of immunoglobulin to cell surface receptors and inhibit the synthesis and/or release of interleukins, thereby decreasing T-lymphocyte blastogenesis and reducing expansion of the primary immune response. The specific cellular mechanisms that act to halt DNA synthesis are thought to be related to inhibition of glucose transport or phosphorylation, retardation of mitosis, and inhibition of protein synthesis. Elimination half-lives for the following age groups have been reported to be: infants and children under 2 years of age: 2.3 to 9.5 hours, 8 to 16 years: 2.82 to 7.5 hours, and adults (age not specified): 3 to 6 hours. The biologic half-life is 36-72 hours. It is primarily metabolized in the liver and excreted by the kidneys.

Toxicity:

	Common Happens to 21-100 children out of every 100	**Occasional** Happens to 5-20 children out of every 100	**Rare** Happens to < 5 children out of every 100
Immediate: Within 1-2 days of receiving drug	Insomnia, hyperphagia	Gastritis	Hyperuricemia
Prompt: Within 2-3 weeks, prior to the next course	Immunosuppression, personality changes (mood swings, euphoria, anxiety, depression), pituitary-adrenal axis suppression, acne (L)	Hyperglycemia, facial erythema, poor wound healing, infections (bacterial, fungal, parasitic, viral), edema	Pancreatitis (L), increased intraocular pressure (L), hypertension, psychosis, vertigo, headache
Delayed: Any time later during therapy	Cushing's syndrome (moon facies, truncal obesity)	Striae and thinning of the skin, easy bruising, muscle weakness, osteopenia	Spontaneous fractures (L), growth suppression, peptic ulcer and GI bleeding, pseudotumor cerebri (increased intracranial pressure with papilledema, headache), aseptic necrosis of the femoral and humeral heads (L), urolithiasis[1] (L)
Late: Any time after completion of treatment		Cataracts (which may be reversible on discontinuation of dexamethasone in children)	
Unknown Frequency and Timing:	**Fetal and teratogenic toxicities**: dexamethasone crosses the placenta with 54% metabolized by enzymes in the placenta. In animal studies, large doses of cortisol administered early in pregnancy produced cleft palate, stillborn fetuses, and decreased fetal size. Chronic maternal ingestion during the first trimester has shown a 1% incidence of cleft palate in humans. There are no reports of dexamethasone excretion into breast milk in humans; however, it is expected due to its low molecular weight that it would partition into breast milk.		

[1] Mainly reported in pediatric patients with ALL. Howard SC et al. Urolithiasis in pediatric patients with acute lymphoblastic leukemia. Leukemia 2003; 17: 541-6.

(L) Toxicity may also occur later.

Formulation and Stability:

Oral:

Available in 0.5 mg, 0.75 mg, 1 mg, 1.5 mg, 2 mg, 4 mg, and 6 mg tablets; liquid formulations are available in 0.5 mg/5 mL and 1 mg/1 mL concentrations. Inactive ingredients vary depending on manufacturer but tablet formulations may include: calcium or magnesium stearate, corn starch, lactose, and various dyes. Liquid formulations may include: 5%-30% alcohol, benzoic acid, sorbitol, sodium saccharin, glycerin, purified water, and various dyes.

Injection:

Dexamethasone Sodium Phosphate Solution for Injection is available as 4 mg/mL (1 mL, 5 mL, and 30 mL vials) and 10 mg/mL (1 mL and 10 mL vial sizes). Vials are available in multi-dose vials as well as unit of use vials and syringes. Inactive ingredients vary depending on manufacturer but include creatinine, sodium citrate, sodium hydroxide to adjust pH, Water for Injection, sodium sulfite, bisulfite and metabisulfite, methyl and propyl paraben, benzyl alcohol, and EDTA.

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

Dexamethasone Sodium Phosphate for Injection may be given IV, or IM undiluted. For IV use, it may be further diluted in dextrose or saline containing solutions. Avoid using benzyl alcohol-containing dexamethasone solutions in neonates. Diluted solutions that contain no preservatives should be used within 24 hours, but maintain stability for at least 14 days in PVC bags at room temperature protected from light.

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

6.6 DOXORUBICIN (05/09/11)

(Adriamycin®) NSC #123127

Source and Pharmacology:
An anthracycline antibiotic isolated from cultures of *Streptomyces peucetius*. The cytotoxic effect of doxorubicin on malignant cells and its toxic effects on various organs are thought to be related to nucleotide base intercalation and cell membrane lipid binding activities of doxorubicin. Intercalation inhibits nucleotide replication and action of DNA and RNA polymerases. The interaction of doxorubicin with topoisomerase II to form DNA-cleavable complexes appears to be an important mechanism of doxorubicin cytocidal activity. Doxorubicin cellular membrane binding may affect a variety of cellular functions. Enzymatic electron reduction of doxorubicin by a variety of oxidases, reductases, and dehydrogenases generate highly reactive species including the hydroxyl free radical (OH•). Free radical formation has been implicated in doxorubicin cardiotoxicity by means of Cu (II) and Fe (III) reduction at the cellular level. Cells treated with doxorubicin have been shown to manifest the characteristic morphologic changes associated with apoptosis or programmed cell death. Doxorubicin-induced apoptosis may be an integral component of the cellular mechanism of action relating to therapeutic effects, toxicities, or both.

Doxorubicin serum decay pattern is multiphasic. The initial distributive $t_{½}$ is approximately 5 minutes suggesting rapid tissue uptake of doxorubicin. The terminal $t_{½}$ of 20 to 48 hours reflects a slow elimination from tissues. Steady-state distribution volumes exceed 20 to 30 L/kg and are indicative of extensive drug uptake into tissues. Plasma clearance is in the range of 8 to 20 mL/min/kg and is predominately by metabolism and biliary excretion. The P450 cytochromes which appear to be involved with doxorubicin metabolism are CYP2D6 and CYP3A4. Approximately 40% of the dose appears in the bile in 5 days, while only 5 to 12% of the drug and its metabolites appear in the urine during the same time period. Binding of doxorubicin and its major metabolite, doxorubicinol, to plasma proteins is about 74 to 76% and is independent of plasma concentration of doxorubicin.

Toxicity:

	Common Happens to 21-100 children out of every 100	**Occasional** Happens to 5-20 children out of every 100	**Rare** Happens to < 5 children out of every 100
Immediate: Within 1-2 days of receiving drug	Nausea, vomiting, pink or red color to urine, sweat, tears, and saliva	Hyperuricemia, facial flushing, sclerosis of the vein	Diarrhea, anorexia, erythematous streaking of the vein (flare reaction), extravasation (rare) but if occurs = local ulceration, anaphylaxis, fever, chills, urticaria, acute arrhythmias
Prompt: Within 2-3 weeks, prior to the next course	Myelosuppression (leukopenia, thrombocytopenia, anemia), alopecia	Mucositis (stomatitis and esophagitis), hepatotoxicity	Radiation recall reactions, conjunctivitis and lacrimation
Delayed:		Cardiomyopathy[1] (CHF occurs in 5-20% at	Cardiomyopathy[1] (CHF occurs in < 5% at cumulative doses

Any time later during therapy		cumulative doses ≥ 450 mg/m²) (L)	≤ 400 mg/m²) (L), ulceration and necrosis of colon, hyper-pigmentation of nail bed and dermal crease, onycholysis
Late: Any time after completion of treatment	Subclinical cardiac dysfunction	CHF (on long term follow up in pediatric patients)	Secondary malignancy (in combination regimens)
Unknown Frequency and Timing:	Fetal and teratogenic toxicities. Carcinogenic and mutagenic effects of doxorubicin have been noted in animal models. Doxorubicin is excreted into breast milk in humans		

[1] *Risk increases with cardiac irradiation, exposure at a young or advanced age.*
(L) Toxicity may also occur later.

Formulation and Stability:
Doxorubicin is available as red-orange lyophilized powder for injection in 10 mg[1], 20 mg[1], 50 mg[1]vials and a preservative-free 2 mg/mL solution in 10 mg[1], 20 mg[1], 50 mg[1], 200 mg[2] vials.
[1]: Contains lactose monohydrate, 0.9 NS, HCl to adjust pH to 3. The Adriamycin RDF® (rapid dissolution formula) also contains methylparaben, 1 mg per each 10 mg of doxorubicin, to enhance dissolution.
[2] Multiple dose vial contains lactose, 0.9% NS, HCl to adjust pH to 3.

Aqueous Solution: Store refrigerated 2°-8°C, (36°-46°F). Protect from light. Retain in carton until contents are used.

Powder for Injection: Store unreconstituted vial at room temperature, 15°-30°C (59°-86°F). Retain in carton until contents are used. Reconstitute with preservative-free NS to a final concentration of 2 mg/mL. After adding the diluent, the vial should be shaken and the contents allowed to dissolve. The reconstituted solution is stable for 7 days at room temperature and 15 days under refrigeration, 2°-8°C (36°-46°F) when protected from light. Doxorubicin further diluted in 50 – 1000 mL of NS or D5W is stable for up to 48 hours at room temperature (25°C) when protected from light.

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

Administer IV through the tubing of rapidly infusing solution of D_5W or 0.9% NaCl preferably into a large vein. Protect the diluted solution from sunlight. To avoid extravasation, the use of a central line is suggested.

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

6.7 ETOPOSIDE -– INJECTION (11/15/16)

(Toposar ®, Etopophos®,VP-16) NSC #141540

Source and Pharmacology:
A semisynthetic derivative of podophyllotoxin that forms a complex with topoisomerase II and DNA which results in single and double strand DNA breaks. Its main effect appears to be in the S and G_2 phase of the cell cycle. The initial $t_{½}$ 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 that 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.

Toxicity:

	Common Happens to 21-100 children out of every 100	**Occasional** Happens to 5-20 children out of every 100	**Rare** Happens to < 5 children out of every 100
Immediate: Within 1-2 days of receiving drug	Nausea, vomiting	Anorexia	Transient hypotension during infusion; anaphylaxis (chills, fever, tachycardia, dyspnea, bronchospasm, hypotension)
Prompt: Within 2-3 weeks, prior to next course	Myelosuppression (anemia, leukopenia), alopecia	Thrombocytopenia, diarrhea, abdominal pain, asthenia, malaise, rashes and urticaria	Peripheral neuropathy, mucositis, hepatotoxicity, chest pain, thrombophlebitis, congestive heart failure, Stevens-Johnson Syndrome, exfoliative dermatitis
Delayed: Any time later during therapy			Dystonia, ovarian failure, amenorrhea, anovulatory cycles, hypomenorrhea, onycholysis of nails
Late: Any time after completion of treatment			Secondary malignancy (preleukemic or leukemic syndromes)
Unknown Frequency and Timing:	Fetal toxicities and teratogenic effects of etoposide have been noted in animals at 1/20th of the human dose. It is unknown whether the drug is excreted in breast milk.		

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

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

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

Etoposide:
Dilute etoposide to a final concentration ≤ 0.4 mg/mL in D5W or NS. Etoposide infusions are stable at room temperature for 96 hours when diluted to concentrations of 0.2 mg/mL; stability is 24 hours

at room temperature with concentrations of 0.4 mg/mL. The time to precipitation is highly unpredictable at concentrations > 0.4 mg/mL. Use in-line filter during infusion secondary to the risk of precipitate formation. However, the use of an in-line filter is not mandatory since etoposide precipitation is unlikely at concentrations of 0.1-0.4 mg/mL. **Do not administer etoposide by rapid intravenous injection.** Slow rate of administration if hypotension occurs.

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

Etoposide Phosphate:
Reconstitute the 100 mg vial with 5 or 10 mL of Sterile Water for Injection, D5W, NS, Bacteriostatic Water for Injection with Benzyl Alcohol, or Bacteriostatic Sodium Chloride for Injection with Benzyl Alcohol for a concentration equivalent to 20 mg/mL or 10 mg/mL etoposide equivalent (22.7 mg/mL or 11.4 mg/mL etoposide phosphate), respectively. **Use diluents without benzyl alcohol for neonates and infants < 2 years of age or patients with hypersensitivity to benzyl alcohol.**

When reconstituted as directed, etoposide phosphate solutions can be stored in glass or plastic containers under refrigeration for 7 days. When reconstituted with a diluent containing a bacteriostatic, store at controlled room temperature for up to 48 hours. Following reconstitution with SWFI, D5W, or NS store at controlled room temperature for up to 24 hours.

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

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

CANADIAN SITES
Etoposide for Injection is available as a 20 mg/mL solution.

Etopophos® (etoposide phosphate) is not commercially available in Canada. Sites may purchase and import the USA commercial supply from Bristol Laboratories via an International Distributor (Pharma Exports LLC, phone: 1-412-885-3700, fax: 1-412-885-8022, email: pharexp@aol.com) under the authority of the protocol's No Objection Letter (NOL). Drug Accountability Log (DAL) must record Lot #'s and expiry dates of shipments received and doses dispensed. Sites may use their own DAL as long as it complies with all elements of ICH GCP and Division 5 of the Food and Drugs Act. Each site is responsible for the procurement (import +/- purchase) of Etoposide Phosphate (Etopophos). Sites may import and manage a single clinical trial supply for multiple protocols as long as each protocol has an NOL and the protocol the patient is registered on is recorded on the DAL.

6.8 FILGRASTIM, TBO-FILGRASTIM, FILGRASTIM-SNDZ (11/15/16)

(Granulocyte Colony-Stimulating Factor, r-metHuG-CSF, G-CSF, Neupogen® Granix®, Zarxio®) NSC #614629

Source and Pharmacology:

Filgrastim is a human granulocyte colony-stimulating factor (G-CSF), produced by recombinant DNA technology. Filgrastim is a 175 amino acid protein with a molecular weight of 18,800 daltons manufactured by recombinant DNA technology utilizing E coli bacteria into which has been inserted the human granulocyte colony stimulating factor gene. It differs from the natural protein in that the N- amino acid is methionine and the protein is not glycosylated. G-CSF is a lineage specific colony-stimulating factor that regulates the production of neutrophils within the bone marrow and affects neutrophil progenitor proliferation, differentiation, and selected end-cell functional activation (including enhanced phagocytic ability, priming of the cellular metabolism associated with respiratory burst, antibody dependent killing, and the increased expression of some functions associated with cell surface antigens.) Filgrastim exhibits nonlinear pharmacokinetics with clearance dependent on filgrastim concentration and neutrophil count. Filgrastim is cleared by the kidney. The elimination half-life is similar for subcutaneous and intravenous administration, approximately 3.5 hours. The time to peak concentration when administered subcutaneously is 2-8 hours

Toxicity:

	Common Happens to 21-100 children out of every 100	**Occasional** Happens to 5-20 children out of every 100	**Rare** Happens to <5 children out of every 100
Immediate: Within 1-2 days of receiving drug		Local irritation at the injection site, headache	Allergic reactions (more common with IV administration than subq):skin (rash, urticaria, facial edema), respiratory (wheezing, dyspnea) and cardiovascular (hypotension, tachycardia), low grade fever
Prompt: Within 2-3 weeks, prior to the next course	Mild to moderate medullary bone pain	Increased: alkaline phosphatase, lactate dehydrogenase and uric acid, thrombocytopenia	Splenomegaly, splenic rupture, rash or exacerbation of pre-existing skin rashes, sickle cell crises in patients with SCD, excessive leukocytosis, Sweet's syndrome (acute febrile neutrophilic dermatosis)
Delayed: Anytime later during therapy			Cutaneous vasculitis, ARDS
Late: Anytime after completion of treatment			MDS or AML (confined to patients with severe chronic neutropenia and long term administration)
Unknown Frequency and Timing:	Fetal toxicities and teratogenic effects of filgrastim in humans are unknown. Conflicting data exist in animal studies and filgrastim is known to pass the placental barrier. It is unknown whether the drug is excreted in breast milk.		

Formulation and Stability:

Neupogen® supplied as a clear solution of 300 mcg/mL in 1 mL or 1.6 mL vials. Neupogen® vials are preservative free single use vials. Discard unused portions of open vials.

Neupogen®, Granix®, and Zarxio® are also available as single use prefilled syringes containing 300 mcg/0.5 mL or 480 mcg/0.8 mL of filgastrim for subcutaneous administration.

Store refrigerated at 2º-8ºC (36º-46ºF). Protect from light. Do not shake. Prior to injection, filgrastim and filgrastim-sndz may be allowed to reach room temperature for a maximum of 24 hours (infusion must be completed within 24 hours of preparation). TBO-filgrastim may be removed from 2°C - 8°C (36°F - 46°F) storage for a single period of up to 5 days between 23°C to 27°C (73°F to 81°F). Avoid freezing and temperatures > 30ºC.

For IV use, dilute filgrastim (Neupogen®) and tbo-filgrastim (Granix®) in D5W only to concentrations >15 mcg/mL. Filgrastim-sndz (Zarxio®) may be diluted in D5W to concentrations between 5 mcg/mL and 15 mcg/mL. At concentrations below 5 and 15 mcg/mL, human serum albumin should be added to make a final albumin concentration of 0.2% (2 mg/mL) in order to minimize the adsorption of filgrastim to plastic infusion containers and equipment for all 3 products (communication on file from Teva Pharmaceuticals USA). Filgrastim or filgrastim-sndz dilutions of 5 mcg/mL or less are not recommended. Tbo-filgrastim dilutions below 2 mcg/mL are not recommended. Diluted filgrastim biosimilar products should be stored at 2º-8ºC (36º-46ºF) and used within 24 hours. Do not shake.

Do not dilute with saline-containing solutions at any time; precipitation will occur.

Guidelines for Administration:
See Treatment, Dose Modifications and Supportive Care sections of the protocol.

Filgrastim biosimilar products should not be administered within 24 hours of (before AND after) chemotherapy.

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

6.9 HYDROCORTISONE – INTRATHECAL (07/30/14)

(Hydrocortisone sodium succinate, Solu-cortef®) NSC #010483

Source and Pharmacology:
Hydrocortisone is a synthetic compound closely related to cortisol. 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. Hydrocortisone is approximately 90% protein bound with a plasma $t_{½}$ of 1-2 hours. The elimination of hydrocortisone from the CNS is prolonged.

Toxicity: (Toxicities for Hydrocortisone Intrathecal and Methotrexate and/or Cytarabine)[1]

	Common Happens to 21-100 children out of every 100	**Occasional** Happens to 5-20 children out of every 100	**Rare** Happens to < 5 children out of every 100
Immediate: Within 1-2 days of receiving drug	Nausea, vomiting, fever, headache	Arachnoiditis: (headache, fever, vomiting, meningismus and pleocytosis)	Rash, anaphylaxis (L), paresis, bleeding into subarachnoid or subdural space (risk > with platelet counts < 20,000), confusion,

			fatigue, disorientation, seizures
Prompt: Within 2-3 weeks, prior to the next course			Myelosuppression, somnolence, ataxia, cranial nerve palsy, transient paraplegia, (L) speech disorders
Delayed: Any time later during therapy, excluding the above condition		Cognitive disturbances (L), learning disabilities (L)	Demyelinating leukoencephalopathy[1] (L), blindness[1]
Late: Any time after the completion of treatment			Progressive CNS deterioration[2]

[1] Toxicity for hydrocortisone alone has not been described
[2] May be enhanced by systemic therapy such as high dose methotrexate or cytarabine and/or cranial irradiation.
(L) Toxicity may also occur later.

Formulation and Stability:
For intrathecal administration, use hydrocortisone sodium succinate 100 mg vial sterile powder for injection **WITHOUT** preservative. Do not reconstitute vial with bacteriostatic water for injection.

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

For recommended mixing directions for Intrathecal triples [Hydrocortisone, Methotrexate and Cytarabine] refer to the monograph for Intrathecal Triples.

Intrathecal Methotrexate and Hydrocortisone:
***Optimal Volume (20 mL)*:** Final concentration of Intrathecal Methotrexate and Hydrocortisone: Methotrexate 1 mg/mL and Hydrocortisone 1 mg/mL
The following is a suggested method for preparing the IT using the 25 mg/mL concentration of preservative free methotrexate. Institutional practices may be used as clinically appropriate.

- Withdraw 0.8 mL (20 mg) of methotrexate 25 mg/mL concentration
- Reconstitute hydrocortisone 100 mg vial with 10 mL of preservative free NS to a concentration of 10 mg/mL. Withdraw 2 mL (20 mg) of hydrocortisone
- Combine 0.8 mL (20 mg) methotrexate and 2 mL (20 mg) hydrocortisone

QS with 17.2 mL of NS for a total volume of 20 mL

***Alternative Volume (12 mL)*:** Final concentration of Intrathecal Methotrexate and Hydrocortisone: Methotrexate 1.5 mg/mL and Hydrocortisone 1.5 mg/mL

The following is a suggested method for preparing the IT using the 25 mg/mL concentration of preservative free methotrexate. Institutional practices may be used as clinically appropriate.
Withdraw 0.72 mL (18 mg) of methotrexate 25 mg/mL concentration

- Reconstitute hydrocortisone 100 mg vial with 10 mL of preservative free NS to a concentration of 10 mg/mL. Withdraw 1.8 mL (18 mg) of hydrocortisone
- Combine 0.72 mL (18 mg) methotrexate and 1.8 mL (18 mg) hydrocortisone
- QS with 9.48 mL of NS for a total volume of 12 mL

Intrathecal Cytarabine and Hydrocortisone:
Optimal Volume (20 mL): Final concentration of Intrathecal Cytarabine and Hydrocortisone: Hydrocortisone 1 mg/mL and Cytarabine 2 mg/mL

The following is a suggested method for preparing the IT. Institutional practices may be used as clinically appropriate.

- Reconstitute Hydrocortisone 100 mg vial with 10 mL of preservative free NS to a concentration of 10 mg/mL. Withdraw 2 mL (20 mg) of hydrocortisone
- Reconstitute Cytarabine 100 mg vial with 5 mL of preservative free NS to a concentration of 20 mg/mL. Withdraw 2 mL (40 mg) of cytarabine.
- Combine 2 mL (20 mg) hydrocortisone and 2 mL (40 mg) of cytarabine.
- QS with 16 mL of NS for a total volume of 20 mL.

Alternative Volume (12 mL): Final concentration of Intrathecal cytarabine and hydrocortisone: Hydrocortisone 1.5 mg/mL and Cytarabine 3.0 mg/mL.
The following is a suggested method for preparing the IT. Institutional practices may be used as clinically appropriate.
Reconstitute Hydrocortisone 100 mg vial with 10 mL of preservative free NS to a concentration of 10 mg/mL. Withdraw 1.8 mL (18 mg) of hydrocortisone
Reconstitute Cytarabine 100 mg vial with 5 mL of preservative free NS to a concentration of 20 mg/mL. Withdraw 1.8 mL (36 mg) of cytarabine
Combine 1.8 mL (18 mg) hydrocortisone and 1.8 mL (36 mg) of cytarabine
QS with 8.4 mL of NS for a total volume of 12 mL

Intrathecal Methotrexate and/or cytarabine and hydrocortisone are stable in preservative free NS for 24 hours at 25ºC but contain no preservative and should be administered as soon as possible after preparation.

Supplier: Hydrocortisone is commercially available. See package insert for further information

6.10 **INTRATHECAL TRIPLES** (Methotrexate/Hydrocortisone/Cytarabine, IT-3) **(05/08/12)**

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 $t_{½}$ of 4.5 and 14 hours respectively. Following IT injection of cytarabine the elimination of the drug from the CSF is biphasic with a $t_{½}$ of 1 and 3.4 hours respectively, which is 8-fold longer than the clearance from plasma. The elimination of hydrocortisone is similarly prolonged.

Intrathecal Triple Therapy (Methotrexate/ Hydrocortisone/Cytarabine) Toxicity:

	Common Happens to 21-100 children out of every 100	Occasional Happens to 5-20 children out of every 100	Rare Happens to < 5 children out of every 100
Immediate: Within 1-2 days of receiving drug	Nausea, vomiting, fever, headache	Arachnoiditis: (headache, fever, vomiting, meningismus and pleocytosis)	Rash, anaphylaxis (L), paresis, bleeding into subarachnoid or subdural space (risk > with platelet counts < 20,000), confusion, fatigue, disorientation, seizures
Prompt:			Myelosuppression, somnolence, ataxia, cranial nerve palsy, transient and

Within 2-3 weeks, prior to the next course			rarely permanent paraplegia (L), speech disorders
Delayed: Any time later during therapy, excluding the above condition		Cognitive disturbances (L), learning disabilities (L)	Demyelinating leukoencephalopathy[1] (L), blindness[1]
Late: Any time after the completion of treatment			Progressive CNS deterioration[1]

[1] *May be enhanced by systemic therapy such as high dose methotrexate or cytarabine and/or cranial irradiation.*
(L) Toxicity may also occur later.

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 succinate 100 mg vial sterile powder for injection.

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

For intrathecal administration, dilute each agent with 5-10 mL preservative free NS, lactated ringers or Elliot's B solution or as per institutional standard of practice. The volume of CSF removed should be equal to at least half the volume delivered.

Patient Age (years)	Doses (MTX/Hydrocortisone/Ara-C)	Recommended volume	10% CSF volume	CSF Volume *
0 – 0.99	7.5 mg / 7.5 mg / 15 mg	5-10 mL	5 mL	50 ± 10 mL (babies)
1 – 1.99	8 mg / 8 mg / 16 mg	5-10 mL	5 mL	50 ± 10 mL (babies)
2 – 2.99	10 mg / 10 mg / 20 mg	5-10 mL	8 mL	80 ± 20 mL (younger children)
3 – 8.99	12 mg / 12 mg / 24 mg	5-10 mL	10 mL	100 ± 20 mL (older children)
9 or greater	15 mg / 15 mg / 30 mg	5-10 mL	13 mL	130 ± 30 mL (adults)

*Rieselbach, *R.E. et.al. Subarachnoid distribution of drugs after lumbar injection. *N Engl J Med* 1962 Dec 20; 267:1273-8

Of note: Larger volumes approximating at least 10% of the CSF volume, isovolumetric delivery, with the patient remaining prone after the procedure may facilitate drug distribution. These procedures have not been validated in clinical trials. They are allowed but not mandated for patients on COG studies.

Intrathecal triples are stable in NS for 24 hours at 25ºC but contain no preservative and should be administered as soon as possible after preparation.

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

6.11 IFOSFAMIDE (05/09/11)

(Isophosphamide, Iphosphamide, Z4942, Ifex®) NSC #109724

Source and Pharmacology:

Ifosfamide is a structural analogue of cyclophosphamide. Ifosfamide requires hepatic microsomal activation (P450 3A isoenzymes) for the production of the reactive 4-hydroxyoxazaphorine intermediate, which serves as a carrier molecule for the ultimate intracellular liberation of acrolein and phosphoramide mustard, which is an active bifunctional alkylating species. Acrolein is thought to be the cause of the hemorrhagic cystitis as seen with cyclophosphamide. Ifosfamide demonstrates dose-dependent pharmacokinetics whereby the terminal half-life ranges from 7 to 16 hours at doses of 1.6-2.4 g/m² to 3.8-5 g/m², respectively. At 1.6-2.4 g/m²/d, 12 to 18% of the dose was excreted as unchanged drug in the urine, whereas at a 5 g/m² single-dose, 61% was excreted in the urine as the parent drug. Evidence also exists to suggest that ifosfamide metabolism is inducible, with more rapid clearance occurring in the second and later doses when a course of therapy is given as fractionated doses over 3 to 5 days. There is more chloroethyl side chain oxidation of ifosfamide (up to 50%) than of cyclophosphamide (< 10%), and the degree of such metabolism is more variable than with cyclophosphamide. Oxidation of the chloroethyl groups produces chloroacetaldehyde, which is thought to be responsible for the neurotoxicity and renal toxicity that have been seen with ifosfamide therapy.

Toxicity:

	Common Happens to 21-100 children out of every 100	**Occasional** Happens to 5-20 children out of every 100	**Rare** Happens to < 5 children out of every 100
Immediate: Within 1-2 days of receiving drug	Nausea & vomiting (acute and delayed)	CNS toxicity (somnolence, depressive psychosis and confusion)	Anorexia, diarrhea, constipation, encephalopathy which may progress to coma (L), seizure, SIADH, phlebitis, hypokalemia
Prompt: Within 2-3 weeks, prior to next course	Leukopenia, alopecia, immune suppression	Thrombocytopenia, anemia, cardiac toxicities (arrhythmia, asymptomatic ECG changes), microscopic hematuria, metabolic acidosis	↑ liver enzymes, ↑ bilirubin, hemorrhagic cystitis with macroscopic hematuria, dysuria, cystitis and urinary frequency (< 5% with mesna and vigorous hydration) (L), bladder fibrosis
Delayed: Any time later during therapy	Gonadal dysfunction: azoospermia or oligospermia (prolonged or permanent)[1] (L)		Renal failure acute or chronic, renal tubular acidosis, Fanconi-like syndrome gonadal dysfunction, ovarian failure[1] (L), CHF
Late: Any time after completion of treatment	Moderate nephrotoxicity (↓ in glomerular filtration rate, renal tubular threshold for phosphate, and serum bicarbonate)		Secondary malignancy, hypophosphatemic rickets
Unknown Frequency and Timing:	Fetal toxicities and teratogenic effects of ifosfamide have been noted in animals. Ifosfamide is excreted into breast milk.		

[1] *Dependent on dose, age, gender and degree of pubertal development at time of treatment*
(L) Toxicity may also occur later.

Formulation and Stability:
Ifosfamide is available in 1 g and 3 g single dose vials of lyophilized white powder without preservatives and as a 50 mg/mL solution in 20 mL and 60 mL vials.

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

Reconstitute ifosfamide lyophilized powder with sterile water for injection or bacteriostatic water for injection (use 20 mL for the 1 g vial and 60 mL for the 3 g vial) to produce a final concentration of

50 mg/mL. Use sterile water for injection without benzyl alcohol for neonates and infants < 2 years of age or patients with hypersensitivity to benzyl alcohol. Although the reconstituted product is stable for 7 days at room temperature and up to 6 weeks under refrigeration, the manufacturer recommends refrigeration and use within 24 hours to reduce the possibility of microbial contamination. Store unreconstituted vials at room temperature 20°-25°C (68°-77°F). Protect from temperatures above 30°C (86° F). Ifosfamide may liquefy at temperatures > 35°C.

Reconstituted solutions of ifosfamide or ifosfamide solution should be diluted further to concentrations of 0.6 to 20 mg/mL in dextrose or saline containing solutions. Such admixtures, when stored in large volume parenteral glass bottles, Viaflex bags or PAB bags, are physically and chemically stable for 1 week at 30°C (86°F) or 6 weeks at 5°C (41°F). The manufacturer recommends refrigeration and use within 24 hours to reduce the possibility of microbial contamination.

Mesna must always be administered in conjunction with ifosfamide. Adequate hydration is required. Achieve urine specific gravity ≤ 1.010 prior to start of ifosfamide. Refer to the Chemotherapy Administration Guidelines for additional information.

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

6.12 LEUCOVORIN CALCIUM (05/09/11)

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

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

Toxicity:

	Common Happens to 21-100 children out of every 100	**Occasional** Happens to 5-20 children out of every 100	**Rare** Happens to <5 children out of every 100
Immediate: Within 1-2 days of receiving drug			Anaphylaxis, urticaria, seizure

Unknown Frequency and timing:	Fetal toxicities and teratogenic effects of leucovorin in humans are unknown. It is unknown whether the drug is excreted in breast milk.

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

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

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

Injection:
Because of the calcium content of the leucovorin solution, no more than 160 mg of leucovorin should be injected intravenously per minute (16 mL of a 10 mg/mL solution per minute). IV leucovorin and sodium bicarbonate are incompatible.

Oral:
Oral leucovorin should be spaced evenly (e.g., every six hours) throughout the day and may be taken without regard to meals. Doses > 25 mg should be given IV due to the saturation of absorption.

Leucovorin should not be administered < 24 hours after intrathecal injections which contain methotrexate unless there are special circumstances.

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

6.13 MERCAPTOPURINE (12/05/16)

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

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) that catalyzes the conversion of MP to several active nucleotide metabolites including thioinosinic acid, a ribonucleotide that 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. Food intake and 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, 6-thiopurine methyltransferase, is also one of the enzymes involved in the metabolism of MP, those individuals who have an inherited deficiency of the enzyme may be unusually sensitive to the myelosuppressive effects of MP and prone to develop rapid bone marrow suppression following the initiation of treatment. Mercaptopurine is excreted in urine as metabolites and some unchanged drug; about half an oral dose has been recovered in 24 hours. A small proportion is excreted over several weeks.

Toxicity:

Incidence	Toxicities
Common (>20% of patients)	Neutrophil count decreased, white blood cell decreased, anorexia, fatigue.
Occasional (4 - 20% of patients)	Diarrhea, nausea, vomiting, oligospermia, infection, fever, platelet count decreased, anemia, mucositis, stomach pain, ulcerative bowel lesion, skin rash, alanine aminotransferase increased, aspartate aminotransferase increased
Rare (≤3% of patients)	Urticaria, skin hyperpigmentation, alopecia, hyperuricemia, hepatic failure, hepatic necrosis, blood bilirubin increased, pulmonary fibrosis, secondary malignant neoplasm, renal toxicity, uricosuria, pancreatitis
Pregnancy and Lactation	**Pregnancy Category D** Mercaptopurine can cause fetal harm, including an increased incidence of abortion and stillbirth. Advise women to avoid becoming pregnant while receiving mercaptopurine. Mercaptopurine was embryo-lethal and teratogenic in several animal species (rat, mouse, rabbit, and hamster). It is not known whether mercaptopurine is excreted in human milk; breastfeeding should be avoided.

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. In the United States, mercaptopurine is also available as an oral suspension in a concentration of 20 mg/mL (2000 mg/100 mL per bottle). The oral suspension is a pink to brown viscous liquid supplied in amber glass multiple-dose bottles with a child resistant closure. It should be stored at 15°-25°C (59°-77°F) in a dry place.

NOTE: the concentration of the commercially available suspension (20 mg/mL) and the compounded suspension (50 mg/mL) are NOT the same; doses should be prescribed in the milligrams required, not mL.

Guidelines for Administration: See Treatment and Dose Modifications sections of the protocol. Mercaptopurine should be taken consistently at the same time every day.

If allopurinol is also given, the oral dose of mercaptopurine should be reduced by 67-75%. Patients with severe myelosuppression should have their thiopurine S-methyltransferase (TPMT) status and/or their thiopurine metabolite concentrations evaluated, so that the dose of mercaptopurine can

be reduced in patients with a TPMT defect. Patients with the rare homozygous deficient TPMT phenotype may tolerate only 1/10th to 1/20th the average mercaptopurine dose. TPMT testing and thiopurine metabolite measurements are commercially available.

Suspension:
For children unable to swallow the tablets whole, a 50 mg/mL oral suspension can be compounded. The suspension is prepared by crushing 50 mercaptopurine 50 mg tablets in a mortar and adding 8.5 mL sterile water for irrigation. The mixture is triturated to form a smooth paste. Next, 16.5 mL simple syrup (pH=7) are added with continuous mixing and finally cherry syrup (pH=7.1) is added to a total volume of 50 mL. The suspension is stable in amber glass bottles at room temperature (19ºC -23ºC) for up to 5 weeks. The suspension should be shaken well before each use. Procedures for proper handling and disposal of cytotoxic drugs should be used when preparing the suspension. (Aliabadi HM, Romanick M, Desai S et al. Effect of buffer and antioxidant on stability of mercaptopurine suspension. *Am J Heath-Syst Pharm.* 65:441-7, 2008.)

Supplier: The tablets are commercially available from various manufacturers. In the United States, the commercially available oral suspension is available through AnovoRx Distribution, LLC (1-888-470-0904). See package insert for further information. **PLEASE NOTE there is a difference in the concentration of the commercially available (20 mg/mL) and extemporaneously compounded (50 mg/mL) oral suspensions.**

6.14 MESNA - INJECTION (11/15/16)

(sodium 2-mercaptoethane sulfonate, UCB 3983, Mesnex®) NSC #113891

Source and Pharmacology:
Mesna was developed as a prophylactic agent to reduce the risk of hemorrhagic cystitis induced by ifosfamide. Mesna is rapidly oxidized to its major metabolite, mesna disulfide (dimesna). Mesna disulfide remains in the intravascular compartment and is rapidly eliminated by the kidneys. In the kidney, the mesna disulfide is reduced to the free thiol compound, mesna, which reacts chemically with the urotoxic ifosfamide metabolites (acrolein and 4-hydroxy-ifosfamide) resulting in their detoxification. The first step in the detoxification process is the binding of mesna to 4-hydroxy-ifosfamide forming a nonurotoxic 4-sulfoethylthioifosfamide. Mesna also binds to the double bonds of acrolein and to other urotoxic metabolites. In multiple human xenograft or rodent tumor model studies, mesna in combination with ifosfamide (at dose ratios of up to 20-fold as single or multiple courses) failed to demonstrate interference with antitumor efficacy.

After an 800 mg dose the half-lives for mesna and dimesna are 0.36 hours and 1.17 hours, respectively. Approximately 32% and 33% of the administered dose was eliminated in the urine in 24 hours as mesna and dimesna, respectively. The majority of the dose recovered was eliminated within 4 hours.

Toxicity[1]:

	Common Happens to 21-100 children out of every 100	**Occasional** Happens to 5-20 children out of every 100	**Rare** Happens to < 5 children out of every 100
Immediate: Within 1-2 days of receiving drug		Nausea, vomiting, stomach pain, fatigue, headache	Facial flushing, fever, pain in arms, legs, and joints, rash, transient hypotension, tachycardia, dizziness, anxiety, confusion, periorbital swelling, anaphylaxis, coughing

Prompt: Within 2-3 weeks, prior to the next course		Diarrhea	
Unknown Frequency and Timing:	Fetal toxicities and teratogenic effects of mesna have not been noted in animals fed 10 times the recommended human doses. There are however no adequate and well-controlled studies in pregnant women. It is not known if mesna or dimesna is excreted into human milk		

[1]*All currently available products in the U.S. are preserved with benzyl alcohol. Benzyl Alcohol has been associated with death in pre-term infants weighing less than 2500 g and receiving 99-405 mg/kg/day. Benzyl alcohol is normally oxidized rapidly to benzoic acid, conjugated with glycine in the liver, and excreted as hippuric acid. In pre-term infants, however, this metabolic pathway may not be well developed. Onset of toxic illness in these infants occurred between several days and a few weeks of age with a characteristic clinical picture that included metabolic acidosis progressing to respiratory distress and gasping respirations. Many infants also had central-nervous-system dysfunction, including convulsions and intracranial hemorrhage; hypotension leading to cardiovascular collapse was a late finding usually preceding death. [For comparison in the ICE regimen of 3000 mg/m²/day of ifosfamide and a daily mesna dose of 60% of the ifosfamide dose = to 1800 mg/m²/day; a child would be expected to receive 18 mL/m²/day of mesna (concentration of 100 mg/mL and 10.4 mg/mL of benzyl alcohol) 187.2 mg/m²/day of benzyl alcohol or 6.24 mg/kg/day.]*

Formulation and Stability:
Mesna for injection is available as 100 mg/mL in 10 mL multidose vials which contain 0.25 mg/mL edetate disodium and sodium hydroxide for pH adjustment. Mesna Injection multidose vials also contain 10.4 mg/mL of benzyl alcohol as a preservative. Store product at controlled room temperature 15º-25°C (68º-77ºF). Mesna is not light-sensitive, but is oxidized to dimesna when exposed to oxygen. Mesna as benzyl alcohol-preserved vials may be stored and used for 8 days.

Guidelines for Administration: See Treatment, Dose Modifications, and Supportive Care sections of the protocol.

For IV administration, dilute mesna to 20 mg/mL with dextrose or saline containing solutions. Mesna may be mixed with ifosfamide or cyclophosphamide. After dilution for administration, mesna is physically and chemically stable for 24 hours at 25ºC (77ºF). Mesna may cause false positive test for urinary ketones.

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

CANADIAN SITES
Preservative-free Mesna is commercially available in Canada from Baxter Corporation (Urometixan®); supplied as a 100mg/mL solution which contains edentate disodium and sodium hydroxide for pH adjustment, 4mL and 10mL single-use ampoules. It is also available from Baxter and Fresenius Kabi Canada in multi-dose vials containing antibacterial preservatives.

6.15 METHOTREXATE - All Routes (02/29/12)
(MTX, amethopterin, Trexall®) NSC #000740

Source and Pharmacology:
A folate analogue that 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/m^2, 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.

Toxicity:

	Common Happens to 21-100 children out of every 100	**Occasional** Happens to 5-20 children out of every 100	**Rare** Happens to <5 children out of every 100
Immediate: Within 1-2 days of receiving drug	Transaminase elevations	Nausea, vomiting, anorexia	Anaphylaxis, chills, fever, dizziness, malaise, drowsiness, blurred vision, acral erythema, urticaria, pruritus, toxic epidermal necrolysis, Stevens-Johnson Syndrome, tumor lysis syndrome, seizures[1], photosensitivity
Prompt: Within 2-3 weeks, prior to the next course		Myelosuppression, stomatitis, gingivitis, photosensitivity, fatigue	Alopecia, folliculitis, acne, renal toxicity (ATN, increased creatinine/BUN, hematuria), enteritis, GI ulceration and bleeding, acute neurotoxicity[1] (headache, drowsiness, aphasia, paresis, blurred vision, transient blindness, dysarthria, hemiparesis, decreased reflexes) diarrhea, conjunctivitis
Delayed: Any time later during therapy, excluding the above conditions		Learning disability[1] (L)	Pneumonitis, pulmonary fibrosis (L), hepatic fibrosis (L), osteonecrosis (L), leukoencephalopathy[1] (L), pericarditis, pericardial effusions, hyperpigmentation of the nails
Late: Any time after the completion of therapy			Progressive CNS deterioration[1]
Unknown Frequency and Timing:	Methotrexate crosses the placenta. Fetal toxicities and teratogenic effects of methotrexate have been noted in humans. The toxicities include: congenital defects, chromosomal abnormalities, severe newborn myelosuppression, low birth weight, abortion, and fetal death. Methotrexate is excreted into breast milk in low concentrations.		

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

Intrathecal Therapy (Methotrexate Single Agent)
Toxicity:

	Common Happens to 21-100 children out of every 100	**Occasional** Happens to 5-20 children out of every 100	**Rare** Happens to < 5 children out of every 100
Immediate: Within 1-2 days of receiving drug	Nausea, headache	Arachnoditis: (headache, fever, vomiting, meningismus, nuchal rigidity, and pleocytosis)	Anaphylaxis, vomiting, seizures (L), malaise, confusion, back pain, rash, bleeding into subarachnoid or subdural space (risk > with platelet counts < 20,000),
Prompt: Within 2-3 weeks, prior to the next course			Myelosuppression, ataxia, somnolence, cranial nerve palsy, subacute myelopathy (paraparesis/paraplegia), speech disorders, pain in the legs, bladder dysfunction
Delayed: Any time later during therapy, excluding the above condition		Cognitive disturbances (L)[1], learning disability (L)[1]	Leukoencephalopathy[1] (L)
Late: Any time after the completion of treatment			Progressive CNS deterioration[1]

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

Formulation & Stability:
Methotrexate for oral use is available as 2.5 mg, 5 mg, 7.5 mg, 10 mg and 15 mg tablets. 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.

Methotrexate for Injection is available as a lyophilized powder for injection in 1000 mg vials. The powder for injection contains approximately 7 mEq sodium in the 1000 mg vial. Methotrexate for Injection is also available as a 25 mg/mL solution in 2, 4, 8, 10, and 40 mL preservative free vials and 2 and 10 mL vials with preservative. The 2, 4, 8, 10, and 40 mL solutions contain approximately 0.43, 0.86, 1.72, 2.15, and 8.6 mEq sodium per vial, respectively. The preserved vials contain 0.9% benzyl alcohol as a preservative.

Sterile methotrexate powder or solution is stable at 20º-25ºC (68º-77ºF); excursions permitted to 15º-30ºC (59º- 86 Fº). Protect from light

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

Oral administration: Food or milk delays absorption and reduces peak concentration. Methotrexate for oral use should preferentially be given on an empty stomach, 1 hour before or 2 hours after food or milk and at the same time each day. Methotrexate injection diluted in water

can be used for oral administration (Marshall PS, Gertner E. Oral administration of an easily prepared solution of injectable methotrexate diluted in water: a comparison of serum concentrations vs methotrexate tablets and clinical utility. *J Rheumatol* 23:455-8, 1996).

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

The 25 mg/mL solution may be given directly for IM administration or further diluted in Saline or Dextrose containing solutions for IV use. **Do not use the preserved solution for high dose methotrexate administration due to risk of benzyl alcohol toxicity.** Methotrexate dilutions are chemically stable for at least 7 days at room temperature but contain no preservative and should be used within 24 hours. Diluted solutions especially those containing bicarbonate exposed to direct sunlight for periods exceeding 4 hours should be protected from light.

High dose methotrexate requires alkalinization of the urine, adequate hydration and leucovorin rescue. Avoid probenecid, penicillins, cephalosporins, aspirin, proton pump inhibitors, and NSAIDS as renal excretion of MTX is inhibited by these agents.

For Intrathecal use: Use **preservative free** 25 mg/mL solution.

For intrathecal administration, dilute with 5-10 mL preservative free NS, lactated Ringer's, or Elliot's B solution as per institutional standard of practice. The volume of CSF removed should be equal to at least half the volume delivered.

Note: When IT therapy and ID MTX are scheduled for the same day, deliver the IT therapy within 6 hours of the beginning of the IV MTX infusion (hour -6 to +6, with 0 being the start of the MTX bolus).

For IT administration, use the preservative free formulation. The volume to be given IT should be in the range of 5-10 mL. The volume of CSF removed should be equal to at least half the volume delivered (see drug monograph).

Note: Larger volumes approximating at least 10% of the CSF volume, isovolumetric delivery, with the patient remaining lying down after the procedure may facilitate drug distribution. These procedures have not been validated in clinical trials. They are allowed but not mandated for patients on COG studies.

Patient Age (years)	Methotrexate dose	Recommended volume	10% CSF volume	CSF Volume *
1–1.99	8 mg	5–10 mL	5 mL	50 ± 10 mL (babies)
2–2.99	10 mg	5-10 mL	8 mL	80 ± 20 mL (younger children)
3–8.99	12 mg	5-10 mL	10 mL	100 ± 20 mL (older children)
9 or greater	15 mg	5-10 mL	13 mL	130 ± 30 mL (adults)

*Rieselbach, R.E. et.al. Subarachnoid distribution of drugs after lumbar injection; N Engl J Med. 1962 Dec 20; 267:1273-8

Of Note: Larger volumes approximating at least 10% of the CSF volume, isovolumetric delivery, with the patient remaining prone after the procedure may facilitate drug distribution. These procedures have not been validated in clinical trials. They are allowed but not mandated for patients on COG studies.

Diluted methotrexate for intrathecal administration is stable for 24 hours at 25ºC but contains no preservative and should be administered as soon as possible after preparation.

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

6.16 PEGASPARGASE (11/15/16)

(PEG-asparaginase, PEGLA, PEG-L-asparaginase, polyethylene glycol-L-asparaginase, Oncaspar®)
NSC #624239

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 $t_{½}$ in adult patients is 5.73 days. The enzyme could not be detected in the urine. The half-life is independent of the dose administered, disease status, renal or hepatic function, age, or gender. In a study of newly diagnosed pediatric patients with ALL who received either a single intramuscular injection of pegaspargase (2500 IU/m^2), *E. coli* L-asparaginase (25000 IU/m^2), or *Erwinia* (25000 IU/m^2), the plasma half-lives for the three forms of L-asparaginase were: 5.73 ± 3.24 days, 1.24 ± 0.17 days, and 0.65 ± 0.13 days respectively. The plasma half-life of pegaspargase is shortened in patients who are previously hypersensitive to native L-asparaginase as compared to non-hypersensitive patients. L-asparaginase is cleared by the reticuloendothelial system and very little is excreted in the urine or bile. Cerebrospinal fluid levels are < 1% of plasma levels.

Toxicity:

	Common Happens to 21-100 children out of every 100	**Occasional** Happens to 5-20 children out of every 100	**Rare** Happens to < 5 children out of every 100
Immediate: Within 1-2 days of receiving drug	Allergic reactions (total likelihood of local, and or systemic reaction especially if previous hypersensitivity reaction to native asparaginase), pain at injection site, weakness, fatigue, diarrhea	Allergic reactions (total likelihood of local, and or systemic reaction if **no** previous hypersensitivity reaction to native asparaginase), rash	Anaphylaxis, hyper/hypotension, tachycardia, periorbital edema, chills, fever, dizziness, dyspnea, bronchospasm, lip edema, arthralgia, myalgia, urticaria, mild nausea/vomiting, abdominal pain, flatulence, somnolence, lethargy, headache, seizures (L), hyperuricemia

Prompt: Within 2-3 weeks, prior to the next course	Hyperammonemia (L), coagulation abnormalities with prolonged PTT, PT and bleeding times (secondary to decreased synthesis of fibrinogen, AT-III & other clotting factors) (L)	Hyperglycemia, abnormal liver function tests, pancreatitis (L), increased serum lipase/amylase	Hemorrhage (L), DIC, thrombosis, anorexia, weight loss, CNS ischemic attacks, edema, azotemia and decreased renal function, mild leukopenia, granulocytopenia, thrombocytopenia, pancytopenia, hemolytic anemia, infections (sepsis with/without septic shock, subacute bacterial endocarditis [SBE], URI), CNS changes including irritability, depression, confusion, EEG changes, hallucinations, coma and stupor, paresthesias, hypertriglyceridemia, hyperlipidemia, Parkinson-like syndrome with tremor and increase in muscular tone, hyperbilirubinemia, chest pain
Delayed: Any time later during therapy			Renal failure, urinary frequency, hemorrhagic cystitis, elevated creatinine and BUN, fatty liver deposits, hepatomegaly, liver failure
Unknown Frequency and Timing:	Animal reproduction studies have not been conducted with pegaspargase. It is not known whether pegaspargase can cause fetal harm when administered to a pregnant woman or can affect reproduction capacity. However, fetal toxicities and teratogenic effects of asparaginase have been noted in animals. It is unknown whether the drug is excreted in breast milk.		

(L)Toxicity may also occur later.

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

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

For IM administration: the volume at a single injection site should be limited to 2 mL. If the volume to be administered is greater than 2 mL, multiple injection sites should be used.

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 of if precipitate is present.

Have available during and after the infusion: antihistamine, epinephrine, oxygen, and IV corticosteroids. Observe patient for ONE hour after administration for signs of hypersensitivity reactions.

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

CANADIAN SITES
Pegaspargase is not commercially available in Canada. Sites may purchase and import the USA commercial supply directly from Baxalta under the authority of the protocol's No Objection Letter (NOL). The Canadian Senior Medical Officer (SMO)'s office is responsible for coordinating the "Fax Back" approval with Health Canada's Biologics and Genetic Therapies Directorate for all lots for use in Canada on behalf of all Canadian sites. A list of approved lot numbers is posted on the C17 website (www.c17.ca) under the protocol titled Oncaspar. If an unapproved lot is received from Baxalta, quarantine the lot and contact the COG Canada Regulatory Affairs Office at 1-780-492-7064. Note: Pegaspargase may have orders placed and Drug Accountability Logs maintained on a multiple protocol basis (Multiple Protocol—Imported Biologic) as long as each protocol has a NOL. Drug Accountability Logs (DAL) must record Lot #'s and expiry dates of shipments received and doses dispensed. Sites may use their own DAL as long as it complies with all elements of ICH GCP and Division 5 of the Food and Drugs Act. Sites may import and manage a single clinical trial supply for multiple protocols as long as each protocol has an NOL and the protocol the patient is registered on is recorded on the DAL.

6.17 THIOGUANINE (12/05/16)

(6-thioguanine, tioguanine, 2-amino-1,7-dihydro-6H-purine-6-thione, WR-1141, Tabloid®, Lanvis®) NSC #752

Source and Pharmacology:
Thioguanine is a purine analogue of the nucleic acid guanine with the substitution of a thiol group in place of the hydroxyl group on guanine. The main intracellular pathway for 6-TG activation is catalyzed by the enzyme hypoxanthine-guanine phosphoribosyl transferase (HGPRT), which catalyzes the conversion of 6-TG to the active nucleotide, 6-thioguanylic acid. The monophosphate nucleotide form of 6-TG inhibits *de novo* purine synthesis and purine interconversion reactions, whereas the nucleotide triphosphate metabolite is incorporated directly into nucleic acids. Incorporation of fraudulent nucleotides into DNA interferes with DNA replication and results in the formation of DNA strand breaks. The net consequence of its action is a sequential blockade of the synthesis and utilization of the purine nucleotides. The relative contribution of each of these actions to the mechanism of cytotoxicity of 6-TG is unclear. The absorption of an oral dose of 6-TG is incomplete and variable, averaging approximately 30% of the administered dose (range: 14% to 46%).

6-TG undergoes deamination by the enzyme guanine deaminase resulting in 6-thioxanthene, which is then oxidized by xanthine oxidase to 6-thiouric acid. In contrast to mercaptopurine, 6-TG is not a direct substrate for xanthine oxidase. Because the inhibition of xanthine oxidase results in the accumulation of 6-thioxanthene, an inactive metabolite, adjustments in 6-TG dosage are not required for patients receiving allopurinol. Since TPMT, 6-thiopurine methyltransferase, is one of the enzymes involved in the deactivation of 6-TG, those individuals who have an inherited deficiency of the enzyme may be unusually sensitive to the myelosuppressive effects of 6-TG and prone to developing rapid bone marrow suppression following the initiation of treatment.

Peak levels occur 2 to 4 hours after oral administration with a median half-life is about 90 minutes (range: 25-240 minutes). Very little unchanged drug is excreted renally.

Toxicity:

	Common Happens to 21-100 children out of every 100	**Occasional** Happens to 5-20 children out of every 100	**Rare** Happens to < 5 children out of every 100
Immediate: Within 1-2 days of receiving drug		Anorexia, nausea, vomiting, diarrhea, malaise	Urticaria, rash, hyperuricemia

Prompt: Within 2-3 weeks, prior to next course	Myelosuppression		Toxic hepatitis (L), increased SGOT (AST)/SGPT (ALT), ataxia, mucositis
Delayed: Anytime later during therapy			Hepatic fibrosis(L), sinusoidal obstruction syndrome (SOS, formerly VOD) (L), hyperbilirubinemia
Unknown Frequency and Timing:	Fetal toxicities and teratogenic effects of thioguanine have been noted in animals. It is unknown whether the drug is excreted in breast milk.		

(L) Toxicity may also occur later.

Formulation and Stability:
Each greenish-yellow, scored tablet contains 40 mg thioguanine. Store at 15°-25°C (59°-77°F) in a dry place.
For patients unable to swallow tablets, a 20 mg/mL oral suspension may be compounded. Crush fifteen (n=15) 40 mg tablets in a mortar and reduce to a fine powder. Add 10 mL methylcellulose 1% in incremental proportions and mix to a uniform paste. Transfer to a graduated cylinder, rinse mortar with simple syrup, and add quantity of simple syrup sufficient to make 30 mL. Dispense in an amber glass bottle and label "shake well" and "refrigerate". If methylcellulose is not available, substitute 15 mL of Ora-Plus in place of the methylcellulose and qs with Ora-Sweet (in place of simple syrup) to a final volume of 30 mL. Both preparations are stable for 63 days at 19° C – 23° C. (Aliabadi HM, Romanick M, Somayah V, et al. Stability of compounded thioguanine oral suspensions. *Am J Health Syst Pharm* 2011;68:1278. Dressman JB, Poust RI. Stability of Allopurinol and Five Antineoplastics in Suspension. *Am J Hosp Pharm* 1983;40(4):616-8.)

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

Thioguanine should be taken consistently at the same time every day.

Substantial dosage reductions may be required in patients with an inherited deficiency of the enzyme thiopurine methyltransferase (TPMT) due to accumulation of active thioguanine metabolites resulting in a higher incidence of myelosuppression.

Supplier: Commercially available. See package insert for more detailed information.

6.18 VINCRISTINE SULFATE (08/16/12)
(Oncovin®, VCR, LCR) NSC #67574

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

Toxicity:

	Common Happens to 21-100 children out of every 100	**Occasional** Happens to 5-20 children out of every 100	**Rare** Happens to < 5 children out of every 100
Immediate: Within 1-2 days of receiving drug		Jaw pain, headache	Extravasation (rare) but if occurs = local ulceration, shortness of breath, and bronchospasm
Prompt: Within 2-3 weeks, prior to the next course	Alopecia, constipation	Weakness, abdominal pain, mild brief myelosuppression (leukopenia, thrombocytopenia, anemia)	Paralytic ileus, ptosis, diplopia, night blindness, hoarseness, vocal cord paralysis, SIADH, seizure, defective sweating
Delayed: Any time later during therapy	Loss of deep tendon reflexes	Peripheral paresthesias including numbness, tingling and pain; clumsiness; wrist drop, foot drop, abnormal gait	Difficulty walking or inability to walk; sinusoidal obstruction syndrome (SOS, formerly VOD) (in combination); blindness, optic atrophy; urinary tract disorders (including bladder atony, dysuria, polyuria, nocturia, and urinary retention); autonomic neuropathy with postural hypotension; 8th cranial nerve damage with dizziness, nystagmus, vertigo and hearing loss
Unknown Frequency and Timing:	Fetal toxicities and teratogenic effects of vincristine (either alone or in combination with other antineoplastic agents) have been noted in humans. The toxicities include: chromosome abnormalities, malformation, pancytopenia, and low birth weight. It is unknown whether the drug is excreted in breast milk.		

Formulation and Stability:
Vincristine is supplied in 1 mL and 2 mL vials in which each mL contains vincristine sulfate 1 mg (1.08 µmol), mannitol 100 mg, SWFI; acetic acid and sodium acetate are added for pH control. The pH of vincristine sulfate injection, *USP* ranges from 3.5 to 5.5. This product is a sterile, preservative free solution. Store refrigerated at 2˚-8˚C or 36˚-46˚F. Protect from light and retain in carton until time of use.

Do not mix with any IV solutions other than those containing dextrose or saline.

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

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

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

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

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

Note: Medication errors have occurred due to confusion between vinCRIStine and vinBLAStine. VinCRIStine is available in a liposomal formulation (vinCRIStine sulfate liposomal injection, VSLI, Marqibo®). The conventional and liposomal formulations are NOT interchangeable; use of the liposomal formulation is not permitted in this trial.

7.0 EVALUATIONS/MATERIAL AND DATA TO BE ACCESSIONED

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 (and up to 1 week for surgery) for valid clinical, patient and family logistical, or facility, procedure and/or anesthesia scheduling issues are acceptable per COG administrative Policy 5.14 (except where explicitly prohibited within the protocol).

7.1 Required & Optional Clinical, Laboratory and Disease Evaluations: Diagnosis, Induction, Consolidation Arm A and Arm B: T-ALL ONLY

STUDIES**	Diagnosis	Induction	Consolidation
Required Observations: Arms A and B T-ALL ONLY			
Hx/PE with VS/Wt (BSA)	X	Weekly	Start of Course
CBC/diff/plts	X	Weekly	Weekly
CSF cell count & cytospin	X	With each IT	With each IT
Bilirubin (total and direct), ALT, AST creatinine	Baseline	Days 1 and 29	Start of Course
Performance Status	Baseline		Prior to Day 1 therapy
Pregnancy Test[1]	Baseline		
Bone Marrow MRD and cytomorphology Assessment	X[$]	End of Induction[5]	End of Consolidation[2,5]
Chest x-ray	Baseline		
Required Observations: Patients receiving bortezomib (Arm B) T-ALL ONLY			
Pulse Oximetry (O2 saturation) and Chest x-ray		X[3]	
Electrolytes including PO_4		Start of Course[6]	
Optional Observations: Arms A and B T-ALL ONLY (All optional studies require patient consent)			
Bone Marrow (BM)/Peripheral Blood (PB) for bortezomib response (ETP-ALL) study (**open to Arm A and B**)[4]	BM: Pre-treatment	PB:Day 1 Hour 0, 6, 24 BM &PB:End of Induction	
Cell Banking[7]	Baseline[7]	End of Induction[7]	
Recommended Observations: Arms A and B T-ALL ONLY			
TPMT and NUDT15 genotype (if available)		During Induction	

[1] Female patients of childbearing potential require a negative pregnancy test prior to starting treatment; sexually active patients must use an acceptable method of birth control

[2] End of consolidation bone marrow is not performed on SR T-ALL patients. It is REQUIRED for T-ALL patients with end Induction BM MRD ≥ 0.01% ONLY

[3] Patients who develop respiratory signs or symptoms as defined in Section 5.2.2 should have pulse oximetry (02 saturation) and Chest x-ray documented within 12 hours prior to each dose of bortezomib.

[4] See Section 15.1 for details. Peripheral blood can be substituted if >80% blasts. Please send AALL1231 specimen transmittal form and institutional immunophenotype report with the sample submission to the laboratory

[5] Patients who are not in a morphologic remission at end of induction or consolidation remain on protocol therapy.

[6] Bortezomib has been associated with hyponatremia, hypophosphatemia, and hypokalemia in some studies. Serum phosphorus should be checked at the beginning of Induction. See Section 5.2.4 for optional recommendations on correction and monitoring.

[7] Done as part of AALL08B1 or APEC14B1. See AALL08B1 or APEC14B1 Manual of Procedures for details. Also note that specimens are requested at time of relapse as part of AALL08B1 or APEC14B1.

7.2 Required & Optional Clinical, Laboratory and Disease Evaluations: Diagnosis, Induction, Consolidation: Arm A and Arm B: T-LLy ONLY

STUDIES**	Diagnosis	Induction	Consolidation
Required Observations: Arms A and B T-LLy ONLY			
Hx/PE with VS/Wt (BSA)	Baseline	Weekly	Start of Course
CBC/diff/plts	Baseline	Weekly	Weekly
CSF cell count & cytospin	X	With each IT	With each IT
Bilirubin (total and direct), ALT, AST, creatinine	Baseline	Days 1 and 29	Start of Course
Performance Status	Baseline		Prior to Day 1 therapy
Pregnancy Test[1]	Baseline		
Disease Evaluation			
Chest & neck CT/Chest x-ray[2]	Baseline[2]	End of Induction[2]	End of Course[2]
Abdomen/Pelvis CT or MRI [8]	Baseline	End of Induction[8]	End of Course[8]
Bone scan[5]	Baseline	End of Induction[5]	End of Course[5]
Diagnostic Biopsy/Cytology[4]	Baseline		End of Course if PD or NR
Bone Marrow MRD Assessment[7]	X		
Bilateral bone marrow aspirate and biopsy cytomorphology [4]	X[7]	End of Induction only if positive at diagnosis	End of Course only if + at end of Induction
Required Observations: Patients receiving bortezomib (Arm B) T-LLy ONLY			
Pulse Oximetry (O2 saturation),chest x-ray[3]		X[3]	
Electrolytes including PO_4		Start of Course[6]	
Optional Observations: Arms A and B T-LLy ONLY (All optional studies require patient consent)			
Cell Banking[7]	Baseline[7]		
Bone Marrow MRD Assessment		End of Induction[9]	
Recommended Observations: Arms A and B T-LLy ONLY			
PET Scan (Recommended)	Diagnosis	End of Induction#	PET Scan (If + at End Induction)
TPMT and NUDT15 genotype (if available)		During Induction	

[1]Female patients of childbearing potential require a negative pregnancy test prior to starting treatment; sexually active patients must use an acceptable method of birth control

[2] Obtain chest CT for all T-LLy patients at Baseline and at end-Induction. The baseline chest CT may be delayed until the patient is stable. If patient has CR at end-Induction, no subsequent chest CT is required but a chest x-ray will be performed at end-Consolidation and end of therapy. If patient does not have CR at end-Induction, a chest CT will be performed at end-Consolidation. Patients who have PD at end of consolidation are off protocol therapy.

[3] Patients who develop respiratory signs or symptoms as defined in Section 5.2.2 should have pulse oximetry (02 saturation) and Chest x-ray documented within 12 hours prior to each dose of bortezomib.

[4] Determined by morphology on bilateral bone marrow aspirates/biopsies (not MRD or flow). Bilateral bone marrow aspirates and biopsies are strongly preferred but not required for study eligibility. A unilateral bone marrow aspirate for morphology and for central MRD bone marrow assessment are required to be eligible.

[5] Bone scan only if patient has bone symptoms; follow-up exams only if baseline demonstrates disease. A PET scan can be used as a substitute for bone scan; however, a bone scan is preferred in patients with bone symptoms.

[6] Bortezomib has been associated with hyponatremia, hypophosphatemia, and hypokalemia in some studies. Phosphorus should be checked at the beginning of Induction. See Section 5.2.3 for optional recommendations on correction and monitoring.

[7] If enrolled on APEC14B1, submit as a part of APEC14B1. If not enrolled on APEC14B1, submit as part of this protocol (see Section 14.2 for details).

[8] Many patients will have no disease below the diaphragm; if the abdominal and pelvic CTs at baseline are negative, no repeat scans are required.

[9] See Section 14.2 for details. # If positive at diagnosis or a residual mass present

7.3 Required Clinical, Laboratory and Disease Evaluations Post-Consolidation Therapy: Arm A-SR and Arm B-SR (T-ALL and T-LLy)

STUDIES**	IM (CMTX)	Delayed Intensification	Maintenance
Required Observations: Arms A-SR and B-SR T-ALL and T-LLy			
Hx/PE with VS/Wt (BSA)	Start of Course	Start of Course	Every 4 weeks
CBC/diff/plts	Prior to each MTX dose	Weekly	Every 4 weeks
CSF cell count & cytospin	With each IT	With each IT	With each IT
Bilirubin, ALT, creatinine	Prior to each MTX dose	Day 1, 29	Prior to each 12 wk cycle
Thiopurine metabolites			As clinically Indicated [1,4]
Required Observations: Patients receiving bortezomib (Arm B-SR) ONLY (T-ALL and T-LLy)			
Pulse Oximetry (O2 saturation) and Chest x-ray		X[3]	
Electrolytes including PO_4		Start of Course[6]	
Required Observations: T-LLy ONLY			
Chest CT/Chest x-ray[2]			Completion of Therapy[2]
Abdomen/Pelvis CT or MRI			Completion of Therapy[7]
Bone scan[5]			Completion of Therapy
Diagnostic Biopsy/Cytology[8]			At relapse[8]
PET scan[#]			Post-treatment[#]
Optional Observations: T-ALL and T-LLy			
Optional Banking/Biology[8]			At relapse[8]

[1] For patients in whom TPMT and/or NUDT15 genotyping was not previously performed (see Section 5.10).

[2] If patient has CR at end-Induction, no subsequent chest CT is required but a chest x-ray will be performed at end-Consolidation and end of therapy. If patient has CR at end-Consolidation, a chest x-ray will be performed at end of therapy. If patient does not have CR at end-Consolidation, a chest CT will be performed at end of therapy. For patients who had PET-CT at diagnosis, a PET-CT can be used instead of standard CT to follow disease response.

Note: Patients who have NR and have not achieved at least a PR at end-Consolidation are off protocol therapy.

[3] Patients who develop respiratory signs or symptoms as defined in Section 5.2.2 should have pulse oximetry (02 saturation) and Chest x-ray documented within 12 hours prior to each dose of bortezomib.

[4] Recommended only for HR-ALL patients during any Maintenance cycle in which 6MP/MTX has been held and restarted or in case of persistent ANC elevation and concern for non-compliance (see Section 5.10).

[5] Bone scan only if patient has bone symptoms; follow-up exams only if baseline demonstrates disease. A PET scan can be used as a substitute for bone scan; however, a bone scan is preferred in patients with bone symptoms.

[6] Bortezomib has been associated with hyponatremia, hypophosphatemia, and hypokalemia in some studies. Serum phosphorus should be checked at the beginning of Delayed Intensification. See Section 5.2.3 for optional recommendations on correction and monitoring.

[7] Many patients will have no disease below the diaphragm; if the abdominal and pelvic CTs at Baseline are negative, no repeat scans are required.

[8] **T-ALL:** Done as part of AALL08B1 or APEC14B1

[8] **T-LLy**: If enrolled on APEC14B1, submit as a part of APEC14B1. If not enrolled on APEC14B1, submit as part of this protocol (see Section 13.6 for details)

#Patients with post treatment residual masses only

7.4 Required Clinical, Laboratory and Disease Evaluations Post-Consolidation Therapy: Arm A-IR and Arm B-IR (T-ALL and T-LLy)

STUDIES**	IM#1 (HD MTX)	Delayed Intensification	IM#2 (CMTX)	Maintenance
Required Observations: Arms A-IR and B-IR (T-ALL and T-LLy)				
Hx/PE with VS/Wt (BSA)	Start of Course	Start of Course	Start of Course	Every 4 weeks
CBC/diff/plts	Prior to each MTX dose	Weekly	Prior to each MTX dose	Every 4 weeks
CSF cell count & cytospin	With each IT	With each IT	With each IT	With each IT
Bilirubin, ALT, creatinine	Prior to each MTX dose	Day 1, 29	Prior to each MTX dose	Prior to each 12 wk cycle
Thiopurine metabolites				As clinically Indicated [1,2]
Required Observations: Patients receiving bortezomib (Arm B-IR) ONLY (T-ALL and T-LLy)				
Pulse Oximetry (O2 saturation) and Chest x-ray		X[8]		
Electrolytes including PO_4		Start of Course[6]		
Required Observations: T-LLy ONLY				
Chest CT/Chest x-ray[3]				Completion of Therapy[3]
Abdomen/Pelvis CT or MRI				Completion of Therapy[4]
Bone scan[5]				Completion of Therapy
Diagnostic Biopsy/Cytology[7]				At relapse[7]
PET scan[#]				Post-treatment[#]
Optional Observations: T-ALL and T-LLy				
Optional Banking/Biology[7]				At relapse[7]

[1]For patients in whom TPMT and/or NUDT15 genotyping was not previously performed (see Section 5.10).

[2]Recommended only for HR-ALL patients during any Maintenance cycle in which 6MP/MTX has been held and restarted or in case of persistent ANC elevation and concern for non-compliance (Section 5.10).

[3]Obtain chest CT for all patients at Baseline and at end-Induction. The baseline chest CT may be delayed until the patient is stable. If patient has CR at end-Induction, no subsequent chest CT is required but a chest x-ray will be performed at end-Consolidation and end of therapy. If patient does not have CR at end-Induction, a chest CT will be performed at end-Consolidation. If patient has CR at end-Consolidation, a chest x-ray will be performed at end of therapy. If patient does not have CR at end-Consolidation, a chest CT will be performed at end of therapy. For patients who had PET-CT at diagnosis, a PET-CT can be used instead of standard CT to follow disease response.

[4] Many patients will have no disease below the diaphragm; if the abdominal and pelvic CTs at Baseline are negative, no repeat scans are required.

[5] Bone scan only if patient has bone symptoms; follow-up exams only if baseline demonstrates disease. . A PET scan can be used as substitute for bone scan; however, a bone scan is preferred in patients with bone symptoms.

[6] Bortezomib has been associated with hyponatremia, hypophosphatemia, and hypokalemia in some studies. Serum phosphorus should be checked at the beginning of Delayed Intensification. See Section 5.2.3 for optional recommendations on correction and monitoring.

[7] **T-ALL:** Done as part of AALL08B1 or APEC14B1

[7] **T-LLy**: If enrolled on APEC14B1, submit as a part of APEC14B1. If not enrolled on APEC14B1, submit as part of this protocol (see Section 13.6 for details)

[8] Patients who develop respiratory signs or symptoms as defined in Section 5.2.2 should have pulse oximetry (02 saturation) and Chest x-ray documented within 12 hours prior to each dose of bortezomib.

Patients with post treatment residual masses only

7.5 Required & Optional Clinical, Laboratory and Disease Evaluations for Arm A-VHR and Arm B-VHR (T-ALL and T-LLy)

STUDIES**	3 HR BFM Blocks	Delayed Intensification	IM (CMTX)	Maintenance
Required Observations: Arms A-VHR and B-VHR				
Hx/PE with VS/Wt (BSA)	Start of each Block	Start of Course	Start of Course	Every 4 weeks
CBC/diff/plts	Start of Course & every 2 days after completion of chemotherapy	Weekly	Prior to each MTX dose	Every 4 weeks
CSF cell count & cytospin	With each IT	With each IT	With each IT	With each IT
Bilirubin, ALT, creatinine	Start of Course	Day 1, 29	Prior to each MTX dose	Prior to each 12 wk cycle
T-ALL only: Bone Marrow MRD & cytomorphology Assessment	End of 3 HR BFM blocks[8,9]			
Thiopurine metabolites				As clinically Indicated [1,2]
Required Observations: Patients receiving bortezomib (Arm B-VHR T-ALL and T-LLy) ONLY				
Pulse Oximetry (O2 saturation) and CXR[3]		X[10]		
Electrolytes including PO_4		Start of Course[6]		
Required Observations: T-LLy ONLY				
Chest CT[3]	End of 3 HR BFM blocks			Completion of Therapy[3]
Abdomen/Pelvis CT or MRI	End of 3 HR BFM blocks			Completion of Therapy[4]
Bone scan[5]	End of 3 HR BFM blocks			Completion of Therapy
Diagnostic Biopsy/Cytology (bilateral Bone Marrow aspirate and biopsy) [8,9]	End of 3 HR BFM blocks if PR or NR			At relapse[8]
PET scan[#]				Post-treatment[#]
Optional Observations: T-ALL and T-LLy				
Optional Banking/Biology [8]				At relapse [8]

[1]For patients in whom TPMT and/or NUDT15 genotyping was not previously performed (see Section 5.10).
[2]Recommended only for HR-ALL patients during any Maintenance cycle in which 6MP/MTX has been held and restarted or in case of persistent ANC elevation and concern for non-compliance (see Section 5.10).
[3]For patients who had PET-CT at diagnosis, a PET-CT can be used instead of standard CT to follow disease response.
[4]Reimage if positive at end of consolidation.
[5] Many patients will have no disease below the diaphragm; if the abdominal and pelvic CTs at Baseline are negative, no repeat scans are required. A PET scan can be used as substitute for bone scan; however, a bone scan is preferred in patients with bone symptoms.
[6] Bone scan only if patient has bone symptoms; follow-up exams only if baseline demonstrates disease.
[7] Bortezomib has been associated with hyponatremia, hypophosphatemia, and hypokalemia in some studies. Serum phosphorus should be checked at the beginning of Delayed Intensification. See Section 5.2.3 for optional recommendations on correction and monitoring.
[8] **T-ALL:** Done as part of AALL08B1 or APEC14B1
T-LLy: If enrolled on APEC14B1, submit as a part of APEC14B1. If not enrolled on APEC14B1, submit as part of this protocol (see Section 13.6 for details)
[9] T-ALL Only. See Section 14.1 for details
[10] Patients who have detectable MRD and/or are not in morphologic remission at the end of 3 HR BFM blocks are removed from protocol therapy
[11] Patients who develop respiratory signs or symptoms as defined in Section 5.2.2 should have pulse oximetry (02 saturation) and Chest x-ray documented within 12 hours prior to each dose of bortezomib.
#Patients with post treatment residual masses only

7.6 **Studies Suggested to be Obtained After Stopping Therapy (T-ALL Patients)**

Note: See COG Late Effects Guidelines for recommended post treatment follow-up: http://www.survivorshipguidelines.org

1st year	PE, CBC/diff/platelets q 4 weeks, CXR, BMA, CSF, as clinically indicated*
2nd year	PE, CBC/diff/ platelets q 2 months CXR, as clinically indicated*
3rd year	PE, CBC/diff/ platelets q 3 months CXR, as clinically indicated*
4th year	PE, CBC/diff/ platelets q 6 months CXR, as clinically indicated*
5th year	PE, CBC/diff/ platelets q 6-12 months

* Obtain at any point after the end of therapy when it is clinically indicated.

7.7 **Studies Suggested to be Obtained After Stopping Therapy (T-LLy Patients)**

See COG Late Effects Guidelines for recommended post treatment follow-up: http://www.survivorshipguidelines.org

1st year	PE, CBC/diff/platelets q 3 months CT of primary site, BMA, CSF, as clinically indicated*
2nd year	PE, CBC/diff/ platelets q 3 months CT of primary site, as clinically indicated*
3rd year	PE, CBC/diff/ platelets q 4 months CT of primary site, as clinically indicated*
4th year	PE, CBC/diff/ platelets q 6 months CT of primary site, as clinically indicated*
5th year	PE, CBC/diff/ platelets q 12 months

* Obtain at any point after the end of therapy when it is clinically indicated.

7.8 **At Relapse**

T-ALL patients who relapse should have samples of bone marrow sent to the Molecular Reference Laboratory for cell banking as part of AALL08B1 or Project:EveryChild (APEC14B1, if open for ALL relapse specimens).

T-LLy patients who enrolled on APEC14B1: T-LLy patients who relapse should have samples of bone marrow sent to the Molecular Reference Laboratory for cell banking as part of Project:EveryChild (APEC14B1).

T-LLy patients who did not enroll on APEC14B1: samples should be sent as part of this protocol (See Section 13.6)

8.0 CRITERIA FOR REMOVAL FROM PROTOCOL THERAPY AND OFF STUDY CRITERIA

8.1 Criteria for Removal from Protocol Therapy

a) Relapse/ progressive disease
b) VHR T-ALL who have an M2/M3 marrow and/or detectable MRD at the end of the 3 HR Intensification Blocks
c) VHR T-LLy with biopsy-proven persistent disease and/or morphologically positive bone marrow at end of 3 HR Intensification Blocks
d) Identified to have Ph+ T-ALL*
e) Identified to have Ph+ T-LLy**
f) Refusal of further protocol therapy by patient/parent/guardian.
g) Completion of planned therapy
h) Physician determines it is in patient's best interest
i) Development of a second malignancy
j) Inevaluable
k) Adverse events requiring removal from protocol therapy

[#]As assessed by CT with persistent active disease by biopsy or morphologically positive bone marrow at end of 3 HR Intensification Blocks

*Ph+ T-ALL patients are not eligible for post-induction therapy on AALL1231 and should be removed from protocol therapy prior to Day 15 of Induction therapy.
**Ph+ T-LLy patients are not eligible for post-induction therapy on AALL1231 and should be removed from protocol therapy prior to or at the end of Induction.

Patients who are off protocol therapy are to be followed until they meet the criteria for Off Study (see below). Follow-up data will be required unless consent was withdrawn.

8.2 Off Study Criteria

a) Death.
b) Lost to follow-up.
c) Patient enrollment onto another COG study with tumor therapeutic intent (e.g., at recurrence) with the exception of COG AALL1421, a Phase 2 study of IV pegcrisantaspase, a pegylated Erwinia asparaginase, as a replacement for pegaspargase in patients with pegaspargase hypersensitivity.
d) Withdrawal of consent for any further data submission.
e) Tenth anniversary of the date the patient was enrolled on this study.

9.0 STATISTICAL CONSIDERATIONS

9.1 Statistical Design and Endpoints

This is a randomized Phase III trial, with patients randomized 1:1 to backbone chemotherapy +/- bortezomib. Patients will be stratified at enrollment as T-ALL or T-LLy and will be randomized within each stratum.

Primary Endpoint: Comparison of EFS between modified ABFM backbone +/- bortezomib in all randomized patients.

Secondary Endpoints:

(a) To compare EFS and cumulative incidence rates for isolated CNS, isolated bone marrow, and combined bone marrow relapse between IR patients on the non-bortezomib containing arm of this study (no CRT) with similar patients on AALL0434 (+ CRT)
(b) To assess toxicities associated with administering modified standard therapy for T-ALL and T-LLy, which includes dexamethasone and additional doses of pegaspargase.

(c) To compare EFS between VHR T-ALL patients treated with HR BFM intensification blocks who become MRD negative and those who remain MRD positive at the end of HR Block 3. Comparison of EFS between VHR T-LLy patients treated with HR BFM intensification blocks who have PR or CR with those who do not respond (NR).

9.2 Patient Accrual and Expected Duration of Trial

Based on accrual rates observed on AALL0434, this study is projected to accrue around 250/year T-ALL and 75/year T-LLy patients. This study is expected to accrue up to a maximum of 1400 patients over a 4.4 year period with a minimum follow up of 3 years, adjusting for ineligible and inevaluable patients, patient withdrawals, and losses due to other reasons.

Projected distribution of accrual by risk group:
Standard Risk (SR) T-ALL: 40-50% of T-ALL patients;
Intermediate Risk (IR) T-ALL: 35-50% of patients;
Very High Risk (VHR) T-ALL: 10-15% of patients;
SR T-LLy: 65% of T-LLy patients;
IR T-LLy: 25% of T-LLy patients; and,
VHR T-LLy: <10% of T-LLy patients.

9.3 Statistical Analysis Methods

9.3.1 Power Calculation for Phase 3 Bortezomib Primary End-point:

With 1200 eligible, evaluable patients randomized to the +/- bortezomib arms, there is 90.5% power to detect improvement in 4-year EFS from 85% to 90% with an alpha of 0.05, using a one-sided log rank test (HR =0.6483) (comparison of the two EFS curves). There is sufficient power to detect a difference if the baseline EFS is different from the projected 85%. A baseline 4 year-EFS of 80% would allow detection of an improvement to 85% (HR=0.7283), with power of 82.1%; and there is 83.4% power to detect an improvement in EFS from 88% to 92% (HR=0.6523). Interim monitoring for efficacy and futility will be performed. The efficacy stopping boundaries to be used will allocate greater importance to the later analyses. The upper boundaries selected are based on the alpha × $(\text{time})^2$ spending function. The study will also be monitored for futility using the method of Freidlin and Korn. The lower boundaries are based on repeated testing of the alternative hypothesis at the 0.05 level (1-sided). The comparative analyses of regimen outcome will occur at approximately 20%, 40%, 60%, 80% and 100% of the projected combined EFS event horizon (217 events assuming baseline EFS rate of 85%) for the overall randomized group.

9.3.1.1 Amendment #6 (05/29/2019)

The COG DSMC recommended permanent closure of the study to accrual during the spring 2019 meeting; primarily due to concerns about the impact the positive results from AALL0434 for the nelarabine randomization could have on the conduct and interpretability of AALL1231. With the permanent closure to accrual as of 05/24/2019, this study will not have the required number of randomized patients (+/- Bortezomib) to have sufficient power to meet the primary objective. Last patient was randomized in November 2017. Per the recommendation of the DSMC, final analyses comparing EFS for the randomized arms, will be conducted based on the 3/31/2020 database freeze.

9.3.2 Secondary End-points:

Power calculation for CNS therapy changes primary end-point: The risk group definitions on this proposed study are different than AALL0434, and EOC MRD data will not be available on many AALL0434 patients. In order to determine if eliminating CRT and the multiple backbone modifications affect EFS and relapse rates, similar populations must be compared.

Any subject on either trial who is all of the following: [<9.99 years old, initial WBC <50,000/mm^3, day 29 MRD <0.1%, CNS1, testicular disease negative, not steroid pretreated] will be eliminated from the analysis, because they did not receive CRT on 0434 and will not receive CRT on AALL1231. We will also eliminate any subject on either trial who is an induction failure (M3 marrow on day 29) or CNS3 because they would have received CRT on 0434 and will still get CRT on AALL1231. Outcomes (EFS) for the remaining patients (about 49% of all T-ALL patients) on both studies will be compared. It is important not only to compare CNS relapse rates but also to compare BM, combined BM and CNS, and total relapse rates, because the elimination of prophylactic CRT may change the distribution of relapse, but not change overall relapse rate. Cumulative incidence rates for isolated CNS, isolated marrow, and combined marrow relapses will be monitored for these patients and compared with that seen for similar patients on AALL0434. Assuming approximately 584 patients (as identified by our definitions earlier excluding various subgroups) in 0434 meet these criteria and received CRT, there will be around the same number of patients who meet these criteria on this study and will not be getting CRT. Outcomes on AALL0434 are blinded at this time, hence the table below gives the power and detectable differences in cumulative incidence rates assuming a range of baseline rates on AALL0434 for the different types of relapse (isolated CNS, isolated marrow, combined marrow+CNS) for the above monitoring, using a one-sided log-rank test with a 5% significance level. Interim monitoring will be done using an alpha x (time)^2 spending function with 5 looks occurring at 20%, 40%, 60%, 80%, and 100% information.

Comparison of 2-year cumulative incidence rates on AALL0434 vs. AALL1231	Hazard ratio	Total expected events	Power
1.5% vs. 4%	2.7	32	81%
2% vs. 4.7%	2.38	39	80%
2.5% vs. 5.5%	2.23	47	81%
3% vs. 6.2%	2.1	54	81%
3.5% vs. 6.8%	1.98	60	80%

Evaluation of the modified backbone: Event-free survival (EFS) of patients treated on the control arm (no bortezomib) of AALL1231 will be compared with the EFS and OS of patients treated on the control arm (no nelarabine and Capizzi MTX with PEG-ASP arm) of the previous study AALL0434. Safety data will also be compared between the two arms.

Evaluation of HR blocks:
The proportion of VHR T-ALL patients with EOC MRD ≥0.1% who become MRD negative (MRD undetectable) after the three high-risk BFM blocks of therapy, will be estimated. EFS for these patients (who continue on chemotherapy) will be compared with those who continue to have detectable MRD and who may receive other treatment options including HSCT. It is anticipated that there will be about 100 – 120 VHR T-ALL patients accrued on this study. With this, the proportion who become MRD negative after the HR blocks can be estimated with a maximum standard error of 5%. It is projected that 80% of patients will become MRD negative after the HR blocks. With small numbers, the comparison of EFS between the two groups above will be essentially descriptive.

There will be a total of about 25 to 30 VHR T-LLy patients on study. Comparison of EFS between VHR T-LLy patients treated with HR BFM intensification blocks who are PR or CR with those who do not respond (NR) will essentially be descriptive due to small patient numbers.

9.3.3 Interim Monitoring

Induction Mortality. It is expected that a small percentage of patients will experience treatment related mortality (TRM) during induction. It is possible that induction mortality may increase based on the modifications that include dexamethasone, an additional dose of PEG-ASP, and +/- bortezomib randomization. Induction deaths on the two randomized arms will be closely monitored. The induction mortality rate on UKALL 2003 was 2.4% for patients treated with the anthracycline containing induction, dexamethasone at 6mg/m^2/day, and an extra dose of pegaspargase on day 18. This TRM rate is quite similar to that seen in other recent studies using a four-drug induction regimen, including AIEOP-BFM ALL 2000 (2% on dexamethasone-containing arm) and COG AALL0232 (2.16% for AYA pts vs. 1.67% for younger pts.[46,47,51] Due to the changes in induction compared to previous trials, the overall induction death rate will be closely monitored. All patients enrolled on study who receive induction will be included in this monitoring. Assuming a 'null' induction mortality rate of 2.5%. A Pocock boundary (truncated at 3 standard deviations) with 7 interim looks (after first 100 patients and then after every 200 patients enrolled, up to the first 1000 patients). The probability of crossing the boundary at any time when the true induction mortality rate is 2.5% is 10%. The boundary is given in the table below. If the boundary is crossed, the study will be temporarily closed for review of deaths and possible modifications to therapy.

Sample size	Number of induction deaths to trigger concern (Pocock boundary, alpha = 10%)	Percent of induction deaths that trigger concern (Pocock boundary, alpha = 10%)
100	7	7.0%
200	11	5.5%
400	17	4.25%
600	23	3.83%
800	29	3.63%
1000	35	3.50%
1200	41	3.42%

The induction death rates on the two randomized arms will also be compared and monitored closely for increased rates on the bortezomib arm. The induction death rates will be monitored using a Pocock type boundary (truncated at 3 standard deviations) to provide a greater chance of stopping early if the death rate looks excessive, using a one-sided test with an alpha of 10%. There will be 6 planned looks after every 200 patients randomized to the two arms. With this plan, there is 80% power to detect a difference if the induction death rates on the two arms are 2% vs. 4% or 3% vs. 5%. Since the induction regimen using dexamethasone and the addition of bortezomib, is hoped to result in some potential improvement in EFS, the above monitoring rule for induction deaths needs always to be considered in conjunction with EFS results (i.e., a slightly increased induction death rate would possibly be acceptable if the observed early EFS results looked promising).

Grade 4-5 infections. It is expected that a percentage of patients will experience Grade 4-5 infections. It is possible that Grade 4-5 infections may increase based on the modifications during induction that include dexamethasone, an additional dose of PEG-ASP, and +/- bortezomib randomization and during DI that include an additional dose of PEG-ASP and +/- bortezomib randomization. In AALL0232, 84.8% of subjects had a grade 3-5 infection during protocol therapy; however, only 13.7% had a grade 4 infection and 1.5% had a grade 5 infection (AALL0232, Study Progress Report, September 30 2011). The overall grade 4+ infection rate together with other toxicities of concern, among all patients will be estimated every six months at the time of interim monitoring to the COG Data Safety Monitoring Committee (DSMC). If the rate seems excessive compared to that seen on earlier studies (AALL0232, AALL0434),

the data will reviewed for possible modifications to therapy. In addition, the grade 4+ infection rates will be compared between the two randomized arms (+/- bortezomib). Significant differences will be brought to the attention of the COG DSMC for review and possible therapy modifications.

Osteonecrosis. It is expected that a modest percentage of patients will experience osteonecrosis (ON). The reported rates of osteonecrosis vary widely between studies and increased incidence occurs with older age and female gender. Three-year ON rates on CCG-1882 were 14.2% +/- 1.3% for children greater than 10 years of age.[180] On CCG-1961, the 5-year ON incidence rates were 9.9% +/-1.5% and 20% +/- 4.3% for the 10 to 15 and 16 to 21 year age groups, respectively.[181] On AALL0232, the rates in children over 10 years were 17.2% for patients randomized to receive dexamethasone and 12.6% on the prednisone arm.[182] On the St Jude Total XV ALL trial, the cumulative incidence of symptomatic ON (grade 2-4) was 14.6% +/- 1.6%.[183] Finally, on UKALL 2003 the rate of ON in intermediate risk patients of all ages, which included most of the T-ALL patients, was 9.4% (Vora, personal communication). As of February 2013, AALL0434 had a total of 85/1406 (6%) grade 2-3 osteonecrosis reported. There were no reports of grade 4 ON. It is possible that osteonecrosis rates may increase based on the increased use of dexamethasone and extra doses of PEG-ASP. The incidence of grade 2-4 symptomatic osteonecrosis will be closely monitored on the trial. Very detailed information is captured on osteonecrosis with a specific case report form on the COG ALL trials and will also be done for this study. Rates of osteonecrosis will be summarized overall, and by age-group and gender in the biannual study progress reports and reviewed by the study committee. Any indication of an increase in rates will be reviewed closely and reported to the COG DSMC for review and possible therapy modifications.

Targeted Toxicities (Bortezomib). Peripheral neuropathy and pulmonary toxicity are of special concern with bortezomib treatment, although these toxicities are more commonly found in adults than children. Both of these will be closely monitored and reported to the DSMC for biannual review and on an *ad hoc* basis as required for judging the safety of bortezomib. In addition, the specific dose modifications and supportive care guidelines are provided in Section 5.2.

Adverse Event Monitoring

The study will be monitored to ensure that bortezomib is feasible and does not result in excessive toxicities or death during induction or DI. In addition, the study will be monitored to ensure changes in the backbone do not result in excessive toxicities. As described above, specific monitoring rules will be followed for TRM, grade 4-5 infections, osteonecrosis, peripheral neuropathy, and pulmonary toxicity. The incidence rates of the following key adverse events (in addition to any Grade 4 non hematologic toxicities) will be estimated across all patient subgroups on this trial.

1. CNS hemorrhage requiring medical intervention (Grade 2 and higher)
2. GI bleed requiring operative or interventional radiology intervention (Grade 3 and higher)
3. Pancreatitis requiring medical intervention (Grades 2 and higher)
4. Transient ischemic attacks (All grades)
6. Stroke (All grades)
7. Encephalopathy (Grade 3 and higher)
8. Neuropathy; motor or sensory, interfering with ADL (Grade 3 and higher)*
9. Seizure (Grade 2 and higher)
10. Allergic reaction (Grade 3 and higher)
11. Ileus (Grade 3 and higher)

12. Mucositis/stomatitis; functional (Grade 3 and higher)
13. Bilirubin (Grade 3 and higher)
14. Thrombosis (Grade 3 and higher)
15. Hyponatremia (Grade 3 and higher)
16. Hypophosphatemia (Grade 3 and higher)
17. Osteonecrosis (Grade 2 and higher)

9.4 Gender and Minority Accrual Estimates

Based on the distribution derived from AALL0434, the gender and minority distribution of the study population is expected to be:

Accrual Targets			
Ethnic Category	**Sex/Gender**		
	Females	**Males**	**Total**
Hispanic or Latino	57	144	201
Not Hispanic or Latino	320	879	1199
Ethnic Category: Total of all subjects	377	1023	*1400
Racial Category			
American Indian or Alaskan Native	1	6	7
Asian	22	56	78
Black or African American	64	148	212
Native Hawaiian or other Pacific Islander	5	8	13
White	285	805	1090
Racial Category: Total of all subjects	377	1023	*1400

* These totals must agree

9.5 Correlative Studies

9.5.1 Minimal Residual Disease Determination at Serial Time Points During Therapy

Patients with newly diagnosed T-ALL will have MRD measured in the bone marrow (BM) at the end of the first block of Induction therapy (Day 29). Those patients (~50%) with MRD levels <0.01% in BM at Day 29 will be considered Standard Risk and assigned to the least intensive cytotoxic therapy. For the remaining patients (~50%), MRD will be assessed in BM at the end of Consolidation (EOC) therapy. Those with MRD <0.1% (~40%) will be considered Intermediate Risk and receive intermediate intensity therapy. The remainder (~10%) will be considered Very High Risk and receive intensified therapy followed by an additional MRD assessment with those positive for MRD taken off study. Specimens from all patients will also be assayed at study entry in order to define an abnormal phenotype that will facilitate detection of MRD.

Part of the diagnostic immunophenotyping at study entry will include the marginal cost for early thymic precursor (ETP) sub classification, a subset whose outcome is an important secondary endpoint of the study. Early studies suggested patients with ETP ALL do poorly, but more recent data suggest the prognosis may not be dire (see Section 2.2 for background and rationale on ETP ALL). It is critical to understand whether or not ETP phenotype independently predicts outcome in T-ALL. Multivariate analysis will be performed to determine if ETP ALL is an independent predictor of poor outcome based on MRD rates at End Induction and End of Consolidation. Preliminary data suggest ETP represents 12.4% of

T-ALL in AALL0434, yielding 118 ETP patients of the 952 T-ALL anticipated on AALL1231. There are few data available to predict the proportion of ETP+ patients that will have MRD <0.1% at EOC. Overall, we expect that ~10% of T-ALL patients will have MRD ≥0.1% at EOC while ~90% will have MRD <0.1%. Given the known higher percentage of end induction MRD+ patients among the ETP+ subset, we hypothesize that ~50% of ETP+ patients (n=59) will have MRD ≥0.1% at EOC (5-fold higher rate than MRD-negative), while ~50% (n=59) will have MRD <0.1%. If the 4-year EFS of these ETP+, EOC MRD <0.1% patients is ~50% or better, it would suggest that ETP patients can be risk stratified based on MRD alone (unless there is a specific therapy available for ETP patients). However, if this assumption is incorrect and ETP+ patients with MRD <0.1% at EOC have an EFS < 50%, it would suggest that MRD alone cannot be used to risk stratify these patients and alternative strategies (stem cell transplant in CR1 or other novel therapies) should be pursued for all ETP patients. Hence, we will see if the lower limit of the 95% confidence interval for the 4-year EFS estimate for ETP+, EOC MRD <0.1% patients exceeds 50%. With a sample size of 59 ETP+, EOC MRD < 0.1% cases (assuming no censoring before 4 years), the lower bound on an exact 95% CI for 4-year EFS will exceed 50% with at least 86% probability (=Prob[number event free at four years is at least 38]) if the true 4-year EFS is at least 70%. The EFS will also be compared between ETP+, EOC MRD<0.1% versus ETP+, EOC MRD≥0.1%. Due to small numbers (59 patients in each group), this comparison will essentially be descriptive.

Patients with T-LLy will have the level of marrow involvement assessed at diagnosis to facilitate risk stratification into Standard and Intermediate risk groups. T-LLy patients will also be assessed for MRD at Day 29, as this study will enroll more T-LLy patients than any to date and offers a unique opportunity to establish risk factors for this understudied patient population. We hypothesize that T-LLy patients with <0.01% MRD at End of Induction will have the best outcome, consistent with existing data in T-ALL. No data exist on the frequency of marrow MRD following therapy for T-LLy, although the frequency of marrow involvement at diagnosis > 0.01% is 66% in AALL0434. Assuming that about 80% of T-LLy patients will have MRD < 0.01% at End of Induction, this yields roughly 198 T-LLy patients on this study with MRD<0.01% and 50 patients with MRD≥0.01%. Assuming an overall 4-year EFS of 80% for T-LLy, there is over 99% power to detect a difference in EFS between the two MRD groups (90% vs. 40% 4-year EFS, HR=0.1155). There is over 98% power to detect a difference in EFS if the EFS rates are 85% and 60% in the two groups (HR=0.3181).

Additional statistical details are included in Appendix XI.

9.5.2 Evaluating Mechanisms of Bortezomib Response and Mechanisms of Bortezomib Resistance in T- ALL

Statistical analysis, including sample sizes are included in Appendix XII.

9.5.3 Identifying Biomarkers and Mechanisms of Chemotherapy Resistance and Response in T-ALL, Focusing on ETP ALL

Statistical analysis, including sample sizes are included in Appendix XII.

10.0 EVALUATION CRITERIA

10.1 Common Terminology Criteria for Adverse Events (CTCAE)

This study will utilize version 4.0 of the CTCAE of the National Cancer Institute (NCI) for toxicity and performance reporting. A copy of the CTCAE version 4.0 can be downloaded from the CTEP website (http://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm). Additionally, toxicities are to be reported on the appropriate case report forms.

Please note: 'CTCAE v4.0' is understood to represent the most current version of CTCAE v4.0 as referenced on the CTEP website (i.e., v4.02 and all subsequent iterations prior to version 5.0).

10.2 T-ALL and T-LLy: Relapse

Any recurrence of disease whether in marrow or extramedullary. Relapse should be histopathologically/biopsy confirmed.

10.2.1 CNS Relapse

Positive cytomorphology and WBC ≥ 5/μL OR clinical signs of CNS leukemia such as facial nerve palsy, brain/eye involvement, or hypothalamic syndrome. If any CSF evaluation shows positive cytomorphology and < 5 WBC/μL, a second CSF evaluation is required within 2-4 weeks. While identification of a leukemic clone in CSF by flow cytometry (CD2, CD3, CD34, or the same T-cell immunophenotypic markers that were identified at diagnosis) or FISH for diagnostic karyotypic abnormality may be useful, definitive evidence of CNS involvement (i.e. WBC ≥ 5/μl or clinical signs of CNS leukemia) is required for a diagnosis of CNS relapse.

10.2.2 Testicular Relapse

Must be documented by testicular biopsy, if not associated with a marrow relapse.

10.2.3 Bone Marrow Relapse

Patients with an M3 marrow at any point after achieving remission.

10.2.4 Combined extramedullary and Bone Marrow Relapse

Patients with a CNS or testicular relapse and a M2 or M3 marrow.

10.3 T-ALL: Response

See definitions in Section 3.3.

10.4 T-LLy: Response

These criteria are derived from published international consensus guidelines.[184,185]

Prior to therapy: Patients will have a bilateral bone marrow biopsies and aspirate, CT of the neck, chest, abdomen and pelvis, CXR, and, if indicated, a bone scan. On Day 29 patients will have a repeat CT of chest and CT of areas of active disease at diagnosis (neck, abdomen, and/or pelvis), CXR and if positive at diagnosis a bone scan. Patients with morphologic evidence of bone marrow disease at diagnosis will repeat the bilateral bone marrow aspirates and biopsies (>5-25% blasts by morphology) at Day 29. At end of consolidation, patients not in a CR at the end of Induction will have repeat CT imaging of active sites of disease and if positive at end of Induction a bone scan. Patients with morphologic evidence of bone marrow disease (>5-25% blasts by morphology) at end of Induction will repeat the bilateral bone marrow aspirates and biopsies at the end of consolidation. VHR patients will have repeat imaging at the end of 3 HR BFM blocks of sites demonstrating active disease at the end of consolidation. Patients with morphologic evidence of bone marrow disease at end of consolidation will have a repeat bilateral bone marrow aspirates and biopsies at the end of the 3 HR BFM blocks. A PET scan is highly recommended but not required at diagnosis, at the end of Induction, and, if there are residual masses, at the end of Consolidation and for VHR patients if there are residual masses at the end of the 3 HR BFM blocks.

Of note, if a PET scan is obtained at baseline, PET imaging should be continued with subsequent response assessment until patient no longer has PET-avid disease. A bone scan is the preferred method to follow bone disease however, PET scan can be used as a substitute for a bone scan in patients with bone symptoms. For patients who had a PET-CT at diagnosis, a PET-CT can be used instead of a regular CT to follow disease response.

10.4.1 Complete Response (CR)

Defined as disappearance of all detectable clinical evidence of disease from all sites as determined by physical examination and appropriate imaging studies. Lymph nodes must have decreased to less or equal 1.5cm. Any macroscopic nodules in any organs detectable by CT should be gone. Bone marrow aspirate/biopsy must be morphologically normal. MRD will be sent on D29 on those patients consenting but will not be used in clinical decision making on this study.

For patients with a previous positive PET scan, PET scan must be negative.

A post-treatment residual mass of any size is considered a CR as long as it is PET negative. A negative PET is required in patients with post-treatment residual masses to be considered a CR. Patients with post-treatment residual masses must be followed by PET and remain PET negative to be considered CR.

Bone lesions that remain positive by MRI/CT and/or PET will be considered CR if there is resolution of all surrounding soft tissue component by the end of Consolidation. No new lesions.

10.4.2 Partial Response (PR)

At least a 50% decrease in the sum of the product of diameters (SPD) of the lesions of up to six of the largest dominant nodes or nodal masses. Splenic and hepatic nodules must decrease by at least 50% in their SPD. No new lesions.

In patients with a positive PET scan prior to therapy, the post-treatment PET must be positive in at least one previously involved site. No new lesions.

10.4.3 Stable Disease (SD) / No response (NR)

Failure to qualify for a PR or PD. No new lesions.

In patients with a positive PET scan prior to therapy, the PET must be positive at prior sites of disease with no new areas of involvement on the post-treatment CT or PET.

10.4.4 Relapsed/Progressive Disease (PD)

Greater than 25% increase in the size of any lesions or appearance of new lesion(s) more than 1.5 cm in any axis. In patients with a positive PET scan prior to therapy, lesions must be PET positive.

11.0 ADVERSE EVENT REPORTING REQUIREMENTS

11.1 Purpose

Adverse event (AE) data collection and reporting, which are required as part of every clinical trial, are done to ensure the safety of patients enrolled in the studies as well as those who will enroll in future studies using similar agents. Certain adverse events must be reported in an expedited manner to allow for timelier monitoring of patient safety and care. The following sections provide information about expedited reporting.

11.2 Determination of Reporting Requirements

Reporting requirements may include the following considerations: 1) whether the patient has received an investigational or commercial agent; 2) the characteristics of the adverse event including the *grade* (severity), the *relationship to the study therapy* (attribution), and the *prior experience* (expectedness) of the adverse event; 3) the Phase (1, 2, or 3) of the trial; and 4) whether or not hospitalization or prolongation of hospitalization was associated with the event.

An investigational agent is a protocol drug administered under an Investigational New Drug Application (IND). In some instances, the investigational agent may be available commercially, but is actually being tested for indications not included in the approved package label.

Commercial agents are those agents not provided under an IND but obtained instead from a commercial source. The NCI, rather than a commercial distributor, may on some occasions distribute commercial agents for a trial.

When a study includes both investigational and commercial agents, the following rules apply.

- *Concurrent administration*: When an investigational agent is used in combination with a commercial agent, the combination is considered to be investigational and expedited reporting of adverse events would follow the guidelines for investigational agents.
- *Sequential administration: When a study includes an investigational agent and a commercial agent on the same study arm, but the commercial agent is given for a period of time prior to starting the investigational agent, expedited reporting of adverse events which occur prior to starting the investigational agent would follow the guidelines for commercial agents. Once therapy with the investigational agent is initiated, all expedited reporting of adverse events follow the investigational agent reporting guidelines.*

11.3 Expedited Reporting Requirements – Serious Adverse Events (SAEs)

To ensure compliance with these regulations/this guidance, as IND/IDE sponsor, NCI requires that AEs be submitted according to the timeframes in the AE reporting tables assigned to the protocol, using the CTEP Adverse Event Reporting System (CTEP-AERS).

Any AE that is serious qualifies for expedited reporting. An AE is defined as any untoward medical occurrence associated with the use of a drug in humans, whether or not considered drug related. A Serious Adverse Event (SAE) is any adverse drug event (experience) occurring at any dose that results in ANY of the following outcomes:

1) Death.
2) A life-threatening adverse drug experience.
3) An adverse event resulting in inpatient hospitalization or prolongation of existing hospitalization (for ≥ 24 hours). This does not include hospitalizations that are part of routine medical practice.
4) A persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions.

5) A congenital anomaly/birth defect.
6) Important Medical Events (IME) that may not result in death, be life threatening, or require hospitalization may be considered a serious adverse drug experience when, based upon medical judgment, they may jeopardize the patient or subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition.

11.4 Special Situations for Expedited Reporting

11.4.1 SAEs Occurring More than 30 Days After Last Dose of Study Drug

Any Serious Adverse Event that occurs more than 30 days after the last administration of the investigational agent/intervention **and** has an attribution of a possible, probable, or definite relationship to the study therapy must be reported according to the CTEP-AERS reporting tables in this protocol.

11.4.2 Persistent or Significant Disabilities/Incapacities

Any AE that results in persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions (formerly referred to as disabilities), congenital anomalies or birth defects, must be reported via CTEP-AERS if it occurs at any time following treatment with an agent under a NCI IND/IDE since these are considered to be serious AEs.

11.4.3 Death

Reportable Categories of Death

- Death attributable to a CTCAE term.
- Death Neonatal: Newborn death occurring during the first 28 days after birth.
- Sudden Death NOS: A sudden (defined as instant or within one hour of the onset of symptoms) or an unobserved cessation of life that cannot be attributed to a CTCAE term associated with Grade 5.
- Death NOS: A cessation of life that cannot be attributed to a CTCAE term associated with Grade 5.
- Death due to progressive disease should be reported as Grade 5 "*Disease progression*" in the system organ class (SOC) "*General disorders and administration site conditions*". Evidence that the death was a manifestation of underlying disease (*e.g.*, radiological changes suggesting tumor growth or progression: clinical deterioration associated with a disease process) should be submitted.

Any death occurring ***within 30 days*** of the last dose, regardless of attribution to the investigational agent/intervention requires expedited reporting within 24 hours.

Any death occurring ***greater than 30 days*** after the last dose of the investigational agent/intervention requires expedited reporting within 24 hours **only if** it is possibly, probably, or definitely related to the investigational agent/intervention.

11.4.4 Secondary Malignancy

A ***secondary malignancy*** is a cancer caused by treatment for a previous malignancy (e.g., treatment with investigational agent/intervention, radiation or chemotherapy). A metastasis of the initial neoplasm is not considered a secondary malignancy.

The NCI requires all secondary malignancies that occur following treatment with an agent under an NCI IND/IDE be reported via CTEP-AERS. Three options are available to describe the event:

- Leukemia secondary to oncology chemotherapy
- Myelodysplastic syndrome
- Treatment related secondary malignancy

Any malignancy possibly related to cancer treatment (including AML/MDS) must also be reported via the routine reporting mechanisms outlined in this protocol.

11.4.5 Second Malignancy:

A **second malignancy** is one unrelated to the treatment of a prior malignancy (and is **NOT** a metastasis from the initial malignancy). Second malignancies require **ONLY** routine reporting via CDUS unless otherwise specified.

11.4.6 Pregnancy, Pregnancy Loss, and Death Neonatal

NOTE: When submitting CTEP-AERS reports for "Pregnancy", "Pregnancy loss", or "Neonatal loss", the Pregnancy Information Form, available at http://ctep.cancer.gov/protocolDevelopment/electronic_applications/docs/PregnancyReportForm.pdf, needs to be completed and faxed along with any additional medical information to (301) 897-7404. The potential risk of exposure of the fetus to the investigational agent(s) or chemotherapy agent(s) should be documented in the "Description of Event" section of the CTEP-AERS report.

11.4.6.1 **Pregnancy**

Patients who become pregnant on study risk intrauterine exposure of the fetus to agents that may be teratogenic. For this reason, pregnancy needs to be reported in an expedited manner via CTEP-AERS as **Grade 3 "*Pregnancy, puerperium and perinatal conditions - Other (pregnancy)*"** under the "***Pregnancy, puerperium and perinatal conditions"*** SOC.

There is a possibility that the sperm of male patients treated on studies involving possible teratogenic agents may have been damaged. For this reason, **pregnancy in partners of men on study needs be reported and followed in the same manner as a patient pregnancy**.

Pregnancy needs to be followed **until the outcome is known**. If the baby is born with a birth defect or anomaly, then a second CTEP-AERS report is required.

11.4.6.2 **Pregnancy Loss (Fetal Death)**

Pregnancy loss is defined in CTCAE as "*Death in utero*." Any Pregnancy loss should be be reported expeditiously, as **Grade 4 "*Pregnancy loss*" under the "*Pregnancy, puerperium and perinatal conditions*" SOC**. Do NOT report a pregnancy loss as a Grade 5 event since CTEP-AERS recognizes any Grade 5 event as a patient death.

11.4.6.3 **Death Neonatal**

Neonatal death, defined in CTCAE as *"Newborn death occurring during the first 28 days after birth*", should be reported expeditiously as **Grade 4, "*Death neonatal*" under the "*General disorders and administration*" SOC, when the death is the result of a patient pregnancy or pregnancy in partners of men on study**. Do NOT report a neonatal death resulting from a patient pregnancy or pregnancy in partners

of men on study as a Grade 5 event since CTEP-AERS recognizes any Grade 5 event as a patient death.

11.5 Reporting Requirements for Specialized AEs

11.5.1 Baseline AEs

Although a pertinent positive finding identified on baseline assessment is not an AE, when possible it is to be documented as "Course Zero" using CTCAE terminology and grade. An expedited AE report is not required if a patient is entered on a protocol with a pre-existing condition (e.g., elevated laboratory value, diarrhea). The baseline AE must be re-assessed throughout the study and reported if it fulfills expedited AE reporting guidelines.

a. If the pre-existing condition worsens in severity, the investigator must reassess the event to determine if an expedited report is required.
b. If the AE resolves and then recurs, the investigator must re-assess the event to determine if an expedited report is required.
c. No modification in grading is to be made to account for abnormalities existing at baseline.

11.5.2 Persistent AEs

A persistent AE is one that extends continuously, without resolution between treatment cycles/courses.

ROUTINE reporting: The AE must be reported only once unless the grade becomes more severe in a subsequent course. If the grade becomes more severe the AE must be reported again with the new grade.
EXPEDITED reporting: The AE must be reported only once unless the grade becomes more severe in the same or a subsequent course.

11.5.3 Recurrent AEs

A recurrent AE is one that occurs and resolves during a cycle/course of therapy and then reoccurs in a later cycle/course.
ROUTINE reporting: An AE that resolves and then recurs during a subsequent cycle/course must be reported by the routine procedures.
EXPEDITED reporting: An AE that resolves and then recurs during a subsequent cycle/course does not require CTEP-AERS reporting unless:

1) The grade increases OR
2) Hospitalization is associated with the recurring AE.

11.6 Exceptions to Expedited Reporting

11.6.1 Specific Protocol Exceptions to Expedited Reporting (SPEER)

SPEER: Is a subset of AEs within the Comprehensive Adverse Events and Potential Risks (CAEPR) that contains a list of events that are considered expected for CTEP-AERS reporting purposes. (Formerly referred to as the Agent Specific Adverse Event List (ASAEL).)

AEs listed on the SPEER should be reported expeditiously by investigators to the NCI via CTEP-AERS ONLY if they exceed the grade of the event listed in parentheses after the event. If the CAEPR is part of a combination IND using multiple investigational agents and has an SAE listed on different SPEERs, use the lower of the grades to determine if expedited reporting is required.

11.6.2 Special Situations as Exceptions to Expedited Reporting

An expedited report may not be required for a specific protocol where an AE is listed as expected. The exception or acceptable reporting procedures will be specified in the protocol. The protocol specific guidelines supersede the NCI Adverse Event Reporting Guidelines. These special situations are listed under the CTEP-AERS reporting Table A for this protocol.

11.7 Reporting Requirements - Investigator Responsibility

Clinical investigators in the treating institutions and ultimately the Study Chair have the primary responsibility for AE identification, documentation, grading, and assignment of attribution to the investigational agent/intervention. It is the responsibility of the treating physician to supply the medical documentation needed to support the expedited AE reports in a timely manner.

Note: All expedited AEs (reported via CTEP-AERS) must also be reported via routine reporting. Routine reporting is accomplished via the Adverse Event (AE) Case Report Form (CRF) within the study database.

11.8 General Instructions for Expedited Reporting via CTEP-AERS

The descriptions and grading scales found in the revised NCI Common Terminology Criteria for Adverse Events (CTCAE) version 5.0 will be utilized for AE reporting. All appropriate treatment areas should have access to a copy of the CTCAE version 5.0. A copy of the CTCAE version 5.0 can be downloaded from the CTEP web site http://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm.

An expedited AE report for all studies utilizing agents under an NCI IND/IDE must be submitted electronically to NCI via CTEP-AERS at: https://eapps-ctep.nci.nih.gov/ctepaers.

In the rare situation where Internet connectivity is disrupted, the 24-hour notification is to be made to the NCI for agents supplied under a CTEP IND by telephone call to (301)897-7497.
In addition, once Internet connectivity is restored, a 24-hour notification that was phoned in must be entered into the electronic CTEP-AERS system by the original submitter of the report at the site.

- Expedited AE reporting timelines are defined as:
  - **24-Hour; 5 Calendar Days -** The AE must initially be reported via CTEP-AERS within 24 hours of learning of the event, followed by a complete expedited report within 5 calendar days of the initial 24-hour report.
  - **7 Calendar Days -** A complete expedited report on the AE must be submitted within 7 calendar days of the investigator learning of the event.
- Any event that results in a persistent or significant incapacity/substantial disruption of the

ability to conduct normal life functions, or a congenital anomaly/birth defect, or is an IME, which based upon the medical judgment of the investigator may jeopardize the patient and require intervention to prevent a serious AE, must be reported via CTEP-AERS **if the event occurs following investigational agent administration.**

- Any death occurring within 30 days of the last dose, regardless of attribution to an agent/intervention under an NCI IND/IDE requires expedited reporting **within 24 hours**.
- Any death occurring greater than 30 days of the last dose with an attribution of possible, probable, or definite to an agent/intervention under an NCI IND/IDE requires expedited reporting **within 24 hours**.

CTEP-AERS Medical Reporting includes the following requirements as part of the report: 1) whether the patient has received at least one dose of an investigational agent on this study; 2) the characteristics of the adverse event including the *grade* (severity), the *relationship to the study therapy* (attribution), and the *prior experience* (expectedness) of the adverse event; 3) the Phase (1, 2, or 3) of the trial; and 4) whether or not hospitalization or prolongation of hospitalization was associated with the event.

Any medical documentation supporting an expedited report (e.g., H & P, admission and/or notes, consultations, ECG results, etc.) MUST be faxed within 48-72 hours to the NCI. NOTE: English is required for supporting documentation submitted to the numbers listed below in order for the NCI to meet the regulatory reporting timelines.

Fax supporting documentation **for AEs related to investigational agents supplied under a CTEP IND** to: (301) 897-7404.

Also: Fax or email supporting documentation to COG for **all** IND studies (Fax # (310) 640-9193; email: COGAERS@childrensoncologygroup.org; Attention: COG AERS Coordinator).

- **ALWAYS include the ticket number on all faxed documents.**
- **Use the NCI protocol number and the protocol-specific patient ID provided during trial registration on all reports.**

11.9 Reporting Table for Late Phase 2 and Phase 3 Studies – Table A

Expedited Reporting Requirements for Adverse Events that Occur on Studies under an IND/IDE within 30 Days of the Last Administration of the Investigational Agent/Intervention [1]

FDA REPORTING REQUIREMENTS FOR SERIOUS ADVERSE EVENTS (21 CFR Part 312)
NOTE: Investigators **MUST** immediately report to the sponsor (NCI) **ANY** Serious Adverse Events, whether or not they are considered related to the investigational agent(s)/intervention (21 CFR 312.64)
An adverse event is considered serious if it results in **ANY** of the following outcomes:

1) Death.
2) A life-threatening adverse event.
3) Any AE that results in inpatient hospitalization or prolongation of existing hospitalization for ≥ 24 hours. This does not include hospitalizations that are part of routine medical practice.
4) A persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions.
5) A congenital anomaly/birth defect.
6) Important Medical Events (IME) that may not result in death, be life threatening, or require hospitalization may be considered serious when, based upon medical judgment, they may jeopardize the patient or subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition. (FDA, 21 CFR 312.32; ICH E2A and ICH E6.)

ALL SERIOUS adverse events that meet the above criteria **MUST** be immediately reported to the NCI via CTEP-AERS within the timeframes detailed in the table below.

<table>
<tr><th>Hospitalization</th><th>Grade 1 Timeframes</th><th>Grade 2 Timeframes</th><th>Grade 3 Timeframes</th><th>Grade 4 & 5 Timeframes</th></tr>
<tr><td>Resulting in Hospitalization ≥ 24 hrs</td><td colspan="3">7 Calendar Days</td><td rowspan="2">24-Hour Notification 5 Calendar Days</td></tr>
<tr><td>Not resulting in Hospitalization ≥ 24 hrs</td><td colspan="2">Not Required</td><td>7 Calendar Days</td></tr>
<tr><td colspan="5">NOTE: Protocol specific exceptions to expedited reporting of serious adverse events are found in the Specific Protocol Exceptions to Expedited Reporting (SPEER) portion of the CAEPR. Additional Special Situations as Exceptions to Expedited Reporting are listed below.

Expedited AE reporting timelines are defined as:
“24-Hour; 5 Calendar Days” - The AE must initially be reported via CTEP-AERS within 24 hours of learning of the AE, followed by a complete expedited report within 5 calendar days of the initial 24-hour notification.
“7 Calendar Days” - A complete expedited report on the AE must be submitted within 7 calendar days of learning of the AE.</td></tr>
<tr><td colspan="5">[1]SAEs that occur more than 30 days after the last administration of investigational agent/intervention and have an attribution of possible, probable, or definite require reporting as follows:
Expedited 24-hour notification followed by complete report within 5 calendar days for:
• All Grade 4, and Grade 5 AEs
Expedited 7 calendar day reports for:
• Grade 2 adverse events resulting in hospitalization or prolongation of hospitalization
• Grade 3 adverse events</td></tr>
</table>

11.10 **Protocol Specific Additional Instructions and Reporting Exceptions**

- All Grade 3-Grade 5 pulmonary toxicities (with the exception of laryngitis) **must** be reported as SAEs. **Sites are required to complete the patient Medidata/Rave screens on-line within 24 hours of any Grade 3-5 pulmonary toxicity.**
- **Grades 1-4 myelosuppression (anemia, neutropenia, thrombocytopenia) do not require expedited reporting.**

11.11 **Reporting of Adverse Events for <u>commercial</u> agents – CTEP-AERS abbreviated pathway**

The following are expedited reporting requirements for adverse events experienced by patients on study who have <u>not</u> received any doses of an investigational agent on this study.
Commercial reporting requirements are provided in Table B.

COG requires the CTEP-AERS report to be submitted **within 7 calendar days** of learning of the event.

<u>Table B</u>
Reporting requirements for adverse events experienced by patients on study who have NOT received any doses of an investigational agent on this study.

CTEP-AERS Reporting Requirements for Adverse Events That Occur During Therapy With a Commercial Agent or Within 30 Days[1]

Attribution	Grade 4		Grade 5
	Unexpected	Expected	
Unrelated or Unlikely			CTEP-AERS
Possible, Probable, Definite	CTEP-AERS		CTEP-AERS
[1]This includes all deaths within 30 days of the last dose of treatment with a commercial agent, regardless of attribution. Any death that occurs more than 30 days after the last dose of treatment with a commercial agent that can be attributed (possibly, probably, or definitely) to the agent and is not due to cancer recurrence must be reported via CTEP-AERS.			

11.12 Routine Adverse Event Reporting

Note: The guidelines below are for routine reporting of study specific adverse events on the COG case report forms and do not affect the requirements for CTEP-AERS reporting.

Routine reporting is accomplished via the Adverse Event (AE) Case Report Form (CRF) within the study database. For this study, routine reporting will include all toxicities reported via CTEP-AERS and all Grade 4 and higher nonhematologic Adverse Events as well as the following specific toxicities:

1. CNS hemorrhage requiring medical intervention (Grade 2 and higher)
2. GI bleed requiring operative or interventional radiology intervention (Grade 3 and higher)
3. Pancreatitis requiring medical intervention (Grades 2 and higher)
4. Transient ischemic attacks (All grades)
6. Stroke (All grades)
7. Encephalopathy (Grade 3 and higher)
8. Neuropathy; motor or sensory, interfering with ADL (Grade 3 and higher)*
9. Seizure (Grade 2 and higher)
10. Allergic reaction (Grade 3 and higher)
11. Ileus (Grade 3 and higher)
12. Mucositis/stomatitis; functional (Grade 3 and higher)
13. Bilirubin (Grade 3 and higher)
14. Thrombosis (Grade 3 and higher)
15. Hyponatremia (Grade 3 and higher)
16. Hypophosphatemia (Grade 3 and higher)
17. Osteonecrosis (Grade 2 and higher)

*Please use Balis scale (Section 5.13.6) grading for neuropathy (motor or sensory).

See Section 5.2 for identification, management and reporting of bortezomib-related toxicities.

12.0 RECORDS AND REPORTING

See the Case Report Forms posted on the COG web site with each protocol under "*Data Collection/Specimens*". A submission schedule is included.

12.1 CDUS

This study will be monitored by the Clinical Data Update System (CDUS). Cumulative CDUS data will be submitted quarterly to CTEP by electronic means. Reports are due January 31, April 30, July 31 and October 31. This is not a responsibility of institutions participating in this trial.

12.2 **CTA/CRADA**

The agent(s) supplied by CTEP, DCTD, NCI used in this protocol is/are provided to the NCI under a Collaborative Agreement (CRADA, CTA, CSA) between the Pharmaceutical Company(ies) (hereinafter referred to as "Collaborator(s)") and the NCI Division of Cancer Treatment and Diagnosis. Therefore, the following obligations/guidelines, in addition to the provisions in the "Intellectual Property Option to Collaborator" (http://ctep.cancer.gov/industryCollaborations2/intellectual_property.htm) contained within the terms of award, apply to the use of the Agent(s) in this study:

1. Agent(s) may not be used for any purpose outside the scope of this protocol, nor can Agent(s) be transferred or licensed to any party not participating in the clinical study. Collaborator(s) data for Agent(s) are confidential and proprietary to Collaborator(s) and shall be maintained as such by the investigators. The protocol documents for studies utilizing Agents contain confidential information and should not be shared or distributed without the permission of the NCI. If a copy of this protocol is requested by a patient or patient's family member participating on the study, the individual should sign a confidentiality agreement. A suitable model agreement can be downloaded from: http://ctep.cancer.gov.

2. For a clinical protocol where there is an investigational Agent used in combination with (an)other Agent(s), each the subject of different Collaborative Agreements, the access to and use of data by each Collaborator shall be as follows (data pertaining to such combination use shall hereinafter be referred to as "Multi-Party Data"):

a. NCI will provide all Collaborators with prior written notice regarding the existence and nature of any agreements governing their collaboration with NCI, the design of the proposed combination protocol, and the existence of any obligations that would tend to restrict NCI's participation in the proposed combination protocol.

b. Each Collaborator shall agree to permit use of the Multi-Party Data from the clinical trial by any other Collaborator solely to the extent necessary to allow said other Collaborator to develop, obtain regulatory approval or commercialize its own Agent.

c. Any Collaborator having the right to use the Multi-Party Data from these trials must agree in writing prior to the commencement of the trials that it will use the Multi-Party Data solely for development, regulatory approval, and commercialization of its own Agent.

3. Clinical Trial Data and Results and Raw Data developed under a Collaborative Agreement will be made available to Collaborator(s), the NCI, and the FDA, as appropriate and unless additional disclosure is required by law or court order as described in the IP Option to Collaborator (http://ctep.cancer.gov/industryCollaborations2/intellectual_property.htm).
Additionally, all Clinical Data and Results and Raw Data will be collected, used and disclosed consistent with all applicable federal statutes and regulations for the protection of human subjects, including, if applicable, the *Standards for Privacy of Individually Identifiable Health Information* set forth in 45 C.F.R. Part 164.

4. When a Collaborator wishes to initiate a data request, the request should first be sent to the NCI, who will then notify the appropriate investigators (Group Chair for Cooperative Group studies, or PI for other studies) of Collaborator's wish to contact them.

5. Any data provided to Collaborator(s) for Phase 3 studies must be in accordance with the guidelines and policies of the responsible Data Monitoring Committee (DMC), if there is a DMC for this clinical trial.

6. Any manuscripts reporting the results of this clinical trial must be provided to CTEP by the Group office for Cooperative Group studies or by the principal investigator for non-Cooperative Group studies for immediate delivery to Collaborator(s) for advisory review and comment prior to submission for publication. Collaborator(s) will have 30 days from the date of receipt for review. Collaborator shall have the right to request that publication be delayed for up to an additional 30 days in order to ensure that Collaborator's confidential and proprietary data, in addition to Collaborator(s)'s intellectual property rights, are protected. Copies of abstracts must be provided to CTEP for forwarding to Collaborator(s) for courtesy review as soon as possible and preferably at least three (3) days prior to submission, but in any case, prior to presentation at the meeting or publication in the proceedings. Press releases and other media presentations must also be forwarded to CTEP prior to release. Copies of any manuscript, abstract and/or press release/ media presentation should be sent to:

   Email: ncicteppubs@mail.nih.gov

   The Regulatory Affairs Branch will then distribute them to Collaborator(s). No publication, manuscript or other form of public disclosure shall contain any of Collaborator's confidential/ proprietary information.

13.0 PATHOLOGY GUIDELINES FOR T-LLy

13.1 Pathology Goals

To provide accurate diagnosis and classification of pediatric lymphoblastic lymphoma through central pathologic review of morphology and immunophenotypic data. **This study is limited to T-cell lymphoblastic lymphoma (Stages II-IV)**. The central review will employ the 2008 World Health Organization (WHO) Lymphoma Classification[98] to facilitate concordance in diagnosis and correlate morphologic, immunophenotypic and cytogenetic data.

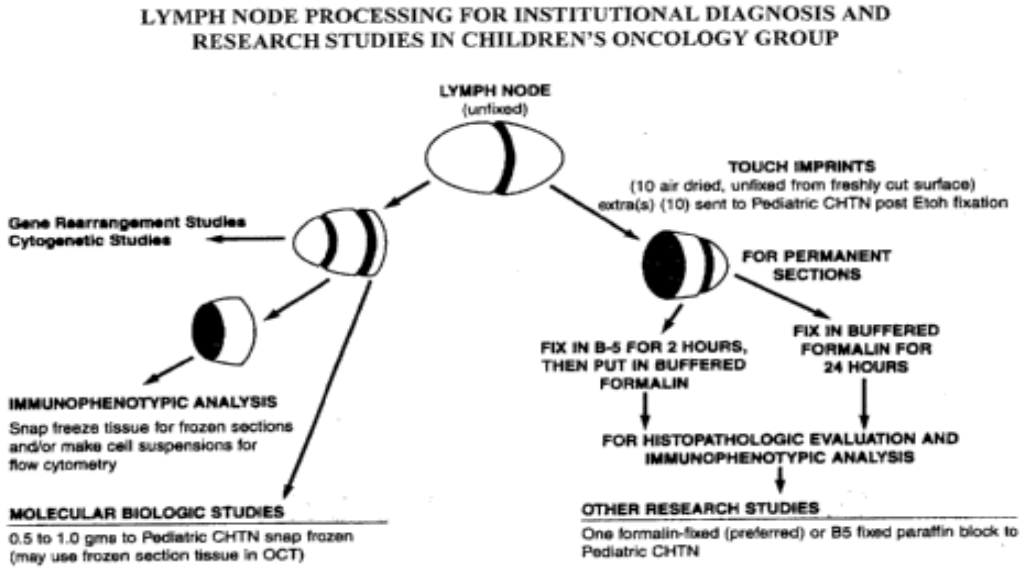
NOTE: Tissue for central review is strongly encouraged but not required for enrollment. The distinction between T-ALL and T-LLy is based on morphology on bone marrow aspirate as determined by local institutions and not by central MRD. Bilateral bone marrow aspirates and biopsies are strongly preferred but not required for study eligibility. A unilateral bone marrow aspirate for morphology and for central MRD bone marrow assessment are required to be eligible.

13.2 Requirements for Handling Tissue or Cytology Specimens at Primary Institutions

13.2.1 Tissue Specimens

Tissue should preferentially, whenever possible, be obtained fresh and delivered immediately to the Pathology Laboratory for optimal handling and distribution (fixation, snap freezing, cytogenetics, etc.). Refer to diagram entitled 'Lymph Node Processing For Institutional Diagnosis And Research Studies In Children's Oncology Group' (Figure 13.1). Submit representative tissue sections for fixation including at least one block with 10% buffered formalin.

Figure 13.1



13.2.2 Cytology Specimens

Cytology or body fluid specimens (i.e. pleural fluid) should be delivered promptly to the pathology laboratory, and handled per primary institutional procedures. Sufficient material should be utilized for morphologic evaluation by cytocentrifuge preparations stained with a Romanowsky stain (i.e. Giemsa or Wright's stains). Provided enough specimen is available, at least one cell block should be prepared with specification of the fixative utilized and the time in fixative

13.3 Immunophenotyping Recommendations for Primary Institutions

For eligibility in this study, the methodology and criteria for immunophenotypic analysis defined by the submitting institution will be accepted. Recognized methods include: paraffin section immunohistochemistry, frozen section immunohistochemistry, cytocentrifuge (cytospin) immunocytochemistry, and flow cytometry.

For eligibility in this study, an extensive panel of antibodies should be employed for immunophenotypic evaluation. This can be done on snap frozen tissue by immunohistochemistry, and body fluid/cytology specimens by flow cytometry or cytocentrifuge (cytospin) immunocytochemistry. This panel of antibodies is listed as follows:

T-Cell: CD1a, CD2, CD3, CD4, CD5, CD7, CD8.
B-Cell: CD19, CD20, Kappa, Lambda.
Myeloid: CD13, CD14, CD33.
Other: CD10, CD25, CD34, CD45, TdT.

The method of TdT evaluation should be specified (i.e. flow cytometry, immunofluorescence, immunohistochemistry).

For cases in which no paraffin embedded tissue has been prepared, and only stained cytospin slides remain available, these cases will be acceptable for protocol submission and pathology review when

adequate immunophenotypic data is available from the primary institution. This situation may occur with cases evaluated by cytospin immunocytochemistry or flow cytometry immunophenotyping.

If specimen is limited, preventing a complete immunophenotypic evaluation, a recommended minimum panel of antibodies should include: CD3, CD5, CD19, CD79a and TdT. If specimen is limited to paraffin embedded tissue only, a preferred panel of antibodies should include at least: CD45RO (UCHL-1), CD79a, and TdT. If additional antibodies that may be utilized in paraffin embedded tissue are available at the primary institution, the panel may include: CD3 (polyclonal), CD43 (Leu22), CD22, PAX5[99,100], and CD45RA (4KB5). If immunophenotyping studies are not available locally, the case may be sent as a consultation case for evaluation including immunophenotyping studies to Dr. Rodney Miles (see address in Section 13.5.6).

13.4 Pathology Staging Criteria

Cerebrospinal Fluid: Leukocyte count greater than or equal to 5/µL, with presence of blasts. TdT evaluation is strongly recommended.

Bone Marrow: The presence of greater than 5% and less than 25% blasts in a bone marrow aspirate, or focal infiltration in a bone marrow biopsy, represents involvement of the marrow by lymphoblastic lymphoma.

13.5 Retrospective Central Pathology Review

13.5.1 Requested Materials

Materials to be submitted for retrospective pathology review to the COG Biopathology Center include the following:

- Initial diagnostic material prior to therapy
- Specimens demonstrating relapse of lymphoma at any time
- Specimens from residual masses demonstrating residual lymphoma or complete response to therapy
- A copy of all final pathology reports (see details in Section 13.5.1d)
- Pathology Data Collection Form
- AALL1231 Transmittal Form

Please label all materials with the patient's COG patient identification number and the institutional pathology number and block number found on the corresponding pathology report. The materials to be submitted are described below and listed in Table 13-1.

a.) Paraffin Blocks

If possible, it is preferred that paraffin blocks be submitted to the COG Biopathology Center. For surgical biopsy specimens, this should include a paraffin block of tissue prepared in 10% Buffered Formalin (as described in Section 13.5.4). For cytology specimens, a paraffin block may be available as a cell block preparation (see Section 13.5.1.5). If paraffin blocks cannot be submitted, then submit twenty (20) unstained sections (4 microns thick) of unbaked slides air-dried at room temperature from one representative block and two (2) H&E stained slides from each block. These sections should be place on sialinized slides (i.e. Fisher Superfrost Plus).

b.) Cytology Slides

When paraffin blocks have not been prepared, a cytologic preparation of one stained, air-dried cytospin slide (i.e. Romanowsky stain such as Giemsa or Wright's stain) and 10 unstained slides should be submitted.

c.) Biopsies of Residual Masses
For these biopsy specimens, send a recut slide (hematoxylin and eosin stain) from all of the paraffin blocks for review. The corresponding pathology report should accompany the slides for review.

d.) Pathology Reports
A copy of all pathology reports on each case should be submitted. This should include: Final reports of diagnostic biopsy and bone marrow specimens (even if negative) All immunophenotyping reports of diagnostic biopsy and bone marrow specimens (if available); also include copies of flow cytometry histograms (if available) Results of any genotypic studies (i.e. gene rearrangement studies) Results of any cytogenetic (karyotypic) analysis

e.) Pathology Data Collection Forms
A separate pathology data collection form (Institutional Pathology Form) should be completed and submitted along with the above materials. Also, indicate the primary institution pathology diagnosis utilizing the WHO Lymphoma Classification[98] on the data collection form.

13.5.2 Transmittal Form
An AALL1231 specimen transmittal form must be submitted along with the pathology review materials.

13.5.3 Biopathology Center Address
All material submitted for central pathology review should be sent via regular mail or using your institutional courier account to:

COG Biopathology Center
Nationwide Children's Hospital
700 Children's Drive, WA 1340*
Columbus, OH 43205
Phone: (614) 722-2865
Fax: (614) 722-2897
Email: BPCParaffinTeam@nationwidechildrens.org

* The room number is required. Packages not listing the room number could be denied and returned to sender.

13.5.4 Paraffin Blocks and Cytologic Slides-Storage/Return
Paraffin blocks and cytologic slides will be retained at the COG Biopathology Center indefinitely, unless the institution requests their return.

13.5.5 Lymphoma Classification
Morphologic evaluation and classification of the study cases will utilize the criteria described in the WHO Lymphoma Classification.[98] Eligible pediatric lymphomas will be classified as precursor T-cell lymphoblastic lymphoma.

13.5.6 Review Pathologists
For any questions regarding the pathology protocol, please contact:

Rodney R. Miles, MD PhD
ARUP Laboratories Hematopathology
500 Chipeta Way
Salt Lake City, UT 84108
Phone: (801) 213-3448 (801) 581-5854

Email: rodney.miles@path.utah.edu

13.6 **Preparation of Tissue Banking Samples at Time of Diagnosis or Relapse- for T-LLy patients not enrolled on Project:EveryChild (APEC14B1)**

At diagnosis, at least one square centimeter of snap frozen tumor is requested in addition to the material required for central review (described in Section 13.5). If more than 1 gram is available, cut tissue into 1 gram aliquots. Wrap each piece of tissue in a separate piece of foil and snap freeze in vapor phase liquid nitrogen (do not submerge the tissue in liquid nitrogen) or cold isopentane. Label the foil with either "P" or "M" to designate whether the tissue submitted is primary or metastatic. Place tissue in the appropriate zip-lock bag (primary or metastatic) and, using a waterproof marker, label the bag with the patient's BPC number, specimen type and date obtained. Document the anatomic site of collection on the transmittal form. Store specimens at -70°C or colder until shipped. Include a transmittal form and pathology with each shipment of specimens.

If tumor tissue is obtained at the time of relapse for clinical purposes, additional material (as described above for diagnosis) is requested for banking and subsequent biologic studies.

The Biopathology Center (BPC) will bank the tissue for future distribution and use including the studies listed above.

13.6.1 Specimen Shipping Instructions

Specimen Procurement Kits for shipping frozen tumor tissue to the BPC are provided upon request. To request a Specimen Procurement Kit, use the following link:

https://ricapps.nationwidechildrens.org/KitManagement/

Select 'AALL1231' from the protocol list to order kits for the submission of frozen tumor tissue from T-LLy patients.

Frozen specimens must be shipped to the BPC, Monday through Thursday for delivery Tuesday through Friday.

1. Before the frozen tissue is placed into the Specimen Procurement Kit, it must first be placed in three separate layers of packaging :
    a. Place the tissue in a zip-lock bag.
    b. Place the zip-lock bag in the biohazard diagnostic envelope. Expel as much air as possible and seal the envelope securely.
    c. Place the biohazard envelope inside the Tyvek envelope. Expel as much air as possible and seal securely.
2. Place the tissue inside the kit compartment with dry ice. Layer the bottom of the compartment with dry ice until it is approximately one-third full. Place the frozen specimens on top of the dry ice. Cover the specimens with the dry ice until the compartment is almost completely full.
3. Place the transmittal form and pathology report inside the compartment.
4. Place the foam lid on top to secure specimens during shipment.
5. Close the outer lid of the Specimen Procurement Kit and tape with filament or other durable sealing tape.
6. Access Kit Management to print a Federal Express shipping label. A blank adhesive label is provided in the Specimen Procurement Kit to use when printing the shipping label.

Attach the shipping label to the top of the kit. Complete the dry ice label (UN 1845). Attach the dry ice and Exempt Human Specimen labels to the side of the kit.. Arrange for Federal Express pick-up per your usual institutional procedure or by calling 1-800-238-5355.

Ship specimens to:
COG Biopathology Center
Nationwide Children's Hospital
700 Children's Drive, Room WA1340*
Columbus, OH 43205
Phone: (614) 722-2865
Email: BPCBank@nationwidechildrens.org

* The room number is required. Packages not listing the room number could be denied and returned to sender.

14.0 MRD ANALYSIS GUIDELINES AND REQUIREMENTS

14.1 Minimal Residual Disease- Required and Optional Specimens: T-ALL patients

The following MRD assessments are **required** for T-ALL patients. Bone marrow will be collected and shipped at the following time points:

- Diagnosis (done as part of AALL08B1 or Project:EveryChild)
- *End of Induction
- *End of Consolidation (IR and VHR patients with end of Induction MRD ≥ 0.01%)
- *End of High Risk Block #3 (for VHR T-ALL patients) in order to assess disease involvement in the bone marrow and for subsequent risk stratification.

***These samples will be shipped to the COG ALL Reference Flow Cytometry Lab using the shipping and handling requirements in Appendix IX.**

NOTE: Day 8 peripheral blood MRD is NOT sent on this study

14.2 Minimal Residual Disease- Required and Optional Specimens: T-LLy Only*

The following MRD assessments are **REQUIRED** for T-LLy patients as a part of AALL1231. Bone marrow will be collected and shipped at the following time point:

- Diagnosis

***NOTE: Only a unilateral specimen should be sent for MRD testing at diagnosis.**

The following MRD assessments are **OPTIONAL** for T-LLy patients as part of AALL1231. Bone marrow will be collected and shipped at the following time point to assess whether MRD can predict EFS and OS in T-LLy:

- **End of Induction

**** These samples will be shipped to the COG ALL Reference Flow Cytometry Lab using the shipping and handling requirements in Appendix IX. Only a unilateral specimen should be sent for MRD testing at end of Induction.**

15.0 SPECIAL STUDIES SPECIMEN REQUIREMENTS

Correlative studies are **optional**, and **not required** for risk-stratification or treatment assignment. These tests are not required for treatment decisions but are essential for advancing the field.

15.1 **Evaluating Mechanisms of Bortezomib Response and Resistance in T-ALL and Identifying Biomarkers and Mechanisms of Chemotherapy Resistance and Response in T-ALL, Focusing on Early Thymic Precursor (ETP) ALL.**

This optional study is open to **all** T-ALL patients (Arm A and B and is NOT limited to patients receiving bortezomib.). Peripheral blood and bone marrow will be collected. Please check the protocol website for updates.

The goal of this study is to understand the mechanisms of bortezomib action and the mechanisms of bortezomib resistance and to compare signaling pathway abnormalities between ETP ALL and non-ETP ALL. Appendix XII provides additional details regarding experimental design.

For consenting patients of Arm A and Arm B, we are requesting a pretreatment and end of Induction bone marrow sample (peripheral blood can be substituted if >80% blasts), and 4 peripheral blood samples. Peripheral blood will be collected at the following time points:

- Pre-treatment
- 4-8 hours (ideally at 6 hours) after initiating Induction chemotherapy
- 22-26 hours (ideally at 24 hours) after initiating Induction chemotherapy
- End of Induction.

If randomized to the bortezomib-containing arm post-treatment samples are timed relative to first dose of bortezomib.

See Appendix X for sample collection, processing, and shipping information for the Horton Lab.

15.2 **OPTIONAL TISSUE BANKING**

15.2.1 Studies of Genomic Variation

There is substantial evidence that both inherited germline constitutional and somatically-acquired ALL-specific genomic variation may contribute to variations in response[186-195] AALL08B1 and successor study Project:EveryChild (APEC14B1) will serve as the mechanism by which materials will be collected and banked for genomic research, all of which must be approved by the ALL Biology committee. The AALL08B1 protocol and the Project:EveryChild (APEC14B1) protocol and Manual of Procedures include information on consent, collection, shipping, processing, analysis, and storage of diagnostic samples. The same guidelines and instructions for collection, shipping, processing, analysis, and storage will be used for subsequent samples, including End of Induction, End of Consolidation, and relapse.

A blood sample at Day 29 is being requested specifically for the purpose of providing constitutional (germline) DNA from each patient. Whenever possible, an aliquot of diagnostic BM or PB will also be set aside for extraction of somatic tumor DNA. In some cases, RNA may be used as the starting material.

COG maintains an ALL cell bank that includes specimens from legacy CCG and POG ALL cell banks, COG ALL classification protocols (AALL03B1 and AALL08B1) and specimens collected prospectively from patients enrolled in current trials, including Project:EveryChild (APEC14B1). Specimens are available to any qualified investigator, from COG and non-COG institutions. Applications are submitted electronically to the Chair of the COG ALL Cell Bank Committee and reviewed by the Cell Bank Committee following established policies. In general, every effort is made to supply qualified investigators with small numbers (10-20) of non-scarce specimens. For large studies and

particularly those that request scarce specimens (e.g., those from low frequency genetic or clinical subsets, relapsed samples or sample sets containing patients of defined outcomes), the committee prioritizes the use of samples based on scientific merit to ensure adequate supply for future research questions.

Specimens from the COG ALL cell bank are supplied to investigators following scientific review and approval of their proposal. Released samples may include vials of cryopreserved cells, or aliquots of nucleic acids, depending upon the needs of the investigator and the supplies that are available. Following scientific approval, investigators must sign a formal letter of collaboration and provide evidence of local IRB approval for the proposed laboratory studies. Any proposal that involves greater than 100 patients must also be approved by CTEP prior to sample release (for banked tissue request form, see https://members.childrensoncologygroup.org/News/Newsitem.asp?ID=11427). The samples that are sent to investigators are coded.

16.0 RADIATION THERAPY GUIDELINES

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 (and up to 1 week for surgery) for valid clinical, patient and family logistical, or facility, procedure and/or anesthesia scheduling issues are acceptable per COG administrative Policy 5.14 (except where explicitly prohibited within the protocol).

Radiation therapy (RT) for patients on COG protocols can only be delivered at approved COG RT facilities (per COG administrative policy 3.9).

16.1 Cranial Radiation Therapy

All CRT will be given during the 1st cycle (first 4 weeks) of Maintenance. See the tables below to determine which subjects will receive CRT.

T-ALL- Cranial Radiation Therapy

	SR	IR	VHR
CNS 1	none	none	CRT (1200 cGy)
CNS 2	none	none	CRT (1200 cGy)
CNS 3	n/a	CRT (1800 cGy)	CRT (1800 cGy)

T-LLy- Cranial Radiation Therapy

	SR	IR	VHR
CNS 1	none	none	none
CNS 2	none	none	none
CNS 3	n/a	CRT (1800 cGy)	CRT (1800 cGy)

16.1.1 Equipment and Calibration

16.1.1.1 Modality

X-ray beams with a nominal energy between 4 and 6 MV are permitted. The use of IMRT is not permitted in this study.

16.1.1.2 Calibration

Calibrations of therapy units used in this protocol shall be verified by the Radiological Physics Center (RPC).

16.1.1.3 Target Volume

The target volume consists of the entire brain and meninges, including the frontal lobe as well as posterior halves of the globes of the eyes, with the optic disc and nerve, extending superior to the vertex and posterior to the occiput. The caudal border will be below the skull base at the C2 vertebral level.

The target volume shall be defined by means of CT or conventional simulation. Care must be taken to avoid shielding the posterior orbit and cribriform plate. In case of conventional simulation, radio-opaque markers should be placed on the surface of the fleshy canthus to aid in localizing this point.

16.1.2 Target Dose

16.1.2.1 Prescription Point

The prescription point in the cranial volume is at or near the center. For multi-convergent beams, the prescription point is usually at intersection of the beam axes. Note: Regardless of the location of central axis, dose should be prescribed at or near the center of the cranial volume (midway between the maximum separation).

16.1.2.2 Dose Definition

Absorbed dose is specified in centigrays (cGy)-to-muscle.

16.1.2.3 Tissue Heterogeneity

No tissue heterogeneity corrections, such as for bone attenuation, will be made.

16.1.2.4 Prescribed Dose and Fractionation

Very High Risk T -ALL patients who are CNS 1 or 2

These patients will receive prophylactic cranial radiation, consisting of a total dose of 1200 cGy given in 8 daily fractions of 150 cGy per fraction, administered Monday through Friday.

All T-ALL and T-LLy who are CNS3

All patients who are CNS3 at diagnosis will receive cranial radiation consisting of a total dose of 1800 cGy given in 10 daily fractions of 180 cGy per fraction, administered Monday through Friday.

16.1.2.5 Dose Uniformity

Dose variations in target volume will be within +7%, -5% of prescription-point dose. (From ICRU Report 50: small high-dose volumes can be excluded from evaluation of dose uniformity but not small low-dose volumes.)

16.1.2.6 Treatment Interruptions

No corrections will be made for treatment interruptions less than 7 days. For interruptions greater than 7 days, contact the Study Radiation Oncology Coordinator.

16.1.3 Treatment Technique

16.1.3.1 Patient Position
It is recommended that the patient be treated in supine with immobilization appropriate for the child such as a face mask and well-fitting headrest.

16.1.3.2 Beam Configuration
The cranial volume is treated with two lateral, equally-weighted photon beams. Fields shall extend at least 1 cm beyond periphery of the scalp.

16.1.3.3 Field Shaping
Field-shaping will be done with blocks which are at least 5 half-value layers (HVLs) thick. Multi-leaf collimators are acceptable.

16.1.2.11 Eye Protection
A simple method to minimize lens irradiation, while treating posterior halves of eyes, is to let central axes of the horizontal cranial beams go through both orbits. The anterior edges of beams are defined by an external block or by an independently controlled collimator and meet at a point 1 cm anterior to frontal lobe meninges. Shielding blocks cover the anterior halves of eyes and protect nose and mouth. Essentially the same geometry can be achieved with by placing central axes through center of head by angling lateral fields so rays through the eyes lie in the same horizontal plane. It is acceptable to use a parallel-opposed beam-pair, without such angling, with shielding blocks that cover the anterior half of proximal eye. The dose to contralateral lens will then increase

16.2 **Testicular Radiation**
T-ALL and T-LLy patients with persistent testicular disease at end-Induction should receive radiation to the testes during Consolidation. A testicular biopsy should be performed if the clinical findings are equivocal. During the first two weeks of Consolidation, testicular radiation therapy will be given at 2400 cGy in 12 once-daily fractions of 200 cGy. Testicular radiation must be started during Consolidation and should be completed before the end of this phase of therapy. **Patients with testicular leukemia at diagnosis that clinically resolves completely by end-Induction, and those that have a negative testicular biopsy at end-Induction will NOT receive testicular radiation.**

16.2.1 Equipment and Calibration

16.2.1.1 Modality
High-energy photon or electron beams are permitted. Selection of energy is determined by dose uniformity criterion, and with electrons, lowest possible energy should be used to spare tissues outside target volume. IMRT is not permitted in this study.

16.2.1.2 Calibration
Calibrations of therapy machines used in this protocol will be verified by the Radiological Physics Center.

16.2.1.3 Target Volume
The target volume consists of testes in the scrotal sac. (Note: The cremasteric reflex may move testes high up in inguinal canal.) The field may be reduced as the palpably enlarged mass decreases in size during treatment.

16.2.2 Target Dose

16.2.2.1 Prescription Point
Prescription point is at or near center of planning target volume.

16.2.2.2 Dose Definition
Absorbed dose is specified as centigrays (cGy)-to-muscle.

16.2.2.3 Prescribed Dose and Fractionation
Total dose to prescription point will be 2400 cGy in 12 daily fractions of 200 cGy per fraction, administered Monday through Friday.

16.2.2.4 Dose Uniformity
Variations of dose within planning target volume will be within +7%, -5% of dose to prescription point. The uniformity requirement can be met with electron beam of appropriate energy provided bolus is used, which is simplest technique. Bolus may also be needed for photon beams to fulfill dose uniformity requirement. (From ICRU Report 50: small high-dose volumes can be excluded from evaluation of dose uniformity, but not small low-dose volumes.)

16.2.2.5 Treatment Interruptions
No corrections will be made for treatment interruptions less than 7 days. For interruptions greater than 7 days, contact the Study Radiation Oncology Coordinator.

16.2.3 Treatment Technique

16.2.3.1 Patient Position
Patient will be treated in supine position.

16.2.3.2 Field Shaping
Field shaping can be done with blocks of at least 5 HVLs thick. Multi-leaf collimators are acceptable.

16.2.3.3 Normal Tissue Sparing
Testes will be supported posteriorly and, if possible, extended caudally in order to minimize perineal irradiation. Field shall not be angled towards perineum. The penis shall be excluded from field by fixing it to skin over the symphysis pubis.

16.3 Quality Assurance Documentation

16.3.1 IROC RHODE ISLAND (FORMERLY QARC) Post Treatment Review
Patients receiving RT on this study will have a review only of the dose delivered. There is no on-treatment review and no target volume review.
Within one week of the completion of radiotherapy, the following data will be submitted:

"RT-2 Radiotherapy Total Dose Record" form.
Copy of patient's radiation therapy chart, including prescription, and daily and cumulative doses.

16.3.2 Data must be sent to

IROC Rhode Island (formerly QARC)
Building B, Suite 201
640 George Washington Highway

Lincoln, RI 02865-4207
Phone: (401) 753-7600
Fax: (401) 753-7601

16.3.3 Questions regarding the dose calculations or documentation should be directed to

COG Protocol Dosimetrist
IROC Rhode Island (formerly QARC)
Building B, Suite 201
640 George Washington Highway
Lincoln, RI 02865-4207
Phone: (401) 753-7600
Fax: (401) 753-7601

16.3.4 Questions regarding the radiation therapy section of this protocol should be directed to the Study Radiation Oncology Coordinator

Samir I. Patel, MD
University of Alberta-Stollery Children's Hospital
11560 University Avenue
Edmonton, AB T6L 6J5
Phone: (780) 432-8518
Fax: (780) 432-8380
E-mail: samir.patel2@albertahealthservices.ca

16.4 Definitions of Deviation in Protocol Performance

16.4.1 Minor Deviation
Dose to prescription point differs from that in protocol between 6% and 10%.

16.4.2 Major Deviation
Dose to the prescription point differs from that in the protocol by more than 10%.

APPENDIX I: THERAPY DELIVERY MAPS – ARM A (T-ALL and T-LLy)

APPENDIX I-A

INDUCTION- Arm A (without bortezomib)

This Induction course is for patients randomized to treatment on Arm A (no bortezomib) See Section 4.3 for details.

Patient name or initials _____ DOB _____

Patients must complete and sign the appropriate informed consent and undergo bortezomib randomization before starting Induction therapy. This Course lasts 5 weeks (35 days) and this Therapy Delivery Map is on **one (1)** page.

DRUG	ROUTE	DOSAGE	DAYS	IMPORTANT NOTES	OBSERVATIONS
Intrathecal Cytarabine (IT ARAC)	IT	Age (yrs) Dose 1 – 1.99 30 mg 2 – 2.99 50 mg ≥ 3 70 mg	Given at time of diagnostic lumbar puncture (LP)* **OR** Day 1	May give prior to randomization **Note age-based dosing**	a. Hx/PE with VS/Wt (BSA), CBC/diff/plts b. Bilirubin (total and direct), ALT, AST creatinine c. CSF cell count & cytospin[2] d. TPMT and NUDT15 genotype (if available) e. BM MRD f. Performance status g. Pregnancy test h. BM cytomorphology i. CXR j. Cell Banking (optional) **T-LLy Only** k. Chest CT l. Abdomen/pelvis CT (or MRI) m. Bone scan n. Diagnostic biopsy/cytology[4] o. PET scan **Optional Studies T-ALL ONLY** Bortezomib Response Study (open to Arms A and B): See Section 15.1 for details p. PB sample q. BM sample **OBTAIN OTHER STUDIES AS REQUIRED FOR GOOD PATIENT CARE**
VinCRIStine (VCR)	IV Push Over 1 min+	1.5 mg/m^2/dose	Days 1, 8, 15 & 22	+ Or infusion via minibag as per institutional policy **Maximum dose of 2 mg**	
Dexamethasone (DEX)	PO (may be given IV)	3 mg/m^2/dose BID	Days 1-28 (no taper)	Total daily dose: 6 mg/m^2/day, divided BID	
DAUNOrubicin (DAUN)	IV Push/infusion Over 1-15 min	25 mg/m^2/dose	Days 1, 8, 15 & 22		
PEG aspargase (PEG-ASP)	IV over 1-2 hours	2500 International units/m^2 **x 1 dose**	Days 4 & 18	Administer through the tubing of a freely infusing solution of D_5W or 0.9% NaCl	
Intrathecal Methotrexate (IT MTX)	IT	Age (yrs) Dose 1 – 1.99 8 mg 2 – 2.99 10 mg 3 – 8.99 12 mg ≥ 9 15 mg	Days 8 & 29 (Days 15 & 22 **for CNS3 ONLY)**	**Note age-based doing** **Please note CNS3**	

Therapy Delivery Map **Ht_____cm** **Wt_____kg** **BSA____m**

Date Due	Date Given	Day	IT ARAC mg	VCR mg	DEX mg mg	DAUN mg	PEG-ASP IU	IT MTX mg	Studies	Comments
			Enter calculated dose above and actual dose administered below							
		-2/-1/0/LP							(a,b,f,g,h,i,j$)@ (k,l,m[8],n)[4,@] (p)[5,^] (e)$	
		1	____mg*	____mg	____mg ____mg	____mg			b,c*, d[11],n[1,5]	
		4					____IU **(1 dose)**			
		8		____mg		____mg		____mg	a, c	
		15		____mg		____mg		____mg#	a, c#,	
		18					____IU **(1 dose)**			
		22		____mg		____mg		____mg#	a, c#	
		29						____mg	a, b,c,e[3],h[10], (i ,k[12],l[7],m[8],o[9]) [4] (p,q)[5]	
		36	**Start next course (Consolidation, Appendix I-B) on Day 36 or when blood count parameters are met (whichever occurs later) Patients with Day 29 marrow M2 or M3 or MRD >5% should start as soon as possible (not wait for Day 36 or count recovery to occur.)**							

*On Day 1 **OR** at the time of diagnostic lumbar puncture (LP) if < than 72 hours from the start of protocol therapy.

$ **T-ALL:** Done as part of AALL08B1 or APEC14B1 **T-LLy**: If enrolled on APEC14B1, submit as a part of APEC14B1. If not enrolled on APEC14B1, submit as part of this protocol (see Section 13.6 for details) #CNS3 patients ONLY @Baseline ^ Pre-treatment

[1] For all patients who consent, collect at hours 0, 6 and 24. See Section 15.1 for details. [2] Obtain with each IT administration

[3] Optional for T-LLy. Required for T-ALL [4] T-LLy ONLY [5]T-ALL ONLY [6] follow-up exams only if baseline demonstrates disease.

[7] Many patients will have no disease below the diaphragm; if the abdominal and pelvic CTs at Baseline are negative, no repeat scans are required.

[8] Bone scan only if patient has bone symptoms; follow-up exams only if baseline demonstrates disease. A PET scan can be used as a substitute for bone scans; however, a bone scan is preferred in patients with bone symptoms.

[9] PET scan is recommended but not required at baseline. If a PET is obtained at baseline, PET imaging should be continued with subsequent response assessments until patient no longer has PET-avid disease.

[10]**T-LLy:** only if positive at diagnosis

[11] To be performed any time during Induction [12]If a PET-CT was obtained at diagnosis, it can be used instead of a regular CT to follow response.

SEE PROTOCOL SECTION 5.0 FOR DOSE MODIFICATIONS. SEE THE COG MEMBER WEBSITE FOR SUPPORTIVE CARE GUIDELINES.

APPENDIX I-B

CONSOLIDATION Arm-A This Consolidation course is for patients on Arm A. See Section 4.6 for details.	_____ Patient name or initials	_____ DOB

Start Consolidation on Day 36 (7 days following Day 29 LP) or when peripheral counts recover with ANC ≥ 750/µL and platelets ≥ 75,000/µL (whichever occurs later). Patients with Day 29 marrow M2 or M3 or MRD >5% should start as soon as possible (not wait for Day 36 or count recovery to occur.) Once Consolidation therapy has begun, interruptions for myelosuppression should occur only at Day 29. Patients with T-ALL and persistent testicular disease at end-Induction will receive XRT to the testes during Consolidation (a testicular biopsy is required if there is any uncertainty regarding the clinical response). Within the first two weeks of Consolidation, testicular radiation therapy will be given at 2400 cGy in 12 once-daily fractions of 200 cGy (See Section 16.2). Testicular radiation must be started during Consolidation and should be completed before the end of this phase of therapy. This Course lasts 8 weeks (56 days) and this Therapy Delivery Map is on **two (2)** pages.

DRUG	ROUTE	DOSAGE	DAYS	IMPORTANT NOTES	OBSERVATIONS
Intrathecal Methotrexate (IT MTX)	IT	Age (yrs) Dose 1 – 1.99 8 mg 2 – 2.99 10 mg 3 – 8.99 12 mg ≥ 9 15 mg	Days 1, 8, 15, 22 **Omit Days 15 & 22 for CNS3 patients ONLY**	**Note age-based dosing** **Please note CNS3**	a. Hx/PE with VS/Wt (BSA) b. CBC/diff/plts c. CSF cell count & cytospin[2] d. Bilirubin (total and direct), ALT, AST creatinine e. Performance Status f. BM MRD[1,4] g. Bone Marrow cytomorphology T-LLy Only h. Chest CT/CXR i. Abdomen/pelvis CT (or MRI), Bone scan, PET scan **OBTAIN OTHER STUDIES AS REQUIRED FOR GOOD PATIENT CARE**
Cyclophosphamide (CPM)	IV over 30-60 minutes	1000 mg/m^2/dose	Days 1 & 29	See Section 4.6 for admin guidelines	
Cytarabine (ARAC)	IV or SubQ	75 mg/m^2/dose	Days 1-4, 8-11, 29-32 & 36-39		
Mercaptopurine (MP)	PO	60 mg/m^2/dose	Days 1-14 & 29-42	See Section 4.6 and Appendix VI for administration guidelines	
VinCRIStine (VCR)	IV Push over 1 min$^+$	1.5 mg/m^2/dose	Days 15, 22, 43 & 50	+ Or infusion via minibag as per institutional policy **Maximum dose of 2 mg**	
Pegaspargase (PEG-ASP)	IV over 1-2 hours	2500 International units/m^2/dose	Days 15 & 43	Administer through the tubing of a freely infusing solution of D_5W or 0.9% NaCl	
T-ALL patients with biopsy proven testicular disease at end-Induction will receive testicular XRT. See Section 4.4 & Section 16.0 for additional details.					

APPENDIX I-B

CONSOLIDATION Arm-A This Consolidation course is for patients on Arm A. See Section 4.6 for details.	_____ Patient name or initials	_____ DOB

Start Consolidation on Day 36 (7 days following Day 29 LP) or when peripheral counts recover with ANC ≥ 750/µL and platelets ≥ 75,000/µL (whichever occurs later). Patients with Day 29 marrow M2 or M3 or MRD >5% should start as soon as possible (not wait for Day 36 or count recovery to occur.) Once Consolidation therapy has begun, interruptions for myelosuppression should occur only at Day 29. Patients with T-ALL and persistent testicular disease at end-Induction will receive XRT to the testes during Consolidation (a testicular biopsy is required if there is any uncertainty regarding the clinical response). Within the first two weeks of Consolidation, testicular radiation therapy will be given at 2400 cGy in 12 once-daily fractions of 200 cGy (See Section 16.2). Testicular radiation must be started during Consolidation and should be completed before the end of this phase of therapy. This Course lasts 8 weeks (56 days) and this Therapy Delivery Map is on **two (2)** pages.

Therapy Delivery Map **Ht_____cm** **Wt_____kg** **BSA____m²**

Date Due	Date Given	Day	IT MTX ____mg		CPM ____mg	ARAC ____mg	MP ____mg	VCR ____mg	PEG-ASP ____IU	Studies	Comments
			CNS3 T-ALL	**All other patients**							
			Enter calculated dose above and actual dose administered below								
		1$	____mg	____mg	____mg	____mg	____mg			a,b,c,d,e[5]	
		2				____mg					
		3				____mg					
		4				____mg					
		8	____mg	____mg		____mg				b,c	
		9				____mg					
		10				____mg					
		11				____mg					
		14									
		15		____mg				____mg	____IU	b,c	
		22		____mg				____mg		b,c	
		29			____mg	____mg	____mg			b	
		30				____mg					
		31				____mg					
		32				____mg					
		36				____mg				b	
		37				____mg					
		38				____mg					
		39				____mg					
		43						____mg	____IU	b	
		50						____mg		b	
		56								b,g[6], (i[7],j[8])[3]	
		57								f[1,4]	
			Start next course (Based on Risk Assignment See Section 4.1.2) on Day 57 or when blood count parameters are met (whichever occurs later).								

[1] End of Consolidation bone marrow is not performed on SR T-ALL patients. It is REQUIRED for T-ALL patients with end Induction BM MRD ≥ 0.01% ONLY

[2] Obtain with each IT administration [3] T-LLy ONLY [4] T-ALL only [5] Prior to Day 1 therapy

[6] T-LLy: Only if positive at end Induction. See section 7.0 for additional details.

[7] If patient has CR at end-Induction perform a CXR. If PR or NR at end-Induction perform a chest CT (If a PET-CT was obtained at diagnosis, it can be used instead of a regular CT to follow response)

[8] If baseline is negative, no repeat scans are required.

[$] T-ALL and T-LLy patients with persistent testicular disease at end-Induction should receive radiation to the testes during Consolidation. See Section 16.2 for details.

SEE PROTOCOL SECTION 5.0 FOR DOSE MODIFICATIONS. SEE THE COG MEMBER WEBSITE FOR SUPPORTIVE CARE GUIDELINES.

THERAPY DELIVERY MAPS – ARM A-SR (T-ALL and T-LLy)

APPENDIX I-C

INTERIM MAINTENANCE with CMTX- Arm A-SR

This IM course is for patients assigned to Arm A-SR. See Section 4.7 for details.

_____ Patient name or initials

_____ DOB

Post-consolidation risk assignment must have been completed as described in Section 4.1. Start when ANC ≥750/µL and platelets ≥75,000/µl. Patients need to meet renal and hepatic function requirements to initiate methotrexate as described in Section 5.10.1, Section 5.10.2, and Section 5.10.3. This Course lasts 8 weeks (56 days) and this Therapy Delivery Map is on **one (1)** page.

DRUG	ROUTE	DOSAGE	DAYS	IMPORTANT NOTES	OBSERVATIONS
VinCRIStine (VCR)	IV push over 1 min+	1.5 mg/m²/dose	Days 1, 11, 21, 31 & 41	+ Or infusion via minibag as per institutional policy **Maximum dose of 2 mg**	a. Hx/PE with VS/Wt (BSA) b. CBC/diff/plts Bilirubin, ALT, creatinine c. CSF cell count & cytospin[1] [1] Obtain with each IT administration **OBTAIN OTHER STUDIES AS REQUIRED FOR GOOD PATIENT CARE**
Methotrexate (CMTX)	IV push over 2-5 mins or IV infusion over 10-15 mins	Starting dose is 100 mg/m² **escalate by 50 mg/m²/dose**	Days 1, 11, 21, 31 & 41	See Section 4.7 and Section 5.10 for details	
Pegaspargase (PEG-ASP)	IV over 1-2 hours	2500 International units/m²/dose	Days 2 & 22	Administer through the tubing of a freely infusing solution of D_5W or 0.9% NaCl	
Intrathecal Methotrexate (IT MTX)	IT	Age (yrs) – Dose 1 – 1.99 – 8 mg 2 – 2.99 – 10 mg 3 – 8.99 – 12 mg ≥ 9 – 15 mg	Days 1 & 31	**Note age-based dosing**	

Therapy Delivery Map **Ht_____cm** **Wt_____kg** **BSA____m²**

Date Due	Date Given	Day	VCR ____mg	IV MTX *(escalating dose)*	PEG-ASP ____IU	IT MTX ____mg	Studies	Comments
			Enter calculated dose above and actual dose administered below					
		1	____mg	____mg		____mg	a,b*,c	
		2			____IU			

		11	____mg	____mg			b*	

		21	____mg	____mg			b*	
		22			____IU			

		31	____mg	____mg		____mg	b*,c	

		41	____mg	____mg			b*	

		56						
		57	**Start next course (Delayed Intensification, Appendix I-D) on Day 57 or when blood count parameters are met (whichever occurs later).**					

[1] Obtain with each IT administration

*To be performed prior to each dose of methotrexate

SEE PROTOCOL SECTION 5.0 FOR DOSE MODIFICATIONS. SEE THE COG MEMBER WEBSITE FOR SUPPORTIVE CARE GUIDELINES.

Page 1 of 1

APPENDIX I-D

DELAYED INTENSIFICATION Arm A-SR (without bortezomib) This Delayed Intensification course is for patients randomized to treatment on Arm A-SR (no bortezomib) See Section 4.8 for details.	Patient name or initials DOB

Patients should have ANC ≥ 750/µL and platelets ≥ 75,000/µL prior to starting therapy. Once Delayed Intensification has begun, it may be interrupted for myelosuppression (ANC ≤ 750/µL and platelets ≤ 75,000/µL) on Day 29 ONLY. Once the Day 1 therapy or Day 29 cyclophosphamide has been given, the remainder of the therapy should not be held solely for myelosuppression, which is expected to occur. Therapy must be interrupted for patients with serious proven or presumed infection and resumed when the signs of infection have abated. Hold Day 29 chemotherapy until ANC ≥ 750/µL and platelets ≥ 75,000/µL. This Course lasts 9 weeks (63 days) and this Therapy Delivery Map is on **two (2)** pages.

DRUG	ROUTE	DOSAGE	DAYS	IMPORTANT NOTES	OBSERVATIONS
VinCRIStine (VCR)	IV push over 1 min+	1.5 mg/m²/dose	Days 1, 8, 15, 43 & 50	+ Or infusion via minibag as per institutional policy **Maximum dose of 2 mg**	a. Hx/PE with VS/Wt (BSA) b. CBC/diff/plts c. CSF cell count & cytospin[1] d. Bilirubin, ALT, creatinine **OBTAIN OTHER STUDIES AS REQUIRED FOR GOOD PATIENT CARE**
Dexamethasone (DEX)	PO (may be given IV)	5 mg/m²/dose BID	Days 1-7 & 15-21	Total daily dose: 10 mg/m²/day, divided BID	
DOXOrubicin (DOXO)	IV Push Over 1-15 min	25 mg/m²/dose	Days 1, 8, & 15		
PEG aspargase (PEG-ASP)	IV over 1-2 hours	2500 International units/m²/dose	Day 4, 18, & 43	Administer through the tubing of a freely infusing solution of D_5W or 0.9% NaCl	
Intrathecal Methotrexate (IT MTX)	IT	Age (yrs) – Dose 1 – 1.99 – 8 mg 2 – 2.99 – 10 mg 3 – 8.99 – 12 mg ≥ 9 – 15 mg	Days 1, 29 & 36	**Note age-based dosing**	

Therapy Delivery Map **Ht_____cm** **Wt_____kg** **BSA_____m²**

Date Due	Date Given	Day	VCR ____mg	DEX ____mg ____mg	DOXO ____mg	PEG-ASP ____IU	IT MTX _____mg	Studies	Comments
				Enter calculated dose above and actual dose administered below					
		1	____mg	____mg ____mg	____mg		_____mg	a,b,c,d	
		2							
		3							
		4				____IU			
		5							
		6							
		7							
		8	____mg		____mg			b	

		15	____mg	____mg ____mg	____mg			b	
		16							
		17							
		18				____IU			
		19							
		20							
		21							
		22						b	
			This therapy delivery map continues on the next page with Day 29.						

[1] Obtain with each IT administration

SEE PROTOCOL SECTION 5.0 FOR DOSE MODIFICATIONS. SEE THE COG MEMBER WEBSITE FOR SUPPORTIVE CARE GUIDELINES.

DELAYED INTENSIFICATION Arm A-SR (without bortezomib)

This Delayed Intensification course is for patients randomized to treatment on Arm A-SR (no bortezomib). See Section 4.8 for details.

Patient name or initials

DOB

DRUG	ROUTE	DOSAGE	DAYS	IMPORTANT NOTES	OBSERVATIONS
VinCRIStine (VCR)	IV push over 1 min+	1.5 mg/m²/dose	Days 1, 8, 15, 43 & 50	+ Or infusion via minibag as per institutional policy **Maximum dose of 2 mg**	a. Hx/PE with VS/Wt (BSA) b. CBC/diff/plts c. CSF cell count & cytospin[1] d. Bilirubin, ALT, creatinine **OBTAIN OTHER STUDIES AS REQUIRED FOR GOOD PATIENT CARE**
Pegaspargase (PEG-ASP)	IV over 1-2 hours	2500 International units/m²/dose	Day 4, 18, & 43	Administer through the tubing of a freely infusing solution of D_5W or 0.9% NaCl	
Cyclophosphamide (CPM)	IV over 30-60 minutes	1000 mg/m²/dose	Day 29	See Section 4.8 for administration guidelines	
Cytarabine (ARAC)	IV over 1-30 min or SubQ	75 mg/m²/dose	Days 29-32 & 36-39		
Thioguanine (TG)	PO	60 mg/m²/dose	Days 29-42	See Section 4.8, Section 5.13 & Appendix VII for administration guidelines	
Intrathecal Methotrexate (IT MTX)	IT	Age (yrs) Dose 1 – 1.99 8 mg 2 – 2.99 10 mg 3 – 8.99 12 mg ≥ 9 15 mg	Days 1, 29 & 36	**Note age-based dosing**	

Therapy Delivery Map **Ht_____cm** **Wt_____kg** **BSA____m²**

Date Due	Date Given	Day	VCR mg	PEG-ASP IU	CPM mg	ARAC mg	TG mg	IT MTX mg	Studies	Comments
			Enter calculated dose above and actual dose administered below							
		29			____mg	_____mg	_____mg	_____mg	b,d,c	
		30								
		31								
		32								
		33								

		36				_____mg		_____mg	b,c	
		37								
		38								
		39								
		40								
		41								
		42								
		43	____mg	____IU					b	
		44								
		45								
		46								
		47								
		48								
		49								
		50	____mg						b	

		57							b	

		63							b	
		64	**Start next course (Maintenance, Appendix I-M) on Day 64 or when blood count parameters are met (whichever occurs later)**							

[1] Obtain with each IT administration

SEE PROTOCOL SECTION 5.0 FOR DOSE MODIFICATIONS. SEE THE COG MEMBER WEBSITE FOR SUPPORTIVE CARE GUIDELINES.

THERAPY DELIVERY MAPS – ARM A-IR (T-ALL and T-LLy)

APPENDIX I-E

INTERIM MAINTENANCE #1 with HDMTX- Arm A-IR

This IM course is only for patients randomized to Arm A-IR. See Section 4.10 for details.

Patient name or initials

DOB

Patients receive this block immediately after consolidation and must meet all of the following criteria: Post-consolidation risk assignment must have been completed as described in Section 4.1. Start when ANC ≥750/µL and platelets ≥75,000/µl. Patients need to meet renal and hepatic function requirements to initiate methotrexate as described in Section 5.9.1, Section 5.9.2, and Section 5.9.3. This Course lasts 8 weeks (56 days) and this Therapy Delivery Map is on **one (1)** page.

DRUG	ROUTE	DOSAGE	DAYS	IMPORTANT NOTES	OBSERVATIONS
High-Dose Methotrexate (HD MTX)	IV over 24 hours	5000 mg/m^2/dose	Days 1, 15, 29 & 43	See Section 5.9 & Appendix IV for administration guidelines. **Note: 2 stage administration**	a. Hx/PE with VS/Wt (BSA) b. CBC/diff/plts Bilirubin, ALT, creatinine c. CSF cell count & cytospin[1] **OBTAIN OTHER STUDIES AS REQUIRED FOR GOOD PATIENT CARE**
Leucovorin (LCV)	PO/IV	15 mg/m^2/dose	42, 48, and 54 hours after the start of the HD MTX infusion	See Section 5.9 and Appendix IV for HD MTX Infusion and Leucovorin rescue guidelines.	
VinCRIStine (VCR)	IV push over 1 min$^+$	1.5 mg/m^2/dose	Days 1, 15, 29 & 43	+ Or infusion via minibag as per institutional policy **Maximum dose of 2 mg**	
Mercaptopurine (MP)	PO	25 mg/m^2/dose	Days 1-56	See Section 4.10, Section 5.11and Appendix VI for administration guidelines	
Intrathecal Methotrexate (IT MTX)	IT	Age (yrs) / Dose 1 – 1.99 / 8 mg 2 – 2.99 / 10 mg 3 – 8.99 / 12 mg ≥ 9 / 15 mg	Days 1 & 29	Deliver within 6 hours of the start of IV MTX (hr -6 to +6) **Note age-based dosing**	

Therapy Delivery Map **Ht_____ cm** **Wt_____ kg** **BSA_____ m^2**

Date Due	Date Given	Day	HD MTX ____mg	LCV	VCR ____mg	MP ____mg	IT MTX ____mg	Studies	Comments
			Enter calculated dose above and actual dose administered below						
		1	____mg		____mg	____mg	____mg	a,b*,c	
		2							
		3		____mg$					
		4							
		5							

		15	____mg		____mg			b*	
		16							
		17		____mg$					
		18							
		19							

		29	____mg		____mg		____mg	b*,c	
		30							
		31		____mg$					
		32							
		33							
		34							

		43	____mg		____mg			b*	
		44							
		45		____mg$					
		46							
		47							

		56							
		57	**Start next course (Delayed Intensification, Appendix I-F) on Day 57 or when blood count parameters are met (whichever occurs later).**						

[1] Obtain with each IT administration

*To be performed prior to each dose of methotrexate

$Please document the number of doses of leucovorin administered

SEE PROTOCOL SECTION 5.0 FOR DOSE MODIFICATIONS. SEE THE COG MEMBER WEBSITE FOR SUPPORTIVE CARE GUIDELINES.

APPENDIX I-F

DELAYED INTENSIFICATION Arm A-IR (without bortezomib)

This Delayed Intensification course is for patients randomized to treatment on Arm A-IR (no bortezomib) See Section 4.8 for details.

Patient name or initials _____

DOB _____

Patients should have ANC ≥ 750/µL and platelets ≥ 75,000/µL prior to starting therapy. Once Delayed Intensification has begun, it may be interrupted for myelosuppression (ANC ≤ 750/µL and platelets ≤ 75,000/µL) on Day 29 ONLY. Once the Day 1 therapy or Day 29 cyclophosphamide has been given, the remainder of the therapy should not be held solely for myelosuppression, which is expected to occur. Therapy must be interrupted for patients with serious proven or presumed infection and resumed when the signs of infection have abated. Hold Day 29 chemotherapy until ANC ≥ 750/µL and platelets ≥ 75,000/µL. This Course lasts 9 weeks (63 days) and this Therapy Delivery Map is on **two (2)** pages.

DRUG	ROUTE	DOSAGE	DAYS	IMPORTANT NOTES	OBSERVATIONS
VinCRIStine (VCR)	IV push over 1 min+	1.5 mg/m²/dose	Days 1, 8, 15, 43 & 50	+ Or infusion via minibag as per institutional policy **Maximum dose of 2 mg**	a. Hx/PE with VS/Wt (BSA) b. CBC/diff/plts c. CSF cell count & cytospin[1] d. Bilirubin, ALT, creatinine **OBTAIN OTHER STUDIES AS REQUIRED FOR GOOD PATIENT CARE**
Dexamethasone (DEX)	PO (May be given IV)	5 mg/m²/dose BID	Days 1-7 & 15-21	Total daily dose: 10 mg/m²/day, divided BID	
DOXOrubicin (DOXO)	IV Push Over 1-15 min	25 mg/m²/dose	Days 1, 8, & 15		
Pegaspargase (PEG-ASP)	IV over 1-2 hours	2500 International units/m²/dose	Day 4, 18, & 43	Administer through the tubing of a freely infusing solution of D_5W or 0.9% NaCl	
Intrathecal Methotrexate (IT MTX)	IT	Age (yrs) – Dose 1 – 1.99 – 8 mg 2 – 2.99 – 10 mg 3 – 8.99 – 12 mg ≥ 9 – 15 mg	Days 1, 29 & 36	**Note age-based dosing**	

Therapy Delivery Map **Ht_____cm** **Wt_____kg** **BSA____m²**

Date Due	Date Given	Day	VCR ____mg	DEX ____mg ____mg	DOXO ____mg	PEG-ASP ____IU	IT MTX _____mg	Studies	Comments
				Enter calculated dose above and actual dose administered below					
		1	mg	____mg ____mg	mg		mg	a,b,c,d	
		2							
		3							
		4				____IU			
		5							
		6							
		7							
		8	____mg		____mg			b	

		15	____mg	____mg ____mg	____mg			b	
		16							
		17							
		18				____IU			
		19							
		20							
		21							
		22						b	
			This therapy delivery map continues on the next page with Day 29.						

[1] Obtain with each IT administration

SEE PROTOCOL SECTION 5.0 FOR DOSE MODIFICATIONS. SEE THE COG MEMBER WEBSITE FOR SUPPORTIVE CARE GUIDELINES.

DELAYED INTENSIFICATION Arm A-IR (without bortezomib)

This Delayed Intensification course is for patients randomized to treatment on Arm A-IR (no bortezomib) See Section 4.8 for details.

Patient name or initials

DOB

DRUG	ROUTE	DOSAGE	DAYS	IMPORTANT NOTES	OBSERVATIONS
VinCRIStine (VCR)	IV push over 1 min+	1.5 mg/m²/dose	Days 1, 8, 15, 43, & 50	+ Or infusion via minibag as per institutional policy **Maximum dose of 2 mg**	a. Hx/PE with VS/Wt (BSA) b. CBC/diff/plts c. CSF cell count & cytospin[1] d. Bilirubin, ALT, creatinine **OBTAIN OTHER STUDIES AS REQUIRED FOR GOOD PATIENT CARE**
Pegaspargase (PEG-ASP)	IV over 1-2 hours	2500 International units/m²/dose	Day 4, 18, & 43	Administer through the tubing of a freely infusing solution of D_5W or 0.9% NaCl	
Intrathecal Methotrexate (IT MTX)	IT	Age (yrs) Dose 1 – 1.99 8 mg 2 – 2.99 10 mg 3 – 8.99 12 mg ≥ 9 15 mg	Days 1, 29 & 36	**Note age-based dosing**	
Cyclophosphamide (CPM)	IV over 30-60 min	1000 mg/m²/dose	Day 29	See Section 4.8 for administration guidelines	
Cytarabine (ARAC)	IV over 1-30 min or SubQ	75 mg/m²/dose	Days 29-32 & 36-39		
Thioguanine (TG)	PO	60 mg/m²/dose	Days 29-42	See Section 4.8, Section 5.13 & Appendix VII for administration guidelines	

Therapy Delivery Map **Ht cm Wt kg BSA m²**

Date Due	Date Given	Day	VCR mg	PEG-ASP IU	IT MTX mg	CPM mg	ARAC mg	TG mg	Studies	Comments
			Enter calculated dose above and actual dose administered below							
		29			mg	mg	mg	mg	b,d,c	
		30								
		31								
		32								
		33								

		36			mg		mg		b,c	
		37								
		38								
		39								
		40								
		41								
		42								
		43	mg	IU					b	
		44								
		45								
		46								
		47								
		48								
		49								
		50	mg						b	

		57							b	

		63							b	
		64	**Start next course (IM #2 CMTX, APPENDIX I-G) on Day 64 or when blood count parameters are met (whichever occurs later)**							

[1] Obtain with each IT administration

SEE PROTOCOL SECTION 5.0 FOR DOSE MODIFICATIONS. SEE THE COG MEMBER WEBSITE FOR SUPPORTIVE CARE GUIDELINES.

APPENDIX I-G

INTERIM MAINTENANCE #2 with CMTX- Arm A-IR This IM course is for patients randomized to Arm A-IR. See Section 4.7 for details.	_____ Patient name or initials _____ DOB

Begin IM when peripheral counts recover with an ANC ≥ 750/µL and platelets ≥ 75,000/µL. Patients need to meet renal and hepatic function requirements to initiate methotrexate as described in Section 5.10. This Course lasts 8 weeks (56 days) and this Therapy Delivery Map is on **one (1)** page.

DRUG	ROUTE	DOSAGE	DAYS	IMPORTANT NOTES	OBSERVATIONS
VinCRIStine (VCR)	IV push over 1 min+	1.5 mg/m²/dose	Days 1, 11, 21, 31 & 41	+ Or infusion via minibag as per institutional policy **Maximum dose of 2 mg**	a. Hx/PE with VS/Wt (BSA) b. CBC/diff/plts Bilirubin, ALT, creatinine c. CSF cell count & cytospin[1] **OBTAIN OTHER STUDIES AS REQUIRED FOR GOOD PATIENT CARE**
Methotrexate (CMTX)	IV push over 2-5 mins or IV infusion over 10-15 mins	Starting dose is 100 mg/m² **escalate by 50 mg/m²/dose**	Days 1, 11, 21, 31 & 41	See Section 4.7 and Section 5.10 for details	
Pegaspargase (PEG-ASP)	IV over 1-2 hours	2500 International units/m²/dose	Days 2 & 22	Administer through the tubing of a freely infusing solution of D_5W or 0.9% NaCl	
Intrathecal Methotrexate (IT MTX)	IT	Age (yrs) — Dose 1 – 1.99 — 8 mg 2 – 2.99 — 10 mg 3 – 8.99 — 12 mg ≥ 9 — 15 mg	Days 1 & 31	**Note age-based dosing**	

Therapy Delivery Map **Ht_____cm** **Wt_____kg** **BSA____m²**

Date Due	Date Given	Day	VCR ____mg	IV MTX *(escalating dose)*	PEG-ASP ____IU	IT MTX ____mg	Studies	Comments
			Enter calculated dose above and actual dose administered below					
		1	____mg	____mg		____mg	a,b*,c	
		2			____IU			

		11	____mg	____mg			b*	

		21	____mg	____mg			b*	
		22			____IU			

		31	____mg	____mg		____mg	b*,c	

		41	____mg	____mg			b*	

		56						
		57	**Start next course (Maintenance Appendix I-M) on Day 57 or when blood count parameters are met (whichever occurs later).**					

[1] Obtain with each IT administration

*To be performed prior to each dose of methotrexate

SEE PROTOCOL SECTION 5.0 FOR DOSE MODIFICATIONS. SEE THE COG MEMBER WEBSITE FOR SUPPORTIVE CARE GUIDELINES.

THERAPY DELIVERY MAPS – ARM A-VHR (T-ALL and T-LLy)

APPENDIX I-H

Intensification Block (1) – Arm A-VHR

This course is only for patients randomized/assigned to treatment on Arm A-VHR. See Section 4.11 for details

Patient name or initials _____ DOB _____

T-ALL patients who are M2 or M3 at the end of Consolidation should proceed directly to Intensification Block 1 without waiting for count recovery or MRD results to proceed. Patients receive this block immediately after consolidation and **must meet all of the following criteria:** Post-consolidation risk assignment must have been completed as described in Section 4.1. **T-ALL only:** Begin Intensification Block #1 after collection of end of consolidation bone marrow for MRD, when ANC ≥750/µL and platelets ≥75,000/µl, and organ function requirements are met as defined in Sections 5.9.1, 5.9.2, and 5.9.3, or whichever occurs later. **T-LLy only:** Start when ANC ≥750/µL and platelets ≥75,000/µl. This Cycle lasts 21 days and this Therapy Delivery Map is on **one (1)** page.

DRUG	ROUTE	DOSAGE	DAYS	IMPORTANT NOTES	OBSERVATIONS
Dexamethasone (DEX)	PO (May be given IV)	10 mg/m^2/dose BID	1-5	Total daily dose: 20 mg/m^2/day, divided BID	a. Hx/PE with VS/Wt (BSA) b. CBC/diff/plts c. CSF cell count & cytospin[1] d. Bilirubin, ALT, creatinine **OBTAIN OTHER STUDIES AS REQUIRED FOR GOOD PATIENT CARE**
High dose methotrexate (HD-MTX)	IV	5000 mg/m^2/dose	Day 1	See Section 5.9 & Appendix IV for administration guidelines	
Leucovorin (LCV)	PO/IV	15 mg/m^2/dose	Days 3-4	Minimum of 3 doses given at 42, 48, and 54 hours after the start of the HD MTX infusion See Section 5.9 and Appendix IV for HD MTX Infusion and Leucovorin rescue guidelines.	
VinCRIStine (VCR)	IV push over 1 min$^+$	1.5 mg/m^2/dose	Days 1 & 6	+ Or infusion via minibag as per institutional policy **Maximum dose of 2 mg**	
Cyclophosphamide (CPM)	IV over 1-6 hours	200 mg/m^2/dose Q12 hrs x5 doses	Days 2-4	See Section 4.11 for administration guidelines	
High dose cytarabine (ARAC)	IV over 3 hours	2000 mg/m^2/dose Q12 hrs x2 doses	Day 5		
Pegaspargase (PEG-ASP)	IV over 1-2 hours	2500 International units/m^2/dose	Day 6	Administer 3 hours after completion of the second HD-AraC infusion through the tubing of a freely infusing solution of D_5W or 0.9% NaCl	
Triple Intrathecal Therapy (ITT): Methotrexate (MTX) Hydrocortisone (HC) Cytarabine (ARAC)	IT	**MTX and HC dosing:** Age (yrs) Dose 1 – 1.99 8 mg 2 – 2.99 10 mg 3 – 8.99 12 mg ≥ 9 15 mg **ARAC dosing:** Age (yrs) Dose 1 – 1.99 16 mg 2 – 2.99 20 mg 3 – 8.99 24 mg ≥ 9 30 mg	Day 1	**Note age-based dosing** **Delivery within 6 hrs of IV MTX infusion**	
Filgrastim (G-CSF)	SubQ	5 mcg/kg/dose	Daily beginning on Day 7	Continue until WBC > 3000/µL	

Enter Cycle # _____ Ht_____cm Wt_____kg BSA____m^2

Date Due	Date Given	Day	DEX ___mg	IV MTX ____mg	LCV ____mg$	VCR ____mg	CPM ____mg	ARAC ____mg	PEG-ASP ____IU	ITT ____mg (MTX) ____mg (HC) ____mg (ARAC)	G-CSF ____mcg	Studies	Comments
			Enter calculated dose above and actual dose administered below										
		1	___mg ___mg	____mg		____mg				____mg (MTX) ____mg (HC) ____mg (ARAC)		a,b,c,d	
		2	___mg ___mg				____mg ___mg						
		3	___mg ___mg		____mg$		____mg ___mg						
		4	___mg ___mg				____mg						
		5	___mg mg					____mg mg					
		6				mg			IU				
		7									____mcg	b*	
		21	**Start next course (Intensification Block 2 Appendix I-I) on Day 22 or when blood count parameters are met (whichever occurs later).**										

[1] Obtain with each IT administration *Every 2 days after completion of chemotherapy until count recovery

$Please document the number of doses of leucovorin administered

SEE PROTOCOL SECTION 5.0 FOR DOSE MODIFICATIONS. SEE THE COG MEMBER WEBSITE FOR SUPPORTIVE CARE GUIDELINES.

APPENDIX I-I

Intensification Block (2) -Arm A-VHR This course is only for patients randomized to treatment on Arm A-VHR .See Section 4.12 for details.	_____ _____ Patient name or initials DOB

Start Day 1 when ANC ≥750/µL and platelets ≥75,000/µL. This Cycle lasts 21 days and this Therapy Delivery Map is on **one (1)** page.

DRUG	ROUTE	DOSAGE	DAYS	IMPORTANT NOTES	OBSERVATIONS
Dexamethasone (DEX)	PO (May be given IV)	10 mg/m^2/dose BID	1-5	Total daily dose: 20 mg/m^2/day, divided BID	a. Hx/PE with VS/Wt (BSA) b. CBC/diff/plts c. CSF cell count & cytospin[1] d. Bilirubin, ALT, creatinine **OBTAIN OTHER STUDIES AS REQUIRED FOR GOOD PATIENT CARE**
High dose methotrexate (HD-MTX)	IV over 24 hours	5000 mg/m^2/dose	Day 1	See Section 5.9 & Appendix IV for administration guidelines	
Leucovorin (LCV)	PO/IV	15 mg/m^2/dose	Days 3-4	Minimum of 3 doses given at 42, 48, and 54 hours after the start of the HD MTX infusion See Section 5.9 and Appendix IV for HD MTX Infusion and Leucovorin rescue guidelines.	
VinCRIStine (VCR)	IV push over 1 min$^+$	1.5 mg/m^2/dose	Days 1 & 6	+ Or infusion via minibag as per institutional policy **Maximum dose of 2 mg**	
Mesna	IV	300 mg/m^2/dose Hour 0, 4, and 8 from start of each ifosfamide infusion	Days 2-4	Hydration following ifosfamide administration: If patient stops methotrexate hydration for whatever reason, need to continue until 12 hours post ifosfamide infusion.	
Ifosfamide (IFOS)	IV over 1 hr	800 mg/m^2/dose Q12 hours x5 doses	Days 2-4	Suggested hydration: Administer 3000 mL/m^2/day (125 mL/m^2/hr) using fluid containing 0.45% NaCl or 0.9% NaCl. Achieve urine specific gravity < 1.010 and urine output > 3 mL/kg/hr prior to start of ifosfamide **Start immediately after HD-MTX**	
DAUNOrubicin (DAUN)	IV Push/infusion Over 1-15 min	30 mg/m^2/dose	Day 5		
Pegaspargase (PEG-ASP)	IV over 1-2 hours	2500 International units/m^2/dose	Day 6	Administer through the tubing of a freely infusing solution of D_5W or 0.9% NaCl	
Triple Intrathecal Therapy (ITT): Methotrexate (MTX) Hydrocortisone (HC) Cytarabine (ARAC)	IT	**MTX and HC dosing:** Age (yrs) Dose 1 – 1.99 8 mg 2 – 2.99 10 mg 3 – 8.99 12 mg ≥ 9 15 mg **ARAC dosing:** Age (yrs) Dose 1 – 1.99 16 mg 2 – 2.99 20 mg 3 – 8.99 24 mg ≥ 9 30 mg	Day 1	**Note age-based dosing** **Delivery within 6 hrs of IV MTX infusion**	
Filgrastim (G-CSF)	SubQ	5 mcg/kg/dose	Daily beginning on Day 7	Continue until WBC > 3000/µL	

Intensification Block (2) -Arm A-VHR

This course is only for patients randomized to treatment on Arm A-VHR .See Section 4.12 for details.

_____ _____
Patient name or initials DOB

Enter Cycle # _____ Ht ____ cm Wt ____ kg BSA ____ m²

Date Due	Date Given	Day	DEX ___mg	IV MTX mg	LCV ___mg$	VCR ____mg	MESNA	IFOS ____mg	DAUN ____mg	PEG-ASP IU	IT MTX mg	IT HC mg	IT ARAC mg	G-CSF ____mcg	Studies	Comments
			Enter calculated dose above and actual dose administered below													
		1	___mg ___mg	___mg		___mg					___mg	___mg	___mg		a,b,c,d	
		2	___mg ___mg				____mg ___mg ____mg ____mg ___mg mg	____mg ___mg								
		3	___mg ___mg		____mg$		____mg ___mg ____mg ____mg ___mg ___mg	____mg ___mg								
		4	___mg ___mg				____mg ___mg ____mg	____mg								
		5	___mg ___mg						___mg							
		6				___mg				___IU						
		7												____mcg	b*	
		21	**Start next course (Intensification Block 3 Appendix I-J) on Day 22 or when blood count parameters are met (whichever occurs later).**													

[1] Obtain with each IT administration *Every 2 days after completion of chemotherapy until count recovery

$Please document the number of doses of leucovorin administered

SEE PROTOCOL SECTION 5.0 FOR DOSE MODIFICATIONS. SEE THE COG MEMBER WEBSITE FOR SUPPORTIVE CARE GUIDELINES.

Page 2 of 2

APPENDIX I-J

Intensification Block (3) Arm A-VHR

This course is only for patients randomized to treatment on Arm A-VHR. See Section 4.13 for details.

Patient name or initials

DOB

Start Day 1 when ANC ≥ 750/µL and platelets ≥ 75,000/µL. HR3 lasts 21 days. This Cycle lasts 21 days and this Therapy Delivery Map is on one (1) page.

DRUG	ROUTE	DOSAGE	DAYS	IMPORTANT NOTES	OBSERVATIONS
Dexamethasone (DEX)	PO (May be given IV)	10 mg/m^2/dose BID	Days 1-5	Total daily dose: 20 mg/m^2/day, divided BID	a. Hx/PE with VS/Wt (BSA) b. CBC/diff/plts c. CSF cell count & cytospin[1] d. Bilirubin, ALT, creatinine, **T-ALL ONLY** e. BM MRD **T-LLy ONLY:** f. Chest CT/CXR[2] g. Abdomen/pelvis CT (or MRI)[2] h. Bone scan[2] i. Diagnostic biopsy/ cytology **OBTAIN OTHER STUDIES AS REQUIRED FOR GOOD PATIENT CARE**
High dose cytarabine (ARAC)	IV over 3 hours	2000 mg/m^2/dose Q12 hours x 4 doses	Days 1-2		
Etoposide (ETOP)	IV over 2 hrs	100 mg/m^2/dose Q12 hours x5 doses	Days 3-5	See Section 4.13 for administration guidelines	
Pegaspargase (PEG-ASP)	IV over 1-2 hours	2500 International units/m^2/dose	Day 6	Administer through the tubing of a freely infusing solution of D_5W or 0.9% NaCl	
Triple Intrathecal Therapy (ITT): Methotrexate (MTX) Hydrocortisone (HC) Cytarabine (ARAC)	IT	**MTX and HC dosing:** Age (yrs) Dose 1 – 1.99 8 mg 2 – 2.99 10 mg 3 – 8.99 12 mg ≥ 9 15 mg **ARAC dosing:** Age (yrs) Dose 1 – 1.99 16 mg 2 – 2.99 20 mg 3 – 8.99 24 mg ≥ 9 30 mg	Day 5	**Note age-based dosing**	
Filgrastim (G-CSF)	SubQ	5 mcg/kg/dose	Daily beginning on Day 7	Continue until WBC > 3000/µL	

Enter Cycle # ____ **Ht** ____ **cm** **Wt** ____ **kg** **BSA** ____ **m^2**

Date Due	Date Given	Day	DEX ___mg	ARAC ____mg	ETOP ____mg	PEG-ASP IU	IT MTX mg	IT HC mg	IT ARAC mg	G-CSF ___mcg	Studies	Comments
			Enter calculated dose above and actual dose administered below									
		1	___mg ___mg	____mg ____mg							a,b,d	
		2	___mg ___mg	____mg ____mg								
		3	___mg ___mg		____mg ___mg							
		4	___mg ___mg		____mg ___mg							
		5	___mg ___mg		____mg		___mg	___mg	___mg		c	
		6				___IU						
		7								mcg		
		21									**T-ALL:** b*, e **T-LLy:** b*, i, (f,g,h)[2]	
Start next course (Delayed Intensification Appendix I-K) on Day 22 or when blood count parameters are met (whichever occurs later).												

[1] Obtain with each IT administration [2] See Section 7.0 for details

*Every 2 days after completion of chemotherapy until count recovery

SEE PROTOCOL SECTION 5.0 FOR DOSE MODIFICATIONS. SEE THE COG MEMBER WEBSITE FOR SUPPORTIVE CARE GUIDELINES.

APPENDIX I-K

DELAYED INTENSIFICATION- Arm A-VHR (without bortezomib)

This Delayed Intensification course is for patients randomized to treatment on Arm A-VHR (no bortezomib). See Section 4.8 for details.

Patient name or initials _____

DOB _____

Patients receive this block immediately after HR Intensification 3 and must meet all of the following criteria: **VHR T-ALL** Post-HR Intensification Block 3 MRD was completed and demonstrated undetectable MRD as described in Section 4.13 Start 7 days after collection of HR Intensification Block 3 bone marrow for MRD or when ANC ≥750/µL and platelets ≥75,000/µl, whichever occurs later. **VHR T-LLy:** Post-HR Intensification Block 3 disease evaluation was completed and demonstrated a radiographic CR OR a radiographic PR and a biopsy showing no residual disease as described in Section 10.4 Start when ANC ≥750/µL and platelets ≥75,000/µl **All patients:** Once Delayed Intensification has begun, it may be interrupted for myelosuppression (ANC ≤ 750/µL and platelets ≤ 75,000/µL) on Day 29 ONLY. Once the Day 1 therapy or Day 29 cyclophosphamide has been given, the remainder of the therapy should not be held solely for myelosuppression, which is expected to occur. Therapy must be interrupted for patients with serious proven or presumed infection and resumed when the signs of infection have abated. Hold Day 29 chemotherapy until ANC ≥ 750/µL and platelets ≥ 75,000/µL. This Course lasts 9 weeks (63 days) and this Therapy Delivery Map is on **two (2)** pages.

DRUG	ROUTE	DOSAGE	DAYS	IMPORTANT NOTES	OBSERVATIONS
VinCRIStine (VCR)	IV push over 1 min+	1.5 mg/m²/dose	Days 1, 8, 15, 43 & 50	+ Or infusion via minibag as per institutional policy **Maximum dose of 2 mg**	a. Hx/PE with VS/Wt (BSA) b. CBC/diff/plts c. CSF cell count & cytospin[1] d. Bilirubin, ALT, creatinine **OBTAIN OTHER STUDIES AS REQUIRED FOR GOOD PATIENT CARE**
Dexamethasone (DEX)	PO (May be given IV)	5 mg/m²/dose BID	Days 1-7 & 15-21	Total daily dose: 10 mg/m²/day, divided BID	
DOXOrubicin (DOXO)	IV Push Over 1-15 min	25 mg/m²/dose	Days 1, 8, & 15		
Pegaspargase (PEG-ASP)	IV over 1-2 hours	2500 International units/m²/dose	Day 4, 18, & 43	Administer through the tubing of a freely infusing solution of D_5W or 0.9% NaCl	
Intrathecal Methotrexate (IT MTX)	IT	Age (yrs) – Dose 1 – 1.99 – 8 mg 2 – 2.99 – 10 mg 3 – 8.99 – 12 mg ≥ 9 – 15 mg	Days 1, 29 & 36	**Note age-based dosing**	

Therapy Delivery Map **Ht_____cm** **Wt_____kg** **BSA____m²**

Date Due	Date Given	Day	VCR ____mg	DEX ____mg ____mg	DOXO ____mg	PEG-ASP ____IU	IT MTX _____mg	Studies	Comments
				Enter calculated dose above and actual dose administered below					
		1	____mg	____mg ____mg	____mg		____mg	a,b,c,d	
		2							
		3							
		4				____IU			
		5							
		6							
		7							
		8	____mg		____mg			b	

		15	____mg	____mg ____mg	____mg			b	
		16							
		17							
		18				____IU			
		19							
		20							
		21							
		22						b	
			This therapy delivery map continues on the next page with Day 29.						

[1] Obtain with each IT administration

SEE PROTOCOL SECTION 5.0 FOR DOSE MODIFICATIONS. SEE THE COG MEMBER WEBSITE FOR SUPPORTIVE CARE GUIDELINES.

DELAYED INTENSIFICATION- Arm A-VHR (without bortezomib)

This Delayed Intensification course is for patients randomized to treatment on Arm A-VHR (no bortezomib). See Section 4.8 for details.

Patient name or initials

DOB

DRUG	ROUTE	DOSAGE	DAYS	IMPORTANT NOTES	OBSERVATIONS
VinCRIStine (VCR)	IV push over 1 min+	1.5 mg/m²/dose	Days 1, 8, 15,43, & 50	+ Or infusion via minibag as per institutional policy **Maximum dose of 2 mg**	a. Hx/PE with VS/Wt (BSA) b. CBC/diff/plts c. CSF cell count & cytospin[1] d. Bilirubin, ALT, creatinine **OBTAIN OTHER STUDIES AS REQUIRED FOR GOOD PATIENT CARE**
Pegaspargase (PEG-ASP)	IV over 1-2 hours	2500 International units/m²/dose	Day 4, 18, & 43	Administer through the tubing of a freely infusing solution of D_5W or 0.9% NaCl	
Intrathecal Methotrexate (IT MTX)	IT	Age (yrs) Dose 1 – 1.99 8 mg 2 – 2.99 10 mg 3 – 8.99 12 mg ≥ 9 15 mg	Days 1, 29 & 36	**Note age-based dosing**	
Cyclophosphamide (CPM)	IV over 30-60 minutes	1000 mg/m²/dose	Day 29	See Section 4.8 for administration guidelines	
Cytarabine (ARAC)	IV over 1-30 minutes or SubQ	75 mg/m²/dose	Days 29-32 & 36-39		
Thioguanine (TG)	PO	60 mg/m²/dose	Days 29-42	See Section 4.8, Section 5.13 & Appendix VII for administration guidelines	

Therapy Delivery Map **Ht_____cm** **Wt_____kg** **BSA____m²**

Date Due	Date Given	Day	VCR ____mg	PEG-ASP ____IU	IT MTX ____mg	CPM ____mg	ARAC ____mg	TG ____mg	Studies	Comments
			Enter calculated dose above and actual dose administered below							
		29			____mg	____mg	____mg	____mg	b,d,c	
		30								
		31								
		32								
		33								

		36			____mg		____mg		b,c	
		37								
		38								
		39								
		40								
		41								
		42								
		43	____mg	____IU					b	
		44								
		45								
		46								
		47								
		48								
		49								
		50	____mg						b	

		57							b	

		63							b	
		64	**Start next course (IM CMTX, APPENDIX I-L) on Day 64 or when blood count parameters are met (whichever occurs later)**							

[1] Obtain with each IT administration

SEE PROTOCOL SECTION 5.0 FOR DOSE MODIFICATIONS. SEE THE COG MEMBER WEBSITE FOR SUPPORTIVE CARE GUIDELINES.

APPENDIX I-L

INTERIM MAINTENANCE (CMTX) – Arm A-VHR This IM course is for patients randomized to Arm A-VHR. See Section 4.7 for details.	_____ Patient name or initials _____ DOB

Begin IM when peripheral counts recover with an ANC ≥ 750/µL and platelets ≥ 75,000/µL. Patients need to meet renal and hepatic function requirements to initiate methotrexate as described in Section 5.10 This Course lasts 8 weeks (56 days) and this Therapy Delivery Map is on **one (1)** page.

DRUG	ROUTE	DOSAGE	DAYS	IMPORTANT NOTES	OBSERVATIONS
VinCRIStine (VCR)	IV push over 1 min+	1.5 mg/m²/dose	Days 1, 11, 21, 31 & 41	+ Or infusion via minibag as per institutional policy **Maximum dose of 2 mg**	a. Hx/PE with VS/Wt (BSA) b. CBC/diff/plts Bilirubin, ALT, creatinine c. CSF cell count & cytospin[1] **OBTAIN OTHER STUDIES AS REQUIRED FOR GOOD PATIENT CARE**
Methotrexate (CMTX)	IV push over 2-5 mins or IV infusion over 10-15 mins	Starting dose is 100 mg/m² **escalate by 50 mg/m²/dose**	Days 1, 11, 21, 31 & 41	See Section 4.7 and Section 5.10 for details	
Pegaspargase (PEG-ASP)	IV over 1-2 hours	2500 International units/m²/dose	Days 2 & 22	Administer through the tubing of a freely infusing solution of D_5W or 0.9% NaCl	
Intrathecal Methotrexate (IT MTX)	IT	Age (yrs) / Dose 1 – 1.99 / 8 mg 2 – 2.99 / 10 mg 3 – 8.99 / 12 mg ≥ 9 / 15 mg	Days 1 & 31	**Note age-based dosing**	

Therapy Delivery Map **Ht_____cm** **Wt_____kg** **BSA____m²**

Date Due	Date Given	Day	VCR mg	IV MTX *(escalating dose)*	PEG-ASP IU	IT MTX mg	Studies	Comments
			Enter calculated dose above and actual dose administered below					
		1	____mg	____mg		____mg	a,b*,c	
		2			____IU			

		11	____mg	____mg			b*	

		21	____mg	____mg			b*	
		22			____IU			

		31	____mg	____mg		____mg	b*,c	

		41	____mg	____mg			b*	

		56						
		57	**Start next course (Maintenance, Appendix I-M) on Day 57 or when blood count parameters are met (whichever occurs later).**					

[1] Obtain with each IT administration

*To be performed prior to each dose of methotrexate

SEE PROTOCOL SECTION 5.0 FOR DOSE MODIFICATIONS. SEE THE COG MEMBER WEBSITE FOR SUPPORTIVE CARE GUIDELINES.

THERAPY DELIVERY MAP – MAINTENANCE ARM A
(T-ALL and T-LLy)

APPENDIX I-M

MAINTENANCE- Arm A

This Maintenance course is for Arm A. See Section 4.14 for details

_____ Patient name or initials _____ DOB

Maintenance begins when peripheral counts recover with ANC ≥ 750/µL and platelets ≥ 75,000/µL. Only Mercaptopurine and PO Methotrexate will be interrupted for myelosuppression as outlined in Section 5.11. This Cycle lasts 12 weeks (84 days) and this Therapy Delivery Map is on **one (1)** page.

DRUG	ROUTE	DOSAGE	DAYS	IMPORTANT NOTES	OBSERVATIONS
VinCRIStine (VCR)	IV push over 1 min+	1.5 mg/m²/dose	Days 1, 29 & 57	+ Or infusion via minibag as per institutional policy **Maximum dose of 2 mg**	a. Hx/PE with VS/Wt (BSA) b. CBC/diff/plts c. CSF cell count & cytospin[1] d. Bilirubin, ALT, creatinine e. Thiopurine metabolites- as clinically indicated f. Optional Banking/Biology **T-LLy ONLY:** g. Chest CT/CXR h. Abdomen/pelvis CT (or MRI) i. Bone scan **OBTAIN OTHER STUDIES AS REQUIRED FOR GOOD PATIENT CARE**
Dexamethasone (DEX)	PO (May be given IV)	3 mg/m²/dose	Days 1-5, 29-33 & 57-61	Total daily dose: 6 mg/m²/day, divided BID	
Mercaptopurine (MP)	PO	75 mg/m²/dose	Days 1-84	See Section 4.14, Section 5.11 and Appendix VI for administration guidelines	
Methotrexate (MTX)	PO	20 mg/m²/dose weekly	Days 8, 15, 22, **29@**, 36, 43, 50, 57, 64, 71 & 78 @**NOTE:** Omit Day 29 of Cycles 1- 4 for SR T-ALL and T-LLy patients and cycles 1- 2 for IR T-ALL and T-LLy patients	See Section 5.11 for suggested starting dose based on TPMT and NUDT15 status (if available) **Please note Day 29**	
Intrathecal Methotrexate (IT MTX)	IT	Age (yrs) / Dose 1 – 1.99 / 8 mg 2 – 2.99 / 10 mg 3 – 8.99 / 12 mg ≥ 9 / 15 mg	Day 1 &Day 29 of Cycles 1- 4 for **SR T-ALL and T-LLy patients ONLY.** Day 29 of Cycles 1-2 **IR T-ALL and T-LLy patients ONLY**	**Note age-based dosing** **Please note Day 29**	

Enter Cycle # _____ Ht _____ cm Wt _____ kg BSA _____ m²

Date Due	Date Given	Day	VCR ___ mg	DEX ___ mg ___ mg	MP ___ mg	PO MTX ___ mg	IT MTX ___ mg	Studies	Comments
			Enter calculated dose above and actual dose administered below						
		1$	___ mg	___ mg ___ mg	___ mg		___ mg	a,b,c,d	
		5							
		8				___ mg			
		15				___ mg			
		22				___ mg			
		29	___ mg	___ mg ___ mg		___ mg@	___ mg&	a,b,c	
		33							
		36				___ mg			
		43				___ mg			
		50				___ mg			
		57	___ mg	___ mg ___ mg		___ mg		a,b	
		61							
		64				___ mg			
		71				___ mg			
		78				___ mg			
		84						f[5] (g[4],h[3],i[3])[2#]	
		85	**Begin next cycle on Day 85 regardless of counts and repeat until two years (for T-ALL girls and all T-LLy pts, regardless of gender) and three years (for T-ALL boys) from the start of Interim Maintenance (see Section 4.14). Only MP & PO MTX will be interrupted for myelosuppression during subsequent Maintenance cycles as outlined in Section 5.11.**						

[1] Obtain with each IT administration [2] T-LLy ONLY [3] If baseline is negative, no repeat scans are required.

[4] If CR at end-Consolidation perform a CXR; If PR or NR at end-Consolidation perform chest CT

[5] Collect ONLY if the patient relapses. **T-ALL**: as a part of AALL08B1 (or APEC14B1 if available to ALL patients) **T-LLy**: submit via APEC14B1 (if enrolled).

$RADIATION THERAPY (T-ALL:IR CNS3 and all VHR patients, T-LLy:CNS3 only) See Section 16.0

 # Only collect at the completion of therapy.

SEE PROTOCOL SECTION 5.0 FOR DOSE MODIFICATIONS. SEE THE COG MEMBER WEBSITE FOR SUPPORTIVE CARE GUIDELINES.

APPENDIX II: THERAPY DELIVERY MAPS – ARM B (T-ALL and T-LLy)

APPENDIX II-A

INDUCTION- Arm B (with bortezomib) This induction is for all patients randomized to treatment on Arm B (with bortezomib). See Section 4.5 for details.	_____ Patient name or initials _____ DOB

Patients must complete and sign the appropriate informed consent and undergo bortezomib randomization before starting Induction therapy. This Course lasts 5 weeks (35 days) and this Therapy Delivery Map is on **two (2)** pages.

DRUG	ROUTE	DOSAGE	DAYS	IMPORTANT NOTES	OBSERVATIONS
Bortezomib (BOR) *IND#*[redacted]	IV push over 3-5 seconds	1.3 mg/m^2/dose	Days 1, 4, 8 & 11	At least 72 hours must have elapsed between doses.	a. Hx/PE with VS/Wt (BSA) CBC/diff/plts b. Bilirubin (total and direct), ALT, AST creatinine c. CSF cell count & cytospin[2] d. Electrolytes including PO_4 e. TPMT and NUDT15 genotype (if available) f. BM MRD g. Performance status h. Pregnancy test i. BM cytomorphology j. CXR k. Pulse oximetry & Chest CXR [7] l. Cell banking **T-LLy only** m. Chest CT n. Abdomen/pelvis CT (or MRI) o. Bone scan p. Diagnostic biopsy/ cytology q. PET scan **Optional Studies T-ALL ONLY** Bortezomib Response Study (open to Arms A and B): See Section 15.1 for details r. PB sample (optional) s. BM sample (optional) **OBTAIN OTHER STUDIES AS REQUIRED FOR GOOD PATIENT CARE**
Intrathecal Cytarabine (IT ARAC)	IT	Age (yrs) / Dose 1 – 1.99 / 30 mg 2 – 2.99 / 50 mg ≥ 3 / 70 mg	Given at time of diagnostic lumbar puncture (LP)* **OR** Day 1	May give prior to randomization **Note age-based dosing**	
VinCRIStine (VCR)	IV Push Over 1 min$^+$	1.5 mg/m^2/dose	Days 1, 8, 15 & 22	+ Or infusion via minibag as per institutional policy **Maximum dose of 2 mg**	
Dexamethasone (DEX)	PO (May be given IV)	3 mg/m^2/dose BID	Days 1-28 (no taper)	Total daily dose: 6 mg/m^2/day, divided BID	
DAUNOrubicin (DAUN)	IV Push/infusion Over 1-15 min	25 mg/m^2/dose	Days 1, 8, 15 & 22		
Pegaspargase (PEG-ASP)	IV over 1-2 hours	2500 International units/m^2/**dose**	Day 4 and 18	Administer through the tubing of a freely infusing solution of D_5W or 0.9% NaCl	
Intrathecal Methotrexate (IT MTX)	IT	Age (yrs) / Dose 1 – 1.99 / 8 mg 2 – 2.99 / 10 mg 3 – 8.99 / 12 mg ≥ 9 / 15 mg	Days 8 & 29 (Days 15 & 22 **for CNS3 ONLY)**	**Note age-based doing** **Please note CNS3**	

INDUCTION- Arm B (with bortezomib)

This induction is for all patients randomized to treatment on Arm B (with bortezomib). See Section 4.5 for details.

_____ Patient name or initials _____ DOB

Therapy Delivery Map **Ht _____ cm** **Wt _____ kg** **BSA ___ m²**

Date Due	Date Given	Day	BOR ____mg	IT ARAC ____mg	VCR ____mg	DEX ____mg ____mg	DAUN ____mg	PEG-ASP ____IU	IT MTX ____mg	Studies	Comments
				Enter calculated dose above and actual dose administered below							
		-2/-1/0/LP*								(a,b,d,g,h,i,j, m, n, o, p)[4@] (r)[5,^] (f, l)[$]	
		1	____mg	____mg	____mg	____mg ____mg	____mg			a,b,c*,d, e[11],k[7],q[4]	
		4	____mg					____IU **(1 dose)**		k[7]	
		8	____mg		____mg		____mg		____mg	a,c, k[7]	
		11	____mg							k[7]	
		15			____mg		____mg		____mg#	a,c#,	
		18						____IU **(1 dose)**			
		22			____mg		____mg		____mg#	a,c#	
		29							____mg	a,b,c,f[3],i[1], (j,m,n[9],o [10], q[12])[4] (r, s)[5]	
		36	**Start next course (Consolidation, Appendix II-B) on Day 36 or when blood count parameters are met (whichever occurs later). Patients with Day 29 marrow M2 or M3 or MRD >5% should start as soon as possible (not wait for Day 36 or count recovery to occur.)**								

*On Day 1 **OR** at the time of diagnostic lumbar puncture (LP) if < than 72 hours from the start of protocol therapy.

^Pre –treatment

$ **T-ALL:** Done as part of AALL08B1 or APEC14B1 **T-LLy**: If enrolled on APEC14B1, submit as a part of APEC14B1. If not enrolled on APEC14B1, submit as part of this protocol (see Section 13.6 for details)

#CNS3 patients ONLY @Baseline

1 **T-LLy:** only if positive at diagnosis

[2] Obtain with each IT administration

[3] Optional for T-LLy. Required for T-ALL

[4] T-LLy ONLY

[5] T-ALL ONLY

[6] To be collected at hours 0, 6 and 24 relative to the first dose of bortezomib. See Section 15.1 for details

[7] Patients who develop respiratory signs or symptoms as defined in Section 5.2.2 should have pulse oximetry (02 saturation) and Chest x-ray documented within 12 hours prior to each dose of bortezomib.

[8] follow-up exams only if baseline demonstrates disease

[9] Many patients will have no disease below the diaphragm; if the abdominal and pelvic CTs at Baseline are negative, no repeat scans are required.

[10] Bone scan only if patient has bone symptoms; follow-up exams only if baseline demonstrates disease.

[11] To be performed any time during Induction

[12] PET scan is recommended but not required at baseline. If a PET is obtained at baseline, PET imaging should be continued with subsequent response assessments until patient no longer has PET-avid disease.

SEE PROTOCOL SECTION 5.0 FOR DOSE MODIFICATIONS. SEE THE COG MEMBER WEBSITE FOR SUPPORTIVE CARE GUIDELINES.

APPENDIX II-B

CONSOLIDATION Arm-B This Consolidation course is for patients on Arm B. See Section 4.6 for details.	_____ _____ Patient name or initials DOB

Start Consolidation on Day 36 (7 days following Day 29 LP) or when peripheral counts recover with ANC ≥ 750/µL and platelets ≥ 75,000/µL (whichever occurs later). Patients with Day 29 marrow M2 or M3 or MRD >5% should start as soon as possible (not wait for Day 36 or count recovery to occur.) Once Consolidation therapy has begun, interruptions for myelosuppression should occur only at Day 29. Patients with T-ALL and persistent testicular disease at end-Induction will receive XRT to the testes during Consolidation (a testicular biopsy is required if there is any uncertainty regarding the clinical response). Within the first two weeks of Consolidation, testicular radiation therapy will be given at 2400 cGy in 12 once-daily fractions of 200 cGy (See Section 16.2). Testicular radiation must be started during Consolidation and should be completed before the end of this phase of therapy. This Course lasts 8 weeks (56 days) and this Therapy Delivery Map is on **one (2)** pages.

DRUG	ROUTE	DOSAGE	DAYS	IMPORTANT NOTES	OBSERVATIONS
Intrathecal Methotrexate (IT MTX)	IT	Age (yrs) Dose 1 – 1.99 8 mg 2 – 2.99 10 mg 3 – 8.99 12 mg ≥ 9 15 mg	Days 1, 8, 15, 22 **Omit Days 15 & 22 for CNS3 patients ONLY**	**Note age-based dosing** **Please note CNS3**	a. Hx/PE with VS/Wt (BSA) b. CBC/diff/plts c. CSF cell count & cytospin[2] d. Bilirubin (total and direct), ALT, AST creatinine e. Performance Status f. BM MRD[1,4] g. Bone Marrow cytomorphology T-LLy Only h. Chest CT/CXR i. Abdomen/pelvis CT (or MRI), Bone scan, PET scan **OBTAIN OTHER STUDIES AS REQUIRED FOR GOOD PATIENT CARE**
Cyclophosphamide (CPM)	IV over 30-60 minutes	1000 mg/m^2/dose	Days 1 & 29	See Section 4.6 for admin guidelines	
Cytarabine (ARAC)	IV or SubQ	75 mg/m^2/dose	Days 1-4, 8-11, 29-32 & 36-39		
Mercaptopurine (MP)	PO	60 mg/m^2/dose	Days 1-14 & 29-42	See Section 4.6 and Appendix VI for administration guidelines	
VinCRIStine (VCR)	IV Push over 1 min$^+$	1.5 mg/m^2/dose	Days 15, 22, 43 & 50	+ Or infusion via minibag as per institutional policy **Maximum dose of 2 mg**	
Pegaspargase (PEG-ASP)	IV over 1-2 hours	2500 International units/m^2/dose	Days 15 & 43	Administer through the tubing of a freely infusing solution of D_5W or 0.9% NaCl	
T-ALL patients with biopsy proven testicular disease at end-Induction will receive testicular XRT. See Section 4.4 & Section 16.0 for additional details.					

CONSOLIDATION Arm-B
This Consolidation course is for patients on Arm B. See Section 4.6 for details.

_____ _____
Patient name or initials DOB

Start Consolidation on Day 36 (7 days following Day 29 LP) or when peripheral counts recover with ANC ≥ 750/µL and platelets ≥ 75,000/µL (whichever occurs later). Patients with Day 29 marrow M2 or M3 or MRD >5% should start as soon as possible (not wait for Day 36 or count recovery to occur.) Once Consolidation therapy has begun, interruptions for myelosuppression should occur only at Day 29. Patients with T-ALL and persistent testicular disease at end-Induction will receive XRT to the testes during Consolidation (a testicular biopsy is required if there is any uncertainty regarding the clinical response). Within the first two weeks of Consolidation, testicular radiation therapy will be given at 2400 cGy in 12 once-daily fractions of 200 cGy (See Section 16.2). Testicular radiation must be started during Consolidation and should be completed before the end of this phase of therapy. This Course lasts 8 weeks (56 days) and this Therapy Delivery Map is on **one (2)** pages.

Therapy Delivery Map **Ht_____cm** **Wt_____kg** **BSA____m²**

Date Due	Date Given	Day	IT MTX ____mg		CPM ____mg	ARAC ____mg	MP ____mg	VCR ____mg	PEG-ASP ____IU	Studies	Comments
			CNS3 T-ALL	**All other patients**							
			Enter calculated dose above and actual dose administered below								
		1$	____mg	____mg	____mg	____mg	____mg			a,b,c,d,e[5]	
		2				____mg					
		3				____mg					
		4				____mg					
		8	____mg	____mg		____mg				b,c	
		9				____mg					
		10				____mg					
		11				____mg					
		15		____mg				____mg	____IU	b,c	
		22		____mg				____mg		b,c	
		29			____mg	____mg	____mg			b	
		30				____mg					
		31				____mg					
		32				____mg					
		36				____mg				b	
		37				____mg					
		38				____mg					
		39				____mg					
		42									
		43						____mg	____IU	b	
		50						____mg		b	
		56								b, g[6], (h[7],i[8])[3]	
		57								f[1,4]	

Start next course (Based on Risk Assignment. See Section 4.1.2) on Day 57 or when blood count parameters are met (whichever occurs later).

1 End of Consolidation bone marrow is not performed on SR T-ALL patients. It is REQUIRED for T-ALL patients with end Induction BM MRD ≥ 0.01% ONLY

2 Obtain with each IT administration 3 T-LLy ONLY 4 T-ALL only 5 Prior to Day 1 therapy

6 T-LLy: Only if positive at end Induction.

7If patient has CR at end-Induction perform a CXR. If PR or NR at end-Induction perform a chest CT

8 If baseline is negative, no repeat scans are required.

$ T-ALL and T-LLy patients with persistent testicular disease at end-Induction should receive radiation to the testes during Consolidation. See Section 16.2 for details.

SEE PROTOCOL SECTION 5.0 FOR DOSE MODIFICATIONS. SEE THE COG MEMBER WEBSITE FOR SUPPORTIVE CARE GUIDELINES.

Page 2 of 2

THERAPY DELIVERY MAPS – ARM B-SR (T-ALL and T-LLy)

APPENDIX II-C

INTERIM MAINTENANCE with CMTX- Arm B-SR This IM course is for patients or assigned to Arm B-SR. See Section 4.7 for details	Patient name or initials DOB

Begin IM when peripheral counts recover with an ANC ≥ 750/µL and platelets ≥ 75,000/µL. Patients need to meet renal and hepatic function requirements to initiate methotrexate as described in Section 5.10. This Course lasts 8 weeks (56 days) and this Therapy Delivery Map is on **one (1)** page.

DRUG	ROUTE	DOSAGE	DAYS	IMPORTANT NOTES	OBSERVATIONS
VinCRIStine (VCR)	IV push over 1 min+	1.5 mg/m²/dose	Days 1, 11, 21, 31 & 41	+ Or infusion via minibag as per institutional policy **Maximum dose of 2 mg**	a. Hx/PE with VS/Wt (BSA) b. CBC/diff/plts Bilirubin, ALT, creatinine c. CSF cell count & cytospin[1] **OBTAIN OTHER STUDIES AS REQUIRED FOR GOOD PATIENT CARE**
Methotrexate (CMTX)	IV push over 2-5 mins or IV infusion over 10-15 mins	Starting dose is 100 mg/m² **escalate by 50 mg/m²/dose**	Days 1, 11, 21, 31 & 41	See Section 4.7 and Section 5.10 for details	
Pegaspargase (PEG-ASP)	IV over 1-2 hours	2500 International units/m²/dose	Days 2 & 22	Administer through the tubing of a freely infusing solution of D_5W or 0.9% NaCl	
Intrathecal Methotrexate (IT MTX)	IT	Age (yrs) — Dose 1 – 1.99 — 8 mg 2 – 2.99 — 10 mg 3 – 8.99 — 12 mg ≥ 9 — 15 mg	Days 1 & 31	**Note age-based dosing**	

Therapy Delivery Map **Ht_____cm** **Wt_____kg** **BSA____m²**

Date Due	Date Given	Day	VCR ____mg	IV MTX *(escalating dose)*	PEG-ASP ____IU	IT MTX ____mg	Studies	Comments
			Enter calculated dose above and actual dose administered below					
		1	____mg	____mg		____mg	a,b*,c	
		2			____IU			

		11	____mg	____mg			b*	

		21	____mg	____mg			b*	
		22			____IU			

		31	____mg	____mg		____mg	b*,c	

		41	____mg	____mg			b*	

		56						
		57	**Start next course (Delayed Intensification, Appendix II-D) on Day 57 or when blood count parameters are met (whichever occurs later).**					

[1] Obtain with each IT administration

*To be performed prior to each dose of methotrexate

SEE PROTOCOL SECTION 5.0 FOR DOSE MODIFICATIONS. SEE THE COG MEMBER WEBSITE FOR SUPPORTIVE CARE GUIDELINES.

APPENDIX II-D

DELAYED INTENSIFICATION Arm B-SR (with bortezomib) This Delayed Intensification (DI) course is for patients randomized to treatment on Arm B-SR (with bortezomib). See Section 4.9 for details	_____ Patient name or initials _____ DOB

Patients should have ANC ≥ 750/µL and platelets ≥ 75,000/µL prior to starting therapy. Once Delayed Intensification has begun, it may be interrupted for myelosuppression (ANC ≤ 750/µL and platelets ≤ 75,000/µL) on Day 29 ONLY. Once the Day 1 therapy or Day 29 cyclophosphamide has been given, the remainder of the therapy should not be held solely for myelosuppression, which is expected to occur. Therapy must be interrupted for patients with serious proven or presumed infection and resumed when the signs of infection have abated. Hold Day 29 chemotherapy until ANC ≥ 750/µL and platelets ≥ 75,000/µL. This Course lasts 9 weeks (63 days) and this Therapy Delivery Map is on **two (2)** pages.

DRUG	ROUTE	DOSAGE	DAYS	IMPORTANT NOTES	OBSERVATIONS
Bortezomib (BOR) *IND#*[redacted]	IV push over 3-5 seconds	1.3 mg/m^2/dose	Days 1, 4, 15 & 18	At least 72 hours must have elapsed between doses.	a. Hx/PE with VS/Wt (BSA) b. CBC/diff/plts c. CSF cell count & cytospin[1] d. Bilirubin, ALT, creatinine e. Electrolytes including PO_4 f. Pulse Oximetry (O2 saturation) and CXR- Patients who develop respiratory signs or symptoms as defined in Section 5.2.2 **OBTAIN OTHER STUDIES AS REQUIRED FOR GOOD PATIENT CARE**
VinCRIStine (VCR)	IV push over 1 min$^+$	1.5 mg/m^2/dose	Days 1, 8, 15, 43 & 50	+ Or infusion via minibag as per institutional policy **Maximum dose of 2 mg**	
Dexamethasone (DEX)	PO (May be given IV)	5 mg/m^2/dose BID	Days 1-7 & 15-21	Total daily dose: 10 mg/m^2/day, divided BID	
DOXOrubicin (DOXO)	IV Push Over 1-15 min	25 mg/m^2/dose	Days 1, 8, & 15		
Pegaspargase (PEG-ASP)	IV over 1-2 hours	2500 International units/m^2/dose	Day 4, 18, & 43	Administer through the tubing of a freely infusing solution of D_5W or 0.9% NaCl	
Intrathecal Methotrexate (IT MTX)	IT	Age (yrs) / Dose 1 – 1.99 / 8 mg 2 – 2.99 / 10 mg 3 – 8.99 / 12 mg ≥ 9 / 15 mg	Days 1,29 & 36	**Note age-based dosing**	

Therapy Delivery Map **Ht_____cm** **Wt_____kg** **BSA____m^2**

Date Due	Date Given	Day	BOR ____mg	VCR ____mg	DEX ____mg ____mg	DOXO ____mg	PEG-ASP ____IU	IT MTX _____mg	Studies	Comments
					Enter calculated dose above and actual dose administered below					
		1	____mg	____mg	____mg ____mg	____mg		_____mg	a,b,c,d,e, f*	
		2								
		3								
		4	____mg				____IU		f*	
		5								
		6								
		7								
		8		____mg		____mg			b	
		15	____mg	____mg	____mg ____mg	____mg			b, f*	
		16								
		17								
		18	____mg				____IU		f*	
		19								
		20								
		21								
		22							b	
				This therapy delivery map continues on the next page with Day 29.						

* Patients who develop respiratory signs or symptoms as defined in Section 5.2.2 should have pulse oximetry (02 saturation) and Chest x-ray documented within 12 hours prior to each dose of bortezomib.

DELAYED INTENSIFICATION Arm B-SR (with bortezomib)

This Delayed Intensification (DI) course is for patients randomized to treatment on Arm B-SR (with bortezomib). See Section 4.9 for details

Patient name or initials

DOB

DRUG	ROUTE	DOSAGE	DAYS	IMPORTANT NOTES	OBSERVATIONS
VinCRIStine (VCR)	IV push over 1 min+	1.5 mg/m²/dose	Days 1, 8, 15, 43 & 50	+ Or infusion via minibag as per institutional policy **Maximum dose of 2 mg**	a. Hx/PE with VS/Wt (BSA) b. CBC/diff/plts c. CSF cell count & cytospin[1] d. Bilirubin, ALT, creatinine e. Electrolytes including PO_4 f. Pulse Oximetry (O2 saturation) and CXR- Patients who develop respiratory signs or symptoms as defined in Section 5.2.2 [1] Obtain with each IT administration **OBTAIN OTHER STUDIES AS REQUIRED FOR GOOD PATIENT CARE**
Pegaspargase (PEG-ASP)	IV over 1-2 hours	2500 International units/m²/dose	Day 4, 18, & 43	Administer through the tubing of a freely infusing solution of D_5W or 0.9% NaCl	
Intrathecal Methotrexate (IT MTX)	IT	Age (yrs) Dose 1 – 1.99 8 mg 2 – 2.99 10 mg 3 – 8.99 12 mg ≥ 9 15 mg	Days 1, 29 & 36	**Note age-based dosing**	
Cyclophosphamide (CPM)	IV over 30-60 minutes	1000 mg/m²/dose	Day 29	See Section 4.9 for administration guidelines	
Cytarabine (ARAC)	IV over 1-30 min or SubQ	75 mg/m²/dose	Days 29-32 & 36-39		
Thioguanine (TG)	PO	60 mg/m²/dose	Days 29-42	See Section 4.9, Section 5.13 & Appendix VII for administration guidelines	

Therapy Delivery Map **Ht_____cm** **Wt_____kg** **BSA____m²**

Date Due	Date Given	Day	VCR ____mg	PEG-ASP ____IU	IT MTX ____mg	CPM ____mg	ARAC ____mg	TG ____mg	Studies	Comments
			Enter calculated dose above and actual dose administered below							
		29			____mg	____mg	____mg	____mg	b,d,c	
		30					↓	↓		
		31								
		32								
		33								
		36			____mg		____mg		b,c	
		37					↓	↓		
		38								
		39								
		40								
		41								
		42								
		43	____mg	____IU					b	
		44								
		45								
		46								
		47								
		48								
		49								
		50	____mg						b	
		57							b	
		63							b	
		64	**Start next course (Maintenance, Appendix II-M) on Day 64 or when blood count parameters are met (whichever occurs later)**							

[1] Obtain with each IT administration

SEE PROTOCOL SECTION 5.0 FOR DOSE MODIFICATIONS. SEE THE COG MEMBER WEBSITE FOR SUPPORTIVE CARE GUIDELINES.

THERAPY DELIVERY MAPS – ARM B-IR (T-ALL and T-LLy)

APPENDIX II-E

INTERIM MAINTENANCE #1 with HDMTX- Arm B-IR

This IM course is only for patients randomized to Arm B-IR. See Section 4.10 for details.

_____ Patient name or initials

_____ DOB

Patients receive this block immediately after consolidation and must meet all of the following criteria: Post-consolidation risk assignment must have been completed as described in Section 4.1. Start when ANC ≥750/µL and platelets ≥75,000/µl. Patients need to meet renal and hepatic function requirements to initiate methotrexate as described in Section 5.9.1, Section 5.9.2, and Section 5.9.3 This Course lasts 8 weeks (56 days) and this Therapy Delivery Map is on **one (1)** page.

DRUG	ROUTE	DOSAGE	DAYS	IMPORTANT NOTES	OBSERVATIONS
High-Dose Methotrexate (HD MTX)	IV over 24 hours	5000 mg/m^2/dose	Days 1, 15, 29 & 43	See Section 5.9 & Appendix IV for administration guidelines **Note: 2 stage administration**	a. Hx/PE with VS/Wt (BSA) b. CBC/diff/plts Bilirubin, ALT, creatinine c. CSF cell count & cytospin[1] **OBTAIN OTHER STUDIES AS REQUIRED FOR GOOD PATIENT CARE**
Leucovorin (LCV)	PO/IV	15 mg/m^2/dose	42, 48, and 54 hours after the start of the HD MTX infusion	See Section 5.9 and Appendix IV for HD MTX Infusion and Leucovorin rescue guidelines.	
VinCRIStine (VCR)	IV push over 1 min$^+$	1.5 mg/m^2/dose	Days 1, 15, 29 & 43	+ Or infusion via minibag as per institutional policy **Maximum dose of 2 mg**	
Mercaptopurine (MP)	PO	25 mg/m^2/dose	Days 1-56	See Section 4.10, Section 5.11 and Appendix VI for administration guidelines	
Intrathecal Methotrexate (IT MTX)	IT	Age (yrs) / Dose 1 – 1.99 / 8 mg 2 – 2.99 / 10 mg 3 – 8.99 / 12 mg ≥ 9 / 15 mg	Days 1 & 29	Deliver within 6 hours of the start of IV MTX (hr -6 to +6) **Note age-based dosing**	

Therapy Delivery Map **Ht_____cm** **Wt_____kg** **BSA____m^2**

Date Due	Date Given	Day	HD MTX ____mg	LCV	VCR ____mg	MP ____mg	IT MTX ____mg	Studies	Comments
			Enter calculated dose above and actual dose administered below						
		1	____mg		____mg	____mg	____mg	a,b*,c	
		2							
		3		____mg$					
		4							
		5							

		15	mg		mg			b*	
		16							
		17		____mg$					
		18							
		19							

		29	____mg		____mg		____mg	b*,c	
		30							
		31		____mg$					
		32							
		33							
		34							

		43	____mg		____mg			b*	
		44							
		45		____mg$					
		46							
		47							

		56							
		57	**Start next course (Delayed Intensification, Appendix II-F) on Day 57 or when blood count parameters are met (whichever occurs later).**						

[1] Obtain with each IT administration *To be performed prior to each dose of methotrexate

$Please document the number of doses of leucovorin administered

SEE PROTOCOL SECTION 5.0 FOR DOSE MODIFICATIONS. SEE THE COG MEMBER WEBSITE FOR SUPPORTIVE CARE GUIDELINES.

APPENDIX II-F

DELAYED INTENSIFICATION Arm B-IR (with bortezomib)

This Delayed Intensification (DI) course is for patients randomized to treatment on Arm B-IR (with bortezomib). See Section 4.9 for details

Patient name or initials _____

DOB _____

Patients should have ANC ≥ 750/µL and platelets ≥ 75,000/µL prior to starting therapy. Once Delayed Intensification has begun, it may be interrupted for myelosuppression (ANC ≤ 750/µL and platelets ≤ 75,000/µL) on Day 29 ONLY. Once the Day 1 therapy or Day 29 cyclophosphamide has been given, the remainder of the therapy should not be held solely for myelosuppression, which is expected to occur. Therapy must be interrupted for patients with serious proven or presumed infection and resumed when the signs of infection have abated. Hold Day 29 chemotherapy until ANC ≥ 750/µL and platelets ≥ 75,000/µL. This Course lasts 9 weeks (63 days) and this Therapy Delivery Map is on **two (2)** pages.

DRUG	ROUTE	DOSAGE	DAYS	IMPORTANT NOTES	OBSERVATIONS
Bortezomib (BOR) *IND#*[redacted]	IV push over 3-5 seconds	1.3 mg/m^2/dose	Days 1, 4, 15 & 18	At least 72 hours must have elapsed between doses.	a. Hx/PE with VS/Wt (BSA) b. CBC/diff/plts c. CSF cell count & cytospin[1] d. Bilirubin, ALT, creatinine e. Electrolytes including PO_4 f. Pulse Oximetry (O2 saturation) and CXR- Patients who develop respiratory signs or symptoms as defined in Section 5.2.2 **OBTAIN OTHER STUDIES AS REQUIRED FOR GOOD PATIENT CARE**
VinCRIStine (VCR)	IV push over 1 min$^+$	1.5 mg/m^2/dose	Days 1, 8, 15, 43 & 50	+ Or infusion via minibag as per institutional policy **Maximum dose of 2 mg**	
Dexamethasone (DEX)	PO (May be given IV)	5 mg/m^2/dose BID	Days 1-7 & 15-21	Total daily dose: 10 mg/m^2/day, divided BID	
DOXOrubicin (DOXO)	IV Push Over 1-15 min	25 mg/m^2/dose	Days 1, 8, & 15		
Pegaspargase (PEG-ASP)	IV over 1-2 hours	2500 International units/m^2/dose	Day 4, 18, & 43	Administer through the tubing of a freely infusing solution of D_5W or 0.9% NaCl	
Intrathecal Methotrexate (IT MTX)	IT	Age (yrs) – Dose 1 – 1.99 – 8 mg 2 – 2.99 – 10 mg 3 – 8.99 – 12 mg ≥ 9 – 15 mg	Days 1, 29 & 36	**Note age-based dosing**	

Therapy Delivery Map **Ht_____cm** **Wt_____kg** **BSA____m^2**

Date Due	Date Given	Day	BOR ____mg	VCR ____mg	DEX ____mg ____mg	DOXO ____mg	PEG-ASP ____IU	IT MTX _____mg	Studies	Comments
					Enter calculated dose above and actual dose administered below					
		1	____mg	____mg	____mg____mg	____mg		_____mg	a,b,c,d,e, f*	
		2								
		3								
		4	____mg				____IU		f*	
		5								
		6								
		7								
		8		____mg		____mg			b	

		15	____mg	____mg	____mg____mg	____mg			b, f*	
		16								
		17								
		18	____mg				____IU		f*	
		19								
		20								
		21								
		22							b	
				This therapy delivery map continues on the next page with Day 29.						

[1] Obtain with each IT administration ^ To be performed prior to first dose of bortezomib

*Patients who develop respiratory signs or symptoms as defined in Section 5.2.2 should have pulse oximetry (02 saturation) and Chest x-ray documented within 12 hours prior to each dose of bortezomib.

SEE PROTOCOL SECTION 5.0 FOR DOSE MODIFICATIONS. SEE THE COG MEMBER WEBSITE FOR SUPPORTIVE CARE GUIDELINES.

DELAYED INTENSIFICATION Arm B-IR (with bortezomib)

This Delayed Intensification (DI) course is for patients randomized to treatment on Arm B-IR (with bortezomib). See Section 4.9 for details

Patient name or initials

DOB

DRUG	ROUTE	DOSAGE	DAYS	IMPORTANT NOTES	OBSERVATIONS
VinCRIStine (VCR)	IV push over 1 min+	1.5 mg/m^2/dose	Days 1, 8, 15, 43 & 50	+ Or infusion via minibag as per institutional policy **Maximum dose of 2 mg**	a. Hx/PE with VS/Wt (BSA) b. CBC/diff/plts c. CSF cell count & cytospin[1] d. Bilirubin, ALT, creatinine e. Electrolytes including PO_4 f. Pulse Oximetry (O2 saturation) and CXR- Patients who develop respiratory signs or symptoms as defined in Section 5.2.2 **OBTAIN OTHER STUDIES AS REQUIRED FOR GOOD PATIENT CARE**
Pegaspargase (PEG-ASP)	IV over 1-2 hours	2500 International units/m^2/dose	Day 4, 18, & 43	Administer through the tubing of a freely infusing solution of D_5W or 0.9% NaCl	
Intrathecal Methotrexate (IT MTX)	IT	Age (yrs) Dose 1 – 1.99 8 mg 2 – 2.99 10 mg 3 – 8.99 12 mg ≥ 9 15 mg	Days 1, 29 & 36	**Note age-based dosing**	
Cyclophosphamide (CPM)	IV over 30-60 minutes	1000 mg/m^2/dose	Day 29	See Section 4.9 for administration guidelines	
Cytarabine (ARAC)	IV or SubQ	75 mg/m^2/dose	Days 29-32 & 36-39		
Thioguanine (TG)	PO	60 mg/m^2/dose	Days 29-42	See Section 4.9, Section 5.13 & Appendix VII for administration guidelines	

Therapy Delivery Map **Ht_____cm** **Wt_____kg** **BSA____m^2**

Date Due	Date Given	Day	VCR mg	PEG-ASP IU	IT MTX mg	CPM mg	ARAC mg	TG mg	Studies	Comments
			Enter calculated dose above and actual dose administered below							
		29			mg	mg	mg	mg	b, c,d,e	
		30								
		31								
		32								
		33								

		36			mg		mg		b,c,e	
		37								
		38								
		39								
		40								
		41								
		42								
		43	mg	IU					b,e	
		44								
		45								
		46								
		47								
		48								
		49								
		50	mg						b,e	

		57							b,e	

		63							b,e	
		64	**Start next course (IM#2 , APPENDIX II-G) on Day 64 or when blood count parameters are met (whichever occurs later)**							

[1] Obtain with each IT administration

SEE PROTOCOL SECTION 5.0 FOR DOSE MODIFICATIONS. SEE THE COG MEMBER WEBSITE FOR SUPPORTIVE CARE GUIDELINES.

APPENDIX II-G

INTERIM MAINTENANCE #2 with CMTX- Arm B-IR This IM course is for patients randomized to Arm B-IR. See Section 4.7 for details	Patient name or initials DOB

Begin IM when peripheral counts recover with an ANC ≥ 750/µL and platelets ≥ 75,000/µL. Patients need to meet renal and hepatic function requirements to initiate methotrexate as described in Section 5.10. This Course lasts 8 weeks (56 days) and this Therapy Delivery Map is on **one (1)** page.

DRUG	ROUTE	DOSAGE	DAYS	IMPORTANT NOTES	OBSERVATIONS
VinCRIStine (VCR)	IV push over 1 min+	1.5 mg/m²/dose	Days 1, 11, 21, 31 & 41	+ Or infusion via minibag as per institutional policy **Maximum dose of 2 mg**	a. Hx/PE with VS/Wt (BSA) b. CBC/diff/plts Bilirubin, ALT, creatinine c. CSF cell count & cytospin[1] **OBTAIN OTHER STUDIES AS REQUIRED FOR GOOD PATIENT CARE**
Methotrexate (CMTX)	IV push over 2-5 mins or IV infusion over 10-15 mins	Starting dose is 100 mg/m² **escalate by 50 mg/m²/dose**	Days 1, 11, 21, 31 & 41	See Section 4.7 and Section 5.10 for details	
Pegaspargase (PEG-ASP)	IV over1-2 hours	2500 International units/m²/dose	Days 2 & 22	Administer through the tubing of a freely infusing solution of D_5W or 0.9% NaCl	
Intrathecal Methotrexate (IT MTX)	IT	Age (yrs) — Dose 1 – 1.99 — 8 mg 2 – 2.99 — 10 mg 3 – 8.99 — 12 mg ≥ 9 — 15 mg	Days 1 & 31	**Note age-based dosing**	

Therapy Delivery Map **Ht_____cm** **Wt_____kg** **BSA____m²**

Date Due	Date Given	Day	VCR mg	IV MTX *(escalating dose)*	PEG-ASP IU	IT MTX mg	Studies	Comments
			Enter calculated dose above and actual dose administered below					
		1	____mg	____mg		____mg	a,b*,c	
		2			____IU			

		11	____mg	____mg			b*	

		21	____mg	____mg			b*	
		22			____IU			

		31	____mg	____mg		____mg	b*,c	

		41	____mg	____mg			b*	

		56						
		57	**Start next course (Maintenance Appendix II-M) on Day 57 or when blood count parameters are met (whichever occurs later).**					

[1] Obtain with each IT administration

*To be performed prior to each dose of methotrexate

SEE PROTOCOL SECTION 5.0 FOR DOSE MODIFICATIONS. SEE THE COG MEMBER WEBSITE FOR SUPPORTIVE CARE GUIDELINES.

THERAPY DELIVERY MAPS – ARM B-VHR (T-ALL and T-LLy)

APPENDIX II-H

Intensification Block (1) - Arm B-VHR This course is only for patients randomized to treatment on Arm B-VHR. See Section 4.11 for details	Patient name or initials DOB

T-ALL patients who are M2 or M3 at the end of Consolidation should proceed directly to Intensification Block 1 without waiting for count recovery or MRD results to proceed. Patients receive this block immediately after consolidation and must meet all of the following criteria: Post-consolidation risk assignment must have been completed as described in Section 4.1. **T-ALL only:** Begin Intenstification Block #1 after collection of end of consolidation bone marrow for MRD. when ANC ≥750/µL and platelets ≥75,000/µl, and organ function requirements are met as definded in Sections 5.9.1, 5.9.2, and 5.9.3, or whichever occurs later. **T-LLy only:** Start when ANC ≥750/µL and platelets ≥75,000/µl. This Cycle lasts 21 days and this Therapy Delivery Map is on **one (1)** page.

DRUG	ROUTE	DOSAGE	DAYS	IMPORTANT NOTES	OBSERVATIONS
Dexamethasone (DEX)	PO (May be given IV)	10 mg/m^2/dose BID	1-5	Total daily dose: 20 mg/m^2/day, divided BID	a. Hx/PE with VS/Wt (BSA) b. CBC/diff/plts c. CSF cell count & cytospin[1] d. Bilirubin, ALT, creatinine **OBTAIN OTHER STUDIES AS REQUIRED FOR GOOD PATIENT CARE**
High dose methotrexate (HD-MTX)	IV	5000 mg/m^2/dose	Day 1	See Section 5.9 & Appendix IV for administration guidelines	
Leucovorin (LCV)	PO/IV	15 mg/m^2/dose	Days 3-4	Minimum of 3 doses given at 42, 48, and 54 hours after the start of the HD MTX infusion See Section 5.9 and Appendix IV for HD MTX Infusion and Leucovorin rescue guidelines.	
VinCRIStine (VCR)	IV push over 1 min$^+$	1.5 mg/m^2/dose	Days 1 & 6	+ Or infusion via minibag as per institutional policy **Maximum dose of 2 mg**	
Cyclophosphamide (CPM)	IV over 1-6 hours	200 mg/m^2/dose Q12 hrs x5 doses	Days 2-4	See Section 4.11 for administration guidelines	
High dose cytarabine (ARAC)	IV over 3 hours	2000 mg/m^2/dose Q12 hours x 2 doses	Day 5		
Pegaspargase (PEG-ASP)	IV over 1-2 hours	2500 International units/m^2/dose	Day 6	Administer 3 hours after completion of the second HD-AraC infusion through the tubing of a freely infusing solution of D_5W or 0.9% NaCl	
Triple Intrathecal Therapy (ITT): Methotrexate (MTX) Hydrocortisone (HC) Cytarabine (ARAC)	IT	**MTX and HC dosing:** Age (yrs) Dose 1 – 1.99 8 mg 2 – 2.99 10 mg 3 – 8.99 12 mg ≥ 9 15 mg **ARAC dosing:** Age (yrs) Dose 1 – 1.99 16 mg 2 – 2.99 20 mg 3 – 8.99 24 mg ≥ 9 30 mg	Day 1	**Note age-based dosing** **Delivery within 6 hrs of IV MTX infusion**	
Filgrastim (G-CSF)	SubQ	5 mcg/kg/dose	Daily beginning on Day 7	Continue until WBC > 3000/µL	

Intensification Block (1) - Arm B-VHR

This course is only for patients randomized to treatment on Arm B-VHR. See Section 4.11 for details

_____
Patient name or initials

_____
DOB

Enter Cycle #____ Ht ____ cm Wt ____ kg BSA ____ m^2

Date Due	Date Given	Day	DEX ___mg	IV MTX ____mg	LCV	VCR ____mg	CPM ____mg	ARAC ____mg	PEG-ASP ____IU	ITT ___mg (MTX) ___mg (HC) ___mg (ARAC)	G-CSF ____mcg	Studies	Comments
			Enter calculated dose above and actual dose administered below										
		1	___mg ___mg	____mg		____mg				____mg (MTX) ____mg (HC) ___mg (ARAC)		a,b,c,d	
		2	___mg ___mg				____mg ___mg						
		3	___mg ___mg		____mg$		____mg ___mg						
		4	___mg ___mg				____mg						
		5	___mg ___mg					____mg ___mg					
		6				____mg			____IU				
		7									____mcg	b*	
		21	**Start next course (Intensification Block 2 Appendix II-I) on Day 22 or when blood count parameters are met (whichever occurs later).**										

*Every 2 days after completion of chemotherapy until count recovery

$Please document the number of doses of leucovorin administered

SEE PROTOCOL SECTION 5.0 FOR DOSE MODIFICATIONS. SEE THE COG MEMBER WEBSITE FOR SUPPORTIVE CARE GUIDELINES.

APPENDIX II-I

Intensification Block (2) Arm B-VHR

This course is only for patients randomized to treatment on Arm B-VHR. See Section 4.12 for details.

_____ Patient name or initials

_____ DOB

Start Day 1 when ANC ≥750/µL and platelets ≥75,000/µL. This Cycle lasts 21 days and this Therapy Delivery Map is on **one (1)** page.

DRUG	ROUTE	DOSAGE	DAYS	IMPORTANT NOTES	OBSERVATIONS
Dexamethasone (DEX)	PO (May be given IV)	10 mg/m^2/dose BID	1-5	Total daily dose: 20 mg/m^2/day, divided BID	a. Hx/PE with VS/Wt (BSA) b. CBC/diff/plts c. CSF cell count & cytospin[1] d. Bilirubin, ALT, creatinine **OBTAIN OTHER STUDIES AS REQUIRED FOR GOOD PATIENT CARE**
High dose methotrexate (HD-MTX)	IV	5000 mg/m^2/dose	Day 1	See Section 5.9 & Appendix IV for administration guidelines	
Leucovorin (LCV)	PO/IV	15 mg/m^2/dose	Days 3-4	Minimum of 3 doses given at 42, 48, and 54 hours after the start of the HD MTX infusion See Section 5.9 and Appendix IV for HD MTX Infusion and Leucovorin rescue guidelines.	
VinCRIStine (VCR)	IV push over 1 min$^+$	1.5 mg/m^2/dose	Days 1 & 6	+ Or infusion via minibag as per institutional policy **Maximum dose of 2 mg**	
Mesna	IV	300 mg/m^2/dose Hour 0, 4, and 8 from start of each ifosfamide infusion	Days 2-4	Hydration following ifosfamide administration: If patient stops methotrexate hydration for whatever reason, need to continue until 12 hours post ifosfamide infusion.	
Ifosfamide (IFOS)	IV over 1 hr	800 mg/m^2/dose Q12 hours x5 doses	Days 2-4	Suggested hydration: Administer 3000 mL/m^2/day (125 mL/m^2/hr) using fluid containing 0.45% NaCl or 0.9% NaCl. Achieve urine specific gravity < 1.010 and urine output > 3 mL/kg/hr prior to start of ifosfamide **Start immediately after HD-MTX**	
DAUNOrubicin (DAUN)	IV Push/infusion Over 1-15 min	30 mg/m^2/dose	Day 5		
Pegaspargase (PEG-ASP)	IV over 1-2 hours	2500 International units/m^2/dose	Day 6	Administer through the tubing of a freely infusing solution of D_5W or 0.9% NaCl	
Triple Intrathecal Therapy (ITT): Methotrexate (MTX) Hydrocortisone (HC) Cytarabine (ARAC)	IT	**MTX and HC dosing:** Age (yrs) Dose 1 – 1.99 8 mg 2 – 2.99 10 mg 3 – 8.99 12 mg ≥ 9 15 mg **ARAC dosing:** Age (yrs) Dose 1 – 1.99 16 mg 2 – 2.99 20 mg 3 – 8.99 24 mg ≥ 9 30 mg	Day 1	**Note age-based dosing** **Delivery within 6 hrs of IV MTX infusion**	
Filgrastim (G-CSF)	SubQ	5 mcg/kg/dose	Daily beginning on Day 7	Continue until WBC > 3000/µL	

Intensification Block (2) Arm B-VHR

This course is only for patients randomized to treatment on Arm B-VHR. See Section 4.12 for details.

_____
Patient name or initials

_____
DOB

Enter Cycle # _____ Ht_____cm Wt_____kg BSA____m^2

Date Due	Date Given	Day	DEX ___mg	IV MTX ____mg	LCV	VCR ____mg	MESNA ____mg	IFOS ____mg	DAUN ____mg	PEG-ASP ____IU	ITT ____mg (MTX) ____mg (HC) ____mg (ARAC)	G-CSF ____mcg	Studies	Comments
			Enter calculated dose above and actual dose administered below											
		1	___mg ____mg	____mg		____mg					____mg (MTX) ____mg (HC) ____mg (ARAC)		a,b,c,d	
		2	___mg ____mg				____mg ____mg ____mg ____mg ____mg mg	____mg ____mg						
		3	___mg ____mg		____mg$		____mg ____mg ____mg ____mg ____mg mg	____mg ____mg						
		4	___mg ____mg				____mg ____mg ____mg	____mg						
		5	___mg ____mg						____mg					
		6				____mg				____IU				
		7										____mcg	b*	
		21	**Start next course (Intensification Block 3 Appendix II-J) on Day 22 or when blood count parameters are met (whichever occurs later).**											

*every 2 days after completion of chemotherapy until count recovery

$Please document the number of doses of leucovorin administered

SEE PROTOCOL SECTION 5.0 FOR DOSE MODIFICATIONS. SEE THE COG MEMBER WEBSITE FOR SUPPORTIVE CARE GUIDELINES.

Page 2 of 2

APPENDIX II-J

Intensification Block (3) Arm B-VHR
This course is only for patients randomized to treatment on Arm B-VHR. See Section 4.13 for details.

_____ Patient name or initials

_____ DOB

This Cycle lasts 21 days and this Therapy Delivery Map is on one (1) page. Start Day 1 when ANC ≥ 750/µL and platelets ≥ 75,000/µL. HR3 lasts 21 days.

DRUG	ROUTE	DOSAGE	DAYS	IMPORTANT NOTES	OBSERVATIONS
Dexamethasone (DEX)	PO (May be given IV)	10 mg/m^2/dose BID	1-5	Total daily dose: 20 mg/m^2/day, divided BID	a. Hx/PE with VS/Wt (BSA) b. CBC/diff/plts c. CSF cell count & cytospin[1] d. Bilirubin, ALT, creatinine **T-ALL ONLY** e. BM MRD **T-LLy ONLY:** f. Chest CT/CXR[2] g. Abdomen/pelvis CT (or MRI)[2] h. Bone scan[2] i. Diagnostic biopsy/cytology[4] [2] See Section 7.0 for details **OBTAIN OTHER STUDIES AS REQUIRED FOR GOOD PATIENT CARE**
High dose cytarabine (ARAC)	IV	2000 mg/m^2/dose Q12 hours x 4 doses	Days 1-2		
Etoposide (ETOP)	IV over 2 hrs	100 mg/m^2/dose Q12 hours x5 doses	Days 3-5	See Section 4.13 for administration guidelines	
Pegaspargase (PEG-ASP)	IV over 1-2 hours	2500 International units/m^2/dose	Day 6	Administer through the tubing of a freely infusing solution of D_5W or 0.9% NaCl	
Triple Intrathecal Therapy (ITT): Methotrexate (MTX) Hydrocortisone (HC) Cytarabine (ARAC)	IT	**MTX and HC dosing:** Age (yrs) Dose 1 – 1.99 8 mg 2 – 2.99 10 mg 3 – 8.99 12 mg ≥ 9 15 mg **ARAC dosing:** Age (yrs) Dose 1 – 1.99 16 mg 2 – 2.99 20 mg 3 – 8.99 24 mg ≥ 9 30 mg	Day 5	**Note age-based dosing**	
Filgrastim (G-CSF)	SubQ	5 mcg /kg/dose	Daily beginning on Day 7	Continue until WBC > 3000/µL	

Enter Cycle #_____ Ht_____cm Wt_____kg BSA____m^2

Date Due	Date Given	Day	DEX ___mg	ARAC ____mg	ETOP ____mg	PEG-ASP ____IU	ITT ____mg (MTX) ____mg (HC) ____mg (ARAC)	G-CSF ___mcg	Studies	Comments
			Enter calculated dose above and actual dose administered below							
		1	___mg ___mg	____mg ____mg					a,b,d	
		2	___mg ____mg	____mg ____mg						
		3	___mg ____mg		____mg ____mg					
		4	___mg ____mg		____mg ____mg					
		5	___mg ____mg		____mg		____mg (MTX) ____mg (HC) ____mg (ARAC)		c	
		6				____IU				
		7						___mcg		
		21							**T-ALL:** b*e **T-LLy:** b*,i, (f,g,h)[2]	
			Start next course (Delayed Intensification Appendix II-K) on Day 22 or when blood count parameters are met (whichever occurs later).							

[1] Obtain with each IT administration

*every 2 days after completion of chemotherapy until count recovery

SEE PROTOCOL SECTION 5.0 FOR DOSE MODIFICATIONS. SEE THE COG MEMBER WEBSITE FOR SUPPORTIVE CARE GUIDELINES.

APPENDIX II-K

DELAYED INTENSIFICATION- Arm B-VHR (with bortezomib)

This Delayed Intensification (DI) course is for patients randomized to treatment on Arm B-VHR (with bortezomib). See Section 4.9 for details

Patient name or initials

DOB

Patients receive this block immediately after HR Intensification 3 and must meet all of the following criteria: **VHR T-ALL** Post-HR Intensification Block 3 MRD was completed and demonstrated undetectable MRD as described in Section 4.13 Start 7 days after collection of HR Intensification Block 3 bone marrow for MRD or when ANC ≥750/µL and platelets ≥75,000/µl, whichever occurs later. **VHR T-LLy:** Post-HR Intensification Block 3 disease evaluation was completed and demonstrated a radiographic CR OR a radiographic PR and a biopsy showing no residual disease as described in Section 10.4 Start when ANC ≥750/µL and platelets ≥75,000/µl **All patients:** Once Delayed Intensification has begun, it may be interrupted for myelosuppression (ANC ≤ 750/µL and platelets ≤ 75,000/µL) on Day 29 ONLY. Once the Day 1 therapy or Day 29 cyclophosphamide has been given, the remainder of the therapy should not be held solely for myelosuppression, which is expected to occur. Therapy must be interrupted for patients with serious proven or presumed infection and resumed when the signs of infection have abated. Hold Day 29 chemotherapy until ANC ≥ 750/µL and platelets ≥ 75,000/µL. This Course lasts 9 weeks (63 days) and this Therapy Delivery Map is on **two (2)** pages.

DRUG	ROUTE	DOSAGE	DAYS	IMPORTANT NOTES	OBSERVATIONS
Bortezomib (BOR) *IND#*[redacted]	IV push over 3-5 seconds	1.3 mg/m^2/dose	Days 1, 4, 15 & 18	At least 72 hours must have elapsed between doses.	a. Hx/PE with VS/Wt (BSA) b. CBC/diff/plts c. CSF cell count & cytospin[1] d. Bilirubin, ALT, creatinine e. Electrolytes including PO_4 f. Pulse Oximetry (O2 saturation) and CXR- Patients who develop respiratory signs or symptoms as defined in Section 5.2.2 **OBTAIN OTHER STUDIES AS REQUIRED FOR GOOD PATIENT CARE**
VinCRIStine (VCR)	IV push over 1 min$^+$	1.5 mg/m^2/dose	Days 1, 8, 15, 43 & 50	+ Or infusion via minibag as per institutional policy **Maximum dose of 2 mg**	
Dexamethasone (DEX)	PO (May be given IV)	5 mg/m^2/dose BID	Days 1-7 & 15-21	Total daily dose: 10 mg/m^2/day, divided BID	
DOXOrubicin (DOXO)	IV Push Over 1-15 min	25 mg/m^2/dose	Days 1, 8, & 15		
Pegaspargase (PEG-ASP)	IV over 1-2 hours	2500 International units/m^2/dose	Day 4, 18, & 43	Administer through the tubing of a freely infusing solution of D_5W or 0.9% NaCl	
Intrathecal Methotrexate (IT MTX)	IT	Age (yrs) Dose 1 – 1.99 8 mg 2 – 2.99 10 mg 3 – 8.99 12 mg ≥ 9 15 mg	Days 1, 29 & 36	**Note age-based dosing**	

Therapy Delivery Map **Ht_____cm** **Wt_____kg** **BSA_____m^2**

Date Due	Date Given	Day	BOR ____mg	VCR ____mg	DEX ____mg ____mg	DOXO ____mg	PEG-ASP ____IU	IT MTX _____mg	Studies	Comments
					Enter calculated dose above and actual dose administered below					
		1	____mg	____mg	____mg ____mg	____mg		_____mg	a,b,c,d,e, f*	
		2								
		3								
		4	____mg				____IU		f*	
		5								
		6								
		7								
		8		____mg		____mg			b	
		15	____mg	____mg	____mg ____mg	____mg			b, f*	
		16								
		17								
		18	____mg				____IU		f*	
		19								
		20								
		21								
		22							b	

This therapy delivery map continues on the next page with Day 29.

[1] Obtain with each IT administration

* Patients who develop respiratory signs or symptoms as defined in Section 5.2.2 should have pulse oximetry (02 saturation) and Chest x-ray documented within 12 hours prior to each dose of bortezomib.

SEE PROTOCOL SECTION 5.0 FOR DOSE MODIFICATIONS. SEE THE COG MEMBER WEBSITE FOR SUPPORTIVE CARE GUIDELINES.

DELAYED INTENSIFICATION- Arm B-VHR (with bortezomib)

This Delayed Intensification (DI) course is for patients randomized to treatment on Arm B-VHR (with bortezomib). See Section 4.9 for details

Patient name or initials

DOB

DRUG	ROUTE	DOSAGE	DAYS	IMPORTANT NOTES	OBSERVATIONS
VinCRIStine (VCR)	IV push over 1 min+	1.5 mg/m^2/dose	Days 1, 8, 15, 43 & 50	+ Or infusion via minibag as per institutional policy **Maximum dose of 2 mg**	a. Hx/PE with VS/Wt (BSA) b. CBC/diff/plts c. CSF cell count & cytospin[1] d. Bilirubin, ALT, creatinine e. Electrolytes including PO_4 f. Pulse Oximetry (O2 saturation) and CXR- Patients who develop respiratory signs or symptoms as defined in Section 5.2.2
Pegaspargase (PEG-ASP)	IV over 1-2 hours	2500 International units/m^2/dose	Day 4, 18, & 43	Administer through the tubing of a freely infusing solution of D_5W or 0.9% NaCl	
Intrathecal Methotrexate (IT MTX)	IT	Age (yrs) Dose 1 – 1.99 8 mg 2 – 2.99 10 mg 3 – 8.99 12 mg ≥ 9 15 mg	Days 1, 29 &36	**Note age-based dosing**	
Cyclophosphamide (CPM)	IV over 30-60 minutes	1000 mg/m^2/dose	Day 29	See Section 4.9 for administration guidelines	
Cytarabine (ARAC)	IV over 1-30 min or SubQ	75 mg/m^2/dose	Days 29-32 & 36-39		
Thioguanine (TG)	PO	60 mg/m^2/dose	Days 29-42	See Section 4.9, Section 5.13 & Appendix VII for administration guidelines	**OBTAIN OTHER STUDIES AS REQUIRED FOR GOOD PATIENT CARE**

Therapy Delivery Map **Ht____cm** **Wt____kg** **BSA____m^2**

Date Due	Date Given	Day	VCR ____mg	PEG-ASP ____IU	IT MTX ____mg	CPM ____mg	ARAC ____mg	TG ____mg	Studies	Comments
			Enter calculated dose above and actual dose administered below							
		29			____mg	____mg	____mg	____mg	b,d,c	
		30								
		31								
		32								
		33								
		36			____mg		____mg		b,c	
		37								
		38								
		39								
		40								
		41								
		42								
		43	____mg	IU					b	
		44								
		45								
		46								
		47								
		48								
		49								
		50	____mg						b	
		57							b	
		63							b	
		64	**Start next course (IM CMTX, APPENDIX II-L) on Day 64 or when blood count parameters are met (whichever occurs later)**							

[1] Obtain with each IT administration

SEE PROTOCOL SECTION 5.0 FOR DOSE MODIFICATIONS. SEE THE COG MEMBER WEBSITE FOR SUPPORTIVE CARE GUIDELINES.

APPENDIX II-L

INTERIM MAINTENANCE (CMTX) – Arm B-VHR This IM course is for patients randomized to Arm B-VHR. See Section 4.7 for details	_____ Patient name or initials _____ DOB

Begin IM when peripheral counts recover with an ANC ≥ 750/µL and platelets ≥ 75,000/µL. Patients need to meet renal and hepatic function requirements to initiate methotrexate as described in Section 5.10. This Course lasts 8 weeks (56 days) and this Therapy Delivery Map is on **one (1)** page.

DRUG	ROUTE	DOSAGE	DAYS	IMPORTANT NOTES	OBSERVATIONS
VinCRIStine (VCR)	IV push over 1 min+	1.5 mg/m²/dose	Days 1, 11, 21, 31 & 41	+ Or infusion via minibag as per institutional policy **Maximum dose of 2 mg**	a. Hx/PE with VS/Wt (BSA) b. CBC/diff/plts Bilirubin, ALT, creatinine c. CSF cell count & cytospin[1] **OBTAIN OTHER STUDIES AS REQUIRED FOR GOOD PATIENT CARE**
Methotrexate (CMTX)	IV push over 2-5 mins or IV infusion over 10-15 mins	Starting dose is 100 mg/m² **escalate by 50 mg/m²/dose**	Days 1, 11, 21, 31 & 41	See Section 4.7 & Section 5.10 for details	
Pegaspargase (PEG-ASP)	IV over 1-2 hours	2500 International units/m²/dose	Days 2 & 22	Administer through the tubing of a freely infusing solution of D_5W or 0.9% NaCl	
Intrathecal Methotrexate (IT MTX)	IT	Age (yrs) – Dose 1 – 1.99 – 8 mg 2 – 2.99 – 10 mg 3 – 8.99 – 12 mg ≥ 9 – 15 mg	Days 1 & 31	**Note age-based dosing**	

Therapy Delivery Map **Ht____ cm** **Wt____ kg** **BSA____ m²**

Date Due	Date Given	Day	VCR ____ mg	IV MTX *(escalating dose)*	PEG-ASP ____ IU	IT MTX ____ mg	Studies	Comments
			Enter calculated dose above and actual dose administered below					
		1	____mg	____mg		____mg	a,b*,c	
		2			____IU			
		11	____mg	____mg			b*	
		21	____mg	____mg			b*	
		22			____IU			
		31	____mg	____mg		____mg	b*,c	
		41	____mg	____mg			b*	
		56						
		57	**Start next course (Maintenance Appendix II-M) on Day 57 or when blood count parameters are met (whichever occurs later).**					

[1] Obtain with each IT administration

*To be performed prior to each dose of methotrexate

SEE PROTOCOL SECTION 5.0 FOR DOSE MODIFICATIONS. SEE THE COG MEMBER WEBSITE FOR SUPPORTIVE CARE GUIDELINES.

Page 1 of 1

THERAPY DELIVERY MAP – MAINTENANCE ARM B
(T-ALL and T-LLy)

APPENDIX II-M

MAINTENANCE- Arm B
This Maintenance course is for Arm B. See Section 4.14 for details

_____ Patient name or initials _____ DOB

Maintenance begins when peripheral counts recover with ANC ≥ 750/µL and platelets ≥ 75,000/µL. Only Mercaptopurine and PO Methotrexate will be interrupted for myelosuppression as outlined in Section 5.11. This Cycle lasts 12 weeks (84 days) and this Therapy Delivery Map is on **one (1)** page.

DRUG	ROUTE	DOSAGE	DAYS	IMPORTANT NOTES	OBSERVATIONS
VinCRIStine (VCR)	IV push over 1 min+	1.5 mg/m²/dose	Days 1, 29 & 57	+ Or infusion via minibag as per institutional policy **Maximum dose of 2 mg**	a. Hx/PE with VS/Wt (BSA) b. CBC/diff/plts c. CSF cell count & cytospin[1] d. Bilirubin, ALT, creatinine e. Thiopurine metabolites- as clinically indicated f. Optional Banking/Biology **T-LLy ONLY:** g. Chest CT/CXR h. Abdomen/pelvis CT i. Bone scan **OBTAIN OTHER STUDIES AS REQUIRED FOR GOOD PATIENT CARE**
Dexamethasone (DEX)	PO (May be given IV)	3 mg/m²/dose	Days 1-5, 29-33 & 57-61	Total daily dose: 6 mg/m²/day, divided BID	
Mercaptopurine (MP)	PO	75 mg/m²/dose	Days 1-84	See Section 4.14, Section 5.11 and Appendix VI for administration guidelines	
Methotrexate (MTX)	PO	20 mg/m²/dose weekly	Days 8, 15, 22, **29@**, 36, 43, 50, 57, 64, 71 & 78 @**NOTE:** Omit Day 29 of Cycles 1- 4 for SR T-ALL and T-LLy patients and cycles 1- 2 for IR T-ALL and T-LLy patients	see Section 5.11 for suggested starting dose based on TPMT and NUDT15 status (if available) **Please note Day 29**	
Intrathecal Methotrexate (IT MTX)	IT	Age (yrs) / Dose 1 – 1.99 / 8 mg 2 – 2.99 / 10 mg 3 – 8.99 / 12 mg ≥ 9 / 15 mg	Day 1 &Day 29 of Cycles 1- 4 for **SR T-ALL and T-LLy patients ONLY.** Day 29 of Cycles 1-2 **IR T-ALL and T-LLy patients ONLY**	**Note age-based dosing** **Please note Day 29**	

Enter Cycle # _____ Ht_____cm Wt_____kg BSA_____m²

Date Due	Date Given	Day	VCR mg	DEX mg mg	MP mg	PO MTX mg	IT MTX mg	Studies	Comments
			Enter calculated dose above and actual dose administered below						
		1$	_____mg	___mg ___mg	____mg		____mg	a,b,c,d	
		5							
		8				____mg			
		15				____mg			
		22				____mg			
		29	_____mg	___mg ___mg		____mg@	____mg&	a,b,c	
		33							
		36				____mg			
		43				____mg			
		50				____mg			
		57	_____mg	___mg ___mg		____mg		a,b	
		61							
		64				____mg			
		71				____mg			
		78				____mg			
		84						f[5] (g[4],h[3],i[3])[2#]	
		85	**Begin next cycle on Day 85 regardless of counts and repeat until two years (for T-ALL girls and all T-LLy pts, regardless of gender) and three years (for T-ALL boys) from the start of Interim Maintenance (see Section 4.14). Only MP & PO MTX will be interrupted for myelosuppression during subsequent Maintenance cycles as outlined in Section 5.11**						

[1] Obtain with each IT administration [2] T-LLy ONLY [3] If baseline is negative, no repeat scans are required.
[4] If CR at end-Consolidation perform a CXR; If PR or NR at end-Consolidation perform chest CT at completion of Maintenance therapy.
[5] Collect ONLY if the patient relapses. **T-ALL**: as a part of AALL08B1 (or APEC14B1 if available to ALL patients) **T-LLy**: submit via APEC14B1 (if enrolled).

[$]RADIATION THERAPY (T-ALL:IR CNS3 and all VHR patients, T-LLy:CNS3 only) See Section 16.0

[#] Only collect at the completion of therapy.

SEE PROTOCOL SECTION 5.0 FOR DOSE MODIFICATIONS. SEE THE COG MEMBER WEBSITE FOR SUPPORTIVE CARE GUIDELINES

APPENDIX III: YOUTH INFORMATION SHEETS

INFORMATION SHEET REGARDING RESEARCH STUDY AALL1231 FOR PATIENTS WITH T-ALL
(for children from 7 through 12 years of age)

A Study of Bortezomib in Children with Newly Diagnosed T-Lymphoblastic Leukemia (T-ALL)

1. We have been talking with you about a type of cancer called T-lymphoblastic leukemia or T-ALL. T-ALL is a type of cancer that only affects T-cells (a special kind of cell of your immune system). T-cell ALL grows in the bone marrow. The bone marrow is inside your bones. It is where your blood is made. After doing tests, we have found that you have this type of cancer.

2. We are asking you to take part in a research study because you have T-ALL. A research study is when doctors work together to try out new ways to help people who are sick. In this study, we are trying to learn more about how to treat T-ALL. We will do this by comparing 2 ways to treat T-ALL. Some of the children and teens in this study will get the usual treatment for T-ALL. Some of the children and teens will get the usual treatment plus a drug called bortezomib. In this study bortezomib is experimental, but is approved by the FDA to treat other conditions. The chemotherapy you get will be decided by chance, like flipping a coin for “heads” or “tails”. We don’t know which way is better. That is why we are doing this study.

3. Children and teens that are part of this study will be treated with chemotherapy. Chemotherapy is a type of medicine that destroys cancer cells. You will have regular blood tests and several bone marrow tests and spinal taps during your treatment. You will also have scans performed to make sure the T-ALL is responding to treatment. The bone marrow tests and spinal taps may hurt some, but medicines will be given to keep it from hurting too much.

4. Sometimes good things can happen to people when they are in a research study. These good things are called ‘benefits’. We hope that a benefit to you of being part of this study is keeping the cancer away for as long as possible, but we don’t know for sure if there is any benefit of being part of this study.

5. Sometimes bad things can happen to people when they are in a research study. These bad things are called ‘risks’. The risks to you from this study are increased toxicities that can cause infections or make it harder for a patient to fight off infections, loss of healthy blood cells and damage to bones or joints. It is also possible that your treatment plan may increase the side effects of treatment without improving the chances of getting rid of the cancer for as long as possible.

6. Your family can choose to be part of this study or not. Your family can also decide to stop being in this study at any time once you start. There may be other treatments for your illness that your doctor can tell you about. Make sure to ask your doctors any questions that you have.

7. We are asking your permission to collect additional blood and bone marrow. We want to see if there are ways to tell how the cancer will respond to treatment. Almost all of these samples will be taken when other standard blood tests and bone marrow tests are done. If there is any blood or bone marrow left over from tests done for this study, we would like to save them for other research tests in the future. You can still take part in this study even if you do not allow us to collect the extra blood or bone marrow samples or save the leftover samples for research.

INFORMATION SHEET REGARDING RESEARCH STUDY AALL1231 FOR PATIENTS WITH T-ALL
(for teens from 13 through 17 years of age)

A Study of Bortezomib in Children with Newly Diagnosed T-Lymphoblastic Leukemia (T-ALL)

1. We have been talking with you about a type of cancer called T-lymphoblastic leukemia or T-ALL. T-ALL is a type of cancer that only affects T-cells (a special kind of cell of your immune system). T-cell ALL grows in the bone marrow. The bone marrow is inside your bones. It is where your blood is made. After doing tests, we have found that you have this type of cancer.

2. We are asking you to take part in a research study because you have T-ALL. A research study is when doctors work together to try out new ways to help people who are sick. In this study, we are trying to learn more about how to treat T-ALL. We will do this by comparing 2 ways to treat T-ALL. Some of the children and teens in this study will get the usual treatment for T-ALL. Some of the children and teens will get the usual treatment plus a drug called bortezomib. In this study bortezomib is experimental, but is approved by the FDA to treat other conditions. The chemotherapy you get will be decided by chance, like flipping a coin for “heads” or “tails”. We don’t know which way is better. That is why we are doing this study.

3. Children and teens that are part of this study will be treated with chemotherapy. Chemotherapy is a type of medicine that destroys cancer cells. You will have regular blood tests and several bone marrow tests and spinal taps during your treatment. You will also have scans performed to make sure the T-ALL is responding to treatment. The bone marrow tests and spinal taps may hurt some, but medicines will be given to keep it from hurting too much.

4. Sometimes good things can happen to people when they are in a research study. These good things are called ‘benefits’. We hope that a benefit to you of being part of this study is keeping the cancer away for as long as possible, but we don’t know for sure if there is any benefit of being part of this study.

5. Sometimes bad things can happen to people when they are in a research study. These bad things are called ‘risks’. The risks to you from this study are increased toxicities that can cause infections or make it harder for a patient to fight off infections, loss of healthy blood cells and damage to bones or joints. It is also possible that adding bortezomib to your treatment plan may increase the side effects of treatment without improving the chances of getting rid of the cancer for as long as possible. Adding bortezomib to your treatment plan could also reduce how well your treatment works.

6. Your family can choose to be part of this study or not. Your family can also decide to stop being in this study at any time once you start. There may be other treatments for your illness that your doctor can tell you about. Make sure to ask your doctors any questions that you have.

7. We are asking your permission to collect additional blood and bone marrow. We want to see if there are ways to tell how the cancer will respond to treatment. Almost all of these samples will be taken when other standard blood tests and bone marrow tests are done. If there is any blood or bone marrow left over from tests done for this study, we would like to save them for other research tests in the future. You can still take part in this study even if you do not allow us to collect the extra blood or bone marrow samples or save the leftover samples for research.

INFORMATION SHEET REGARDING RESEARCH STUDY AALL1231 FOR PATIENTS WITH T-LLy
(for children from 7 through 12 years of age)

A Study of Bortezomib in Children with Newly Diagnosed T-Lymphoblastic Lymphoma (T-LLy)

1. We have been talking with you about a type of cancer called T-lymphoblastic lymphoma or T-LLy. T-LLy is a type of cancer that occurs in the lymph system, which is made up of the lymph nodes and other lymph tissue throughout the body. Lymph tissue makes and stores infection-fighting white blood cells called lymphocytes. These cells become cancerous when a subject has lymphoma. After doing tests, we have found that you have this type of cancer.

2. We are asking you to take part in a research study because you have T-LLy. A research study is when doctors work together to try out new ways to help people who are sick. In this study, we are trying to learn more about how to treat T-LLy. We will do this by comparing 2 ways to treat T-LLy. Some of the children and teens in this study will get the usual treatment for T-LLy. Some of the children and teens will get the usual treatment plus a drug called bortezomib. In this study bortezomib is experimental, but is approved by the FDA to treat other conditions. The chemotherapy you get will be decided by chance, like flipping a coin for “heads” or “tails”. We don’t know which way is better. That is why we are doing this study.

3. Children and teens that are part of this study will be treated with chemotherapy. Chemotherapy is a type of medicine that destroys cancer cells. You will have regular blood tests and several bone marrow tests and spinal taps during your treatment. You will also have scans performed to make sure the T-LLy is responding to treatment. The bone marrow tests and spinal taps may hurt some, but medicines will be given to keep it from hurting too much.

4. Sometimes good things can happen to people when they are in a research study. These good things are called ‘benefits’. We hope that a benefit to you of being part of this study is keeping the cancer away for as long as possible, but we don’t know for sure if there is any benefit of being part of this study.

5. Sometimes bad things can happen to people when they are in a research study. These bad things are called ‘risks’. The risks to you from this study are increased toxicities that can cause infections or make it harder for a patient to fight off infections, loss of healthy blood cells and damage to bones or joints. It is also possible that your treatment plan may increase the side effects of treatment without improving the chances of getting rid of the cancer for as long as possible.

6. Your family can choose to be part of this study or not. Your family can also decide to stop being in this study at any time once you start. There may be other treatments for your illness that your doctor can tell you about. Make sure to ask your doctors any questions that you have.

7. We are asking your permission to collect additional blood and bone marrow. We want to see if there are ways to tell how the cancer will respond to treatment. Almost all of these samples will be taken when other standard blood tests and bone marrow tests are done. If there is any blood or bone marrow left over from tests done for this study, we would like to save them for other research tests in the future. You can still take part in this study even if you do not allow us to collect the extra blood or bone marrow samples or save the leftover samples for research.

INFORMATION SHEET REGARDING RESEARCH STUDY AALL1231 FOR PATIENTS WITH T- LLy
(for teens from 13 through 17 years of age)

A Study of Bortezomib in Children with Newly Diagnosed T-Lymphoblastic Lymphoma (T-LLy)

1. We have been talking with you about a type of cancer called T-lymphoblastic lymphoma or T-LLy. T-LLy is a type of cancer that occurs in the lymph system, which is made up of the lymph nodes and other lymph tissue throughout the body. Lymph tissue makes and stores infection-fighting white blood cells called lymphocytes. These cells become cancerous when a subject has lymphoma. After doing tests, we have found that you have this type of cancer.

2. We are asking you to take part in a research study because you have T-LLy. A research study is when doctors work together to try out new ways to help people who are sick. In this study, we are trying to learn more about how to treat T-LLy. We will do this by comparing 2 ways to treat T-LLy. Some of the children and teens in this study will get the usual treatment for T-LLy. Some of the children and teens will get the usual treatment plus a drug called bortezomib. In this study, bortezomib is experimental, but is approved by the FDA to treat other conditions. The chemotherapy you get will be decided by chance, like flipping a coin for “heads” or “tails”. We don’t know which way is better. That is why we are doing this study.

3. Children and teens that are part of this study will be treated with chemotherapy. Chemotherapy is a type of medicine that destroys cancer cells. You will have regular blood tests and several bone marrow tests and spinal taps during your treatment. You will also have scans performed to make sure the T-LLy is responding to treatment. The bone marrow tests and spinal taps may hurt some, but medicines will be given to keep it from hurting too much.

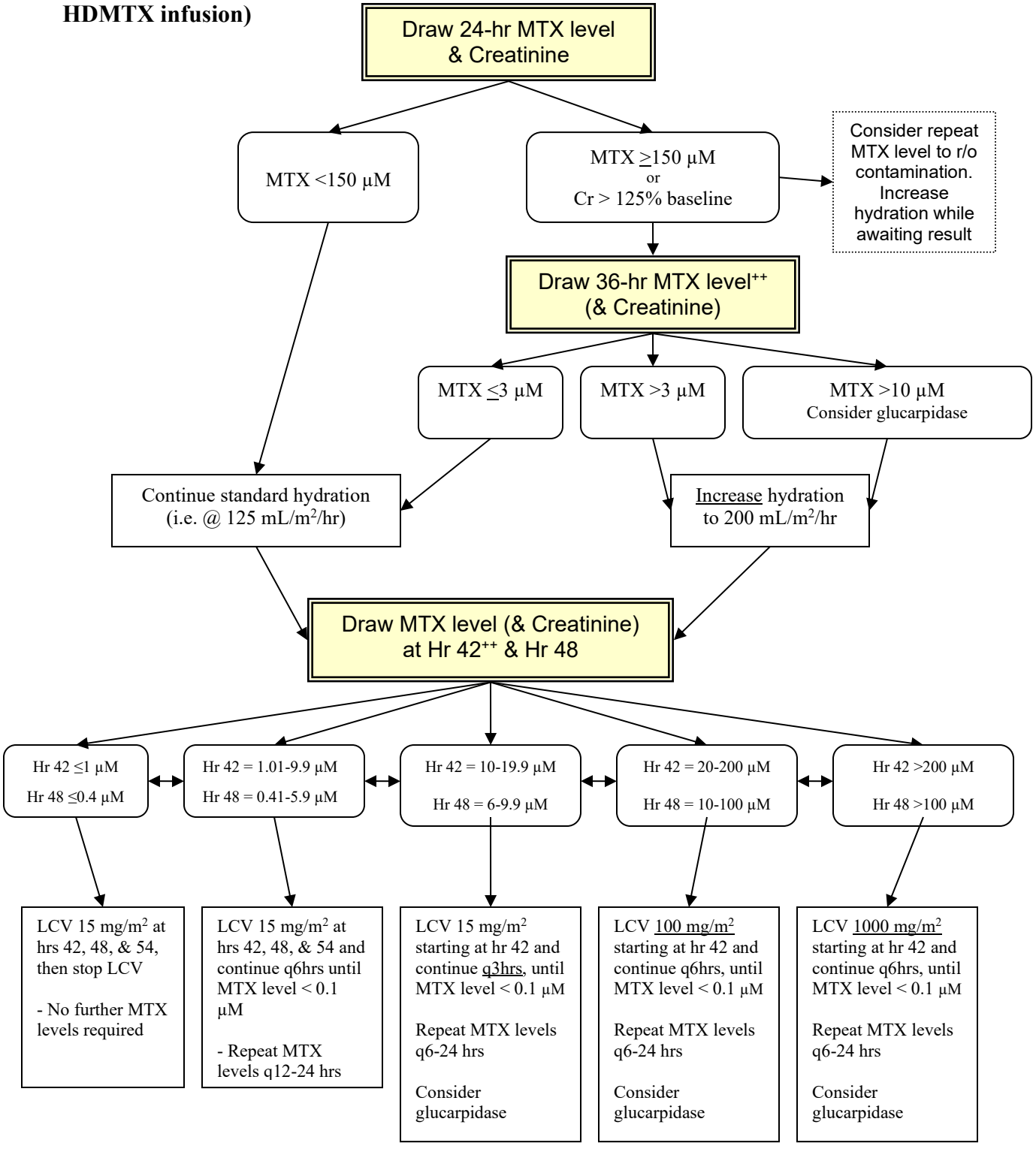
4. Sometimes good things can happen to people when they are in a research study. These good things are called ‘benefits’. We hope that a benefit to you of being part of this study is keeping the cancer away for as long as possible, but we don’t know for sure if there is any benefit of being part of this study.

5. Sometimes bad things can happen to people when they are in a research study. These bad things are called ‘risks’. The risks to you from this study are increased toxicities that can cause infections or make it harder for a patient to fight off infections, loss of healthy blood cells and damage to bones or joints. It is also possible that adding bortezomib to your treatment plan may increase the side effects of treatment without improving the chances of getting rid of the cancer for as long as possible. Adding bortezomib to your treatment plan could also reduce how well your treatment works.

6. Your family can choose to be part of this study or not. Your family can also decide to stop being in this study at any time once you start. There may be other treatments for your illness that your doctor can tell you about. Make sure to ask your doctors any questions that you have.

7. We are asking your permission to collect additional blood and bone marrow. We want to see if there are ways to tell how the cancer will respond to treatment. Almost all of these samples will be taken when other standard blood tests and bone marrow tests are done. If there is any blood or bone marrow left over from tests done for this study, we would like to save them for other research tests in the future. You can still take part in this study even if you do not allow us to collect the extra blood or bone marrow samples or save the leftover samples for research.

APPENDIX IV: HIGH-DOSE METHOTREXATE FLOWCHART
(Please refer to Section 5.9 for complete details; all levels are timed from the start of the HDMTX infusion)



++ If the level is high at hour 36 or 42, but then the patient "catches up" and the level falls to the expected values of ≤1 and/or ≤ 0.4 µM at hours 42 and 48, respectively, resume standard leucovorin and hydration as long as urine output remains satisfactory.

APPENDIX V: CYP3A4 SUBSTRATES, INDUCERS, AND INHIBITORS

This is not an inclusive list. Because the lists of these agents are constantly changing, it is important to regularly consult a frequently-updated medical references.

CYP3A4 substrates	Strong Inhibitors[1]	Moderate Inhibitors	Weak Inhibitors	Inducers
alfentanil[4,5]	atazanavir	aprepitant	alprazolam	armodafinil
amiodarone[4]	boceprevir	atazanavir	amiodarone	barbiturates
aprepitant/fosaprepitant[5]	clarithromycin	cimetidine	atorvastatin	bosentan
benzodiazepines	cobicistat	conivaptan	bicalutamide	carbamazepine
bortezomib	darunavir	crizotinib	cilostazol	deferasirox
brentuximab	conivaptan	cyclosporine	cimetidine	echinacea
budesonide[5]	delavirdine	diltiazem	ciprofloxacin	efavirenz
calcium channel blockers	grapefruit[3]	dronedarone	cyclosporine	etravirine
cisapride	grapefruit juice[3]	erythromycin	fluvoxamine	fosphenytoin
citalopram/escitalopram	indinavir	fluconazole	isoniazid	glucocorticoids[2]
glucocorticoids[2]	itraconazole	fluvoxamine	nicardipine	modafinil
conivaptan[5]	ketoconazole	fosamprenavir	propofol	nafcillin
crizotinib	lopinavir/ritonavir	fosaprepitant	quinidine	nevirapine
cyclosporine[4]	nefazodone	grapefruit[3]	sertraline	oxcarbazepine
cyclophosphamide	nelfinavir	grapefruit	tacrolimus	phenobarbital
dapsone	posaconazole	juice[3]	ranolazine	phenytoin
darifenacin[5]	ritonavir	imatinib		pioglitazone
darunavir	saquinavir	mifepristone		primidone
dasatinib[5]	telaprevir	nilotinib		rifabutin
dihydroergotamine	telithromycin	verapamil		rifampin
docetaxel	voriconazole			rifapentin
doxorubicin				ritonavir
dronedarone[5]				St. John's wort
eletriptan[5]				topiramate
ergotamine[4]				
erlotinib				
esomeprazole				
estrogens				
etoposide				
everolimus[5]				
felodipine[5]				
fentanyl[4]				
fosaprepitant				
gefitinib				
haloperidol				
HIV antiretrovirals				
HMG Co-A inhibitors[5]				
ifosfamide				
imatinib				
indinavir[5]				
irinotecan				
itraconazole				

ketoconazole lansoprazole lapatinib losartan lovastatin[5] lurasidone[5] macrolide antibiotics maraviroc[5] medroxyprogesterone methadone midazolam modafinil monteleukast nefazodone nilotinib nisoldipine[5] omeprazole ondansetron paclitaxel pazopanib quetiapine[5] quinidine saquinavir[5] sildenafil simvastatin[5] sirolimus[4,5] sunitinib tacrolimus[4,5] telaprevir tamoxifen temsirolimus teniposide tetracycline tipranavir[5] tolvaptan[5] triazolam[5] trimethoprim vardenafil[5] vinca alkaloids zolpidem				

[1]Certain fruits and fruit juices (star fruit, Seville oranges, pomegranate, ginko, goldenseal) may inhibit CYP 3A4 isozyme, however, the degree of that inhibition is unknown.
[2]Refer to Section 6.5 and 6.9 regarding use of corticosteroids.
[3]The effect of grapefruit juice (strong vs moderate CYP3A4 inhibition) varies widely among brands and is concentration-, dose-, and preparation-dependent.
[4]Narrow therapeutic range substrates
[5]Sensitive substrates

APPENDIX VI: MERCAPTOPURINE DOSING TABLE

Note: The Mercaptopurine dosing nomograms in this appendix only apply to the tablet formulation.

MERCAPTOPURINE 25 mg/m²

Body Surface Area (m^2)*	Daily Dose (d) for 7 days (1 tablet = 50 mg)	Cumulative Weekly Dose
0.36 - 0.49	½ tab / d x 3	75 mg/wk
0.50 - 0.64	½ tab / d x 4	100 mg/wk
0.65 - 0.78	½ tab / d x 5	125 mg/wk
0.79 - 0.92	½ tab / d x 6	150 mg/wk
0.93 – 1.07	½ tab / d x 7	175 mg/wk
1.08 – 1.21	1 tab / d x 1; ½ tab / d x 6	200 mg/wk
1.22 – 1.35	1 tab / d x 2; ½ tab / d x 5	225 mg/wk
1.36 – 1.49	1 tab / d x 3; ½ tab / d x 4	250 mg/wk
1.50 – 1.64	1 tab / d x 4; ½ tab / d x 3	275 mg/wk
1.65 – 1.78	1 tab / d x 5; ½ tab / d x 2	300 mg/wk
1.79 – 1.92	1 tab / d x 6; ½ tab / d x 1	325 mg/wk
1.93 – 2.07	1 tab / d x 7	350 mg/wk
2.08 – 2.21	1½ tab / d x 1; 1 tab / d x 6	375 mg/wk
2.22 - 2.35	1½ tab / d x 2; 1 tab / d x 5	400 mg/wk
2.36 – 2.49	1½ tab / d x 3; 1 tab / d x 4	425 mg/wk
2.50 – 2.64	1½ tab / d x 4; 1 tab / d x 3	450 mg/wk
2.65 – 2.78	1½ tab / d x 5; 1 tab / d x 2	475 mg/wk
2.79 – 2.92	1½ tab / d x 6; 1 tab / d x 1	500 mg/wk
2.93 – 3.00*	1½ tab / d x 7	525 mg/wk

*****_Patients exceeding a BSA of 3.00 m^2 should have their MP doses calculated on actual BSA with no maximum dose._

MERCAPTOPURINE 60 mg/m²

Body Surface Area (m²)*	Daily Dose (d) for 7 days (1 tablet = 50 mg)	Cumulative Weekly Dose
0.33 - 0.38	½ tab / d x 6	150 mg/wk
0.39 - 0.44	½ tab / d x 7	175 mg/wk
0.45 - 0.50	½ tab / d x 6; 1 tab / d x 1	200 mg/wk
0.51 - 0.56	½ tab / d x 5; 1 tab / d x 2	225 mg/wk
0.57 - 0.62	½ tab / d x 4; 1 tab / d x 3	250 mg/wk
0.63 - 0.68	1 tab / d x 4; ½ tab / d x 3	275 mg/wk
0.69 - 0.74	1 tab / d x 5; ½ tab / d x 2	300 mg/wk
0.75 - 0.80	1 tab / d x 6; ½ tab / d x 1	325 mg/wk
0.81 - 0.86	1 tab / d x 7	350 mg/wk
0.87 - 0.92	1 tab / d x 6; 1½ tab / d x 1	375 mg/wk
0.93 - 0.98	1 tab / d x 5; 1½ tab / d x 2	400 mg/wk
0.99 - 1.04	1 tab / d x 4; 1½ tab / d x 3	425 mg/wk
1.05 - 1.10	1½ tab / d x 4; 1 tab / d x 3	450 mg/wk
1.11 - 1.16	1½ tab / d x 5; 1 tab / d x 2	475 mg/wk
1.17 - 1.22	1½ tab / d x 6; 1 tab / d x 1	500 mg/wk
1.23 - 1.27	1½ tab / d x 7	525 mg/wk
1.28 - 1.33	1½ tab / d x 6; 2 tab / d x 1	550 mg/wk
1.34 - 1.39	1½ tab / d x 5; 2 tab / d x 2	575 mg/wk
1.40 - 1.45	1½ tab / d x 4; 2 tab / d x 3	600 mg/wk
1.46 - 1.51	2 tab / d x 4; 1½ tab / d x 3	625 mg/wk
1.52 - 1.57	2 tab / d x 5; 1½ tab / d x 2	650 mg/wk
1.58 - 1.63	2 tab / d x 6; 1½ tab / d x 1	675 mg/wk
1.64 - 1.69	2 tab / d x 7	700 mg/wk
1.70 - 1.75	2 tab / d x 6; 2½ tab / d x 1	725 mg/wk

1.76 - 1.81	2 tab / d x 5; 2½ tab / d x 2	750 mg/wk
1.82 - 1.87	2 tab / d x 4; 2½ tab / d x 3	775 mg/wk
1.88 - 1.93	2½ tab / d x 4; 2 tab / d x 3	800 mg/wk
1.94 - 1.99	2½ tab / d x 5; 2 tab / d x 2	825 mg/wk
2.00 - 2.05	2½ tab / d x 6; 2 tab / d x 1	850 mg/wk
2.06 - 2.11	2½ tab/ d x 7	875 mg/wk
2.12 - 2.17	2½ tab/ d x 6; 3 tab / d x 1	900 mg/wk
2.18 - 2.23	2½ tab/ d x 5; 3 tab / d x 2	925 mg/wk
2.24 - 2.29	2½ tab/ d x 4; 3 tab / d x 3	950 mg/wk
2.30 - 2.35	3 tab/ d x 4; 2½ tab / d x 3	975 mg/wk
2.36 - 2.41	3 tab/ d x 5; 2½ tab / d x 2	1000 mg/wk
2.42 - 2.47	3 tab/ d x 6; 2½ tab / d x 1	1025 mg/wk
2.48 - 2.52	3 tab/ d x 7	1050 mg/wk
2.53 - 2.58	3 tab/ d x 6; 3½ tab / d x 1	1075 mg/wk
2.59 - 2.64	3 tab/ d x 5; 3½ tab / d x 2	1100 mg/wk
2.65 - 2.70	3 tab/ d x 4; 3½ tab / d x 3	1125 mg/wk
2.71 - 2.76	3½ tab/ d x 4; 3 tab / d x 3	1150 mg/wk
2.77 - 2.82	3½ tab/ d x 5; 3 tab / d x 2	1175 mg/wk
2.83 - 2.88	3½ tab/ d x 6; 3 tab / d x 1	1200 mg/wk
2.89 - 2.94	3½ tab/ d x 7	1225 mg/wk
2.95 - 3.00	3½ tab/ d x 6; 4 tab / d x 1	1250 mg/wk

**Patients exceeding a BSA of 3.00 m^2 should have their MP doses calculated on actual BSA with no maximum dose.*

MERCAPTOPURINE 75 mg/m²

Body Surface Area (m²)*	Daily Dose (d) for 7 days (1 tablet = 50 mg)	Cumulative Weekly Dose
0.36 - 0.40	½ tab / d x 6; 1 tab / d x 1	200 mg/wk
0.41 - 0.45	½ tab / d x 5; 1 tab / d x 2	225 mg/wk
0.46 - 0.49	½ tab / d x 4; 1 tab / d x 3	250 mg/wk
0.50 - 0.54	1 tab / d x 4; ½ tab / d x 3	275 mg/wk
0.55 - 0.59	1 tab / d x 5; ½ tab / d x 2	300 mg/wk
0.60 - 0.64	1 tab / d x 6; ½ tab / d x 1	325 mg/wk
0.65 - 0.69	1 tab / day	350 mg/wk
0.70 - 0.73	1 tab / d x 6; 1½ tab / d x 1	375 mg/wk
0.74 - 0.78	1 tab / d x 5; 1½ tab / d x 2	400 mg/wk
0.79 - 0.83	1 tab / d x 4; 1½ tab / d x 3	425 mg/wk
0.84 - 0.88	1½ tab / d x 4; 1 tab / d x 3	450 mg/wk
0.89 - 0.92	1½ tab / d x 5; 1 tab / d x 2	475 mg/wk
0.93 - 0.97	1½ tab / d x 6; 1 tab /d x 1	500 mg/wk
0.98 - 1.02	1½ tab / day	525 mg/wk
1.03 - 1.07	1½ tab / d x 6; 2 tab / d x 1	550 mg/wk
1.08 - 1.11	1½ tab / d x 5; 2 tab / d x 2	575 mg/wk
1.12 - 1.16	1½ tab / d x 4; 2 tab / d x 3	600 mg/wk
1.17 - 1.21	2 tab / d x 4; 1½ tab / d x 3	625 mg/wk
1.22 - 1.26	2 tab / d x 5; 1½ tab / d x 2	650 mg/wk
1.27 - 1.30	2 tab / d x 6; 1½ tab / d x 1	675 mg/wk
1.31 - 1.35	2 tab / day	700 mg/wk
1.36 - 1.40	2 tab / d x 6; 2½ tab / d x 1	725 mg/wk
1.41 - 1.45	2 tab / d x 5; 2½ tab / d x 2	750 mg/wk
1.46 – 1.49	2 tab / d x 4; 2½ tab / d x 3	775 mg/wk
1.50 – 1.54	2½ tab/ d x 4; 2 tab / d x 3	800 mg/wk
1.55 – 1.59	2½ tab/ d x 5; 2 tab / d x 2	825 mg/wk
1.60 – 1.64	2½ tab/ d x 6; 2 tab / d x 1	850 mg/wk
1.65 – 1.69	2½ tab/ d	875 mg/wk
1.70 – 1.73	2½ tab/ d x 6; 3 tab / d x 1	900 mg/wk
1.74 – 1.78	2½ tab/ d x 5; 3 tab / d x 2	925 mg/wk
1.79 – 1.83	2½ tab/ d x 4; 3 tab / d x 3	950 mg/wk
1.84 – 1.88	3 tab/ d x 4; 2½ tab / d x 3	975 mg/wk
1.89 – 1.92	3 tab/ d x 5; 2½ tab / d x 2	1000 mg/wk
1.93 – 1.97	3 tab/ d x 6; 2½ tab / d x 1	1025 mg/wk
1.98 – 2.02	3 tab/ d x 7	1050 mg/wk
2.03 – 2.07	3 tab/ d x 6; 3½ tab / d x 1	1075 mg/wk
2.08 – 2.11	3 tab/ d x 5; 3½ tab / d x 2	1100 mg/wk
2.12 – 2.16	3 tab/ d x 4; 3½ tab / d x 3	1125 mg/wk
2.17 – 2.21	3½ tab/ d x 4; 3 tab / d x 3	1150 mg/wk
2.22 – 2.26	3½ tab/ d x 5; 3 tab / d x 2	1175 mg/wk
2.27 – 2.30	3½ tab/ d x 6; 3 tab / d x 1	1200 mg/wk
2.31 – 2.35	3½ tab/ d x 7	1225 mg/wk
2.36 – 2.40	3½ tab/ d x 6; 4 tab / d x 1	1250 mg/wk
2.41 – 2.45	3½ tab/ d x 5; 4 tab / d x 2	1275 mg/wk

2.46 – 2.49	3½ tab/ d x 4; 4 tab / d x 3	1300 mg/wk
2.50 – 2.54	4 tab/ d x 4; 3½ tab / d x 3	1325 mg/wk
2.55 – 2.59	4 tab/ d x 5; 3½ tab / d x 2	1350 mg/wk
2.60 – 2.64	4 tab/ d x 6; 3½ tab / d x 1	1375 mg/wk
2.65 – 2.69	4 tab/ d x 7	1400 mg/wk
2.70 – 2.73	4 tab/ d x 6; 4½ tab / d x 1	1425 mg/wk
2.74 – 2.78	4 tab/ d x 5; 4½ tab / d x 2	1450 mg/wk
2.79 – 2.83	4 tab/ d x 4; 4½ tab / d x 3	1475 mg/wk
2.84 – 2.88	4½ tab/ d x 4; 4 tab / d x 3	1500 mg/wk
2.89 – 2.92	4½ tab/ d x 5; 4 tab / d x 2	1525 mg/wk
2.93 – 2.97	4½ tab/ d x 6; 4 tab / d x 1	1550 mg/wk
2.98 – 3.00	4½ tab/ d x 7	1575 mg/wk

**Patients exceeding a BSA of 3.00 m^2 should have their MP doses calculated on actual BSA with no maximum dose.*

APPENDIX VII: THIOGUANINE DOSING TABLE

THIOGUANINE 60 mg/m²

Body Surface Area (m²)*	Daily Dose (d) for 7 days (1 tablet = 40 mg)	Cumulative Weekly Dose
0.31 - 0.35	½ tab / d x 7	140 mg/wk
0.36 - 0.40	½ tab / d x 6; 1 tab / d x 1	160 mg/wk
0.41 - 0.45	½ tab / d x 5; 1 tab / d x 2	180 mg/wk
0.46 - 0.49	½ tab / d x 4; 1 tab / d x 3	200 mg/wk
0.50 - 0.54	1 tab / d x 4; ½ tab / d x 3	220 mg/wk
0.55 - 0.59	1 tab / d x 5; ½ tab / d x 2	240 mg/wk
0.60 - 0.64	1 tab/ d x 6; ½ tab / d x 1	260 mg/wk
0.65 - 0.69	1 tab / day	280 mg/wk
0.70 - 0.73	1 tab / d x 6; 1½ tab / d x 1	300 mg/wk
0.74 - 0.78	1 tab / d x 5; 1½ tab / d x 2	320 mg/wk
0.79 - 0.83	1 tab/ d x 4; 1½ tab / d x 3	340 mg/wk
0.84 - 0.88	1½ tab / d x 4; 1 tab / d x 3	360 mg/wk
0.89 - 0.92	1½ tab / d x 5; 1 tab /d x 2	380 mg/wk
0.93 - 0.97	1½ tab / d x 6; 1 tab / d x 1	400 mg/wk
0.98 - 1.02	1½ tab /day	420 mg/wk
1.03 - 1.07	1½ tab / d x 6; 2 tab / d x 1	440 mg/wk
1.08 - 1.11	1½ tab / d x 5; 2 tab / d x 2	460 mg/wk
1.12 - 1.16	1½ tab / d x 4; 2 tab / d x 3	480 mg/wk
1.17 - 1.21	2 tab / d x 4; 1½ tab / d x 3	500 mg/wk
1.22 - 1.26	2 tab / d x 5; 1½ tab / d x 2	520 mg/wk
1.27 - 1.30	2 tab / d x 6; 1½ tab / d x 1	540 mg/wk
1.31 - 1.35	2 tab / day	560 mg/wk
1.36 - 1.40	2 tab / d x 6; 2½ tab / d x 1	580 mg/wk
1.41 - 1.45	2 tab / d x 5; 2½ tab / d x 2	600 mg/wk
1.46 - 1.49	2 tab / d x 4; 2½ tab / d x 3	620 mg/wk
1.50 - 1.54	2½ tab / d x 4; 2 tab / d x 3	640 mg/wk
1.55 - 1.59	2½ tab / d x 5; 2 tab / d x 2	660 mg/wk
1.60 - 1.64	2½ tab / d x 6; 2 tab / d x 1	680 mg/wk
1.65 - 1.69	2½ tab / d	700 mg/wk
1.70 - 1.73	2½ tab / d x 6; 3 tab / d x 1	720 mg/wk
1.74 - 1.78	2½ tab / d x 5; 3 tab / d x 2	740 mg/wk
1.79 - 1.83	2½ tab / d x 4; 3 tab / d x 3	760 mg/wk
1.84 - 1.88	3 tab / d x 4; 2½ tab / d x 3	780 mg/wk
1.89 - 1.92	3 tab / d x 5; 2½ tab / d x 2	800 mg/wk
1.93 - 1.97	3 tab / d x 6; 2½ tab / d x 1	820 mg/wk
1.98 - 2.02	3 tab / d x 7	840 mg/wk
2.03 - 2.07	3 tab / d x 6; 3½ tab / d x 1	860 mg/wk
2.08 - 2.11	3 tab / d x 5; 3½ tab / d x 2	880 mg/wk
2.12 - 2.16	3 tab / d x 4; 3½ tab / d x 3	900 mg/wk
2.17 - 2.21	3½ tab / d x 4; 3 tab / d x 3	920 mg/wk
2.22 - 2.26	3½ tab / d x 5; 3 tab / d x 2	940 mg/wk
2.27 – 2.30	3½ tab / d x 6; 3 tab / d x 1	960 mg/wk

2.31 – 2.35	3½ tab / d x 7	980 mg/wk
2.36 – 2.40	3½ tab / d x 6; 4 tab / d x 1	1000 mg/wk
2.41 – 2.45	3½ tab / d x 5; 4 tab / d x 2	1020 mg/wk
2.46 – 2.49	3½ tab / d x 4; 4 tab / d x 3	1040 mg/wk
2.50 – 2.54	4 tab / d x 4; 3½ tab / d x 3	1060 mg/wk
2.55 – 2.59	4 tab / d x 5; 3½ tab / d x 2	1080 mg/wk
2.60 – 2.64	4 tab / d x 6; 3½ tab / d x 1	1100 mg/wk
2.65 – 2.69	4 tab / d x 7	1120 mg/wk
2.70 – 2.73	4 tab / d x 6; 4½ tab / d x 1	1140 mg/wk
2.74 – 2.78	4 tab / d x 5; 4½ tab / d x 2	1160 mg/wk
2.79 – 2.83	4 tab / d x 4; 4½ tab / d x 3	1180 mg/wk
2.84 – 2.88	4½ tab / d x 4; 4 tab / d x 3	1200 mg/wk
2.89 – 2.92	4½ tab / d x 5; 4 tab / d x 2	1220 mg/wk
2.93 – 2.97	4½ tab / d x 6; 4 tab / d x 1	1240 mg/wk
2.98 – 3.00	4½ tab / d x 7	1260 mg/wk

**Patients exceeding a BSA of 3.00 m^2 should have their TG doses calculated on actual BSA with no maximum dose.*

APPENDIX VIII: STAGING CLASSIFICATION OF CHILDHOOD NON-HODGKIN LYMPHOMA

Modified from Murphy [Seminars in Oncology (1980) 7; 332-339]

Stage	Criteria for Extent of Disease
Localized	
I	A single tumor (extranodal) or single anatomic area (nodal) with the exclusion of mediastinum or abdomen
II	A single tumor (extranodal) with regional node involvement
Disseminated	
III	Two single tumors (extranodal) on opposite sides of the diaphragm. Two or more nodal areas above and below the diaphragm. All primary intra-thoracic tumors (mediastinal, pleural, thymic) All extensive primary intra-abdominal disease. All paraspinal or epidural tumors, regardless of other tumor site(s)
IV	Any of the above with initial CNS and/or bone marrow involvement

Enumeration of Number of Regions of Nodal Involvement

Each of these twenty regions is counted separately for purposes of determining number of sites of involvement.

Peripheral Regions

Right neck; cervical, supraclavicular, occipital, and pre-auricular
Left neck; cervical, supraclavicular, occipital, and pre-auricular
Right infraclavicular
Left infraclavicular
Right axilla and pectoral
Left axilla and pectoral
Right epitrochlear and brachial
Left epitrochlear and brachial

APPENDIX IX: MINIMAL RESIDUAL DISEASE-SAMPLE SHIPPING REQUIREMENTS

MRD samples will be shipped to the COG ALL Reference Flow Cytometry Lab using the shipping and handling requirements below.

Samples are to be shipped to Dr. Brent Wood at the University of Washington, Flow Cytometry Laboratory. The AALL1231 Specimen Transmittal Form is to be submitted with each sample submitted to the COG Reference Laboratory. The specimen transmittal form information should always include the name and telephone number of a person designated by the PI to receive calls from the Reference Laboratory directors. The PI's FAX number must also be noted on each sample inclusion form. Because clinical recommendations will be made on these samples, **always** include the patient's name, birth date and COG number on any sample submitted. This is a CLIA requirement. COG ALL Reference Laboratories may be unable to analyze specimens if adequate patient identifiers are not provided.

Samples for the Reference Laboratories are to be collected in special 15 mL conical tubes (SM) containing EDTA/RPMI as the anticoagulant and media diluent. These tubes will be prepared in the Reference Laboratories and mailed in batches to each participating institution, where they can be stored frozen at -20°C until use. Tubes are stable for 3 months if refrigerated and stable for 1 year if frozen.

To request prepared and pre-packaged sample shipping tubes, click on the following link:

https://ricapps.nationwidechildrens.org/KitManagement/

Select 'AALL08B1' from the protocol list to order the shipping tubes required for MRD samples.

Bone Marrow Collection Procedures for Reference Laboratories:

a. Collect 1-2 ml of BM from the 1st pull into a syringe and transfer the specimen immediately into the 15 mL shipping media conical tube with RPMI/EDTA. Collection of marrow volumes beyond 2 ml or use of marrow other than the 1st pull will result in hemodilution and may effect quantitation of MRD.
b. Mix well. Up to 5 mL of BM can be placed in one 15 mL tube with RPMI/EDTA. If you don't have shipping media tubes, you can place the BM into large purple EDTA tubes that are commonly available in most hospitals. However, the viability of the cells is enhanced in the shipping media tubes.
c. 1-2 mL of BM will be sufficient for analysis either at diagnosis or following therapy.

16.1.2 Sample Shipping

Bone marrow samples for MRD studies will be shipped to one place:

Western Flow Cytometry Reference Laboratory
Brent Wood, MD, PhD
Seattle Cancer Care Alliance
Hematopathology Laboratory, Room G7-800
825 Eastlake Ave. E.
Seattle, WA 98109-1028
Phone: 206-288-7060
FAX: 206-288-7127

SAMPLES THAT ARE EXPECTED TO BE DELAYED FOR MORE THAN 48 HOURS—PLACE A COLD PACK (NOT ICE PACK) IN SHIPMENT. ALL TUBES SHOULD BE LABELED WITH AT LEAST TWO PATIENT IDENTIFIERS, INCLUDING THE NAME AND THE COG

NUMBER. IN ADDITION, AN AALL1231 SPECIMEN TRANSMITTAL FORM AVAILABLE IN RAVE SHOULD ALWAYS BE SUBMITTED WITH EACH SAMPLE.

Call Reference Laboratories only when shipping a sample to be delivered on Saturday.

Samples for the Flow Cytometry Reference Laboratory should be mailed by FEDERAL EXPRESS PRIORITY (DELIVERY BEFORE 10 AM) using the COG Federal Express account number available at: https://members.childrensoncologygroup.org/_files/reference/FEDEXmemo.pdf

APPENDIX X: EVALUATING MECHANISMS OF BORTEZOMIB RESPONSE AND RESISTANCE IN T-ALL AND IDENTIFYING BIOMARKERS AND MECHANISMS OF CHEMOTHERAPY RESISTANCE AND RESPONSE IN T-ALL, FOCUSING ON ETP ALL

Eligible samples:

All pre-treatment bone marrow samples from both arms of the study (Arm A and Arm B) are eligible. Peripheral blood samples should be sent to the Horton lab only if the patient samples meet the following criteria:

Eligible patients must have an initial absolute blast count of **at least 1000 lymphoblasts/µL**. To calculate the absolute blast percentage, multiply the total WBC by the % peripheral blasts:

(WBC)(% blast)(1000) = absolute blast count/µL

As an example, if the patient has a WBC of 10 and 50% blasts, the absolute blast count is:
(10)(.5)(1000) = 5000/µL

If the initial % blasts is unknown, send peripheral blood samples only if the total WBC is more than 10,000 cell/µL and notify the Horton lab of the % blast as soon as available (contact information provided below).

Samples must be received by the Horton lab **no later than 72h after collection.** Bone marrow and peripheral blood samples can be batched if they will arrive within 72h. If samples will not arrive within 72h, please send the bone marrow separately from peripheral blood samples. Day 1 peripheral blood samples should be shipped together. The Horton lab can accept Saturday shipments if we are contacted ahead of time. Please contact Gaye Jenkins or other Horton lab representative (832-824-4676) for alternative address and shipping information for Saturday delivery.

Sample collection time points:

	On -study	Day 1, Hour 0	Day 1, Hour 6	Day 1, Hour 24	End of Induction
Bone Marrow (Induction only)	5 mL* (peripheral blood can be substituted if >80% blasts) #				5 ml* (peripheral blood can be substituted if >30% blasts)
Peripheral Blood (Induction only)		5 mL* (before start of systemic chemotherapy)	5 mL* (6h following the start of systemic chemotherapy)	5 mL* (24h following the start of systemic chemotherapy)	5 mL* (same day as end of induction bone marrow)

 # If the blast count is <80% and bone marrow is not available, please call Dr. Horton or Gaye Jenkins to discuss on a case-by-case basis.

***Sample Collection:**

1. **Bone marrow**: send in heparin or ACDA tube (ACDA preferred). Can also be sent diluted 1:1 in shipping media. Do not send bone marrow samples in Cell Save tubes.

2. **Peripheral blood: For all Day 1 samples,** Collect 5 mL sample into the CellSave tubes (3 mL) and heparin tubes (2 mL). Collect sample into collection tubes directly, do not transfer a heparinized sample into the Cell Save tube; the Cell Save fixative will not work if the sample has already been heparinized. Either lithium heparin or sodium heparin is acceptable. **Do not use lithium heparin PST (plasma separator tubes).** End-of Induction samples should be sent in heparin tubes only.

Shipping Note: Samples collected on Saturday and Sunday can be shipped Monday for Tuesday arrival. See below for information on obtaining and shipping samples in ThermoSafe containers.

Specimen Requirements:
Store samples in refrigerator until shipment.
CellSave tubes will be provided by the Horton lab to each institution.
To obtain more CellSave tubes, contact the Horton lab at the numbers provided below.
If the CellSave tubes are not available, submit entire 5 mL sample in heparin tubes. Note that the **sample integrity is greatly enhanced by the use of CellSave tubes.**

- Each sample should be clearly labeled to include the 6 digit COG number as well as the 4 digit treatment accession number; study number (AALL1231), date and time sample was drawn.

On the AALL1231 Specimen Transmittal Form in the Medidata/Rave system record the exact time and date that the sample is drawn along with the exact start time for administration of systemic chemotherapy. Please note the WBC and % blasts on the specimen transmittal form.
Please include a copy of the AALL1231 specimen transmittal form and institutional immunophenotype report with the sample submission to the laboratory. Please also fax a copy of the institutional immunophenotype report to FAX #: (832) 825-1206.

Note: it is acceptable for blood to be collected from a central line.

Shipping Requirements:
Prior to sample collection, please contact Dr. Horton at (832) 824-4269 or Gaye Jenkins/Horton lab at (832) 824-4676 for ThermoSafe shipping containers. These containers maintain biology samples at a constant temperature and are recommended, but not required, for biology sample shipment. Shipment of peripheral blood samples should not be delayed for receipt of shipping containers.

If Thermo-Safe shipping container is not available:
- Place collection tubes in a primary container. Wrap each collection tube separately to protect from breakage during shipment. Place the container in a Styrofoam box.
- Please place an **ice pack** in the primary container. During the non-winter months (April-October) add additional ice packs to the Styrofoam box to assure the samples stays cold during shipment.
- Package sample as appropriate for biologic material.

For all samples, including those in ThermoSafe containers:
Include a large ice pack in the ThermoSafe containers.
- Include a copy of the submitted (in Rave) AALL1231 Specimen Transmittal Form with each shipment.
- **If possible, please** send a copy of the **bone marrow immunophenotype report with the first peripheral blood sample.** If this is not possible, please send the report that day via fax (832-825-1206) or email to Dr. Horton at tmhorton@txccc.org and Gaye Jenkins at gnjenkin@txccc.org (Please strip unnecessary identifiers)

- **Ship the sample by Federal Express Priority Overnight delivery to:**

  Dr. Terzah Horton c/o Gaye Jenkins
  Feigin Center, Suite 760.01
  1102 Bates St.
  Baylor College of Medicine
  Houston, TX 77030
  832-824-4676

  FedEx account # 296621072
  Add air bill comment: TCH CC# 3332

- Notify Gaye Jenkins or Horton lab representative **prior** to shipment of the sample. Phone: (832) 824-4676. Please email the Fed-Ex tracking number to the email addresses above if prior notification is not possible.
- If possible, do not ship samples for delivery on a weekend or holiday. Please contact the Horton lab for special instructions if samples are collected on a Friday.

Appendix XI: Minimal Residual Disease (MRD): Description and Characterization of Assay

Description of populations for testing

Patients with newly diagnosed T-ALL will have MRD measured in the bone marrow (BM) at the end of the first block of Induction therapy (Day 29). Those patients (~50%) with MRD levels < 0.01% in BM at Day 29 will be considered Standard Risk and assigned to the least intensive cytotoxic therapy. For the remaining patients (~50%), MRD will be assessed in BM at the end of Consolidation (EOC) therapy. Those with MRD < 0.1% (~40%) will be considered Intermediate Risk and receive intermediate intensity therapy. The remainder (~10%) will be considered Very High Risk and receive intensified therapy followed by an additional MRD assessment with those positive for MRD taken off study. Specimens from all patients will also be assayed at study entry in order to define an abnormal phenotype that will facilitate detection of MRD.

Part of the diagnostic immunophenotyping at study entry will include the marginal cost for early thymic precursor (ETP) subclassification, a subset having a poor outcome[70] and whose outcome is an important secondary endpoint of the study. Data from AIEOP-BFM 2000 suggest that while ETP ALL is often associated with poor prognosis, it may not be an independent risk factor for poor outcome, as most patients with ETP ALL who do poorly are identifiable by poor response to chemotherapy (PPR, Induction failure, or MRD positivity).[38] It is critical to understand whether or not ETP phenotype independently predicts outcome in T-ALL as these patients may need alternative therapy or HSCT for cure. Multivariate analysis will be performed to determine if ETP ALL is an independent predictor of poor outcome based on MRD rates at end Induction and end of Consolidation. Preliminary data suggest ETP represents 12.4% of T-ALL in AALL0434, yielding 118 ETP patients of the 952 T-ALL anticipated on AALL1231. There are few data available to predict the proportion of ETP+ patients that will have MRD < 0.1% at EOC. Overall, we expect that ~10% of T-ALL patients will have MRD ≥ 0.1% at EOC, while ~90% will have MRD <0.1%. Given the known higher percentage of end of Induction MRD+ patients among the ETP+ subset, we hypothesize that ~50% of ETP+ patients (n=59) will have MRD ≥ 0.1% at EOC (5-fold higher rate than MRD-negative), while ~50% (n=59) will have MRD <0.1%. If the 4-year EFS of these ETP+, EOC MRD <0.1% patients is ~50% or better, it would suggest that ETP patients can be risk stratified based on MRD alone (unless there is a specific therapy available for ETP patients). However, if this assumption is incorrect and ETP+ patients with MRD < 0.1% at EOC have an EFS < 50%, it would suggest that MRD alone cannot be used to risk stratify these patients and alternative strategies (stem cell transplant in CR1 or other novel therapies) should be pursued for all ETP patients. Hence, we will see if the lower limit of the 95% confidence interval for the 4-year EFS estimate for ETP+, EOC MRD < 0.1% patients exceeds 50%.

Patients with T-LLy will have the level of marrow involvement assessed at diagnosis to facilitate risk stratification into Standard and Intermediate risk groups. All T-LLy patients will also be assessed for MRD at Day 29, as this study will enroll more T-LLy patients than any to date and offers a unique opportunity to establish risk factors for this understudied patient population. We hypothesize that T-LLy patients with <0.01% MRD at end of Induction will have the best outcome, consistent with existing data in T-ALL. No data exist on the frequency of marrow MRD following therapy for T-LLy, although the frequency of marrow involvement at diagnosis > 0.01% is 66% in AALL0434. Assuming that about 80% of T-LLy patients will have MRD < 0.01% at end of Induction, this yields roughly 198 T-LLy patients on AALL1231. This will enable us to estimate the 4-year EFS for MRD negative T-LLy patients with a maximum standard error of 3.6%.

Laboratory for MRD Testing

The MRD studies will be performed solely in the flow cytometry laboratory located in the Hematopathology Laboratory at the University of Washington in Seattle directed by Dr. Brent Wood. This laboratory has more than 5 years of experience performing this assay on nearly 1,400 pediatric patients enrolled on the

ongoing COG T-ALL trial AALL0434. In addition, for the past 8 years this laboratory has generated MRD results used for risk assignment on COG frontline B-lineage ALL trials for more than 10,000 pediatric patients.

Technical description of the assay
Analyte and platform: The assay detects leukemic cells at high sensitivity against a background of normal peripheral blood or marrow cells using multiparameter flow cytometry. It is based on the principle that leukemic cells express cellular antigens at levels that differ from those seen in normal cells.[196-198] Cells are stained with antibodies that have been previously shown to be informative for this purpose, and that have been conjugated to different fluorochromes designed to maximize the resolution between normal and abnormal cells. Specifically, the antibody combinations used are: CD16 PB/cCD3 FITC/CD7 PE/CD56 PE-Cy5/CD5 PE-Cy7/CD38 A594/sCD3 FITC/CD45 APC-H7 and CD8 BV421/CD48 FITC/CD5 PE/CD34 PE-TR/CD16+56 PE-Cy5/CD3 PE-Cy7/CD4 A594/CD7 APC/CD45 APC-H7 are used to identify leukemic cells. Evaluation for ETP type will be performed by using the following antibody combination in a single tube for pretreatment samples only: HLA-DR PB, CD7 FITC, CD13+CD33 PE, CD117 PE-Cy5, CD34 PE-Cy7, CD38 A594, CD1a APC, CD45 APC-H7. Stained cells are analyzed on a LSRII flow cytometer (Becton Dickinson, San Jose, CA). The proportion of leukemic MRD cells is expressed as a percentage of T/NK cells in each of the two tubes. An additional tube containing the DNA binding dye Syto16 along with CD7 and CD45 is used to calculate the proportion of T/NK cells as a percentage of total nucleated cells, which, when combined with results from the other two tubes allows computation of MRD as a percent of all nucleated cells. Finally, CD45 and side scatter is used to exclude granulocytes from the denominator so that the final value for MRD is expressed as a percent of mononuclear cells. By acquiring at least 750,000 events, it is possible to detect leukemic cells with a routine sensitivity of 0.01%, better in a subset of cases. This assay is currently used to determine prognostically significant thresholds for the on-going AALL0434 clinical trial.

Specimens and processing: Either PB or BM is used for the assays described here. Samples are drawn at local institutional sites and sent in anticoagulated tissue culture medium provided to the local institutions and processed in most cases within 24 hours of collection. Aliquots of whole PB or BM are stained with the combinations of antibodies noted above followed by simultaneous red blood cell lysis with ammonium chloride and fixation using a small amount of formaldehyde. Finally the sample is washed once and listmode data acquired on the flow cytometry.

Scoring procedures/criteria for positive/cut-points: Listmode data are analyzed in third party software using a hierarchical gating strategy with visual interpretation of multiple bivariate dot plots performed by Dr. Wood. Leukemic populations identified are analytically isolated from background normal cells using Boolean logic and based on the principle that leukemic cells express antigens in a pattern different from that seen during normal maturation of that lineage. For patients with T-ALL, the combination of a cut-point of < 0.01% leukemic cells in BM at Day 29 after initiation of chemotherapy and <0.1% leukemic cells in BM at end of Consolidation will be used to identify patients with a standard risk of relapse. For the remaining patients a cut-point of ≥ 0.1% leukemic cells at the end of Consolidation therapy will be used to identify patients at very high risk of relapse. Very high-risk patients that remain MRD positive ≥ 0.01% after intensification of therapy will be removed from the trial for additional therapy. For patients with T-LLy, patients with < 1% BM involvement at diagnosis will be considered Standard Risk and those with ≥ 1% BM involvement at diagnosis will be considered Intermediate or Very High risk depending on CT scan at Day 29. Blasts will be scored for ETP type using the published definition,[70] i.e. lack of expression of CD1a and CD8, expression of one or more of CD13, CD33, CD34, CD117 or HLA-DR, and CD5 expression 1 log lower than the mature T cells or < 75% positive.

Background: Minimal Residual Disease (MRD) is known to be a powerful prognostic factor in childhood B lineage ALL.[36,37,199-205] Only limited data is available for T-ALL, most of it based on relatively small numbers of patients using PCR methodology.[206-210] The largest and most comprehensive study to date of 484 pediatric patients[38] demonstrates that MRD negativity (< 0.01%) by PCR at end of Induction is the most favorable prognostic factor followed by negativity at end of Consolidation (< 0.01%) regardless of MRD positivity at end of Induction. The presence of MRD ≥ 0.1% at end of Consolidation is associated with an increased risk of relapse. Moreover, patients with T-lymphoblastic lymphoma (T-LLy) who have minimal disseminated disease detected by flow cytometry at the time of diagnosis have an adverse outcome.[211] These studies clearly demonstrate that MRD is prognostically important in T-ALL/T-LLy, although confirmation by other large clinical trials is needed.

Flow cytometric approaches to MRD detection in T-ALL have the potential advantage that they are informative in the vast majority of cases, including those where T–cell receptor rearrangements are not present or not detected by PCR.[212] The studies published involve small patient cohorts and largely rely either on the expression of immature antigens, e.g. TdT, CD99, CD1a, or CD34, on T cells outside of the thymus[213-219] or on the absence of surface CD3 expression in the presence of cytoplasmic CD3, an aberrant T cell immunophenotype provided NK cells are effectively excluded.[215] Although the use of TdT and/or CD99 positivity in conjunction with cCD3 is a common strategy, we have shown that following Induction chemotherapy down-regulation of both is very common[220] and can lead to either underestimation or false negative results. The studies that have examined the correlation between MRD detection by Flow cytometry or PCR in T-ALL involve small patient cohorts but show a positive correlation between the techniques, although to a somewhat lesser degree than for B-ALL[41,214,219] perhaps reflecting the reliance of the flow cytometric methods used in those studies on inconsistently expressed immature antigens. Taken together, the data suggest that MRD in T-ALL can be detected by flow cytometry and that it correlates with PCR assays that have been shown to correlate with clinical outcome.

The assay to be employed on AALL1231 is the same as that currently in use on AALL0434 and does not rely on the immature antigens TdT or CD99. The assay uses a combination of more broadly expressed T cell antigens known to be both differentially expressed and more stable in T-ALL,[220] discordant expression of surface and cytoplasmic CD3 with NK cell exclusion,[215] and decreased to absent expression of CD48. The latter has been identified by our group (ASH abstract 2011) as a consistent finding in T-ALL at diagnosis that is relatively stable after Induction chemotherapy. This approach improves upon the published assays and employs a novel antigen in a high level (8-9 color) context that should improve both sensitivity and specificity through exclusion of non-leukemic populations via improved antigenic correlation.
Reference methods for minimal residual disease assays are not available, consequently determination of assay performance characteristics is problematic. Nevertheless, correlation of this flow cytometric assay with a PCR-based high-throughput sequencing assay (HTS) shows a high degree of correlation to a level of roughly 0.01%, suggesting a sensitivity of 0.01%. Those samples positive at any level by flow cytometry show no false positives in comparison with HTS, while flow cytometry negative samples show a single false negative just above 0.01% by HTS, suggesting a high degree of specificity above the level of 0.01%. Independent enumeration from the two tubes of the flow cytometric assay also shows a high degree of correlation without false positives or negatives in either direction, further suggesting a high degree of specificity. Given the absence of proficiency testing programs for this assay, blinded samples will be exchanged with another laboratory 3 times per year and concordance demonstrated.

Expected population distribution: On AALL0434, this assay has identified that roughly 50% of T-ALL patients lack detectable MRD (< 0.01%) at Day 29 in BM, suggesting a group at standard risk for relapse. Similarly, on AALL0434 roughly 10% of patients are estimated to have detectable MRD (≥ 0.1%) at the end of Consolidation, the only caveat being that on AALL0434 only patients with MRD ≥ 1% at Day 29 were assessed for MRD at end of Consolidation. The remaining roughly 40% of patients are considered to have an intermediate risk of relapse. On AALL0434, ETP patients represent 12.4% of the cohort. For T-

LLy, roughly 65% are estimated to have BM involvement <1% at diagnosis and are considered Standard Risk with the remaining roughly 35% of patients being Intermediate or Very High risk.
Cut-point rationale: The Day 29 (0.01%) and end of Consolidation (0.1%) cut-points are the same as those that have been shown to be prognostically important at their respective time points in a prior BFM study[38] and are confirmed to be prognostically important in T-ALL in a preliminary review of outcome data from AALL0434. For T-LLy, the cut-point of 1% is the same as that currently in use in AALL0434.

Result Access: Results from these analyses will be provided to investigators via the Children's Oncology Group clinical data system, using the identical established procedure already developed to communicate results in our frontline trials

Analytical performance: Our flow cytometric assay for minimal residual disease has been used in nearly 1,000 children with T-ALL enrolled in the on-going AALL0434 COG clinical trial for which outcome is not yet available. Consequently, we are not yet able to demonstrate the relationship of MRD to clinical outcome using this assay, but will do so when the outcome data is made available. Given our experience with a B-ALL MRD assay of similar design that has been shown to be highly prognostically significant (unpublished from AALL0232), the published data with assays of this type to date and the correlation of those assays with prognostically significant PCR assays, we are confident the assay will stratify patients for relapse risk. Comparison of this assay with PCR as a surrogate for outcome is not easily done, as the PCR assays are very labor and resource intensive and the group does not have access. However, we have done a limited correlation of this assay with a next-generation sequencing MRD assay (see attached Integrated Assay application) showing good correlation down to the range of 0.01% to 0.1% without false positives,[221] shown in Figure 1.

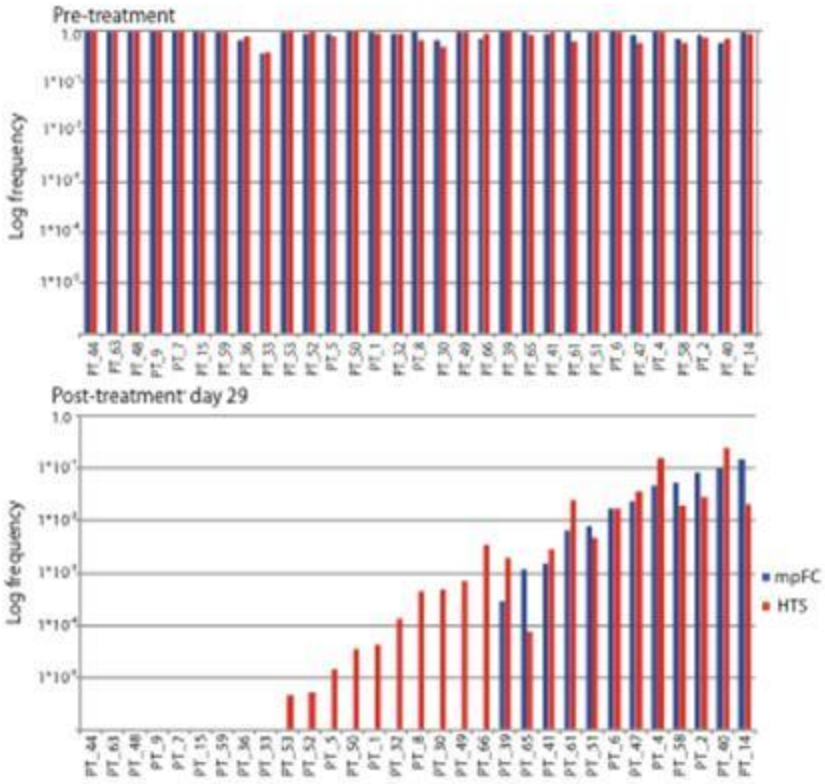


Figure 1. Identification of leukemic populations prior to and following therapy by the flow cytometric assay (mpFC) and next generation sequencing (HTS). Sequence data are reported as the frequency of the clonal sequence of total rearranged T cell sequences; Flow cytometry data are reported as the T-ALL frequency of total T cells including all CD7+ T/NK events.

We are unable to assess the precision of this assay in the usual fashion of having multiple replicates because the amount of sample available for repeat testing is limited. However, we have taken advantage of the fact that we use two different antibody combinations to calculate MRD, and can demonstrate a high degree of concordance between the two measurements, as shown in Figure 2. The small subset of mildly discordant samples is due the inability of one antibody combination to completely define the MRD population, which is evident at the time of analysis and would not lead to errors in reporting.

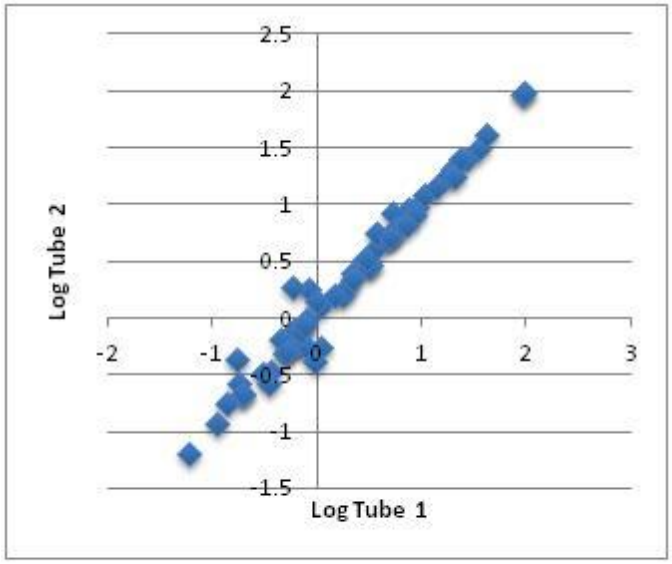


Figure 2. Correlation of MRD levels between two tubes used for MRD detection

The success rate of this assay in our hands is excellent. Less than 1% of samples received give indeterminate results in our assay. We do not have any extensive data on any critical preanalytical variables that have an impact on the performance characteristics of this test. Obviously, quality of the marrow initially obtained could have an impact if the specimen is significantly hemodiluted. While we do not have any direct means to measure hemodilution, we can use the proportion of nucleated red cells recovered in our sample as a surrogate for marrow quality. In preliminary analyses for a conceptually similar assay used for B-lineage ALL MRD monitoring we were unable to demonstrate an outcome difference between patients who lacked MRD but had low levels of nRBCs compared to those with high levels, suggesting that hemodilution in routinely obtained samples does not cause a significant underestimate of the MRD-positive patient population.

To standardize our ability to define ETP T-ALL, a cohort of cases of possible ETP T-ALL were independently reviewed by Dr. Dario Campana, who published the description of the ETP immunophenotype, and Dr. Wood. Discordant cases were reviewed and interpretive criteria refined. Applying these criteria to 790 cases of ETP from AALL0434 provided a frequency of ETP of 12.4%, which agrees very well with published frequency of 12.6%.[70]

APPENDIX XII: ADDITIONAL INFORMATION FOR PROPOSED BIOLOGY STUDIES: STUDIES OF PROTEASOME ACTIVITY, PROTEOMICS, AND SIGNAL TRANSDUCTION

Biology Objectives 1.3.2 and 1.3.3

I. HYPOTHESIS AND BACKGROUND:

The cornerstone of therapy in acute lymphoblastic leukemia (ALL) is risk stratification of patients based upon genetic alterations and minimal residual disease (MRD). While this strategy has proven beneficial in pre-B ALL, prognostic associations with genetic aberrations in T-ALL are not sufficiently compelling to contribute to risk-adapted therapy, likely due to the significant heterogeneity of mutations in this disease[222]. The AALL1231 clinical trial is testing the novel hypothesis that the addition of proteasome inhibitor therapy to an augmented BFM (ABFM) backbone will improve T-ALL event free survival (EFS). Recent data suggests that the many genetic subtypes of T-ALL, including the early thymic progenitor (ETP) phenotype, may converge on a more limited set of deregulated signaling pathways and cellular processes.[223] However, the frequencies with which signaling pathways are deregulated, how they impact prognosis, or how they modulate chemotherapy responses, are poorly understood. With better understanding and validation, these biochemical aberrations could be used clinically to identify patients with a "high risk" molecular signature that would benefit from more intensive chemotherapy.

Because DNA mutation analysis and gene expression profiling (GEP) have to date resulted in only modest gains in predictive power in T- ALL, we propose using protein analysis methods to understand the biochemical underpinnings of T- ALL. Our **long-term goal** is to identify and validate protein cell stress and/or signaling alterations that can be used alone or incorporated with GEP into a single, simple, and robust molecular classification system to aid in risk stratification and inform the design of future clinical trials incorporating targeted agents. If successful, the results of this research will move the study forward from an **integrated study to an integral study** that can be incorporated into future T-ALL clinical trials. The **overall objective** of this study is to determine whether proteasome and/or altered signal transduction patterns can identify protein expression profiles that predict response to bortezomib-containing therapy or are prognostic of clinical outcome. Based on our strong preliminary data, our **central hypothesis** is that proteasome alterations, protein cell stress activation, and/or the pattern of signaling networks will predict therapy response and allow us to identify patients with a "high-risk" protein expression pattern that could benefit from more intense chemotherapy. The **rationale** for this work is that a better understanding of the role of biochemical alterations in T-ALL will result in the optimization of simple, robust assays that will aid in pediatric ALL risk stratification and personalization of chemotherapy, ultimately improving clinical outcome. We will test our central hypothesis with the following **specific aims**:

Specific Aim 1: To determine if changes in proteasome function or cell stress protein expression patterns can predict bortezomib response and drug resistance in T-ALL.

a) To delineate the mechanisms of bortezomib action and resistance in T-ALL to determine if proteasome alterations correlate with clinical response (as measured by minimal residual disease (MRD) or outcome (EFS).
b) To determine if reverse phase protein lysate arrays (RPPA) predict chemotherapy response or resistance.
    1. To determine if protein cell stress pathways, such as the unfolded protein response, are active at baseline and how activity changes in response to chemotherapy +/- bortezomib,
    2. To define a putative "high risk" protein expression profile that identifies patients receiving either standard or intermediate risk therapy that would benefit from the more intense chemotherapy.
    3. To determine if RPPA protein expression profiles correlate with treatment group or T-ALL subtypes.

Specific Aim 2: To identify biomarkers and mechanisms of chemotherapy resistance and response in T-ALL, focusing on ETP-ALL.

a) To use single cell phosphoflow cytometry (SCPFC) to determine if differences in constitutive activation of the MAPK, PI3K/AKT/mTOR, and/or JAK/Stat pathways between ETP and non-ETP T cell subtypes will serve as a biomarker of chemotherapy response or resistance.
b) To determine if augmented NFκB signaling predicts response to induction chemotherapy with or without bortezomib in ETP and non-ETP T-ALL.
c) To use RPPA and SCPFC to build and validate multivariate classifiers to predict patient response to chemotherapy and determine risk of relapse.

II. SIGNIFICANCE: Previous research for prognostic risk factors in pediatric ALL have mostly focused on the characterization of genetic abnormalities.[224] Many of the new biologic agents being introduced into T-ALL therapy, however, directly target protein activation. We expect that the results of this research work will enable us to determine if cell stress pathways such as the UPR or altered signaling networks can identify protein biomarkers that predict response to induction therapy with Vincristine, Dexamethasone, Asparaginase, doxorubicin (VXAD) +/- the proteasome inhibitor (PI) bortezomib. Specifically, our goal is to develop a "high-risk" protein classifier that identifies patients with a higher risk of relapse in the standard and intermediate risk group. If these patients can be identified with a valid molecular classifier, this could directly result in improved therapy and outcome, as higher risk patients would be assigned to more intensive therapy. There are also significant gaps in our understanding of the role of the UPR and other cell stress pathways in response to T-ALL treatment. Successful completion of the aims described will better define the role of these biochemical pathways in response to chemotherapy. Although the primary objective of the clinical trial is to determine if bortezomib-containing therapy increases long-term survival, the objective of this work is to develop protein expression classifiers that provide useful predictive and prognostic information whether or not bortezomib is found to be an effective agent. This contribution will be significant because it will add the predictive power of protein alterations to the armamentarium of genetic mutations currently used to assess relapse risk, increasing the power of risk stratification and identifying patients most likely to benefit from therapies that target deregulated cell signaling pathways in T-ALL.

This research will also have a significant positive impact on future research for three reasons. First, successful completion of this application will define the contribution of alterations in signaling and cell stress proteins to treatment outcomes, an objective within the NIH mission to improve the diagnosis and treatment of cancer. Second, markers of protein cell stress pathway activation could be used to determine the mechanism of action of biologic agents that directly alter protein homeostasis such as proteasome inhibitors (PI) and tyrosine kinase inhibitors (TKI) in appropriate T-ALL subgroups. Third, the results of this study could enable the future development of simple, robust diagnostic assays testing specific proteins or protein clusters that predict response to (VXAD) induction. This will allow for further refinements in risk stratification and, by predicting response to VXAD alone or to PI-containing therapy, will lead to the further development of personalized T-ALL treatment.

III. INNOVATION: Prior research has demonstrated that mRNA abundance does not always correlate with protein expression,[225] highlighting a need for new approaches to assess protein signal transduction in multi-institution trials. There is precedence for this approach. Previous proteomic studies in ALL have utilized prospective cohorts to show that proteasome activity is prognostic of clinical outcome (Horton AACR abstract 2014) and that SCPFC can predict response to therapy in both ALL[226,227] and AML[228-230]. RPPA is also predictive of response duration in adult ALL.[231] *The proposed research is innovative because, with the successful completion of these aims, we will demonstrate that RPPA and SCPFC can transition into simple robust assays that can be applied to large sample sets in multi-institution clinical trials. The data generated from this application will also provide prospective validation of clinical usefulness of the RPPA and SCPFC techniques in the setting of a phase 3 clinical trial.*

Our preliminary data (see below) indicates that RPPA and SCPFC are both feasible and informative in the setting of a multi-institutional clinical trial. Proteomic data from either static (RPPA) or dynamic (SCPFC) molecular diagnostics, in combination with genomic data, could become key assays for response prediction and risk stratification in T-cell ALL where utility of genetic classifiers has been limited. Since gene-expression profiling (GEP) data can be combined with protein array data, there also is the potential to develop more in-depth and robust models of therapy response and relapse risk.[232,233] *The innovation of this study will be in creating the foundation for combined proteome and transcriptome analyses in future trials*.

IV. JUSTIFICATION FOR PROSPECTIVE COLLECTION OF FRESH SAMPLES

Shipping and handling delays are commonplace in multi-institutional clinical trials. Although most samples can be shipped overnight, samples are frequently delayed up to 72h over weekends. Although DNA mutation analysis and gene expression profiling (GEP) can be performed on frozen samples after shipping, GEP and mutation analyses have (to date) limited predictive power in T-ALL. We propose a broader approach using protein analysis methods to understand the biochemical underpinnings of T-ALL. Our long-term goal is to develop a simple, robust protein analysis that could either be incorporated with GEP/ mutation analysis, or used alone as a complementary tool for risk stratification.

Both of the aims of this study will require prospective collection of *fresh* lymphoblasts. This has been demonstrated for RPPA (see below) prior to the initiation of a similar study in AAML1031. A very similar set of experiments was reviewed by both the COG AML Biology committee and by the NCI, which is currently providing R01 funding for the project. Although SCPFC can theoretically be performed on banked specimens, multiple laboratories (C. Mullighan, J. Dick, D. Teachey, R. Lock, M. Hermiston, and A. Fernando, personal communications) have found that T-ALL samples, particularly those of the ETP phenotype, have extremely poor viability and yields upon thawing relative to pre-B ALL samples. For example, the yield upon thawing of 10 AALL0434 T-ALL samples from the COG tissue bank ranged from 3.8-17.8% (mean 6.94% viable cells, equivalent to 0.44 to $2.9x10^6$ cells/vial) (Shimano and Hermiston, unpublished observations; Fig 2A). This is in contrast to adequate viability (>70% of samples have >90% viability) when B-ALL are cells thawed from the COG tissue bank. Similarly, the Teachey laboratory found that only 30% of banked ETP-ALL cells had viability greater than 90% post thaw. While all of these high post-thaw viability samples engrafted successfully in Nod Scid Gamma (NSG) mice, none of the remaining 70% of samples with poor post-thaw viability engrafted.

Based on these observations and our belief that understanding the biology of ETP and high-risk T-ALL at a functional level is critical for identification of protein pathways resulting in chemotherapy resistance, we propose to perform these analyses on fresh samples. Since RPPA and SCPFC both require fresh cells, it will also provide a unique opportunity to compare these methods and to determine if 1) one of the two is more robust in a Phase 3 clinical trial, and 2) if the assays provide complementary information. Use of fresh cells will also enable us to achieve our long-term goal of developing a simple, robust protein classifier that can be combined with mutation analysis and GEP to aid in risk stratification. The objective of the classifier is to identify patients with a "high-risk" molecular signature that have been placed in either the SR or IR risk group, thus improving risk stratification and increasing EFS in these groups. Because sample availability has greatly limited our ability to study T-ALL biology to date, the use of fresh tissue will enable a comprehensive biochemical analysis in both non-ETP and ETP-ALL subtypes.

A. Feasibility of collecting pre-treatment bone marrow samples from multiple sites for SCPFC analysis:

To evaluate the feasibility of shipping fresh T-ALL cells for SCPFC, Dr. Brent Wood shipped 10 pairs of fresh ETP and non-ETP ALL samples (left-over after diagnostic flow cytometric phenotyping and MRD analyses) to the Hermiston Lab at UCSF over a six-month period. Even with delays of 72 hours, viability remained uniformly good (range 81-98%) and robust signaling responses could be obtained on all samples (Figure 1B). Moreover, preliminary data indicates that engraftment rates in NSG mice are quite high (data not shown).

Together, these data indicate that it is feasible to collect pre-treatment samples in the setting of samples shipped from multiple sites.

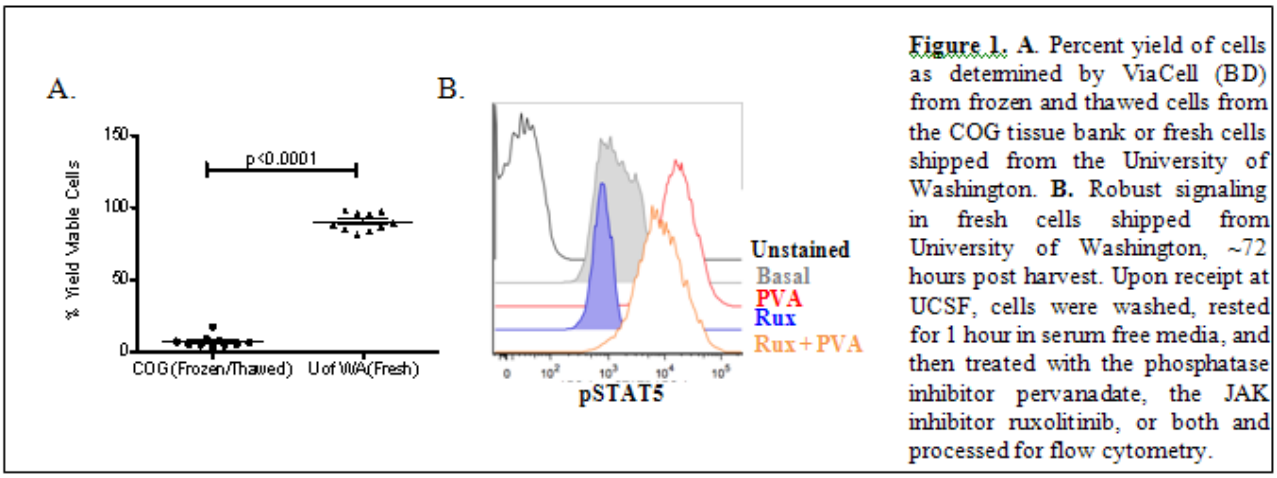


Figure 1. A. Percent yield of cells as determined by ViaCell (BD) from frozen and thawed cells from the COG tissue bank or fresh cells shipped from the University of Washington. **B.** Robust signaling in fresh cells shipped from University of Washington, ~72 hours post harvest. Upon receipt at UCSF, cells were washed, rested for 1 hour in serum free media, and then treated with the phosphatase inhibitor pervanadate, the JAK inhibitor ruxolitinib, or both and processed for flow cytometry.

B. Feasibility of collecting pre-treatment peripheral blood and bone marrow samples from multiple sites for RPPA analysis: To test the magnitude of the effect of shipping and handling delays on protein expression, we generated two custom protein arrays, one to test the stability of protein expression in heparin tubes (Table 1 and figure 2A) and a second to test protein stability in CellSave (CS) preservative tubes (Veridex) following treatment (Figure 2B). In the first analysis, 7 pediatric AML samples were processed either on site or after shipment by air courier from New York University (Horton, manuscript in preparation). Shipping was done with peripheral blood in heparin tubes and lysates were prepared under 5 conditions as shown in Table 1. We found that 16 of 18 proteins (Groups 1-3) (Fig. 2A) showed either no change or a decrease in protein concentration that could be calibrated if samples were processed within <72h. These data indicate that reliable signaling information can be obtained from shipped cells.

Table 1: AML Sample: Test of Shipping Conditions

Sample Treatment / Sample	Tx:1 Same day processing	Tx 2: Held at 4° C	Tx 3: Held at room temp	Tx 4: Shipped at 4° C	Tx 5: Shipped at room temp
Sample 2-PB[1]	X	24h	24h	24h	24h
Sample 3-BM	X	72h	72h	72h	72h
Sample 4-PB	X	72h	72h	72h	72h
Sample 5-BM	X	24h	24h	24h	24h
Sample 5-PB	X	48h	48h	48h	48h
Sample 6-BM	X	24h	24h	24h	24h
Sample 7-PB	X	24h	24h	24h	24h

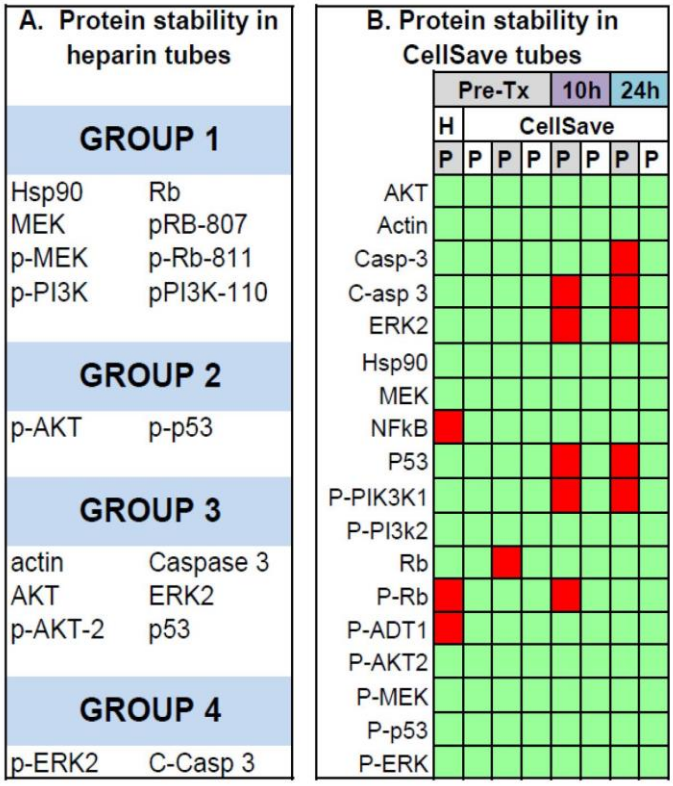


Figure 2:

A. Protein stability in pediatric AML. Samples were collected in heparin tubes and shipped over 24-72h. Changes in mean protein concentration were normalized and compared to the range of each protein expression in 544 adults with AML (normalization from -3 to +3). **Group 1:** Minimal/no change (<0.5 normalized log2 protein concentration) with shipping. **Group 2:** Predictable change (0.5-1 unit decrease) with shipping. **Group 3:** Time sensitive: Minimal change if process <72h from collection. **Group 4:** Unpredictable changes (>0.5 units) independent of shipment time.

B. Protein stability in CellSave preservation tubes: Control lanes (ln) 1-2: Normalized protein conc. - heparin (H) vs. CellSave in pre-tx samples. Significant differences noted in red; no significant difference in green. Ln 3-8: Comparison of protein concentration before, and at 10h and 24h after ADE therapy. Ln3-4: pre-treatment; Ln 5-6: 10h after treatment; Ln 7-8: 24h post-treatment. Gray P= unadjusted p value by Student's t test; White P= adjusted p value after correction for multiple comparisons using false discovery rate method.

C. <u>Justification for prospective collection of non-frozen samples in Cell Save preservation tubes for RPPA analysis</u>. Using a second patient cohort, the second array compared protein quality of lysates prepared from fresh cells versus frozen pellets (Tables 2 and 3). This array was done based on preliminary data in the Kornblau lab, using heparin tubes for pre-treatment samples, that demonstrating that frozen samples underwent significant sample degradation (Figure 3). **This data indicated that RPPA lysates must be made with fresh T-ALL samples,** and that the use of cryopreserved samples would result in sample degradation and potentially misleading results**.**

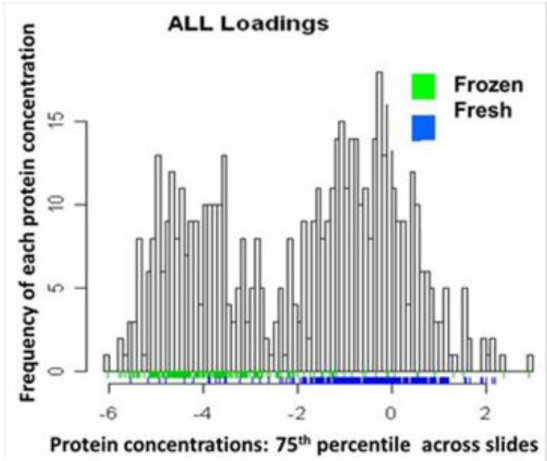


Figure 3: Protein degradation with freezing prior to RPPA lysate preparation: Protein expression of freshly made protein lysates (blue) vs. Proteins lysates made from cyropreserved cells (green). The 75th percentile of all proteins across the slide are compared between slides made from lysates prepared from fresh samples. vs. lysates made after samples had been cryopreserved and used at a later time. Each bar represents the frequency of the relative concentration for each protein from the 2 arrays

To determine if collection of fresh material in CS tubes alone would be sufficient for sample preservation in shipped samples, our second custom RPPA (Tables 2 and 3) compared CS tubes with immediate processing to those collected before treatment, 6h and 24h after treatment (n=23) with and without freezing. Protein expression between groups was compared using Student's t tests analyzing 17 validated RPPA antibodies. Although pre-treatment samples showed little difference in protein expression after shipping (Table 2, left panel) post-treatment samples demonstrated highly significant differences (Table 2, right panel). We then compared protein expression shipped in CS tubes without freezing (Table 3) where there are only a few significant differences between CS samples processed immediately and those processed with non-frozen samples (Table 3, right panel). A summary of the statistically post-treatment using fresh lysates are shown in Figure 2B. Based on

these analyses, we concluded that 1) the effects of shipping and time delays <72h should be minimal and should not confound protein expression analysis, and 2) CS preservation tubes processed within 72h can protect protein expression from changes during shipping. **Taken together, these data indicate that RPPA, SCPFC, the UPR and other cell stress proteins is both feasible and reliable as long as samples are collected prospectively and lysates prepared prior to cryopreservation.**

Table 2: Differences between protein expression using frozen lysates: CS immediate processing vs. CS processed after a 24-72h delay.

	Pre-tx		6h Post		24h Post	
Protein	p	adjusted p	p	adjusted p	p	adjusted p
AKT	0.24	0.38	0.000233	0.00035	0.000523	0.00086
Actin	0.16	0.33	0.000433	0.0006	0.000275	0.00071
Caspase_3	0.40	0.51	0.000135	0.0003	0.000036	0.00036
Caspase_3_cl	0.14	0.32	0.000008	0.00007	0.000121	0.00051
ERK2_	0.32	0.45	0.000571	0.00073	0.000399	0.00086
Hsp90	0.20	0.36	0.000103	0.00026	0.000506	0.00086
MEK	0.13	0.32	0.000197	0.00035	0.000040	0.00036
NF_kB	0.33	0.45	0.000653	0.00078	0.001630	0.00244
P53	0.10	0.32	0.000229	0.00035	0.000141	0.00051
pPI3K110	0.07	0.32	0.000065	0.00023	0.000195	0.00059
pPI3K85	0.14	0.32	0.001386	0.00156	0.002756	0.00331
Rb	0.04	0.32	0.000028	0.00013	0.000096	0.00051
pRb	0.02	0.32	0.000021	0.00013	0.000512	0.00086
p_AKT308	0.14	0.32	0.000217	0.00035	0.002068	0.00286
p_AKT	0.50	0.56	0.000087	0.00026	0.003824	0.00430
p_MEK	0.97	0.97	0.020626	0.02184	0.133027	0.14085
p_P5	0.74	0.78	0.000006	0.00007	0.002342	0.00301
p_erk	0.49	0.56	0.103560	0.10356	0.514352	0.51435
	n=23	n=17	n=23	n=17	n=22	n=16

Significant differences (<0.05) are highlighted 'Normalizedmean protein concentrations for each protein comparedusing Student'st test.'' Adjustedp determinedby false discovery rate method to adjust for multiple comparisons.

Table 3: Differences between protein expression using non-frozen lysates: CS processed immediate processing vs. CS processed after a 24-72h delay.

CellSave tubes					
pretreatment		6h post-treatment		24h post-treatment	
imm	delay	imm	delay	imm	delay
raw data		raw data		raw data	
p	adjusted p	p	adjusted p	p	adjusted p
0.35	0.58	0.10	0.14	0.06	0.13
0.34	0.58	0.07	0.14	0.03	0.11
0.27	0.54	0.09	0.14	0.04	0.11
0.68	0.77	0.09	0.14	0.15	0.21
0.25	0.54	0.10	0.14	0.04	0.11
0.16	0.49	0.04	0.14	0.02	0.11
0.15	0.49	0.05	0.14	0.03	0.11
0.46	0.59	0.10	0.14	0.08	0.13
0.40	0.59	0.20	0.23	0.10	0.15
0.16	0.49	0.04	0.14	0.01	0.11
0.23	0.54	0.03	0.14	0.03	0.11
0.14	0.49	0.08	0.14	0.06	0.13
0.04	0.49	0.03	0.14	0.08	0.13
0.15	0.49	0.08	0.14	0.35	0.43
0.46	0.59	0.12	0.15	0.18	0.23
0.97	0.99	0.67	0.67	0.65	0.68
0.99	0.99	0.14	0.17	0.43	0.48
0.63	0.76	0.53	0.56	0.73	0.73
23	22	23	22	23	22

Significant differences (<0.05) are highlighted 'Normalizedmean protein concentrations for each protein comparedusing Student'st test.'' Adjustedp determinedby false discovery rate method to adjust for multiple comparisons.

V. APPROACH

A. Sample collection, sample size and prioritization: The COG AALL1231 study will be a Phase 3 study which will randomize pediatric and adolescent/young adult (AYA) T-cell ALL and T-cell lymphoblastic lymphoma (T-LL) patients to receive either standard chemotherapy (modified BFM=VXAD) or standard therapy with bortezomib (VXAD-B), with the goal of obtaining 952 eligible, evaluable patients with T-ALL. T-LL patients will be excluded from this biology study.

Bone marrow (5cc at start of study and 5cc at end of Induction (Day 29) in heparinized tubes) will be collected for proteasome and SCPFC analysis. Peripheral blood (PB, 3mL in CS tube, 2 ml in a heparinized tube) will be collected for each eligible patient prior to the start of therapy and at 6 hours (h) and 24h following start of treatment. One requirement for eligibility for biology studies will be to have an absolute blast count (ABC) of at least 1000 cells/µL to have sufficient lymphoblasts for RPPA, SCPFC and proteasome analysis. We estimate that approximately 80% of patients will have sufficient PB lymphoblasts based on the historical control trial AALL0434.Based on bone marrow collection in AALL07P1 and AAML07P1, we estimate that we will receive at least 50% of pretreatment marrows. Based on the Horton lab collection of similar samples on the COG phase 3 AML trial AAML1031, eligible, evaluable and usable peripheral blood were obtained from 36% of enrolled patients. *Based on the percentage of patients with eligible blasts in PB and collection rates from the COG AML study AAML1031, we estimate that we will receive 476 eligible, evaluable, and usable bone marrow samples (238/tx group), and 342 PB samples from T-ALL patients (171/treatment (tx) group).* This will be the sample size for comparing cellular signaling pathways in treatment groups (VXAD +/-B) and in ETP vs. non-ETP patients.

SCPFC will be done from both bone marrow and peripheral blood. Assuming ETP will occur in 12.4% of patients, the samples size for ETP and non-ETP will be quite different. If marrow is received from 50% of eligible patients (estimate from AAML1031 and AALL07P1) we will have 476 marrow samples (238/tx group) with 59 ETP/417 non-ETP patients (30 ETP/tx group, 208 non ETP/tx group). Although the sample size will be smaller, we will also plan to examine changes in PB protein expression in both ETP and non-ETP groups. For peripheral blood we will have 342 patients, 42 of which will be ETP and 300 non-ETP.

Table 4: Prioritization of experiments for peripheral blood and bone marrow collection

Experiment	Aim	Lymphoblast # required/[a]	Sorting	Est blood volume	Tube type[b]	Timepoint (s)	Priority
RPPA	1	$8x10^5$	yes	1-2 mL	CS	0h, 6h, 24h	1
SCPFC	1&2	$1x10^7$	no	1-2 mL	Hep	0h, 6h, 24h	1
Proteasome expression[c]	1a	$5x10^6$	yes	1mL	CS	0h, Day 29[d]	2
Proteasome activity[c]	1a	$3x10^6$	yes	1 mL	Hep	0h, Day 29[d]	2
UPR-qRT-PCR	1	$2x10^6$	yes	0.5 mL	Hep	0h, 6h, 24h	3
UPR protein	1a	$1x10^7$	yes	1mL	CS	0h, 6h, 24h	3

a: #= number, sx = sample; b: CS =CellSave preservation tubes (Veridex), c. proteasome assays will be done with bone marrow prior to treatment and with bone marrow at the end of Induction (Day 29).

For assessment of proteasome activity/expression (Priority 2), we will use BM (pre-treatment and End of Induction (Day 29). Proteasome activity will be compared to response to therapy (as determined by End of Induction (Day 29) MRD with a cutoff at 0.01%) and EFS at 4 year. For bone marrow samples, the sample size is estimated to be 476 (238 Day 29 MRD >=0.01% /238 MRD <0.01%). Changes over time (pre-treatment vs. Day 29 bone marrow) will have a sample size of 238 (119/MRD status), accounting for the 50% drop-out rate of Day 29 bone marrow compared to pre-treatment bone marrow. It is likely that we will have sufficient sample size to compare baseline proteasome activity by ETP status (59 ETP/ 417 non-ETP).

UPR studies will be done with peripheral blood, and bone marrow if extra remains (Priority 3). For UPR protein analysis, we estimate that 25% of the "UPR patients" will have sufficient material for analysis,[234] resulting in a sample size of 86 patients (43 patients/tx group).

For ease of collection and processing at individual sites, all samples will be sent to the Horton lab where the will be divided and subsequently sent to the Hermiston laboratory (Figure 4). The estimate of lymphoblasts per samples will vary significantly depending on the absolute blast count of the sample and the processing required for analysis (e.g., requirement for cell sorting). As in previous studies, we have limited our blood collection volume to 5 mL (2mL heparin, 3mL CS preservation tubes).

B. Workflow. Figure 4 demonstrates the workflow for sample analysis. WBC number and viability will be determined upon receipt of shipped samples using an automated fluorescence cell counter. Cells will then be processed as previously described [235] with the addition of non-T cell depletion using magnetic separation prior to processing for RPPA lysates proteasome, and UPR analysis. Depletion will not be performed on heparin samples for SCPFC analysis or (if excess sample) for xenograft engraftment. Protein pellets and protein lysates will be prepared on day of receipt. Because sample availability has greatly limited our ability to study ETP biology to date, xenografts will be established in NSG mice from any leftover cells not required for these assays. Any samples emerging from these xenografts will be deposited in the BioPathology Center to be banked for future use.

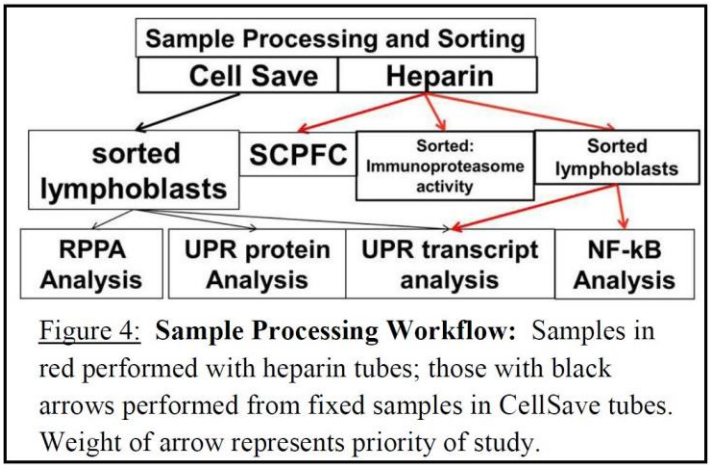


Figure 4: **Sample Processing Workflow:** Samples in red performed with heparin tubes; those with black arrows performed from fixed samples in CellSave tubes. Weight of arrow represents priority of study.

C. Study Team: We have developed a very experienced team of experts with expertise in the areas of focus in these correlative studies: David Teachey (T-cell biology), R. Sifers (UPR), S. Kornblau (RPPA), M. Hermiston (biochemical analysis in T-ALL) and T. Horton, (protein homeostatis). Dr. Horton has conducted several COG clinical trials with bortezomib in relapsed leukemia and lymphoma.[235-240] She will combine her expertise with specialists in the UPR, RPPA and SCPFC that, as a team, are uniquely placed to evaluate protein cell stress pathways within a multi-site clinical trial. We have assembled a statistical team that will be led by M. Devidas, head of the COG statistical core. Protein array analysis and UPR will be done by A. Tsimelzon and C. Ahern (Baylor College of Medicine) and K. Coombes (MD Anderson); SCPFC analysis will be done by C. Delgado-Martin, M. Hermiston and A. Olshen at UCSF; proteasome analysis will be performed by the laboratory of Jacqueline Cloos (VU Medical Center, Amsterdam). Dr. Devidas will review all statistics and aid in the correlation of array results with clinical outcome.

D. Specific Aim 1: **To determine if changes in proteasome function or cell stress protein expression patterns can predict bortezomib response and drug resistance in T- ALL**

1.) Subaim 1a. To delineate the mechanisms of bortezomib action and resistance in T-ALL to determine if proteasome alterations correlate with clinical response (Day 29 MRD) or outcome (EFS).

a. **Overview**

Baseline UPR activation is associated with improved 5y-EFS in adults with AML.[241] There is also evidence that response to PI therapy correlates with UPR activation.[242] However, while *in vitro* studies have shown that UPR activation can induce T-ALL cell apoptosis,[243-245] UPR activation following T-ALL chemotherapy has not been examined in patients. This aim will examine whether baseline UPR activation, or changes that occur in the first hours of VXAD +/- bortezomib can determine if induction of UPR effectors can predicts response to VXAD +/- bortezomib chemotherapy in T-ALL. As part of our goal of identifying prognostic and predictive protein cell stress biomarkers, the objective of this aim will specifically focus on defining the role of the UPR in pediatric T-ALL cell apoptosis and the utility of UPR activation as a indicator of chemotherapy response. We will test the working hypothesis that activation of the UPR pathway will enhance VXAD-mediated apoptosis. Such findings would be of importance, because it will allow, for the first time, the use of UPR proteins as predictors of chemotherapy response.

Several prior studies have examined mechanisms of bortezomib action and resistance in pediatric leukemia. Three signal transduction pathways are of interest based on preliminary data and feasibility from our lab and others: 1) the response of the proteasome to proteasome inhibitor (PI) therapy,[239,246] 2) the activation of the endoplasmic reticulum (ER)-mediated unfolded protein response (UPR), and 3) the activation of the NF-κB pathway.

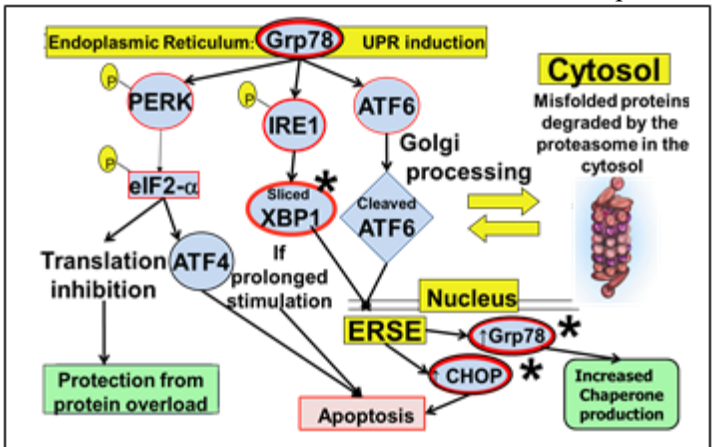


Figure 5: Activation of the UPR: The UPR sensor Grp78 activates three UPR paths, all of which can trigger apoptosis if protein folding capacity if overwhelmed. Proteins with asterisks: UPR transcripts that will be tested on all samples. Methods: UPR transcripts (red circles) will be quantified by qRT-PCR and UPR proteins (black circles) by immunoblots. Antibodies have been optimized for use with clinical samples. ERSE = endoplasmic reticulum response element.

1) Overview of proteasome expression and activity in pediatric leukemia: Although PI therapy has had encouraging results in multiple myeloma and follicular large B cell lymhoma, its efficacy is often limited by the development of proteasome resistance. Although proteasome resistance in T-ALL can result from proteasome mutations,[247] they can also correlate with change in proteasome capacity.[248] The Cloos laboratory has recently shown that

immunoproteasome activity is higher in T-ALL lymphoblasts than in pre-B cells and AML, both in cell lines and in primary patient samples.[239] In a recent collaboration between the Cloos and Horton laboratories, we discovered that patients having higher immunoproteasome expression were more likely to respond to bortezomib, as measured by CR2 after cycle 1 of therapy (see preliminary data below).[246]

2) Overview of the unfolded protein response (UPR): Studies have shown that pathways regulating cell stress, the ubiquitin-proteasome pathway, and the UPR are integrally linked. Damaged proteins awaiting proteasome degradation accumulate in the ER and induce expression of the UPR sensor protein Grp78 (Fig 5).[249,250] Grp78 activates three effector branches of the UPR (Figure 5): the pancreatic kinase like ER kinase (PERK)/eIF2-α pathway (left), the IRE1/XBP1 pathway (middle), and the AFT6 pathway (right).[243] Phospho (P)-eIF2α inhibits global translation, while spliced (s)XBP1 and cleaved (c) AFT6 induce the transcription of chaperone proteins that assist with protein folding.[251] However, if cellular damage is too extensive for repair, UPR effectors (Grp78, IRE1, sXBP1 and CHOP) trigger apoptosis.[245,252]

3. The role of NF-κB activation: NF-κB activity was shown to be inhibited in early trials of bortezomib in pediatric leukemia.[235] Recent data with a larger sample sample size (n=61) collected from patients enrolled on both Phase 2 trials of bortezomib in pediatric leukemia (AAML07P1 and AALL07P1) have shown that, although NF-κB is decreased in many patients, the decrease does not correlate with clinical response. However, whether this holds true in T-ALL has not been adressed. Due to the analytic limitations of the NF-κB ELISA in prior studies, NF-κB analysis will be performed using SCPFC in Aim 2b.

b. **Rationale and preliminary data**

1) Immunoproteasome preliminary data: Results from Jacqueline Cloos (VU Medical Center, Amsterdam, the Netherlands) and the Horton lab have shown that the ratio of immunoproteasome to proteasomes vary widely in pediatric leukemia patients and that increased proteasome activity correlated with increased sensitivity to PI inhibitors including bortezomib.[239] We have expanded this analysis to determine if proteasome activity correlated with response to bortezomib-containing chemotherapy (Figure 6). Samples collected on the Phase 2 clinical trials AALL07P1 and AAML07P1 were assessed for proteasome activity and expression. This study showed, for the first time, that the **baseline ratio of immunoproteasome to proteasome expression correlated with response to therapy** (post-induction CR).[246] Patients that reached a complete remission (CR) following induction, had 2-fold higher ratios of both β5i/β5 and β1i/β1subunit expression compared to patients who did not (p=0.019 and p=0.022). We also observed increased ratios of pre-treatment subunit-

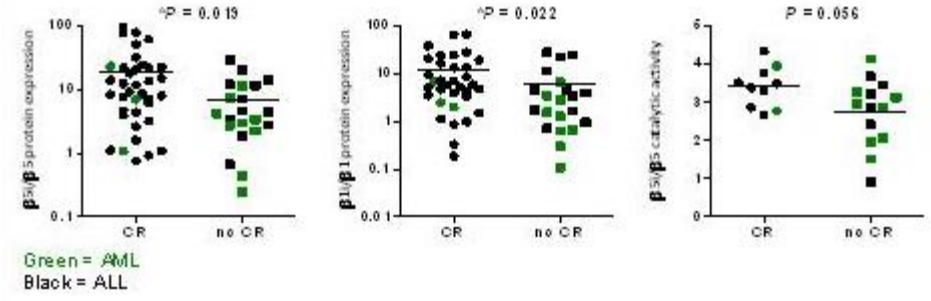


Figure 6: Ratio of immunoproteasome to proteasome protein subunit expression and catalytic activity in patients with CR after induction.

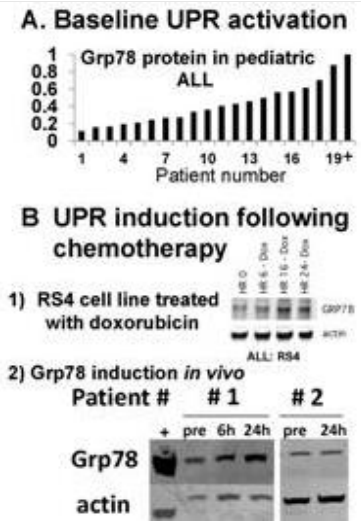


Figure 7: UPR activation in ALL: **A.** The UPR chaperone Grp78 protein is variably expressed at baseline in pediatric ALL patients. **B.** UPR activation (as measured by increases in the UPR sensor Grp78) is seen in both cell lines and in some (but not all) primary ALL patients following VPAD. + control: RAW cell.

specific catalytic activity of β5i/β5 were observed in patients that reached CR (n=10) compared to those who did not (n=14).

2) UPR activation: Similar to adult AML, we **hypothesize** that patients with an intact UPR pathway will have a better response to VXAD +/- B,[241,253] and that patients sensitive to bortezomib will have robust UPR activation following chemotherapy. Preliminary data from the Horton lab shows that baseline UPR activation, as determined by Grp78 protein expression, varies widely between ALL samples (Fig 7A) (Horton et. al., manuscript in preparation). UPR activation after chemotherapy (VPAD; P = prednisone) was also seen in 1/7 pediatric ALL patients (Fig 7B).

c. **Experimental Design**

Subaim 1a will focus on known candidate signal transduction pathways involved with the response to bortezomib. We will evaluate UPR activation using both transcript analysis (qRT-PCR) and protein analysis (immunoblots). Although UPR qRT-PCR does not equate with protein expression, it does reflect UPR pathway activation and activation of proteins that can trigger apoptosis. It also requires limited cell numbers ($2x10^6$) and will be done on most eligible patients (in the context of being Priority 3). Also, qRT-PCR for most UPR transcripts can be performed using CS tubes. Although the yield is decreased by approximately 50%, the results are similar (Horton, unpublished results). Based on data from AAML1031, we estimate that 25% of patients will have sufficient sample for UPR protein analysis by immunoblot.

1) Determine if there is evidence of baseline UPR activation: Since an overwhelmed UPR can trigger apoptosis,[252] we hypothesize that baseline induction of the UPR could indicate cell stress and correlate with therapy response (MRD) and clinical outcome (EFS).[241] We will assess baseline UPR activation by quantitating sXBP1 (an early event in UPR activation), the UPR sensor GRP78, and the UPR terminal effector CHOP. Initial assessment will be by qRT-PCR, as this requires the fewest number of cells and can be done on all eligible, evaluable, and usable patients (n=342). Baseline GRP78 and CHOP will be compared to normal lymphocytes and ALL cell lines. sXBP1 will be the primary measure of baseline UPR activation. Transcript expression will be compared by response (MRD) and and outcome (EFS). We expect that baseline UPR expression will be more common in treatment responders based on our preliminary data; however, groups will also be compared over the first day of treatment to determine if changes over time are more predictive of response.

2) Determine if chemotherapy (VXAD+/-B) induces the UPR. In addition to baseline UPR activation, we will also assess UPR activation following treatment, allowing us to correlate both baseline and induced UPR activation with clinical outcome. UPR effectors (sXBP1,GRP78, CHOP) will be assessed during day 1 of therapy (0h, 6h, and 24h). Our working hypothesis is that chemotherapy will activate the UPR(i.e. increase over time from 0h to 6h or 24h) in some, but not all, patients and that UPR activation will be more pronounced in the patients receiving bortezomib-containing chemotherapy. This is based on our preliminary data (Fig 7) a In patients that have evidence of UPR activation following treatment, we will determine which of the three UPR pathways is activated. We have optimized 7 antibodies for detection of UPR immunoblots (Grp78, PERK, p-eIF2-α, IRE-1, XBP1, CHOP and ATF4) and 11 primer sets for qRT-PCR quantitation of UPR transcripts (above +spliced Xbp1, p-PERK, ATF6, and calreticulin) (Fig 5). We expect that chemotherapy will induce UPR transcripts and effector proteins and result in increased spliced (s)Xbp1, an indicator of UPR activation and an apoptosis trigger.

3) Assess the prevalence of UPR activation in ETP-ALL: Prior work indicates that baseline UPR activation in adult AML is more frequent in high-risk cytogenetic phenotypes such as monosomy 7 and del5q.[241] However, the extent of UPR activation is unknown in ETP-ALL. We expect that UPR activation will be more pronounced in ETP-ALL; however it is possible that only the non-apoptotic pathways (p-eIF2α down-reguation and chaperone induction) will be common and that effector molecules may not be triggered, as seen in Waldenstroms macroglobulinemia.[254] This will be assessed by qRT-PCR and (in patients with sufficient material) immunoblots.

4) Assess UPR activation by treatment and response groups: Baseline UPR activation, as well as UPR induction following chemotherapy, will be stratified by treatment groups. Our working hypothesis is that VXAD+B treated patients will have more sustained activation of protein cell stress pathways, including terminal UPR proteins that trigger apoptosis such as Grp78 (through caspase 7), CHOP (through caspase 4), and sXbp1 (via JNK).[244] We also expect to find that patients responding to therapy (VXAD+/-B) will have evidence of increased sXBP1 transcripts, sustained IRE1 activation, and increased Grp78 and CHOP.[244,245,251] These proteins will be more fully evaluated in the context of other protein cell stress pathways in SA1b. Our goal is to develop a protein expression classifier that can predict response to VXAD+/-B, and to determine if protein classifiers can also identify SR and IR patients likely to benefit from more intense chemotherapy based on their 'high-risk" protein signature.

d.) **Statistical methods and justification**

1) Sample size- Please see Section V for sample size assumptions and justification.

In addition to determining the effect sizes of assays between treatment groups, we will also compare with early response to treatment (MRD) and T-ALL phenotype (ETP vs non-ETP). For the analysis of data across time (0h, 6h and 24h) we do not know *a priori* the most relevant time points to compare. Short-term differences could have returned to baseline by 24h, and may be most accurately estimated by changes between 0h and 6h. Long-term reactions would be best measured by 0h-24h comparison. Complex changes, such as increase at 6h and decreases by 24h (NF-κB) are best measured using all three time points as descriptors. However, for simplicity we have provided the affect sizes for changes from 0h to 24h in Table 5.

Table 5: Effect-size (detectable differences) based on sample size for proteasome activity and UPR*

1. Proteasome studies (done with BM)	**Samples size (max)**	**Effect size ***	**2. UPR studies**	**Sx. Size (max)**	**Effect size ***
Baseline and change (pretx to Day 29) in proteasome activity by treatment arm (VXLD vs VXLD+B)	476 (238/tx group) 238 (119/tx group)	0.257 0.365	Baseline PCR-(BM) by ETP	476 (59 ETP/ 417 non-ETP	0.391
Baseline and change (pretx to Day 29) in proteasome activity by response (MRD status)	476 (238/MRD grp 238 (119/MRD grp	0.257 0.365	PCR-D1change-(PB) by ETP	342 (42 ETP/ 300 non-ETP	0.463
Baseline and change (pretx to Day 29) in proteasome activity by ETP status (12.4% ETP/ 87.6% non ETP)	476 (59 ETP/417 non-ETP 238 (30 ETP/208 non ETP)	0.391 0.549	Baseline UPR protein (PB) UPR Protein change D1 PB	86 (43/tx grp) 70 (35/tx grp	0.611 0.679

Assumptions: alpha = 0.05, beta = 0.2.Abbreviations: tx = treatment, w= with, Day 29 = End induction, PCR = qRT-PCR. grp= group

* Effect size = standardized mean difference of either 1) the measurement at baseline, or 2) the change in measurement over time as noted. With this effect size, the study will have at least 80% power at 2-sided significance level of 0.05 to detect such difference given the corresponding sample size.

Effect sizes in previous studies of the proteasome, the UPR and RPPA have demonstrated that the effect sizes listed in Table 5 and Table 6 (below) are smaller than those needed to detect statistically significant differences in prior leukemia studies.

- Proteasome expression measurements from pediatric patients enrolled in either AALL07P1 (n=46) or AAML07P1 (n-12) showed a 2-fold difference in proteasome subunit expression in those that had reached a complete remission (CR) following induction vs. those that had not, for both β5i/β5 and β1i/β1 subunit expression (p=0.019 and p=0.022).

- A previous UPR study (n=105)[238] in adult AML determined that the activation of the UPR, as measured by the presence of spliced XPB1, correlated with relapse after CR1 (n=88 sXPB1+, n=17 sXBP1 neg, p=0.0182)
- Work with RPPA in adult AML have been able to detect $\log_2$ differences of 0.03 and greater (p=0.041, n=205). Differences as small as $\log_2$ 0.01 have been shown to be statistically different in larger data sets (n=511).[231]

2. SubAim 1b. To determine if reverse phase protein lysate arrays (RPPA) predict chemotherapy response or resistance.

1. To determine if protein cell stress pathways, such as the unfolded protein response, are active at baseline and how activity changes in response to ABFM chemotherapy +/- bortezomib,
2. To define a putative "high risk" protein expression profile that identifies patients receiving either standard or intermediate risk therapy that would benefit from the more intense chemotherapy.
3. To determine if RPPA protein expression profiles correlate with treatment group or T-ALL subtypes.

a) Rationale: In Sub-aim 1b, we will use a biochemical approach to identify protein combinations that are prognostic in T-ALL. This subaim is more comprehensive than Specific aim 1a, and will use a "candidate biomarker approach" to examine proteins previously identified as prognostic in either adult or pediatric acute leukemia.

b) Preliminary data:

1. RPPA feasibility**:** The Kornblau lab has analyzed one cohort of adult ALL and two cohorts of adult AML using RPPA. The first, with 539 samples from 258 patients, was probed with 51 antibodies;[255] the second, with 747 samples from 539 patients, was probed with 231 antibodies.[234] A subsequent array for adult ALL used 194 sampes with 131 antibodies. These studies demonstrated that there were recurrent patterns of protein expression that correlated with outcome (Fig. 6). In the AML, RPPAs proteins constellations correlated with cytogenetic subtype and EFS. In ALL, RPPA protein clusters correlated with CR duration. Prelminary analysis of pediatric ALL and AML samples is ongoing.

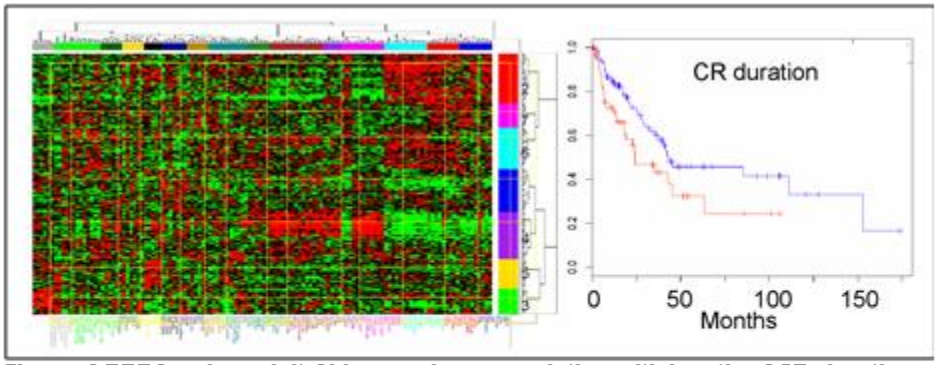


Figure 6 RPPA using adult ALL samples: correlation with length of CR duration: Heat map and outcomes for protein expression profiles from 194 adults with ALL. Left: Protein expression profiles from 194 adults formed 7 protein expression clusters that correlate with cytogenetic subtypes. Right: Protein expression profiles and outcome: Patients divide into 2 groups, those with better complete response (CR) duration (blue line, median 45 months) and those with a shorter CR duration (red line, median 22 months) (p=0.034).

The Kornblau lab recently published an analysis of proteins expression clusters with 180 antibodies, using only 150,000 CD34+CD38- AML cells per sample.[240] **No other currently available technology can analyze as many proteins with as little material.** RPPA analysis for this patient cohort consisted of 260 validated antibodies for the proteins involved in 25 different protein functional groups (i.e. PI3K/AKT, JAK/STAT, WNT/β-catenin, UPR, apoptosis, autophagy, cell cycle regulation integrins and adhesion proteins) that are known to be deregulated in acute leukemia, or are involved in DNA damage repair.[240] The Kornblau lab, in collaboration with the K Coombes (MDACC statistics) and the Qutub laboratory (Rice University) have recently developed statistical methodology to generate interaction networks from the RPPA datasets in combination with known networks from the literature, and can map differences in expression onto these interactome maps, thereby revealing the variations in expression that are present between different types of leukemia, or alternatively, between different treatment or response groups.

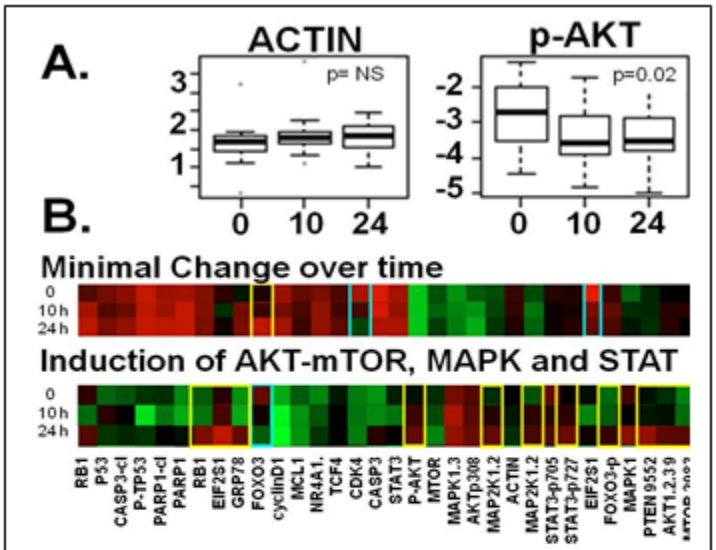


Figure 7: Results from feasibility test set using AAML1031 samples. **A.** Mean protein concentrations from 17 patients were compared for 32 proteins. There were minimal changes in in actin over time, whereas there was a significant decrease in phospho-AKT following treatment (one-way ANOVA). **B:** Heat map of the heterogeneity of protein expression between samples. The top panel shows minimal change in protein expression, whereas the patient in the bottom panel shows both increases (yellow boxes) and decreases

2) **Antibody validation:** RPPA antibodies have gone through extensive testing prior to inclusion on the array,[234,241,255] including both analytic validation and assay validation with clinically relevant samples.[256,257] Antibodies validated for RPPA use demonstrate specificity of signal and, in the case of phosphorylation or cleavage sensitive antibodies, context-specific validation has been performed in baseline and stimulated samples.[255] Validation steps have included: 1) antibody specificity as determined by immunoblot, 2) appropriate induction of phosphorylation/cleavage in response to known inducing agents, 3) correlation of RPPA signal with immunoblot expression ($R \geq 0.5$), 4) acceptable sigmoidal curve fit of signal with sample dilution (analyzed using SuperCurve),[255] 5) variable slope normalization[258] and, in cases of high background, 6) topographical normalization. Slides with unacceptable variances are redone. RPPA has acceptable intra-assay and interassay variability, with intra-assay coefficients of variation (COV) of 6-15% and established interassay reproducibility. We have established standard operating procedures (SOPs) for sample processing, cell sorting and RPPA, and laboratory staff in both the Horton and Kornblau labs are experienced with the procedure.

3) Clinical trial feasibility test set**:** Our preliminary data shows that we can reliably assess post-chemotherapy protein activation by analyzing samples collected during Day 1 of therapy. Seventeen patients from the AAML1031 clinical trial were tested by RPPA (Fig 7) using both heparin and CS tubes. Using a test set of 32 validated RPPA antibodies, we assessed protein activation following ADE therapy. We have determined that 1) CellSave tubes preserve protein expression profiles post-treatment, as shown by the relative stability of actin expression, but a decrease in p-AKT (Fig 7A); and 2) that we can detect the heterogeneous patterns of changes in protein expression over time, with some patients having minimal changes over time (Figure 7B, top panel), whereas other patients have induction of several key regulatory proteins, including AKT, m-TOR, MAPkinase and the FOXO3 pathway (Figure 7B, bottom panel) following treatment.

RPPA analysis for this patient cohort will consist of 260 validated antibodies for proteins involved in 25 -protein functional groups including PI3K-AKT, JAK-STAT, Wnt-β-catenin, apoptosis, autophagy, cell

cycle regulation, DNA damage response, integrins and adhesion proteins.[259] The Kornblau lab and K Coombes (MDACC) along with the Qutub lab (Rice University) have recently developed statistical methodology to generate interaction networks from RPPA datasets in combination with known networks from the literature and can map differences in expression onto interactome maps, thereby revealing the variations in expression that are present between different leukemia response groups, subtypes, or treatment regimens. [260]

c. Experimental design

1) Assessment of dynamic changes in lymphoblast protein activation following chemotherapy: Peripheral blood will be collected in CellSave tubes at three time points on the first day of therapy (0h, 6h, and 24h) and lysates prepared from 200,000 cells for RPPA analysis. Samples will be divided into a training set and test set of roughly equal size. For training set evaluation 171 patient samples (85/treatment group) will undergo serial dilutions and will be spotted onto a single array in duplicate with appropriate controls (cell lines, cell lines treated with chemotherapy, normal PBMC). Replicate slides will be probed with 260 validated antibodies (1 antibody/slide) and normalized protein concentrations determined using SuperCurve.[255]

Our statistical analysis will involve three complementary approaches to examine the dynamic changes that occur in the T-ALL blasts in response to therapy over time. The first will use unsupervised hierarchical clustering ("class discovery") methods to look for protein expression clustering in the training set. We will determine what signatures, or signature components, are static and which are dynamic in response to therapy, and the time course of these changes. Next we will apply the thresher method to limit the dataset to those proteins that are changing dynamically and to define the dynamic principal components from the relevant functionally related protein groups.[260] Finally we will map the observed dynamic changes onto interaction networks, derived from the static data and the known literature, to graphically visualize how each functionally related protein group, and it's direct and indirectly connected members, are being modulated.

By comparing the analysis seen between the different therapies we can discriminate what pathways are differentially modulated by bortezomib, compared to the standard therapy arm, and what are the critical components of each of dynamically modulated protein functional group. Correlation of these protein expression patterns with therapy outcome can reveal which are defensive responses being engaged by the leukemic blasts to evade therapy. Identification of these resistance associated defensive adaptations will suggest further therapies to employ to block bortezomib resistance and improve efficacy. Both static changes (pre-treatment) and dynamic changes over time will be assessed for each protein as well as for each protein functional group. Baseline protein activation, as well as changes over time, will be compared based on 4 year EFS as the primary response endpoint. We expect that chemotherapy responsiveness will differ between protein expression clusters. We will also determine if protein expression varies within T-ALL subgroups. As in adults, we expect that pediatric RPPA profiles will correctly identify T-ALL subgroups[234] as well as identify defensive resistance mechanisms not only for bortezomib-containing therapy, but for VXAD therapy as well, showing that **this body of work will lead to integral studies whether or not bortezomib is an effective agent.** . The training set will be used to develop a protein expression classifier to predict VXAD +/- bortezomib response in the test set.

d. **Statistical methods**

1) Sample size adjustment for drop-outs

We are requesting sample (BM (n=476) and PB (n=342)) for RPPA (BM) and SCPFC analysis (BM and PB). Additional samples will account for two potential sources of sample drop-out. First, we anticipate that approximately 20% of samples identified will have insufficient or unusable sample for RPPA and SCPFC analysis (non-viable samples, collected in wrong tubes, only partially collected sample set, damaged material, etc) Second, during RPPA and SCPFC construction, there will be additional samples that are inevaluable, either due to loss of tissue spots on the RPPA array, inadequate T-ALL cells for UPR analysis, or inadequate sample using quality control criteria (5%). The drop-out rate in our prior RPPA studies using local samples for the same analyses was approximately 5% (Horton lab, unpublished data). An historical drop-out rate of

approximately 5% is also supported by the leukemia RPPA literature.[261] UPR qRT-PCR dropout rate will depend on the quality of the RNA.

The sample size will be drawn from the same samples set for both RPPA and SCPFC. As concluded by Simon et al.,[243] "genes that have low variation across the entire set of samples would be difficult to use for prognostic prediction in clinical situations". Therefore, we will demonstrate statistical power in cell signaling node identification using the median and 90% percentile. We will adjust for multiple comparisons (total 6 signal transduction nodes) using the Holm's method.[262]

2) RPPA analysis

As discussed in SA 1a, there are multiple comparisons of interest. We predict that there will be sufficient power to detect at significant differences between groups as shown below (Table 6). Methods for RPPA and SCPFC analysis have been previously described,[255] Both unsupervised and supervised clustering analyses of the data sets will be completed as outlined below.

a) Unsupervised hierarchical clustering analysis

Unsupervised clustering of all RPPA and SCPFC data will be performed to identify biologically-defined subgroups that will be correlated with clinical outcomes (MRD and EFS). Independent of potential outcome correlations of clusters, this approach will also identify biologically-defined T-ALL subgroups, aiding in a development of biologically-based (i.e. targeted) therapies for these patient populations. Unsupervised clustering analysis will be performed as previously described.[234,263]

b) Stratification of RPPA protein expression profiles by treatment group

A similar clustering analysis as delineated above will be applied to training sets stratified by treatment group. The training set will include 171 patients. With a 1:1 randomization between treatment groups, it is expected that 85 patients will have received each treatment. compare protein expression patterns in VXAD responders (4yr EFS-no event) to responders in the VXAD-B group (4yr EFS-no event). We will specifically look for protein patterns in VXAD non-responders that are also found in VXAD-B responders, suggesting proteins that predict patients likely to respond to bortezomib-containing chemotherapy. Analysis of protein expression clusters by treatment group will also determine if there are protein classifiers that can differentiate response between treatments.

Table 6: Effect-size (detectable differences) based on sample size for each experiment (two-sample 2-sided t-test, alpha=5%, power=80%)

1. RPPA studies	**Samples size (max)**	**Effect-Size**
Baseline RPPA by tx group	342 (171/ tx group)	0.304
Changes in RPPA at D1 by tx group	280 (140/ tx group)	0.336
Baseline RPPA by response groups (4-yr EFS)	342 (291 non-event/51event)	0.426
Changes in RPPA at D1 by response groups (4 yr EFS)	280 (238 non-event/42 event)	0.471
Baseline RPPA by response groups (Day 29 MRD)	342 (171 >=0.01%/ 171 <0.01%)	0.304
Changes in RPPA at D1 by response groups (Day 29 MRD)	280 (140 >=0.01%/ 140 <0.01%)	0.336
Baseline RPPA by **ETP** vs. non-ETP	342 (42 ETP/300 not)	0.463
Changes in RPPA by ETP vs. non-ETP	280 (35 ETP/245 not)	0.508

Tx = treatment, D1 = Day 1, Day 29 MRD: < or >0.01% at end of induction.

One of the advantages of this study is the ability to analyze protein expression patterns over time during the first day of treatment. By comparison of patients that receive VXAD to VXAD-B, we expect be able to 1) determine if there are proteins induced in VXAD responders that are not present in VXAD responders; these proteins might be putative predictors of bortezomib response. The expression of baseline UPR proteins has been linked to bortezomib response in similar tumor types[242,254] however, this study is unique in its ability to assess UPR activation over time in patients randomly assigned to VXAD+/-B therapy. We will also look for

changes in the protein expression patterns of VXAD non-responders that are no longer present in VXAD-B non-responders, indicating another set of proteins that could potentially identify markers of response to bortezomib. Based on accrual in the AAML1031 study, we estimate that we will have Day 1 samples on 82% of patients (approximately 280 samples, 140 samples/tx group), allowing for sufficient samples to make comparisons of protein expression over time between treatment groups.

c) Supervised gene expression analysis by response (1y-EFS)

We will analyze pediatric T-ALL patients as a group to determine if RPPA provides prognostic/predictive information independent of known prognostic variables and information that is complementary to existing prognostic markers. To determine the effect of current disease risk group stratification on the prognostic value of the RPPA and SCPFC protein profiles, EFS data will be analyzed adjusted for treatment arm and ETP status.

d) Building an outcome predictor

Prior data from the Kornblau lab[234,264] and others suggest that protein expression profiles between VXAD responders and VXAD-B responders will differ; RPPA and SCPFC (see aim 2) will likely identify specific biochemical pathways that predict response to bortezomib-containing chemotherapy, such as the UPR[265] and other cell stress pathways, including autophagy,[265,266] and DNA damage response.[267] Based on preliminary data from the Hermiston lab and others we also will likely identify changes in specific signal transduction pathways (NF-κB, JAK/STAT and NOTCH) that will track with clinical outcome. We will build candidate classifiers in a stepwise fashion:

i. We will first look at dynamic changes in protein activations following chemotherapy in sorted lymphoblasts. The purpose is to discover natural groupings based on proteomic data only, without any information about outcome, to compare those groups with other known classifications and then to perform bioinformatics analysis to identify pathways active in each of the newly identified clusters

ii. Once clinical outcomes become available, we will analyze the difference in protein profiles between VXAD and VXAD-B treatment groups as they relate to clinical outcome. This part will include different unsupervised methods, group comparisons and will search for differentially expressed proteins and pathways.

iii. Finally, we will create a classifier(s) to predict clinical outcome. We will use outcome variables MRD status (Day 29, 0.01% cutoff) and 4-year EFS) We may have different classifiers for different outcome variables as well as for different risk groups and T-ALL subtypes.

As with other high-dimensional data, RPPA data analysis will include low-level and high-level analysis:

iv. Low-level analysis, relative protein levels will be determined by interpolation of dilution curves from the global "standard curve" using the R package "SuperCurve".[268] (http://bioinformatics. mdanderon.org/Software/OOMPA/ Current/SuperCurve-manual.pdf). After normalization for protein loading and appropriate transformation, assessments of quality will be performed.[234,255] To the maximum extent possible, samples that will be directly compared to each other will be printed on the same slide to avoid possible batch effects.

v. High-level analysis: To determine clusters (classes) with potentially different outcome or treatment targets based on protein expression and activation, we will use Hierarchical Clustering, Principal Component Analysis (PCA), Self Organizing Maps (SOM) and other class discovery methods.[269] Cluster stability will be accessed using reproducibility measures, including a modified GAP,[270] known as stability GAP (prototype clustering) (Qutub et al, manuscript in review), [271] as well as robustness and discrepancy indices.[272]

Differentially expressed proteins will be found using paired t-test as well as repeated measures, mixed effect ANOVA and ANOVA with contrasts. We will use the Bonferroni correction to account for multiple comparisons in the RPPA analysis.[273]

To determine if dynamic changes in specific protein pathways predict chemoresistance, we will use different regression and classification methods: SOM (when no outcome is used), class prediction methods such as logistic regression, Support Vector Machine,[274] Random Forest,[275] Binary Tree Prediction, Bayesian Compound Covariate Predictor, Discriminant analysis (http://linus.nci.nih.gov/techreport/Manual32.pdf), and

finally, Cox proportional hazard models for survival analysis. We will use different boosting methods[276] to combine different models for the purpose of producing more accurate classification and regression models.

All patients with evaluable sample data will be randomized 1:1 into training and testing sets. We will use cross-validation and permutation methods to estimate the model accuracy and to select a preferable model using the training dataset. To provide an unbiased estimate of the model accuracy on the training set, the model construction, including selection of proteins, will be repeated from scratch for each iteration. The selected classifier or regression model will be validated using the test dataset.

To perform high level statistical analysis, as well as for visualization and integration of the results, we will use different software packages. The software will include but not be limited by Bioconductor (http://www.bioconductor.org), Partek (http://www.partek.com), BRB Array Tools (http://linus.nci.nih.gov/BRB-ArrayTools.htm, dChip (http://biosun1.harvard.edu/complab/dchip), SAS (http://www.sas.com), and R (http://www.r-project.org). We will follow new developments and use new software packages if more advanced ones are developed by the time of analysis. We will use scatter plots, multidimensional scaling, PCA, hierarchical clustering, volcano plots and other visualization methods for visualization of initial data and for results.

e) Integration of RPPA and SCPFC results to build an integrated protein expression classifier:
All patients with evaluable RPPA and SCPFC (aim 2) will be included in the analysis plan for this aim. Protein expression levels as continuous variables (determined by RPPA) will be correlated with each SCPFC nodal output using ANOVA statistics for each protein. P-values will be adjusted for multiple comparisons using the Bonferroni method. We will also analyze GEP and protein datasets using other methods previously developed for the integration of genomic and proteomic data, including linear correlation analysis[277] and cluster analyses[278] as reviewed in Cox et al.[279] These methods will enable us to determine the utility of transcript profiling for prediction of protein expression levels.[280] Data will also be integrated at the level of protein interaction networks as previously described.[263,281-283]

f) Assessment of protein classifiers:
For this aim, all T-ALL samples with usable RPPA and SCPFC (aim 2) analysis will be evaluable (n≈476) and will be included in the analysis plan. In addition to comparison of the ROC performance characteristics, we will also be identifying classifiers whose operating characteristics minimize the false positive rate (1-specificity) while having an acceptable true positive rate (sensitivity). A cutoff point for the classifier will be selected that maximizes the likelihood ratio (LR) of identifying standard and intermediate risk patients that have a "high-risk" protein classifier (i.e. an acceptable true positive rate (> 50%) with a low false positive rate (< 10-20% for this study). This will identify SR and IR patients in need of additional therapy, while avoiding potential overtreatment of SR and IR patients that have been falsely identified as high-risk by the molecular profile.

g) Determination of clinical usefulness:
Our objective is to improve the detection of the patients with a high risk signature in the SR and IR groups that would ultimately relapse, with the objective of improving SR and IR EFS. In this study we will assess 476 patients for aberrations in protein cell stress pathways. Of this number, it is estimated that 90% will be either LR or IR (n=428); this will be the sample set available for analysis. Depending on the change in EFS for those correctly identified as having the putative high risk protein expression classifier, Table 7 demonstrates the relative risks that can be used to identify the clinical usefulness of applying the protein expression classifier to the SR and IR patients (overall 4-year EFS 89%).

Table 7: Range of 4-yr EFS and relative risk reduction that will be used to determine the clinical usefulness of the protein profile classifier (n=428; alpha=5%, one-sided log-rank test)

Proportion of patients classifier negative (sample size in classifier - and + grps)	EFS associated with classifier negative group	EFS associated with classifier positive group	Relative Risk classifier+ /classifier (-) Signature	Power

0.5 (n1=214, n2=214)	0.97 0.95	0.81 0.83	6.92 3.63	>0.99 0.99
0.6 (n1=256, n2=172)	0.95 0.94	0.80 0.81	4.35 3.41	>0.99 0.99
0.7 (n1=299, n2=129)	0.93 0.92	0.80 0.82	3.08 2.38	0.99 0.92
0.8 (n1=342, n2=86)	0.92 0.91	0.77 0.81	3.13 2.23	0.99 0.86

The table above will be used to calculate the sample size in each scenario that would lead to a high probability of correctly identifying a significant difference between patients with the high-risk protein classifier in the SR and IR groups. Our goal would be to identify a classification signature that outperforms the current method of risk stratification.

E. Specific Aim 2: To identify biomarkers and mechanisms of chemotherapy resistance and response in T-ALL, focusing on ETP ALL.

Overview: The objective of this aim is to systematically evaluate the biochemical signature of T-ALL leukemic blasts at diagnosis and in response to chemotherapy in order to identify a biomarker for patients at high risk of relapse. T ALL is a genetically heterogeneous disease with variable responses to therapy. The behavior of a given patient's leukemia and its response to therapy are determined by the accumulation of genetic mutations and epigenetic alterations. To date, the genetic heterogeneity of T-ALL has precluded risk-adapted therapy based on genetic abnormalities. (Epi)genetic aberrations are manifest biochemically as changes in signal transduction and cellular responses. However, recent data suggests that genetic abnormalities in T-ALL may converge on a more limited set of biochemical abnormalities. We predict that these biochemical signatures may serve as biomarkers predictive of chemotherapy response or resistance. We will focus on the ETP, a newly identified phenotype of T ALL with a particularly poor prognosis in some reports[284] in order to identify new biomarkers for enhanced risk stratification and better treatment strategies for high-risk T-ALL patients.

We will test two hypotheses: 1) that the signaling network profile of patients with ETP ALL will be distinct from non-ETP ALL and 2) that these differences in signal network wiring will predict whether the leukemia responds to chemotherapy (with or without bortezomib).

The rationale for this work is two-fold. First, ETP ALL blasts frequently have mutations more commonly associated with myeloid leukemia, including mutations in histone-modifying proteins and in genes that regulate RAS and cytokine receptor signaling. Our approach will be to delineate signaling in 1) the basal state, 2) in response to *in vitro* stimulation, or 3) in response to exposure to chemotherapy using SCPFC. In light of the observed biologic and clinical heterogeneity of T-ALL and the recent trend towards the development of "targeted" therapeutics, identification of patients likely to benefit from biologic therapies such as bortezomib would allow consideration at diagnosis for including these alternative therapeutic strategies. Second, successful completion of this aim will also allow us to identify a high-risk classifier of chemotherapy resistance in a large clinical trial and to directly compare the predictive power of this technology with RPPA (SA1). Our expectation is that understanding the biochemical aberrations that reflect the genetic and epigenetic alterations in the leukemic blasts will enable identification of biomarkers that predict risk of relapse and inform the use of targeted agents in future clinical trials. Given that intracellular staining of protein epitopes is a mainstay of the diagnostic evaluation of T-ALL (e.g., TDT and cytoplasmic CD3 staining), addition of a protein biomarker to future clinical trials should be technically feasible as an integral study.

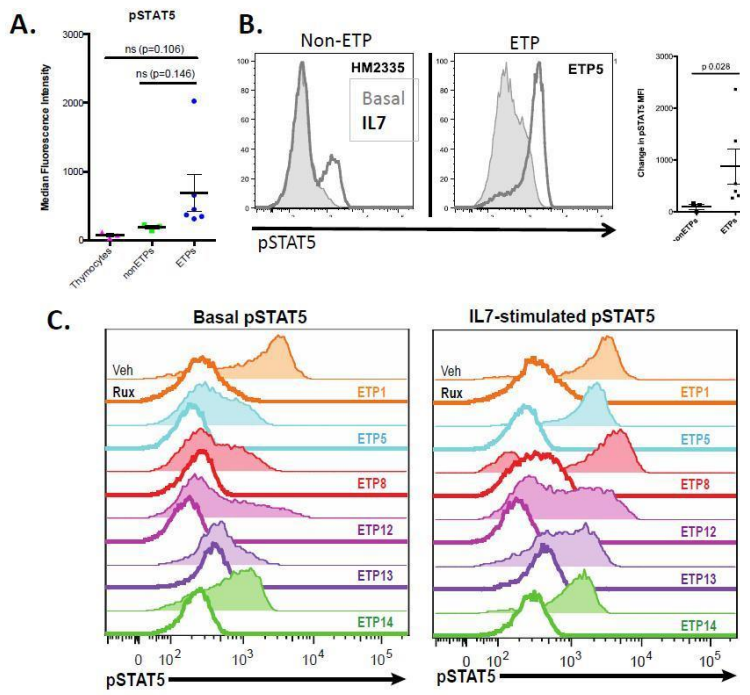


Figure 9. A. Basal levels of pSTAT5 are elevated in ETP-ALL. Plot of mean fluorescence intensity (MFI) for basal pSTAT5 in ETP and non ETP lymphoblasts**. B.** Representative histograms of pSTAT5 levels in the basal state (grey, filled) and after stimulation with Il7 for 20 min (Black, open). Graph depicts change in MFI between stimulated and basal for several independent samples. p-values calculated using the non-parametric Mann-Whitney test**. C.** The JAK1/2 inhibitor ruxolitinib blocks hyperactive pSTAT5 in the basal state (left panel) and in response to IL7 stimulation (right panel). Filled line, vehicle treatment, open line, ruxolitnib treatment.

1. Subaim 2a. To determine if differences in activation of the NF-κB, MAPK, PI3K/AKT/mTOR, and/or JAK/Stat pathways between ETP and non-ETP T cell subtypes will serve as indicator of chemotherapy resistance.

a. Rationale and preliminary data. Multi-parameter phosphoflow cytometry represents a powerful and highly sensitive approach for analyzing and interpreting post-translational protein modifications (e.g. phosphorylation, acetylation, cleavage etc.) at the single cell level in minimal sample size ($0.5x10^6$ cells/assay). Measurements are made on endogenous proteins before and after exposure to extracellular modulators such as growth factors, cytokines and drugs. This enables a comprehensive, dynamic, and functional assessment of signaling pathways within heterogeneous populations of primary cells.[285] SCPFC has been used to demonstrate that hyperactivation of STAT5 and PI3K/mTOR pathway signaling is a common feature of human CRLF2-rearranged pre B-ALL.[227] This approach has also been successfully applied to pediatric AML bone marrow samples to identify and validate a classifier for prediction of response and to build a single cell network profiling (SCNP) classifier in adult AML and pediatric AML studies have confirmed the importance of the JAK/STAT pathway.[230] These classifiers are now being validated in a phase three prospective clinical trial (AML1031). While these studies

demonstrate the feasibility and clinical utility of SCPFC, this approach has yet to be systematically applied to pediatric T-ALL.

Hyperactivation of signaling networks in ETP ALL. The Mullighan laboratory performed a comprehensive genetic analysis of ETP ALL.[284,286,287] Activating mutations in signaling proteins and inactivating mutations in proteins regulating hematopoietic development were common. The Hermiston laboratory performed a biochemical analysis of a subset of these leukemias using SCPFC. Relative to normal human thymocytes, the MAPK, PI3K/AKT/mTOR pathway and the JAK/STAT pathways were hyper-responsive to stimulation.[284,286,287] There was excellent correlation between genetic mutations and the occurring phenotype. For example, IL7 receptor mutations were associated with constitutive pSTAT5 activation while T-ALL samples with Ras pathway mutations were not.[284,286,287]

Evidence of common signaling changes despite heterogeneous genetic alterations in ETP T-ALL. Interestingly, gene expression profiling suggested hyperactivation of the JAK/STAT pathway in ETP ALL whether or not there was an underlying cytokine signaling pathway mutation. This was tested using SCPFC. Regardless of their mutation status, ETP lymphoblasts were hyper-responsive to IL-7 stimulation relative to normal thymocytes or non-ETP T-ALL (FIG 9A). Activation of pSTAT5 in the basal state or in response to IL7 could be completely inhibited by the JAK1/2 inhibitor ruxolitinib *in vitro* and correlated (FIG 9B) (Maude et al., manuscript submitted). These data suggest that hyperactivation of the IL7/JAK/STAT pathway may be a common feature of ETP-ALL and support the notion that multiple genetic (or epigenetic) mutations may converge on a more limited set of deregulated cell signaling pathways.

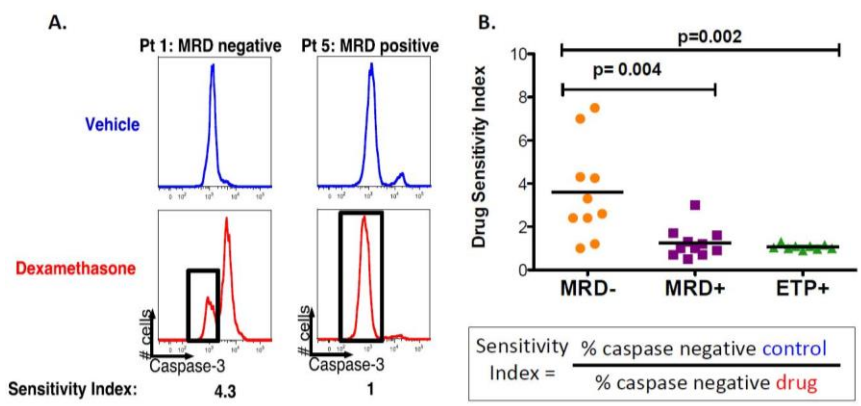


Figure 10. Interrogating chemotherapy resistance and response with SCPFC. A. Representative MRD negative and MRD positive diagnostic samples from AALL0434 were incubated with vehicle or 10 uM dexamethasone for 48 hours *in vitro* and analyzed by phosphoflow cytometry. Histograms depict Caspase-3 induction. **B.** Drug sensitivity index was calculated for each diagnosic sample. Statistical significance was determined using the Student T test.

Correlation of MRD status with *in vitro* response to dexamethasone. To test the hypothesis that analysis of signaling profiles in response to conventional and/or targeted therapeutics *in vitro* could facilitate identification of resistant T-ALL/LL at diagnosis or relapse and facilitate choice of optimal therapy in high-risk patients, we successfully developed a Caspase-3 (a marker of commitment to apoptosis) based assay to monitor response to drug therapy *in vitro* (Figure 10B). An advantage of this approach is the ability to gate on the viable, chemotherapy resistant, Caspase-3 negative cells to further interrogate mechanisms of chemotherapy resistance. Because the upfront prednisone response can be predictive of outcome, especially in T-ALL,[288] we tested the hypothesis that *in vitro* sensitivity to dexamethasone would correlate with prognosis as defined by end of induction MRD status. As anticipated based upon clinical response, MRD negative samples were quite sensitive to drug while the majority of ETP and MRD positive samples were resistant (FIG 10 A, B). Interestingly, dexamethasone resistance did not correlate with expression of the glucocorticoid receptor (data not shown), suggesting that other mechanisms are mediating drug resistance.

4.) T-ALL cells rewire their signaling networks in response to genotoxic stress. The mechanisms mediating chemotherapy resistance in T-ALL are unclear It is clear in normal biologic systems that cells upregulate multiple signaling pathways in response to stress. We hypothesized that chemotherapy resistant cells might similarly rewire their signaling networks in response to genotoxic stress. In addition to the obvious advantage of requiring many fewer cells, an advantage of SCPFC relative to traditional biochemistry using western blots is the opportunity to gate on drug-resistant, Caspase-3 negative cells and to interrogate signaling networks that are activated or inhibited in response to drug. We find that chemotherapy resistant cells upregulate the MAPK and PI3K/AKT/S6 pathways as well as pro-survival proteins such as survivin in T-ALL samples when exposed to genotoxic chemotherapy (FIG 11 A, B). Similar signal network rewiring has been reported as a mechanism of chemotherapy resistance in breast cancer[289]

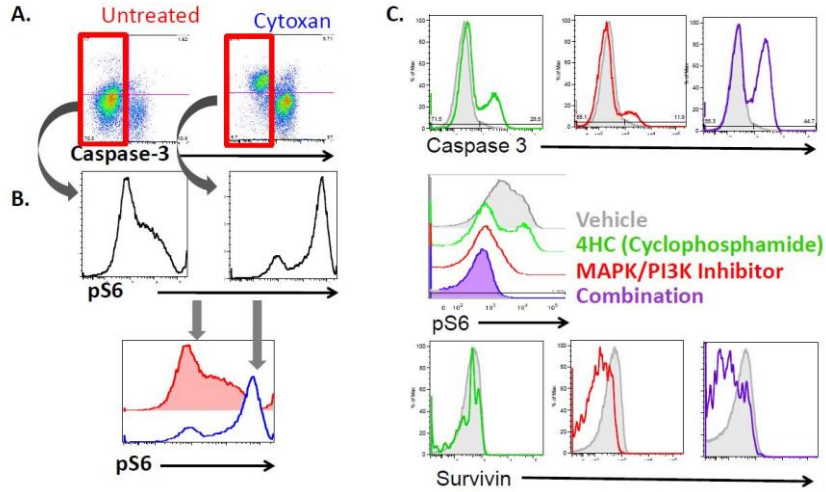


Figure 11. A. T-ALL cells were incubated with vehicle of 4-HC (the active metabolite of cyclophosphamide) for 24 hours and processed for SCPFC.An advantage of SCOFC is the ability to gate on the Caspase-3 negative cells and interrogate mechanism of chemotherapy resistance. **B.** Caspase-3 negative rewire their signaling networks and upregulate pS6 in response to genotoxic stress .**C.** Top, ALL cells were treated with vehicle, 4HC (the active metabolite of cyclophosphamide), and/or a dual mTOR/PI3K inhibitor. Combination therapy shows enhanced cell death relative to either agent alone. Gating on Caspase-3 negative cells (middle and lower panels) shows inhibition of pS6 with inhibitor and correlation with downregulationof the pro-apoptotic mediator Survivin.

5. Targeted inhibitors prevent network rewiring and restore chemosensitivity. Network rewiring can be prevented with the addition of the appropriate targeted inhibitor. Interestingly, our preliminary data of chemotherapy resistant T-ALL (as defined by end-induction MRD) suggests that despite adequate inhibition of the target and downregulation of the pro-survival protein Survivin, targeted agents generally are not cytotoxic as monotherapy. However, when combined with standard genotoxic chemotherapy, a synergistic effect is often seen. SCPFC enables analysis of the mechanistic basis for this observation. In the presence of targeted inhibitor, cells are no longer able to rewire their signaling networks, leading to downregulation of survivin and other pro-survival proteins and increased chemosensitivity when cytotoxic agents are added (FIG 11C). These data argue that identifying the signaling pathways that are hyperactivated in the basal state or after exposure to chemotherapy could be of clinical benefit in determining which targeted therapy would be most beneficial for a given patient. Thus, a goal of this aim will be to determine the relative frequencies that common signaling pathways are deregulated in ETP-TALL.

Experimental design.

1.) Compare signal network wiring in the basal state and in response to IL7 or targeted inhibitors in ETP and non-ETP T-ALL. The goal of this experiment is to determine whether alterations in single network profiles can be identified and whether these correlate with MRD status and/or EFS. Diagnostic samples (either fresh bone marrow or peripheral blood, provided the blast count is greater than 1000 cells/HPF, collected and shipped in heparinized tubes) will be subject to SCPFC using established methods[287]. Briefly, cells will processed on a ficol gradient to remove red blood cells and cellular debris, rested 30 minutes in serum free media, treated with

vehicle, signal pathway inhibitors, or IL7 for 20 minutes, then rapidly fixed, permeablized, and stained with fluorescently conjugated monoclonal antibodies.

Based on a conservative estimate of the number of pretreatment lymphooblasts obtained from 2 mL PBMC (2-5x10^6 cells), we estimate that we will have sufficient sample to test a minimum of 10 biological conditions (modulators, kinetic time points), with 2 to 3 proteins measured in each condition, resulting in approximately 25 nodes representing relevant leukemia biology. The proteins assessed by SCNP will include the same proteins analyzed by RPPA. We will focus on the MAPK (pERK1/2, pMEK), JAK/STAT (pSTAT5), and PI3K/Akt/mTOR (pAKT, pS6) pathways and treat cells with a inhibitors specific to each of these key signaling nodes (i.e., a MEK inhibitor, ruxolitinib (JAK1 inhibitor), or rapamycin (mTOR inhibitor). The phosphatase inhibitor pervanadate will be used as a positive control. These pathways were chosen based on the high frequency of dysregulation in T-ALL and ETP ALL. Over 20% of T-ALL have aberrant PI3K/Akt/mTOR signaling.[290] Early data suggest over 40% of ETP ALL samples have mutations in genes that regulate MAPK and that JAK/STAT pathway activation may be a universal feature of this T-ALL subset.[287] To identify leukemic blasts, cells will be stained with monoclonal antibodies (previously validated for SCPFC[287]) that recognize cell surface proteins (e.g., CD3, CD5, CD7) and signaling proteins (pS6, p-Akt, p-MEK1/2, p-ERK1/2, active NF-κB). Activated Caspase-3 will be used to identify cells committed to apoptosis. The well characterized T-LBL cell line Jurkat and normal human thymocytes (obtained under an IRB approved protocol) at the same developmental stage as defined by cell surface staining will be used as positive controls. Cells will be processed on a FACSverse 2 housed in the Hermiston laboratory. An advantage of the FACSverse is that it contains an algorithm for controlling for day-to-day machine variability. Inclusion of the well-characterized Jurkat cell line with each sample analysis will be used to control for any potential technical variability.

B. Evaluate in vitro responsivness to dexamethasone. The goal of this experiment is to validate whether *in vitro* resistance to dexamethasone correlates with MRD responsiveness. We will also test whether chemotherapy resistant cells upregulate specific signaling pathways in response to dexamethasone exposure. After ficol, 0.5x10^6 cells/ml will be incubated in vehicle or 5μM dexamethasone in complete media for 48 hours and then processed as outlined above for flow cytometry. Dexamethasone sensitive and resistant CEM T-ALL cells with well defined signal network rewiring responses to steroid will be used as positive and negative controls.

Data analysis We will analyze the data by gating on the leukemia population (as defined by forward and side scatter characteristics and cell surface marker expression), eliminating doublets, and then identifying the Caspase-3 negative, viable cell population using FlowJo v.9.3.1 and Cytobank software. The mean fluorescence intensity (MFI) will then be calculated for each phosphoepitope. Samples will be comparing to each other and among subsets (e.g., ETP to non-ETP, end consolidation MRD+ to MRD-). The arcsinh ratio for each plot and the fold-change in intensity relative to the basal state will be calculated in Cytobank and used to generate heat maps for subsequent unsupervised and supervised clustering with the goal of identifying a single cell network profiling (SCPFC) classifier (see statistics section below). For the dexamethasone experiment, induction of Caspase-3 will be monitored in vehicle and drug treated cells by phosphoflow cytometry. A drug sensitivity index will be calculated as the ratio of non-apoptotic (Caspase 3 negative) cells in control vehicle treated vs. drug-treated samples. Additive, synergistic, or antagonistic effects will be calculated using the universal response surface approach.[291] Multivariate analyses will be used to define the parameters that best discriminate patient populations. We expect that signal network wiring will be distinct in ETP-ALL relative to non-ETP ALL. Given that chemotherapy resistance reflects, at least in part the phenotypic consequences of genetic and epigenetic alterations in the leukemic blasts, we also anticipate that patients with persistent MRD at the end of induction and consolidation will have distinct network wiring relative to MRD negative patients, and that these phospho-signatures will correlate with EFS.

Statistical methods and justification: The objective of the statistical analysis In Aim 2a is to provide an assessment of baseline signal network wiring prior to chemotherapy in ETP versus non-ETP T-ALL and to determine whether it correlates with MRD status at the end of induction at 0.01% cutoff and/or or clinical outcome (4-year EFS).

a) SCNP sample size: Based on analysis described in Aim 1, we expect approximately 50% of patients to provide BM and 36% to provide PBMC samples. Therefore, we estimate that we will have evaluable BM samples from 476 patients and PBMC samples from 342 patients (171/treatment group) for power estimations. Given an incidence of 12.4% ETP, we estimate that we will have evaluable BM samples from 59 ETP ALL patients and evaluable PBMC samples from 42 patients.

b) Definition of test and training sets: The statistics team, led by M. Devidas will randomize patients in approximately equal numbers to either the training or test (validation) set. The training set will be used to develop the SCNP-derived diagnostic classifier that will produce a score for each patient indicating the predicted likelihood of response or relapse. The training data will also be used to determine the decision rule (cutpoint) that will be applied to the classifier score to assign patients to one of two groups: e.g. MRD >0.01% vs. MRD <0.01%, or event vs. non-event based on 4 year EFS. The final diagnostic classifier and final decision rule will be referred to as the SCPFC classifier (DX_{SCNP}). The ability of DX_{SCNP} to predict patient's response (or relapse) will be evaluated by applying DX_{SCNP} to predict outcomes for patients in the test set. Estimates of accuracy of these classifiers will be made in the training set using out of bag (OOB) sample data for multiple bootstrapped samples. This will provide an updated estimate of the power calculation at the end of training set analysis.

c) Determine if SCNP can predict response or relapse risk in each treatment group: Differences in the classifiers among patients treated with VXAD vs. VXAD+B will be explored. Based on the estimated performance of these classifiers in the training set, a decision will be made if independent classifiers are needed for response prediction within each treatment group. Logistic regression will be used to model treatment response within each treatment group.

Specific Aim 2b. To determine if augmented NFkB signaling predicts response to induction chemotherapy +/- bortezomib.

Rationale and preliminary data: Nuclear Factor kappa B (NF-κB) is a family of transcription factors that plays an important role in cancer development by preventing apoptosis and facilitating tumor cell growth. Consistent with this, a small study of primary pediatric leukemia cases, including thirteen T-ALL cases, found evidence of constitutive NF-κB pathway activation in more than 90% of cases. [31,89] This is perhaps not surprising since NF-κB is a target of constitutively active NOTCH or PTEN/AKT/mTOR pathways, which are each mutated in over 50% to T-ALL cases. However, whether activation of the NF-κB pathway is a 'driver' mutation in these leukemias and/or contributes to chemotherapy resistance is not known. Interestingly, in other systems tumor cells in which NF-κB is constitutively active, cells are highly resistant to chemotherapy and radiation, but inhibition of NF-κB sensitizes these cells to apoptosis.[82] It is therefore of great interest to understand how deregulated NF-κB signaling can drive disease progression and to explore NF-κB inhibitors as a potential therapeutic strategy. We will test the hypothesis that activation of the NF-κB pathway correlates with chemotherapy responsiveness as measured by MRD at end induction and consolidation and EFS at four years.

Bortezomib is a proteasome inhibitor that prevents NF-κB activity by blocking proteasome-mediated degradation of IκB, a requirement for NF-κB nuclear translocation. However, despite its use in clinical trials for a variety of cancers, how bortezomib exerts its antitumor activity remains incompletely understood. Therefore there is an unmet need to study how bortezomib affects cell proliferation in T-ALL at a molecular level. Recent studies show that bortezomib can overcome or reverse chemoresistance in multiple myeloma (by sensitizing tumor cells to traditional chemotherapy drugs resulting in increased apoptosis.[19] We have developed a flow based assay to evaluate NF-κB activity (FIG 12). NF-κB activity was shown to be inhibited in early trials of bortezomib in pediatric leukemia.[235] Recent data with a larger sample sample size (n=61) collected

from patients enrolled on both Phase II trials of bortezomib in pediatric leukemia (AAML07P1 and AALL07P1) have shown that, although NF-κB is decreased in most patients, the decrease did not correlate with clinical response (CR2).[246] Whether or not this is true for T-ALL has yet to be established as there were only six T-ALL patients on AALL07P1.

Experimental Design. *Determine whether NF-κB activation at baseline and after exposure to induction chemotherapy +/- bortezomib correlates with MRD status at end induction and/or consolidation.*

We will evaluate NF-κB activation status at baseline and at 6h and 24 hours after exposure to bortezomib using the methods in subaim2A. As notch status and activation of PI3K pathway shown to enhance activation of the NF-κB pathway, we will include antisera to pAKT and pS6, components of the PI3K/mTOR signaling network, to test whether increased levels of these phosphoepitopes correlates NF-κB activation.

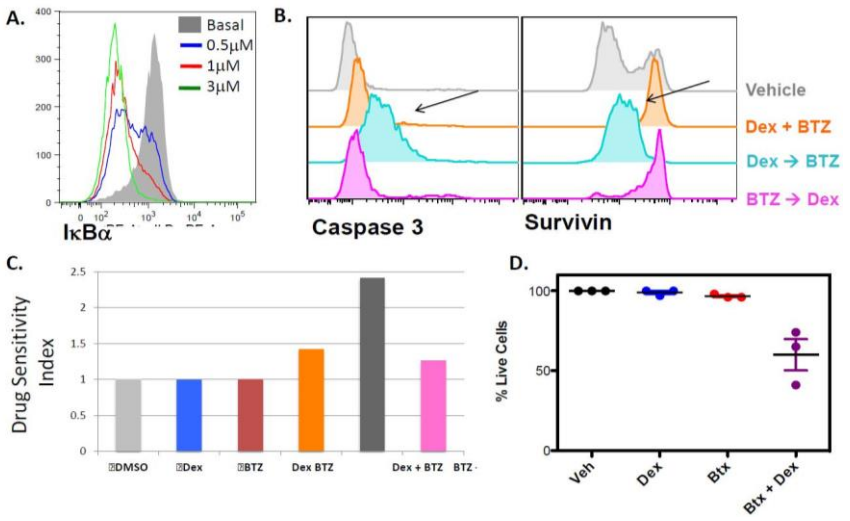


Figure 12. A. Flow-based NF-κB assay. Progressive degradation of IkBa after 20 minutes of stimulation with increasing doses of TNFalpha. **B.** The ETP-like cell line Loucey was incubated with the drugs in the order shown (24 hours drug one followed by 24 hours with drug 1 and 2). Survivin levels were evaluated in the Caspase3 negative population. Note that bortezomib induces efficient down regulation of survivin only when the cells have been pre-treated with dexamethasone first. **C.** . Induction of caspase 3 was used to calculate the drug sensitivity index. **D.** ETP xenograft samples are sensitive to the combination of borteomib and dexamethasone but not either agent alone.

Determine whether exposure to in vitro exposure to bortezomib increases sensitivity to dexamethasone. The goal of this experiment is to use SCPFC to determine if exposure to dexamethasone and/or bortezomib induces differences in signal transduction network wiring that predict chemotherapy resistance in ETP and non-ETP T-ALL cell subtypes. The Caspase-3 phosphoflow cytometry assay and the data analysis strategy described in Aim 2a will be used to assess apoptosis. An advantage of multiparameter flow is the ability to gate on Caspase-3 negative (i.e., drug resistant cells). This will allow us to interrogate potential mechanisms of resistance by comparing expression profiles of known target proteins (such as the cell survival mediators survivin, Bcl2, Noxa and PUMA) before and after treatment. The URSA method will be used to determine whether combinational drug treatments exhibit an additive, synergistic, or antagonistic effect. [291]

Statistical methods and justification. The statistical approaches described in Aim 1 and 2a will be applied here. We expect that bortezomib-containing chemotherapy VXAD-B will sensitize T-ALL cells to chemotherapy treatment and that combinational treatment will enhance apoptosis compared to treating cells with VXAD.

b. **Specific Aim 2c: Compare the performance characteristics of an SCNP classifier to alternative classifiers developed with RPPA data or other clinical/molecular data.**

1) *Approach:* As both RPPA and SCNP can be used a predictive models of response to therapy or risk of relapse, we plan to compare the performance characteristics of both methods. The SCNP training set will

be used to develop a predictor of response (or relapse) based on clinical variables (DX_{Clin}) that are reported in the literature to correlate to relapse. By developing and applying DX_{Clin} in this manner, the study will provide an estimate of the true predictive utility of clinical/laboratory variables compared to RPPA. This will facilitate a fair comparison of the relative contribution of DX_{SCNP}, DX_{RPPA} and DX_{Clin} to the prediction of response or relapse.

2) *Statistical hypothesis and test of significance*: Our primary hypothesis is that the empiric area under the receiver operating characteristic (ROC) curve (AUC) will be significantly greater than 0.5, based on the continuous score from the pre-specified classifier, where higher scores indicate greater probability of continuous complete response at 4 years. Our secondary hypothesis is that DX_{SCNP} contributes significantly to the prediction of treatment response after accounting for clinical variables, T-ALL subtypes and RPPA data. Based on performance characteristics of SCPFC based classifiers in previous studies,[228] we expect accuracy measured by area under the receiver operator characteristic curve (AU_{ROC}) for the prediction of response to be in the range of 0.75 to 0.85 and for the prediction of relapse to be in the range of 0.7 to 0.8. Assuming an equal split of the evaluable samples into training and validation sets, we expect 172 samples in the test set. Under an alternative hypothesis that the true AUC is 0.70, a 1-sided 0.05 level test of the null hypothesis that the AUC = 0.5 has approximately 95% power when there are 26 events (relapse (n = 23) or induction failure (n = 3)) and 146 controls. If separate classifiers are deemed necessary for each treatment group, with approximately 10-15% patients in relapse and 85-90% patients who do not for each treatment group (~85% expected 4 year EFS in VXAD and ~90% expected 4 year EFS in VXAD-B), approximately 80% power is expected for the primary objective if the true area under the ROC curve for each classifier is greater than 0.7.

Expected Results: We expect to be able to provide prospective "validation of clinical usefulness" for both the RPPA and the SCNP method by determining the operating characteristics of both RPPA and SCNP using this data set. Using the same set of prospectively collected samples, we expect to be able to directly compare the predictive power of the RPPA and SCNP classifiers. When successfully completed, the results will significantly advance our knowledge of the predictive capability of two methods and allow for the development of a simple, robust classifier that can aid with therapy risk stratification early in therapy. It will also allow us to determine which SR and IR groups have a "high-risk" protein expression profile that may benefit from more intensive (HR) therapy.

APPENDIX XIII: CTEP AND CTSU REGISTRATION PROCEDURES

CTEP REGISTRATION PROCEDURES

CTEP Investigator Registration Procedures

Food and Drug Administration (FDA) regulations and National Cancer Institute (NCI) policy require all investigators participating in any NCI-sponsored clinical trial to register and to renew their registration annually.

Registration requires the submission of:

- a completed ***Statement of Investigator Form*** (FDA Form 1572) with an original signature
- a current Curriculum Vitae (CV)
- a completed and signed ***Supplemental Investigator Data Form*** (IDF)
- a completed ***Financial Disclosure Form*** (FDF) with an original signature

Fillable PDF forms and additional information can be found on the CTEP website at <http://ctep.cancer.gov/investigatorResources/investigator_registration.htm>. For questions, please contact the ***CTEP Investigator Registration Help Desk*** by email at <pmbregpend@ctep.nci.nih.gov>.

CTEP Associate Registration Procedures / CTEP-IAM Account

The Cancer Therapy Evaluation Program (CTEP) Identity and Access Management (IAM) application is a web-based application intended for use by both Investigators (i.e., all physicians involved in the conduct of NCI-sponsored clinical trials) and Associates (i.e., all staff involved in the conduct of NCI-sponsored clinical trials).

Associates will use the CTEP-IAM application to register (both initial registration and annual re-registration) with CTEP and to obtain a user account.

Investigators will use the CTEP-IAM application to obtain a user account only. (See CTEP Investigator Registration Procedures above for information on registering with CTEP as an Investigator, which must be completed before a CTEP-IAM account can be requested.)

An active CTEP-IAM user account will be needed to access all CTEP and CTSU (Cancer Trials Support Unit) websites and applications, including the CTSU members' website.

Additional information can be found on the CTEP website at <http://ctep.cancer.gov/branches/pmb/associate_registration.htm>. For questions, please contact the ***CTEP Associate Registration Help Desk*** by email at <ctepreghelp@ctep.nci.nih.gov>.

CTSU REGISTRATION PROCEDURES

This study is supported by the NCI Cancer Trials Support Unit (CTSU).

Requirements for AALL1231 Site Registration:

- CTSU IRB Certification (for sites not participating via the CIRB)
- CTSU IRB/Regulatory Approval Transmittal Sheet (for sites not participating via the NCI CIRB)

Submitting Regulatory Documents:

Submit completed forms along with a copy of your IRB Approval to the CTSU Regulatory Office, where they will be entered and tracked in the CTSU RSS.

Regulatory Submission Portal: www.ctsu.org (members' area) → Regulatory Tab →Regulatory Submission

When applicable, original documents should be mailed to:

CTSU Regulatory Office
1818 Market Street, Suite 1100
Philadelphia, PA 19103

Institutions with patients waiting that are unable to use the Portal should alert the CTSU Regulatory Office immediately at 1-866-651-2878 in order to receive further instruction and support.

Checking Your Site's Registration Status:

Check the status of your site's registration packets by querying the RSS site registration status page of the members' section of the CTSU website. (Note: Sites will not receive formal notification of regulatory approval from the CTSU Regulatory Office.)

- Go to https://www.ctsu.org and log in to the members' area using your CTEP-IAM username and password
- Click on the Regulatory tab at the top of your screen
- Click on the Site Registration tab
- Enter your 5-character CTEP Institution Code and click on Go

APPENDIX XIV POSSIBLE DRUG INTERACTIONS

The lists below do not include everything that may interact with chemotherapy. Study Subjects and/or their Parents should be encouraged to talk to their doctors before starting any new medications, using over-the-counter medicines, or herbal supplements and before making a significant change in diet. Supplements may come in many forms, such as teas, drinks, juices, liquids, drops, capsules, pills, or dried herbs. All forms should be avoided.

Bortezomib

Drugs that may interact with bortezomib

- Antibiotics
  - Clarithromycin, erythromycin, nafcillin, rifabutin, rifampin, telithromycin, tetracycline
- Antidepressants and antipsychotics
  - Aripiprazole, citalopram, clozapine, escitalopram, fluoxetine, nefazodone, sertraline
- Antifungals
  - Fluconazole, itraconazole, ketoconazole, posaconazole, voriconazole
- Antiretrovirals and antivirals
  - Atazanavir, boceprevir, darunavir, delaviridine, efavirenz, etravirine, fosamprenavir, indinavir, lopinavir, nelfinavir, nevirapine, ritonavir, saquinavir, telaprevir
- Anti-seizure medications
  - Carbamazepine, oxcarbazepine, phenobarbital, phenytoin, primidone
- Heart medications
  - Amiodarone, clopidogrel, diltiazem, nicardipine, verapamil
- Some chemotherapy (be sure to talk to your doctor about this)
- Many other drugs, including the following:
  - Aprepitant, bosentan, cimetidine, cyclosporine, deferasirox, esomeprazole, haloperidol, ivacaftor, lomitapide, mifepristone, omeprazole, pimozide

Food and supplements that may interact with bortezomib*

- Echinacea
- Grapefruit, grapefruit juice, Star fruit, Seville oranges
- Green tea or a major component of green tea called ECGC
- St. John's Wort
- Vitamin C, ascorbic acid, or multivitamins/minerals containing vitamin C or ascorbic acid
- Drinks, food, supplements, or vitamins containing "flavonoids" or other "antioxidants"

Green tea, ascorbic acid (vitamin C), and other antioxidants may decrease the activity of bortezomib. To avoid the interaction, you should stop taking the following products/foods from 1 day before the start of bortezomib until 3 days after the last dose of bortezomib:

1. Green tea and its components
2. Vitamin products containing vitamin C and antioxidants
3. Foods with high vitamin C content, such as fruits
4. Herbal products and any products containing flavonoids or other antioxidant compounds

Drinking grapefruit juice or eating grapefruit may increase the concentration of bortezomib in the blood. Therefore, eating grapefruit or drinking its juice should be avoided for the duration of treatment with bortezomib.

Cyclophosphamide

Drugs that may interact with cyclophosphamide
• Allopurinol • Chloramphenicol • Cyclosporine • Digoxin • Etanercept • Hydrochlorothiazide • Indomethacin • Nevirapine • Pentostatin • Warfarin

Food and supplements that may interact with cyclophosphamide*
• St. John's Wort • Drinks, food, supplements, or vitamins containing "flavonoids" or other "antioxidants"

Daunorubicin

Drugs that may interact with daunorubicin
• Some antibiotics and antifungals (clarithromycin, erythromycin, itraconazole, ketoconazole) • Some antiepileptics (carbamazepine, phenobarbital, phenytoin, fosphenytoin) • Some antiretrovirals (darunavir, lopinavir; nelfinavir, ritonavir, saquinavir, telaprevir, tenofovir, tipranavir) • Some heart medications (amiodarone, carvedilol, digoxin, dronedarone, nicardipine, propranolol, verapamil) • Other agents, such as atorvastatin, clozapine, cyclosporine, dexamethasone, ivacaftor, leflunomide, natalizumab, nefazodone, progesterone, rifampin, tacrolimus, tofacitinib, and trazodone

Food and supplements that may interact with daunorubicin*
• Echinacea • Grapefruit, grapefruit juice, Seville oranges, star fruit • St. John's Wort • Drinks, food, supplements, or vitamins containing "flavonoids" or other "antioxidants"

Dexamethasone

Drugs that may interact with dexamethasone
• Antibiotics ○ Ciprofloxacin, levofloxacin, moxifloxacin, clarithromycin, erythromycin, nafcillin, rifabutin, rifampin, telithromycin • Antidepressants and antipsychotics ○ Aripiprazole, buproprion, citalopram, clozapine, escitalopram, fluvoxamine, lurasidone, nefazodone, quetiapine • Antifungals ○ Caspofungin, fluconazole, itraconazole, ketoconazole, posaconazole, voriconazole • Arthritis medications ○ Leflunomide, tofacitinib • Anti-rejection medications ○ Cyclosporine, sirolimus, tacrolimus • Antiretrovirals and antivirals ○ Atazanavir, boceprevir, darunavir, delaviridine, efavirenz, etravirine, fosamprenavir, indinavir, lopinavir, nelfinavir, nevirapine, rilpivirine, ritonavir, saquinavir, Stribild, telaprevir, tipranavir • Anti-seizure medications ○ Carbamazepine, oxcarbazepine, phenobarbital, phenytoin, primidone • Heart medications ○ Amiodarone, amlodipine, dronedenarone, verapamil • Some chemotherapy (be sure to talk to your doctor about this) • Some oral contraceptives or birth control medications • Many other drugs, including the following: ○ Aprepitant, artemether/lumefantine, aspirin, deferasirox, ibuprofen, ivacaftor, lomitapide, mifepristone, natalizumab, nimodipine, praziquantel, warfarin

Food and supplements that may interact with dexamethasone*
• Echinacea • St. John's Wort • Grapefruit, grapefruit juice, Seville oranges, star fruit

Doxorubicin

Drugs that may interact with doxorubicin
• Some antiepileptics (carbamazepine, oxcarbazepine, phenobarbital, phenytoin, fosphenytoin) • Some antiretrovirals (stavudine, zidovudine) • Other agents, such as clozapine, cyclosporine, verapamil, and warfarin

Food and supplements that may interact with doxorubicin*
• Echinacea • Glucosamine

- St. John's Wort
- Grapefruit, grapefruit juice, Seville oranges, star fruit
- Drinks, food, supplements, or vitamins containing "flavonoids" or other "antioxidants"

Etoposide

Drugs that may interact with etoposide

- Antibiotics
  - Clarithromycin, erythromycin, nafcillin, rifabutin, rifampin, telithromycin
- Antidepressants and antipsychotics
  - Aripiprazole, clozapine, nefazodone
- Antifungals
  - Fluconazole, itraconazole, ketoconazole, posaconazole, voriconazole
- Arthritis medications
  - Leflunomide, tofacitinib
- Anti-rejection medications
  - Cyclosporine, tacrolimus
- Antiretrovirals and antivirals
  - Atazanavir, boceprevir, darunavir, delaviridine, efavirenz, etravirine, fosamprenavir, indinavir, lopinavir, nelfinavir, nevirapine, ritonavir, saquinavir, Stribild, telaprevir, tipranavir
- Anti-seizure medications
  - Carbamazepine, oxcarbazepine, phenobarbital, phenytoin, primidone
- Heart medications
  - Amiodarone, dronedenarone, verapamil
- Some chemotherapy (be sure to talk to your doctor about this)
- Many other drugs, including the following:
  - Aprepitant, atovaquone, bosentan, deferasirox, dexamethasone, ivacaftor, lomitapide, mifepristone, natalizumab, pimozide, sitaxentan

Food and supplements that may interact with etoposide*

- Echinacea
- Glucosamine
- St. John's Wort
- Grapefruit, grapefruit juice, Seville oranges, star fruit

Hydrocortisone

Drugs that may interact with hydrocortisone
• Arthritis medications o Leflunomide, tofacitinib • Antiretrovirals and antivirals o Darunavir, lopinavir, nelfinavir, ritonavir, saquinavir, telaprevir, tenofovir, tipranivir • Anti-seizure medications o Carbamazepine, phenobarbital, phenytoin, primidone • Heart medications o Amiodarone, carvedilol, dronedarone, verapamil • Some chemotherapy (be sure to talk to your doctor about this) • Some oral contraceptives or birth control medications • Many other drugs, including the following: o Aprepitant, aripiprazole, clarithromycin, cyclosporine, deferasirox, itraconazole, ivacaftor, ketoconazole, mifepristone, natalizumab, nefazodone, rifampin, tacrolimus, trazodone, warfarin
Food and supplements that may interact with hydrocortisone*
• Echinacea • St. John's Wort • Grapefruit, grapefruit juice, Seville oranges, star fruit

Ifosfamide

Drugs that may interact with ifosfamide
• Antibiotics o Clarithromycin, erythromycin, nafcillin, rifabutin, rifampin, telithromycin • Antidepressants and antipsychotics o Citalopram, clozapine, escitalopram, fluvoxamine, lurasidone, nefazodone, paliperidone, quetiapine, thioridizine, ziprasidone • Antifungals o Fluconazole, itraconazole, ketoconazole, posaconazole, voriconazole • Arthritis medications o Leflunomide, tofacitinib • Anti-rejection medications o Cyclosporine • Antiretrovirals and antivirals o Atazanavir, boceprevir, darunavir, delaviridine, efavirenz, etravirine, fosamprenavir, indinavir, lopinavir, nelfinavir, nevirapine, ritonavir, saquinavir, Stribild, telaprevir, tipranavir • Anti-seizure medications o Carbamazepine, oxcarbazepine, phenobarbital, phenytoin, primidone • Heart medications o Amiodarone, dronedenarone, verapamil

- Stomach and reflux medications
  - Esomeprazole, omeprazole
- Some chemotherapy (be sure to talk to your doctor about this)
- Many other drugs, including the following:
  - Bosentan, sitaxentan, aprepitant, dexamethasone, lomitapide, mifepristone, natalizumab, pimozide

Food and supplements that may interact with ifosfamide*
• Echinacea • St. John's Wort • Grapefruit, grapefruit juice, Seville oranges, star fruit

Mercaptopurine

Drugs that may interact with mercaptopurine
○ Artiritis medications: leflunomide, tofacitinib ○ Other medications, such as allopurinol, azathioprine, clozapine, febuxostat, natalizumab, olsalazine, sulfasalazine, warfarin

Food and supplements that may interact with mercaptopurine*
• Echinacea

Methotrexate (by mouth or by vein).

Drugs that may interact with methotrexate
• Some antibiotics (amoxicillin, Bactrim, chloramphenicol, ciprofloxacin, penicillin, piperacillin, tetracycline) • Some anti-inflammatory drugs (aspirin, acetaminophen, ibuprofen, naproxen, ketorolac) • Some heartburn medications (esomeprazole, lansoprazole, omeprazole, pantoprazole) • Several other specific agents, including the following: amiodarone, clozapine, cyclosporine, eltrombopag, leflunomide, phenytoin, pimecrolimus, probenecid, pyrimethamine, retinoids, theophylline, warfain

Food and supplements that may interact with methotrexate*
• Alcohol • Echinacea • Some vitamins, including those that contain folic acid or high doses of vitamin C

Pegaspargase

Drugs that may interact with pegaspargase
○ Leflunomide, natalizumab, tofacitinib

Food and supplements that may interact with pegaspargase*
• Echinacea

Thioguanine

Drugs that may interact with thioguanine
○ Arthritis medications: leflunomide, tofacitinib ○ Other medications, such as allopurinol, clozapine, natalizumab, olsalazine, sulfasalazine

Food and supplements that may interact with thioguanine*
• Echinacea

Vincristine

Drugs that may interact with vincristine
• Antibiotics ○ Clarithromycin, erythromycin, nafcillin, rifabutin, rifampin, telithromycin • Antidepressants and antipsychotics ○ Aripiprazole, nefazodone, trazodone • Antifungals ○ Fluconazole, itraconazole, ketoconazole, posaconazole, voriconazole • Arthritis medications ○ Leflunomide, tocilizumab, tofacitinib • Anti-rejection medications ○ Cyclosporine, tacrolimus • Antiretrovirals and antivirals ○ Atazanavir, boceprevir, darunavir, delaviridine, efavirenz, etravirine, fosamprenavir, indinavir, lopinavir, nelfinavir, nevirapine, ritonavir, saquinavir, Stribild, telaprevir, tenofovir, tipranavir • Anti-seizure medications

○ Carbamazepine, oxcarbazepine, phenobarbital, phenytoin, primidone • Heart medications ○ Amiodarone, digoxin, dronedenarone, propranolol, verapamil • Some chemotherapy (be sure to talk to your doctor about this) • Many other drugs, including the following: ○ Aprepitant, deferasirox, ivacaftor, lomitapide, mifepristone, natalizumab, pimozide, warfarin

Food and supplements that may interact with vincristine*
• Echinacea • St. John's Wort • Grapefruit, grapefruit juice, Seville oranges, star fruit

REFERENCES

1. Borowitz MJ, Dowell BL, Boyett JM, et al: Clinicopathologic aspects of E rosette negative T cell acute lymphocytic leukemia: a Pediatric Oncology Group study. Journal of clinical oncology : official journal of the American Society of Clinical Oncology 4:170-7, 1986
2. Crist WM, Shuster JJ, Falletta J, et al: Clinical features and outcome in childhood T-cell leukemia-lymphoma according to stage of thymocyte differentiation: a Pediatric Oncology Group Study. Blood 72:1891-7, 1988
3. Pui CH: Childhood leukemias. The New England journal of medicine 332:1618-30, 1995
4. Uckun FM, Sensel MG, Sun L, et al: Biology and treatment of childhood T-lineage acute lymphoblastic leukemia. Blood 91:735-46, 1998
5. Gaynon PS, Angiolillo AL, Carroll WL, et al: Long-term results of the children's cancer group studies for childhood acute lymphoblastic leukemia 1983-2002: a Children's Oncology Group Report. Leukemia : official journal of the Leukemia Society of America, Leukemia Research Fund, U.K 24:285-97, 2010
6. Salzer WL, Devidas M, Carroll WL, et al: Long-term results of the pediatric oncology group studies for childhood acute lymphoblastic leukemia 1984-2001: a report from the children's oncology group. Leukemia : official journal of the Leukemia Society of America, Leukemia Research Fund, U.K 24:355-70, 2010
7. Moricke A, Zimmermann M, Reiter A, et al: Long-term results of five consecutive trials in childhood acute lymphoblastic leukemia performed by the ALL-BFM study group from 1981 to 2000. Leukemia : official journal of the Leukemia Society of America, Leukemia Research Fund, U.K 24:265-84, 2010
8. Silverman LB, Stevenson KE, O'Brien JE, et al: Long-term results of Dana-Farber Cancer Institute ALL Consortium protocols for children with newly diagnosed acute lymphoblastic leukemia (1985-2000). Leukemia : official journal of the Leukemia Society of America, Leukemia Research Fund, U.K 24:320-34, 2010
9. Reiter A, Schrappe M, Ludwig WD, et al: Intensive ALL-type therapy without local radiotherapy provides a 90% event-free survival for children with T-cell lymphoblastic lymphoma: a BFM group report. Blood 95:416-21, 2000
10. Amylon MD, Shuster J, Pullen J, et al: Intensive high-dose asparaginase consolidation improves survival for pediatric patients with T cell acute lymphoblastic leukemia and advanced stage lymphoblastic lymphoma: a Pediatric Oncology Group study. Leukemia : official journal of the Leukemia Society of America, Leukemia Research Fund, U.K 13:335-42, 1999
11. Sandlund JT, Downing JR, Crist WM: Non-Hodgkin's lymphoma in childhood. The New England journal of medicine 334:1238-48, 1996
12. 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]. Journal of clinical oncology : official journal of the American Society of Clinical Oncology 26:3971-8, 2008
13. Gaynon PS, Harris RE, Altman AJ, et al: Bone marrow transplantation versus prolonged intensive chemotherapy for children with acute lymphoblastic leukemia and an initial bone marrow relapse within 12 months of the completion of primary therapy: Children's Oncology Group study CCG-1941. Journal of clinical oncology : official journal of the American Society of Clinical Oncology 24:3150-6, 2006
14. 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-Munster Group 87. Journal of clinical oncology : official journal of the American Society of Clinical Oncology 23:7942-50, 2005

15. Houghton PJ, Morton CL, Kolb EA, et al: Initial testing (stage 1) of the proteasome inhibitor bortezomib by the pediatric preclinical testing program. Pediatric blood & cancer 50:37-45, 2008
16. Vilimas T, Mascarenhas J, Palomero T, et al: Targeting the NF-kappaB signaling pathway in Notch1-induced T-cell leukemia. Nature medicine 13:70-7, 2007
17. Horton TM, Gannavarapu A, Blaney SM, et al: Bortezomib interactions with chemotherapy agents in acute leukemia in vitro. Cancer chemotherapy and pharmacology 58:13-23, 2006
18. Chauhan D, Li G, Auclair D, et al: 2-Methoxyestardiol and bortezomib/proteasome-inhibitor overcome dexamethasone-resistance in multiple myeloma cells by modulating Heat Shock Protein-27. Apoptosis : an international journal on programmed cell death 9:149-55, 2004
19. Mitsiades N, Mitsiades CS, Richardson PG, et al: The proteasome inhibitor PS-341 potentiates sensitivity of multiple myeloma cells to conventional chemotherapeutic agents: therapeutic applications. Blood 101:2377-80, 2003
20. Ma MH, Yang HH, Parker K, et al: The proteasome inhibitor PS-341 markedly enhances sensitivity of multiple myeloma tumor cells to chemotherapeutic agents. Clinical cancer research : an official journal of the American Association for Cancer Research 9:1136-44, 2003
21. An J, Sun Y, Fisher M, et al: Antitumor effects of bortezomib (PS-341) on primary effusion lymphomas. Leukemia : official journal of the Leukemia Society of America, Leukemia Research Fund, U.K 18:1699-704, 2004
22. Zheng B, Zhou R, Gong Y, et al: Proteasome inhibitor bortezomib overcomes P-gp-mediated multidrug resistance in resistant leukemic cell lines. International journal of laboratory hematology 34:237-47, 2012
23. Junk S, Lauten M, Cario G, et al: Proteasome inhibitor bortezomib induces apoptosis in prednisone-resistant childhood acute lymphoblastic leukemia cells. Blood ASH Annual Meeting Abstracts. 114:991, 2009
24. Jeremias I, Kupatt C, Baumann B, et al: Inhibition of nuclear factor kappaB activation attenuates apoptosis resistance in lymphoid cells. Blood 91:4624-31, 1998
25. Orlowski RZ, Stinchcombe TE, Mitchell BS, et al: Phase I trial of the proteasome inhibitor PS-341 in patients with refractory hematologic malignancies. Journal of clinical oncology : official journal of the American Society of Clinical Oncology 20:4420-7, 2002
26. Cortes J, Thomas D, Koller C, et al: Phase I study of bortezomib in refractory or relapsed acute leukemias. Clinical cancer research : an official journal of the American Association for Cancer Research 10:3371-6, 2004
27. Horton TM, Pati D, Plon SE, et al: A phase 1 study of the proteasome inhibitor bortezomib in pediatric patients with refractory leukemia: a Children's Oncology Group study. Clinical cancer research : an official journal of the American Association for Cancer Research 13:1516-22, 2007
28. Lee J, Suh C, Kang HJ, et al: Phase I study of proteasome inhibitor bortezomib plus CHOP in patients with advanced, aggressive T-cell or NK/T-cell lymphoma. Annals of oncology : official journal of the European Society for Medical Oncology / ESMO 19:2079-83, 2008
29. Zinzani PL, Musuraca G, Tani M, et al: Phase II trial of proteasome inhibitor bortezomib in patients with relapsed or refractory cutaneous T-cell lymphoma. Journal of clinical oncology : official journal of the American Society of Clinical Oncology 25:4293-7, 2007
30. Falchook GS, Duvic M, Hong DS, et al: Age-stratified phase I trial of a combination of bortezomib, gemcitabine, and liposomal doxorubicin in patients with advanced malignancies. Cancer chemotherapy and pharmacology 69:1117-26, 2012
31. Hu X, Xu J, Sun A, et al: Successful T-cell acute lymphoblastic leukemia treatment with proteasome inhibitor bortezomib based on evaluation of nuclear factor-kappaB activity. Leukemia & lymphoma 52:2393-5, 2011
32. Orlowski RZ, Voorhees PM, Garcia RA, et al: Phase 1 trial of the proteasome inhibitor bortezomib and pegylated liposomal doxorubicin in patients with advanced hematologic malignancies. Blood 105:3058-65, 2005

33. Messinger Y, Gaynon P, Raetz E, et al: Phase I study of bortezomib combined with chemotherapy in children with relapsed childhood acute lymphoblastic leukemia (ALL): a report from the therapeutic advances in childhood leukemia (TACL) consortium. Pediatric blood & cancer 55:254-9, 2010
34. Horton T, Lu X, O'Brien M, et al: Bortezomib reinduction therapy to improve response rates in pediatric ALL in first relapse: A Children's Oncology Group (COG) study (AALL07P1). ASCO Annual Meeting Abstracts. June 17, 2013: 10003
35. Kim SJ, Yoon DH, Kang HJ, et al: Bortezomib in combination with CHOP as first-line treatment for patients with stage III/IV peripheral T-cell lymphomas: A multicentre, single-arm, phase 2 trial. European journal of cancer:epub ahead of print, 2012
36. Borowitz MJ, Devidas M, Hunger SP, et al: Clinical significance of minimal residual disease in childhood acute lymphoblastic leukemia and its relationship to other prognostic factors: a Children's Oncology Group study. Blood 111:5477-85, 2008
37. Conter V, Bartram CR, Valsecchi MG, et al: Molecular response to treatment redefines all prognostic factors in children and adolescents with B-cell precursor acute lymphoblastic leukemia: results in 3184 patients of the AIEOP-BFM ALL 2000 study. Blood 115:3206-14, 2010
38. Schrappe M, Valsecchi MG, Bartram CR, et al: Late MRD response determines relapse risk overall and in subsets of childhood T-cell ALL: results of the AIEOP-BFM-ALL 2000 study. Blood 118:2077-84, 2011
39. Campana D: Progress of minimal residual disease studies in childhood acute leukemia. Current hematologic malignancy reports 5:169-76, 2010
40. Neale GA, Coustan-Smith E, Stow P, et al: Comparative analysis of flow cytometry and polymerase chain reaction for the detection of minimal residual disease in childhood acute lymphoblastic leukemia. Leukemia : official journal of the Leukemia Society of America, Leukemia Research Fund, U.K 18:934-8, 2004
41. Kerst G, Kreyenberg H, Roth C, et al: Concurrent detection of minimal residual disease (MRD) in childhood acute lymphoblastic leukaemia by flow cytometry and real-time PCR. British journal of haematology 128:774-82, 2005
42. Irving J, Jesson J, Virgo P, et al: Establishment and validation of a standard protocol for the detection of minimal residual disease in B lineage childhood acute lymphoblastic leukemia by flow cytometry in a multi-center setting. Haematologica 94:870-4, 2009
43. Vora A, Goulden N, Wade R, et al: Treatment reduction for children and young adults with low-risk acute lymphoblastic leukaemia defined by minimal residual disease (UKALL 2003): a randomised controlled trial. The Lancet Oncology 14:199-209, 2013
44. Vora A, Wade R, Mitchell C, et al: Efficacy and toxicity of pegylated asparaginase in the treatment of children and young adults with acute lymphoblastic leukemia: results of the united kingdom medical research council (MRC) trial UKALL 2003. Blood (ASH Annual Meeting Abstracts) 112:Abstract 909, 2008
45. Vora A, Wade A, Mitchell C, et al: Improved outcome for children and young adult with T-cell acute lymphoblastic leukaemia (ALL): results of the united kingdom medical research council (MRC) trial UKALL 2003. Blood (ASH Annual Meeting Abstracts) 112:Abstract 908, 2008
46. Goulden(PI): UKALL 2011 Trial v1.0. August 2011
47. Larsen EC, Salzer WL, Nachman JB, et al: Treatment toxicity in adolescents and young adult (AYA) patients compared with younger patients treated for high risk B-precursor acute lymphoblastic leukemia (HR-ALL): a report from the children's oncology group study AALL0232. ASH Annual Meeting Abstr 118:1510, 2011
48. Mattano LA, Jr., Devidas M, Winick N, et al: Effects of dexamethasone (DEX) vs prednisone (PDN) and high-dose methotrexate (HD-MTX) vs Capizzi Methotrexate/Asparaginase (C-MTX/ASNase) on osteonecrosis (ON) incidence in children and young adults with high risk acute

lymphoblastic leukemia (HR-ALL): A report from the Children's Oncology Group (COG). Blood. ASH Annual Meeting Abstracts 120:665, 2012
49. Hurwitz CA, Silverman LB, Schorin MA, et al: Substituting dexamethasone for prednisone complicates remission induction in children with acute lymphoblastic leukemia. Cancer 88:1964-9, 2000
50. Winick(PI): POG 9905 Protocol. Version 3B, September 2003
51. Schrappe M, Zimmerman M, Moricke A, et al: Dexamethasone in induction can eliminate one third of all relapses in childhood acute lymphoblastic leukemia (ALL): results of an international randomized trial in 3655 patients (trial AIEOP-BFM ALL 2000). ASH Annual Meeting Abstr 112:7, 2008
52. Moricke A, Zimmerman M, Schrauder A, et al: No influence of the incidence of osteonecrosis when dexamethasone replaces prednisone during induction treatment for childhood ALL: results of trial ALL-BFM 2000. Blood. ASH Annual Meeting Abstracts 112:899, 2008
53. 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. Journal of clinical oncology : official journal of the American Society of Clinical Oncology 31:1202-10, 2013
54. Pui CH, Campana D, Pei D, et al: Treating childhood acute lymphoblastic leukemia without cranial irradiation. The New England journal of medicine 360:2730-41, 2009
55. Pui CH, Howard SC: Current management and challenges of malignant disease in the CNS in paediatric leukaemia. The Lancet Oncology 9:257-68, 2008
56. Seibel NL, Steinherz PG, Sather HN, et al: Early postinduction intensification therapy improves survival for children and adolescents with high-risk acute lymphoblastic leukemia: a report from the Children's Oncology Group. Blood 111:2548-55, 2008
57. Winter(PI): AALL0434 Trial (Amendment 6). October 2010
58. Pui CH, Cheng C, Leung W, et al: Extended follow-up of long-term survivors of childhood acute lymphoblastic leukemia. The New England journal of medicine 349:640-9, 2003
59. Vilmer E, Suciu S, Ferster A, et al: Long-term results of three randomized trials (58831, 58832, 58881) in childhood acute lymphoblastic leukemia: a CLCG-EORTC report. Children Leukemia Cooperative Group. Leukemia : official journal of the Leukemia Society of America, Leukemia Research Fund, U.K 14:2257-66, 2000
60. De Moerloose B, Suciu S, Ferster A, et al: Low CNS Relapse Incidence without radiotherapy and improvement of outcome: results of subsequent EORTC-CLG 58881 and 58951 Trials in Pediatric T-cell acute lymphoblastic leukemia. ASH Annual Meeting Abstr:133, 2012
61. Pui CH, Sandlund JT, Pei D, et al: Improved outcome for children with acute lymphoblastic leukemia: results of Total Therapy Study XIIIB at St Jude Children's Research Hospital. Blood 104:2690-6, 2004
62. Veerman AJ, Kamps WA, van den Berg H, et al: Dexamethasone-based therapy for childhood acute lymphoblastic leukaemia: results of the prospective Dutch Childhood Oncology Group (DCOG) protocol ALL-9 (1997-2004). The Lancet Oncology 10:957-66, 2009
63. Stark B, Avrahami G, Nirel R, et al: Extended triple intrathecal therapy in children with T-cell acute lymphoblastic leukaemia: a report from the Israeli National ALL-Studies. British journal of haematology 147:113-24, 2009
64. Uyttebroeck A, Suciu S, Laureys G, et al: Treatment of childhood T-cell lymphoblastic lymphoma according to the strategy for acute lymphoblastic leukaemia, without radiotherapy: long term results of the EORTC CLG 58881 trial. European journal of cancer 44:840-6, 2008
65. Schrappe M, Hunger SP, Pui CH, et al: Outcomes after induction failure in childhood acute lymphoblastic leukemia. The New England journal of medicine 366:1371-81, 2012

66. Moricke A, Reiter A, Zimmermann M, et al: Risk-adjusted therapy of acute lymphoblastic leukemia can decrease treatment burden and improve survival: treatment results of 2169 unselected pediatric and adolescent patients enrolled in the trial ALL-BFM 95. Blood 111:4477-89, 2008
67. Arico M, Valsecchi MG, Conter V, et al: Improved outcome in high-risk childhood acute lymphoblastic leukemia defined by prednisone-poor response treated with double Berlin-Frankfurt-Muenster protocol II. Blood 100:420-6, 2002
68. Moricke A, Zimmermann M, Valsecchi MG, et al: Dexamethasone vs. prednisone in induction treatment of pediatric ALL: results of the randomized trial AIEOP-BFM ALL 2000. Blood, 2016
69. Vora A, Goulden N, Mitchell C, et al: Augmented post-remission therapy for a minimal residual disease-defined high-risk subgroup of children and young people with clinical standard-risk and intermediate-risk acute lymphoblastic leukaemia (UKALL 2003): a randomised controlled trial. Lancet Oncol 15:809-18, 2014
70. Coustan-Smith E, Mullighan CG, Onciu M, et al: Early T-cell precursor leukaemia: a subtype of very high-risk acute lymphoblastic leukaemia. The Lancet Oncology 10:147-56, 2009
71. Zhang J, Ding L, Holmfeldt L, et al: The genetic basis of early T-cell precursor acute lymphoblastic leukaemia. Nature 481:157-63, 2012
72. Inukai T, Kiyokawa N, Campana D, et al: Clinical significance of early T-cell precursor acute lymphoblastic leukaemia: results of the Tokyo Children's Cancer Study Group Study L99-15. British journal of haematology 156:358-65, 2012
73. Patrick K, Wade R, Goulden N, et al: Characteristics and outcome of children and young adults with early T-cell precursor (ETP) ALL treated on UKALL 2003. . ASH Annual Meeting Abstracts. Abstract 0058, 2013
74. Adams J: Proteasome inhibition in cancer: development of PS-341. Seminars in oncology 28:613-9, 2001
75. Meusser B, Hirsch C, Jarosch E, et al: ERAD: the long road to destruction. Nature cell biology 7:766-72, 2005
76. Voorhees PM, Orlowski RZ: The proteasome and proteasome inhibitors in cancer therapy. Annual review of pharmacology and toxicology 46:189-213, 2006
77. Kumatori A, Tanaka K, Inamura N, et al: Abnormally high expression of proteasomes in human leukemic cells. Proceedings of the National Academy of Sciences of the United States of America 87:7071-5, 1990
78. Landis-Piwowar KR, Milacic V, Chen D, et al: The proteasome as a potential target for novel anticancer drugs and chemosensitizers. Drug resistance updates : reviews and commentaries in antimicrobial and anticancer chemotherapy 9:263-73, 2006
79. Vink J, Cloos J, Kaspers GJ: Proteasome inhibition as novel treatment strategy in leukaemia. British journal of haematology 134:253-62, 2006
80. Schenkein D: Proteasome inhibitors in the treatment of B-cell malignancies. Clinical lymphoma 3:49-55, 2002
81. Guzman ML, Neering SJ, Upchurch D, et al: Nuclear factor-kappaB is constitutively activated in primitive human acute myelogenous leukemia cells. Blood 98:2301-7, 2001
82. Guzman ML, Swiderski CF, Howard DS, et al: Preferential induction of apoptosis for primary human leukemic stem cells. Proceedings of the National Academy of Sciences of the United States of America 99:16220-5, 2002
83. Servida F, Soligo D, Delia D, et al: Sensitivity of human multiple myelomas and myeloid leukemias to the proteasome inhibitor I. Leukemia : official journal of the Leukemia Society of America, Leukemia Research Fund, U.K 19:2324-31, 2005
84. Masdehors P, Merle-Beral H, Maloum K, et al: Deregulation of the ubiquitin system and p53 proteolysis modify the apoptotic response in B-CLL lymphocytes. Blood 96:269-74, 2000

85. Rajkumar SV, Richardson PG, Hideshima T, et al: Proteasome inhibition as a novel therapeutic target in human cancer. Journal of clinical oncology : official journal of the American Society of Clinical Oncology 23:630-9, 2005
86. Adams J, Palombella VJ, Sausville EA, et al: Proteasome inhibitors: a novel class of potent and effective antitumor agents. Cancer research 59:2615-22, 1999
87. Wang CY, Cusack JC, Jr., Liu R, et al: Control of inducible chemoresistance: enhanced anti-tumor therapy through increased apoptosis by inhibition of NF-kappaB. Nature medicine 5:412-7, 1999
88. Karin M, Cao Y, Greten FR, et al: NF-kappaB in cancer: from innocent bystander to major culprit. Nature reviews. Cancer 2:301-10, 2002
89. Kordes U, Krappmann D, Heissmeyer V, et al: Transcription factor NF-kappaB is constitutively activated in acute lymphoblastic leukemia cells. Leukemia : official journal of the Leukemia Society of America, Leukemia Research Fund, U.K 14:399-402, 2000
90. An WG, Hwang SG, Trepel JB, et al: Protease inhibitor-induced apoptosis: accumulation of wt p53, p21WAF1/CIP1, and induction of apoptosis are independent markers of proteasome inhibition. Leukemia : official journal of the Leukemia Society of America, Leukemia Research Fund, U.K 14:1276-83, 2000
91. Munzert G, Kirchner D, Ottmann O, et al: Constitutive NF-kappab/Rel activation in philadelphia chromosome positive (Ph+) acute lymphoblastic leukemia (ALL). Leukemia & lymphoma 45:1181-4, 2004
92. Hironaka N, Mochida K, Mori N, et al: Tax-independent constitutive IkappaB kinase activation in adult T-cell leukemia cells. Neoplasia 6:266-78, 2004
93. Mori N, Fujii M, Ikeda S, et al: Constitutive activation of NF-kappaB in primary adult T-cell leukemia cells. Blood 93:2360-8, 1999
94. Guo D, Teng Q, Ji C: NOTCH and phosphatidylinositide 3-kinase/phosphatase and tensin homolog deleted on chromosome ten/AKT/mammalian target of rapamycin (mTOR) signaling in T-cell development and T-cell acute lymphoblastic leukemia. Leukemia & lymphoma 52:1200-10, 2011
95. Cheng J, Phong B, Wilson DC, et al: Akt fine-tunes NF-kappaB-dependent gene expression during T cell activation. The Journal of biological chemistry 286:36076-85, 2011
96. Hideshima T, Ikeda H, Chauhan D, et al: Bortezomib induces canonical nuclear factor-kappaB activation in multiple myeloma cells. Blood 114:1046-52, 2009
97. Li C, Chen S, Yue P, et al: Proteasome inhibitor PS-341 (bortezomib) induces calpain-dependent IkappaB(alpha) degradation. The Journal of biological chemistry 285:16096-104, 2010
98. Chaturvedi MM, Sung B, Yadav VR, et al: NF-kappaB addiction and its role in cancer: 'one size does not fit all'. Oncogene 30:1615-30, 2011
99. Li ZW, Chen H, Campbell RA, et al: NF-kappaB in the pathogenesis and treatment of multiple myeloma. Current opinion in hematology 15:391-9, 2008
100. Roccaro AM, Vacca A, Ribatti D: Bortezomib in the treatment of cancer. Recent patents on anti-cancer drug discovery 1:397-403, 2006
101. Abayomi EA, Sissolak G, Jacobs P: Use of novel proteosome inhibitors as a therapeutic strategy in lymphomas current experience and emerging concepts. Transfusion and apheresis science : official journal of the World Apheresis Association : official journal of the European Society for Haemapheresis 37:85-92, 2007
102. Zavrski I, Jakob C, Kaiser M, et al: Molecular and clinical aspects of proteasome inhibition in the treatment of cancer. Recent results in cancer research. Fortschritte der Krebsforschung. Progres dans les recherches sur le cancer 176:165-76, 2007
103. Birkenkamp KU, Geugien M, Schepers H, et al: Constitutive NF-kappaB DNA-binding activity in AML is frequently mediated by a Ras/PI3-K/PKB-dependent pathway. Leukemia : official journal of the Leukemia Society of America, Leukemia Research Fund, U.K 18:103-12, 2004

104. Romano MF, Petrella A, Bisogni R, et al: Effect of NF-kappaB/Rel inhibition on spontaneous vs chemotherapy-induced apoptosis in AML and normal cord blood CD34+ cells. Leukemia : official journal of the Leukemia Society of America, Leukemia Research Fund, U.K 17:1190-2, 2003
105. Watanabe K, Kubota M, Hamahata K, et al: Prevention of etoposide-induced apoptosis by proteasome inhibitors in a human leukemic cell line but not in fresh acute leukemia blasts. A differential role of NF-kappab activation. Biochemical pharmacology 60:823-30, 2000
106. Gatto S, Scappini B, Pham L, et al: The proteasome inhibitor PS-341 inhibits growth and induces apoptosis in Bcr/Abl-positive cell lines sensitive and resistant to imatinib mesylate. Haematologica 88:853-63, 2003
107. Minderman H, Zhou Y, O'Loughlin KL, et al: Bortezomib activity and in vitro interactions with anthracyclines and cytarabine in acute myeloid leukemia cells are independent of multidrug resistance mechanisms and p53 status. Cancer chemotherapy and pharmacology 60:245-55, 2007
108. Weigert O, Pastore A, Rieken M, et al: Sequence-dependent synergy of the proteasome inhibitor bortezomib and cytarabine in mantle cell lymphoma. Leukemia : official journal of the Leukemia Society of America, Leukemia Research Fund, U.K 21:524-8, 2007
109. Satou Y, Nosaka K, Koya Y, et al: Proteasome inhibitor, bortezomib, potently inhibits the growth of adult T-cell leukemia cells both in vivo and in vitro. Leukemia : official journal of the Leukemia Society of America, Leukemia Research Fund, U.K 18:1357-63, 2004
110. Nasr R, El-Sabban ME, Karam JA, et al: Efficacy and mechanism of action of the proteasome inhibitor PS-341 in T-cell lymphomas and HTLV-I associated adult T-cell leukemia/lymphoma. Oncogene 24:419-30, 2005
111. Sayers TJ, Brooks AD, Koh CY, et al: The proteasome inhibitor PS-341 sensitizes neoplastic cells to TRAIL-mediated apoptosis by reducing levels of c-FLIP. Blood 102:303-10, 2003
112. Naujokat C, Sezer O, Zinke H, et al: Proteasome inhibitors induced caspase-dependent apoptosis and accumulation of p21WAF1/Cip1 in human immature leukemic cells. European journal of haematology 65:221-36, 2000
113. Greco WR, Park HS, Rustum YM: Application of a new approach for the quantitation of drug synergism to the combination of cis-diamminedichloroplatinum and 1-beta-D-arabinofuranosylcytosine. Cancer research 50:5318-27, 1990
114. Richardson PG, Hideshima T, Mitsiades C, et al: Proteasome inhibition in hematologic malignancies. Annals of medicine 36:304-14, 2004
115. Kane RC, Bross PF, Farrell AT, et al: Velcade: U.S. FDA approval for the treatment of multiple myeloma progressing on prior therapy. The oncologist 8:508-13, 2003
116. Papandreou CN, Daliani DD, Nix D, et al: Phase I trial of the proteasome inhibitor bortezomib in patients with advanced solid tumors with observations in androgen-independent prostate cancer. Journal of clinical oncology : official journal of the American Society of Clinical Oncology 22:2108-21, 2004
117. Barr PM, Fu P, Lazarus HM, et al: Phase I trial of fludarabine, bortezomib and rituximab for relapsed and refractory indolent and mantle cell non-Hodgkin lymphoma. British journal of haematology 147:89-96, 2009
118. Richardson PG, Barlogie B, Berenson J, et al: A phase 2 study of bortezomib in relapsed, refractory myeloma. The New England journal of medicine 348:2609-17, 2003
119. O'Connor OA, Wright J, Moskowitz C, et al: Phase II clinical experience with the novel proteasome inhibitor bortezomib in patients with indolent non-Hodgkin's lymphoma and mantle cell lymphoma. Journal of clinical oncology : official journal of the American Society of Clinical Oncology 23:676-84, 2005
120. Goy A, Younes A, McLaughlin P, et al: Phase II study of proteasome inhibitor bortezomib in relapsed or refractory B-cell non-Hodgkin's lymphoma. Journal of clinical oncology : official journal of the American Society of Clinical Oncology 23:667-75, 2005

121. Strauss SJ, Maharaj L, Hoare S, et al: Bortezomib therapy in patients with relapsed or refractory lymphoma: potential correlation of in vitro sensitivity and tumor necrosis factor alpha response with clinical activity. Journal of clinical oncology : official journal of the American Society of Clinical Oncology 24:2105-12, 2006
122. Dunleavy K, Janik J, Gea-Banacloche J, et al: A phase I/II study of bortezomib alone and bortezomib with dose-adjusted EPOCH chemotherapy in relapsed or refractory aggressive B-cell lymphoma. Blood 104:1385, 2004
123. de Vos S, Goy A, Dakhil SR, et al: Multicenter randomized phase II study of weekly or twice-weekly bortezomib plus rituximab in patients with relapsed or refractory follicular or marginal-zone B-cell lymphoma. Journal of clinical oncology : official journal of the American Society of Clinical Oncology 27:5023-30, 2009
124. Ruan J, Martin P, Furman RR, et al: Bortezomib plus CHOP-rituximab for previously untreated diffuse large B-cell lymphoma and mantle cell lymphoma. Journal of clinical oncology : official journal of the American Society of Clinical Oncology 29:690-7, 2011
125. Attar EC, De Angelo DJ, Supko JG, et al: Phase I and pharmacokinetic study of bortezomib in combination with idarubicin and cytarabine in patients with acute myelogenous leukemia. Clinical cancer research : an official journal of the American Association for Cancer Research 14:1446-54, 2008
126. Fowler N, Kahl BS, Lee P, et al: Bortezomib, bendamustine, and rituximab in patients with relapsed or refractory follicular lymphoma: the phase II VERTICAL study. Journal of clinical oncology : official journal of the American Society of Clinical Oncology 29:3389-95, 2011
127. Kim SJ, Yoon DH, Kang HJ, et al: Bortezomib in combination with CHOP as first-line treatment for patients with stage III/IV peripheral T-cell lymphomas: A multicentre, single-arm, phase 2 trial. European journal of cancer 48:3223-3231, 2012
128. Blaney SM, Bernstein M, Neville K, et al: Phase I study of the proteasome inhibitor bortezomib in pediatric patients with refractory solid tumors: a Children's Oncology Group study (ADVL0015). Journal of clinical oncology : official journal of the American Society of Clinical Oncology 22:4804-9, 2004
129. Messinger YH, Gaynon PS, Sposto R, et al: Bortezomib with chemotherapy is highly active in advanced B-precursor acute lymphoblastic leukemia: Therapeutic Advances in Childhood Leukemia & Lymphoma (TACL) Study. Blood 120:285-290, 2012
130. Horton T, Drachtman R, Chen L, et al: A phase II study of bortezomib with ifosfamide and vinorelbine in pediatric patients with refractory/recurrent Hodgkin disease (HD): A Children's Oncology Group (COG) study. J clin oncol. ASCO Annual Meeting abstracts 28:9537, 2010
131. Lipton A, Uzzo R, Amato RJ, et al: The science and practice of bone health in oncology: managing bone loss and metastasis in patients with solid tumors. Journal of the National Comprehensive Cancer Network : JNCCN 7 Suppl 7:S1-29; quiz S30, 2009
132. Zangari M, Terpos E, Zhan F, et al: Impact of bortezomib on bone health in myeloma: a review of current evidence. Cancer treatment reviews 38:968-80, 2012
133. San Miguel JF, Schlag R, Khuageva NK, et al: Bortezomib plus melphalan and prednisone for initial treatment of multiple myeloma. The New England journal of medicine 359:906-17, 2008
134. Kyle RA, Yee GC, Somerfield MR, et al: American Society of Clinical Oncology 2007 clinical practice guideline update on the role of bisphosphonates in multiple myeloma. Journal of clinical oncology : official journal of the American Society of Clinical Oncology 25:2464-72, 2007
135. Golden EB, Lam PY, Kardosh A, et al: Green tea polyphenols block the anticancer effects of bortezomib and other boronic acid-based proteasome inhibitors. Blood 113:5927-37, 2009
136. Perrone G, Hideshima T, Ikeda H, et al: Ascorbic acid inhibits antitumor activity of bortezomib in vivo. Leukemia 23:1679-86, 2009

137. Bannerman B, Xu L, Jones M, et al: Preclinical evaluation of the antitumor activity of bortezomib in combination with vitamin C or with epigallocatechin gallate, a component of green tea. Cancer Chemother Pharmacol, 2011
138. Duval M, Suciu S, Ferster A, et al: Comparison of Escherichia coli-asparaginase with Erwinia-asparaginase in the treatment of childhood lymphoid malignancies: results of a randomized European Organisation for Research and Treatment of Cancer-Children's Leukemia Group phase 3 trial. Blood 99:2734-9, 2002
139. Silverman LB, Levy DE, Dalton VK ea: Outcome of Dana-Farber cancer institute (DFCI) consortium protocol 95-01 for children with newly diagnosed acute lymphoblastic leukemia (ALL). . ASH Annual Meeting Abstracts 104(11):679, 2004
140. Silverman LB, Gelber RD, Dalton VK, et al: Improved outcome for children with acute lymphoblastic leukemia: results of Dana-Farber Consortium Protocol 91-01. Blood 97:1211-8, 2001
141. Avramis VI, Sencer S, Periclou AP, et al: A randomized comparison of native Escherichia coli asparaginase and polyethylene glycol conjugated asparaginase for treatment of children with newly diagnosed standard-risk acute lymphoblastic leukemia: a Children's Cancer Group study. Blood 99:1986-94, 2002
142. Brenner DE, Wiernick PH, Wesley M, et al: Acute doxorubicin toxicity. Relationship to pretreatment liver function, response, and pharmacokinetics in patients with acute nonmlumphocytic leukemia. Cancer 53:1042-1048, 1984
143. Langer T, Martus P, Ottensmeier H, et al: CNS late-effects after ALL therapy in childhood. Part III: neuropsychological performance in long-term survivors of childhood ALL: impairments of concentration, attention, and memory. Med Pediatr Oncol 38(5):320-8, 2002
144. Mulhern RK, Palmer SL: Neurocognitive late effects in pediatric cancer. Curr Probl Cancer 27(4):177-97, 2003
145. Harila-Saari AH, Paakko EL, Vainionpaa LK, et al: A longitudinal magnetic resonance imaging study of the brain in survivors in childhood acute lymphoblastic leukemia. Cancer 83(12):2608-17, 1998
146. Asato R, Akiyama Y, Ito M, et al: Nuclear magnetic resonance abnormalities of the cerebral white matter in children with acute lymphoblastic leukemia and malignant lymphoma during and after central nervous system prophylactic treatment with intrathecal methotrexate. Cancer 70(7):1997-2004, 1992
147. Iuvone L, Mariotti P, Colosimo C, et al: Long-term cognitive outcome, brain computed tomography scan, and magnetic resonance imaging in children cured for acute lymphoblastic leukemia. . Cancer 95(12):2562-70, 2002
148. Paakko E, Vainionpaa L, Pyhtinen J, et al: Minor changes on cranial MRI during treatment in children with acute lymphoblastic leukaemia. Neuroradiology 38(3):264-8, 1996
149. Biti GP, Magrini SM, Villari N, et al: Brain damage after treatment for acute lymphoblastic leukemia. A report on 34 patients with special regard to MRI findings. Acta Oncol 28(2):253-6, 1989
150. Wilson DA, Nitschke R, Bowman ME, et al: Transient white matter changes on MR images in children undergoing chemotherapy for acute lymphocytic leukemia: correlation with neuropsychologic deficiencies. Radiology 180(1):205-9, 1991
151. Schrappe M, Reiter A, Ludwig WD, et al: Improved outcome in childhood acute lymphoblastic leukemia despite reduced use of anthracyclines and cranial radiotherapy: results of trial ALL-BFM 90. German-Austrian-Swiss ALL-BFM Study Group. Blood 95(11):3310-22, 2000
152. Brouwers P, Riccardi R, Fedio P, et al: Long-term neuropsychologic sequelae of childhood leukemia: correlation with CT brain scan abnormalities. J Pediatr Hematol/Oncol 106(5):723-8, 1985

153. Rollins N, Winick N, Bash R, et al: Acute methotrexate neurotoxicity: findings on diffusion-weighted imaging and correlation with clinical outcome. AJNR Am J Neuroradiol 25(10):1688-95, 2004
154. Laitt RD, Chambers EJ, Goddard PR, et al: Magnetic resonance imaging and magnetic resonance angiography in long term survivors of acute lymphoblastic leukemia treated with cranial irradiation. Cancer 76(10):1846-52, 1995
155. Kramer JH, Norman D, Brant-Zawadzki M, et al: Absence of white matter changes on magnetic resonance imaging in children treated with CNS prophylaxis therapy for leukemia. Cancer 61(5):928-30, 1988
156. Winick NJ, Bowman WP, Kamen BA, et al: Unexpected acute neurologic toxicity in the treatment of children with acute lymphoblastic leukemia. J Natl Cancer Inst. 84(4):252-6, 1992
157. Bleyer WA: Neurologic sequelae of methotrexate and ionizing radiation: a new classification. Cancer Treat Rep 65 89-98, 1981
158. Meadows AT, Gordon J, Massari DJ, et al: Declines in IQ scores and cognitive dysfunctions in children with acute lymphocytic leukaemia treated with cranial irradiation. Lancet 2(8254):1015-8, 1981
159. Williams JM, Ochs J, Davis KS, et al: The subacute effects of CNS prophylaxis for acute lymphoblastic leukemia on neuropsychological performance: a comparison of four protocols. Arch Clin Neuropsychol 1(2):183-92, 1986
160. Mulhern RK, Fairclough D, Ochs J: A prospective comparison of neuropsychologic performance of children surviving leukemia who received 18-Gy, 24-Gy, or no cranial irradiation. J Clin Oncol. 9(8):1348-56, 1991
161. Steinberg GK, Kunis D, DeLaPaz R, et al: Neuroprotection following focal cerebral ischaemia with the NMDA antagonist dextromethorphan, has a favourable dose response profile. Neurol Res. 15(3):174-80, 1993
162. Steinberg GK, Bell TE, Yenari MA: Dose escalation safety and tolerance study of the N-methyl-D-aspartate antagonist dextromethorphan in neurosurgery patients. J Neurosurg 84(5):860-6, 1996
163. Blin O, Azulay JP, Desnuelle C, et al: A controlled one-year trial of dextromethorphan in amyotrophic lateral sclerosis. Clin Neuropharmacol 19(2):189-92, 1996
164. Gredal O, Werdelin L, Bak S, et al: A clinical trial of dextromethorphan in amyotrophic lateral sclerosis. Acta Neurol Scand 96(1):8-13, 1997
165. Hollander D, Pradas J, Kaplan R, et al: High-dose dextromethorphan in amyotrophic lateral sclerosis: phase I safety and pharmacokinetic studies. Ann Neurol. 36(6):920-4, 1994
166. Widemann BC, Balis FM, Murphy RF, et al: Carboxypeptidase-G2, thymidine, and leucovorin rescue in cancer patients with methotrexate-induced renal dysfunction. J Clin Oncol 15:2125-34, 1997
167. DeAngelis LM, Tong WP, Lin S, et al: Carboxypeptidase G2 rescue after high-dose methotrexate. J Clin Oncol 14:2145-9, 1996
168. Weinshilboum R. M, Sladek S. L: Mercaptopurine pharmacogenetics: monogenic inheritance of erythrocyte thiopurine methyltransferase activity. Am.J.Hum.Genet 32:651-662, 1980
169. Bostrom B, Erdmann G: Cellular pharmacology of 6-mercaptopurine in acute lymphoblastic leukemia. Am.J.Pediatr.Hematol.Oncol. 15:80-86, 1993
170. Weinshilboum R: Thiopurine pharmacogenetics: clinical and molecular studies of thiopurine methyltransferase. Drug Metab Dispos 29:601-605, 2001
171. Lennard L, Lewis I. J, Michelagnoli M, et al: Thiopurine methyltransferase deficiency in childhood lymphoblastic leukaemia: 6-mercaptopurine dosage strategies. Med.Pediatr.Oncol. 29:252-255, 1997
172. Relling M. V, Hancock M. L, Rivera G. K, et al: Mercaptopurine therapy intolerance and heterozygosity at the thiopurine S-methyltransferase gene locus. J.Natl.Cancer Inst 91:2001-2008, 1999

173. 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
174. Yates C. R, Krynetski E. Y, Loennechen T, et al: Molecular diagnosis of thiopurine S-methyltransferase deficiency: genetic basis for azathioprine and mercaptopurine intolerance. Ann.Intern.Med 126:608-614, 1997
175. Otterness D, Szumlanski C, Lennard L, et al: Human thiopurine methyltransferase pharmacogenetics: gene sequence polymorphisms. Clin.Pharmacol.Ther 62:60-73, 1997
176. Hon Y. Y, Fessing M. Y, Pui C.-H, et al: Polymorphism of the thiopurine S-methyltransferase gene in African Americans. Hum.Mol.Genet 8:371-376, 1999
177. Krynetski E. Y, Evans W. E: Genetic polymorphism of thiopurine S-methyltransferase: molecular mechanisms and clinical importance. Pharmacology 61:136-146, 2000
178. Van den Berg HW, Desai ZR, Wilson R, et al: The pharmacokinetics of vincristine in man: reduced drug clearance associated with raised serum alkaline phosphatase and dose-limited elimination. Cancer Chemother Pharmacol 8(2):215-9, 1982
179. Microtubule-Targeting Agents, The Chemotherapy Source Book, Perry, MC (ed.), Publisher: Williams and Wilkins, Baltimore, MD, 387-425 (391). 2nd edition.
180. Mattano LA, Jr., Sather HN, Trigg ME, et al: Osteonecrosis as a complication of treating acute lymphoblastic leukemia in children: a report from the Children's Cancer Group. Journal of clinical oncology : official journal of the American Society of Clinical Oncology 18:3262-72, 2000
181. Mattano LA, Jr., Devidas M, Nachman JB, et al: Effect of alternate-week versus continuous dexamethasone scheduling on the risk of osteonecrosis in paediatric patients with acute lymphoblastic leukaemia: results from the CCG-1961 randomised cohort trial. The Lancet Oncology 13:906-15, 2012
182. Mattano LA, Jr., Nachman J, Devidas M: Increased incidence of osteonecrosis with a dexamethasone induction for high risk acute lymphoblastic leukemia: a report from the children's oncology group. Blood ASH Annual Meeting Abstracts:898, 2008
183. Kawedia JD, Kaste SC, Pei D, et al: Pharmacokinetic, pharmacodynamic, and pharmacogenetic determinants of osteonecrosis in children with acute lymphoblastic leukemia. Blood 117:2340-7; quiz 2556, 2011
184. Cheson BD, Pfistner B, Juweid ME, et al: Revised response criteria for malignant lymphoma. Journal of clinical oncology : official journal of the American Society of Clinical Oncology 25:579-86, 2007
185. Cheson BD, Horning SJ, Coiffier B, et al: Report of an international workshop to standardize response criteria for non-Hodgkin's lymphomas. NCI Sponsored International Working Group. Journal of clinical oncology : official journal of the American Society of Clinical Oncology 17:1244, 1999
186. Kishi S, Cheng C, French D, et al: Ancestry and pharmacogenetics of antileukemic drug toxicity. Blood 109:4151-7, 2007
187. Yang JJ, Cheng C, Yang W, et al: Genome-wide interrogation of germline genetic variation associated with treatment response in childhood acute lymphoblastic leukemia. JAMA 301:393-403, 2009
188. Davies SM, Borowitz MJ, Rosner GL, et al: Pharmacogenetics of minimal residual disease response in children with B-precursor acute lymphoblastic leukemia: a report from the Children's Oncology Group. Blood 111:2984-90, 2008
189. Krajinovic M, Labuda D, Sinnett D: Glutathione S-transferase P1 genetic polymorphisms and susceptibility to childhood acute lymphoblastic leukaemia. Pharmacogenetics 12(8):655-8, 2002
190. Ansari M, Sauty G, Labuda M, et al: Polymorphisms in multidrug resistance-associated protein gene 4 is associated with outcome in childhood acute lymphoblastic leukemia. Blood 114:1383-6, 2009

191. Davies SM, Bhatia S, Ross JA, et al: Glutathione S-transferase genotypes, genetic susceptibility, and outcome of therapy in childhood acute lymphoblastic leukemia. Blood 100:67-71, 2002
192. Stanulla M, Schaeffeler E, Flohr T, et al: Thiopurine methyltransferase (TPMT) genotype and early treatment response to mercaptopurine in childhood acute lymphoblastic leukemia. JAMA 293:1485-9, 2005
193. Rocha JC, Cheng C, Liu W, et al: Pharmacogenetics of outcome in children with acute lymphoblastic leukemia. Blood 105:4752-8, 2005
194. Aplenc R, Glatfelter W, Han P, et al: CYP3A genotypes and treatment response in paediatric acute lymphoblastic leukaemia. Br J Haematol 122:240-4, 2003
195. French D, Yang W, Cheng C, et al: Acquired variation outweighs inherited variation in whole genome analysis of methotrexate polyglutamate accumulation in leukemia. Blood 113:4512-20, 2009
196. Dworzak MN, Fritsch G, Panzer-Grumayer ER, et al: Detection of residual disease in pediatric B-cell precursor acute lymphoblastic leukemia by comparative phenotype mapping: method and significance. Leuk Lymphoma 38:295-308, 2000
197. Lucio P, Gaipa G, van Lochem EG, et al: BIOMED-I concerted action report: flow cytometric immunophenotyping of precursor B-ALL with standardized triple-stainings. BIOMED-1 Concerted Action Investigation of Minimal Residual Disease in Acute Leukemia: International Standardization and Clinical Evaluation. Leukemia 15:1185-92, 2001
198. Weir EG, Cowan K, LeBeau P, et al: A limited antibody panel can distinguish B-precursor acute lymphoblastic leukemia from normal B precursors with four color flow cytometry: implications for residual disease detection. Leukemia 13:558-67, 1999
199. van Dongen JJ, Seriu T, Panzer-Grumayer ER, et al: Prognostic value of minimal residual disease in acute lymphoblastic leukaemia in childhood. Lancet 352:1731-8, 1998
200. Campana D, Neale GA, Coustan-Smith E, et al: Detection of minimal residual disease in acute lymphoblastic leukemia: the St Jude experience. Leukemia 15:278-9, 2001
201. Coustan-Smith E, Sancho J, Behm FG, et al: Prognostic importance of measuring early clearance of leukemic cells by flow cytometry in childhood acute lymphoblastic leukemia. Blood 100:52-8, 2002
202. Dworzak MN, Froschl G, Printz D, et al: Prognostic significance and modalities of flow cytometric minimal residual disease detection in childhood acute lymphoblastic leukemia. Blood 99:1952-8, 2002
203. Basso G, Veltroni M, Valsecchi MG, et al: Risk of relapse of childhood acute lymphoblastic leukemia is predicted by flow cytometric measurement of residual disease on day 15 bone marrow. J Clin Oncol 27:5168-74, 2009
204. Van der Velden VH, Corral L, Valsecchi MG, et al: Prognostic significance of minimal residual disease in infants with acute lymphoblastic leukemia treated within the Interfant-99 protocol. Leukemia 23:1073-9, 2009
205. Conter V, Arico M, Basso G, et al: Long-term results of the Italian Association of Pediatric Hematology and Oncology (AIEOP) Studies 82, 87, 88, 91 and 95 for childhood acute lymphoblastic leukemia. Leukemia 24:255-64, 2010
206. Willemse MJ, Seriu T, Hettinger K, et al: Detection of minimal residual disease identifies differences in treatment response between T-ALL and precursor B-ALL. Blood 99:4386-93, 2002
207. Szczepanski T, Pongers-Willemse MJ, Langerak AW, et al: Ig heavy chain gene rearrangements in T-cell acute lymphoblastic leukemia exhibit predominant DH6-19 and DH7-27 gene usage, can result in complete V-D-J rearrangements, and are rare in T-cell receptor alpha beta lineage. Blood 93:4079-85, 1999
208. Bruggemann M, van der Velden VH, Raff T, et al: Rearranged T-cell receptor beta genes represent powerful targets for quantification of minimal residual disease in childhood and adult T-cell acute lymphoblastic leukemia. Leukemia 18:709-19, 2004

209. Neale GA, Menarguez J, Kitchingman GR, et al: Detection of minimal residual disease in T-cell acute lymphoblastic leukemia using polymerase chain reaction predicts impending relapse. Blood 78:739-47, 1991
210. Dibenedetto SP, Lo Nigro L, Mayer SP, et al: Detectable molecular residual disease at the beginning of maintenance therapy indicates poor outcome in children with T-cell acute lymphoblastic leukemia. Blood 90:1226-32, 1997
211. Coustan-Smith E, Sandlund JT, Perkins SL, et al: Minimal disseminated disease in childhood T-cell lymphoblastic lymphoma: a report from the children's oncology group. J Clin Oncol 27:3533-9, 2009
212. Robillard N, Cave H, Mechinaud F, et al: Four-color flow cytometry bypasses limitations of IG/TCR polymerase chain reaction for minimal residual disease detection in certain subsets of children with acute lymphoblastic leukemia. Haematologica 90:1516-23, 2005
213. Bjorklund E, Mazur J, Soderhall S, et al: Flow cytometric follow-up of minimal residual disease in bone marrow gives prognostic information in children with acute lymphoblastic leukemia. Leukemia 17:138-48, 2003
214. Thorn I, Forestier E, Botling J, et al: Minimal residual disease assessment in childhood acute lymphoblastic leukaemia: a Swedish multi-centre study comparing real-time polymerase chain reaction and multicolour flow cytometry. Br J Haematol 152:743-53, 2011
215. Dworzak MN, Froschl G, Printz D, et al: CD99 expression in T-lineage ALL: implications for flow cytometric detection of minimal residual disease. Leukemia 18:703-8, 2004
216. Coustan-Smith E, Behm FG, Sanchez J, et al: Immunological detection of minimal residual disease in children with acute lymphoblastic leukaemia. Lancet 351:550-4, 1998
217. Coustan-Smith E, Sancho J, Hancock ML, et al: Clinical importance of minimal residual disease in childhood acute lymphoblastic leukemia. Blood 96:2691-6, 2000
218. Porwit-MacDonald A, Bjorklund E, Lucio P, et al: BIOMED-1 concerted action report: flow cytometric characterization of CD7+ cell subsets in normal bone marrow as a basis for the diagnosis and follow-up of T cell acute lymphoblastic leukemia (T-ALL). Leukemia 14:816-25, 2000
219. Gaipa G, Cazzaniga G, Valsecchi MG, et al: Time point-dependent concordance of flow cytometry and real-time quantitative polymerase chain reaction for minimal residual disease detection in childhood acute lymphoblastic leukemia. Haematologica 97:1582-93, 2012
220. Roshal M, Fromm JR, Winter S, et al: Immaturity associated antigens are lost during induction for T cell lymphoblastic leukemia: implications for minimal residual disease detection. Cytometry B Clin Cytom 78:139-46, 2010
221. Wu D, Sherwood A, Fromm JR, et al: High-throughput sequencing detects minimal residual disease in acute T lymphoblastic leukemia. Sci Transl Med 4:134ra63, 2012
222. Pui CH, Behm FG, Singh B, et al: Heterogeneity of presenting features and their relation to treatment outcome in 120 children with T-cell acute lymphoblastic leukemia. Blood 75:174-179, 1990
223. Haydu JE, Ferrando AA: Early T-cell precursor acute lymphoblastic leukaemia. Curr.Opin.Hematol. 20:369-373, 2013
224. Pui CH, Carroll WL, Meshinchi S, et al: Biology, Risk Stratification, and Therapy of Pediatric Acute Leukemias: An Update. J Clin Oncol. [epub ahead of print]:1, 2011
225. Gygi SP, Rochon Y, Franza BR, et al: Correlation between protein and mRNA abundance in yeast. Mol.Cell biol. 19:1720-1730, 1999
226. Maurer BJ, Dolai S, Delgado-Martin C, et al: Targeting the Jak/Stat signaling pathway is highly effective in xinograft models of early T cell precursor (ETP) acute lymphoblastic leukemia (ALL). Proc AACR:997, 2014
227. Tasian SK, Doral MY, Borowitz MJ, et al: Aberrant STAT5 and PI3K/mTOR pathway signaling occurs in human CRLF2-rearranged B-precursor acute lymphoblastic leukemia. Blood 120:833-842, 2012

228. Lacayo NJ, Alonzo TA, Gayko U: Single cell network profiling (SCNP)-based classifier to predict response to induction therapy in pediatric patients with de novo acute myeloid leukemia (AML): validation study results. Blood 118:3544, 2011
229. Lacayo NJ, Cohen A, Wesfall M: Single cell network profiling (SCNP) signatures predict response to induction therapy and relapse risk in pediatric patients with acute myeloid leukemia: a Children's Oncology Group (COG) study POG-9421. Blood 116:a954, 2010
230. Redell MS, Ruiz MJ, Gerbing RB, et al: FACS analysis of Stat3/5 signaling reveals sensitivity to G-CSF and IL-6 as a significant prognostic factor in pediatric AML: a Children's Oncology Group report. Blood 121:1083-1093, 2013
231. Kornblau SM, KR. C: Use of reverse phase protein microarrays to study protein expression in leukemia: technical and methodological lessons learned. In: Korf U, ed. Methods Mol Biol 785. Humana Press 141-142, 2011
232. Greenbaum D, Colangelo C, Williams K, et al: Comparing protein abundance and mRNA expression levels on a genomic scale. Genome Biol. 4:117, 2003
233. Jansen R, Greenbaum D, Gerstein M: Relating whole-genome expression data with protein-protein interactions. Genome Res 12:37-46, 2002
234. Kornblau SM, Tibes R, Qiu YH, et al: Functional proteomic profiling of AML predicts response and survival. Blood 113:154-164, 2009
235. Horton TM, Pati D, Plon SE, et al: A Phase 1 Study of the Proteasome Inhibitor Bortezomib in Pediatric Patients with Refractory Leukemia: a Children's Oncology Group Study. Clin Cancer Res 13:1516-1522, 2007
236. Horton TM, Sheehan A, Lopez-Terrada D, et al: Analysis of NF-B pathway proteins in pediatric Hodgkin lymphoma: correlations with EBV status and clinical outcome: A Children's Oncology Group (COG) Study. Lymphoma, 2012
237. Horton TM, Perentesis JM, Gamis AS, et al: A phase 2 study of bortezomib combined with reinduction chemotherapy in children and young adults with recurrent, refractory or secondary acute myeloid leukemia: A Children's Oncology Group (COG) study. Pediatr.Blood Cancer, 2014
238. Muscal JA, Thompson PA, Horton TM, et al: A phase I trial of vorinostat and bortezomib in children with refractory or recurrent solid tumors: a Children's Oncology Group phase I consortium study (ADVL0916). Pediatr.Blood Cancer 60:390-395, 2013
239. Niewerth D, Franke NE, Jansen G, et al: Higher ratio immune versus constitutive proteasome level as novel indicator of sensitivity of pediatric acute leukemia cells to proteasome inhibitors. Haematologica 98:1896-1904, 2013
240. Horton TM, Lu X, O'Brian MM, et al: Bortezomib reinduction therapy to improve response rates in pediatric ALL in first relapse: A Children's Oncology Group (COG) study (AALL07P1). Journal of Clinical Oncology 31:a10003, 2013
241. Schardt JA, Weber D, Eyholzer M, et al: Activation of the unfolded protein response is associated with favorable prognosis in acute myeloid leukemia. Clin.Cancer Res. 15:3834-3841, 2009
242. Ling SC, Lau EK, Al-Shabeeb A, et al: Response of myeloma to the proteasome inhibitor bortezomib is correlated with the unfolded protein response regulator XBP-1. Haematologica 97:64-72, 2012
243. Scriven P, Brown NJ, Pockley AG, et al: The unfolded protein response and cancer: a brighter future unfolding? J.Mol.Med. 85:331-341, 2007
244. Liu Y, Ye Y: Proteostasis regulation at the endoplasmic reticulum: a new perturbation site for targeted cancer therapy. Cell Res 21:867-883, 2011
245. Hetz C, Martinon F, Rodriguez D, et al: The unfolded protein response: integrating stress signals through the stress sensor IRE1alpha. Physiol Rev. 91:1219-1243, 2011
246. Niewerth D, Kaspers GJL, Jansen G, et al: Ratios of immunoproteasome over constitutive proteasome expression are an indicator for sensitivity to bortezmoib-onctainin rinduction chemotherapy in pediatric relaped ALL and AML. Proc AACR:LB-169, 2014

247. Oerlemans R, Franke NE, Assaraf YG, et al: Molecular basis of bortezomib resistance: proteasome subunit beta5 (PSMB5) gene mutation and overexpression of PSMB5 protein. Blood 112:2489-2499, 2008
248. Lu S, Chen Z, Yang J, et al: Overexpression of the PSMB5 gene contributes to bortezomib resistance in T-lymphoblastic lymphoma/leukemia cells derived from Jurkat line. Exp.Hematol. 36:1278-1284, 2008
249. Lee AS: GRP78 induction in cancer: therapeutic and prognostic implications. Cancer Res. 67:3496-3499, 2007
250. Li J, Ni M, Lee B, et al: The unfolded protein response regulator GRP78/BiP is required for endoplasmic reticulum integrity and stress-induced autophagy in mammalian cells. Cell Death.Differ. 15:1460-1471, 2008
251. Ron D, Harding H: eIF2-alpha phosphorylation in cellular stress responses and disease, in Hershey J, Mathews M, Sonenberg N (eds): Translational Control. Cold Spring Harbor, NY, Cold Spring Harbor Laboratory Press, 2007, pp 345-368
252. Kim R, Emi M, Tanabe K, et al: Role of the unfolded protein response in cell death. Apoptosis. 11:5-13, 2006
253. Schardt JA, Mueller BU, Pabst T: Activation of the unfolded protein response in human acute myeloid leukemia. Methods Enzymol. 489:227-243, 2011
254. Leleu X, Xu L, Jia X, et al: Endoplasmic reticulum stress is a target for therapy in Waldenstrom macroglobulinemia1. Blood 113:626-634, 2009
255. Tibes R, Qiu Y, Lu Y, et al: Reverse phase protein array: validation of a novel proteomic technology and utility for analysis of primary leukemia specimens and hematopoietic stem cells. Mol.Cancer Ther. 5:2512-2521, 2006
256. Cummings J, Raynaud F, Jones L, et al: Fit-for-purpose biomarker method validation for application in clinical trials of anticancer drugs. Br.J.Cancer 103:1313-1317, 2010
257. Dancey JE, Dobbin KK, Groshen S, et al: Guidelines for the development and incorporation of biomarker studies in early clinical trials of novel agents. Clin.Cancer Res. 16:1745-1755, 2010
258. Neeley ES, Kornblau SM, Coombes KR, et al: Variable slope normalization of reverse phase protein arrays. Bioinformatics. 25:1384-1389, 2009
259. Kornblau SM, Qutub A, Yao H, et al: Proteomic profiling identifies distinct protein patterns in acute myelogenous leukemia CD34+. PLoS.One. 8:e78453, 2013
260. Kornblau SM, Qutub A, KR. C: Hippo Pathway (HP) Activity In Acute Myelogenous Leukemia (AML): Different Prognostic Implications Of TAZ versus YAP Inactivation By Phosphorylation [abstract]. Blood 122:1337, 2013
261. Spurrier B, Ramalingam S, Nishizuka S: Reverse-phase protein lysate microarrays for cell signaling analysis. Nat.Protoc. 3:1796-1808, 2008
262. Holm S: A simple sequentially rejective multiple test procedure. Scandinavian Journal of Statistics 6:70, 1979
263. Kornblau SM, Minden MD, Rosen DB, et al: Dynamic single-cell network profiles in acute myelogenous leukemia are associated with pateint response to standard induction therapy. Clin Cancer Res 16:3721-3733, 2010
264. Kornblau SM, Qiu Y, Zhang N, et al: Proteomic Profiling In CD34+CD38- Stem Cells and CD34+ Cells From 185 Myelodysplasia Patients Using Reverse Phase Proteins Arrays (RPPA) Reveals Recurrent Proteins Expression Signatures with Prognostic Implications. Blood 116:2126a, 2010
265. Schewe DM, guirre-Ghiso JA: Inhibition of eIF2alpha dephosphorylation maximizes bortezomib efficiency and eliminates quiescent multiple myeloma cells surviving proteasome inhibitor therapy. Cancer Res 69:1545-1552, 2009
266. Ding WX, Ni HM, Gao W, et al: Linking of autophagy to ubiquitin-proteasome system is important for the regulation of endoplasmic reticulum stress and cell viability. Am.J.Pathol. 171:513-524, 2007

267. Ramadan K, Meerang M: Degradation-linked ubiquitin signal and proteasome are integral components of DNA double strand break repair: New perspectives for anti-cancer therapy. FEBS Lett. 585:2868-2875, 2011
268. Hu J, He X, Baggerly KA, et al: Non-parametric quantification of protein lysate arrays. Bioinformatics. 23:1986-1994, 2007
269. Dupuy A, Simon RM: Critical review of published microarray studies for cancer outcome and guidelines on statistical analysis and reporting. J.Natl.Cancer Inst. 99:147-157, 2007
270. Tibshirani R, Walther G, Hastie T: Estimating the number of data clusters via the gap statistic. J.R.Statist.Doc.B 63:10-20, 2001
271. Hu CW K, SM, Slater JH, Qutub AA.: Prototype Clustering: An improved stability-based approach to estimate the optimal number of clusters. Submitted
272. McShane LM, Radmacher MD, Freidlin B, et al: Methods for assessing reproducibility of clustering patterns observed in analyses of microarray data. Bioinformatics 18:1462-1469, 2002
273. benjamini Y, Hochberg Y: Methods of assessing reporducibiolity of clustering patterns observed in analysis of microarray data. Journal of the Royal Statistical Society B 57:289-300, 1995
274. Chih-Wei H, Chih-Jen L: A comparison of methods for multiclass support vector machines. Neural Networks, IEEE Transactions 13:415-425, 2002
275. Statnikov A, Wang L, Aliferis CF: A comprehensive comparison of random forests and support vector machines for microarray-based cancer classification. BMC.Bioinformatics 9:319, 2008
276. McCaffrey DF, Ridgeway G, Morral AR: Propensity score estimation with boosted regression for evaluating causal effects in observational studies. Psychol.Methods 9:403-425, 2004
277. Urbanczyk-Wochniak E, Willmitzer L, Fernie AR: Integrating profiling data: using linear correlation to reveal coregulation of transcript and metabolites. Methods Mol.Biol. 358:77-85, 2007
278. Quackenbush J: Microarray data normalization and transformation. Nat.Genet. 32 Suppl:496-501, 2002
279. Cox B, Kislinger T, Emili A: Integrating gene and protein expression data: pattern analysis and profile mining. Methods 35:303-314, 2005
280. Shankavaram UT, Reinhold WC, Nishizuka S, et al: Transcript and protein expression profiles of the NCI-60 cancer cell panel: an integromic microarray study. Mol Cancer Ther. 6:820-832, 2007
281. Perco P, Muhlberger I, Mayer G, et al: Linking transcriptomic and proteomic data on the level of protein interaction networks. Electrophoresis 31:1780-1789, 2010
282. Kornblau SM, Covey T, Putta S, et al: Signaling changes in the stem cell factor-AKT-S6 pathway in diagnostic AML samples are associated with disease relapse. Blood Cancer J 1:e3, 2011
283. Cohen AC, Huang YW, Cesano A, et al: Single-cell network profiling (SCNP) reveals age- and disease-based heterogeneity in healthy individuals and in patients with low risk (LR) myelodysplastic syndrome (MDS) [abstract]. Blood 118:a337, 2011
284. Coustan-Smith E, Mullighan CG, Onciu M, et al: Early T-cell precursor leukaemia: a subtype of very high-risk acute lymphoblastic leukaemia. Lancet Oncol. 10:147-156, 2009
285. Covey T, Putta S, Cesano A: Single cell network profiling: mapping drug and target interactions. assay drug devel technol 8:321-343, 2010
286. Mullighan CG, Downing JR: Global genomic characterization of acute lymphoblastic leukemia. Semin.Hematol. 46:3-15, 2009
287. Zhang J, Ding L, Holmfeldt L, et al: The genetic basis of early T-cell precursor acute lymphoblastic leukaemia. Nature 481:157-163, 2012
288. Cario G, Fetz A, Bretscher C, et al: Initial leukemic gene expression profiles of patients with poor in vivo prednisone response are similar to those of blasts persisting under prednisone treatment in childhood acute lymphoblastic leukemia. Ann Hematol 87:709-16, 2008
289. Lee MJ, Ye AS, Gardino AK, et al: Sequential application of anticancer drugs enhances cell death by rewiring apoptotic signaling networks. Cell 149:780-94, 2012

290. Van Vlierberghe P, Ferrando A: The molecular basis of T cell acute lymphoblastic leukemia. J Clin Invest 122:3398-406, 2012
291. Greco WR, Park HS, Rustum YM: Application of a new approach for the quantitation of drug synergism to the combination of cis-diamminedichloroplatinum and 1-beta-D-arabinofuranosylcytosine. Cancer Res 50:5318-5327, 1990